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Biology and Management of the fruit piercing moth *Serrodes partita* in citrus orchards

Submitted in fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

at

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by

Tapiwa Mushore

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Declaration

I confirm that all the work presented in this thesis is entirely my own, and it has not been previously submitted for examination at any other university. This thesis is being submitted to Rhodes University in fulfilment of the requirements for the degree of Doctor of Philosophy.

Tapiwa Mushore

[December 2023]

Dedication

In Loving Memory of my dear mother Maria Mushore

July 4, 1958 - August 26, 2023

To the woman whose unwavering belief and support ignited the flame of my academic journey, my dearest mother, Maria. You were my constant pillar of strength, motivating me to reach new heights even when faced with challenges. Your enduring faith in my abilities fuelled my perseverance, and it is with a heavy heart and profound gratitude that I dedicate this thesis to you.

"*Ukaita zvakanaka haungafarewo here*

chido chemwana kufadza mubereki.

Ruva rakanaka hariirarame, anopera rumwe rumwe

Ndangariro haibvisi hupenyu,

as inozadza mukaha.

Dai manga muripo ndinoziva manga muchazofara"

In your absence, I carry forward the lessons of resilience, determination, and the pursuit of excellence that you instilled in me. Though you are no longer with me in person, your spirit lives on in every page of this work. This achievement is not just mine but a testament to the sacrifices you made and the dreams you nurtured. It is because of you that I never lacked. You raised us well.

As I navigate the academic landscape, I will forever cherish the memories of your encouragement, love, and boundless support. This thesis stands as a tribute to the woman who shaped my aspirations and made the impossible seem within reach.

Abstract

The fruit-piercing moth, *Serrodes partita* (Fabricius) (Lepidoptera: Erebidae), is a polyphagous, multivoltine pest of citrus. This insect has a distinct geographical separation between its larval and adult stages, each with different feeding patterns. During the larval stage, it primarily acts as a forest defoliator, feeding mostly on Jacket plum, *Pappea capensis* Eckl. & Zeyh. (Sapindaceae). In contrast, the adult stage of this moth feeds on both tropical and subtropical fruit, including citrus and can cause serious economic losses. The adult moth uses its sclerotised proboscis to pierce the skin of ripening or ripe fruit, from which it extracts the juice. This piercing action initiates a fermentation process within the fruit, attracting other secondary-feeding moths, commonly referred to as fruit-sucking moths. As a result of the feeding activity, the affected fruit eventually rot, drop to the ground, and become unsuitable for the market. *Serrodes partita* exhibits an outbreak life strategy, reoccurring every 5 to 10 years. In South Africa's Eastern Cape Province, specifically in the Upper Kat River Valley, citrus growers have expressed concerns about the impact of this moth on soft citrus (Satsumas and Clementines). This raises the possibility of a shift in the population dynamics of *S. partita*, where these occurrences become more frequent and less sporadic. Such a trend poses a significant threat to fruit arboriculture in the Eastern Cape region.

Currently, there are limited management strategies available for managing fruit-piecing moths. The use of pesticides is not a feasible option for ripe or nearly ripe fruit, and is ineffective against the adult moth. Alternative control methods, such as orchard netting and light barriers, either come with high costs or are impractical for large-scale citrus production. Given the limited range of management options, combined with the moth's tendency for sudden outbreaks, citrus growers find themselves without effective means to manage this pest. The objective of this study was, therefore, to investigate the biology of *S. partita* and explore various control options to effectively manage this pest. Research focused on the biology and laboratory rearing of larval stages of *S. partita*. The flight behaviour, feeding patterns, and

preferences of adult *S. partita* within citrus orchards were also explored. The aim was to elucidate key fundamental aspects, including whether the same population frequents a particular orchard, and if infestations within orchards exhibit a specific direction. Lure type and lure presentation method trials were conducted to determine the most effective lure and trap design. Seasonal monitoring of *S. partita* in soft citrus orchards was conducted over three years to determine its outbreak status in the Committee's Drift area and the role of weather variables in the activity of the moth. Damage assessments were also conducted alongside monitoring to determine the level of damage inflicted by *S. partita*. Natural enemies associated with *S. partita* were explored to determine the prevalence and causes of mortality in late instars during laboratory rearing.

Rearing *S. partita* on an artificial diet was unsuccessful despite several modifications. The moth, however, completed its entire life cycle on its natural host, *P. capensis* in the laboratory. The total life cycle from egg to adult took 80.7 ± 3.6 days, the larval stage lasted 52.3 ± 2.8 days, and the pupal stage lasted 25.8 ± 3.6 days at 21°C. The investigation into the biology of *S. partita* also brought attention to the most susceptible stages of its growth, with high mortality rates recorded among neonates and late instars.

The findings of the study revealed directional patterns of moth infestations, with higher numbers observed at the orchard's periphery leading towards natural vegetation. This raises the prospect of using sacrificial rows on the edge of a citrus orchard to concentrate moth feeding damage during outbreak years. Using a mark and recapture technique, the study showed that a relatively small proportion (4.5 %) of moths tended to revisit the same orchard. The moths strongly preferred damaged fruit (85 %) over undamaged fruit. Visible damage (rotting symptoms) typically became apparent within 3 to 5 days. Satsumas had a higher number of feeding scars (2.1) than Clementines (1.08), highlighting their susceptibility. The study also established that, on average, pierced soft citrus fruit takes about four days to display symptoms of decay.

Synthetic proprietary Australian lures were ineffective at attracting the moth, whereas fresh bananas proved to be a successful lure. Furthermore, the addition of both Agar and Super absorbent polymer showed promise as thickening agents to enhance the longevity of fresh bananas in traps. The effectiveness of various trap designs was compared, including the funnel trap, delta trap, bucket trap, and circular trap, in capturing fruit-feeding moths. The funnel trap performed best as it captured the most moths, followed by the delta trap, Lynfield trap and disc trap, respectively. Additionally, an electronic enhancement to the funnel trap, incorporating a zapper element, improved efficiency. However, efforts to exploit both visual and olfactory cues through the inclusion of an Ultraviolet (UV) light component did not improve its effectiveness.

No extensive outbreaks were recorded during the study; however, population variations of *S. partita* populations were recorded. Annual trends showed two population peaks, with the first peak recorded from December to March, while the second peak was recorded from April to July. The activity of the moths also differed across different months, with the highest peaks recorded in May, while no moths were recorded from August to November. Both cultivar type and farm location did not influence the occurrence of the moth. Meanwhile cumulative weather parameters (rainfall, temperature and humidity) from the four months prior to occurrence influenced the activity of *S. partita*. Temperature determined the timing of the outbreak, while rainfall determined the magnitude of the outbreak. Damage assessment showed very low fruit damage by *S. partita* throughout the monitoring period. Varying levels of infestation by a tachinid fly, 4 % and 35 %, were recorded for 2021 and 2022, respectively. The tachinid parasitoid could not be identified at the species level. A novel baculovirus, tentatively classified as *S. partita* NPV (SepaNPV), was identified as the larval mortality causative agent. This study enhanced our understanding of *S. partita's* biology and population dynamics, providing valuable insights for developing effective management strategies against this economically impactful citrus pest. Future research should focus on refining control measures and addressing the challenges of the adult moth's elusive nature.

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List of Abbreviations

AGE	Agarose Gel Electrophoresis
°C	Degrees Celsius
µl	Microlitre
AICc	Akaike Information Criterion correction
ANOVA	Analysis of variance
CGA	Citrus Growers Association
cld	Compact letter display
cm	Centimetres
cm^2	Square centimetre
cm^3	Cubic centimetre
df	Degrees of freedom
DHARMA	Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models
DNA	Deoxyribonucleic Acid
EAD	Electroantennographic Detection
EIL	Economic Injury Level
ETL	Economic Threshold level
EU	European Union
FAO	Food Agriculture Organisation
g	Gram
GC-MS	Gas Chromatography-Mass Spectrometry
gDNA	Genomic deoxyribonucleic acid
GLM	General Linear Model
GLMM	Generalized Linear Mixed Model
glmmTMB	Generalized Linear Mixed Models using Template Model Builder

ha	Hectare
IPM	Integrated Pest Management
ITCZ	Inter Tropical Convergence Zones
km	Kilometre
L	Litre
lef-8	Late expression factor 8
lef-9	Late expression factor 9
LSD	Least Significant Difference
mg	Milligram
ml	Millilitre
MuMin	Multi-Model Inference
NPV	Nucleopolyhedrovirus
PCR	Polymerase chain reaction
polh/gan	Polyhedrin/granulin
rpm	Revolutions per minute
SDA	Sabouraud Dextrose Agar
SDS	Sodium dodecyl sulfate
UK	United Kingdom
USDA	United States Department of Agriculture
UV	Ultraviolet
w_i	Akaike Weights
ZAR	South African Rand

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CHAPTER 1

General introduction

Problem statement

The fruit-piercing moth, *Serrodes partita* (Fabricius) (Lepidoptera: Erebidae), is a polyphagous, multivoltine pest that poses a significant threat to citrus crops. The adult moth pierces the rind of ripe or ripening soft citrus fruit and sucks the juices, making the fruit vulnerable to secondary fungal infections, which cause the fruit to rot and eventually drop. In South Africa, pest occurrences have historically been sporadic, with outbreaks occurring at least once every decade. However, recent reports suggest a shift in occurrence from sporadic to frequent events, especially in citrus-growing regions of the Eastern Cape Province. This change in pest status may be due to environmental changes such as rising temperatures, lower rainfall, and extended fruiting seasons. Current pest management practices for fruit-piercing moth are useful only for small orchards but are ineffective for large-scale production. Given the significant value of citrus to the South African economy, there is a need to develop pest management solutions with a unique and competitive sustainable advantage. This literature review provides an overview of the South African citrus industry, which sets the context for the study, explores the concept of pest population dynamics and outbreak pests, and evaluates the impact of the fruit-piercing moth on citrus production. The review further synthesises existing research on the biology, ecology, and management strategies of fruit-piercing moth in South Africa. This study aims to gain insight into the biology and ecology of *S. partita*, particularly its population dynamics and flight behaviour, to develop adaptable pest management strategies that can be used in large-scale citrus production.

1.1 The South African citrus industry: past, present and future perspectives

1.1.1 Brief history

The South African citrus industry boasts a rich history that dates back to the 17th century when European settlers, led by Jan van Riebeek, were sent to the Cape to establish a Fort and plant a garden to provide fruit and vegetables for the East India Company. The industry remained small until the 19th century when settlers planted individual orange and "naartjie" (soft citrus) trees in the Eastern Cape and what was then Natal. The industry experienced a significant boost in 1850 when the first budded trees were imported from Brazil, later grafted by a Grahamstown farmer, and distributed throughout the Cape Province. This marked the second stage of development and the beginning of commercial cultivation. In 1875, Cecil John Rhodes, the then Cape Minister, brought in trained horticulturists from California to further develop the industry (Mather 1999).

Mather & Greenberg (2003) and Ndou (2012) provided an overview of the history of the citrus industry in South Africa. The citrus industry initially progressed slowly, but after World War I, it began to grow significantly. Between 1920 and 1930, over 500,000 Valencia late orange trees were planted, with the English market serving as the primary consumer. The World War II outbreak significantly impacted the industry and prompted the establishment of the Citrus Board in 1939 to assist farmers. The sector was revitalised post-war as the United Kingdom (UK) markets turned to South Africa for citrus exports. The Citrus Board established the Citrus Exchange to further facilitate fruit export. A return on investment was realised as the money from grower levies was used to establish a nursery system, and research and extension officers were stationed in major growing regions. The single-channel export system that the Citrus Exchange provided gave southern African citrus a dominant position in the overseas market, enabling it to supply the right fruit to the right market.

In 1997, the Marketing of Agricultural Products Act No 46 was implemented, promoting market deregulation. While this led to a focus on quality in line with overseas demands, it initially had negative consequences for the citrus industry (Ndou 2012). Several marketing agents offered higher returns and quicker payments, prompting farmers to leave the Citrus Exchange facility. This led to market fragmentation and, ultimately, loss of market power overseas (Mather & Greenberg 2003). To support growers in research, production, marketing, and market access, the Citrus Growers Association (CGA) was formed in 1997, representing 15 grower regions in South Africa, Zimbabwe, and Eswatini, with fruits from the latter two countries exported through South Africa. This initiative again unified the industry, enabling southern African citrus to strengthen its position in export markets.

1.1.2 Citrus production and export

Citrus cultivation has spread throughout South Africa, particularly in Limpopo, Eastern Cape, Western Cape, Mpumalanga, and KwaZulu-Natal (CGA 2022). This growth was driven by the attractive return on investment, especially for lemons and easy peelers, which offer high profit margins. South Africa emerged as one of the leading citrus exporting countries globally, with the industry expected to play a significant role in creating jobs in the agriculture sector (Genis 2017). Notably, the industry has experienced remarkable growth in the past decade, with exports increasing from 1.5 million tonnes in 2012 to 2.5 million tonnes in 2021 (World Citrus Organisation 2022). The sector supplies a wide range of citrus varieties within the following types: orange, soft citrus, lime, and grapefruit (CGA 2022). Citrus trees are grown across various regions in southern Africa, and each region has unique edaphic and climatic conditions (Bedford 1998, Zekri 2011). The country's diverse climatic conditions allow a wide range of citrus products to be produced over extended periods. Limpopo, Mpumalanga, Eastern Cape, and Western Cape are the main production areas in South Africa, while Zimbabwe and Eswatini have smaller citrus production volumes. Production varies significantly based on climate, with the cooler growing regions in the Western and Eastern Cape focused on Navel

oranges and lemons (CGA 2022). Citrus production for export is in its infancy in Botswana and Namibia.

South Africa has over 100,000 hectares of citrus orchards, with oranges making up 47 % of the planted area. Tangerines/Mandarins (soft citrus), lemons, and grapefruit account for 26 %, 18 %, and 9 %, respectively (CGA 2022). Oranges are the country's main citrus export, constituting 51 % of the total, while soft citrus accounts for 21 %, with lemons and grapefruit making up the rest. Soft citrus production has experienced significant growth in recent years due to higher global demand and more favourable profit margins than other citrus types. Most of the soft citrus production takes place in the Western Cape (37 %), Limpopo (28 %), and Eastern Cape (25 %) (Chisoro-Dube & Roberts 2021, CGA 2022). Despite being able to respond to consumer demand for soft citrus, growers face numerous challenges that affect other citrus varieties as well, such as high input costs, shipping expenses, and inadequate infrastructure. Additionally, there are concerns about weakening demand in critical soft citrus markets such as the European Union (EU) and the UK, owing to inflationary pressures on consumers and sluggish economic growth (USDA 2022). The situation could be exacerbated by pest outbreaks specific to soft citrus. Each citrus-growing region has its own set of major and minor pests, which are primarily influenced by prevailing climatic conditions, particularly temperature and humidity (Skendžić et al. 2021). Citrus pest populations vary by cultivar type and growing region due to differences in environmental factors and the presence of natural enemies (Bedford 1998, Grout & Moore 2015).

1.2.3 South African citrus pests

Severe pest infestations can permanently damage trees and may result in significant loss of productive orchards. Moreover, damaged fruit is less desirable for the market, particularly in South Africa, where citrus is export-oriented, and consumers expect fruit with excellent external quality and few blemishes (Grout & Moore 2015). To successfully manage pests, it is crucial to have a comprehensive understanding of their strategies. Citrus is vulnerable to

different pests at different stages of its growth, particularly at flowering and fruiting stages. In fruit crops like citrus, pest behaviour is best understood within injury guilds (Klem & Zaspel 2019).

One of the insect pest guilds found in citrus is the stand reducers/defoliators, which cause an immediate loss of biomass and decrease the plant's photosynthetic ability (Boote 1983, Pedigo & Rice 2009, Klem & Zaspel 2019). An example of a significant stand reducer associated with citrus is the Citrus thrips, *Scirtothrips aurantii* Faure (Thysanoptera: Thripidae). It attacks new growth, causing leaf deformation and severely curtailing leaf development, as the damaged leaf curls and twists at high thrips population densities (Bedford 1998). Certain insects' consumption of leaf mass can have a detrimental impact on the absolute photosynthetic potential of the leaf canopy, directly affecting the plant's growth (Boote 1983, Pedigo & Rice 2009). This group of pests includes leaf-rolling larvae, which cause the leaves to curl, providing shelter from predators and adverse conditions and leaf miners, which feed between the inner and lower tissues of the leaf. Biting and chewing insects that feed directly on the leaves are also included (Johnson & Tripplehorn 2005, Kadioglu et al. 2012). The larvae of the citrus swallowtail butterfly, *Papilio demodocus* Esper (Lepidoptera: Papilionidae), are a prime example of leaf-mass consumers, as they chew on the leaves, reducing the plant's ability to photosynthesise and stunting its growth. In cases where the infestation is severe, trees may not recover, although in most instances, they do (Table 1.1) (Bedford 1998).

Table 1.1: Summary of South African citrus pests and their respective feeding guilds (Bedford 1998, Grout & Moore 2015).

Injury Guild	Order/Family	Common name	Species name	Other guilds[1]
Stand reducers	**Orthoptera**			
	Pyrgomorphidae	Bush stink locust	*Phymateus leprosus* Fabricius	LC
		Elegant grasshopper	*Zonocerus elegans* Thunberg	LC
	Hemiptera			
	Coreidae	Large black tip wilter	*Anoplocnemis curvipes* Fabricius	LC, FC
	Diaspididae	Red scale	*Aonidiella aurantii* Maskell	
		Circular purple scale	*Chrysomphalus aonidum* L.	
		Punnule scale	*Chrysomphalus pinnulifer* Maskell	
		Black thread scale	*Ischnaspis longirostris* Signoret	
		Citrus mussel scale	*Lepidosaphes beckii* Newman	AS, FF
		Long mussel scale	*Lepidosaphes gloverii* Packard	AS, FF
	Coccidae	White wax scale	*Ceroplastes breviacuda* Hail	AS
		Citrus wax scale	*Ceroplastes destructor* Newstead	AS
		Green soft scale	*Pulvinaria aethiopica* De Lotto	AS
		Black scale	*Saissetia olieae* Oliver	AS
Leaf consumers	**Coleoptera**			
	Chrysomelidae	Blue-green citrus beetle	*Colasposoma fulgidum* Lefevre	FF
	Curculionidae	Fullers rose beetle	*Pantomorus cervinus* Boheman	FF
	Lepidoptera			
	Totricidae	Apple leaf roller	*Lozotaenia capensana* Walker	FF
		Citrus leafminer	*Phyllocnistis citrella* Stainton	FF
	Gracillariidae	Mocker swallowtail	*Papilio dardanus* Stoll	
	Papillionidae	Citrus swallowtail	*Papilio demodocus* Esper	

		Green-banded swallowtail	*Papilio nireus lyaeus* Doubleday	
Assimilate sappers	**Hemiptera**			
	Flatidae		*Decipha* sp.	FF
	Cicadellidae	Green citrus leafhopper	*Empoasca distinguenda* Paoli	FF
		Citrus leafhopper	*Penthimiola bella* Stal	FF
	Triozidae	Citrus triozid	*Trioza erytreae* Del Guerico	
	Aleyrodidae	Spiny blackfly	*Aleurocanthus spiniferus* Quaintance	
		Spiny blackfly	*Aleurocanthus zizyphi* Priesner & Hosny	
		Citrus blackfly	*Aleurocanthus woglumi* Ashby	
		Woolly whitefly	*Aleurocanthus floccosus* Maskel	
	Aphididae	Cotton aphid	*Aphis gossypii* Glover	
		Spriea aphid	*Aphis spiraecola* Patch	
		Brown citrus aphid	*Toxoptera aurantii* Boyer de Fonsocolombe	
		Black citrus aphid	*Toxoptera citricida* Kirkaldy	
	Monophlebidae	Cottony cushion scale	*Icerya purchasi* Maskell	
	Pseudococcidae		*Delottococcus aberiae* De Lotto	FF
		Stripped mealybug	*Ferrisia virgata* Cockerell	FF
		Karro thorn mealybug	*Nipaecoccus viridis* Newstead	FF
		Oleander mealybug	*Paracoccus burnerae* Brian	FF
		citrus mealybug	*Planococcus citri* Risso	FF
		citrophilus mealybug	*Pseudococcus calceolariae* Maskell	FF
		long-tailed mealybug	*Pseudococcus longispinus* Targoni Tozzetti	FF
	Thysanoptera			
	Thripidae	Greenhouse thrips	*Heliothrips haemorrhoidalis* Bouche	FF
		Citrus thrips	*Scirtothrips aurantii* Faure	FF
	Arachnida: Acari	Citrus bud mite	*Aceria sheldoni* Ewing	SR, FF
		Red spider mite	*Tetranychus urticae* C L Koch	SR, FF
		Citrus red mite	*Panonychus citri* McGregor	SR, FF
		Grey mite	*Calcareous citrifolia* Keifer	SR, FF

		Silver mite	*Phyllocoptruta oleivora* Ashmead	SR, FF
Blossom and Fruit Feeders	**Lepidoptera**			
	Totricidae	False codling moth	*Thaumatotibia leucotreta* Meyrick	
	Praydidae	Citrus flower moth	*Prays citri* Milliere	
	Pyralidae	Carob moth	*Ectomyelois ceratoniae* Zeller	
	Geometridae	Citrus looper	*Ascotis reciprocaria* Walker	LC
	Noctuidae	Fruit-piercing moth	*Serrodes partita* Fabricius	
		Fruit-sucking moth	*Achaea lienardi* de Biosduval	
		African Bollworm	*Helicoverpa armigera* Hübner	LC
	Diptera			
	Tephritidae	Oriental fruit fly	*Bactrocera dorsalis* Hendel	
		Mediterranean fruit fly	*Ceratitis capitata* Wiedmann	
		Natal fruit fly	*Ceratitis rosa* Karsch	

Other guilds: AS = Assimilate sappers; LC = Leaf-mass consumers; FF = fruit feeders; SR Stand reducers.

Assimilate-sappers are a common group of pests consisting of piercing-sucking insects that extract plant nutrients before they are translocated to plant tissues (Boote 1983, Pedigo et al. 1986). Some sappers may inject toxic substances into the plant to facilitate feeding. For example, the red spider mite *Tetranychus urticae* Koch (Acarina: Tetrachanyidae) is a typical citrus sapper that is particularly troublesome in the hot and dry summers of the Western Cape region (Bedford 1998). Other examples of sappers include the Woolly whitefly *Aleurothrixus floccosus* Maskell (Hemiptera: Aleyrodidae) and the cotton aphid *Aphis gossypii* Glover (Hemiptera: Aphididae) (Grout & Moore 2015).

The fruit-feeding guild is another significant group of pests that occur on citrus. Fruit-feeding pests can directly damage the host plant's reproductive organs, such as buds, flowers, and fruits (Pedigo & Rice 2009, Klem & Zaspel 2019). Fruit flies, such as the oriental fruit fly *Bactrocera dorsalis* Hendel, the Mediterranean fruit fly *Ceratitis capitata* Wiedemann, and the Natal fruit fly *Ceratitis rosa* Karsch (Diptera: Tephritidae), puncture fruits and deposit eggs, leading to fruit decay due to maggot feeding and secondary bacterial infection (Grout & Moore 2015). Another agriculturally significant group of fruit feeders are the fruit-piercing moths, which have a specially adapted proboscis to pierce fruits and suck the juice. This pest behaviour is unique in the fruit-feeding guild (Denton et al. 1989, 1990, 1999).

1.2 Dynamic patterns and ecological impacts of outbreak pests

1.2.1 Classification of pest based on occurrence frequency

Pests can also be categorised based on their frequency of occurrence as regular or outbreak pests. Regular pests occur more frequently and are associated with a particular plant species. On the other hand, outbreak pests show rapid changes in densities periodically, causing

extensive damage to their host plants (Price et al. 2011). Moreover, outbreaks of pests are often characterised by spatial synchronisation of oscillations among geographically separated populations (Liebhold et al. 2004, Johnson & Tripplehorn 2005). This suggests that outbreaks are driven by environmental perturbations known as the "Moran" effect (Haynes et al. 2013). The frequency of outbreaks varies among species, with some exhibiting cyclic outbreaks every 8-11 years, while others have longer cycles lasting 28-35 years (Myers 1998, Price et al. 2011). Outbreak phases are relatively short-lived and less frequent compared to non-outbreak phases (Price & Hunter 2005). The duration of outbreaks varies among different pests, lasting only a few weeks in the case of *Spodoptera exempta* Walker (Noctuidae: Lepidoptera) and several months for the Desert locust *Schistocerca gregaria* Forsskål (Orthoptera: Acrididae) (Rose et al. 1995, Adhikari 2020).

1.2.2 Phases of pest outbreaks

Outbreak pest population dynamics are never stable as they exhibit different forms of equilibrium points of cyclic oscillations between two points (low and high) (Wallner 1987). Elkinton & Liebhold (1990) proposed four phases during an outbreak: innocuous, release, outbreak and decline. The innocuous phases are characterised by a very low population in a given location at a given time, making individuals scarce to encounter between outbreaks. The release phase is when the population density starts to increase over time. Depending on the insect pest, this may take a few months to a few years.

Eventually, the population reaches a threshold where the damage becomes evident on a large scale. Outbreaks tend to occur synchronously with high pest densities across vast geographical areas (Speight et al. 2008). During the outbreak phase, pests exhibit prolific reproduction, and in some cases, density-dependent polymorphism is observed. The African armyworm, *Spodoptera exempta*, displays passive and active phases that differ in movement, growth, and reproduction. The passive phase has slow growth and sluggish movement, enabling survival

during dry seasons, while the active phase is highly mobile, voracious, and matures rapidly, often leading to outbreaks (Rose et al. 1995, Bouaichi & Simpson 2003).

1.2.3 Factors influencing pest outbreaks

1.2.3.1 The Allee effect

Pest outbreaks are influenced by different factors on multiple scales (Taylor & Hastings 2005). The establishment of initial colonies before an outbreak occurs is influenced by the Allee effect, as described by Allee (1932). Species subject to the Allee effect experience reduced fitness when population density is low (Tobin et al. 2009). The impact of the Allee effect on outbreak pests is particularly pronounced during the innocuous phase, characterised by low population densities. At such low densities, the likelihood of successful mating for females is significantly diminished, increasing the risk of local extinction even if no management strategies are implemented (Tobin et al. 2012). In addition to inbreeding depression, certain insect species employ cooperative feeding techniques, like mass attacks, to overcome plant defence strategies (Raffa & Berryman 1983, Raffa et al. 2008). Failure to overcome these defences triggers the Allee effect, as demonstrated by the mass feeding behaviour of the Spruce bark beetle *Ips typographus* L. (Coleoptera: Curculionidae). When the density of beetle attacks is low, trees respond by confining the beetles within necrotic lesions that contain toxic compounds. However, once the attacks surpass a critical level, the tree's defensive capacity becomes depleted (Mattson & Haack 1987, Haack 2001).

1.2.3.2 Natural enemies

Natural enemies play a crucial role in regulating outbreak dynamics, although determining cause and effect between natural enemies and outbreak pests can be challenging. Nevertheless, examining the relationship between insect populations and natural enemy changes can provide valuable insights into how they interact (Price et al. 2011, Schowalter 2011). Typically, insect herbivore populations peak first, followed by a peak in natural enemies with a time lag of at

least one generation. The length of this lag phase can drive populations up or down, depending on the strength of the herbivore-predator association (Begon et al. 2006). However, complex interactions often contribute to outbreaks. For example, Price (2003) proposed that herbivorous insects are caught between the "devil" of natural enemies and the "blue sea" of plant food and nutrients.

1.2.3.3 Host plants

While many studies have focussed on the vertical interaction between natural enemies and herbivores, significant horizontal interactions within trophic levels play a role in plant-herbivore interactions (Wool 2002, Price 2003, Price et al. 2011). Host plants influence the occurrence of outbreaks through variations in food quality and quantity, as well as through indirect effects on herbivores. For example, the winter moth *Operophtera brumata* L. (Lepidoptera: Geometridae) feeds on young foliage, which has phenology adapted to maximise larval survival by providing nitrogen-rich food. Nitrogen availability is critical to female winter moths' growth rate and fecundity (Kaplan & Denno 2007). In addition to direct effects on herbivores, host plants can indirectly affect pest populations through their phytochemicals. For instance, the Gypsy moth *Lymantria dispar* L. (Lepidoptera: Erebidae) is known to preferentially attack oak trees, which have high concentrations of tannins and phenolic compounds that help to inhibit the Gypsy moth nucleopolyhedrovirus, resulting in higher survival rates of the moth larvae on oak trees compared to other tree species (Foster et al. 1992, Foster & Harris 1997).

1.2.3.4 Weather influence

Rainfall patterns and seasonal temperatures are known to determine the distribution of organisms in space. Weather parameters affect the development rate, fitness level of activity, phenology, distribution, size and continuity of insect pests (Altieri et al. 1984, Haynes et al. 2013). Temperature is the most determining factor in outbreaks as insects are poikilothermic (derive body temperature from the environment). They have a temperature range known as the

tolerable zone within which they can survive optimally, with different optimal temperature ranges for various functions (Carbognin et al. 2023). Exposure to temperatures at the extremes of this zone can lead to mortality, with most insects having an upper temperature tolerance between 40 to 50°C. While some pests adapt to hot, dry conditions through summer diapause, the absolute minimum temperature for insect survival is likely below -30°C. Temperature influences insect function, lifespan, and flight behaviour and can affect wind patterns, influencing insect dispersion and, ultimately, outbreaks (Haynes et al. 2014). Meanwhile, the moisture content in insect habitats directly affects their survival and indirectly influences pest populations by impacting crop growth. Environmental moisture, including atmospheric humidity and rainfall, affects pest water balance and susceptibility to diseases, with wet conditions facilitating the spread of pathogens. Heavy rainfall can cause mortality directly or indirectly, while rain also alters host susceptibility to wind-borne insects and disease vectors (Wallner 1987, Zheng et al. 2011, Prasad et al. 2013).

1.2.4 Migration patterns of outbreak pests

Outbreaks often lead to deteriorating conditions, such as a resource shortage, triggering insect migration. Migration is an evolutionary pattern gained over time under regular driving forces in a given space (Chapman et al. 2011). This phenomenon involves the movement of insects from one area to another in search of food or more suitable breeding grounds and is observed not only in outbreak pests but also in many regular field crop pests (Cant et al. 2005). There are two types of migration flights: appetitive and non-appetitive. Appetitive flight is observed in insects that fly solely to search for resources, mates, or oviposition sites. It terminates once the sought-after resource is encountered or they need to escape predators (Chapman et al. 2011). Non-appetitive migratory flights, on the other hand, are persistent, non-meandering, undistracted, and commonly observed in outbreak pests (Sappington 2018). The termination of migration is based on environmental cues, such as the onset of dusk/dawn or endogenous signals (Begon et al. 2006). Once the migratory flight is terminated, appetitive behaviour is no longer suppressed, and the insect begins searching for resources (Price et al. 2011).

The level of migration in insects is often influenced by factors such as population densities and food availability. Butterflies and moths have segregated larval and adult habitats and are more mobile than other species (Farrow & McDonald 1987). The fall armyworm *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) is incapable of diapause and breeds continuously throughout the year. It migrates from winter breeding sites to locations as far as 3000 km away (Gao et al. 2020). On the other hand, monarch butterflies are capable of diapause and often have a residual population at a localised breeding ground. They migrate and spread outward due to their high mobility and fecundity (Drake & Reynolds 2012, Gao et al. 2020). Insects with low dispersal ability tend to have short and restricted outbreaks, although in some cases, wind can aid their migration. For instance, the green peach aphid *Myzus persicae* Sulzer (Hemiptera: Aphididae) uses airborne migration to move long distances, although most move locally (Ahmed et al. 2019). Understanding migration patterns, sources, and pathways can lead to better forecasting and control of outbreak pests (Hu et al. 2020).

1.3 Fruit-feeding moths: fruit-piercing and fruit-sucking moths

Fruit-feeding moths in the Family Erebidae are significant agricultural pests found throughout the tropical and subtropical regions in Africa, Southeast Asia, Australia, and the Pacific islands (Bänziger 1982, Leong & Keuh 2011). These moths feed on various ripe or ripening fruits, including citrus, pomegranates, kiwis, figs, bananas, peaches, apples and pears (Hargreaves 1936, Cochereau 1969, Bänziger 1982, Leroy et al. 2021). Jack (1922) proposed two different categories of fruit-feeding moths, fruit-sucking moths (unmodified proboscis) and fruit-piercing moths (modified proboscis). Fruit-piercing moths, as defined by Bänziger (1982), are species that can create holes through the skin or rind of fruits. Klem & Zaspel (2019) and Leroy et al. (2021) proposed three categories, based on a species-piercing ability, to classify fruit-piercing moths. The first category comprises moths that can pierce thin, soft-skinned fruit, such as tomatoes, and the second category includes moths that can pierce soft-skinned fruits, such

as guavas. The final category comprises moths with strong and sclerotised proboscises, enabling them to penetrate hard-skinned fruit like oranges. The host range of these moth species is determined by their piercing ability (Leroy et al. 2021).

These pests were observed damaging citrus fruits as early as 1869, according to the observations made by the French botanist Thozet (Baptist 1944). Since then, fruit-piercing moths have received considerable attention as pests of economic importance due to improved fruit varieties with soft rind, more intensive cultivation methods in orchards, and the destruction of natural forests (Bänziger 1982, Klem & Zaspel 2019). Fruit-piercing moths are unique among agricultural pests in their mode of injury, which is specialised and distinct from other pests (Denton et al. 1990, Leroy et al. 2021). Unlike most other lepidopteran pests, fruit-piercing moths cause damage to the harvestable yield during the adult stage rather than the larval stage (Leong & Kueh 2011). Fruit-piercing moths exclusively attack fruit and do not harm any other part of the plant that produces the fruit (Zaspel et al. 2016)).

1.3.2 Systematics and nomenclature

The Superfamily Noctuoidea (pest clade) comprises six strongly supported groups (Families), namely Oenosandridae, Notodontidae, Eutelidae, Nolidae, Noctuidae, and Erebidae (Mitchell et al. 2005, Zahiri et al. 2012). The classification of Noctuoidea is based on four principal divisions that rely on the position of vein M2 on the forewing and wing venation (Miller 1991). The first group (trifid) is based on the proximal marginal vein's origin of the M2 vein. In the second group (quadrifid), the M2 originates closer to vein M3 and includes the Family Erebidae (Mitchell et al. 2000, Fibiger & Lafontaine 2005, Lafontaine & Fibiger 2006). Zahiri et al. (2011) discovered 18 well-supported clades (subfamilies) within this family. The subfamily Erebinae, the most taxonomically complex and species-rich clade, possesses tympanic organs that detect echolocating bats and other predators (Fullard & Napoleone 2001). The current classification of Erebinae follows the molecular phylogenetic classification by Zahiri et al. (2012), who used eight synapomorphies from previous studies by Fibiger & Lafontaine (2005)

and Lafontaine & Fibiger (2006) as diagnostic features. Zahiri et al. (2012) identified 19 tribes in the subfamily Erebinae.

1.3.2 Fruit-piercing moths in Southern Africa

Fruit-piercing moths were initially documented in southern Africa in the early 20th century (Jack 1916, 1922). While approximately 30 species of fruit-feeding moths exist in the region, only ten species from specific genera, including *Eudocima*, *Oraesia*, *Egybolis*, *Pericyma*, and *Serrodes*, have been identified as primary fruit-piercers (Bedford 1998, Johannsmeier 1998, Goddard et al. 2019). Among these, the genus *Eudocima* comprises three species, with *Eudocima phalonia* Clerk predominantly feeding on citrus across various regions including the Eastern Cape, KwaZulu-Natal, and Lowveld in South Africa, as well as Namibia, Botswana, and Zimbabwe (Neubecker 1966, Johannsmeier 1998). *Eudocima divitiosa* Walker is found in KwaZulu-Natal and Mpumalanga provinces in South Africa, with reports of occurrence in East and West Africa. Additionally, *E. materna* L. has a wide distribution spanning South Africa, Ethiopia, South Asia, and Australia (Pinhey 1975, Leroy et al. 2021). The activity of Eudocima spp. in commercial citrus orchards is very limited in South Africa (Johannsmeier 1998).

The genus *Oraesia,* formerly known as *Calpe*, comprises three species: *Oraesia provocans* Walker, *O. triobliqua* Saalmüller, and *O. emarginata* F.. These species have been observed feeding on citrus in Zimbabwe and South Africa, ranging from the Western Cape to Mpumalanga through the Eastern Cape and KwaZulu-Natal provinces (Bosch 1971a, Johannsmeier 1976). Another genus, *Egybolis*, is represented by a single species in southern Africa: *Egybolis vaillantina* Stoll, which feeds on deciduous fruit, including citrus, in KwaZulu-Natal (Johannsmeier & Kok 1978). The fourth genus of fruit-piercing moth, *Pericyma*, includes *Pericyma artifusa* Hampson, *P. mendax* Walker, and *P. scandulata* Felder & Rigenhofer. While these moths have been reported feeding on citrus in West Africa, they have not been observed doing so in southern Africa. They are found in KwaZulu-Natal and Mpumalanga in South Africa, as well as in Zimbabwe and Botswana.

1.3.3 Target pest: *Serrodes partita*

The genus *Serrodes* is represented in South Africa by the fruit-piercing moth *S. partita,* a polyphagous, multivoltine fruit pest of tropical and deciduous fruit (Jack 1916, Swart 1969, Johannsmeier 2001, Moore 2010). *Serrodes partita* has a narrow adult host range in comparison with other fruit-piercing moth species. It is regarded as a soft fruit piercer as it lacks heavily sclerotised proboscis in comparison with *Eudocima* sp. (Goddard et al. 2019). *Serrodes partita* is only considered a major pest in southern Africa on pears, apples, peaches, apricots, and nectarines (Kriegler 1958). The other species of *Serrodes*, *S. campana* has been recorded in other areas feeding on fruit, albeit in low numbers. In most cases it is often found co-occurring with other fruit piercing moths (Cochereau 1977, Fay & Halfpapp 2006).

The species has been known by different names, *S. inara* Cramer (1979), *S. campana* Guene (1852), *Ophiusa bassignum* Walker (1858) and *S. campana* (Pinhey 1975, Johannsmeier 1998). *Serrodes* is an old-Tropics genus of Indo-Malayan, Australian and African descent (Holloway 2005, Zahiri et al. 2023). It is within the subfamily Erebinae in the tribe Cocytiini with four other genera *Averuthina*, *Ophyx, Avatha* and *Cocytia* (Holloway 2005, Zahiri et al. 2011, 2012). The genus was described by Moore (1887), based on *S. campana* as the type species. The adult moth has a distinctive forewing pattern, consisting of three sections: a straight, carved postmedial line, a large paler area with a component of a dark triangle, and a grey basal section. The hindwing is dull with a more uniform underside. Male moths have ciliate antennae and strongly tufted tibiae, while female moths have setaceous antennae and thick legs with dense pilose (Holloway 2005).

1.3.2 Distribution of *Serrodes*

Serrodes sp. occur in southern, western, eastern and central Southern Africa, with records from India, Sri Lanka, Indonesia and Australia (Figure 1.1) (Hargreaves 1936, Golding 1946, Grout

& Moore 2015). It is found in most parts of South Africa, where the larvae develop on wild host plants in the Karoo (Johannsmeier 1998).

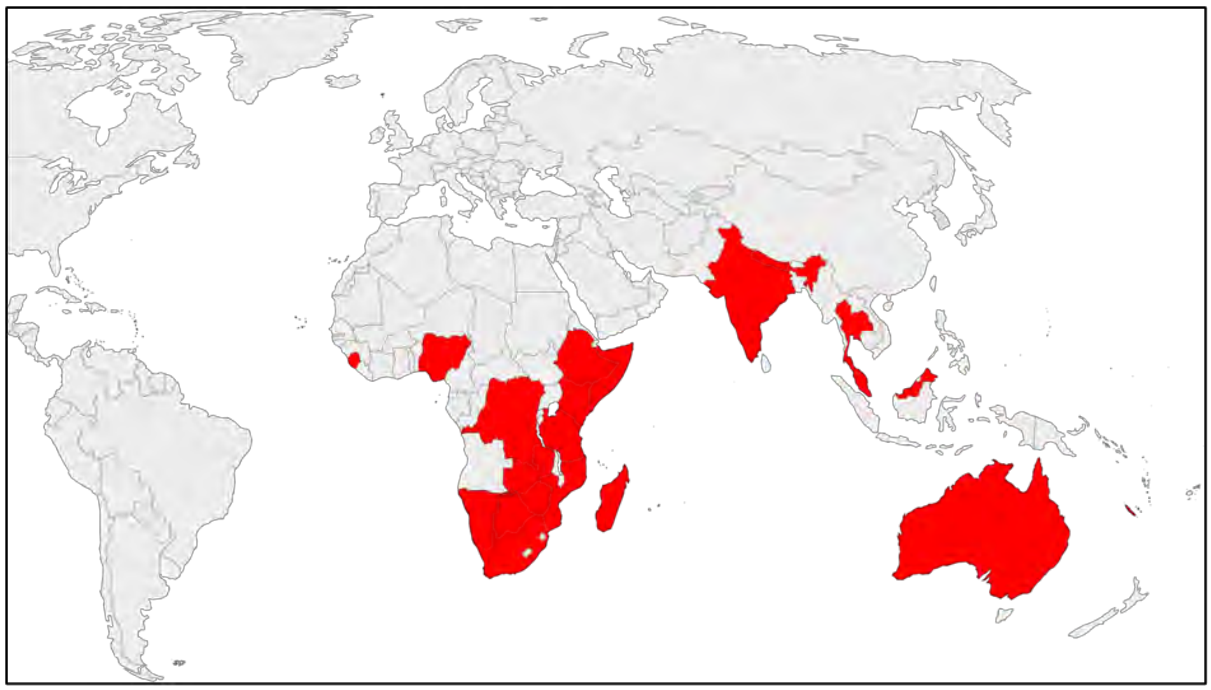


Figure 1.1: Distribution of the genus *Serrodes* (Hargreaves 1936, Golding 1946, Pinhey 1975, Johannsmeier 1998, Grout & Moore 2015, Zahiri et al. 2023).

1.3.3 Biology of *Serrodes partita*

Serrodes partita moths are nocturnal with pale brown-grey forewings with three characteristic black spots that form a thin broken "V" or triangular shape near the base of the wing (Johannsmeier 1998). The forewing has a straight postmedial line with a tooth on the outer margin, which is not distinct (Figure 1.2a). It has sub-basal dark red-brown spots on the costa with a line from its lower edge. The hindwing is pale yellowish-brown. The eggs are laid singly or in batches on the back of host plants, where they hatch into neonates (Figure 1.2d). The moth takes 83 – 91 days to develop from egg to adult. The eggs take two to three days to hatch. The adult has a life span of 53 days (Jack 1922, Taylor 1951, Kriegler 1962, Taylor 1965a). The

larvae of *S. partita* are thick and semi-looped with sixteen prolegs and two pointed dorsal tubercules on the twelfth segment (Figure 1.2c). They are greyish green to dirty brown dotted with pre-anal dorsal tubercles. The larvae are nocturnal, feeding exclusively on foliage. The pupa is dark brown to dull black, and adults eclose after 20 to 33 days (Figure 1.2b) (Kriegler 1962, Taylor 1965a). The adult moth is active at night from 7 pm – 1 am, and activity declines thereafter (Whitehead & Rust 1972).

Figure 1.2: Adult *Serrodes partita* feeding on soft citrus (a), pupa (b), larva (c) and eggs (d) (Photo Credits: David Taylor).

1.3.4 Host plants

The larvae of *S. partita* are highly polyphagous, feeding on a variety of plants. Jacket plums, including *Pappea capensis* Eckl. & Zeyh (Sapindaceae), are its preferential host species (Taylor 1965a, Pinhey 1975). there is very high abundance of this tree especially in the Karoo. *Serrodes partita* also feeds on other plants, including *Deinbollia oblongifolia* E. May. ex Arn, *Deinbollia pinnata* Schum. & Thonn. (Sapindaceae), *Prunus persica* (L.) Batsch (Rosaceae), *Sapindus trifoliatus* L., *Sapindus Saponaria* L., *Dimocarpus longan* Lour, *Schleichera oleosa*

Lour (Oleaceae), *Grewia occidentalis* L (Rutaceae), *Acacia* spp. (Fabaceae), *Eucalyptus globulus* Labill, and *Leptospermum laevigatum* (Gaertn.) F.Muell (Myrtaceae) (Platt 1921, Taylor 1946, 1965a, Pinhey 1975). Meanwhile, the adult feeds mainly on peaches and apricots, but apples, citrus, plums, figs, loquats and grapes are also attacked (Jack 1922, Hargreaves 1936, Swart 1969, Swart et al. 1975).

1.3.5 Adult feeding and damage

Serrodes partita has a specialised proboscis that features sharp-tipped erectile structures, hooks and spines (Swart 1969, Goddard et al. 2019). The erectile structures comprise a socket and an acute shaft corresponding to the sensilla. At the same time, the hooks serve as homologues of sensilla basilica, and the spines correspond to the dorsal ligulae of the galea linkage (Figure 1.3) (Krenn 2010). Johannsmeier (1976) described the sophisticated technique by which the fruit is pierced. Once the moth lands on the fruit, it presses the proboscis tip against the skin and rapidly vibrates it while oscillating its head, resulting in drilling a small hole as the hooks anchor the proboscis. The proboscis penetrates the fruit via antiparallel movements of the galeae and tilting movements of the head to compensate for the proboscis's sideward bending when one galea is pushed forward. A bulging of the cuticle extends the erectile structures due to increased haemolymph pressure inside the galea, resulting from pumping, much like the uncoiling process. The erected structures of one galea provide resistance, enabling the other galea to be pushed deeper into the tissue. After feeding, the haemolymph pressure inside the galeae is reduced by opening the stipes valve. The flexion of the erectile structures reverse, and antiparallel movements of the galeae retract the proboscis (Swart 1969, Johannsmeier 1976, Klem & Zaspel 2019).

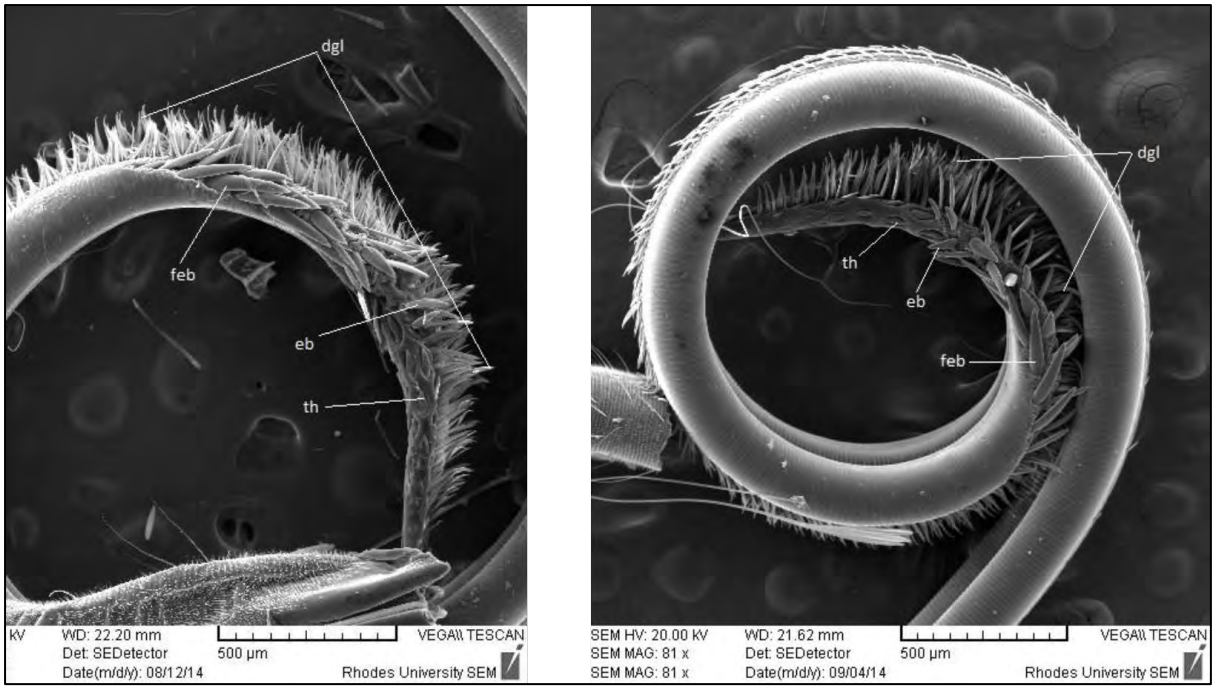


Figure 1.3: Scanning electron micrographs of *Serrodes partita* (A) male and (B) female showing erectile barbs (eb), tearing hooks (th), furcate erectile barbs (feb)), serrate ridge (sr) and dorsal galeal linkages (dgl) (Figure adapted from Goddard et al 2019).

The proboscis penetrates about 15 mm into the fruit, resulting in the honeycombing and sponginess of the tissue beneath the skin. This feeding activity often causes juice to leak from the punctured site, which can discolour the fruit. (Fay 2002). In high population densities, both ripe and unripe fruit may be targeted. Piercing leads to the admission of air into the fruit, triggering fermentation that attracts vinegar flies (*Drosophila* spp.) to lay their eggs. Hargreaves (1936) gave a concise description of the results of piercing citrus fruit. The puncture is followed by premature ripening from the point of injury, with as many as 10 holes observed per fruit. Depending on the climate, it may take 1-2 weeks for the fruit to fall, which can moderate fungal development. High temperature and humidity tend to accelerate fruit senescence. The fruit is vulnerable to secondary damage by fungal species, such as *Fusarium* sp. and *Oospora* sp., which travel internally from the puncture site to the button. Fruit fall only occurs after the button has been affected, so damaged fruit may still be harvested and rot during holding, in packhouses, or, worse yet, during transit to the market.

1.3.6 Economic impact

Estimating the economic impact of fruit-piercing moth damage accurately is complicated by several factors, including the masking effect of fruit fall and secondary invasion (Fay 2002). The situation is further complicated in the case of *S. partita* by the presence of other fruit-piercing moth species co-occurring, particularly in citrus crops (Johannsmeier 1998). Whitehead & Rust (1971) noted that adult moths are concentrated on the leeward edge of the orchard, with population densities peaking during the first three hours after dark. While sampling ripening fruit in citrus orchards can provide insight into the extent of damage, Grout & Moore (2015) highlighted that inspecting for moth damage in orchards may be challenging to justify, given the typically sporadic occurrence of the problem. Instead, they suggested nightly inspections of susceptible fruit during ripening. The potential damage of an outbreak depends on its size and nature, with most outbreaks in South Africa occurring in the Eastern Cape Province (Rust & Myburgh 1986). For example, the 1999 outbreak affected citrus in the Sundays River Valley, Gamtoos River Valley, and Knysna, resulting in estimated losses of 20 %, 30 %, and 50 %, respectively, for Miho Wase Satsumas. Early ripening cultivars, such as Satsumas and Clementines, are the most susceptible to *S. partita*. In 2009, another outbreak occurred in the Sundays River Valley and Kat River Valley. Each outbreak of *S. partita* often precedes that of *Achaea lineardi* Boisduval (Lepidoptera: Noctuidae), a fruit-sucking moth (Moore 2010). *Serrodes partita*, acts as a primary fruit piercer, initiates piercing, enabling secondary fruit-feeding moths to access and feed on the fruit.

1.3.7 Epidemic outbreaks

Literature on the management of *S. partita* in South Africa reveals gaps in research between outbreak years, occurring in 1922 – 1929, 1958 – 1963, 1966 – 1975, 1986 – 1988, 1999, and 2009 – 2012 (Jack 1922, Kriegler 1958, Myburgh 1963a, Whitehead & Rust 1972, Johannsmeier 1998, Moore 2010). Rust & Myburgh (1986) termed the occurrence of *S. partita* as sporadic, with epidemic outbreaks happening every 5 – 10 years. These outbreaks start off

as larval outbreaks on natural host plants before migrating to orchards as adults. These migrations occur over great distances towards fruit-bearing orchards (Johannsmeier 1998). The adult moths are nocturnal feeders and do not remain in orchards after feeding; rather, they return to hide in thickets where they remain immobile during the day (Johannsmeier 1976). Although the moths are relatively bigger, they are strong fliers capable of migrating up to 500 km (Whitehead & Rust 1971, Johannsmeier 1998).

Researchers have explored the causes of *S. partita* outbreaks and presented varying explanations. Whitehead & Rust (1971) reported that good rainfall drives these epidemic outbreaks in the Karoo by promoting the larval host's flush. Whitehead, as described in an obituary by Robertson & Cochrane (2005), conducted a four-year study on the prevalence of *S. partita* in the Karoo and discovered that larval survival in substantial numbers relies on the availability of young foliage during their first instar. He also noted that a healthy leaf flush supporting young larval development is cyclic in the Karoo and occurs every eight years. On the other hand, Bosch (1971a) attributed outbreaks to the southward movement of the Inter-Tropical Convergence Zone (ITCZ), which brings rain to Zimbabwe and proposed forecasting outbreaks based on knowledge of migratory routes and weather data. However, recent reports suggest that *S. partita* infestations are becoming more frequent in South Africa rather than occurring as epidemic outbreaks (Goddard 2016). Bosch (1970), in her earlier research, classified orchard invasion into three categories: i) damage caused by resident moths, ii) damage due to migrant moths that have established temporary breeding grounds, and iii) damage caused by migrant moths. Despite these findings, the causes of occurrence and the shift in outbreak dynamics remain unclear and requires further investigation.

1.3.8 Management of *Serrodes partita*: The South African experience

Managing *S. partita* in orchards poses a substantial challenge, since the larvae develop on native hosts, which may be far from the orchards where the adults eventually feed. Traditional management approaches primarily target orchard pests that either complete their entire life

cycle within the orchard or have larval stages restricted to the orchard. Management efforts are, therefore, rendered ineffective by the adult moth's feeding behaviour, as it exclusively visits the orchard during the night and does not stay on the fruit for an extended period.

Additionally, *S. partita* is an outbreak pest with a strong migration tendency, making it challenging to manage effectively. Pesticides have been explored for the management of *S. partita*, but their effectiveness has been limited and they pose significant health risks, particularly when applied at fruit ripening just before harvesting (Johannsmeier 1998). Studies such as those by Kriegler (1957) and Johannsmeier (1976) reported some repellent effects of cover sprays containing DDT and Parathion on canning peaches. However, these pesticides raise health concerns and are not highly effective due to the moths' strong resistance and feeding behaviour (Kriegler 1962, Johannsmeier 1998). Additionally, Whitehead & Rust (1971) and Moore (2010) warned against pesticide use citing their inefficacy and required withholding periods to meet market residue level regulations.

1.3.8.1 Biological control

Several significant pest complexes have been described for fruit-piercing moths, with Cochereau (1974) reporting 20 recorded parasitoids on *Achaea* sp. in India and Asia. The most common natural enemies recorded for fruit-piercing moths are egg parasitoids, larval parasitoids and predators (Leroy et al. 2021). Egg parasitoids primarily come from four families: Encrytidae, Eulophidae, Ichneumonidae and Braconidae (Heraty 2017). Three genera *Ooencyrtus* sp, *Telonomus* sp. and *Trichogramma* sp. have been the most studied on fruit-piercing moth (Leroy et al. 2021). The first two have been reported to be more efficient on egg clusters, while the latter had high parasitism in Guam (Denton et al. 1989). *Trichogramma* sp. have been reported on *Achaea* sp. eggs in Mauritius (Cochereau 1974).

According to Myburgh et al. (1973), larvae of *S. partita* are parasitised by a tachinid fly, achieving up to 80 % parasitism during outbreak years. However, when larval numbers are low, parasitism has been recorded to be as low as 1 % (Johannsmeier 1998). In New Caledonia,

the endoparasitoid tachinid fly, *Winthemia caledoniae* (Mensi), is the most important parasitoid of *Eudocima phalonia* L.. Parasitism ranges from 25 – 100 % after the outbreak peak (Cochereau 1977). Despite this, the tachinid fly's efficacy as a biocontrol agent is limited, as it peaks at the outbreak's end when moth numbers decline (Fay 2002). Bats have also been associated with the occurrence of *S. partita* in the field (Hargreaves 1936, Johannsmeier 1998). Johannsmeier (1976) noted that moths scatter in response to bats flying through orchards, which could be due to the sensitivity to ultrasonic pulses on the tympanic organs of noctuid moths. Miyata (2005) found bird-damaged specimens of *S. campana* and other fruit-piercing moth species, suggesting the potential for biological control of *S. partita*. However, the biological control options for *S. partita* remain largely unexplored.

1.3.8.2 Lights

Understanding the phototropism of the noctuid moth is a potential way to control infestations, but it requires significant investment and specialised facilities (Johannsmeier 1998, Leroy et al. 2021). Nomura et al. (1965) found that light traps were ineffective at catching fruit-piercing moths, but fruits located near the light source were not damaged. Whitehead & Rust (1967) pioneered using light as a repellent against fruit-piercing moths in South Africa. Subsequently, they found that illuminating peach and plum orchards with pressure lamps, mercury vapour lamps, or yellow lights could reduce *S. partita* infestations by 50-90 % (Whitehead & Rust 1971). Bosch (1971b) reported that fluorescent mercury lamps emitting light exceeding 0.2 lumens could reduce infestations to less than 1.5 %. Whitehead & Rust (1972) further demonstrated that illuminating both sides of an orchard could increase efficiency, reducing infestations by up to 78 %. However, the lights must be turned on before dusk since moths are not easily disturbed once feeding starts (Whitehead & Rust 1972).

According to Johannsmeier (1998), light inactivates most noctuid moths at night, causing them to become quiescent, whereas it induces physiological changes in the eyes of others, leading them to spiral upward and away from the light (Hofmeyr 2003, Moore 2010). Green-yellow

spectrum incandescent light repels *S. partita*, whereas UV light generally attracts moths (Johannsmeier 1998, Bhumannavar & Viraktamath 2012). Neubecker (1966) found that using UV light on a Robinson trap yielded little success. The effect of UV light attraction could be further explored with other trap designs.

1.3.8.3 Chemical control through attraction and repellence

Non-essential oils have been exploited as repellents of fruit-piercing moths in orchards. Neem oil repelled *Eudocima materna* in the laboratory but not under field conditions. Although essential oils possess some repellent activity, they can be phytotoxic if applied directly on plants; hence, they must be used cautiously (Bhumannavar & Viraktamath 2012, Leroy et al. 2021). Bosch (1971a) reported some success in repelling *S. partita* with citronella oil. However, repellents are generally ineffective against fruit-piercing moths, considering the odour of ripening fruit, which tends to mask the repellent odours (Baptist 1944). Odour neutralisation refers to the lack of recognition of olfactory molecules, while repellence is the recognition of repulsive or unpleasant molecules (Leroy et al. 2021). In various pest models, neutralising odours with oil emulsions has been suggested (Balikai et al. 2011, Leong & Keuh 2011). By burning oil and different plant materials, direct fumigation through smoking can also mask orchard odours (Baptist 1944). Masking/neutralising odours is effective but subject to abiotic factors (wind and rain), which can reduce efficiency and must be repeated every night throughout the fruiting season (Klem & Zaspel 2019).

The idea of managing fruit-piercing moths with fruit lures was first recommended by Susainathan (1924), who suggested luring with poisoned fruits. Gunn (1929) developed a classical trap for managing *S. partita* and other fruit-feeding moths in South Africa. The traps were made of paraffin cans cut open on two sides with treacle or brown sugar syrup poisoned with lead arsenate or sodium arsenate. This mixture became popularly known as the “South African lure” but was only effective against *A. lineardi*, a fruit-sucking moth. Baptist (1944) used a mixture of water, unrefined sugar and fruit pulp in wide-mouth bottles with limited

success. Since then, several authors have explored fruit lures to attract fruit-piercing moths. Reddy et al. (2007) discovered a strong preference for bananas and guava. Fullard et al. (2012) tested different fruits against *S. partita* and concluded that banana was the most attractive fruit lure. The study was further corroborated by Goddard (2016), who conducted field trials in the Eastern Cape, South Africa, to monitor *S. partita* with ripe bananas and different synthetically produced components of bananas. Fresh banana was the most attractive lure for *S. partita* and other fruit-feeding moths. These studies have established feeding preferences that could be exploited for *S. partita* management. There is a need to isolate and formulate synthetic lures as a robust and more sustainable management tactic. To this end, Fay & Halfpapp (2003) developed a patent for artificial bait for *Eudocima* sp.. These baits were tested in citrus and showed considerable promise with an estimated reported reduced damage by 75-85 % (Fay 2002). Recently, Mallikarjun et al. (2019) and Mallikarjun & Thippaiah (2019) explored electroantennographic responses of *Eudocima martena* to fruit extracts of host plants and female pheromones. These approaches could potentially be adapted for *S. partita* management.

1.8.3.4 Other methods

Various methods for managing the fruit-piercing moth exist, yet they have not been implemented in South African citrus orchards due to factors such as the sporadic nature of the pest, method inefficiency, cost, adherence to citrus production guidelines, and the scale of production. *S. partita* is known for its clumsy flight, and once it begins feeding, it is not easily disturbed (Whitehead & Rust, 1971; Johannsmeier, 1976). Handpicking has been proposed as a control measure for the fruit-piercing moth (Leroy et al., 2021). Although hand picking is feasible due to the large size and clumsiness of the adult moth, it is rendered ineffective by the vast scale of citrus production and the pressure from pest populations (Taylor 1965, Johannsmeier 1976).

Large-scale nylon nets with mesh sizes of 1-2.5 cm^2 have been recommended for managing *E. phalonia* in other countries, such as Australia, Samoa, and Japan, where they have proven

successful (Cochereau 1969, Yoon & Kim 1977, Ali & Vargo 1990, Fay 2002). While nylon nets in citrus production serve multiple purposes, including reducing light intensity, wind speed, sunburn, temperature buffering, and protection against hail and bird damage (Raveh et al. 2003, Wachsmann et al. 2014), their effectiveness in excluding *S. partita* access to orchards has not been studied. Moreover, the primary challenge for citrus growers remains the cost of erecting and maintaining these nets.

Another method for controlling fruit-piercing moths involves managing fruit crops through careful consideration of planting patterns, harvesting periods, and cultivar selection. Planting trees in a compact scheme, particularly on the leeward side of orchards, has been suggested to deter moth encroachment (Cochereau 1969, Fay & Halfpapp 1993). Adjusting planting patterns and orchard orientation, with susceptible cultivars positioned at the centre, can also help reduce damage, especially since *S. partita* is known to target soft citrus cultivars (Moore 2010). Additionally, using citrus cultivars with ripening periods that do not coincide with moth occurrence, as proposed by Cochereau (1974) and Fay & Halfpapp (1993), showed promise. However, implementing this strategy may be challenging as citrus growers may be reluctant to forgo highly lucrative but susceptible cultivars like Satsumas and Clementines (Moore 2010). Alternatively, early harvesting, as suggested by Fay & Halfpapp (2006), presents another potential management practice for *S. partita*. Nevertheless, the South African citrus industry is heavily regulated, with fruit typically harvested only after reaching specific colour and acid-sugar ratio criteria.

1.4 Aims of the Study

Reports by citrus growers in the Eastern Cape province, specifically in the Kat River valley, suggest frequent occurrence of the moth as opposed to usual seasonal outbreaks. As a result, this moth has become a severe threat to the soft citrus industry in the area. A review of the current pest management practices for the fruit-piercing moth, *S. partita*, showed that some strategies might positively affect smaller orchards but present many disadvantages.

Furthermore, they lack specificity and could negatively impact the environment and non-target pests. Therefore, there is a need for innovative, sustainable, and environmentally friendly strategies that control the problem while preventing the erosion of biodiversity. The use of an attractive lure (banana lure trap) has shown to be promising but impractical in the long-term. The biology and chemical ecology of *S. partita* have not been well investigated to develop synthetic lures. Therefore, this study aimed to examine the biology and chemical ecology of *S. partita* to develop monitoring and management tactics. The specific objectives of this study were to: (i) gain a comprehensive understanding of the biology and laboratory rearing of *S. partita*. Cultivating this species in a controlled environment facilitates investigations into its host preferences and mating behaviour, critical for the development of pheromones; (ii) investigate feeding preferences concerning fruit cultivar, fruit maturity stage, and fruit quality to guide management strategies effectively; (iii) explore lure type and lure presentation techniques in both monitoring and management of fruit-piercing moth; (iv) conduct seasonal abundance monitoring and assess the occurrence and distribution of fruit-piercing moths in susceptible orchards across multiple seasons. This analysis aims to determine the occurrence status, distinguishing between sporadic and frequent occurrences as well as determine the role of climatic factors in occurrences; (v) explore the natural enemies of *S. partita* to gain deeper insights into their role in outbreaks and their potential for managing outbreaks.

CHAPTER 2

Biology and rearing of the fruit-piecing moth, *Serrodes partita*

2.1 Introduction

Serrodes partita, poses a significant threat to soft citrus, *Citrus reticulata*, particularly in the Eastern Cape, South Africa (Moore 2010, Chapter 1). This destructive pest causes direct damage to the fruit, due to its specially adapted proboscis (Swart et al. 1975, Johannsmeier 1976, Goddard et al. 2019). The puncture made by the insect serves as a secondary infection site for microorganisms, especially fungi, leading to fruit decay (Hargreaves 1936, Johannsmeier 1976). Given the impact of this pest on soft citrus production and the potential for post-harvest losses, research focused on reducing the population of both adult and larval stages of *S. partita* holds great potential for reducing crop losses.

Pest biology and plant-pest interactions are crucial in developing management practices (Faust 2008). Understanding the fruit-piercing moths' life history, and ecology is therefore necessary for a successful management programme (Bänziger 1982, Bawaskar et al. 2018). Several studies have been conducted on the biology and morphology of *S. partita*. Notable contributions include the works of Taylor (1951), Neubecker (1962, 1966), Swart (1969), and Johannsmeier (1976). Despite these efforts, our understanding of the species' biology and ecology remains limited. One of the challenges in studying *S. partita* is its nature as an outbreak pest. It primarily manifests during outbreaks, making conducting continuous and accurate research on its biology and ecology difficult. Due to this sporadic occurrence, studying the pest's life cycle and ecological interactions becomes more complex.

Previous attempts have been made to rear fruit-feeding moths, yielding varying degrees of success. Bot (1967) pioneered mass-rearing techniques for fruit-sucking moths, specifically *Achaea finita* and *A. catella,* which pose less threat to fruit crops and primarily target castor beans. Iwabuchi et al. (1979) achieved continuous larval rearing of the fruit-piercing moth, *O. emarginata*, in laboratory settings in Japan for over 10 generations on artificial diet. Currently, there is no established artificial diet or rearing protocol for *S. partita*. Initial efforts were made by Bot (1966) who attempted to rear a semi-looper on a diet intended for *S. partita*. Additionally, Hewitt et al. (1969) isolated a phagostimulant, quebrachitol, from *Pappea capensis*, but little progress has been made since then. The absence of an artificial diet for *S. partita* necessitates larval rearing in large spaces and the use of field-collected natural host leaves, *P. capensis*, to maintain insects in the laboratory. Unfortunately, this approach introduces sanitation challenges and therefore increases the risk of disease incidence. Consequently, these constraints hinder research on pheromone development and impede the assessment of different management strategies. Artificial diets are crucial in advancing research and technology for pest control and evaluating various management approaches (Cohen 2015, 2018). For example, Moore et al. (2014) successfully developed an artificial diet for the false codling moth, *T. leucotreta*, which has since been widely used in numerous studies to assess different pest management strategies.

Sexing insects is a crucial aspect of entomological studies. The ability to differentiate between male and female insects is not only vital for artificial rearing and developing sterile insect technology, but it also plays a significant role in various other studies, such as pesticide screening, biological control investigations, and research on sex-ratios and behavioural responses to semiochemicals and mass trapping (Chen et al. 2020, Ramaya et al. 2020). Typically, adult stages are preferred for sexing due to their relatively easier identification. Researchers rely on various morphological characteristics such as overall body and abdomen size, forewing markings, pupal weight, and antennae appearance to distinguish between male and female adult Lepidoptera. However, it's worth noting that these distinguishing features,

except for antennal structure, may vary significantly depending on factors such as food quality, host plants, oviposition termination in females, and generation time. Moreover, determining the sex of insects before they reach the adult stage is essential for subsequent experiments that necessitate the segregation of males and females, such as developing sex pheromones to manage *S. partita*. In some species, the pupal stage provides suitable cues for sex determination. Researchers have successfully identified the sex of pupae by considering factors such as weight, thickness, and the number of posterior abdominal segments (Raj et al. 2019). Notable studies by Hanife (2005), Tuncer & Aker (2017) and Raj et al. (2019) have utilised these methods to accurately sex pupae.

The main objective of this component of the study was to develop a rearing protocol and provide a detailed description of the biology of *S. partita*. The specific objectives were: (i) to establish an efficient rearing procedure that can simplify mass production and facilitate experimental studies on the biology, physiology, behaviour, and potential control methods of *S. partita*, (ii) to accurately describe the life history of *S. partita* through head capsule measurements of the larvae and determine its generation time, (iii) to quantify the degree of sexual dimorphism in the pupal and adult stages, particularly in the genital area, by examining the type, density, and dimensions of sensilla. This information will contribute to a better understanding of the biology of this pest.

2.2 Materials and Methods

2.2.1 Collection sites and insect collection

The study was conducted in the Eastern Cape Province of South Africa, ideally suited for Navel orange production. Three distinct study areas were selected for the research: two citrus-growing regions and a study area in Steytlerville within the Karoo region. These areas were chosen specifically due to the presence of both adult and larval stages of *S. partita*. Each study site encompassed various farms. The first study site was in the Committee's Drift area, a part of the historically significant Kat River Valley. The valley relies heavily on citrus production, serving as a major source of employment in the region (CGA 2007). The Committee's Drift area includes three farms on the Upper side of the Kat River Valley along the Keiskamma River, adjacent to the Great Fish River nature reserve. Within the Amathole District, two farms, namely Naudeshoek and Siyamila, fall under the jurisdiction of the Ngqushwa Municipality, while Ripplemead Farm is located in the Nkonkobe Municipality (Figure 2.1).

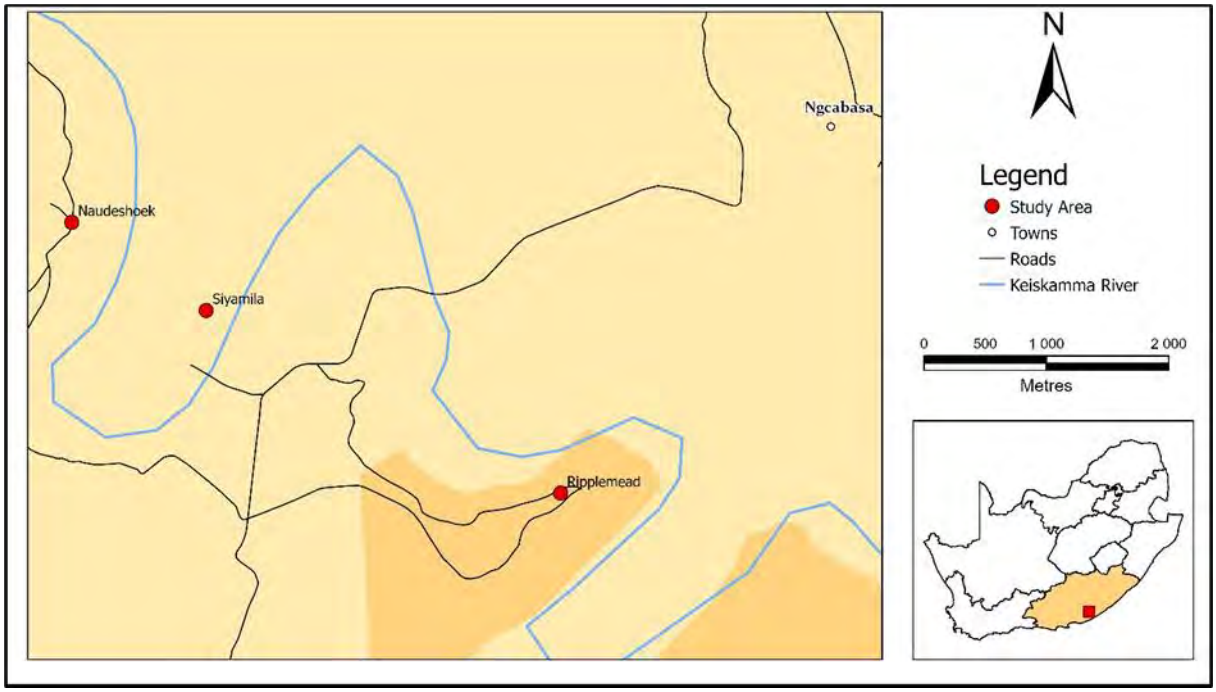


Figure 2.1: The Committee's Drift study area showing the three citrus farms. Different shades represent different municipalities.

The study also encompassed two other main sites: The Gamtoos River Valley and the Karoo near Steytlerville. The Gamtoos River Valley produces approximately 6 600 hectares of citrus land and boasts an impressive annual export of about nine million cartons. Situated in the Kouga Municipality, west of Nelson Mandela Bay, and falling under the Sarah Baartman district, the Gamtoos River Valley is supported by two supply towns, Hankey and Patensie, both accessible via the R331 road. For the Gamtoos River Valley, seven farms were carefully selected based on their cultivation of susceptible citrus cultivars, specifically Satsuma Mandarins (Table 2.1). These farms were chosen due to their potential as host sites for *S. partita* adults.

The other study site was in the Karoo, close to Steytlerville, directly opposite the Gamtoos River Valley, with the Baviaans mountain range acting as a natural barrier between the two regions. The sites in the Karoo were chosen based on reports of *S. partita* larval presence and the abundance of suitable larval host trees. Within the Karoo study area, two farms were used, located in the Baviaans Municipality on either side of the Gamtoos River. The first, Hopedale, primarily focused on sheep farming, while the second, Royal Karoo, served as a game reserve (Table 2.1). The last farm, Phindy Farm, was in the Sundays River Municipality, easily accessible from the R75 road (Figure 2.2).

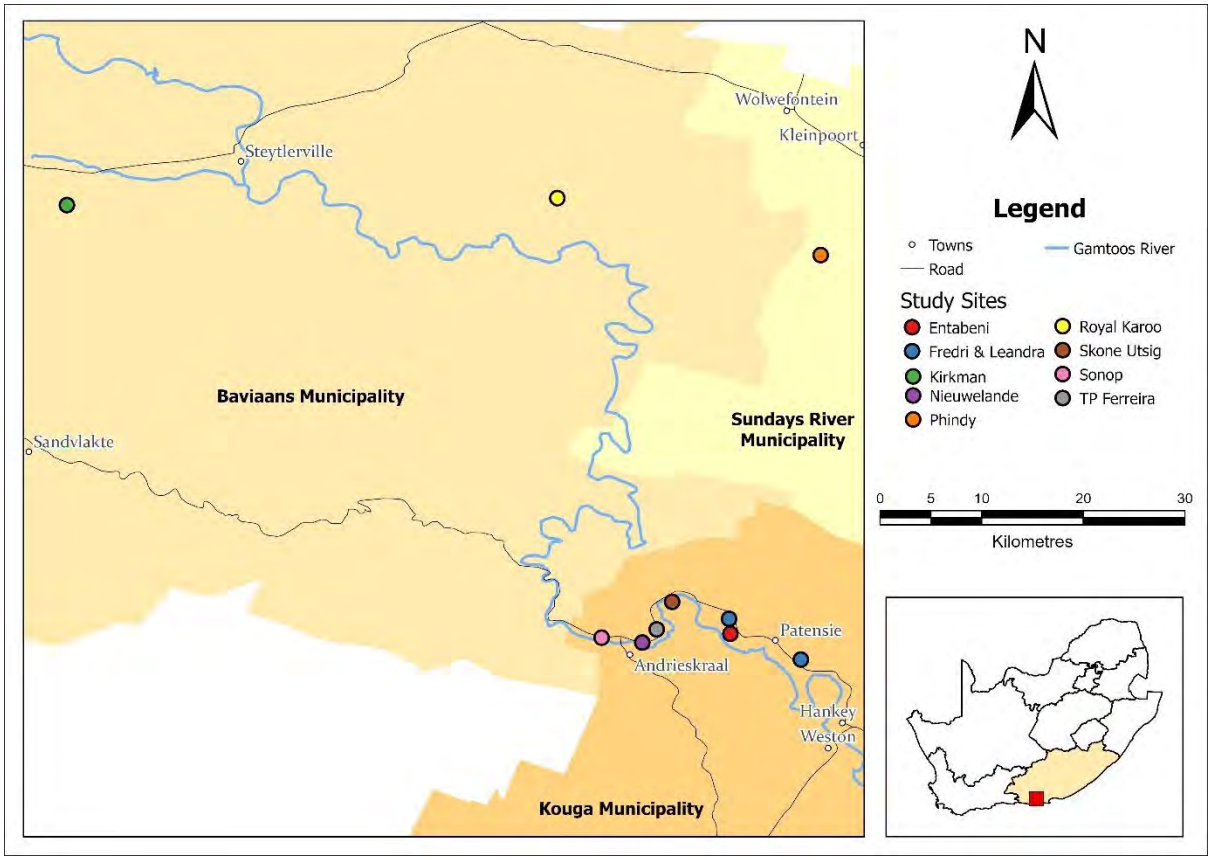


Figure 2.2: Patensie and Steytlerville study sites. Different shades represent different municipalities.

Insect collection differed from one study site to the next depending on the occurrence and developmental stage required. Larvae and pupae were collected mainly in Steytlerville and on one farm in Committee's Drift while adults were collected exclusively on citrus farms (Table 2.1).

Table 2.1: Physical description of study sites, including cultivars and number of orchards used per site.

Citrus sites			
Farm	**Location (coordinates)**	**Number of orchards**	**Cultivars**
Naudeshoek	Committee's drift (33°00'49"S; 26°95'32"E)	7	Clementines, Satsumas and Nardocotts
Ripplemead	Committee's drift (33°02'47"S; 26°98'91"E)	5	Clementines and Nardocotts
Similar	Committee's drift (33°01'13"S; 26°96'30"E)	5	Clementines, Satsumas and Nardocotts
Entabeni	Patensie (33.75'23"S; 24°77'61"E)	1	Satsuma Mandarins
Fredri & Leandra	Patensie (33°77'47"S; 24°83'75"E)	3	Satsuma Mandarins
Niuewelande	Patensie (33°75'98"S; 24°69'94"E)	1	Satsuma Mandarins
Skone Uitsig	Patensie (33°72'45"S; 24°72'54"E)	1	Satsuma Mandarins
Sonop	Patensie (33°75'56"S; 24°66'41"E)	2	Satsuma Mandarins
TP Ferreira	Patensie (33°74'84"S; 24°71'19"E)	2	Satsuma Mandarins
Non-citrus sites			
Farm	**Location**		**Type of farm**
Hopedale	Steytlerville (33°37'92"S; 24°19'85"E)		Game/Sheep farm
Phindy	Steytlerville (33°42'28"S; 24°85'47"E)		Sheep farm
Royal Karoo	Steytlerville (33°37'31"S; 24°62'54"E)		Game farm

Serrodes partita moths were collected in the field from both the Committee's Drift and Patensie study areas during the night using the hand collection method as they were feeding. The collection of adult moths proved relatively straightforward, as they are not easily disturbed once they begin feeding. Once captured, the moths were carefully placed into small cages for safe transportation to the laboratory. In addition to moths, larvae and pupae were collected from Steytlerville and Siyamila farms in the Committee's Drift area (Figure 2.1). It is known that *S. partita* larvae descend from the trees during the daytime to seek refuge from potential predators as well as undergo pupation (Taylor 1951, Johannsmeier 1998). A proactive approach was employed to gather larvae and pupae, involving a thorough search of the leaf litter beneath trees exhibiting feeding damage. The larvae of *S. partita* have a cryptic nature and tend to aggregate in specific areas. In addition, the pupae create silk cocoons covered by soil and leaf

litter, making them more challenging to locate. A spade was used to carefully move the leaf litter to gather these insects until the first larvae or pupae were found. Subsequently, bare hands were employed for the search to minimise the risk of causing harm to the insects. Both larvae and pupae were then transported to the laboratory, where the larvae were allowed to undergo pupation.

The adult *S. partita* collected from the field were placed in cages along with branches of *P. capensis* to facilitate egg laying. Four soft citrus fruits, either Satsumas or Clementines depending on the season, were also provided in the cages as a food source and were replaced every five days. Fruit was acquired from the orchard or local Pick n Pay supermarket. The cages were kept in the laboratory at ± 25°C until the eggs hatched. Due to the difficulty of removing the eggs from the cages or leaves, they were left in the cages until hatching. Once hatched, the neonates were transferred to fresh leaf material or prepared artificial diets using a fine paintbrush. The adult moths were moved from their original cages to a new cage after laying eggs. The time taken for the eggs to hatch was recorded.

2.2.2 Larval rearing on artificial diet

Three treatment diets, i.e. the bollworm diet used in the rearing of *Helicorvepa zea* Boddie, and other Noctuid moths, the *Plusia acuta* (Walker) diet adapted from Bot (1966) and the legume bean diet Hervet et al. (2016) used in rearing several Lepidoptera species, symbolised as D1, D2 and D3, respectively, were prepared according to their suggested formulations (Table 2.2). When no feeding was observed, diets were modified by adding dry leaf plant extract and later ethanolic extracts of the larval host plant, *Pappea capensis* which may act as a potential feeding stimulant (Hewitt et al. 1969).

The bollworm diet (D1), was prepared following a step-by-step procedure. First, soya flour, brewer's yeast, and agar were autoclaved individually at 121°C for 20 minutes. Thereafter the ingredients were combined in 836 ml boiling water and mixed thoroughly in a blender. The temperature was then allowed to cool to 60°C before adding wheat germ and mixing

simultaneously to ensure a thorough and even distribution of the ingredients within the diet. Ascorbic acid, propionic acid, phosphoric acid, methylparaben (antimicrobial agent), and sorbic acid were mixed thoroughly to complete the formulation. The prepared diet was then poured into a 2-litre plastic container and left to set at room temperature in the laboratory. Once set, the diet was used immediately, and the remainder was sealed and stored in a refrigerator at 4°C for a month until it was required for use during the experiment.

Table 2.2: Ingredients of the three artificial diets that were tested for rearing *Serrodes partita* larvae (Bot 1966, Hervet et al. 2016, Yucel & Genic 2018).

Ingredients	D1	D2	D3
Soya Flour	55.2 g	-	-
Brewer's Yeast	32.1 g	24 g	40 g
Agar	31.2 g	5 g	8 g
Wheat Germ	38.5 g	20 g	-
Vitamin free casein	-	4 g	-
Soaked beans	-	-	265 g
Maize grits	-	-	26.5 g
Ascorbic acid	2.26 g	2 g	4 g
Propionic acid	0.85 ml	-	-
Phosphoric acid	0.081 ml	-	-
Methyl paraben	2.2 g	0.8	2.5 g
Sorbic acid	2.2 g	-	1. 25 g
Cholesterol	-	0.08 g	-
Formalin	-	-	2.5 ml
Choline chloride	-	0.8 g	-
Water	836 ml	150 ml	800 ml

The *P. acuta* looper diet (D2), was prepared following the method described by Bot (1966). Initially, a thorough mixture of wheat germ, casein, and yeast was created. Next, methylparaben and cholesterol were dissolved in 20 ml of 95 % ethyl alcohol and gently stirred into the mixture, ensuring even moisture distribution. The alcohol was then evaporated through constant stirring in a steam bath or by spreading the mixture thinly in a fume hood at 25°C for 10 minutes. Afterwards, the dry mixture was brought back to room temperature, and agar was carefully blended. In a separate step, inositol, choline chloride, and ascorbic acid were dissolved in 150 ml water and then stirred into the mixture, resulting in a thin, gruel-like consistency. This mixture was poured into a 2-litre plastic container and left undisturbed for about half an hour until a soft, light brown crust formed on the medium. The container was then sealed tightly and set aside for 24 hours, allowing the medium to regain its moisture content, after which it was refrigerated at 4°C until it was used in experiments.

In the preparation of the legume sugar bean diet (D3), the beans underwent a preliminary step by soaking them in distilled water for 24 hours. Once soaked, they were boiled until reaching a soft consistency. Then, 265 g of the soaked and boiled beans were macerated using a blender in 500 ml of distilled water. In a separate container, yeast, sorbic acid, ascorbic acid, nipagin, and formalin were mixed in 350 ml of water before being combined with the macerated beans. Following this, small maize grits (0.5 – 1mm) were added and thoroughly mixed. Simultaneously, agar was sterilised in 500 ml of distilled water at 121°C for 30 minutes and then allowed to cool to 60°C before being blended with the bean meal mixture. The final diet composition was then poured into 2-litre plastic containers and stored in a refrigerator at 4°C.

Fresh diet blocks measuring 5 cm^3 were carefully cut from the prepared diet and transferred to 70 ml plastic transparent tubs, equipped with ventilation holes. To facilitate easy removal of the diet blocks and reduce desiccation, small pieces of Parafilm M® (Bemis Company Incorporated, USA) (9 cm^2) were placed both beneath and on top of each block. This arrangement was implemented to allow the caterpillars to feed while positioned on the underside of the upper parafilm, mimicking their behaviour under a plant leaf in natural

conditions. Since *S. partita* did not exhibit cannibalistic behaviour, each rearing tub contained five individual larvae. The neonates were transferred to the rearing containers from the top of rearing cages, where they were suspended using a fine brush. To create sheltered areas and diminish the diurnal activity of the caterpillars, variously sized pieces of brown paper were introduced into the tubs, some laid flat and others slightly crumpled.

2.2.2.1 Preparation of Pappea capensis *and ethanolic extracts as potential phagostimulants*

Since *S. partita* failed to feed on all the artificial diets, host plant material and ethanolic extract were prepared to stimulate feeding of *S. partita* on artificial diets. The host plant material was prepared following the method described by Bakaze et al. (2018). To produce *P. capensis* powder, young leaves were first chopped into small slices, each measuring about 1 cm^2. These slices were then spread out in trays and left to dry in the sun for a period of five days. Throughout this drying process, the material was regularly turned to ensure uniform drying. Prior to pounding, the dried leaf material underwent further drying in an oven set at 70°C for an hour to achieve thorough dryness. The leaves were then carefully ground using a ceramic pestle and porcelain mortar, transforming them into a fine powder. If the powder was not used immediately, it was stored in airtight containers in a refrigerator until required, for a maximum period of three months. The powder was added to comprise 10 % of the final volume of the diet.

Ethanolic extracts were prepared using a modified method based on Hewitt et al. (1969). Fresh *P. capensis* leaves were carefully collected and weighed, with 150 g of leaves subsequently crushed in a blender until they formed a pulp-like consistency. This pulp was then combined with an equal volume of ethanol and left for 48 hours in a water bath set at 50°C. After the designated period, the alcohol was filtered out using filter paper and a pump. Next, an additional 150 ml of ethanol was added to the leaf pulp and left for another 24 hours at 50°C. The ethanol was filtered out, the pulp was discarded, and the ethanol extracts from both steps

were combined. These combined extracts were concentrated to 20 % of their original volume by allowing them to stand in an open bottle in a 50°C water bath for 48 hours. This process reduced the volume from 300 ml to approximately 60 ml of concentrated ethanol extracts. These concentrated ethanol extracts were added to constitute 10 % of the final diet volume.

2.2.3 Larval rearing on the host plant

, A cohort of caterpillars was raised on their natural food source, fresh *P. capensis* leaves. Branches with leaf material were collected and then cut into smaller pieces using secateurs. The small branches (8 – 10 cm long) were then securely inserted into a 5 cm^3 Styrofoam cube. This cube was placed in water within a 10 ml plastic tub, covered with a fine mesh to prevent the larvae from escaping. During the early stages (first three instars), the leaf material was changed every five days as the larvae consumed more food as they grew. As the caterpillars continued to develop, they required more substantial branches inserted into vermiculite-filled plastic cups. The addition of water ensured the leaves remained fresh and prevented wilting. The vermiculite provided support for the branches and acted as a substrate for pupation when the time came. With the larger branches in place, the setup was transferred to cages in the laboratory, and the leaf material was now changed every 3 – 4 days to maintain a fresh food supply.

The width of the head capsules was measured under a dissecting microscope (Leica EZ4D, Leica Microsystems, Wetzlar, Germany) at 10 – 30× magnification at five-day intervals to minimise disturbance until the pre-pupal stages. These data were used to determine the number of instars in larval development. Additionally, the number of survivors, as well as the duration of larval and pupal stages, were carefully noted and recorded. To distinguish between male and female pupae of *S. partita,* morphological differences were observed under a microscope, focusing on the genital area of the abdomen. Confirmation was made by dissecting the adult male and female moths.

2.2.4 Data analysis

This study used descriptive statistics to calculate the moth's average incubation and development time. Descriptive statistics allow calculation of averages, ranges and variability in both incubation time and development time. Head capsule width measurements were used to determine the larval instar, according to Dyar's rule (Dyar 1890, Mohammadi et al. 2010, Thakur 2016).

2.3 Results

2.3.1 Larval rearing

The larvae of *S. partita* showed a complete failure to establish on all three diets, including the modified diets, at every life stage. Although limited feeding was observed on the modified bollworm diet with ethanolic extracts added, the larvae did not survive beyond the third day. Successful rearing from egg to adult was only achieved on the natural host plant, *P. capensis*. The highest level of mortality was observed from the 1st to the 2nd instar (Table 2.3).

Table 2.3: Percentage mortality of *Serrodes partita* larvae at different life stages, reared in the laboratory on *Pappea capensis*.

Year	2021						2023					
Instar	1	2	3	4	5	6	1	2	3	4	5	6
No of larvae	250	23	21	17	3	1	400	44	39	34	4	3
% Mortality	90.8	8.7	19.0	82.4	66.7		89.0	11.4	12.8	88.2	25.0	

2.3.2 Life history study

The life cycle of *S. partita*, from egg to adult, was completed in 80.7 ± 3.6 days, with the larval stage spanning about 52.3 ± 2.8 days (n = 4). The pupal stage lasted for approximately 25.8 ± 3.6 days (n = 20) at 21°C. Throughout the development, six instars were recorded (Table 2.4).

Table 2.4: Average head capsule width of *Serrodes partita* larvae at different instars.

Larval instar	Head capsule width (mean ± SD (mm)	Number of insects	Dyar's rule growth ratio
1	0.41 ± 0.03	14	1.68
2	0.69 ± 0.04	12	1.74
3	1.20 ± 0.07	11	1.50
4	1.84 ± 0.10	14	1.28
5	2.31 ± 0.09	5	1.32
6	3.04 ± 0.04	3	-

2.3.2.1 Eggs

The eggs of *S. partita* were laid individually on various parts of the host plant material, including both sides of leaves, branches, and net material. These circular eggs possessed a firmly attached ventral base, securing them to the substrate. When freshly laid, the eggs appeared translucent at the bottom and featured a small brown patch at the top (Figure 2.3a). Synchronisation of egg-laying occurred in different cages over three days, with hatching taking place on the third day after being laid at 21°C. On average, the eggs had a diameter of 0.87 ± 0.04 mm (n = 20).

2.3.2.2 Larvae

The first instar larvae of *S. partita* are initially translucent but quickly gain colour once they start feeding. Their head is light brown in colour, while their body is adorned with long hairy setae (Figure 2.3b). The larvae possess six pairs of prolegs, with three pairs situated anteriorly and the remaining three posteriorly. The last pair of legs is situated right at the posterior edge, creating a hooked appearance. Upon hatching, the larvae instinctively move to the top of the rearing cages and host plant material, suspending themselves upside down using the last three pairs of legs. When disturbed, they swiftly repel down using a silk thread.

As they enter the second instar, the larvae turn dark green, and the head darkens. The length and number of setae are reduced (Figure 2.3c). In the third instar, the larvae maintain a dark green shade with black dots covering the body, from which setae protrude (Figure 2.3d). As they reach the fourth instar, a greyish-green shade begins to emerge, and the head becomes more pronounced. Fifth and sixth instar larvae are similar. They assume a greyish appearance with a subtle hint of green shade. The larvae now possess a prominent hump, visible just after the third pair of prolegs (Figure 2.3e).

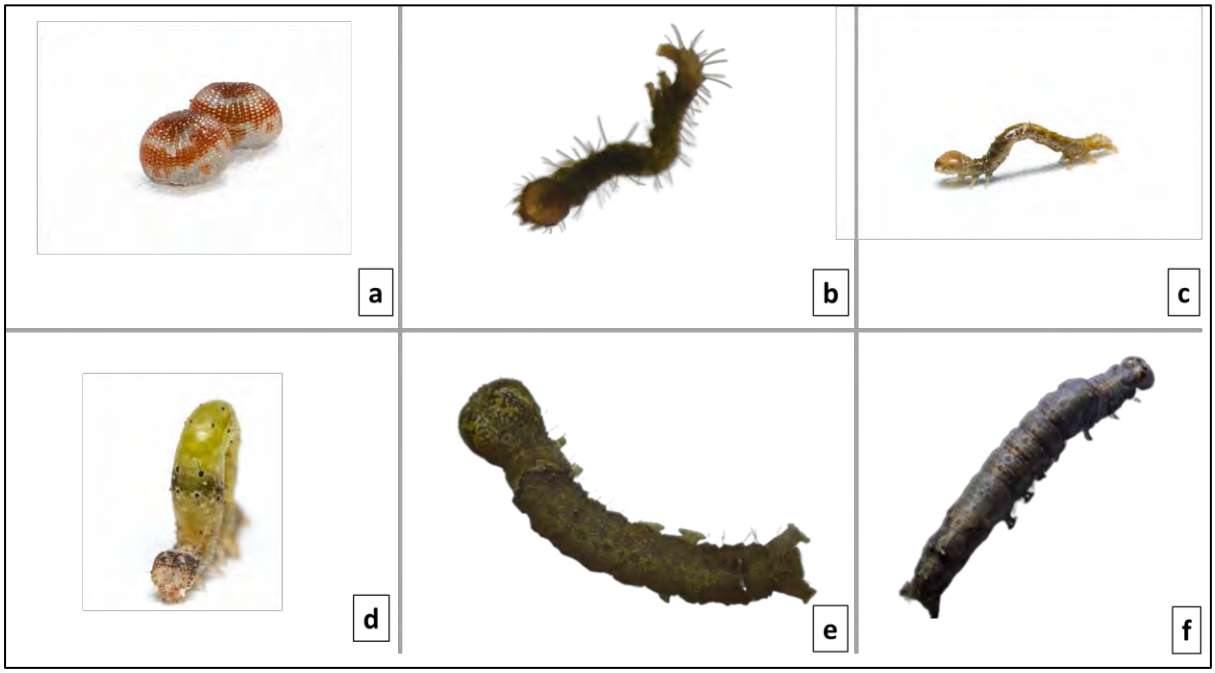


Figure 2.3: Life stages of *Serrodes partita* from eggs to the last instar. a) eggs; b) first instar with pronounced setae; c) second instar; d) third instar; e) fourth instar; f) sixth instar (Photo credits: David Taylor and Tahnee Bennett).

2.3.2.3 Pupae

As the larvae neared the pupation stage, they descended from the branches where they had been feeding and began pupating on the vermiculite substrate. During this process, they formed a cocoon using silk interwoven with vermiculite. In nature, the cocoon would comprise silk and leaf litter. The pupae exhibited a dark brown colouration, which intensified with age, and they

featured distinct wing pads visible on the thorax (Figure 2.4a). The anterior end of the body appeared blunt, while the posterior end was conical in shape. To differentiate between male and female pupae, attention was paid to the location of the genital slits. The sexual differences were visible from the ventral side under a dissecting microscope or magnifying glass. The male genital slit is located on the middle section of the 9th abdominal segment (Figure 2.4b & c). In contrast, the female genital slit is on the anterior edge of the 8th abdominal segment (Figure 2.4d & e).

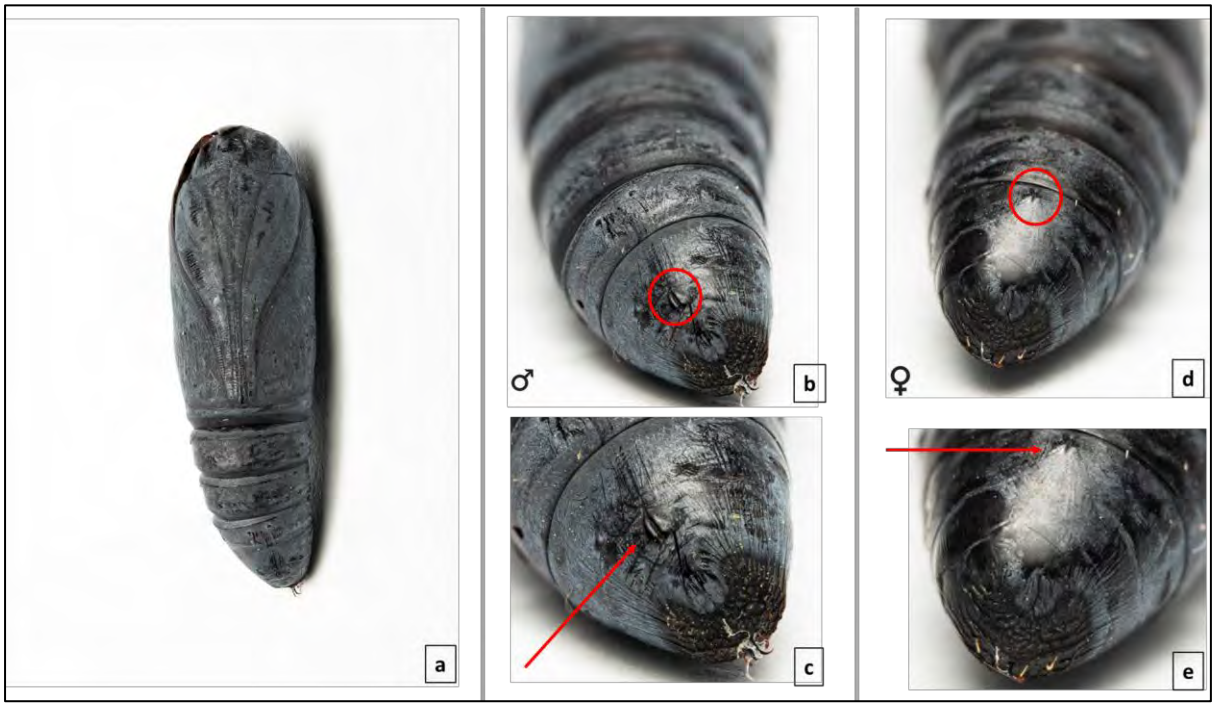


Figure 2.4: a) Pupa of *Serrodes partita* with wing pads visible; b & c) male pupa; d & e) female pupa. Red arrows and circles show the position of the genital slits. (Photo credit: David Taylor).

2.3.2.4 Adult

The adult males and females exhibit only slightly noticeable sexual dimorphism. Males are slightly bigger than females. Moreover, the male possesses a stouter body with a broad posterior, whereas the female's posterior end tapers to a point. Antennal sexual dimorphism was evident in *S. partita* adults. Both males and females have filiform antennae, but upon closer

examination, it was observed that the males possess more setae than females. The presence of additional setae on the male antennae distinguishes them from the females, contributing to the overall sexual dimorphism in the species (Figure 2.5).

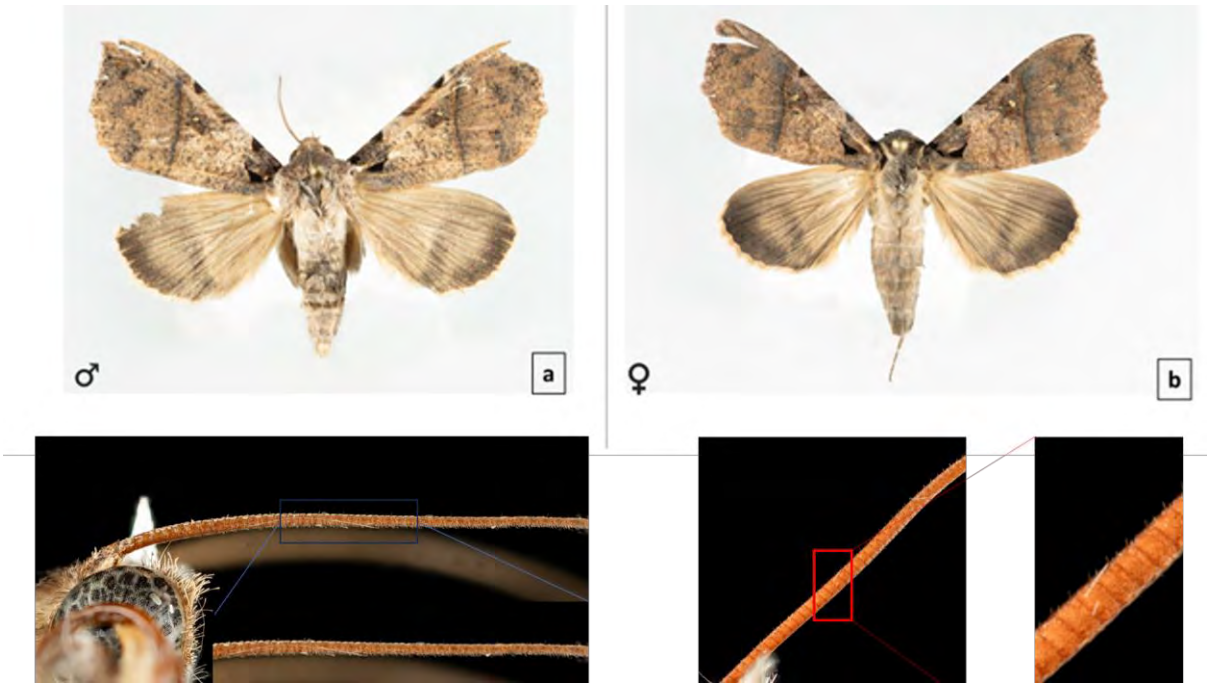


Figure 2.5: Male (a) and female (b) adult *Serrodes partita* with zoomed antennae (Photo credit: David Taylor).

2.4 Discussion

Serrodes partita larvae showed a consistent failure to establish on all tested artificial diets, including modified versions. The inability to thrive on artificial diets can stem from various factors, such as issues of poor palatability, suitability, feeding behaviour, toxin accumulation, nutritional complexity, and the absence of essential nutrients (Cohen 2015). Since very little to no feeding was observed during the study, it is indicative that the primary cause of failure to establish could be the lack of a proper feeding stimulant. A phagostimulant, as defined by Morales-Ramos et al. (2023), is a chemical compound that triggers insect-feeding responses. Interestingly, these substances might lack inherent nutritional value. Notably, Firlej et al.

(2006) enhanced the weight gain of the mirid mite-predator *Hyaliodes vitripennis* (Say) (Hemiptera: Miridae) by introducing the phagostimulant β-sitosterol into an artificial diet.

Previous research by Hewitt et al. (1969) successfully demonstrated that *S. partita* larvae require a feeding stimulant called quebrachitol, ideally at 12 % to 15 % concentration. Quebrachitol, an optically active methoxy analogue of Inositol (Yodthong et al. 2018), occurs naturally but in very limited amounts in the larval host plant of *S. partita* (Hewitt 1969). Due to the difficulty and high cost associated with isolating this compound, attempts were made to introduce ethanolic extracts and crude plant extracts into the diet; unfortunately, they failed to elicit feeding responses. Further investigations into alternative derivatives of quebrachitol, such as Inositol, are necessary to identify potential feeding stimulants for *S. partita* larvae. Inositol and sucrose have been identified as feeding stimulants in some insects (Morales-Ramos et al. 2023). This research may unlock the successful establishment of *S. partita* on artificial diets. Alternatively, a carcass milling technique to analyse the nutrient composition of insects could be used (Morales-Ramos et al. 2023). This technique has been used to successfully improve the artificial diet of *Eldana saccharina* Walker (Lepidoptera: Pyralidae) (Ngomane et al. 2022).

Successful larval rearing was achieved on the natural host, but a significant increase in mortality was observed during the first instar. Zalucki et al. (2002) conducted a comprehensive review of neonate mortality across 80 Lepidoptera species, revealing mortality rates ranging from 9 % to as high as 96 %. Various factors have been suggested as potential causes for high neonate mortality, including predators, pathogens, parasitoids, and environmental factors (Price et al. 2011). However, since the mortality was observed in laboratory-reared insects, external factors might have been responsible for the observed mortality. Plant traits, such as leaf toughness and tannin levels, emerge as the most probable causes of high neonate mortality (Zalucki et al. 2002, Cribb et al. 2010). Cochereau (1969) reported high neonate mortality of *Eudocima phalonia* when egg-laying coincides with drought. Additionally, mortality was observed at the fourth instar, with Swart (1975) reporting high mortality due to an unknown

pathogenic agent, which was most likely the novel insect virus identified in this study (Chapter 3).

The life history findings in this study are consistent with previous research conducted on *S partita* in South Africa by Taylor (1951) and Neubecker (1962). They reported a total life span ranging from 83 to 91 days, with larval life stages spanning 51 to 58 days and pupal stages lasting from 20 to 33 days. In Taylor's (1951) study, seven instars were recorded, while in this current study, six instars were recorded. Generally, the number of instars is constant within a species (Salgado & Saastamoinen 2019, Kivelä et al. 2020). However, some species have had instances where the number of instars varies due to environmental factors. Under poor environmental conditions, larvae may undergo additional instars to attain the size threshold required for metamorphosis (Esperk et al. 2013, Grunert et al. 2015). This could explain the disparity in findings between this study and earlier studies. The variation in instar numbers recorded in this study might be attributed to the specific environmental conditions in the laboratory experienced by the larvae during their growth and development. Moreover, measurements were taken every fifth day to minimise disturbance hence an instar stage might have been missed.

Pupal sexual dimorphism was observed in *S. partita* based on the location of genital slits. Sexing pupae based on the position of genital slits was described by Tuncer & Aker (2017) on *Hyphantria cunea* Drury (Lepidoptera: Erebidae). Antennal specialisations and sexual dimorphism are common traits in adult insects, particularly in Lepidoptera. According to Faucheux (1999), adults often exhibit a strong degree of antennal specialisation and sexual dimorphism, which is believed to be of adaptive significance for male perception of female sex pheromones. Typically, males have elaborate pectinate or bipectinate antennae, while females retain the filiform type (Schulz et al. 1990, Hansson et al. 1995, Hallberg et al. 2003). This sexual dimorphism is evident in most families of Lepidoptera and can manifest at various morphological levels (Hallberg et al. 2003). In the case of adult *S. partita*, antennal sexual dimorphism was observed, with males having more setae than females. Insect antennae house

several types of sensilla with diverse functions that play crucial roles in various aspects of adult life, including recognising food, hosts, oviposition sites, sexual partners, and identifying conspecifics (Schulz et al. 1990, Hallem et al. 2006). More setae in males suggest that they might use them for perceiving female sex pheromones (Johnson et al. 2022). These findings contribute to our understanding of the fascinating adaptations and behaviours in their interactions with their environment and conspecifics.

In conclusion, future research efforts can focus on methods to extract larval phagostimulants of *S. partita* that can be used to enhance diet. Furthermore, understanding the factors behind larval mortality, which was particularly high in neonate and fourth instars, recorded in laboratory-reared insects, might aid in our understanding of the *S. partita* outbreak phenomena. It may also enable the development of targeted strategies to mitigate mortality, potentially improving the overall survival rate of *S. partita*. Additionally, insights into sexual dimorphism and sensory mechanisms enhance our understanding of the species' reproductive strategies and ecological interactions with the potential for developing sex pheromones. Considering the findings from this study, they can be used to further refine the approach to developing a rearing protocol for *S. partita.*

CHAPTER 3

Refining *Serrodes partita* feeding choice, patterns and damage severity

3.1 Introduction

Serrodes partita is recognised as an outbreak migratory pest that predominantly affects soft citrus (Johannsmeier 1998, Moore 2010). Even during non-outbreak years, moth occurrences were observed, as Whitehead (1972) noted. Bosch (1971a) suggested that *Oraesia* sp. (*Calpe* sp.) moths in non-outbreak years might stem from non-migratory resident moth populations that exhibit persistent behaviour. A study by Whitehead & Rust (1971) reported that less than 5 % of marked moths returned to the same orchard, corroborating Johannsmeier's (1976) findings of low-marked moth returns. Additionally, Whitehead & Rust (1972) documented extensive infestations on the leeward side of large apricot orchards in the Little Karoo region. Significant damage was observed on the northern side of orchards located south of the Swartberg Range. This observation led to the implementation of concentrated light barriers on the leeward edge of the orchard to exclude moths. Subsequent experimentation revealed that illuminating more than one side of the orchard reduced moth populations. This indicated that moths did not originate exclusively from the leeward side. In contrast, Bosch (1971a) achieved nearly complete protection against *Oraesia* sp. Guenée by surrounding the entire vineyard with a barrier of mercury vapour lamps.

Recent occurrences of moth activity in the Eastern Cape have underscored the need for deeper investigation into their residency patterns. Such an inquiry is essential to develop enhanced management strategies, which could involve either "lure and kill" approaches for residential moth populations or exclusion-based methods for migratory moth populations. No comprehensive studies have been undertaken to elucidate the host-seeking behaviour of *S.*

partita. The existing body of research on the fruit-piercing moth *S. partita* in South Africa has primarily concentrated on monitoring and control measures, with less emphasis on understanding the moth's host-seeking tendencies.

As outlined by Hargreaves (1936), citrus is susceptible to fruit-piercing moth damage approximately 4 to 5 weeks before reaching maturity, occasionally experiencing attacks even earlier. Cochereau (1977) noted that fruit-piercing moths can target Navels and Mandarins even in their green and highly acidic stages, with feeding activity intensifying as the fruit nears maturity. In some instances, unripe and overly ripe fruit are susceptible to damage when fully ripe fruit is unavailable (Bänziger 1982), particularly during high moth densities (Kumar & Lal 1983, Fay 2002). Notably, these moths display a preference for intact ripe fruit as opposed to damaged or fallen fruit (Bänziger 1982). Fay & Halfpapp (2006) demonstrated that fruit-feeding moth preferences can be determined based on fruit colour as an indicator of ripeness. Despite this, the susceptibility of *S. partita's* host has not yet been experimentally tested. Moreover, accurately estimating the economic ramifications of fruit-piercing moth damage remains challenging due to potential confounding factors such as fruit drop and secondary infestations.

Another challenge posed by *S. partita* feeding is the delayed visibility of damage, which does not immediately manifest after feeding (Johannsmeier 1976). A lag period exists between fruit-piercing activity and the subsequent onset of fruit rot. Hargreaves (1936) reported that this lag phase, varying between 1 to 2 weeks, is contingent upon climate conditions and the cultivar, particularly in oranges. However, the precise interval between *S. partita* damage on soft citrus and the emergence of damage symptoms has yet to be definitively established.

In the Eastern Cape region, damaged fruit has to be taken out during harvest and in packhouses, a process further assisted by ethylene degreening (Moore 2010). Particularly for Satsuma Mandarins, ethylene degreening is employed to facilitate fruit colour transformation. This degreening process can span up to five days and is influenced by ethylene concentration,

temperature, humidity, and air circulation (Porat 2008). Following the 1999 outbreak, packhouses in the Sundays River Valley culled approximately 10 to 15 % of affected fruit, while Gamtoos and Kat River Valley saw culling rates of 5 % and 3 to 4 %, respectively (Moore 2010). Reducing or omitting the degreening period increases the likelihood of packaged pierced fruit not exhibiting visible rotting symptoms. Therefore, there is a need to establish a period between picking and packing susceptible cultivars during outbreaks.

Against this backdrop, the primary aim of this study was to understand the flight behaviour, feeding tendencies, and preferences of *S. partita* within Eastern Cape citrus orchards. The insights garnered from this investigation are poised to provide valuable guidance on orchestrating effective monitoring and management strategies and identifying optimal timings for implementing these interventions. Additionally, the study aided in accurately attributing fruit damage to the actual pest, facilitating the identification of the most vulnerable citrus varieties. Moreover, a significant focus of the study was to establish the temporal interval between inflicted damage and the onset of observable rotting symptoms. The study's specific objectives encompass several key dimensions: (i) discerning whether a consistent population frequents a particular orchard, (ii) investigating potential directional trends within infestations across orchards, (iii) evaluating the susceptibility of different soft citrus cultivars to *S. partita* damage, (iv) delving into *S. partita* feeding preferences by assessing the role of fruit maturity and physical soundness (as indicated by the presence or absence of physical deformities), and (v) pinpointing the temporal gap between fruit damage and the manifestation of symptomatic changes.

3.2 Materials and Methods

3.2.1 Mass trapping

To assess whether moths exhibited directional tendencies when congregating in a specific orchard, a mass trapping experiment was conducted at TP Ferreira Farm (33°74'84"S; 24°71'19" E) in Patensie. Two Satsuma Mandarin orchards were selected for the study due to their strategic placement and susceptibility to *S. partita* damage. One orchard (referred to as orchard 21) was situated alongside the Gamtoos River, while the other orchard (designated as orchard 7) was located on the opposite side of the valley, adjacent to the veld (Figure 3.1).

Figure 3.1: Orchard locations for mass trapping. One orchard is situated adjacent to the river, while the other is adjacent to the veld. Red numbers indicate orchard numbers.

Each trap was lured with fresh bananas, which are commercial hybrids of *Musa acuminata* Colla (Musaceae). Funnel traps were strategically positioned around the orchard. The monitoring protocol involved inspecting the traps at 3 to 4-day intervals, during which the traps were emptied and the banana lure was replaced. A total of 50 traps were used in Orchard 7. Twenty were placed along each row on both the Eastern and Western sides. On the Northern side, a configuration of 10 traps was arranged along the last row, with a spacing of 6 m between each trap (Figure 3.2a). Similarly, the identical trap arrangement was replicated in Orchard 21 with 28 traps. The traps were evenly divided between the Eastern and Western sides (Fig 3.2b).

Figure 3.2: Mass trapping of moths in the orchards. (a) Arrangement of traps along three sides of the orchard. (b) Arrangement of traps along two sides of the orchard.

3.2.2 Mark and recapture

A mark and recapture study was conducted at TP Ferreira farm in the Gamtoos Valley to investigate whether moths continued to come to the same orchard night after night. Moths were captured at night between 18h00 and 23h00 using a hand capture method, while peeled bananas were strategically placed also to help attract them. The moths were captured as they oriented towards the fruit or while feeding. Blue and pink UV powder were applied in a plastic tub to mark the captured moths. After marking, the moths were placed in cages and released 10 m away from the edge of the orchard at the end of each night (Figure 3.3). The marking process was repeated twice on consecutive days. Ten funnel traps were placed on the orchard's edge at 12 m apart and left in place for five consecutive days to recapture the marked moths. Any recaptured moths were subjected to ultraviolet light to detect the presence of UV powder. The proportion of recaptured individuals was then used to estimate the rate at which moths returned to the same orchards. Throughout the mark and recapture period, the weather was characterised by wet and humid conditions with occasional slight rain showers.

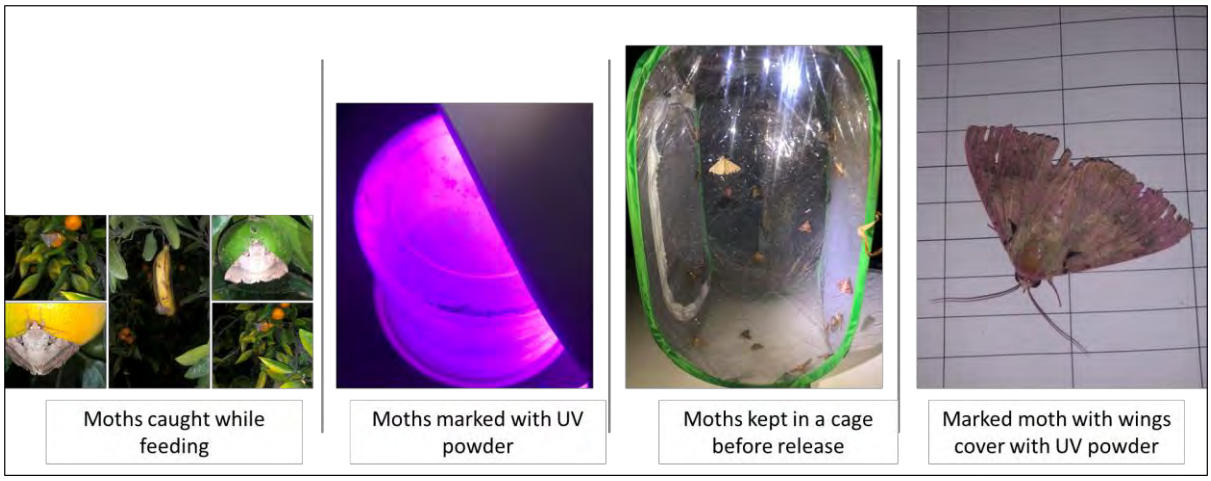


Figure 3.3: The mark and recapture procedure used for *Serrodes partita* in a soft citrus orchard.

3.2.3 *Serrodes partita* feeding assessments: investigating feeding patterns

The evaluation of adult *S. partita* feeding preferences was conducted across three Satsuma Mandarin orchards in Patensie over a span of four weeks in 2023. Orchard assessments took place between 18h30 and 23h00 using battery-powered headlamps (magneto®, 120 lumens). This method involved a systematic traversal between rows of trees, during which observations were made regarding the condition of the fruit being fed on and the condition of four other adjacent fruits. To assess this, a scoring mechanism adapted from Fay & Halfpapp (2006) was employed. The scoring system encompassed five distinct fruit categories designed to assign fruit scores based on their ripening physical status (Table 3.1).

Table 3.1: Fruit rating on maturity and quality scoring as described by Fay and Halfpapp (2006).

Rating	A visual showcase of various fruits	Fruit Description
1		**Green** – no sign of yellowing or colour change
2		**Colouring** – signs of yellow but colour change incomplete

3		**Ripe** – uniformly yellow or orange coloured and regarded as harvestable
4		**Overripe** – beyond the commercial harvest stage (usually dark orange in colour and easily detached from the tree
5		**Damaged** – generally split rind or evidence of bird or insect feeding. Damaged fruit could be green, colouring or ripe.

A ratio system (R) determined the relationship between the fruit being fed on and the four adjacent fruits. An average score was obtained from the four adjacent fruit scores and then divided by the corresponding score of the fruit being fed on where;

R > 1, fruit being fed on is more ripe/damaged than adjacent fruits.

R = 1, fruit being fed on has a similar score (green, colouring, ripe, overripe or damaged) with adjacent fruits.

R < 1, fruit fed on is less ripe/damaged than adjacent fruits.

3.2.4 Vulnerability of two soft citrus cultivars to *Serrodes partita* damage

Soft citrus cultivars, namely Satsuma Mandarins and Clementines, mature at different stages, posing a challenge in identifying the most susceptible variety to *S. partita* damage. Satsumas demonstrate very early maturation, while Clementines fall within the mid-early ripening category. The study was conducted in Committee's Drift and Patensie between 2022 and 2023. The comparison of the count of moth feeding scars per fruit was employed as the yardstick to evaluate the susceptibility of different citrus cultivars to *S. partita* damage. For this assessment, fruits exhibiting characteristic signs of fruit feeding damage by *S. partita* were chosen. These had feeding punctures surrounded by soft, damp, brownish necrotic ring tissue (Figure 3.4). They were randomly collected from the orchard floor on the trees leading to the edge of the leeward side as it contained the most damaged fruit. Information concerning the cultivar and the count of feeding scars was recorded. To confirm fruit-piercing moth damage, the collected fruits were promptly dissected from the centre of the rot. The presence of an empty cavity beneath the rind was used as a criterion to validate fruit-piercing moth damage, as outlined by Hargreaves (1936) and Bosch (1970).

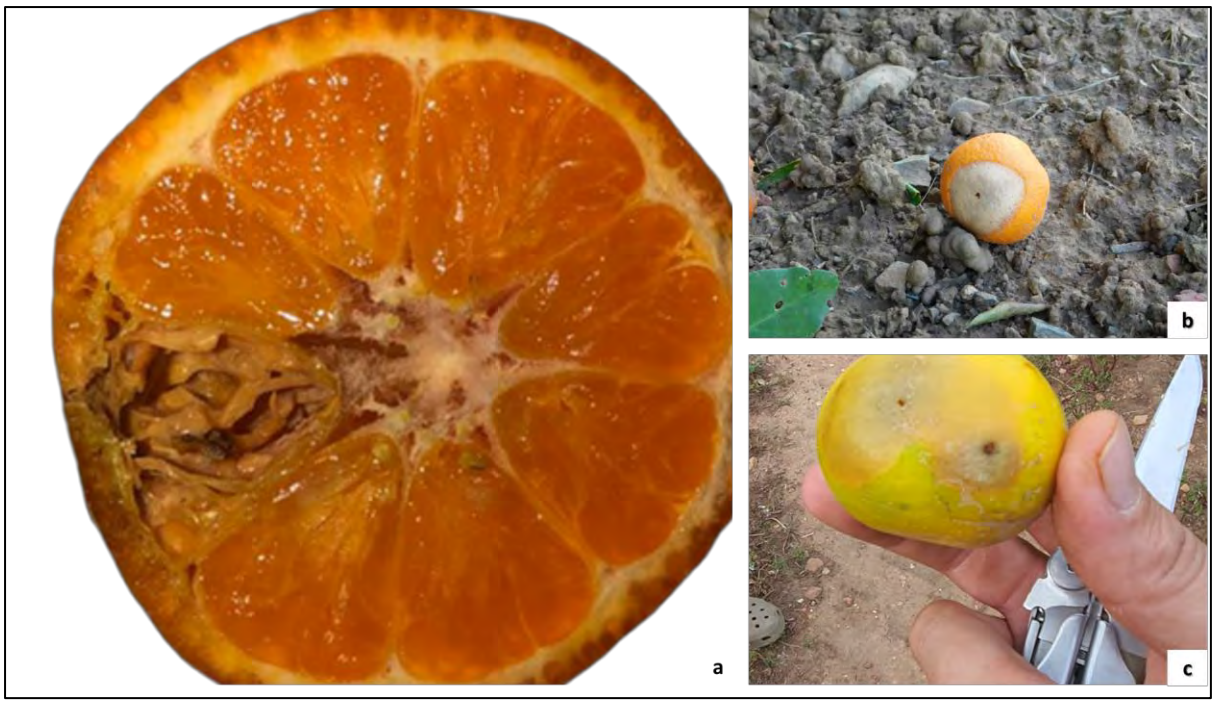


Figure 3.4: Fruit-feeding scars caused by *Serrodes partita* on soft citrus. (a) Internal damage showing the empty space where juice has been extracted, (b) Clementine damage and (c) Satsuma Mandarin damage. (Photo credit: David Taylor and Wayne Kirkman).

3.2.5 Effects of fruit-piercing on the incubation period of symptoms

Damage interval trials were conducted concurrently with feeding assessment trials to establish the interval between the feeding and the emergence of feeding-related symptoms. Damage trials were carried out following the methodology originally established by Hargreaves (1936). However, in contrast to their simulations involving sterile needles in field conditions, our trials utilised actual fruit-piercing moths under controlled laboratory conditions. During the nocturnal survey sessions, fruits being actively fed upon, excluding those damaged, were gathered and taken to the laboratory. The fruit cultivar and the number of days until observable damage symptoms—specifically, the onset of visible rotting symptoms appeared were noted. The identification of damage symptoms was based on the presence of evident fruit rot (Figure 3.5b). Some fed-upon fruits did not exhibit rotting symptoms; rather, they shrunk and displayed visible rind shrivelling. However, these cases were not considered.

Since the number of fruits collected directly from the field was limited due to most feeding being observed on already damaged fruits, laboratory-based feeding simulations were also executed. These simulations involved using laboratory-reared adult moths. *Serrodes partita* adults were hand collected while feeding on soft citrus at Naudeshoek Farm (33°00’49"S; 26°95'32"E). These adults were then brought to the laboratory and placed in rearing cages (60 × 60 × 90 cm) made of cloth screen for egg-laying. The experimental setup was initiated by subjecting adult moths to a 24-hour period of food deprivation, ensuring that feeding commenced immediately upon food reintroduction. Subsequently, each fruit was exposed to a single adult moth, initiating the feeding process. This usually took two to five minutes, and the feeding was considered complete after the moth moved away from the fruit. After feeding, the fruit were placed on trays (50 × 35cm) with 12 fruit evenly spaced on the tray and left at room temperature (21 - 24°C). (Figure 3.5a). The number of days taken between the feeding event and the appearance of symptoms was recorded.

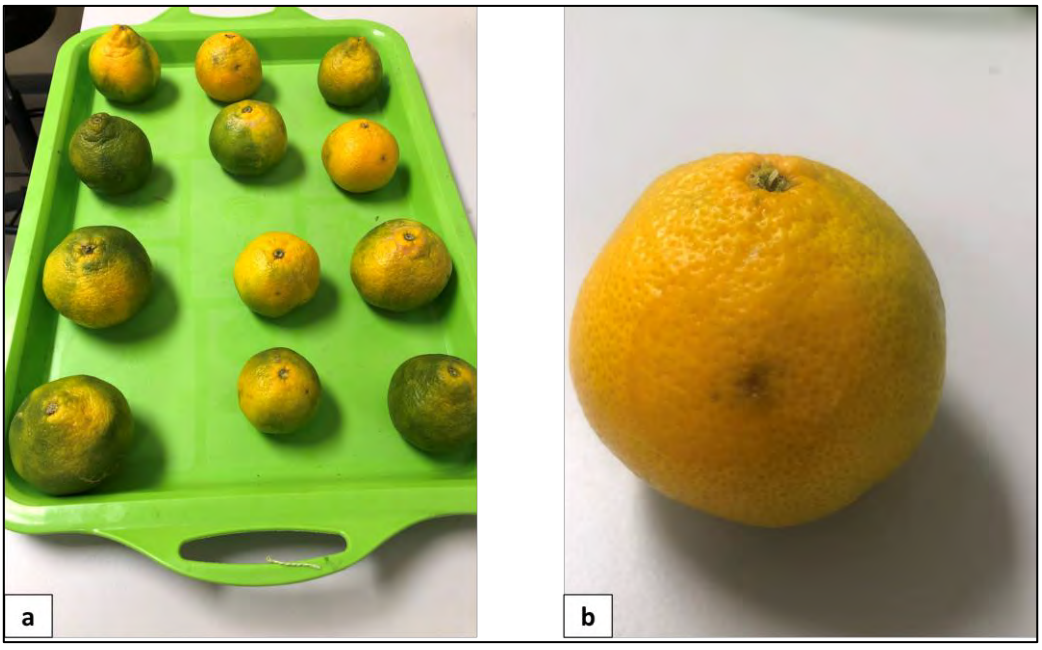


Figure 3.5: (a) Experimental arrangement depicting the monitored fruits for assessing damage symptoms. (b) Illustration of a fruit displaying the initial signs of damage symptoms.

3.2.6 Data analysis

The data analysis was conducted using R software version 4.2.3 (R Core Team 2023). Mass trapping data and lure presentation failed the normality test using the Shapiro-Wilk test. Mass trapping data was analysed by generalized linear mixed effects models following the approach outlined by Bolker et al. (2009). The models were formulated, incorporating two fixed effects: cardinal points and days. A Poisson regression model utilising the "lme4" package (Bates et al., 2015) was used. The generalized linear model (GLM) framework, using the "glm" function within the "stats" package, was adopted (R Core Team 2023) to fit the model. Lure presentation data were analysed using the "glmmTMB" package (Bolker et al., 2009), fitting a generalized linear mixed-effects model with a negative binomial distribution. All model diagnostics were performed using the "DHARMa" package (Hartig & Lohse 2020).

The data from feeding assessments were tested for normality and homogeneity of variance using the Shapiro-Wilk and Bartlett tests. The data were then analysed using one-way analysis of variance (ANOVA). Prior to analysis, the normality of the percentage of fruit fed was assessed using the Shapiro-Wilk test and the homogeneity of variance was examined using the Bartlett test. To determine specific group differences, post hoc tests were performed using the "emmeans" function. Visualisation of multiple comparisons was achieved through the application of the compact letter display procedure ("cld"), implementing a Tukey adjustment with a significance level (α) set at 0.05. This visualisation method facilitated a clear representation of the outcomes from numerous comparisons. Wilcoxon signed-rank tests were employed to assess the significance of differences in the symptom incubation period and feeding scars data. This non-parametric test was chosen due to the non-normal distribution of the data, as confirmed by Shapiro-Wilk tests ($P < 0.05$).

3.3 Results

3.3.1 Mass trapping

In the veld adjacent orchard (orchard 7), there were significant differences in *S. partita* in trap catches from different orientations. The northern side (veld adjacent side) had the highest number of moths caught ($\chi 2 = 27.13$; df = 2; $P < 0.0001$). There were also significant differences in the number of moths caught on different days, with the highest moth catches on days 7 and 13 ($\chi 2 = 111.62$; df = 4; $P < 0.0001$). However, there were no substantial differences in the interaction between the trap orientation and day ($\chi 2 = 13.54$; df = 8; $P > 0.05$).

The river adjacent orchard (orchard 21) exhibited significant differences in the number of *S. partita* caught on different days, with the highest trap catches on days 7 and 13 ($\chi 2 = 45.81$; df = 4; $P < 0.0001$). Significant differences were also observed in trap orientation, with the eastern side (river adjacent side) having the highest trap catches ($\chi 2 = 4.58$; df = 1; $P < 0.001$). An interaction was observed between trap orientation and day ($\chi 2 = 10.09$; df = 4; $P = 0.04857$), with the highest number of *S. partita* being caught on days 7 and 13 from the eastern side (Figure 3.6).

3.3.2 Mark and recapture

During the study, 67 moths were captured, marked, and released on the same night for two consecutive days. Over the following five consecutive days, 89 additional moths were caught in the traps. Among these newly caught moths, only three were found to be marked individuals, resulting in a return rate of 4.5 %.

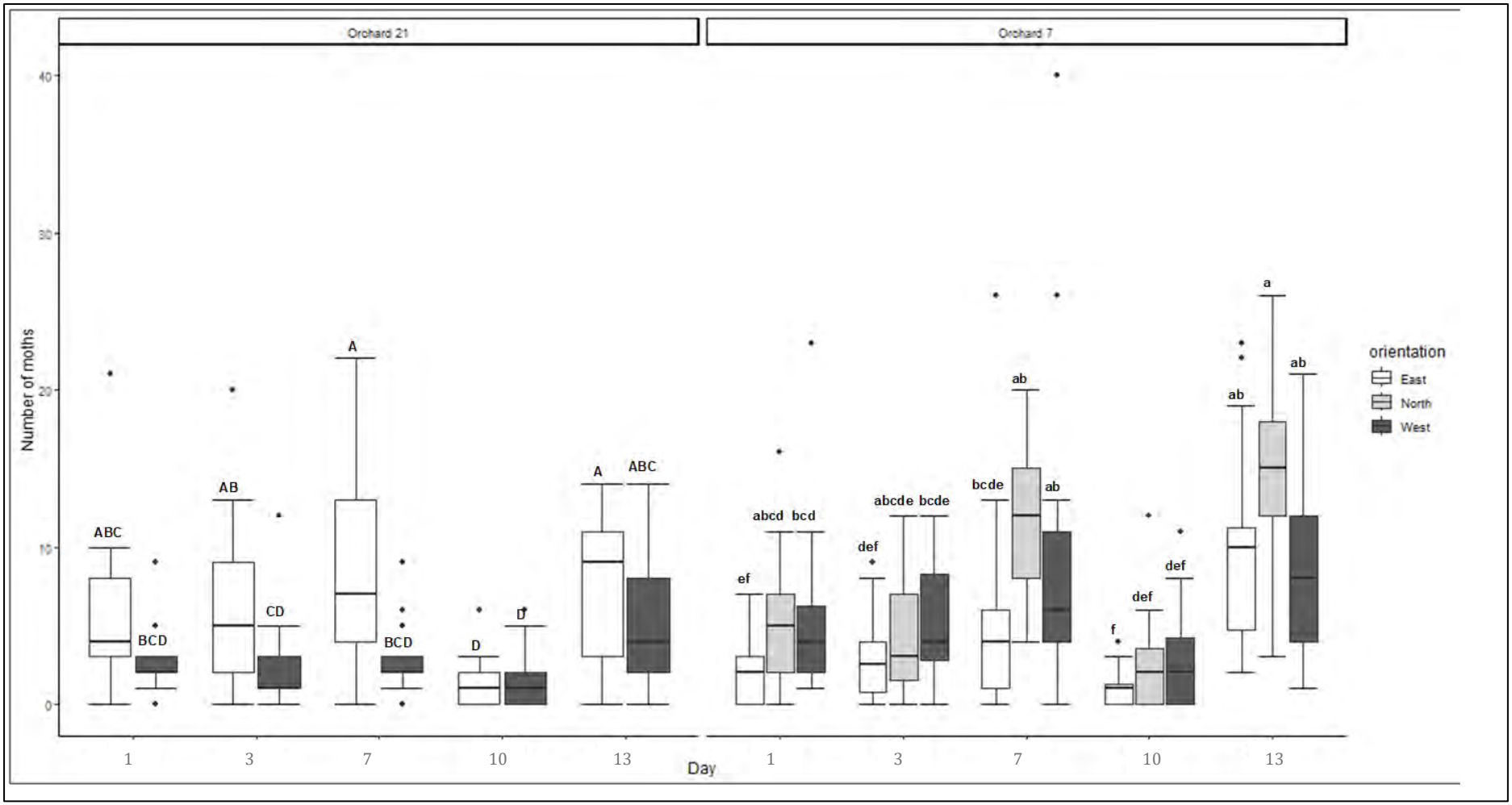


Figure 3.6: Box plots of *Serrodes partita* trap catches in Orchard 7 and Orchard 21 across a 13-day period and different orientations. The same letters above plots indicate no significant difference in trap catches within each orchard (Tukey HSD, $P < 0.05$).

3.3.3 *Serrodes partita* feeding assessment: investigating feeding patterns

An average ratio of 2.16 was obtained when the average score of adjacent fruit was expressed as a ratio of the average score of pierced fruit, indicating that the fruit became more desirable for piercing as they ripened or were damaged. Furthermore, there were significant differences between different conditions of pierced fruit ($F_{(4, 40)} = 426.5$; $P < 0.05$). Damaged fruit were significantly more fed on than undamaged fruit (Figure 3.7).

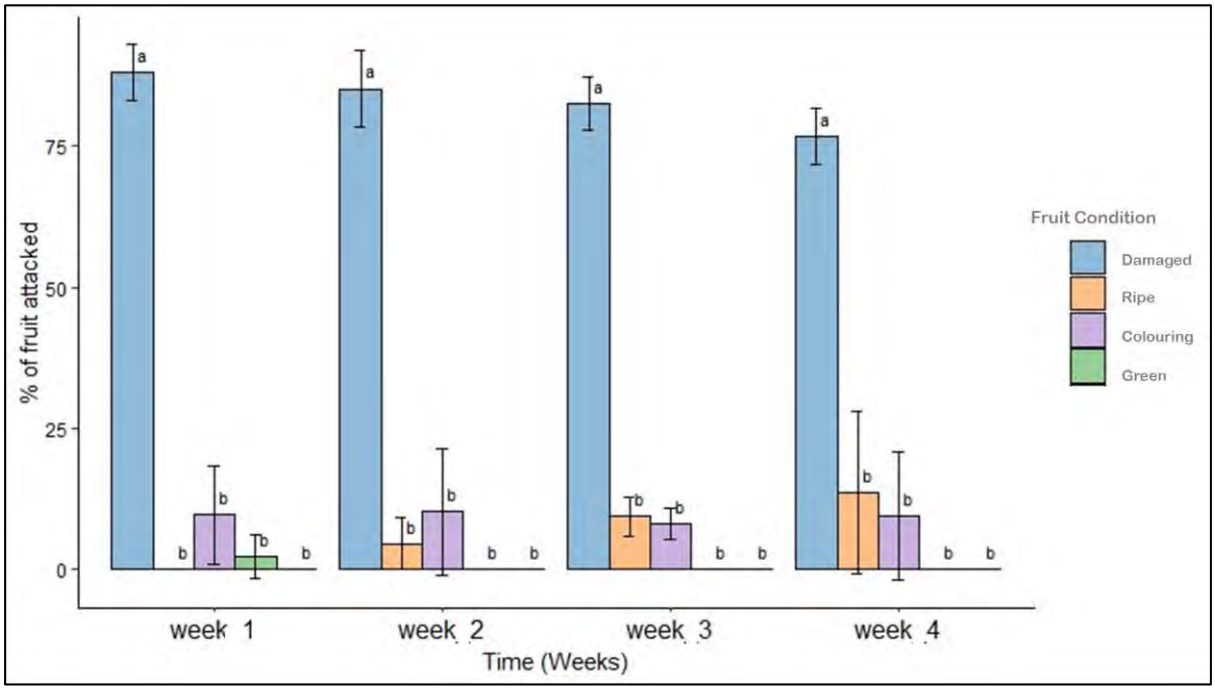


Figure 3.7: Trends in soft citrus fruit damage by *Serrodes partita* over weekly monitoring in Patensie (mean ± SD). Statistically significant variations in fruit condition targeted at each monitoring interval are denoted by different letters (Tukey HSD, $P < 0.05$).

3.3.4 Vulnerability of two soft citrus cultivars to *Serrodes partita* damage

The Mann-Whitney U test was conducted to determine whether there is a difference in the number of feeding scars between Satsumas (Mdn = 2) and Clementines (Mdn = 1). Satsumas had significantly more feeding scars on average (2.1) as compared to Clementines (1.08) ($W = 2170.5$, $P < 0.005$) (Figure 3.8).

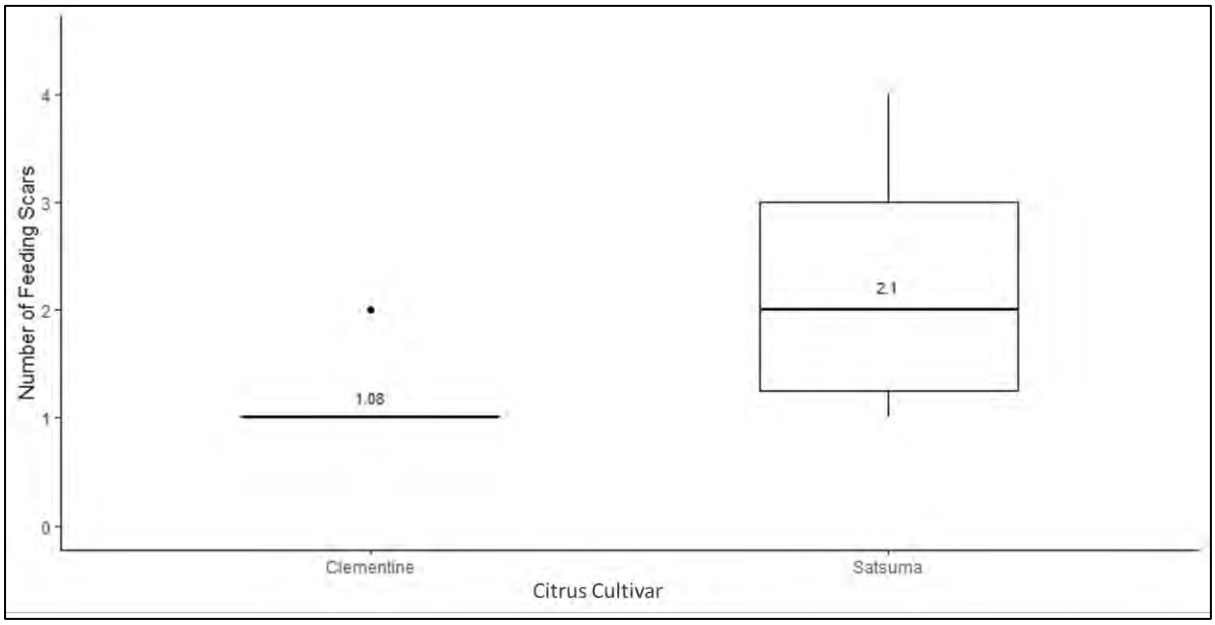


Figure 3.8: Box plots illustrating the severity of feeding by *Serrodes partita* through quantifying feeding scars on two susceptible soft citrus cultivars: Satsumas and Clementines. Outliers are represented by black dots.

3.3.5 Effects of fruit-piercing on the incubation period of symptoms

A Wilcoxon signed-ranks test showed that there were no significant differences ($Z = 394.5$, $P = 0.21$) between the Satsumas (Mdn = 3) and the Clementines (Mdn = 4). Satsumas took an average of 3.46 days to show symptoms, while Clementines took an average of 3.65 days to show rotting symptoms (Figure 3.9).

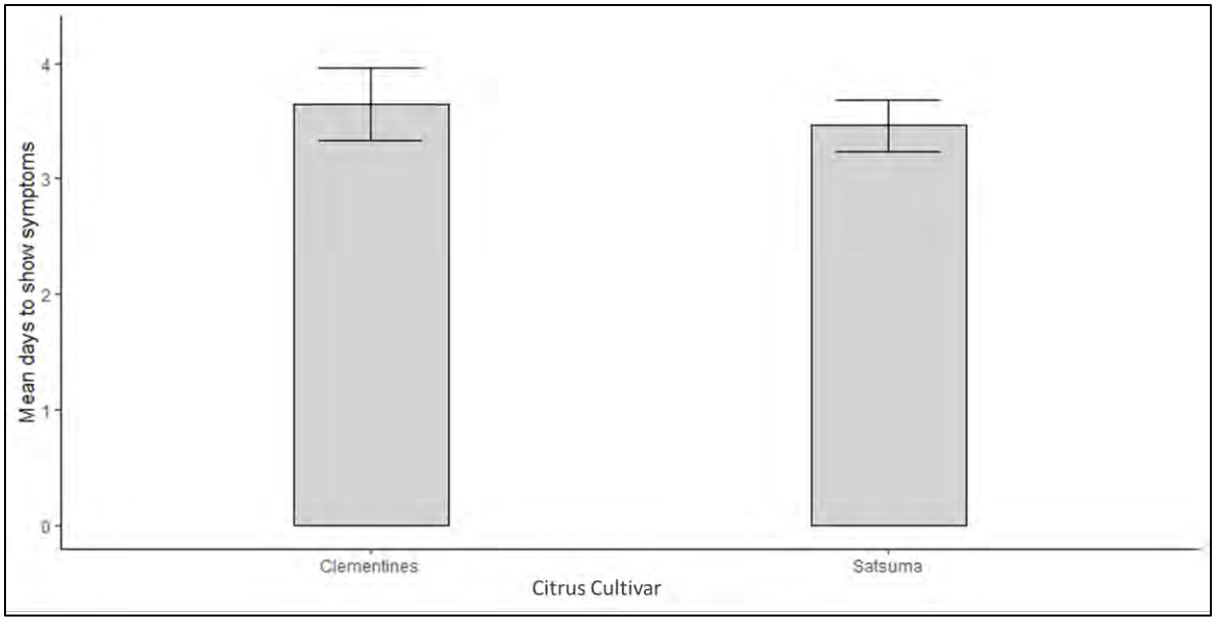


Figure 3.9: Average number of days for rotting symptoms to appear in two citrus cultivars (Satsumas and Clementines) after piercing by *Serrodes partita* under laboratory conditions. Error bars represent 95 % confidence intervals.

3.4 Discussion

In this study, a low proportion of moths returned to the same orchard, consistent with previous findings by Johannsmeier (1976), who also noted low proportions (< 6 %) of returns by fruit-feeding moths *Achaea* sp. L. and *Sphingomorpha chlorea* Cramer in peach orchards. This observation highlights the potential dispersal behaviour of the species. It suggests that a significant portion of the population might move to other locations rather than remain in the same area. It is essential to consider that the low number of insect species used in this study might be misleading, as other potential food sources, such as other ripening fruit in the Gamtoos Valley, could have influenced the moth's choices. However, the finding that some moths did return to the same orchard indicates the possible presence of resident moth populations, as opposed to migratory populations. Resident moth populations visit the same food sources at night and seek shelter outside the orchards during the day (Bosch 1971b).

During mass trapping, we observed the highest moth activity along the boundaries adjoining both the river and the veld. This observation suggests the potential for inter-habitat movement of *S. partita* between the orchard and its surrounding environment. The presence of fruit-piercing moths in each area is influenced by several factors, including the availability of larval food sources, suitable hiding places for adult moths, and prevailing climate conditions (Leroy et al., 2021). Roland et al. (2012) and Leroy et al. (2021) have reported that adult fruit-piercing moths, such as *E. phalonia* tend to seek refuge in vineyards, reeds, and shaded areas during daylight hours. The river valley and veld adjacent to the orchards in the Gamtoos region provide an ideal habitat conducive to this behaviour. Interestingly, in Australia, fruit-piercing moths have been reported to undertake extensive migrations between their breeding sites and fruit orchards (Sands et al., 1991). Conversely, observations by Cochereau (1969) in New Caledonia revealed that moths tend to move relatively shorter distances between their larval host plants and orchards. These findings suggest that these are residential moth populations which contribute to the seasonal prevalence of *S. partita*, particularly in the Gamtoos Valley.

While the highest moth catches were indeed recorded on the sides adjacent to both the river and the veld, it is noteworthy that significant numbers were also recorded from other sides of the orchards. This finding aligns with the observations made by Bosch (1971a) and Whitehead & Rust (1972), who successfully reduced infestations by employing illumination on multiple sides of the orchard. It becomes crucial to consider placing traps around the entire orchard to enhance mass trapping and mass annihilation strategies for more effective pest management. However, a greater concentration of traps should be strategically positioned on the sides that are more exposed to the external environment.

Serrodes partita feeding behaviour was found to be more pronounced on fruit that had physical damage. This contrasts with the observations made by Fay & Halfpapp (2006), who reported no substantial distinction between damaged fruit and fruits at varying ripening stages by *E. phalonia* in Australia. Nevertheless, they document escalated damage to ripening fruit as the harvesting period approached, spanning the eight weeks preceding harvest. This aligns with

the present study's findings, wherein fruits showed a general trend of becoming more susceptible as they ripen. Fay & Halfpapp (2006) noted instances of *S. campana* feeding on damaged fruit in their study. However, it's important to acknowledge that they reported relatively low numbers of *S. campana* compared to other fruit-piercing moth species; hence, they could not make substantial conclusions. Additionally, their research focused on navels and grapefruit, which fall under the category of hard citrus fruits. In contrast, *Serrodes* sp. is categorised as a "soft fruit-piercer," as described by Kriegler (1958).

The tendency for *S. partita* to exhibit a preference for physically damaged fruit could be attributed to the fact that compromised rinds offer easier access to the fruit juice. *Serrodes partita* does not prefer hard-skinned citrus cultivars such as navels but instead soft-skinned fruits (Kriegler 1958). Consequently, fruits featuring compromised rinds are more conducive to feeding due to the reduced effort required to access the juice. Additionally, in physically damaged fruit, the exposure of fruit juices could lead to the heightened release of volatile organic compounds, which might otherwise be hindered by an intact rind. This, in turn, could intensify the attractiveness of the fruit to *S. partita*, potentially explaining the observed increased preference for feeding on physically damaged fruit.

During this study, it was observed that Satsuma Mandarins often exhibit multiple feeding scars, a phenomenon corroborated by a separate investigation conducted by Jayanathi et al. (2020) in India. In their research, Jayanathi et al. (2020) uncovered that *E. martena*, a fruit-piercing moth, displays a preference for feeding on previously pierced pomegranate fruits that have already been subjected to feeding by other moths. Their findings led them to conclude that moths are attracted to the distinctive scent characteristics of damaged fruits. *Serrodes partita* tends to coexist with fruit-sucking moths, such as *Achaea* spp., which feed on the fruit through the existing pierced hole (Moore 2010). This observed pattern hints at the possibility that damaged fruits emit volatile organic compounds that serve as attractants, luring the moths to feed on the same fruit. This dynamic may explain why physically damaged fruits become enticing targets for these moths, as they appear to possess attributes that are particularly attractive to their

feeding behaviours. Hargreaves (1936) reported the presence of as many as ten punctures per fruit and suggested that the extent of puncturing is influenced by the number of moths present and the specific cultivar type. While the findings were not conclusive, the observation of a higher number of punctures in Satsumas compared to Clementines suggests that Satsumas may be more susceptible to damage by *S. partita*.

Both Satsumas and Clementines were shown to display rotting symptoms within four days after piercing. It's noteworthy that injuries inflicted by *S. partita* on citrus, occurring weeks prior to harvest, can often be easily discerned during the picking or packhouse stages. However, damage occurring closer to the ripening of fruits, particularly just before harvest, may escape detection by the naked eye and go unnoticed during the packing process (Gunn 1929, Hargreaves 1936, Fay & Halfpapp 2006). Hargreaves (1936) reported that piercing was followed by premature yellowing from the point of injury, which typically took four to five days to manifest on navels and grapefruit. Additionally, they noted that the fruit displayed signs of rotting around the point of injury, which became visible after approximately two weeks. The extent of incidence and rotting, however, is contingent on various factors, including climatic conditions, cultivar type, and the degree of ripeness. In the Eastern Cape, Satsuma Mandarins usually undergo a degreening process lasting 24 to 72 hours to meet the standards required for the EU, USA, and Japanese markets, depending on their ripening status (Morales et al. 2022). Increasing the time interval between picking and packing can prove beneficial in mitigating post-packing losses, particularly following encounters with high moth populations.

In conjunction with previous research, the findings of this study provide valuable insights into the dispersal behaviour of moths within citrus orchards. However, it is important to acknowledge the potential influence of other available food sources and the necessity for more extensive and refined mark-and-recapture exercises in various citrus-growing regions of the Eastern Cape. These additional studies can contribute significantly to a deeper understanding of the dynamics of fruit-piercing moth species within a given area. The presence of suitable habitats and nearby larval hosts supports the notion that *S. partita* may have adopted a

residential lifestyle. Consequently, it is crucial to adapt management strategies accordingly to address this behaviour effectively. Building upon the findings of this study, the observed time interval from moth feeding damage to the onset of symptoms, coupled with the general increase in damage as fruits ripen, underscores the importance for growers to implement a holding period of at least four days between picking and packing to facilitate the removal of damaged fruit.

In light of the observations made by Fay & Halfpapp (2006), who raised questions regarding the handling of damaged and overripe fruit, it is noteworthy that in this study, over 85 % of the fruit consumed by the moths was damaged. Therefore, it is in the growers' best interest to leave damaged fruit as decoys. These decoys can be strategically placed in the orchards, particularly along the edges where fruit-piercing moth activity is most pronounced. Leaving damaged fruit in orchards requires careful consideration, as it may attract other citrus pests, such as fruit flies or False codling moth, which have more severe consequences due to their phytosanitary status (Grout & Moore 2015, Manrakhan 2022). Fruit flies prefer to lay their eggs on compromised fruit rinds to enhance larval survivability (Manrakhan 2022), necessitating weekly sanitation to remove all damaged fruit from the orchard as per management guidelines for these pests. However, the threat posed by leaving damaged fruit can potentially be mitigated by strategically leaving decoy fruit only on the orchard edges, particularly if the pressure from *S. partita* is high. There is a need to assess whether sanitation guidelines for both fruit flies and False codling moth can be revised to address the presence of *S. partita*. Moreover, there remains the need to determine the optimal number of traps required to control *S. partita* in citrus orchards effectively. This aspect warrants further investigation to develop more precise and efficient pest management strategies.

CHAPTER 4

Optimising lure type and lure presentation strategies for effective monitoring and management of the fruit-piercing moth *Serrodes partita* in citrus orchards

4.1 Introduction

The fruit-piercing moth, *S. partita*, poses a significant threat as a polyphagous pest to tropical and subtropical fruits such as citrus (Johannsmeier 1998). Addressing this pest necessitates the development of innovative, sustainable, and eco-friendly strategies that effectively manage the problem without compromising biodiversity, especially because this is an indigenous species to the region. Currently, the challenge lies in the limited availability of efficient control methods for *S. partita*. Traditional chemical control measures are unsuitable due to the moth's feeding behaviour, concerns over pesticide residues during harvest, and potential environmental impact (Moore 2010). Alternative integrated pest management techniques have been proposed for the management of this moth. These include the deployment of orchard nets, either on individual trees or across entire orchards, as recommended by Fay (2002). Additionally, researchers have explored orchard illumination using green-yellow light (550 – 580 nm), as reported by multiple studies (Whitehead & Rust 1967, 1972, Bosch 1971b, Fay 1995), demonstrating a notable reduction in infestation. However, both strategies entail significant inputs that are expensive for citrus growers. As such, the pursuit of effective and financially viable solutions remains a critical objective in the ongoing efforts to mitigate the impact of *S. partita* on citrus and other fruits.

Baits are luring substances strategically deployed, serving as effective tools to attract, and in some cases, kill target organisms if combined with a toxicant. Their success hinges on

exploiting the olfactory responses of these organisms to entice and capture them. Among the diverse approaches, food-based baits have the most potential for insect monitoring and control. These formulations comprise attractive feeding materials combined with toxicants that ingeniously lure insects into a trap. When tailored to specific pests, they are environmentally safe (Fay & Halfpapp 2001). The application of baiting strategies in managing fruit-piercing moths has garnered substantial attention from past research. Early studies employed brown sugar syrup infused with pesticides (Gunn 1929, Weddel 1944). Other researchers experimented with a blend of sugar syrup, overripe fruit pulp (predominantly banana), and alcohol (Baptist 1944, Kriegler 1957, Yoon & Kim 1977, Dodia et al. 1986). Notably, Reddy et al. (2007) reported banana as the most efficacious lure among 14 different fruits. Despite these advancements, the inconsistency of olfactory release in crude organic food lures, as cited by Fay & Halfpapp (2001), and thus their ability to compete with natural fruit odours within orchards remains a challenge.

In the past, Bosch (1971b) embarked on pioneering efforts to utilise synthetic lures, employing butyl-acetate, and reported modest attraction in laboratory settings for an undisclosed moth species. A significant advancement emerged through the work of Fay & Halfpapp (2003), who devised a proprietary bait using esters, aldehydes, and alcohols -mimicking the alluring scents of ripening fruit - with sugar and a toxicant encapsulated within a gel matrix. In a comparative study by Fullard et al. (2012) three synthetic lures and molasses were evaluated against fresh bananas. Again, bananas were found to be the most effective lure. This was further substantiated by Goddard (2016), who conducted field trials targeting *S. partita* using ripe bananas alongside various generically synthesised components mimicking banana volatiles, as elucidated by Thaiphanit & Anprung (2010). The pattern persisted, affirming the efficacy of fresh ripe banana as the most effective lure, while the generically formulated volatiles struggled to elicit moth attraction.

Monitoring the elusive *S. partita* is challenging due to its nocturnal habits. Whitehead & Rust (1971) proposed a method of nocturnal moth counting on twenty trees during feeding periods.

However, Whitehead & Rust (1972) later reported that this procedure proved arduous, particularly during high infestation. Neubecker (1966) experimented with Ultraviolet (UV) light and Robinson traps for monitoring purposes but encountered limited success, which was attributed to trap inadequacies. In a contrasting approach, Blackmer & Cañas (2005) noted that augmenting attraction via both visual and olfactory host-locating cues can yield superior outcomes. The evolution of lure presentation techniques for combating the fruit-piercing moth has been a recent focal point in research, as highlighted by Roland et al. (2012). Several methodologies have focussed on the lure-and-kill strategy (Fay 2002). Addressing lure longevity and presentation quality of fresh bananas, Reddy et al. (2007) explored the use of gelling or thickening agents, namely agar, Phytagel™, and agarose, contained within sausage casings. They observed that both agar and Phytagel™ outperformed agarose, yielding significantly higher moth attraction.

Trap design is particularly important within monitoring and mass trapping systems, together with the selection of attractants. The uniqueness of each pest insect species necessitates tailored trap configurations, an approach underscored by Bell et al. (2019), aligning trap structure with individual behavioural traits. The efficacy of traps hinges on a spectrum of biological factors, including insect size, flight capacity, and the habitat of host plants (Barak et al. 1990, Tinzaara et al. 2005, Lemay et al. 2022). Moreover, environmental variables such as rainfall, relative humidity, temperature, and wind speed and direction, as evidenced by Endler & Day (2006) and Chemnitz et al. (2020), can impede the flight abilities of insects. Consequently, these factors hold the potential to curtail trap efficiency, emphasising the need for rigorous evaluations involving both controlled laboratory conditions and field settings. Studies by Fullard et al. (2012) and Goddard (2016) have concentrated on evaluating different lures using a funnel trap configuration. This apparatus features a suspended lure positioned atop the trap, complemented by a lethal agent (dichlorvos) stationed at its base. The combination of a refined trap design and carefully selected lures has the potential to advance the monitoring and management of the fruit-piercing moth.

The concept of lure presentation in the management of fruit-piercing moths has received less attention than the actual lure. To date, several traps including Gunn traps, Robinson traps, funnel traps and other homemade traps have been used for catching fruit-piercing moths (Gunn 1929, Neubecker 1966, Johannsmeier 1998). No comparison has been made to compare the trapping efficiency of these traps. The objective of this chapter is to further evaluate lure type and lure presentation techniques to enhance the monitoring and management of *S. partita*. A synthetic lure that performs equally to or better than fresh banana would be ideal for long-term management of *S. partita*. The specific objectives were: (i) evaluating the efficacy of synthetic lures in comparison to fresh pulped banana; (ii) evaluating diverse gelling agents' effectiveness in combination with pulped banana; and (iii) scrutinising alternative trap designs that may exceed the efficiency of the conventional funnel trap, with a concentrated focus on integrating UV light and food lures within these novel configurations. Lure presentation trials sought to find the ideal trap design that is readily available for monitoring and, ultimately, control of *S. partita*.

4.2 Materials and Methods

4.2.1 Lure evaluation

4.2.1.1 Lure preparation

Four different lures were prepared and evaluated for attractiveness to *S. partita*. The first lure consisted of fresh banana pulp introduced into a yellow plastic cage topped with a lid to act as a lure dispenser. The second lure was a commercially available banana essence (Robertsons banana flavour essence) procured from the local supermarket. It was prepared by pipetting 10 ml of banana essence into a glass beaker containing 500 ml of water. This mixture was then augmented with a food colourant and 5 g of a super absorbent polymer (I-CAT Pretoria, South Africa) and mixed until a dense slurry formed. The attractant mix was fed through a sausage-loading machine, which facilitated its loading into a 22 mm diameter sausage casing. Once a

substantial length of casing was filled, they were tied off into individual sausages approximately 70 mm in length, each comprising around 50 ml of the mixture. The ends were tied with white cotton strings to prevent the slurry from leaking. Prior to their utilisation, the sausages were coated with cheese wax to reduce desiccation in the field. The cheese wax was first heated in a beaker on an electric stove until it melted. Once melted the cheese wax was poured on the prepared sausages. All lures were prepared the day before use, while any surplus was refrigerated uncoated with the cheese wax at -25°C for a week before subsequent use. Frozen sausages were allowed to thaw before coating with cheese wax.

The remaining two lures under scrutiny were the Australian proprietary lure formulation (Patent AU2002331417A) developed by Fay & Halfpapp (2003). These lures, denoted as Australian lure 1 and Australian lure 2, respectively, consist of four-component and seven-component variants. The various attractants were first measured using micropipettes in a volumetric flask containing paraffin oil. Subsequently, half of the necessary water volume was added to a large beaker with sugar and a coloured food dye (to distinguish between different lures). Instant gel in starch form was introduced to the beaker while the mixture was constantly stirred to achieve complete dissolution. To ensure longevity, sodium benzoate, a preservative, was separately dissolved in 100 ml of hot water and incorporated into the beaker. The attractants within the volumetric flask were then mixed with the beaker contents, with thorough stirring for three minutes. The super absorbent polymer was then added gradually with continuous stirring until the mixture within the beaker transformed into a dense slurry. After five minutes the attractant mix was ready for loading into the sausage casings as described above. If they were destined for extended storage, they were frozen unwaxed within transparent plastic containers and stored in a freezer at conditions until needed (Figure 4.1). For application, freshly prepared or thawed sausages were individually dipped into molten cheese wax to achieve complete exterior coverage of the lure.

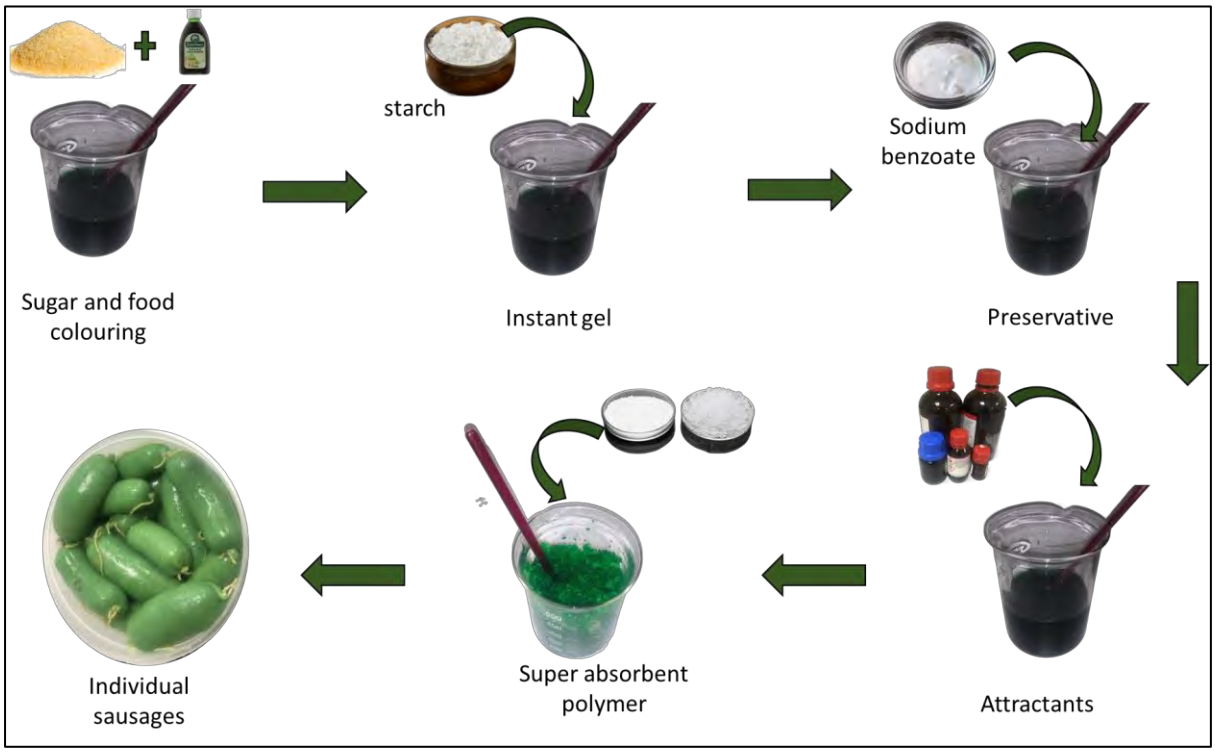


Figure 4.1: Step-by-step lure preparation procedure for the Australian proprietary lures.

4.2.1.2 Evaluation trials

The evaluation of lures followed the methodology outlined by Fullard et al. (2012) and Goddard (2016), employing a funnel trap setup. In this approach, individual sausages were loaded into a lure dispenser positioned at the trap's upper section, after which the dispenser was securely closed. At the trap's base, a dichlorvos tablet was positioned to function as a fumigant, killing moths that fell into the trap. The traps were positioned starting from the second row within the orchard to shield the traps from undesirable environmental influences, such as excessive wind and dust. They were deliberately placed on the leeward side of the orchards as this area is most prone to *S. partita* infestations, as reported by Whitehead & Rust (1972). The traps were stationed on the more shaded southern side of the selected trees to prevent rapid desiccation. Each trap was placed on a tree approximately 1.5 – 1.8 m above ground level. They were spaced 12 m apart, strategically positioned in a uniform pattern throughout the orchard. This arrangement was chosen to minimise the potential for lure competition while still

ensuring proximity for the lures to engage the same moth population. This arrangement facilitated a fair and unbiased comparison of lure effectiveness across the experimental setup.

Lure evaluation took place in March 2023 within susceptible Satsuma Mandarin orchards located in Patensie (Chapter 2, Figure 2.1) in the Gamtoos River Valley. A completely randomised design consisting of four distinct treatments, each replicated six times, was employed. The aim was to compare the effectiveness of banana essence and two variants of Australian lures against the benchmark of fresh pulped banana. The traps were monitored at three-day intervals, during which the captured moth count was recorded. During monitoring, the traps were emptied, and the lures were replaced. The traps underwent a clockwise rotation, as described by Cruz-Esteban et al. (2021) to avert potential bias, contributing to the impartiality of the assessment process.

4.2.2 Evaluation of lure thickening agents

Two distinct thickening and gelling agents, namely agar and superabsorbent polymer, underwent evaluation in comparison to fresh pulped banana. The agar-based thickened lure was prepared following the procedure outlined by Reddy et al. (2007). The methodology involved initially weighing 250 g of fresh pulped banana, subsequently crushed into a puree utilising a 2 L Phillips HR214/90 blender. Meanwhile, 5 g of agar was combined with 250 ml of water, this mixture was then heated and concurrently stirred until the agar fully dissolved, achieving a thickened consistency. Subsequently, the agar was incorporated into the banana puree within the blender, ensuring a thorough mixture. This mixture was then allowed to cool at room temperature, and upon solidification, it underwent blending once more to achieve uniform, small sizes, facilitating their loading into sausage casings. The resulting sausages were tied into individual sausages, secured using cotton string and subsequently stored in a refrigerated environment in preparation for future usage.

The superabsorbent polymer-thickened lure followed a similar preparation method to the agar lure, albeit with minor differences. Fresh pulped banana (250 g), a few drops of coloured food

dye and 250 ml of water were blended to create a banana puree. Once a consistent texture was achieved, the mixture was transferred to a 1 L beaker. Superabsorbent polymer (5 g) was gradually introduced while stirring, resulting in the formation of a dense slurry. This slurry was then loaded into sausage casings, each casing securely tied to create individual sausages. Following this, the sausages were stored in a refrigerator at 4°C (Figure 4.2).

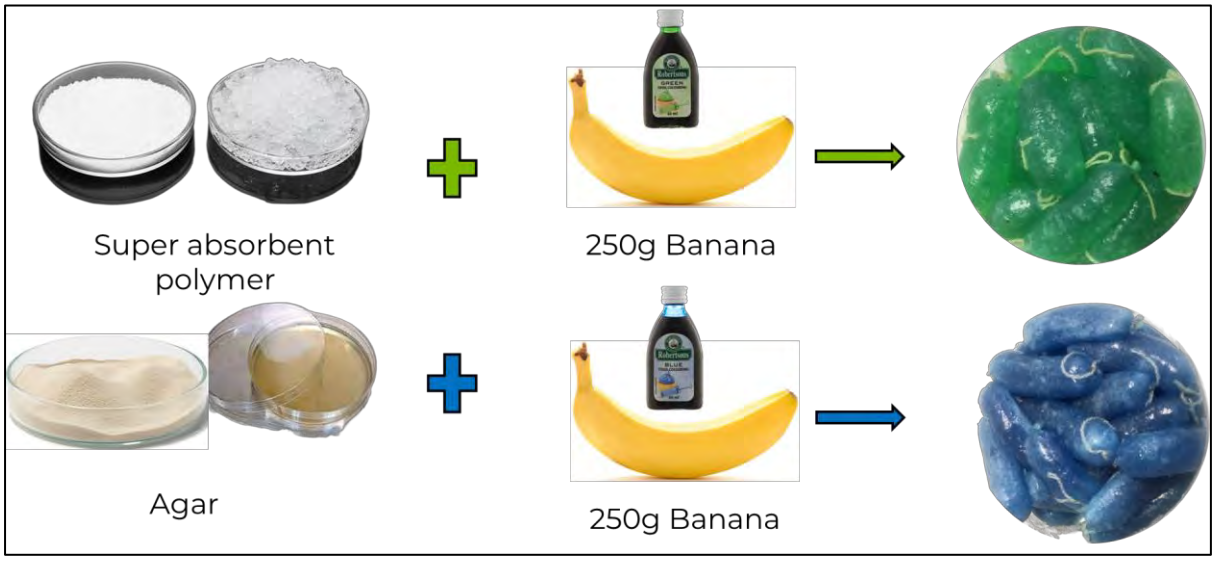


Figure 4.2: The lure making process for both agar and super absorbent polymer thickened lures.

These formulated lures underwent assessment for their attraction of *S. partita* in contrast to fresh pulped banana. The evaluation protocol remained consistent with the approach employed in prior lure assessment procedures. The trials were conducted in the Committee's Drift area of the Eastern Cape Province in susceptible Clementine orchards. A completely randomised design was employed with three different treatments, each replicated six times. For a course of three weeks, a weekly schedule was maintained for trap inspection, during which the count of fruit-feeding moths was recorded. After each assessment session, the lures were replaced, and the traps underwent a clockwise rotation at each lure replacement cycle.

4.2.3 Lure presentation

4.2.3.1 Experiment 1 – trap designs

Lure presentation involved a series of sequential experiments, each building upon the preceding one. Throughout these trials, fresh pulped banana was employed as the lure, dispensed via attachments affixed to the traps. The initial experiment entailed evaluating various trap designs to determine their efficacy in capturing fruit-feeding moths. The first trap employed was the yellow funnel trap system, distinguished by three robust components effortlessly interlocking. It consisted of a bottom collecting container, a funnel shaped cap (both 16.5 cm diameter × 8 cm height) and a circular roof (16.5 cm diameter). This trap was suspended using the top dome lid attachment. Inside this trap, a small plastic cage within the dome lid held the bananas, as the lure dispenser. Each trap had a dichlorvos block within the bucket, serving to kill the trapped insects (Figure 4.3a).

The second trap design was the yellow delta trap, featuring a pre-glued white cardboard sheet with a grid pattern, commonly referred to as a sticky liner, positioned at the bottom to shield it from dust and humidity (Figure 4.3b). This delta trap configuration is widely employed in agricultural orchards for the monitoring of several Lepidoptera pests (Cruz et al. 2012, Farr & Wills 2012, Caparros Megido et al. 2013, Cruz-Esteban et al. 2021). The third trap introduced a modified iteration of the bucket trap concept, adapted from the McPhail traps used for monitoring fruit flies (Potamitis et al. 2015, Doitsidis et al. 2017). This version featured a transparent plastic container with yellow lid, measuring 15 cm in length with a diameter of 7.5 cm. On its sides, there were two 5 cm diameter holes to facilitate moth entry (Figure 4.3c). The lure was strategically positioned within the top of the lid, suspended therein. A dichlorvos block was placed inside, serving as a killing agent. This trap design aimed to investigate a more cost-effective approach to the funnel trap.

The fourth trap incorporated a downward-facing circular blue disc configuration. It had a diameter of 22 cm, the trap's edges curved downwards, providing protection against water

infiltration. The underside of the disc was coated with sticky glue to trap insects. At the top, two small holes allowed for the insertion of a hanging wire protruding from the back of the trap (Figure 4.3d). This wire offered stability to prevent tilting of the trap. The core concept behind this design was to leverage the sticky surface while simultaneously safeguarding the lure from environmental elements.

Figure 4.3: Different traps assessed for efficacy for monitoring and controlling the *Serrodes partita*. a) the funnel trap, b) the delta trap c) the modified bucket trap, d) circular disc.

Field tests were conducted in April 2022 within Satsuma orchards located in Patensie, followed by a repetition in May 2022 within Clementine orchards situated in Committee's Drift. For optimal placement, the traps were strategically positioned at the peripheries of the orchards, focusing on the leeward side. In areas where the orchards directly faced the leeward side, the traps occupied the second row, while in orchard sections adjacent to the leeward side, the traps were placed in every alternative row from the second row. The first experimental setup in Patensie employed a completely randomised design, comprising four treatments replicated nine times each. The second experimental setup in Committee's Drift had four treatments replicated

10 times. This framework facilitated the comparison of the four traps in terms of their efficiency, as gauged by the number of adult fruit-feeding moths captured. The monitoring protocol involved weekly inspections of trap captures, with documentation of fruit-feeding moth numbers. Following each assessment, the traps were emptied and underwent a clockwise rotation to prevent potential biases. The lure (fresh banana) was changed each time as well.

4.2.3.2 Experiment 2 – enhancing funnel trap efficiency

The second experiment took place in Patensie during March 2023, with the aim of enhancing the funnel trap's efficacy by capitalising on both the visual and olfactory senses of *S. partita.* An electric trap comprising a shock grid/zapper and UV light both powered by battery and a solar panel was devised to achieve this (Figure 4.4). The UV light element served as the visual attractant for the moths, while the zapper was designed to deliver a shock upon their approach. These electric components were seamlessly integrated with a modified funnel trap. Comparative evaluation pitted this electric trap against the conventional funnel trap, both employing pulped bananas as lure. The study featured two treatments, the funnel trap and the electric trap, each replicated five times. The traps underwent inspection every three days, during which the lure was replaced for a period of 15 days. The traps were systematically rotated to negate any potential sources of bias.

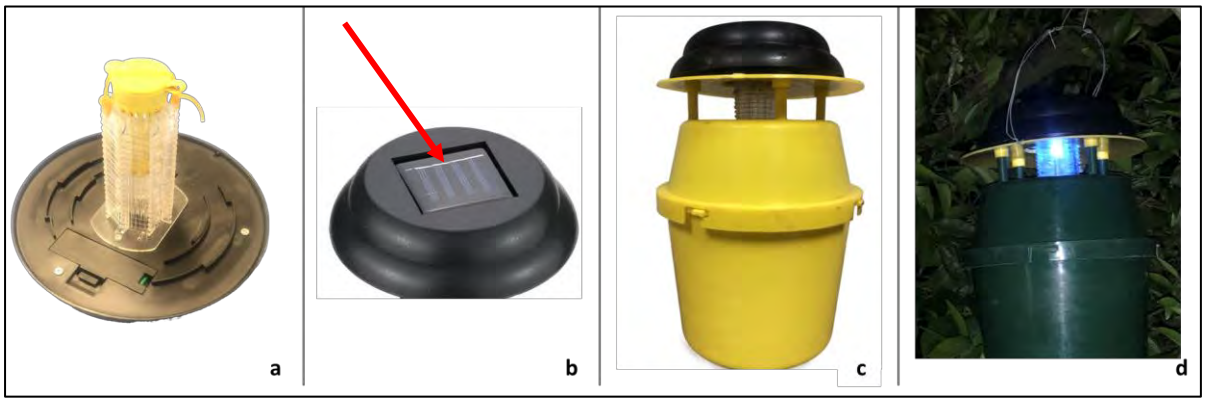


Figure 4.4: Outline of the electric trap a) the shock grid/zapper with food lure attached, b)

the solar panel, c) the electric components mounted on a modified funnel trap and d) an activated trap in the field with UV light.

4.2.3.3Experiment 3 – electric trap enhancement

The third experiment took place at Naudeshoek, Siyamila, and Ripplemead farms within Committee's Drift. A similar electric trap configuration to the one previously mentioned was employed, equipped with a shock grid but omitting the UV light component. This modified electric trap underwent evaluation against the conventional funnel trap, both featuring pulped banana as lure. The study adopted a completely randomised design, encompassing two treatments, the funnel trap and the modified electric trap, each replicated five times. The assessment regimen encompassed weekly trap inspections recording the number of moths caught per trap for four weeks. In line with the preceding experiments, both lure replacement and rotational adjustments of the traps were systematically conducted after each observation to ensure a robust and unbiased evaluation.

4.3.4 Data analysis

The data analysis was conducted using R software version 4.2.3 (R Core Team 2023). Prior to analysis, the normality of number of moths per trap data were assessed using the Shapiro-Wilk test and the homogeneity of variance was examined using the Bartlett test. For the analysis of lure evaluation and lure presentation trials, generalized linear mixed effects models following the approach outlined by Bolker et al. (2009) were used. The models were formulated, incorporating two fixed effects: lure type and species for lure evaluation trials and trap and species for lure presentation trials. To analyse lure evaluation and lure thickening agents, a Poisson regression model using the "lme4" package (Bates et al. 2015) was used. The generalized linear model (GLM) framework, using the "glm" function within the "stats" package was adopted R Core Team (2023) to fit the model. Lure presentation data were analysed using the "glmmTMB" package (Bolker et al. 2009) fitting a generalized linear mixed-

effects model with a negative binomial distribution. All model diagnostics were performed using the "DHARMa" package (Hartig & Lohse 2020).

Analysis of variance (ANOVA) Type III was subsequently conducted to evaluate the significance of the interaction terms within the model. This analysis enabled the assessment of the collective impact of interactions between lure/trap type and species on the variability observed in the captured moth counts. The Nagelkerke's pseudo-R-squared value was computed to assess the model goodness of fit. Post hoc tests were performed using the "*emmeans*" function to determine specific group differences. Visualisation of multiple comparisons were achieved through the application of the compact letter display procedure ("*cld*"), implementing a Tukey adjustment with a significance level (α) set at 0.05. This visualisation method facilitated a clear representation of the outcomes from numerous comparisons.

4.3 Results

4.3.1 Lure evaluation

The number of captured moths showed marked sensitivity to the variety of lure employed ($\chi 2 = 77.14$; df = 3; $P < 0.001$). Furthermore, significant differences in the types of species captured were evident ($\chi 2 = 6.931$; df = 1; $P < 0.001$). Notably, both the type of lure and species exerted a significant influence on the overall number of moths captured ($\chi 2 = 10.1$; df = 3; $P < 0.001$). Intriguingly, fresh banana emerged as the most efficacious lure, while both Australian proprietary lures failed to attract moths (Figure 4.5).

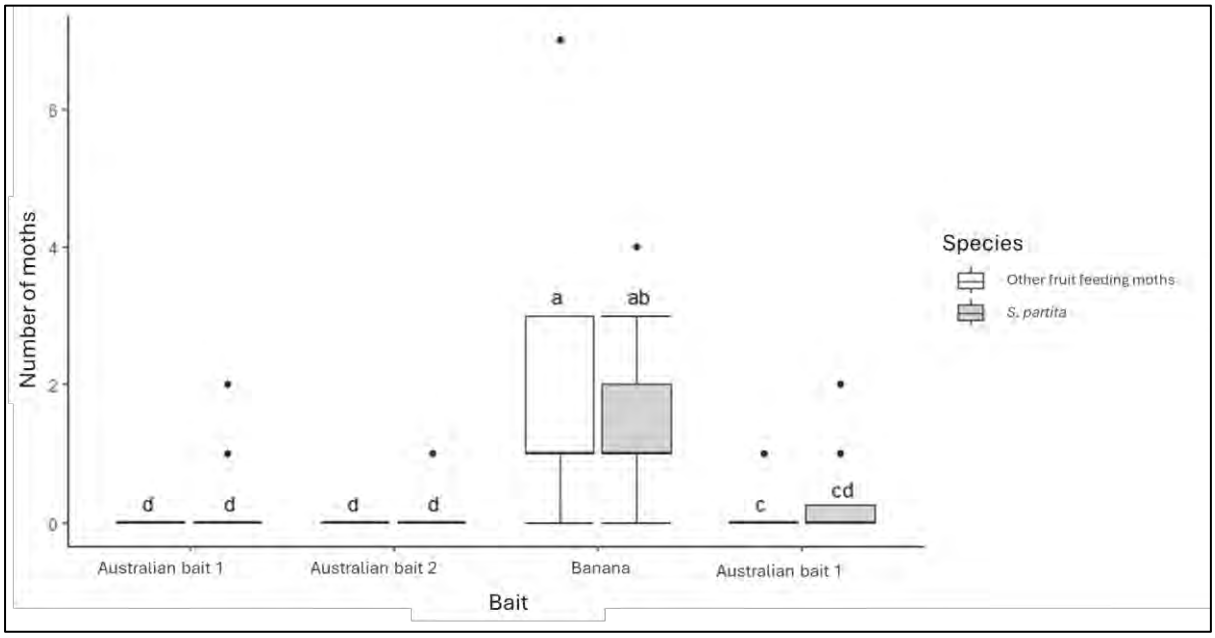


Figure 4.5: Responses of *Serrodes partita* and other fruit-feeding moths to various lures under field conditions. Identical letters above plots denote no significant difference between lures (Tukey HSD, $P < 0.05$).

4.3.2 Evaluation of lure thickening agents

During the assessment of lure thickening agents, the population of *S. partita* was notably limited, contrasting with the higher population of other fruit-feeding moths. Significant differences were observed between the number of *S. partita* and other fruit-feeding moths ($\chi 2$

= 192.14; df = 1; $P < 0.0001$). Specifically, the combination of agar with banana proved more attractive to fruit-feeding moths compared to the combination of super absorbent polymer with banana ($\chi 2 = 31.98$; df = 2; $P < 0.05$). However, no significant differences were recorded between the efficacy of fresh banana alone and the combination of either agar or super absorbent polymer. An absence of interaction was noted between species and lure type ($\chi 2 = 1.84$; df = 1; $P = 0.40$) (Figure 4.6).

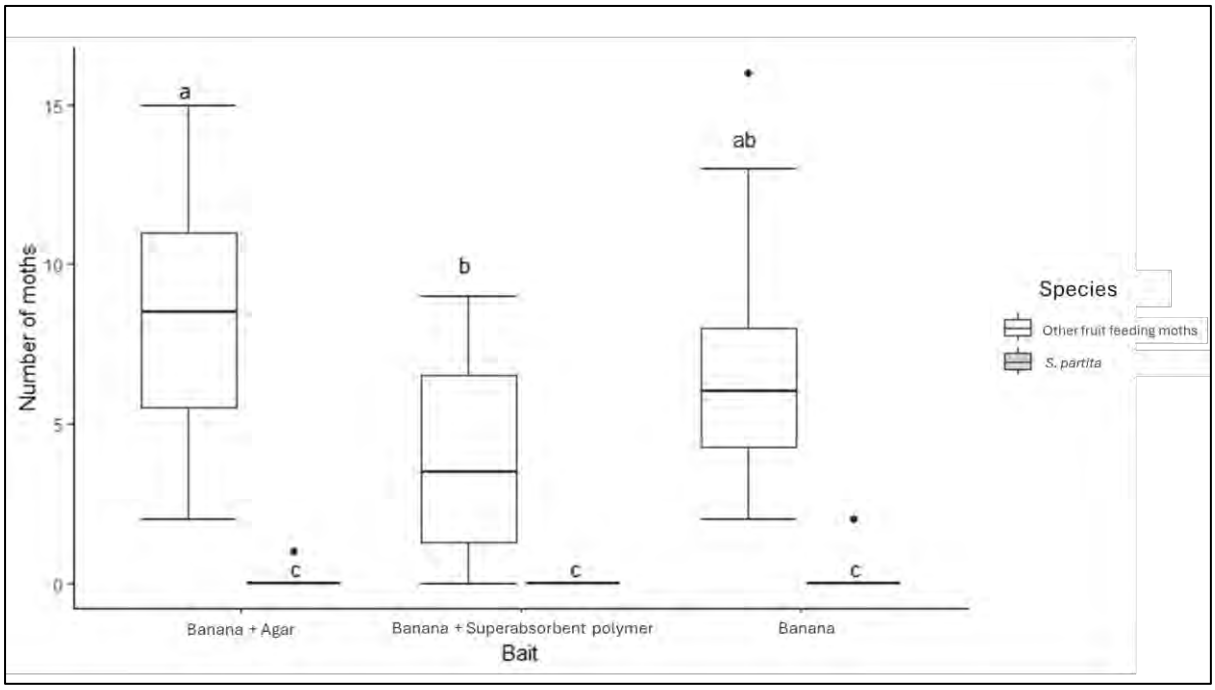


Figure 4.6: Response of *Serrodes partita* and other fruit-feeding moths to different gelling agents and fresh banana. The same letters above plots indicate no significant difference between lures (Tukey HSD, $P < 0.05$).

4.3.3 Lure presentation

4.3.3.1 Experiment 1– trap designs

Lure presentation trials yielded significant differences in trap attraction for both *S. partita* and other fruit-feeding moths ($\chi 2 = 30.61$; df = 1; $P < 0.001$). The funnel trap demonstrated the highest efficacy in catching both *S. partita* and other fruit-feeding moths. The delta trap emerged as the second most proficient trapper succeeded by the bucket trap, while the disc trap

failed to catch moths ($\chi2 = 386.6$; df = 3; $P < 0.001$). Notably, substantial differences were also recorded in the interaction between species and trap type ($\chi2 = 15.09$; df = 3; $P < 0.001$) (Figure 4.7).

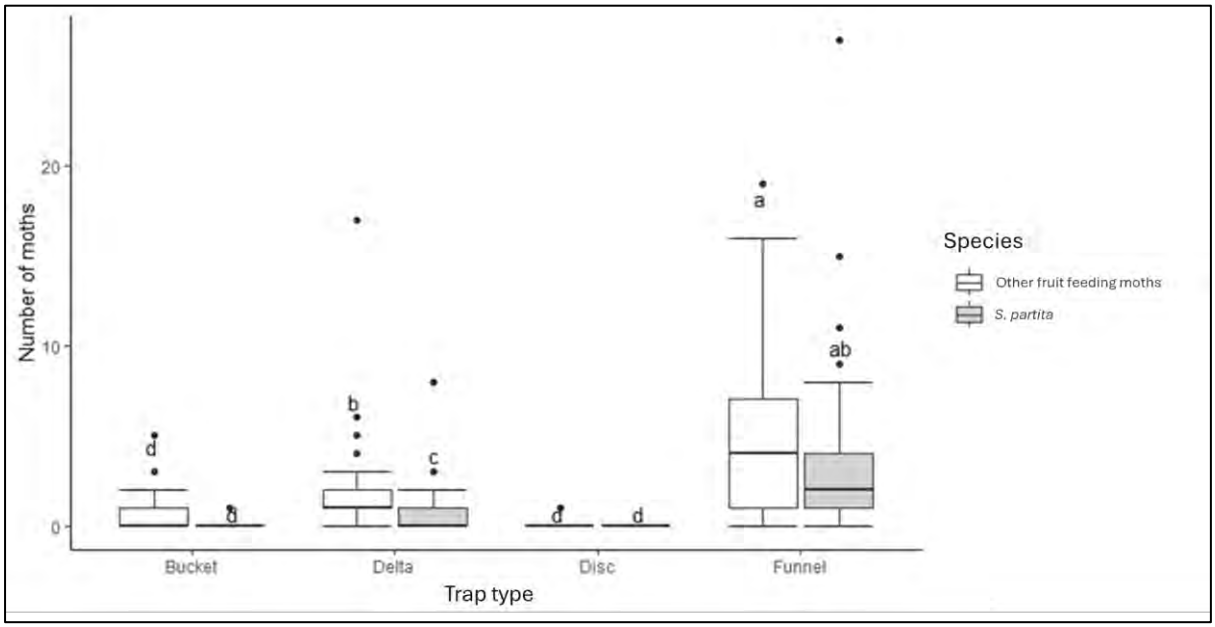


Figure 4.7: Response of *Serrodes partita* and other fruit-feeding moths to different traps with fresh banana lure under field conditions. The same letters above plots indicate no significant difference between traps (Tukey HSD, $P < 0.05$).

4.3.3.2 Experiment 2– enhancing funnel trap efficiency

No statistically significant differences were found between the funnel trap and the electric trap in terms of their efficacy to catch both *S. partita* and other fruit-feeding moths ($\chi2 = 0.98$; df = 1; $P = 0.32$). Similarly, no notable variations were recorded in the interaction between trap type and species of moths caught per trap ($\chi2 = 0.5$; df = 1; $P = 0.48$). Furthermore, no significant differences emerged in the number of species caught per trap ($\chi2 = 0.21$; df = 1; $P = 0.13$) (Figure 4.8).

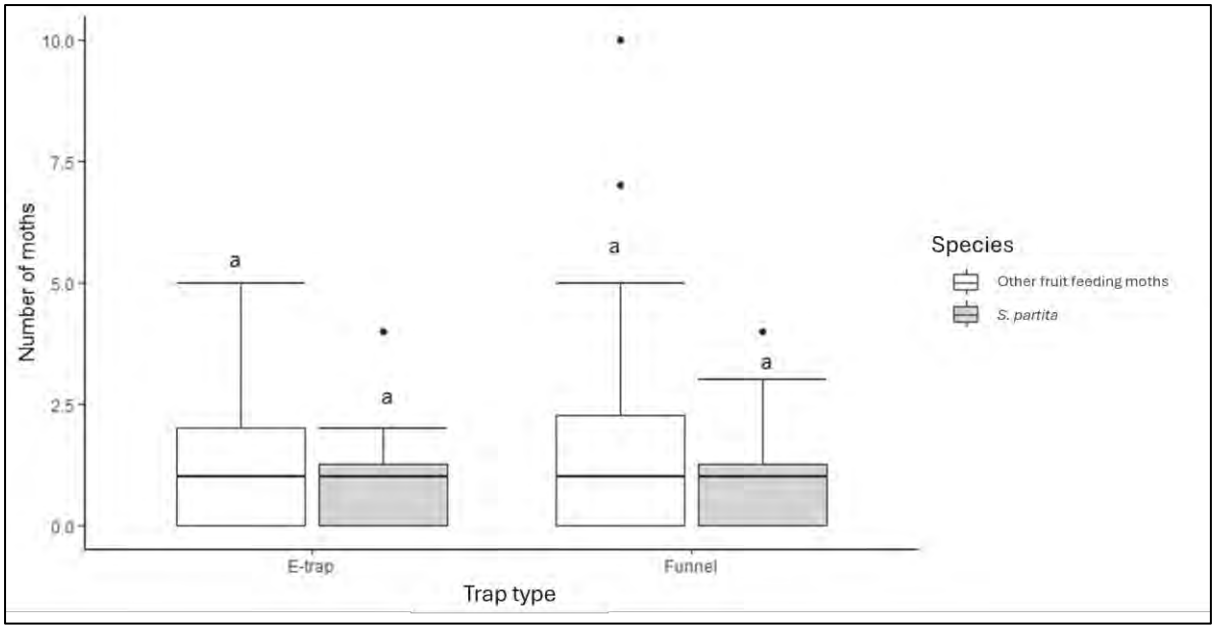


Figure 4.8: Response of *Serrodes partita* and other fruit-feeding moths to an electric and funnel traps with fresh banana lure. The same letters above plots indicate no significant difference between traps (Tukey HSD, $P < 0.05$).

4.3.3.3 Experiment 3– electric trap enhancement

During the evaluation of the electric trap without the UV light component and the funnel trap, low populations of *S. partita* were observed in contrast to populations of other fruit-feeding moths. Notably, the electric trap outperformed the funnel trap as the most effective moth catcher ($\chi 2 = 71.51$; df = 1; $P < 0.001$). Moreover, a discernible difference in the types of species caught was evident, with a significantly higher count of fruit-feeding moths captured, compared to *S. partita* ($\chi 2 = 90.41$; df = 1; $P < 0.001$). However, there was no interaction between the species and trap type ($\chi 2 = 3.08$; df = 1; $P = 0.80$) (Figure 4.9).

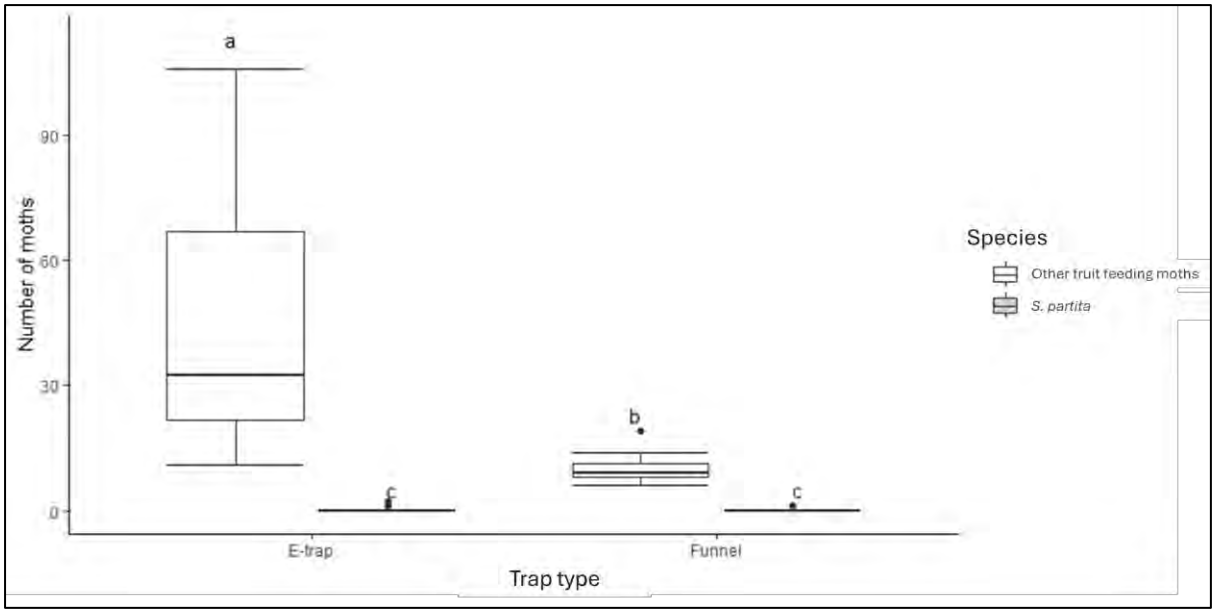


Figure 4.9: Response of *Serrodes partita* and other fruit-feeding moths to electric and funnel traps with fresh banana lure. The same letters above plots indicate no significant difference between traps (Tukey HSD, $P < 0.05$).

4.4 Discussion

Fresh banana was identified as the most effective lure for attracting both *S. partita* and other fruit-feeding moths. This finding aligns with previous research by Denton et al. (1990), Fay & Halfpapp (2001), Reddy et al. (2007), Fullard et al. (2012) and Goddard (2016) who evaluated banana along with synthetic lure substitutes and various fruits. However, the Australian proprietary lures failed to elicit responses from both *S. partita* and other fruit-feeding moths present in citrus orchards. It is important to mention that these proprietary lures were specifically formulated for *Eudocima phalonia*, a different fruit-piercing moth species. This difference shows how insect species respond to contrasting lures, and fruit flies (Diptera: Tephritidae) confirm this level of specificity . Synthetic lures play a crucial role in managing fruit flies, with different species exhibiting varying degrees of sensitivity to different lures. For instance, males of *Bactrocera* spp. display positive responses to methyl eugenol, a naturally occurring compound present in various plants and fruits (El-Sayed et al. 2009, Manrakhan et al. 2021, Ahmad et al. 2023). In contrast, males of *Ceratitis* spp. are drawn to Trimedlure, a

synthetic compound that mimics the volatile organic compounds found in ripe or fermenting fruit (Bayoumy et al. 2019, Manrakhan et al. 2021).

The proprietary bait was found to be highly attractive to *E. salaminia* and *E. phalonia*, but less effective in attracting *E. materna*, despite its abundance in the orchards (Fay 2002). This then reflects on the sensitivity of the moths to different bait formulations. Moreover, different species of fruit-piercing insects may exhibit varying levels of sensitivity to synthetic lures, a phenomenon likely influenced by their distant phylogenetic relationships. Both *S. partita* and *E. phalonia* have evolved through convergent evolution, adapting to the same ecological niche characterised by their specialised barbed proboscis for fruit feeding (Swart 1969, Goddard et al. 2019, Klem & Zaspel 2019). Despite this shared ecological trait, they are not closely related from a phylogenetic standpoint. Both species belong to the Family Erebidae, but they fall within different subfamilies. *Eudocima phalonia* belongs to the subfamily Calpinae, specifically the tribe Calpini, which contains most fruit-piercing moth species (Zahiri et al. 2011, 2012, Klem & Zaspel 2019). In contrast, *S. partita* is classified in the subfamily Erebinae, tribe Cocytiini (Lafontaine & Fibiger 2006, Zahiri et al. 2012, 2023). This taxonomic difference in their lineage might contribute to explaining the failure of the proprietary lures to attract *S. partita*.

Alternatively, it is possible that the quantity of the odour, rather than the quality, was responsible. Ripe bananas may emit overwhelming odours compared to the tested synthetic lure and other fruits examined in previous studies by Reddy et al. (2007), Fullard et al. (2012), and Goddard et al. (2019). While the lures were specifically designed for use as free-hanging lures (Fay & Halfpapp 2003), traps were used to assess and quantify the effectiveness of these lures by capturing attracted moths. However, the traps may have inadvertently acted as an additional barrier to the odour plume released. Hence, it is necessary to consider augmenting the volatile quantities of the Australian proprietary lure to counteract trap hindrances and try to align it with the quantity of banana volatiles. Amplifying the volatile quantity could potentially mitigate the preference of *S. partita* for damaged fruit feeding (Chapter 3). For the

moth to be attracted by the lure/bait, it must surpass the attractiveness of the damaged fruit it would typically feed on.

Agar emerged as the most effective gelling agent, exhibiting a greater capacity to attract moths than the super absorbent polymer. This aligns with the findings of Reddy et al. (2007), who highlighted its substantial attractiveness when used as a thickening agent. Super absorbent polymers are materials characterised by their hydrophilic nature and remarkable water-absorption capabilities (Zohuriaan-Mehr 2008). Behavioural observations at night revealed that fruit-feeding moths displayed a tendency to engage with the lure for a considerable amount of time. An ideal gelling agent should, therefore, be able to maintain the lure in an aqueous state. Use of superabsorbent polymer for lure presentation was ideal. This choice mirrors the approach taken by Fay & Halfpapp (2003) in their synthetic lure formulation. However, it is worth noting that, despite its advantageous properties for lure presentation, the polymer showed a tendency to leak from the sausage packaging. This could explain its relatively lower performance compared to Agar, which forms a moisture-retentive gel without compromising lure stability or causing leakage. Alternatively, leakage could be reduced by adding more super absorbent polymer.

The funnel trap proved to be the most effective among the various trap designs that were evaluated. This outcome is unsurprising given the widespread use of funnel traps for monitoring adult Lepidoptera, including the fall armyworm *Spodoptera frugiperda* (Ikemoto et al. 2021). As already mentioned, *S. partita* and other fruit-feeding moths tend to feed on the lure for a considerable amount of time. The distinctive design of the funnel trap facilitates the gradual emission of chemicals from the dichlorvos tablet (fumigant). This emission weakens the moths, ultimately causing them to descend into the trap. Importantly, once an insect enters the trap and falls, flight resumption is repressed, allowing for easy collection and subsequent processing of captured moths. In contrast, sticky traps such as delta and disc traps—employed in this study, traditionally find application in managing flies and thrips rather than moths, as highlighted (Shimoda & Honda 2013). Sticky traps present certain challenges when monitoring

fruit-feeding moths. Their size becomes limiting, as the adhesive surface rapidly becomes covered after capturing only a few moths, hindering further captures. Additionally, the need to frequently replace the sticky material upon changing traps introduces further logistical challenges. The bucket trap's performance fell short of optimal, likely due to moths managing to escape. Unlike the funnel trap, the inclusion of an entry hole on the side also allowed for a potential escape route.

Field observations also highlighted certain limitations in the effectiveness of the funnel trap. Notably, a portion of the captured moths managed to escape the trap once their feeding activity concluded, prompting a consideration for enhancing the funnel trap's performance using an electric trap. While much of the focus in electric trap development has centred around automating catch counting rather than optimising trap efficiency (Preti et al. 2021, Rigakis et al. 2021), other electric traps featuring electric grids and UV lights have demonstrated effectiveness in managing moths and beetles, particularly within greenhouse environments (Tazawa 2009). In this study, an electric trap was introduced to take the advantageous features of the conventional funnel trap, while reducing its limitations. Notably, the electric trap showcased superior performance to the traditional funnel trap. Unlike the latter, the electric trap prevents the moth from landing and feeding. Instead, moths are immobilised upon encountering the electrified grid. The electric shock administered does not result in immediate mortality; rather, the dichlorvos tablet contained within the trap is responsible for achieving eventual insect mortality.

No significant differences were recorded when the electric trap equipped with a UV light component was compared against the traditional funnel trap. However, significant differences emerged when the UV light component was omitted from the trap design. Interestingly, Neubecker (1966) incorporated UV light into a Robinson trap but reported limited success. *Serrodes partita* is known to be repelled by light within the green-yellow spectrum (Johannsmeier 1998). This prompts consideration that UV light might have a subtle repellent impact on *S. partita* and other fruit-feeding moth species.

In conclusion, further efforts are warranted to identify the specific volatile organic compounds present in fresh bananas that are responsible for attracting *S. partita* and other fruit-feeding moths. This could be facilitated through electroantennography studies or the strategic manipulation of ratios within the Australian lure formulations to optimise *S. partita* attraction. Equally vital to an effective lure is the selection of a proficient thickening agent. Moreover, comprehensive longevity trials are needed to ascertain whether both the agar and the super-absorbent polymer can extend the shelf life of fresh banana by preventing rapid desiccation. The observed efficacy of the funnel trap in this study corroborates its established role in monitoring adult Lepidoptera. In contrast, while sticky traps exhibit efficacy for specific pests, their use with fruit-feeding moths is hampered by size and maintenance limitations. Although the electric trap shows substantial promise, its optimisation for commercial use remains an ongoing endeavour. Moreover, there is need for testing and further refining of other trap designs, such as the Gamgee trap developed in Australia. The use of field cages can be adopted to help develop better lures as they help remove trap impediments. The intricate interplay of factors encompassing light sensitivity, trap design, and species behaviour necessitates in-depth exploration for a comprehensive understanding of moth attraction and repulsion mechanisms. On a broader scale, the lure and kill concept holds significant potential for the management of *S. partita* in citrus orchards, offering a promising approach to enhance pest control strategies.

CHAPTER 5

Monitoring fruit-piercing moth *Serrodes partita* activity in the Kat River Valley, Eastern Cape

5.1 Introduction

The fruit-piercing moth, *S. partita*, has historically exhibited outbreaks occurring at intervals of 5 to 10 years in South Africa (Myburgh 1963a, Johannsmeier 1998, Robertson & Cochrane 2005). However, recent reports suggest a shift towards a more seasonal occurrence of *S. partita* instead of these periodic outbreaks (Moore 2010, Goddard et al. 2019). More regular outbreaks pose a substantial and pressing issue for soft citrus growers, particularly in the Eastern Cape Province. This shift in occurrence has also been observed in other fruit-piercing moth species, particularly populations of *Eudocima* spp. in the Pacific region (Roland et al. 2012, Leroy et al. 2021). The causes behind these seasonal fluctuations leading to outbreaks of fruit-piercing moths are multifaceted and unclear (Bänziger 1982). Various factors, such as weather patterns, host plant availability, and natural predators, have been suggested as potential triggers for fruit-piercing moth outbreaks (Cochereau 1977). Fay (2004) attributed the shift in fruit-piercing moth occurrences to a recent increase in fruit production and extended fruiting periods. While increases in fruit-piercing moth populations and activity may coincide with the primary fruiting season in their respective regions, the availability of larval host plants remains a crucial contributing factor (Bänziger 1982, Leong & Kueh 2011).

Plant phenology plays a crucial role in driving recurring events related to herbivorous insect occurrence, as highlighted by various studies (Gross & Werner 1983, Nyambo 1988, Fagundes et al. 2018), and the availability of high-quality larval host plants, particularly young shoots, has been identified as a critical factor that enhances the survival of Lepidoptera neonates (Zalucki et al. 2002). This phenomenon is observed in outbreak pests like the Tent caterpillar

Malacosoma disstria Hübner (Lepidoptera: Lasiocampidae), where egg hatching concurs with the blooming of their larval host, such as the Star magnolia *Magnolia stellata* (Siebold & Zucc.) Maxim (Magnoliaceae). The same is also reported in the case of the Gypsy moth *Lymantria dispar,* where egg hatching coincides with the emergence of Redbud *Cercis canadensis* Duhamel (Fabaceae) (Herms & Mattson 1992, Herms 2004). Cochereau (1969) reported an outbreak of *E. phalonia* that followed a severe drought. This outbreak followed a flush of young leaves on the larval host plant, accompanied by a substantial increase in the eggs observed after rainfall. Similarly, *S. partita* has been reported to follow rainfall events that trigger a flush of *P. capensis* in the Karoo region in the centre of the country (Taylor 1965b, Johannsmeier 1998, Robertson & Cochrane 2005).

Predicting the occurrence of *S. partita* using host plant phenology presents a considerable challenge, due to the extensive geographic range of the larval host plant and the variable climatic conditions throughout their range. Furthermore, the larvae are difficult to spot, as they develop on a host that may be far away from fruit growing areas (Cochereau 1974). Several authors have noted that fruit-piercing moths tend to emerge at the onset of the rainy season, even though fruit may be available all year round (Hargreaves 1936, Golding 1946, Cochereau 1974, Ngampongsai et al. 2005). Outbreaks and fruit-piercing activity of *Eudocima* spp. have been reported to synchronise with the rainy season in India (Bhumannavar and Viraktamath, 2012) and Australia (Sands & Schotz 1988). In Sri Lanka, Baptist (1944) recorded a minor peak in population during June and July and a more significant peak between November and December. However, this pattern was inconsistent, as *Eudocima* populations in Fiji exhibit the opposite trend, with the lowest numbers during the wet season and a peak between June and August, culminating in July (Kumar & Lal 1983). These observations align with those made in Sierra Leone (Hargreaves 1936, Leong & Kueh 2011) and Sarawak, Malaysia, where populations reached their highest during the dry months and subsided during the wet season.

In addition to rainfall, Yela & Holyoak (1997) have highlighted the role of temperature fluctuations in modulating the activity period of fruit-piercing moths. The sensitivity of insect

physiology to temperature is well-documented, given its influence on metabolic processes and reproduction (Dukes et al. 2009, Skendžić et al. 2021). Consequently, temperature affects various aspects of insect biology, including consumption rate, fecundity, generation time, geographic distribution, and population size (Shrestha 2019). Sands et al. (1991) demonstrated how temperature impacts Australia's fruit-feeding moth *Eudocima salaminia* Cramer. They observed that both feeding and oviposition increased with increasing temperatures. Besides temperature, humidity is also considered a primary factor affecting insect growth, development and survival (Carbognin et al. 2023). Relative humidity is significantly related to temperature as both can affect insects, especially during early development (Tochen et al. 2016). In addition, humidity also affects oviposition and adult activity, as there is increased adult activity and oviposition at high humidity, while at low humidity, the opposite is observed (Schmidt et al. 2018). By examining the combined effects of rainfall, temperature and humidity on *S. partita*, it may be possible to predict its occurrence better, ultimately facilitating informed decision-making in pest management strategies.

While various authors have reported the predominant feeding habits of *S. partita* on soft-citrus cultivars, particularly emphasising damage within Satsuma Mandarin (Kriegler 1958, Myburgh et al. 1973, Johannsmeier 1998, Moore 2010), there remains a notable gap in research. No prior studies have ventured to assess the varietal susceptibility and behaviour of *S. partita* across distinct soft citrus cultivars. Furthermore, the presence of any potential bias toward specific farms within a given locality has not been thoroughly investigated. Given the factors outlined earlier, this chapter aimed to examine the annual patterns of *S. partita* pest activity over three seasons in the Kat River Valley, with the goal of developing predictive capabilities for outbreak occurrences. Specifically, the chapter aimed to (i) conduct a detailed analysis of the pest population dynamics, including identifying peak activity periods, (ii) assess the influence of locality and cultivar on the occurrence of *S. partita*, (iii) investigate the impact of rainfall, temperature, and humidity, on the recurrence of the moth, and (iv) establish a correlation between trap catches and the level of damage recorded in orchards.

5.2 Materials and Methods

5.2.1 Study area: Location and orchard characteristics

The study was conducted from May 2021 to August 2023 in the Kat River Valley, Committee's Drift area, in the Eastern Cape. The study area encompassed three citrus farms between the Great Fish River Nature Reserve and the Keiskamma River (Figure 5.1). The Fort Beaufort weather station, near the study area, served as the source for weather data. Three-year weather data from 2020 to 2022 showed that the region experienced an average annual rainfall of 614 ± 67 mm and an average annual temperature of 18.9 ± 3.5°C (see section 5.3.3).

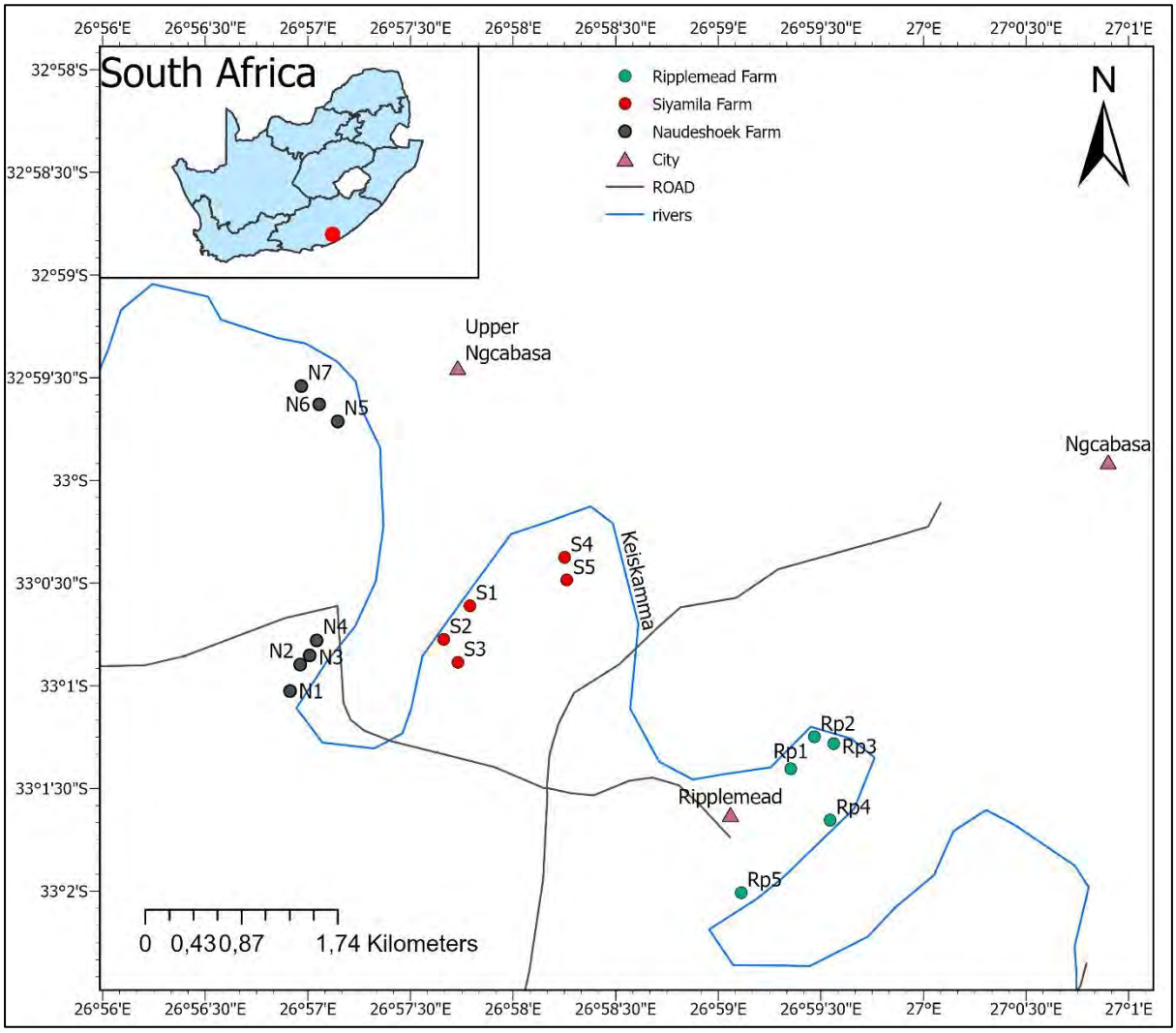


Figure 5.1: The location of orchards with monitoring traps in each of the three farms, Ripplemead, Siyamila and Naudeshoek.

Monitoring was conducted in all susceptible orchards, seven soft citrus orchards located at Naudeshoek Farm, five orchards at Siyamila Farm, and an additional five at Ripplemead Farm. The selection of orchards was deliberate, considering susceptibility and variations of different cultivars (all Mandarins) and the age of the orchards (Table 5.1).

Table 5.1: Characteristics of orchards used for monitoring activity of *Serrodes partita.*

Farm	Orchard Number	Orchard size (ha)	Year planted	Cultivar
Naudeshoek	NH1C	0.7	1989	Clementine
	NH2C	1.05	1989	Clementine
	NH3C	1.38	1989	Clementine
	NH4C	3.14	1989	Clementine
	NH31B	1.33	2016	Nardocott
	NH30	4.22	2016	Nardocott
	NH29	2.98	2016	Nardocott
Ripplemead	R1	1.6	2018	Orogrande
	R2	2.53	1989	Clementine
	R3	2.89	1989	Clementine
	R4	3.62	2016	Nardocott
	R5	1.1	2016	Nardocott
Siyamila	SM13C	4.15	1988	Clementine
	SM14C	3.3	1988	Clementine
	SM02C	0.83	1988	Clementine
	SM16M	4.80	2016	Nardocott
	SM17M	4.15	2016	Nardocott

5.2.2 Monitoring

The data collection method followed the protocol outlined by Goddard (2016). Yellow funnel traps (Insect Science™, Tzaneen, South Africa) were employed as the primary trapping mechanism. Ripe bananas were utilised as an attractant (Figure 5.2). Fresh bananas were procured weekly from a local produce shop and manually mashed to create a lumpy pulp. This pulp was then placed inside the plastic cages and integrated into the lid of each trap. The

decision to use freshly squashed bananas as an attractant was based on findings from Chapter 4 and previous studies by Fullard et al. (2012) and Goddard (2016), which demonstrated that this approach was highly effective in attracting fruit-feeding moths. Each trap was equipped with a dichlorvos block placed inside the collection bucket to ensure that trapped insects could be identified without harm. This block served the purpose of euthanising the captured insects without causing damage. The blocks were changed every eight weeks.

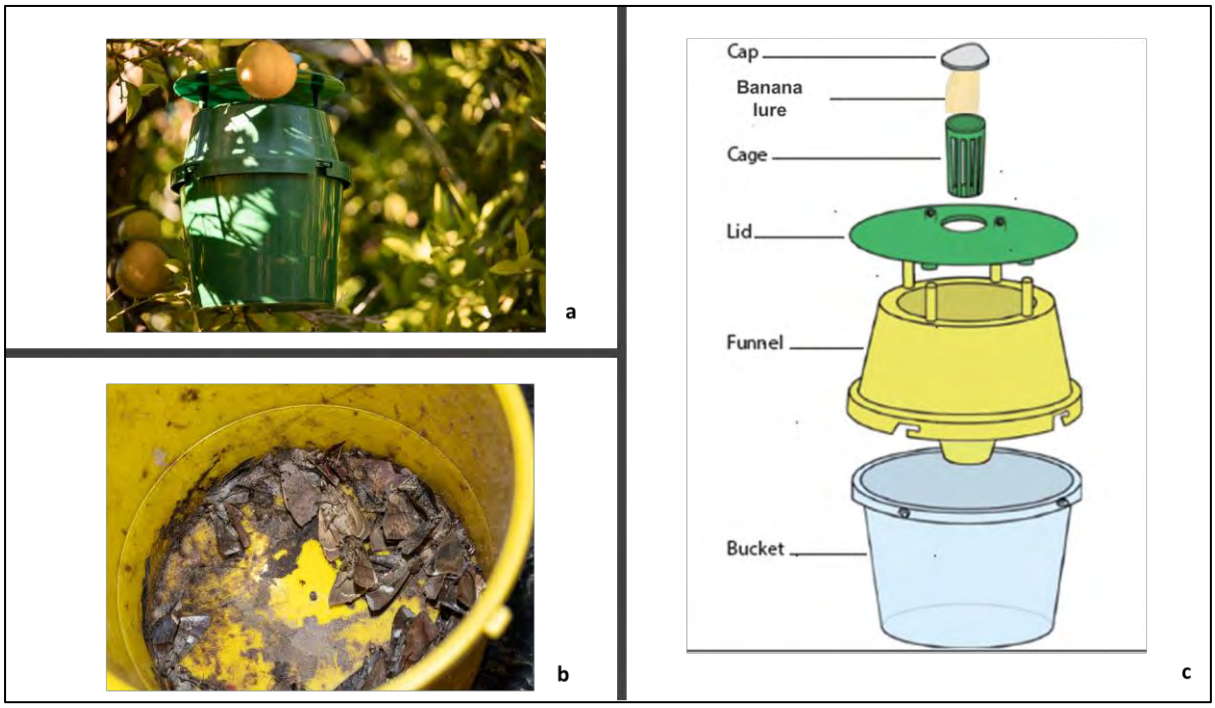


Figure 5.2: The monitoring trap: a) fully assembled trap, b) the bucket from the trap showing captured moths and c) outline of the funnel trap with its various components (Photo credit: David Taylor).

The traps were strategically positioned along the orchard perimeters, specifically on the leeward side, where the observed damage was most pronounced. The placement of traps was adjusted based on the orchard's orientation relative to the leeward side. Typically, one trap was hung on the second tree in the second row, approximately 1.5 m above ground. The traps were monitored throughout the study at varying intervals to capture seasonal fluctuations. Specifically, during the citrus ripening period, from mid-January to the end of August, traps

were monitored weekly. In contrast, during the off-season period extending from August to December, monitoring was conducted monthly. The number of *S. partita* moths caught was recorded and lures were changed during each monitoring event. The specified schedule was adhered to as closely as possible, with minor adjustments made when necessary due to accessibility and prevailing weather conditions.

5.2.3 Weather data

Weather data, including rainfall, maximum and minimum temperatures, and humidity from January 2020 to August 2023, was sourced from the South African Weather Services. The Fort Beaufort weather station data were selected for analysis as it was the closest available station to the study area. The weather data were subsequently compared with the monthly trap catches of *S. partita* in each orchard. A lagged data approach, as detailed by Haggis (1996), was employed to assess the relationship between trap catches and preceding weather conditions. Using lagged data involved utilising historical weather data from the past to help elucidate and predict patterns and trends in *S. partita* activity. Different weather parameters were then utilised to explore potential correlations with recorded trap catches. Various combinations of historical data from current and previous months were employed to construct distinct models. These models were compared to identify the best model to determine the correlation with trap catches.

5.2.4 Fruit damage assessment

Fruit damage on the trees was monitored weekly, commencing a few weeks before harvesting began and continuing until after harvest in all the surveyed orchards. The fruit damage survey was conducted by inspecting 20 fruit on 10 trees within each orchard, following the methodology outlined by Fullard et al. (2012) and Goddard (2016). The damage evaluation followed guidelines similar to those of Goddard (2016) and Fullard et al. (2012). The recorded damage was categorised into several distinct types, including damage attributed to fruit-piercing moths, birds/mechanical factors (like splitting or damage caused by machinery, such as tractors), diseased fruit (including conditions like citrus scab and misshapen fruit), and a

category labelled as "other," which encompassed damage that was undefined, such as sunburn, or caused by other pests like red scale or mealybugs. Identifying fruit-piercing moth damage was reliable, as the damage patterns are distinct and specific to this pest (see Chapter 3, section 3.2.4). Subsequently, fruit-piercing moth damage in each orchard was compared with the weekly trap catches of *S. partita* reported in the traps.

5.2.5 Data analysis

Data analysis was done using R software version 4.2.3 (R Core Team 2023). The trap catch data for *S. partita* were analysed using generalized linear mixed-effects models, following the methodology outlined by Bolker et al. (2009). Comprehensive global models were established, incorporating seven fixed effects: year, month, cultivar, farm, rainfall, temperature, and humidity, along with their respective interactions (Table 5.2). These fixed effects were chosen by their significance and relevance to the study. Specifically, farm and cultivar were selected based on their localities and susceptibility, while year and month were chosen to determine their seasonality patterns. Furthermore, the inclusion of weather variables was driven by the biological knowledge of the study species, as highlighted by Taylor (1965a) and Johannsmeier (1998). The orchard variable was introduced as a random factor. A lagged approach was employed to determine the correlation between average monthly trap catches and corresponding weather parameters.

An information-theoretic model selection approach was employed to identify the most influential model affecting *S. partita* trap catches. This process involved fitting 14 models, which were then ranked based on Akaike's information criterion corrected for small sample sizes (AICc) scores, to identify the most plausible model. This plausible model was recognised as the one with the lowest AICc, as per Harrison et al. (2018). Once the best model was identified, the relative importance of fixed effects was determined through two methods: (1) model averaging, along with the calculation of conditional parameter estimates, and (2) the

summation of wi (Akaike weights) across all models within the plausible model set in which each predictor appeared, following the principles described by Burnham and Anderson (2003).

The models were specified using the "glmmTMB" package, which involved fitting a generalized linear mixed-effects model with a negative binomial distribution to account for overdispersion in the data. Model diagnostics were conducted using the "DHARMa" package (Hartig & Lohse 2020), and model selection was executed using the 'MuMIn' package (Barton 2015). For data visualisation, plots were generated using Microsoft Excel 365 (Microsoft Corporation, Redmond, WA, USA).

Table 5.2: Description, data type and range of values for fixed effects that influenced the occurrence of *Serrodes partita.*

Model term	Description	Data type	Range
Year	Data collected across three different years	Yearly	2021 - 2023
Month	Twelve months of the year	Monthly	January - December
Cultivar	Three different susceptible soft citrus cultivars	Categorical	
Farm	Three different farms	Categorical	
Rainfall	Total monthly rainfall	Continuous	0 – 150
Temperature	Mean monthly temperature	Continuous	0 – 32
Humidity	Mean monthly humidity	Continuous	20 – 80

5.3 Results

5.3.1 Model selection

Akaike information criterion corrected values obtained from the 14 models ranged from 1748.88 – 1999.31. Model 11 (Previous + 2nd previous + 3rd previous + 4th previous month)

had the lowest AICc value, followed by model 4 (3rd previous month). Model 11 was chosen as the best plausible model (Table 5.3).

Table 5.3: Akaike information criterion corrected (AICc) values from 14 different models that were evaluated to determine the best model. The best model (lowest AICc) is highlighted in bold.

Model	Description	df	AICc
1	Current month	13	1999.31
2	Previous month	13	1933.53
3	2nd previous month	13	1885.67
4	3rd previous month	13	1785.263
5	4th previous month	13	1890.88
6	current + Previous	13	1945.92
7	current + 2nd previous	13	1917.52
8	current + 3rd previous	13	1858.20
9	Previous + 2nd previous	13	1892.31
10	Previous + 2nd previous + 3rd previous	13	1803.43
11	**Previous + 2nd previous + 3rd previous + 4th previous**	**13**	**1749.88***
12	2nd previous + 3rd previous	13	1905.23
13	2nd previous + 3rd previous + 4th previous	13	1892.51
14	3rd previous + 4th previous	13	1814.55

A stepwise procedure was systematically conducted using model 11 to select the most optimal set of models, which encompassed a combination of fixed effects and their interactions. The primary criterion for model selection was the AICc values, which helped identify the most informative and parsimonious models. In assessing the importance of each predictor variable, with a particular focus on the weather data variables, the analysis revealed the following key

insights. The best model, which exhibited the highest explanatory power for *S. partita* trap catches, pinpointed temperature as the most influential factor. Furthermore, the analysis highlighted that both rainfall and temperature exerted an impact on trap catches. Additionally, the interaction between rainfall and temperature was a significant factor explaining differences in trap catches. The second-best model also presented an interaction effect, specifically between temperature and humidity (Table 5.4).

Table 5.4 Plausible models (ΔAICc < 1770) explaining *Serrodes partita* trap catches. Parameter estimates are provided for each model term across all plausible models based on Maximum Likelihood estimation.

Model rank	1	2	3	4	5	6	7
ΔAICc	0	0.03	1.7	11.31	12.92	17.21	18.23
Akaike weight (w_i)	1749.8	1749.9	1741.5	1761.2	1761.8	1767.1	1768.1
Cultivar		+	+		+		
Humidity	0.33	-0.45	0.33	0.51	0.51	0.62	
Rainfall	0.05	0.08	0.05	0.01	0.01	0.01	0.1
Temperature	0.94	-1.57	0.93	0.16	0.16		0.17
Year	+	+	+	+	+	+	+
Humidity: temp		0.04					
Rain: temp	0.003	0.004	0.003				0.005

5.3.2 Monitoring

A total of 786 adult *S. partita* moths were captured during the monitoring period from May 2021 to the end of July 2023 from the 17 orchards across the three different farms. Most of the moths were captured in 2021 and the captures decreased progressively each year. There were significant differences in trap catches across different years ($\chi 2 = 14.85$; df = 2; $P < 0.0001$). The average number of moths caught also differed across different months ($\chi 2 = 83.05$; df =

11; $P < 0.0001$) with a significant interaction between the years and months ($\chi 2 = 66.04$; df = 13; $P < 0.0001$) (Figure 5.3). The annual trends in trap activity showed two peaks of varying magnitudes. The first peak was recorded in December, followed by a decrease in January and February of both 2021 and 2022. The second peak was recorded in May for all three years, with a steady decline in June and July before terminating in August. There was little to no moth activity from August to November 2021 and 2022 (Figure 5.3).

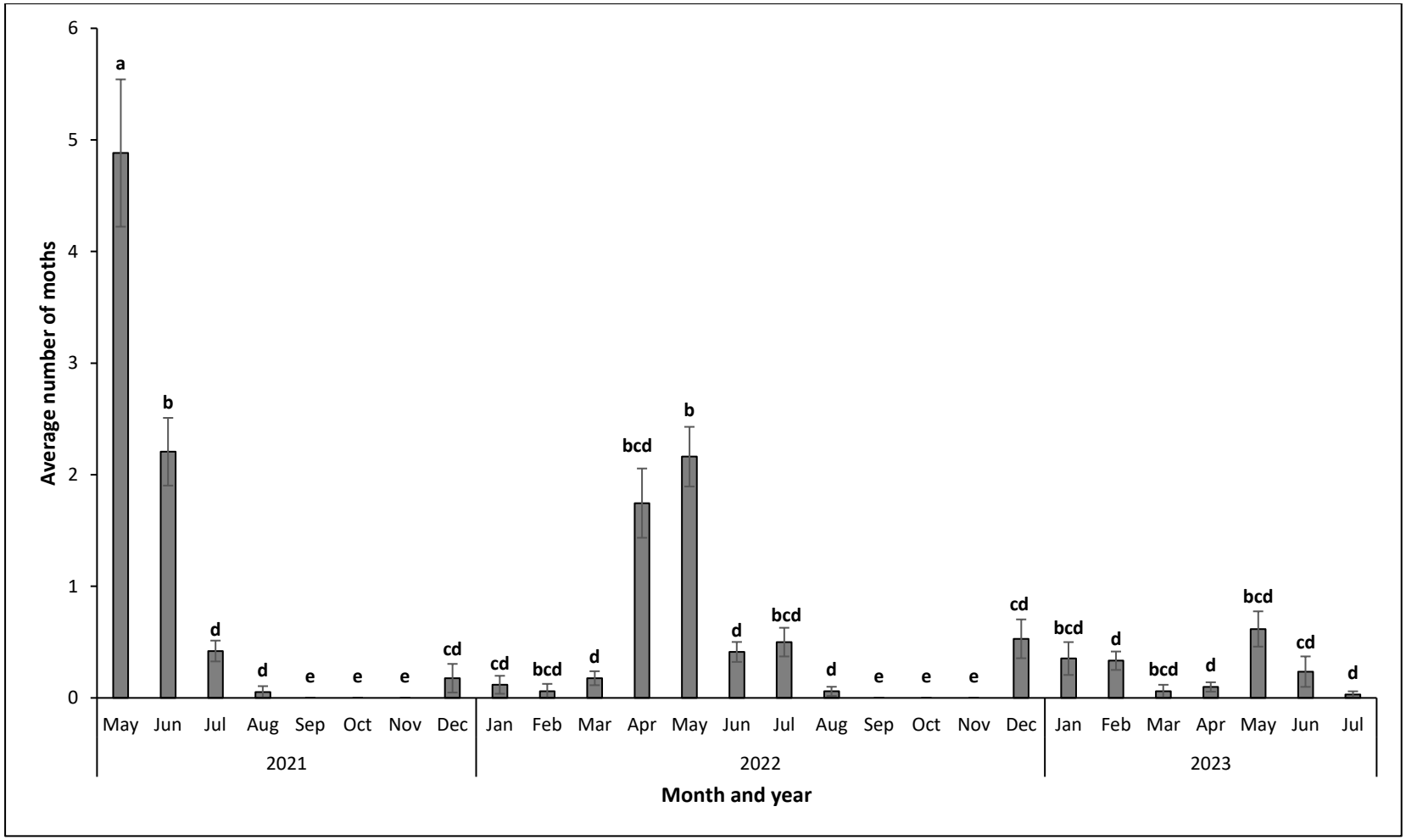


Figure 5.3: Mean monthly trap captures of *Serrodes partita*, grouped by year. Error Bars ± SD. Bars with the same letters not significantly different (Tukey HSD, $P < 0.05$).

There were no statistically significant differences in trap catches between the three soft citrus cultivars ($\chi 2 = 1.46$; df = 2; $P = 0.48$). Moth activity was first recorded in the Clementines in December, Orongrades in January, and Nardocotts in April (Figure 5.4). There were also no significant differences in trap catches between the different farms ($\chi 2 = 3.64$; df = 2; $P = 0.16$). Moth activity was first recorded at Siyamila Farm and Naudeshoek Farm in December. At Ripplemead Farm, moths were recorded later (Figure 5.4).

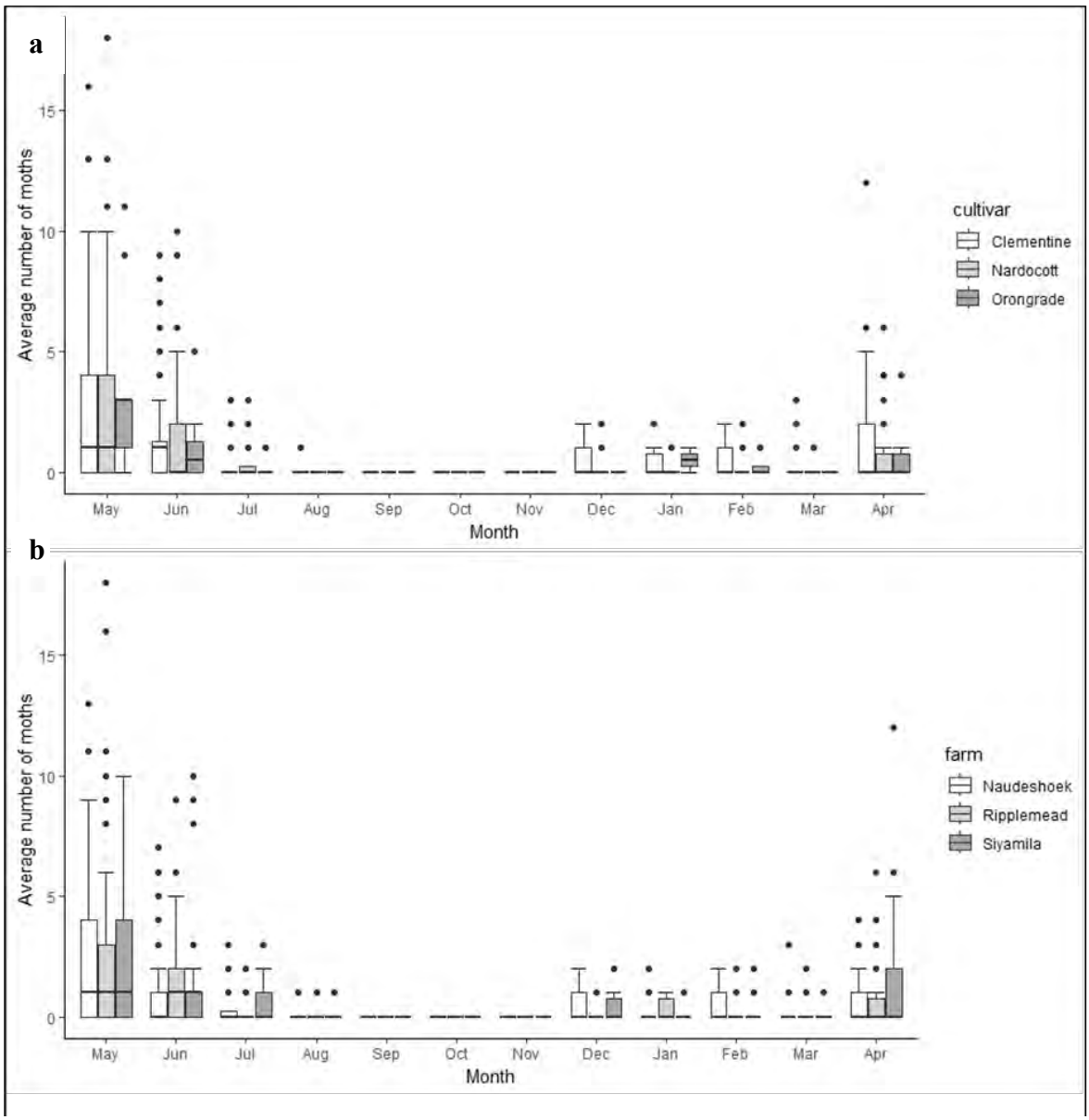


Figure 5.4: Box plots of mean monthly trap catches from the three year monitoring period

of *Serrodes partita* in the Committee's Drift area grouped by a) cultivar and b) farm. Dots represent outliers.

There was a significant interaction between farm and year in the mean monthly trap catches of *S. partita* ($\chi 2$ = 14.62; df = 3; $P < 0.0001$). The highest moth activity was recorded in Nardocotts in 2021, with equal distribution across all three cultivars in 2022. In 2023, the lowest average number of moths was recorded in the Nardocotts (Figure 5.5).

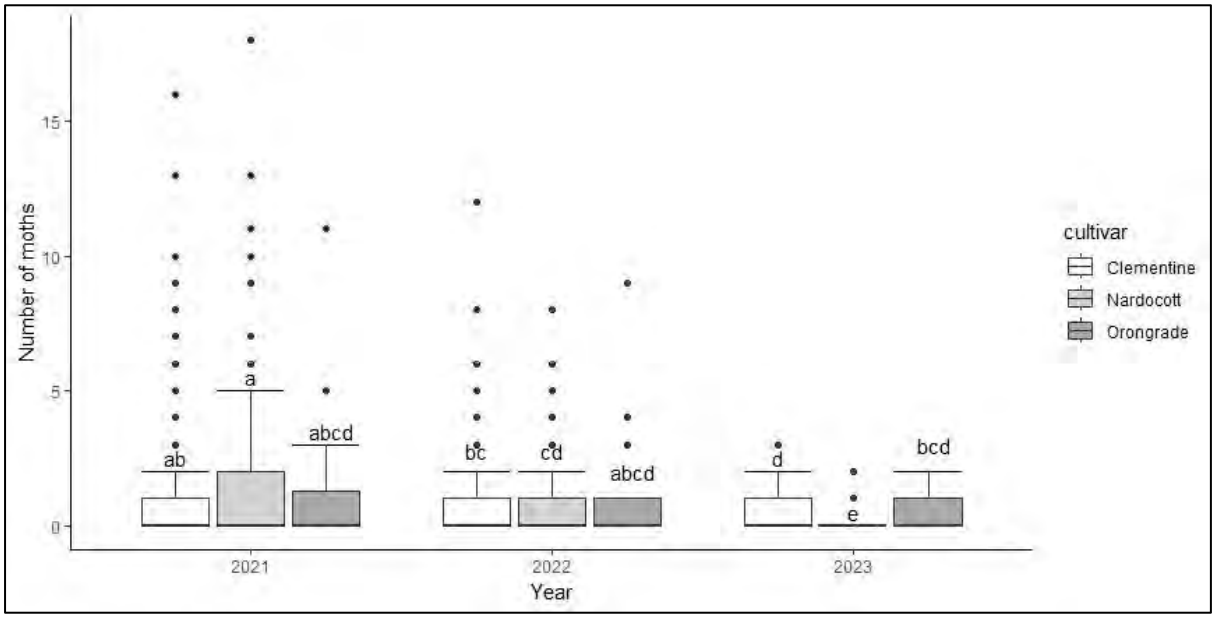


Figure 5.5: Boxplots of annual average number of *Serrodes partita* moths caught from Committee's Drift area grouped by different cultivars. Dots represent outliers, and the same letters above the bars show no significant difference (Tukey HSD, $P < 0.05$).

5.3.3 Effect of weather on trap catches

The weather data obtained from the Fort Beaufort weather station revealed significant variations in rainfall and temperature patterns across different years and months from January 2020 to August 2023. Notably, the data showed a distinct seasonal pattern, with higher levels of rainfall occurring primarily between September and May. In March 2020, the station recorded the highest rainfall, 154.2 mm. Conversely, the lowest rainfall was recorded during June and July in the same year. In addition to rainfall fluctuations, the data also indicated cyclic

temperature changes, with higher temperatures consistently recorded from November to February in each successive year. The maximum temperature peaked in January 2022, with a reading of 32.2°C. On the other hand, the lowest minimum temperature was documented in July 2022, measuring just 4.3°C (Figure 5.6). These findings show the dynamic and seasonal nature of weather conditions in the study region, with potential implications for the study's analysis and interpretation.

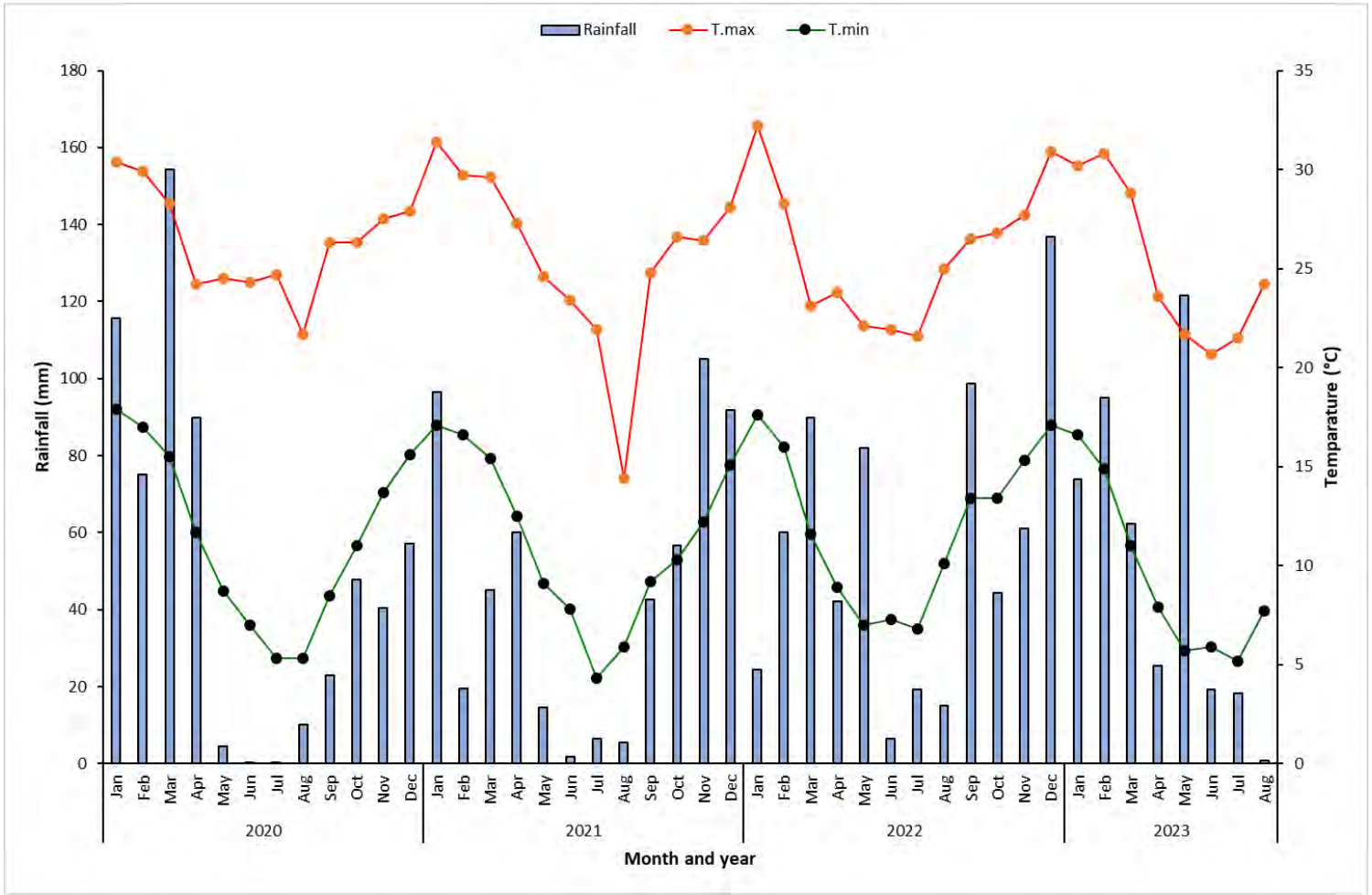


Figure 5.6: Weather data of the study area showing monthly rainfall patterns and maximum and minimum temperatures from Jan 2020 – Aug 2023.

The analysis revealed that both cumulative lagged rainfall and temperature played a significant role in affecting *S. partita* trap catches. Specifically, a cumulative lag of four months, spanning from the previous month to the fourth month, substantially impacted the trap catches recorded. This means that high cumulative rainfall increased the number of moths caught in traps over this period. The highest average trap catch was recorded when the cumulative total rainfall reached 221 mm. High rainfall was associated with *S. partita* activity. However, as the cumulative rainfall increased each year from 2021 to 2023, the trap catches were reduced. This trend indicates that highest rainfall does not necessarily correlate with highest insect activity.

Similar patterns were recorded for temperature, where the cumulative average of the preceding four months significantly influenced *S. partita* activity. Moth activity was recorded when the average temperature exceeded 17°C (Figure 5.7). This temperature threshold seems to be a key driver for increased *S. partita* activity. In contrast, no clear and discernible pattern was observed between average relative humidity and moth activity (Figure 5.8). Relative humidity remained consistently high throughout the year, with only minor variations. High humidity levels were associated with the high activity of *S. partita* (Figure 5.8). There was very little difference between high and low humidity and temperature, suggesting that humidity levels alone did not significantly impact *S. partita* trap catches, in contrast to the pronounced effects of rainfall and temperature.

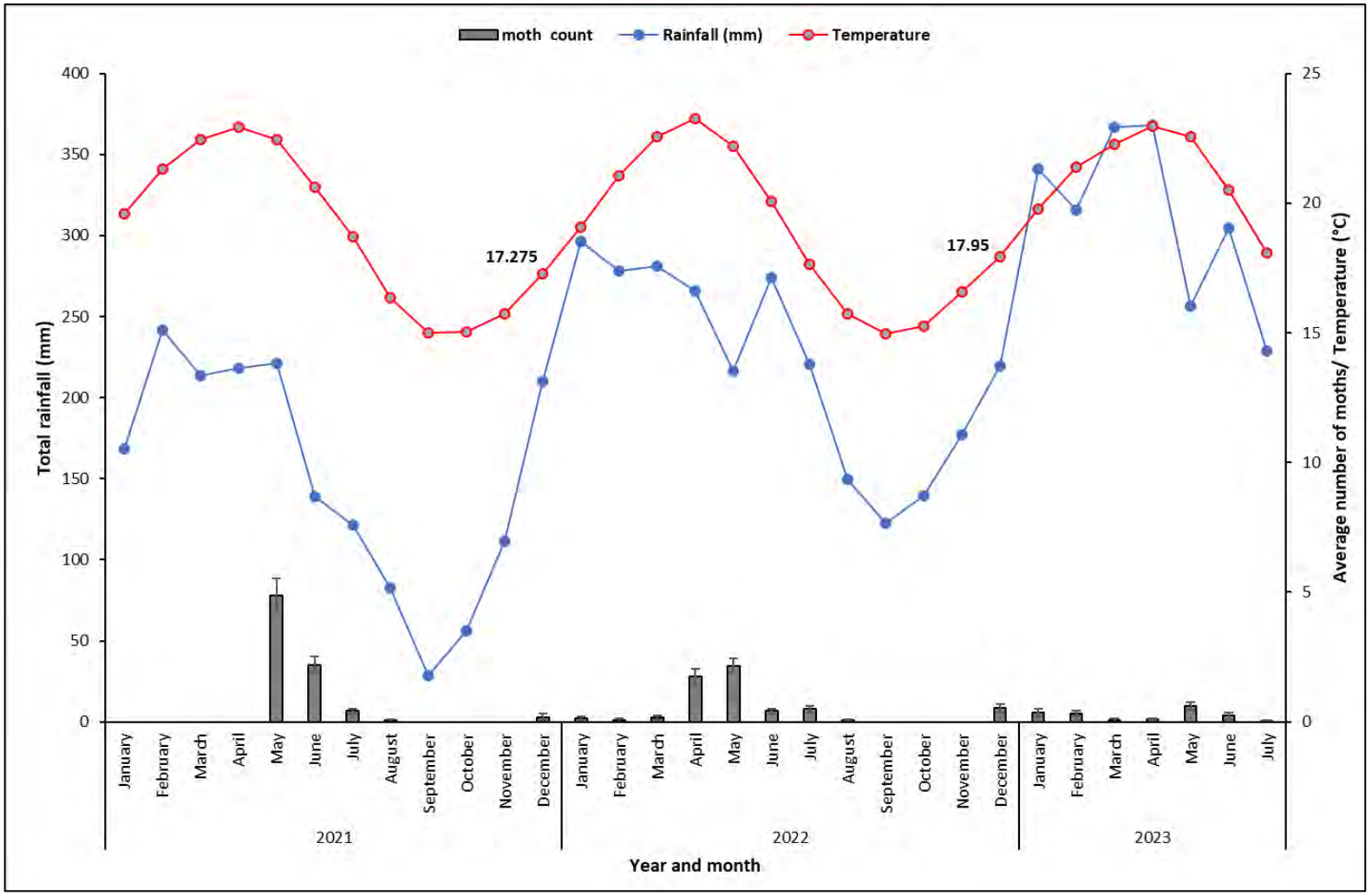

moth count
Rainfall (mm)
Temperature
Total rainfall (mm)
Average number of moths/ Temperature (°C)
17.275
17.95
January
February
March
April
May
June
July
August
September
October
November
December
2021
2022
2023
Year and month

Figure 5.7: Relationship between monthly trap catches of *Serrodes partita* from Committee's Drift area and corresponding lagged total monthly rainfall and average monthly temperature from four previous months. Error bars represent standard errors.

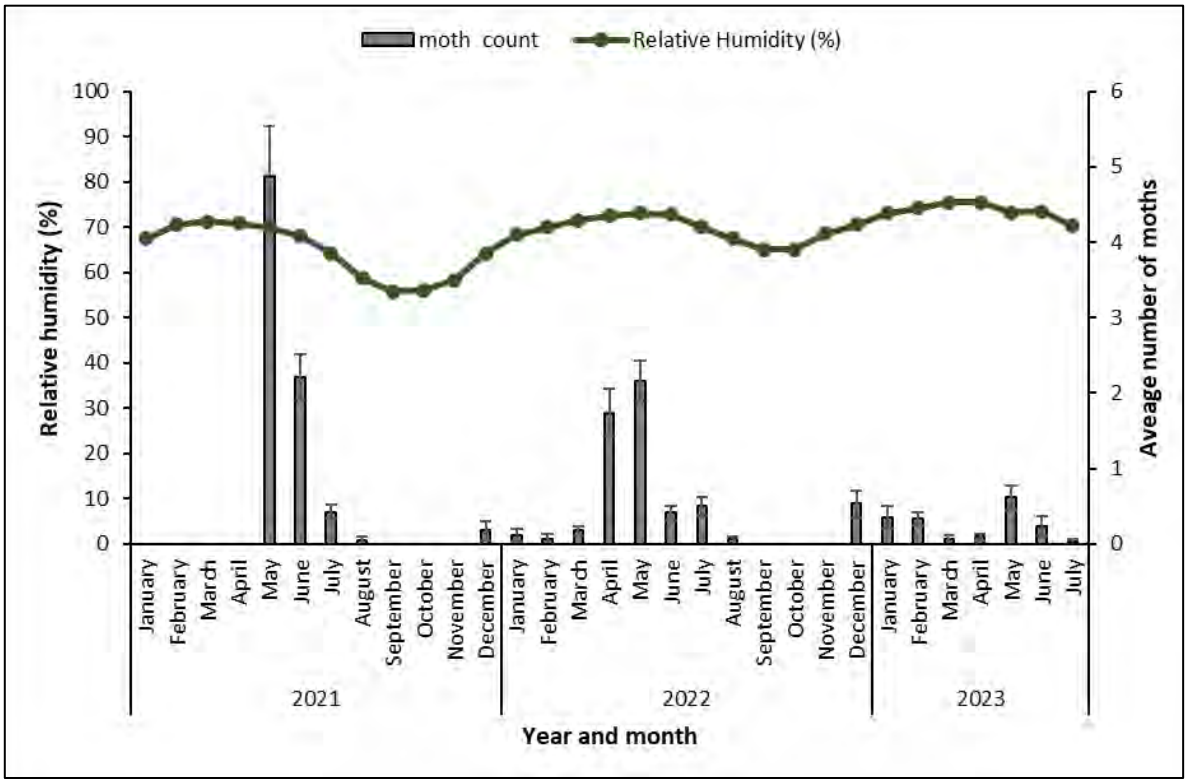


Figure 5.7: Relationship between monthly trap catches of *Serrodes partita* from Committee's Drift and corresponding average monthly humidity from four previous months. Error bars represent standard errors.

5.4 Fruit damage assessment

Throughout the monitoring period, damage caused by *S. partita* remained exceptionally low, with recorded fruit-piercing moth damage mostly on Clementines at Naudeshoek Farm. Naudeshoek had piecing moth damage of 0.1 % followed by Ripplemead with 0.03 % and Siyamila Farm with 0.02 %. The scarcity of fruit damage from piercing made it unfeasible to establish a meaningful correlation with trap catches. Efforts to conduct damage assessments

were undertaken solely in 2021, a year when moth numbers were notably high. In contrast, the subsequent years, 2022 and 2023, witnessed a progressive decline in moth numbers.

5.4 Discussion

The populations of *S. partita* in soft citrus orchards displayed a distinct pattern, commencing in December and levels gradually decreasing by August the following year. Throughout this period, there were fluctuations in the population, characterised by varying peaks and magnitudes. Notably, there was no moth activity recorded between September and November. This pattern of moth activity aligns with findings from other studies. Fay & Halfpapp (2002) documented a similar absence of moth activity in orchards between September and October in Queensland, Australia, and Sands et al. (1991) reported no trap catches of the fruit-piercing moth *E. salaminia* Cramer between the first week of July and December in Australia. This period of inactivity coincides with no fruit in the orchards. Soft citrus cultivars are typically harvested between February and July, with some late-maturing cultivars extending the harvest season into September (CGA 2017). This information supports the hypothesis proposed by Fay (2002) that an extended fruiting season is linked to increased moth activity. It suggests that the availability of fruit in the orchards plays a role in influencing the activity patterns of these moths.

The study recorded two distinct population peaks in fruit-piercing moth activity in both 2022 and 2023. This pattern was also recorded in other studies by Baptist (1944) and Leong & Kueh (2011). These peaks were classified into an initial peak observed from December to February and a slater peak from April to July. This trend was more evident in 2023, strongly indicating the presence of two distinct moth populations with temporal separation. In the years 2021 to 2022, the early peak appeared smaller compared to the later one, suggesting a correlation between these primary and secondary peaks. Additionally, the span between these peaks is approximately 3 to 4 months, which aligns with the generation time of *S. partita*, further emphasising the connection between them. Many factors that vary across time and space can influence insect population dynamics. In the case of *S. partita*, its presence has been linked to the flush of its natural host plant, *P. capensis* (Myburgh 1963b, Whitehead & Rust 1972, Robertson & Cochrane 2005). *Pappea capensis* occurs in the Karoo, where the vegetation is

known to undergo two different flushes: summer and spring flush (Kesler 1993). The first population peak could be attributed to a spring flush of the host plant, while the secondary peak might be the result of a summer flush.

Additionally, the prolonged duration of the second peak, as opposed to the first peak, may be influenced by temperature variations. Insect population peaks are often regulated by temperature, as highlighted in studies by Régnière et al. (2012) and Djaman et al. (2019). The first peak, associated with high temperatures, occurs during warmer weather, while the second peak occurs during winter months, characterised by lower temperatures. These cooler temperatures may extend the life cycle of the moths, contributing to the extended activity period recorded during the secondary peak. The egg-laying behaviour of *S. partita* displayed synchrony among various individuals, as documented in Chapter 2. This synchronisation in egg-laying likely contributes to the recorded coordinated peaks. These two distinct peaks and their temporal separation could be attributed to a complex interplay of factors, including host plant flushes and temperature fluctuations, highlighting the intricate dynamics governing the population of *S. partita*.

The study found no significant differences in moth activity among the three farms. This observation is likely because all three farms are situated in the same locality and produce the same cultivars. Consequently, they are exposed to the same moth populations and environmental conditions. However, interestingly, initial moth activity was recorded at Naudeshoek Farm, located upriver on the Keiskamma River. This was followed by Siyamila Farm, situated in the middle region and Ripplemead Farm, located downriver, recorded moth activity much later. This pattern suggests a gradual movement of the moths from the easterly direction (upriver) towards the westerly direction (downriver) over time. The differences in the timing of moth activity between the farms might be influenced by various factors, including local environmental conditions, vegetation, and possibly the prevailing wind patterns that could facilitate the movement of moths along the river. While there were no significant overall differences in moth activity between the farms, the temporal variations in their activity suggest

a spatial pattern that could be related to the geographic layout and environmental conditions within the study area.

The study also demonstrated the relationship between the occurrence of moths and fruit maturation through the activity of *S. partita* in different cultivars. Early-season cultivars, Clementines, were found to be associated with the initial moth activity, while late-season cultivars, like Nardocotts, were linked to *S. partita* activity later in the season. This finding strongly suggests that moth activity aligns with the maturation and ripening of fruit. In December and January, most fruit is still green or ripening. It's important to note that fruit becomes more susceptible to damage by fruit-piercing moths as it ripens. Research by Cochereau (1969), Bänziger (1982) and Fay & Halfpapp (2006) has shown that green fruit can be fed upon by these moths up to eight weeks before harvest when it begins to colour. This makes the fruit vulnerable to moth infestation as it reaches its ripened state. The high moth activity recorded in Nardocotts in 2021 can be attributed to their late-season nature, which results in a more extended period of susceptibility to moth activity. Additionally, monitoring commenced mid-season in May, coinciding with peak activity in these late-season cultivars.

As reported by previous studies, fruit-piercing moth activity has consistently peaked during the wet season (Hargreaves 1936, Baptist 1944, Ngampongsai et al. 2005). These findings are in line with the results of this study, which recorded that the presence of *S. partita* correlated with rainfall. Nevertheless, there was an overlap in moth activity between dry months (May to August) and wet months (December to March). This overlap likely arises from moths recorded in the orchards maturing during the preceding wet season. Additionally, there have been reports in the literature, such as Hargreaves (1936) and Leong & Kueh (2011), of fruit-piercing moth activity during the dry months, indicating a more complex seasonal pattern. Overall, these findings suggest that the activity and behaviour of *S. partita* can vary depending on environmental factors and may not be entirely restricted to a specific season. Alternatively, these findings suggest a lag from which the moth develops through larval stages during favourable (wet) seasons, while adult activity will be recorded later.

Ngampongsai et al. (2005) reported no significant correlation between rainfall and fruit-piercing moth activity in citrus orchards. In a study conducted by Goddard (2016), correlations were identified between weekly precipitation and weekly trap catches in certain study areas, while in other study areas, no such correlations were recorded. Ngampongsai et al. (2005) suggested that previous months' rainfall might have triggered the outbreaks. This study established a noteworthy correlation between *S. partita* and cumulative total lagged rainfall from the prior month to the fourth previous month, further explaining fruit-piercing moth activity during drier periods. The lag approach agrees with the life cycle of *S. partita,* which takes up to 90 days from egg to adult (Taylor 1951, Neubecker 1962, Chapter 2, section 2.3.2.1). The lag is the period when the host plant flushes and the moth develops. The lag approach was used to demonstrate that African armyworm *S. exempta* outbreaks were significantly associated with the rainfall preceding the official onset of the rainy season (Haggis 1996).

This discovery supports the long-standing hypothesis regarding the relationship between *S. partita* outbreaks and climatic factors. Bosch (1970) also emphasised that seasonal rains played a pivotal role in governing these outbreaks. Meanwhile, Whitehead (cited in Robertson & Cochrane 2005) was convinced that *S. partita* outbreaks in the Karoo result only when adequate rainfall occurs in November, promoting a good flush of host plants. This observation implies that *S. partita'*s occurrence is not solely governed by host plant availability but is also indirectly influenced by rainfall patterns that impact its host plants. Furthermore, Jack (1922) reported that fruit-piercing moth species tend to proliferate in southern Zimbabwe after heavy rains and during the subsequent wet summers. Webb (1953) and Taylor (1965a) detailed the occurrences of outbreaks involving *A. liernardi* and other unnamed Lepidoptera in the Eastern Cape, triggered by a specific sequence of events: late rains following a period of drought that led to extensive outbreaks. They posited that drought-induced delays in the spring flush of host plants caused it to coincide with the arrival of summer rains, resulting in extensive outbreaks.

Additionally, Webb (1953) suggested that late-summer egg-laying is facilitated by elevated night temperatures, which provide suitable conditions for oviposition. This concept found support in research conducted by Sands et al. (1991), which reported that temperatures below 16°C prevented oviposition in *E. salaminia* in Australia. Our study has contributed to this knowledge by establishing a link between adult *S. partita* activity and high average temperatures, specifically from the prior month to the fourth previous month, with temperatures exceeding 17°C. Furthermore, Sands et al. (1991) noted that larval development of *E. salaminia* required 246 day-degrees for larval development and 233 day-degrees for pupation. This insight underscores the central role of temperature in the occurrence of *S. partita*.

Relative humidity displayed a consistent pattern, like rainfall and temperature, where high humidity levels were associated with increased moth activity. Although the specific role of humidity in fruit-piercing moth activity remains largely unexplored, it is likely intertwined with rainfall and temperature, as established in other studies. Carbognin et al. (2023) provided evidence of the joint influence of temperature and humidity on pest outbreaks in *Diatraea saccharalis* Fabricius. Similarly, Tamiru et al. (2012) highlighted the significance of temperature and humidity in affecting the growth and fecundity of *Chilo partellus* Swinhoe. In this study, *S. partita* occurrence was notably linked to high levels of rainfall, temperature, and humidity. At the same time, low extremes of these weather variables were associated with minimal or no moth activity. This aligns with Webb's (1953) observations regarding the sudden disappearance of *A. lienardi* after heavy infestations, possibly due to low temperatures hindering oviposition. It is plausible that a combination of unfavourable weather conditions for egg-laying may explain this phenomenon.

Establishing a correlation between trap catches and *S. partita* was challenging due to the low numbers of recorded *S. partita* during the study. Moreover, the method employed for assessing damage by counting damaged fruit on the trees presents certain limitations. Orchards are subject to sanitation practices where farmers remove all damaged fruit from the trees, a practice which is mandatory for compliance with export requirements for certain markets (Hattingh et

al. 2020). Consequently, if damage assessments are conducted after sanitation, the recorded numbers may not accurately reflect the true extent of damage. Additionally, limiting damage assessment to fruit still on the tree overlooks the fruit that may have fallen to the ground. Leong & Kueh (2011) conducted damage assessments by counting the number of fruit-piercing moth-damaged fruit on the ground. Fay & Halfpapp (2006) suggested counting moths during night surveys, which can be labour-intensive. Notably, *S. partita* predominantly feeds on damaged fruit, rather than undamaged fruit (Chapter 3). This implies that fruit-piercing moth damage may not accurately represent trap catches or the actual moth population. Establishing a correlation may be more feasible under conditions of high moth populations.

In conclusion, the research conducted by Webb (1953), Taylor (1965b), Sands et al. (1991), Ngampongsai et al. (2005), Goddard (2016) and other studies provides valuable insights into the complex factors influencing outbreaks of fruit-piercing moths. These outbreaks appear to be intricately linked to the interplay of rainfall patterns and drought, with late rains following dry spells triggering extensive outbreaks. Furthermore, the role of temperature in shaping the occurrence of these moths is evident, as elevated night temperatures facilitate late-summer egg-laying. Moreover, as demonstrated in other pest-related studies, the interplay of humidity with temperature and rainfall emphasises the need for a comprehensive understanding of these climatic factors in predicting fruit-piercing moth populations. This study adds to this understanding by revealing a significant association between adult *S. partita* activity and high average temperatures, rainfall and humidity over a specific time frame. This highlights the importance of climate and weather patterns in the dynamics of moth populations, shedding light on their life cycles and potential implications for pest management and ecological studies. The study also highlighted the complexity of establishing direct correlations in cases where moth populations are relatively small or influenced by specific behavioural factors like feeding preferences.

CHAPTER 6

Exploring natural enemies of the fruit-piercing moth, *Serrodes partita*, in the Eastern Cape, South Africa

6.1 Introduction

Density-dependent and density-independent factors influence insect pest population dynamics. Among these, natural enemies play a significant role in determining the occurrence and distribution of pests (Price et al. 2011). The factors exert delayed density-dependent mortality, contributing to the cyclical nature of pest populations (Alalouni et al. 2013). Natural enemies can take the form of predators, parasitoids, or pathogens. During their immature life cycle, they rely on host tissues as their primary source of nourishment, but as adults, they typically lead separate lives, focused on reproduction. Parasitoids exhibit high host specificity, selecting and targeting hosts based on kairomone cues associated with the host or host plant (Colazza & Wajnberg 2013). They lay their eggs inside or on the surface of host eggs, larvae, or pupae, depending on the specific characteristics of each parasitoid species. The resulting immature parasitoids can develop either inside (endoparasitoid) or outside (ectoparasitoid) the host as solitary or gregarious species, feeding on host tissue or haemolymph (Heraty 2017). This feeding behaviour may immediately arrest the host's development (idiobiont parasitoids) or allow it to continue growing and feeding (koinobiont parasitoids). The host is usually killed when the parasitoid emerges or shortly after that. Tens of thousands of parasitoid species have been identified, many of which play crucial roles in controlling and reducing insect host population densities (Cusumano et al. 2016, Abram et al. 2019).

On the other hand, pathogens encompass microorganisms such as viruses, bacteria, fungi, and nematodes. These pathogens occur naturally under specific conditions, such as high humidity in the case of certain pathogens and can significantly reduce pest populations (Cossentine

2009). The utilisation of pathogens as microbial control agents primarily relies on inundative application methods. Among these agents, *Bacillus thuringiensis*, a bacterium, is widely employed for managing Lepidoptera, Coleoptera, and Diptera populations (Ruiu 2015). In recent years, baculoviruses have gained popularity as biocontrol components in IPM strategies. These viruses offer several advantages, including their lower toxicity to the environment, compared to conventional pesticides, as well as their non-pathogenic nature to humans (Knox et al. 2015, Afolami & Oladunmoye 2017).

Baculoviruses are a sophisticated type of insect pathogen. They have developed effective survival strategies to target, harm, and eliminate specific insect species, while utilising the host's tissues to reproduce. Baculoviruses have been discovered in numerous insect species, primarily those belonging to the Lepidoptera, Diptera and Hymenoptera orders (Jehle et al. 2006, Cossentine 2009). They establish persistent infections and can cause widespread epizootics. The Baculoviridae is a family of insect viruses with double-stranded DNA. They produce budded virions, enveloped by cell-derived membranes and occlusion-derived virions, embedded in proteinaceous occlusion bodies (OBs). The occlusion bodies are responsible for horizontal transmission from one insect to another. Pest populations decline drastically following viral epizootics. (Wennmann et al. 2018).

To establish effective and sustainable pest management practices, it is crucial to gain a comprehensive understanding of the ecological dynamics of *S. partita* and any natural enemies. While several studies have focused on the economic impact of *S. partita* as a fruit-feeding insect (Whitehead & Rust 1967, 1972, Swart et al. 1975, Rust & Myburgh 1986, Johannsmeier 1998) very few have been focused on its larval bionomics. The development of successful forecasting and management strategies for controlling outbreaks of pests relies on understanding the factors that influence their occurrence (Skendžić et al. 2021). There remains a knowledge gap concerning the role of natural enemies in *S. partita*'s population dynamics.

During outbreaks, the larval feeding behaviour of *S. partita* leads to significant defoliation of Jacket plum trees, which could classify it as an indigenous forest pest. In this regard, it shares similarities with other forest pests, such as the Gypsy moth, *Lymantria dispar* in terms of feeding habits and outbreak occurrence. However, in the Gypsy moth example, two pathogens, the fungus *Entomophaga maimaiga* Humber, Shimazu, and Soper (Entomophthorales: Entomophthoraceae), and the *Alphabaculovirus lydisparis*, commonly known as the *L. dispar* multiple nucleopolyhedrovirus (LdMNPV) have been identified as responsible for population declines, particularly at high pest populations (Alalouni et al. 2013, Reilly et al. 2014, Myers & Cory 2016). Baculoviruses have also been discovered in other outbreak pests, such as the western tent caterpillar *Malacosoma californicum* Pluviale, the African armyworm *Spodoptera exempta*, and the monarch butterfly *Danaus plexippus* (Myers & Cory 2016). Swart et al. (1975) highlighted larval mortalities of *S. partita* and hypothesised the causative agent to be either a bacteria or virus, but it was not identified. Viral infections are related to increased host insect population densities, with low detection in low-density populations. It is highly suggestive that *S. partita* larval stages may be susceptible to a baculovirus.

Most of the research on the role of natural enemies on fruit-piercing moth species has been focused on parasitoids and predators. Several attempts have been made towards biological control of fruit-piercing moths in the genus *Eudocima.* These include the egg parasitoids *Telenomous* sp. (Hymenoptera: Scalionidae) and *Ooencyrtus* sp. (Encyrtidae) as well as the tachinid fly, *Winthemia caledoniae* Mensil (Diptera: Tachinidae) (Cochereau 1974, Denton et al. 1990, Vargas-Fonseca et al. 2020, Leroy et al. 2021). However, no study has been dedicated to identifying and characterising parasitoids associated with *S. partita*, hence, the need for such research.

This study aimed to investigate the natural enemies associated with *S. partita*; larval parasitoids and entomopathogens. This research aimed to enhance our understanding of the ecological dynamics and develop potential management strategies for *S. partita*. The study was divided into two parts. The first part of the study aimed to describe and confirm parasitism in *S. partita*

larvae and determine the rate of parasitism under natural conditions. The second part of the study involved bioprospecting for insect viruses, specifically targeting symptomatic larval cadavers of *S. partita*. This investigation aimed to discover and characterise potential novel insect viruses, associated with the pest.

6.2 Materials and Methods

6.2.1 Parasitism of *Serrodes partita*

Late instar larvae and pupae of *S. partita* were collected from the field in both May 2021 and March 2022 to assess the level of parasitism. *Serrodes partita* has a wide geographic range in the Eastern Cape Karoo, yet it typically manifests in small bands or patches during non-outbreak years, making early instars challenging to detect (Figure 6.1). Additionally, these early instars cause minimal visible damage, with significant damage on host plants only becoming apparent in late instars. Therefore, efforts were directed towards targeting late instars for surveillance, as they are easier to detect due to the significant damage they cause on host plants. The study relied heavily on farmers and landowners for outbreak detection. Often, reports were received only after the insect has completed its development.

The collection sites were Steytlerville and Kat River Valley, located in the Eastern Cape region of South Africa. The larvae aggregate in the leaf litter of *P. capensis* trees during the day, feeding in the tree canopy only at night. Once collected, the larvae were reared on *P. capensis* leaves until they reached the pupal stage. The pupae were then transferred into individual transparent 350 ml containers with perforated tops and monitored daily to identify any seclusion of parasitoids. The containers were kept in the laboratory at room temperature (21 - 24°C). Parasitised pupae changed colour, shifting from light brown or dark brown to black. The presence of entry holes on the pupal casings further confirmed parasitism as described by Dindo & Nakamura (2018). The parasitism rate for each year was calculated by expressing the

number of parasitised individuals as a percentage of the total number of collected larvae and pupae. In 2022, the parasitised pupae were also sexed to gather more detailed information.

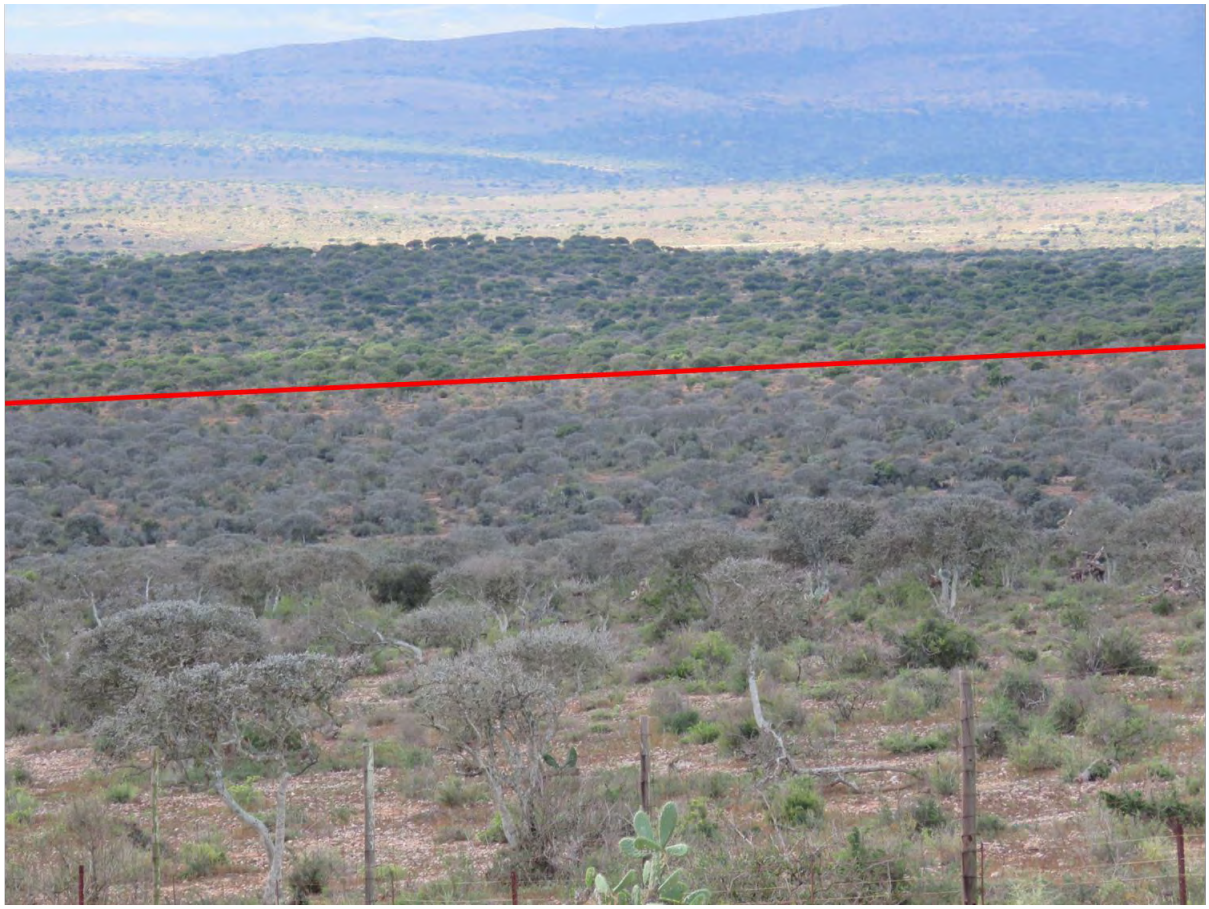


Figure 6.1: Defoliated trees in Steytlerville Karoo veld, the site of larval occurrence of *Serrodes partita*. The red line delineates between the occurrence band (bottom) and the non-outbreak band (top). The occurrence band is characterized by complete defoliation of leaves.

6.2.2 Bioprospecting for insect viruses

6.2.2.1 Insect collection and rearing

Insect collection and larval rearing was done using the protocol described in Chapter 2 section 2.2 and 2.3

6.2.2.2 Symptomology

The rearing containers were checked daily for larval mortality until pupation. Larvae were checked for virus symptoms, including a change in behaviour and appearance, particularly colour, as described by Opoku-Debrah et al. (2013). Both dead and dying larvae collected and stored in the freezer at -4°C before virus isolation.

6.2.2.3 Purification of occlusion bodies

A method described by Dhladhla (2012) was employed for the purification of occlusion bodies. Insect cadaver samples weighing 200 mg were homogenised in 1 ml of 0.1 % sodium dodecyl sulfate (SDS) solution, and then centrifuged at 100 ×*g* for 30 seconds. The resulting supernatant was carefully collected, and 1 ml of 0.1 % SDS was added to the pellet before centrifuging again at 100 ×*g* for 30 seconds. The supernatant was once again collected, pooled with the first collection, and subjected to a higher centrifugation speed of 10,000 ×*g* for 10 minutes. The supernatant was discarded and the resulting pellet resuspended in 1 ml of 0.01 % SDS. The resuspended sample was centrifuged at 10,000 ×*g* for 10 minutes. The supernatant was again discarded and the resulting pellet resuspended in 1 ml of distilled water. The resuspended sample was centrifuged at 10,000 ×*g* for 20 minutes. Finally, the pellet was collected and resuspended in 500 µl of distilled water.

6.2.2.4 Genomic DNA extraction

Genomic DNA (gDNA) was extracted using the Zymo-Quick DNA™ MiniPrep Plus Kit (Zymo Research, USA). The OBs were prepared for DNA extraction by adding 90 µl of 1M Na_2CO_3 to 200 µl of OBs and incubated at 37°C for 30 minutes. To this, 120 µl Tris-HCL (1M, pH 6.8) was added to the sample to correct the pH. After preparation, DNA was extracted using the steps outlined in the Zymo-Quick DNA^{TM} Miniprep Plus Kit. DNA was visualised on 1 % agarose gel electrophoresis run at 80 V for 30 minutes in 1× TAE buffer (40 mM Tris-acetate,

20 mM acetic acid, 1 mM EDTA) with ethidium bromide (0.5 µg/ml). Images were captured using a ChemiDoc™ XRS+ with Image Lab™ Software (Bio-Rad Laboratories, USA).

6.2.2.5 PCR amplification of the polh/gran, lef-8 and lef-9 genes

Polymerase chain reactions were performed to amplify the *polh/gran*, *lef-8* and *lef-9* genes using degenerate oligonucleotides designed by Lange et al. (2004) (Table 6.1). The reaction mixture comprised 12.5 µl Amplicon Taq 2× Ready mix DNA polymerase (Ampliqon, Denmark), 1 µl forward primer, 1 µl reverse primer and 1 µl of template DNA. Reactions were made up to a total volume of 25 µl using ddH_2O. The *Betabaculovirus cryleucotretae* (Cryptophlebia leucotreta granulovirus (CrleGV)) DNA was also amplified alongside the sample DNA as a positive control. A negative control reaction with no template was also included with each gene amplified.

Table 6.1: Oligonucleotide primer pairs used in PCR and sequencing reactions of viral genes (Lange et al. 2004).

Target gene	Primer name	Sequence (5' – 3')	Amplicon size
Polh/gran	prPH-1	TGTAAAACGACGGCCAGTNRCNGARGAYCCNTT	507 – 510
	prPH-2	CAGGAAACAGCTATGACCDGGNGCRAAYTCYTT	
Lef-8	prL8-1	TGTAAAACGACGGCCAGTCAYGGHGARATGAC	681 – 771
	prL9-2	CAGGAAACAGCTATGACCAYRTAS$_1$GGRTCYTCS$_2$GC	
Lef-9	prL9-1	CAGGAAACAGCTATGACCAARAAYGGITAYGCB$_3$G	258 – 273
	prL9-2	TGTAAAACGACGGCCAGTTTG$_4$TCDCCRTCRCARTC	

B = C, G, or T; D = A, G, or T; H = A, C, or T; N = C, A, T, or G; R = A or G; S = C or G; Y = C or T; I = Inosin.
Underlined nucleotides indicate standard sequencing primers (-21) M13F and (-29) M13R.

Different cycling parameters were used for each gene as described by Lange et al. (2004), with some minor modifications. For the *polh/gran* genes, the PCR consisted of cycling parameters with an initial denaturation step of 95°C for 3 minutes and 36 cycles of 95°C for 30 seconds,

50°C for 1 minute, 72°C for 1 minute, and a final extension step 72°C for 10 minutes. Amplification reactions for the *lef-8* gene were performed with an initial denaturation step of 95°C for 4 minutes and 40 cycles of 95°C for 2 minutes, 58°C for 2 minutes 30 seconds, 72°C for 1 minute, and a final extension step of 72°C for 5 minutes. The PCR reactions for the *lef-9* gene were performed with an initial denaturation step of 95°C for 4 minutes and 35 cycles of 95°C for 2 minutes, 55°C for 1 minute, 72°C for 1 minute, and a final extension step of 72°C for 2 minutes. Amplicons were visualised by 1 % agarose gel electrophoresis at 80 V for 30 minutes in 1× TAE and gel images were captured as mentioned above.

The samples were then sent to Inqaba Biotechnical Industries Pty Ltd (South Africa) for sequencing using the M13F and M13R sequencing primers. The gene sequences were analysed separately using Chromas Software version 2.6.6 Techenelsium (Australia) with ambiguous nucleotide correction. The corrected sequences were imported into Benchling Overwatch Research Limited (San Francisco, USA) to align and assemble the forward and reverse sequences into a single consensus sequence. The resulting consensus sequences were then submitted to BLAST (NCBI, USA).

6.2.3 Data analysis

A phylogenetic analysis was done using concatenated polh/gran, lef-8 and lef-9 gene sequences generated in this study and for several others obtained from GenBank (NCBI). The analysis used the maximum likelihood method in MEGA-XI (v 11.0.13), with the model parameters selected using the model testing tool in MEGA.

6.3 Results

6.3.1 Parasitism of *Serrodes partita*

A high occurrence of *S. partita* in the Karoo was observed in 2021, with extensive defoliation of *P. capensis*. The collection of larval and pupal stages took place late in the season. The late

instars of *S. partita* are parasitised by a tachinid fly, and the parasitism is confirmed after pupation (Figure 6.1a). Among 121 larvae and pupae collected in May and June 2021, only six were parasitised by a tachinid fly (4 %), indicating low parasitism. In 2022, the occurrence of *S. partita* was more isolated, reported in much fewer locations than the previous year. The larvae collected in 2022 (31) were only collected in one location, (Steytlerville), which had notably higher parasitism at 35 % (Table 6.2).

Table 6.2: Parasitism of *Serrodes partita* on larvae and pupa collected in 2021 and 2022 by a tachinid fly in the Eastern Cape, South Africa.

	2021	**2022**
Larvae collected	28	31
Pupae collected	93	0
Number parasitised	6	11 (4 males, 7 females)
% parasitism	4 %	35 %

Parasitised pupae changed from light to dark brown and became rigid. An entry hole was observed on the posterior margin of the pupa (Figure 6.1). The tachinid fly was identified at the Albany Museum, South Africa, to be from the subfamily Tachininae and the tribe Linnaemyini.

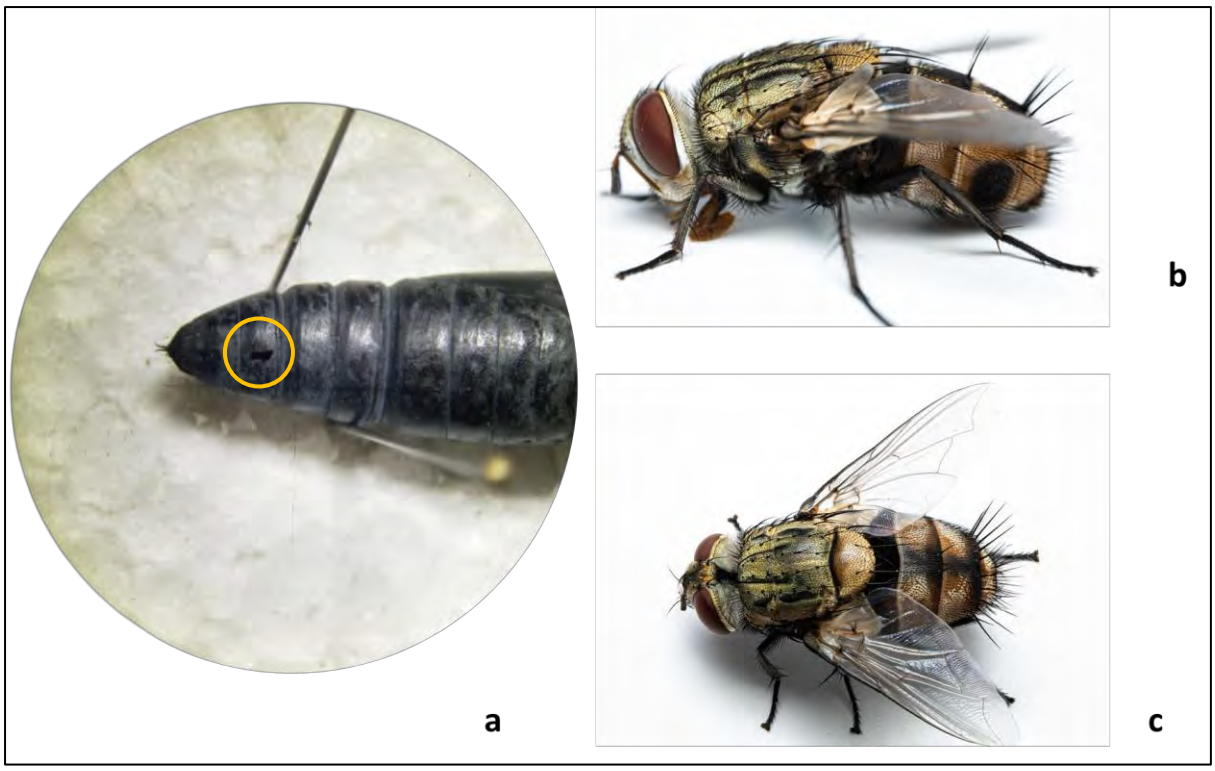


Figure 6.2: a) Parasitised *Serrodes partita* pupa with the parasitoid entry hole showing; (b & c) lateral and dorsal view of the tachinid fly (Photo Credit: David Taylor).

6.3.2 Bioprospecting for insect viruses

6.3.2.1 Symptomology

Infected larvae were observed to change colour from greyish green to light pinkish. They were also reduced in size compared to non-infected larvae of the same instar (Figure 6.3). They were sluggish in movement, rarely moving unless disturbed, and would loosely cling to branches such that sudden movement of the branch would dislodge them. Dead larvae were found loosely hanging on the branches or on the vermiculite substrate that anchored the fresh branches. The cadavers were observed to be squishy and could tear easily, a symptomatic characteristic of baculoviral infections (Mushobodzi et al. 2005).

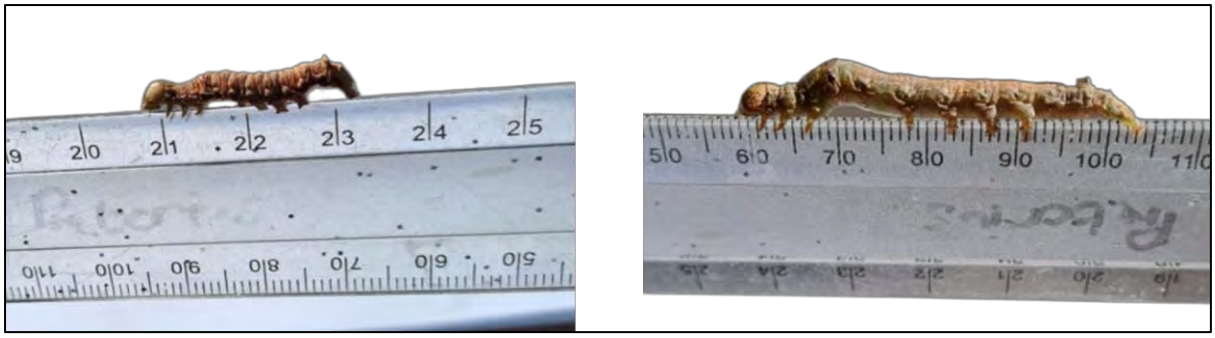


Figure 6.3: *Serrodes partita* larvae, showing symptomatic reduction in late instars, size virus-infected (left) and healthy (right) larvae.

6.3.3.2 Genomic DNA extraction

Genomic DNA was successfully extracted, following the purification of OBs from symptomatic larvae. It was confirmed by high molecular weight bands around the 20,000 bp marker, however, the actual size of the DNA could not be determined, due to size limitations of the DNA ladder used and gel resolution (Figure 6.4).

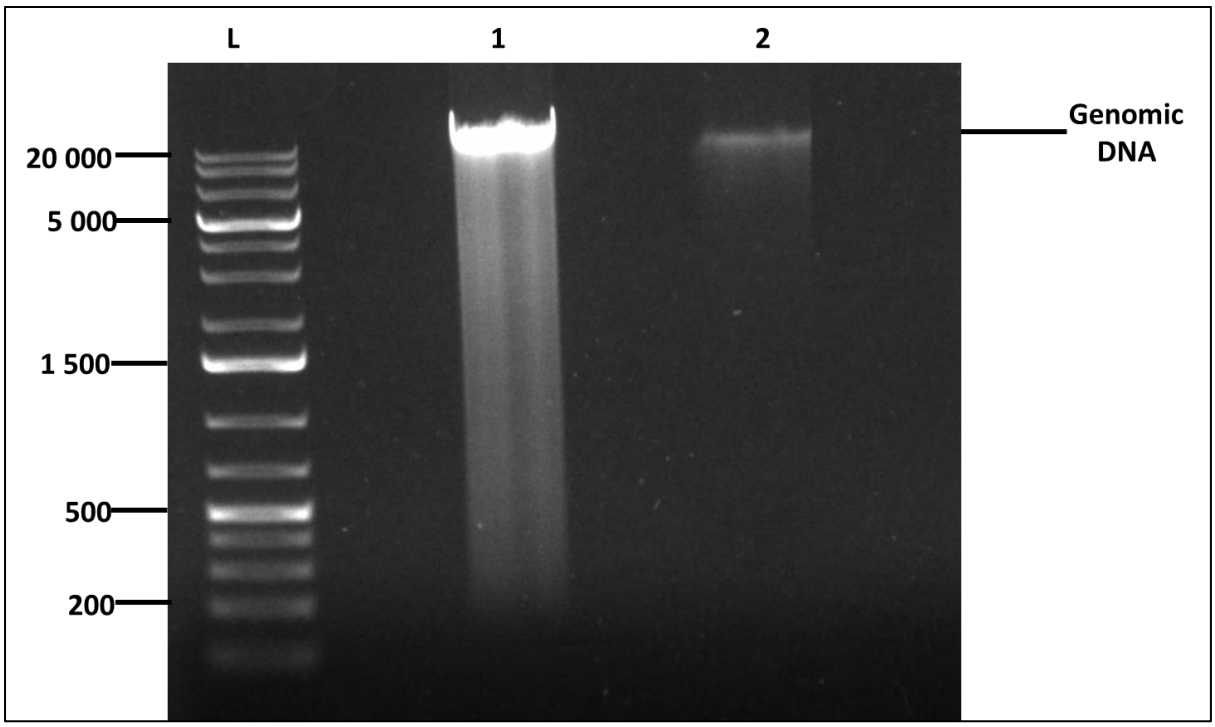


Figure 6.4: Extracted gDNA run on a 1 % agarose gel. Lane L – GeneRuler™ 1 kb Plus

DNA ladder (Thermo Scientific, USA); Lane 1 and Lane 2 – extracted genomic DNA from two virus-symptomatic *Serrodes partita* larval samples.

6.3.2.3 Amplification of the polh/gran, lef-8 and lef-9 gene sequences

The *polh/gran* gene was successfully amplified using the extracted gDNA as template. The CrleGV DNA was also successfully amplified as a positive control. PCR amplification produced dense, bright bands for both the gDNA extracted from sample 2 and CrleGV control in lanes 2 and 3 respectively (Figure 6.5). Non-specific binding was observed in lanes 1 and 3 for both the gDNA extracted from sample 1 and CrleGV control. The oligonucleotides used, produce a partial *polh/gran* gene amplicon with an estimated size of 510 bp (Table 6.1).

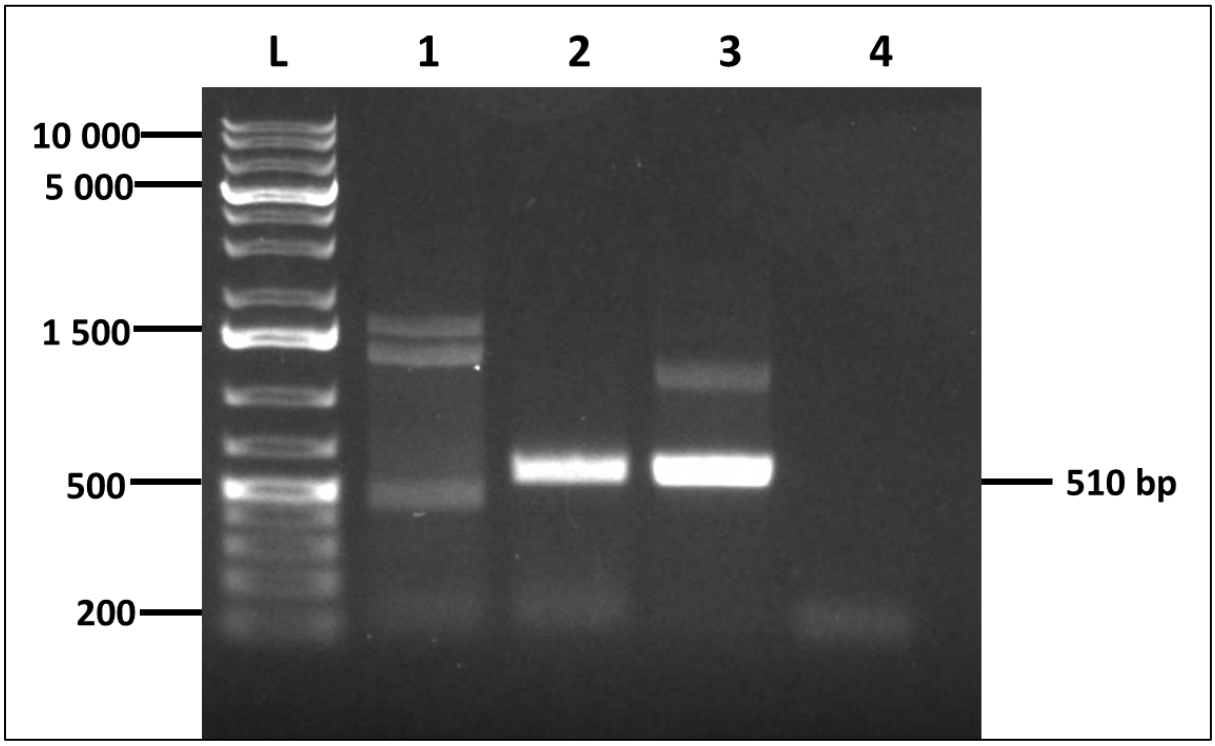


Figure 6.5: PCR amplification of the *polh/gran* gene using gDNA extracted from *S. partita* and CrleGV OBs on 1 % agarose gel. Lane L – GeneRuler™ 1 kb Plus DNA Ladder; Lane 1 – *Serrodes partita* gDNA sample 1; Lane 2 – *Serrodes partita* gDNA sample 2; Lane 3 – CrleGV gDNA (+ve control); Lane 4 – No template control.

The *lef-8* gene was successfully amplified by PCR amplification using gDNA extracted from OBs purified from *S. partita* larvae and the CrleGV gDNA. The partial amplification of the *lef-*

8 gene produced a bright band at an estimated size of 743 bp for the CrleGV DNA and gDNA extracted from sample 2, as shown in lanes 1 and 2 (Figure 6.6). Missing lanes were skipped during loading.

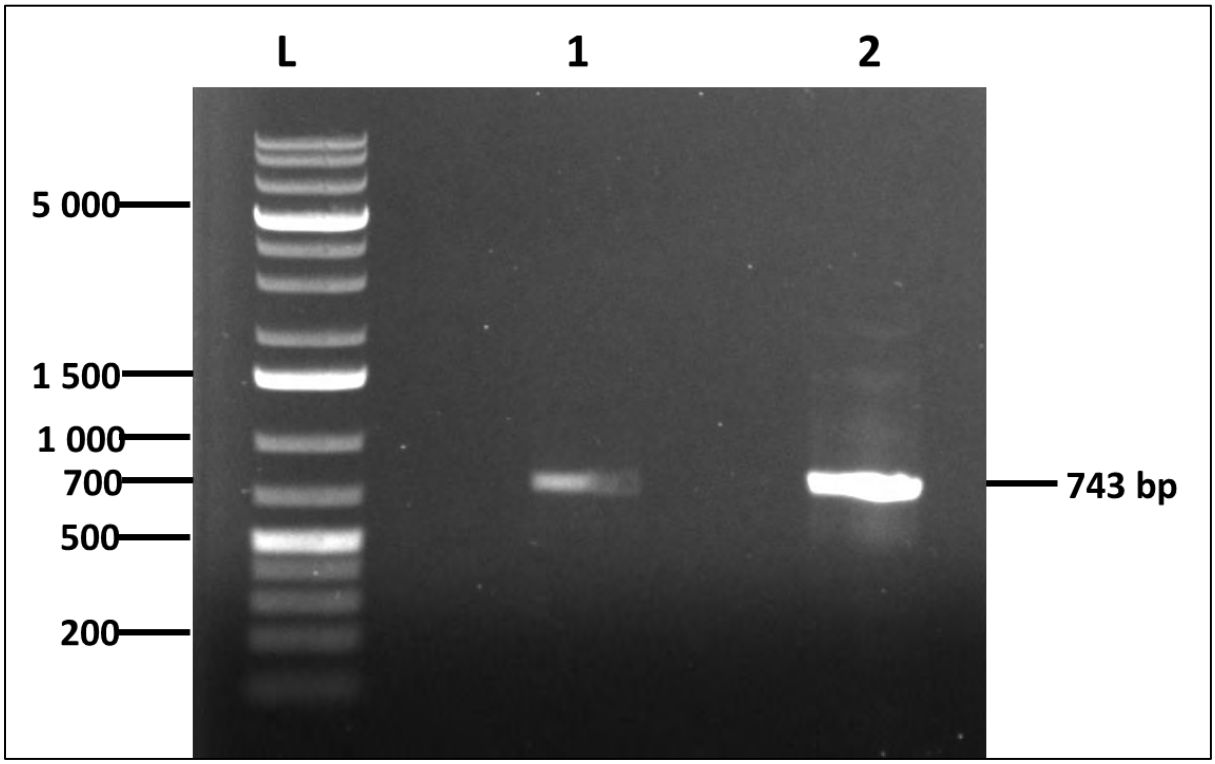


Figure 6.6: PCR amplification of the *lef-8* gene using gDNA extracted from *S. partita* and CrleGV OBs on 1 % agarose gel. Lane L – GeneRuler™ 1 kb Plus DNA Ladder; Lane 1 – *Serrodes partita* gDNA Sample 2; Lane 2 – CrleGV (+ve control).

The *lef-9* gene was also amplified using the extracted gDNA and the CrleGV gDNA as a positive control. Amplification produced bands for both the gDNA extracted from sample 2 and for the positive control, CrleGV, as shown in lanes 2 and 3 respectively (Figure 6.7). Non-specific amplification was again observed for the gDNA extracted from sample 1, as seen in lane 1. No amplification occurred in the negative control shown in lane 4. A partial amplicon for the *lef-9* gene was expected, with an estimated size of 280 bp.

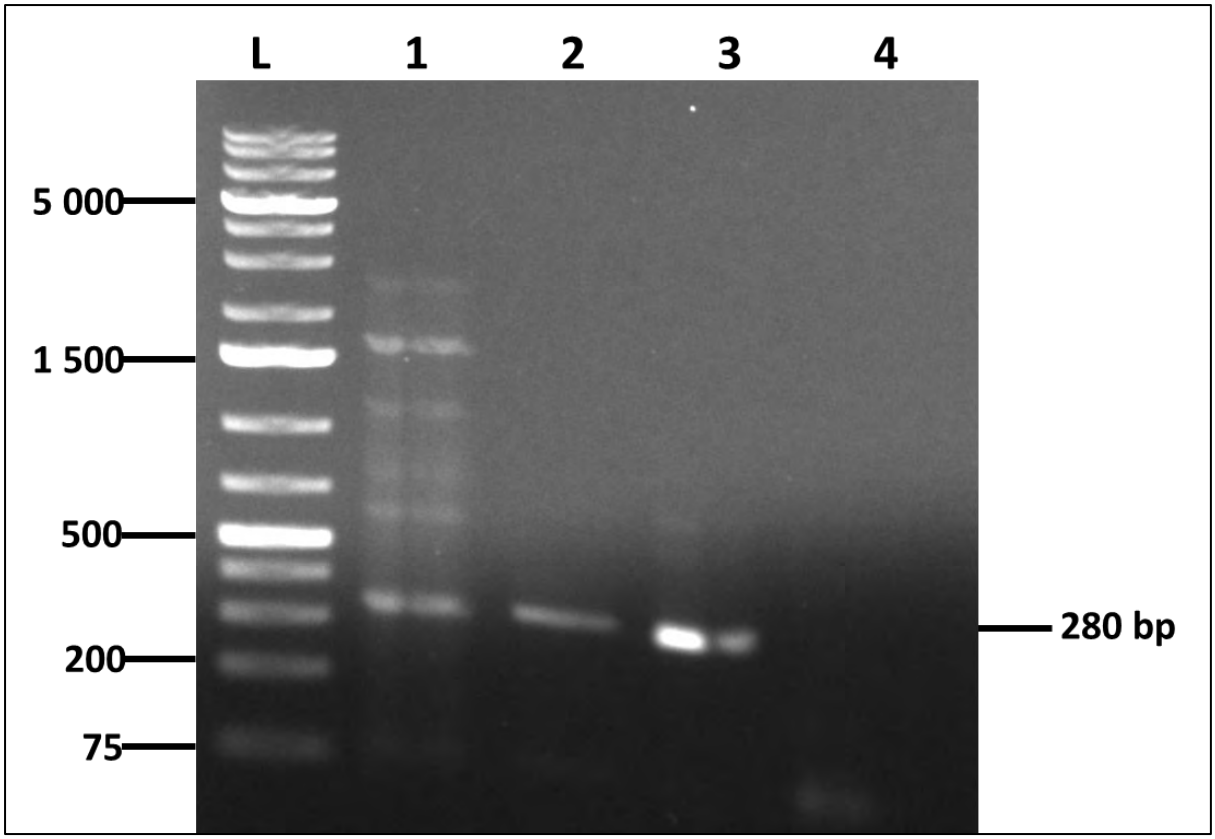


Figure 6.7: PCR amplification of the *lef-9* gene using gDNA extracted from *Serrodes partita* and CrleGV OBs on 1 % agarose gel. Lane L – GeneRuler™ 1 kb Plus DNA Ladder; Lane 1 – *Serrodes partita* gDNA sample 1; Lane 2 – *Serrodes partita* gDNA sample 2; Lane 3 – CrleGV gDNA (+ve control); Lane 4 – No template control.

6.3.3.4 Analysis of the polh/gran, lef-8 and lef-9 sequences

Amplicons generated using the sample 2 gDNA were Sanger sequenced in both the forward and reverse directions. The resulting nucleotide sequences for the *polh/gran*, *lef-8* and *lef-9* genes were analysed separately via Chromas v2.6.6 with ambiguous nucleotides manually corrected. The corrected sequences were imported into Benchling for pairwise alignment. A consensus sequence was assembled from the forward and reverse sequences for each gene respectively. The sequences were submitted to BLAST on GenBank (NCBI), with all three sequences resulting in searches with less than 85 % identity scores (Table 6.3). These nucleotide sequences exhibited significant homology with various alphabaculoviruses, such as

LdMNPV) or *Alphabaculovirus penudae* (Perina nuda NPV), many of which infect insects in the Noctuidae superfamily of Lepidoptera (Table 6.3).

Table 6.3: BLAST analysis of the *polh/gran, lef-8,* and *lef-9* gene sequences of the *Serrodes partita* virus isolate showing the top hits of each gene.

Gene	Binominal species name	Percentage Identity (%)	E value	Accession
polh/gran	*Alphabaculovirus penudae*	81.4	0.00	U22824.1
	Alphabaculovirus pendibubdae	82.04	0.00	OP891564.1
	Alphabaculovirus orpseudotsugatae	81.54	0.00	NC_001875.2
	Alphabaculovirus spliturae	81.21	0.00	X94437.1
lef-8	*Alphabaculovirus trini*	79.26	0.00	MH577296.1
	Alphabaculovirus lydisparis	71.8	0.00	MK089451.1
lef-9	*Alphabaculovirus leseparatae*	77.93	0.00	NC_008348.1

6.3.3.5 Phylogenetic analysis of the polh/gran, lef-8 and lef-9 genes

The *S. partita* virus isolate clustered among other alphabaculoviruses (nucleopolyhedroviruses), following phylogenetic analysis of the aligned concatenated nucleotide sequences of the three genes: *polh/gran*, *lef-8* and *lef-9* of the isolate from this study and other baculoviruses identified from the BLAST analysis (Figure 6.8). A maximum likelihood tree was created in MEGA-XI with 1000 bootstrap replicates. Model analysis identified the General Time reversible model as the ideal model, resulting in the lowest Bayesian information criterion (BIC) score.

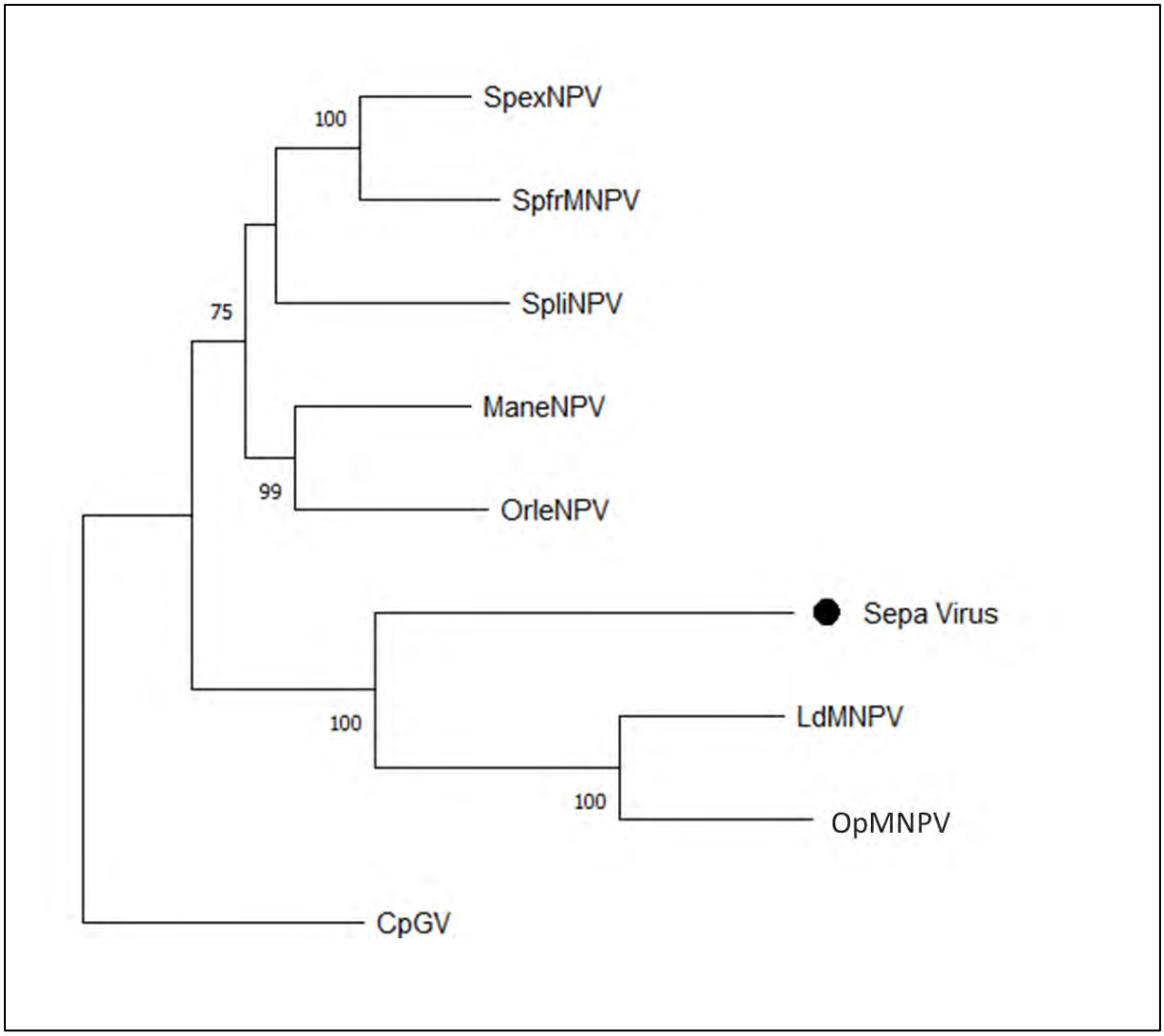


Figure 6.8: Cladogram showing phylogenetic analysis of the *Serrodes partita* virus with other baculoviruses based on the concatenated sequences of the *polh/gran*, *lef-8* and *lef-9* sequence. *Betabaculovirus cypomonellae* CpGV was set as an outgroup to root the tree.

6.4 Discussion

Serrodes partita exhibits a wide geographic distribution in the Karoo region of the Eastern Cape Province of South Africa, displaying sporadic occurrence and a cryptic habit (Johannsmeier 2001). During non-outbreak years, the population remains extremely low, making detecting larval stages and conducting comprehensive surveys challenging. The information regarding parasitoids of *S. partita* covered in this study is preliminary in nature. Although a single parasitoid species was observed, the limited number of collected insect specimens hindered drawing informed conclusions about the rate of parasitism. Nonetheless, the study confirmed the presence of the tachinid fly as the predominant larval parasitoid, as previously highlighted by Johannsmeier (1998). Myburgh et al. (1973) observed parasitism rates on *S. partita* as high as 80 % by this tachinid fly during outbreak years. There is need to definitively identify the parasitoid to species level. The tachinid family is the largest family of Diptera and consists of mostly endoparasitoids of larval Lepidoptera (Dai et al. 2022). They are good potential biocontrol agents and have been used successfully in the management of *Helicoverpa armigera* and *Operophtera brumata* (Cherry et al. 2003, Broadley et al. 2018).

The *S. partita* virus was found to cluster among other Alphabaculovirus, forming a monophyletic clade alongside LdMNPV and OpMNPV, with branches having strong bootstrap support of 100. The grouping of the *S. partita* virus with other alphabaculoviruses supports its classification as *S. partita* NPV (SepaNPV), however, additional analysis that includes a greater diversity of baculovirus species is required. The recovery of an alphabaculovirus associated with *S. partita* is surprising, considering the scarce occurrence nature of this insect. Baculoviruses are known to be transmitted horizontally, particularly at high population densities. Infection levels rise as host density surpasses a theoretical threshold (Chapman et al. 2015). Given that the larvae used in this experiment were reared in the laboratory, it seems that there is a possibility of vertical transmission from mother to offspring or a combination of both vertical and horizontal transmission. Vertical transmission can occur through trans-ovum contamination (external to the egg) or transovarial transmission (within the egg) (Cory 2015).

In the case of *S. partita*, vertical transmission could occur if the larval stages consume OBs but manage to pupate before succumbing to the virus, resulting in sublethal-infected adults that pass the virus on to their offspring (Myers & Cory 2016). The offspring of sublethal-infected adults could exhibit either an active infection that proves lethal or a covert infection that is transmitted to the next generation (Cooper et al. 2003). Covert infections have been observed in *S. exempta*, where all field-collected insects tested positive for *Alphabaculovirus spexemptae* (*Spex*NPV). Furthermore, virus DNA has been detected in insect colonies, continuously maintained in the laboratory for five years (Vilaplana et al. 2010).

Covert, non-symptomatic insect infections can transition into active infections, leading to mortality through a stochastic process. Lepidopteran larval stages harbour overt lethal disease, which becomes expressed following certain triggering events, such as food availability, density changes, or humidity variations. The density-dependent hypothesis proposes that high population conditions induce stress, making insects more susceptible to diseases. Opoku-Debrah et al. (2013) applied this concept by using overcrowding to induce latent infections and successfully isolated five new CrleGV isolates from *T. leucotreta.* However, it is important to note that this density-dependent praxis may not be universally observed in all lepidopteran species, as there are exceptions (Myers & Cory 2016). Reilly & Hajek (2008) observed a negative relationship between host density and viral susceptibility in *L. dispar,* indicating that lower densities could also result in higher vulnerability to viral infections. Similarly, Reeson et al. (2000) found lower virus mortalities and transmission rates in gregariously reared larvae of *S. exempta*, compared to solitary-reared larvae. The larvae were reared at very low population densities in the present study, yet they exhibited active infections. This suggests that the triggering event responsible for activating the latent infections might not be density-related but could be influenced by other factor such as food stress. Moreover, since they were reared on plant material, this may have been contaminated with the virus leading to horizontal transmission.

Utilising a baculovirus as a control strategy against *S. partita* poses significant challenges due to the ecological characteristics, scale, and sporadic nature of its outbreaks. Aerial application over expansive areas would be necessary for effectively targeting the virus at the larval stages. However, this approach presents multiple challenges, with cost being a prominent concern, alongside logistics and accessibility. It is important to note that the larval host of *S. partita* is typically found far away from citrus orchards, from where the adults migrate and become pests (Johannsmeier 1998). Additionally, while the damage inflicted on the larval host can be extensive, it is not economically significant, as the affected trees often recover after rainfall. Consequently, the need for control measures for the sake of the larval host is not justified. The use of LdMNPV has been explored in the United States as a potential solution against a more severe forest defoliator, the Gypsy moth. However, it has not gained popularity, primarily due to the high costs associated with formulation and application (Ruiu 2015).

Baculoviruses can be used as "stressor" virus to manage other pests. It has been observed that latent NPVs in insect larvae can transition to more virulent forms when subjected to foreign viruses. Cooper et al. (2003) demonstrated this in forest tent caterpillars, *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae), when challenged with the western tent caterpillar virus. The efficacy of LdMNPV was assessed by Shapiro & Shepard (2006) as a synergistic agent alongside other baculoviruses on *S. exigua* and *S. frugiperda* resulting in reduced lethal concentration 50 (LC_{50}) values under laboratory conditions. This specific baculovirus isolate could also be evaluated for its potential synergistic effects in managing other pests. Further research and evaluation through dose-response assays are necessary to explore the suitability and effectiveness of the identified baculovirus as a biocontrol agent or stressor virus in pest management.

The simultaneous occurrence of parasitoids and baculoviruses is common in many host species, resulting in positive and negative impacts. Certain parasitoids can reduce the pathogenicity of baculoviruses, while host baculoviruses can also decrease parasitoid population densities (Cossentine 2009). The survival and development of parasitoids on larval hosts depend on the

timing of parasitism and infection (Abbas 2020). Arai et al. (2018) observed a discriminatory behaviour of *Meteorus gyrator* Walker (Hymenoptera: Braconidae) towards healthy infected larvae of *Lacanobia oleracea* L. (Lepidoptera: Noctuidae) over infected hosts in tomato plants. They found a lower parasitism rate in hosts infected by *Betabaculovirus lacoleraceae* (Lacanobia oleracea granulovirus) than in non-infected hosts. Parasitoids, however, have also been implicated in the transmission of baculoviruses (Mathews et al. 2004, Cossentine 2009). Arai et al. (2018) reported the transmission of *Heliothis virescens* ascovirus by the parasitoid *Meteorus pulchricornis* Wesmael (Hymenoptera: Braconidae) from infected larvae of *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). Furthermore, the incidence of LdMNPV in Gypsy moth in the United States was found to be positively correlated with the incidence of parasitoids (Dwyer & Elkinton 1995). However, the relationship between the tachinid fly and the baculovirus has yet to be established. Further research is needed to elucidate the interactions and dynamics between these organisms.

Based on the results, it appears that both the tachinid fly and baculovirus discovered in this study play significant roles in the outbreak dynamics of *S. partita*, either through regulating outbreaks or terminating them. Cyclic outbreak occurrences have been observed to be mediated by parasitoids, pathogens and host plants (Sasaki & Godfray 1999, Dickermann et al. 2002). The tachinid fly is a biocontrol agent for the larval stages of *S. partita*. However, further investigation is required to understand its life history, host specificity, and the feasibility of mass rearing of the host for in vivo mass production of the parasitoid and virus for inundative use against *S. partita*. Additionally, more research is needed to examine the natural enemy complex, including egg, larval, and pupal parasitoids, and other entomopathogens. These studies will contribute to a more comprehensive understanding of the ecological dynamics and potential management strategies for forecasting and controlling *S. partita* outbreaks.

CHAPTER 7

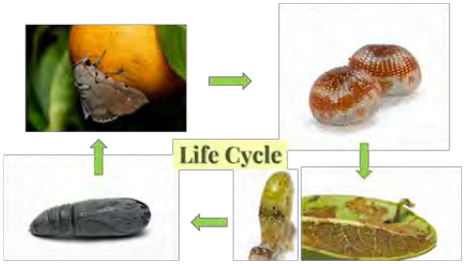
General Discussion

7.1 Thesis summary

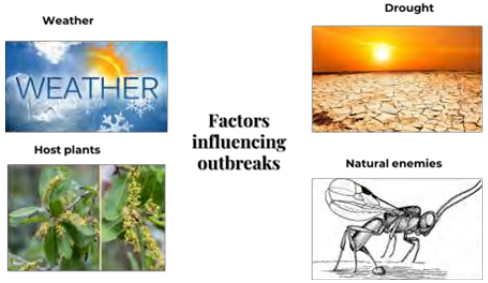
The fruit-piercing moth *S. partita* possesses the ability to puncture the rind of fruits, particularly soft citrus varieties, using its proboscis to extract juice. These puncture holes serve as vulnerable entry points for various saprophytic fungi, ultimately resulting in fruit rot and subsequent fruit drop. This moth exhibits an outbreak lifestyle within citrus orchards, often following larval outbreaks on natural hosts. Additionally, it displays adult migration behaviour as it seeks out new food sources. Despite its significant impact on fruit crops, effective management practices for this moth remain limited. Therefore, this study sought to comprehensively explore the biology and ecology of *S. partita*, with a particular focus on its population dynamics, flight patterns, and feeding behaviour. Furthermore, the occurrence pattern of this moth was examined to identify any shifts and ascertain the role played by weather patterns in its occurrence. Various management options, including the potential contribution of natural enemies, were also investigated. The findings of this study, together with recommendations for future studies, are presented in a graphical summary (Figure 7.1), offering a visual representation of the key insights into the biology, ecology, and management of *S. partita*. These aspects, including population dynamics, flight patterns, feeding behaviour, occurrence patterns, weather influences, and management options, will be discussed in detail throughout the subsequent sections of this discusssion.

Biology and management of *Serrodes partita in citrus orchards*

Adult pest of soft citrus
Outbreak occurrences



Damage



Management


Weather forecasting

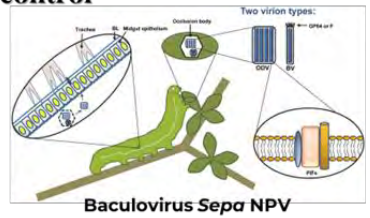

Larval host monitoring

Outbreaks Forecasting and Monitoring


Banana bait monitoring


Pheromone monitoring

Biological control

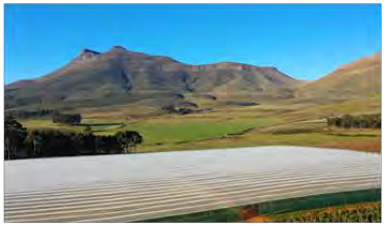



Bats in the orchard


Potential egg parasitoids

Management by Exclusion


Orchard illumination



Orchard netting

Cultural Control

Other management options

Lure and kill (food baits)
Larval host suppression
Orchard layout
Holding period after harvest
Pack shed monitoring

Figure 7.1: Graphical overview of the biology and management strategies for *Serrodes partita.* This illustration highlights the moth's life cycle, citrus damage, outbreak influencers, and various management options, including monitoring, biological, cultural, physical control, and other methods.

7.2 Monitoring pest populations: *Serrodes partita* pest population dynamics

Monitoring of outbreak pests poses a considerable challenge, given their unpredictable nature (Brown et al. 2022, Buchaillot et al. 2022). In East Africa, the monitoring and control of the African armyworm, involves various methods, such as light traps (Tucker 1984), pheromone traps, radar warnings, and rainstorms (Broza et al. 1991). Meanwhile, Tanzania adopts a community-based approach to prevent caterpillar outbreaks and their subsequent impacts (Mushobodzi et al. 2005). Addressing the need for comprehensive monitoring and timely management, the Food and Agriculture Organization (FAO) has recently launched a project aimed at establishing 2,400 monitoring sites across six east African countries (Banjaw 2023). Meanwhile, monitoring activities are conducted in the breeding sites of the Desert locust, *Schistocerca gregaria*, aimed at averting and effectively handling unforeseen outbreaks (Dinku et al. 2010). Favourable conditions, such as rainfall in desert regions, facilitates locust aggregation, leading to the formation of hopper bands that ultimately evolve into swarms (Van Huis et al. 2007). To monitor weather patterns and desert locust populations, FAO employs a remote sensing approach via the Desert Locust Information Services based in Rome (Cressman 2013).

Detecting outbreaks of *S. partita*, however, is even more challenging, due to larval development on natural hosts, often located in a considerable distance from citrus orchards. *Serrodes partita* also occupies a wide geographic range and exhibits unpredictable adult migration patterns, further complicating monitoring efforts. In addition, *S. partita* shares habitats with other fruit-feeding moths, especially fruit-sucking varieties that aren't primary

fruit feeders. This similarity leads to misidentification by citrus growers, who may mistakenly report sightings of fruit-sucking moths as *S. partita*. Pest outbreaks are typically the result of a complex interplay of multiple factors. In the case of *S. partita*, our study and other researchers' findings underscore the influence of various factors. These factors include drought conditions, weather patterns encompassing rainfall, temperature, humidity, the availability of larval and adult host plants, and the presence of natural enemies. Late rainfall after drought conditions promote the growth of larval host plants conducive to larval development. At the same time, rainfall, temperature, and relative humidity play a role in facilitating the growth of *S. partita*. This pattern has been reported in other fruit-piercing moth species, including *Eudocima phalonia* in New Caledonia and India (Leroy et al. 2021, Ramkumar et al. 2023).

7.2.1 Role of drought and larval host

This study recorded no extensive outbreaks, as the incidence of *S. partita* in citrus orchards remained low over the three-year study period. Similarly, other researchers have reported varying levels of *S. partita* activity during non-outbreak years, as reported by Taylor (1965a) and Goddard (2016). Myburgh (1963) reported that a low incidence of *S. partita* has minimal economic impact. Low incidences of *S. partita* have also been noted in the Pacific Islands and New Caledonia, where it has been reported to cause very little damage (Comstock 1963). In conjunction with these low incidence rates, our study showed that *S. partita* primarily feeds on already-damaged fruit, supporting the work by Goddard (2019), who showed that *S. partita* has mandibles that are not heavily sclerotized, unlike other fruit-piercing moths, hence, it has a strong bias towards damaged fruit.

Historical records show that extensive outbreaks of *S. partita*, however, cause severe economic damage, with reports in 1928 (Gunn 1929), 1951-52 (Kriegler 1958), 1966-67 (Whitehead & Rust 1972) and 2009 (Moore 2010). These outbreaks have been observed with both fruit-piercing and fruit-sucking moths and have a typical pattern of following drought periods, as highlighted by Webb (1953), Taylor (1965b) and Cochereau (1972). According to Webb

(1953) and Taylor (1965b), egg-laying is delayed until the first rainfall, to coincide with the flush of larval host plants. This subsequently enhances larval survival rates.

Moreover, the phenomenon of severe pest upsurges following drought has been well-documented in other pest species, such as the desert locust *S. gregaria*, (Ibrahim 2001), and in *L. migratoria* (Dempster 1963, Lecoq & Sukirno 1999). Several hypotheses have been put forward to explain this pattern. Cochereau (1974) suggested that drought conditions could lead to the demise of natural enemies, allowing the moth population to thrive unchecked post-drought. Drought negatively affects natural enemies and reduces virulence of insect pathogens due to reduced rainfall and humidity (Pimbert & Srivastava 1991, Bao et al. 2019). Pimbert & Srivastava (1991), reported that prolonged deficits of rainfall deficits resulted in *Helicoverpa armigera* Hübner population growth due to reduced population and efficacy and natural enemies. Drought therefore likely impacts the egg and larval parasitoids as well as reduces virulence of the identified SepaNPV. Meanwhile, White (1976) attributed it to the concentration of amino acids in drought-stressed plants. Additionally, Birch (1958) proposed that drought might increase the mineralisation of nitrogen in the soil, thereby boosting the nutritional value of leaves during the initial flush after the rains. Considering the findings of this study, particularly the role of larval host plants and the impact of natural enemies on *S. partita*, it is likely that all these factors collectively contribute to the modulation of outbreaks in this moth species.

7.2.1 Role of climate

The study revealed an interesting relationship between pest levels and weather factors, where temperature governs the timing of pest outbreaks, while rainfall influences the magnitude of these outbreaks. Rainfall exerts a dual impact on the occurrence of *S. partita*, directly by affecting the insect's biology and indirectly influencing its larval host plants. It's worth noting that the role of host plants in mediating the presence of other fruit-piercing moth species has also been well-documented in previous research (Cochereau 1977, Bänziger 1982, Leroy et al.

2021). Cochereau (1972) reported high mortality rates among neonate larvae, highlighting the role played by host plants in fruit-piecing moth outbreaks. The study further reported low larval populations when egg-laying occurred before the emergence of a new flush of larval host plants, *Erythrina* sp. (Fabaceae). During laboratory-rearing efforts in this study, high neonate mortality was recorded, further confirming the influence of host plants on the occurrence of *S. partita*. These findings align with earlier hypotheses regarding the impact of rainfall on the natural host of *S. partita*, *P. capensis* (Jack 1922, Bosch 1970, Whitehead & Rust 1972, Rust & Myburgh 1986, Johannsmeier 1998).

Seasonal monitoring showed an association between rainfall and the activity of *S. partita.* A trend emerged as the period from 2021 to 2023 saw an increase in rainfall corresponding to a decrease in moths. It became evident that an increase in rainfall had a negative effect on the occurrence of moths. Although manifesting in *S. partita*, this pattern is not unique to this species and has been documented in other moth species (Lecoq & Sukirno 1999). It is well-established that rainfall can impact the pupation success of Lepidoptera (Zheng et al. 2011). *Serrodes partita* undergoes pupation in the soil, enclosed within a silk cocoon made up of a mixture of soil and leaf litter (Taylor 1951, 1965a, Chapter 2). Rainfall events during the pupation stage can lead to the disintegration of the protective silk cocoon resulting in pupal mortality. Prasad et al. (2013) conducted an experiment involving simulated rainfall events on *S. litura* and reported a decline in adult moth emergence from pupal stages with increased rainfall. Meanwhile, Zheng et al. (2012) found that *S. exigua* larvae preferred pupating in soils with less than 25 % moisture content. Murray & Zalucki (1990) also reported low emergence of *Helicorvepa punctigera* Hübner (Lepidoptera: Noctuidae) in dry soils. While rainfall's influence on host plants is well-established, it is also highly likely that it impacts the pupation success of *S. partita*, as evidenced by the reduced adult activity observed in 2022 and 2023 compared to 2021, which experienced lower levels of rainfall. However, this conclusion is still premature, as historical reports link high rainfall with extensive outbreaks. the premise for this

is that high rainfall influences extreme flushing on larval hosts leading to extraordinary build-up of larval populations. Therefore, there is still a need for more monitoring efforts.

In addition to its potential impact on pupation success, rainfall may also have a bearing on larval survival. As Harvey & Mallya (1995) noted, rainfall can increase larval mortality in *S. exempta* by dislodging larvae from host plants and facilitating insect pathogen proliferation. The larval stages of fruit-piercing moths exhibit an inactive behaviour during the daytime, taking refuge beneath leaf litter, bark, and stones (Taylor 1951, Johannsmeier 1998). Cochereau (1974) attributed this behaviour to an adaptation to avoid desiccation, emphasising the pivotal role of humidity in their development. High humidity levels were associated with the occurrence of *S. partita*. However, it is noteworthy that both rainfall and humidity coupled with the aggregation behaviour, create conditions conducive to the spread of pathogens. This study unveiled the discovery of a novel baculovirus associated with *S. partita*. Before this research, Myburgh (1963b) and Swart et al. (1975) reported larval mortalities for which bacteria were considered potential causative agents. The findings from this study confirm virus infections, hence their speculations on the identity of the pathogen were probably incorrect. The virus is also likely to influence *S. partita* activity and could play a role in managing outbreaks.

7.2.3 Pest status of *Serrodes partita*

Concerns have been raised about a shift in the occurrence pattern of *S. partita*, transitioning from sporadic outbreaks to more permanent populations. Climate change has been identified as the primary driver for changes in pest population dynamics (Phophi et al. 2020, Skendžić et al. 2021, Subedi et al. 2023). Climate change is associated with rising temperatures, creating new ecological niches that enable insects to expand into previously unaffected regions (Pureswaran et al. 2018). The Diamondback moth, *Plutella xylostella* L., has been reported in Arctic and Norwegian islands, extending its range by 800 km from its previous recorded location in Western Russia (Coulson et al. 2002). In the United States, range expansion has

also been documented for the corn earworm, *H. zea*, and the Cotton bollworm, *H. armigera*, attributed to heightened winter survival rates, resulting from climate change (Diffenbaugh et al. 2008). Additionally, elevated temperatures alleviate thermal constraints on population dynamics, contributing to increased infestations (Lehmann et al. 2020). The ambient temperature hypothesis suggests that rising temperatures lead to expanded population sizes, subsequently influencing species composition in the dynamic equilibrium (Menéndez et al. 2007).

The monitoring of *S. partita* in the Committees Drift area, showed that the moth is present in the area every season, albeit at low densities and with low damage levels. The pest population dynamics therefore have not changed but the location of the orchards might have influenced change in pest status. The uniqueness of the area compared to other citrus-growing regions in the Eastern Cape Province, lies in the planting of citrus amid a high population of larval host plants, *P. capensis*. The area likely hosts a resident moth population, as suggested by Bosch (1970), exhibiting varying levels of occurrences that, in some years, may lead to damage in soft citrus. Cochereau (1974) and Fay & Halfpapp (2006) noted that orchards situated near breeding sites of fruit-piercing moths tend to experience the most damage. Cochereau (1972) suggests that the proximity of orchards to feeding sites facilitates the movement of moths between hosts. Therefore, it is recommended to implement host suppression measures whenever feasible to effectively address this issue, as highlighted by Cochereau (1972). The unique combination of adjacent larval and adult host plants in this area encourages the proliferation and persistence of *S. partita*. *Serrodes partita* larval hosts span a large geographic area, a citizen science approach of using landowners/farmers to report larval outbreaks could also be employed. The larval host is predominantly fed on by *S. partita*, any feeding could be attributed to the moth, hence correct identification.

7.3 Management of fruit-piercing moth *Serrodes partita*

7.3.1 Mass trapping – lure and kill

Despite its inherent limitations, the bucket funnel trap was the most successful of all traps tested. Mass trapping (Chapter 3) has proven effective when employing funnel traps with fresh banana as lures, which should be changed every 3 to 4 days. Both synthetic lures failed to attract *S. partita*. However, gelling agents such as agar and a superabsorbent polymer can be employed to extend the longevity of fresh banana as a lure. Field trials and mass-trapping initiatives have revealed that areas of the orchard adjacent to natural features like rivers or natural bushveld experience the highest moth activity. To optimise the effectiveness of these traps, their placement should be more concentrated on the leeward side or areas adjacent to the external landscape, in line with findings presented by Cochereau (1969), Whitehead & Rust (1972) and Fay & Halfpapp (2006). Furthermore, reports from Cochereau (1972) and Johannsmeier (1976) have emphasised the fruit-piercing moth's tendency to seek refuge in bushes and surrounding forests during the day, returning to the same orchard at night. Although this study noted a low rate of moth return, *S. partita* likely employs a similar strategy.

7.3.2 Cultural control – exploiting feeding habits

Another potential strategy for managing *S. partita* involves exploiting its feeding habits, especially when dealing with high moth populations. This study has revealed a distinct preference for *S. partita* to target previously damaged fruit. Citrus growers may want to implement the strategic retention of damaged fruit as sacrificial fruit within their orchards when faced with an infestation of *S. partita*. This approach has been suggested by other researchers as well, including Myburgh (1963b), Cochereau (1974), Fay & Halfpapp (2006) and Jayanathi et al. (2020). However, this method presents a challenge, as orchard sanitation practices typically mandate removing and disposing of all damaged fruit (Hattingh et al. 2020). Furthermore, leaving damaged fruit in the orchard has potential to increase incidence of fungal

diseases such as green and blue mould (Ismail & Zhang 2004). Citrus growers are recommended to remove and destroy any decaying, damaged, or infested fruit, whether still on the trees or lying on the orchard floor on a weekly basis. This practice is recommended for controlling fruit fly, FCM, and pathogens (Du Toit 1998, Hofmeyr 2003, Moore & Kirkman 2008).

The data from this study indicate that fruit-piercing moth activity is concentrated on the trees at the orchard's periphery (Chapter 3). To address this, a potential solution is to leave damaged fruit on two to three rows leading to the edge of the orchard as sacrificial fruit. Additionally, early-season cultivars like Satsuma Mandarins and Clementines are susceptible to bird damage from fruit-eating birds, such as Mouse Birds and Cape White-eyes (Moore & Manrakhan 2008). These bird-damaged fruit on the outer rows can also be intentionally left as sacrificial fruit for *S. partita*. Furthermore, Satsumas are prone to sunburn due to high temperatures and intense sunlight (Verreynne & van der Merwe 2011, Park et al. 2022). Sunburnt fruit is not marketable, and in cases where bird damage is absent, this sunburnt fruit can be intentionally cut and left as sacrificial fruit as well. This approach is cost-effective and can be practised in conjunction with mass trapping. Notably, it need not be applied throughout the season but specifically when dealing with high moth populations in conjunction with FCM, fruit fly and fungal disease incidence. This entails citrus pest control guidelines in soft citrus orchards, particularly in the Eastern Cape, where *S. partita* occurs.

7.3.3 Larval host suppression

A proactive strategy for managing fruit-piercing moths involves larval host suppression by completely removing host plants near orchards. This method is aimed at discouraging moths from settling outside orchards after feeding but instead encourages them to return to their breeding sites (Cochereau 1972, 1974). In addition, Bosch (1970) highlighted that residential moths pose more challenges than migrating moths, which visit orchards without making subsequent returns. Successful larval host suppression was carried out by eliminating the host

plants of *E. phalonia* in New Caledonia, reducing damage from 100 % in neighbouring areas to 42 % (Cochereau 1972).

Nevertheless, this approach has considerable challenges, particularly in the Kat River Valley and Committees Drift areas. The persistence of the fruit-piercing moth problem in these areas has been linked to the presence of hosts, *P. capensis,* near orchards. Removing all the larval host plants is nearly impossible since it is the dominant tree species. Furthermore, the associated costs and the presence of these trees in a Nature reserve adjacent to farms complicate the removal process. Additionally, *P. capensis* serves as a valuable source of fodder for livestock (Mng'omba et al. 2007), further complicating the idea of eliminating it. Considering these challenges, a more practical approach involves inundative releases of natural enemies, such as the tachinid fly or aerial baculovirus sprays during an outbreak or predicted outbreak years. Moreover, since *S. partita* is migratory and can travel long distances to orchards (Johannsmeier 1998), this approach can only reduce fruit-piercing moth activity within an area. However, complete eradication of the moth may remain elusive, hence more focus should be on adult control in orchards.

7.4 Recommendations for Future Studies

7.4.1 Continuous monitoring

Forecasting *S. partita* outbreaks presents a significant challenge, due to the species' expansive geographic range and the variability in outbreak sizes, among other factors. This study's findings make it difficult to draw firm conclusions regarding potential shifts in the population dynamics of *S. partita* within the Kat River Valley. The study area chosen for seasonal monitoring includes orchards close to larval host plants, aligning with the observation made by Fay & Halfpapp (2006) that orchards adjacent to breeding sites tend to experience higher levels of damage than those located further away.

Our understanding of *S. partita* remains limited, primarily because research efforts have been concentrated on outbreak years, with substantial gaps during non-outbreak years. Therefore, there is a compelling need for continuous monitoring of *S. partita* across all citrus-growing regions in the Eastern Cape, particularly in the Kat River Valley and the Gamtoos River Valley. Long-term monitoring data can provide insights into trends in *S. partita's* behaviour over time, enhancing our comprehension of the precise mechanisms underlying extensive outbreaks and facilitating the development of more accurate prediction models. Developing predictive models often requires several years' worth of data, as exemplified in the case of *S. exempta* (Tucker 1984, Harvey & Mallya 1995, Haggis 1996). Additionally, it can further validate some of the conclusions derived from this study.

Reports have indicated that extensive outbreaks of *S. partita* tend to follow a cyclical pattern, typically occurring every 5 to 10 years (Myburgh 1963a, Johannsmeier 1998, Robertson & Cochrane 2005). Continuous monitoring is essential to determine the persistence of these cycles and use them as a predictive tool for outbreaks. When moth populations are low, assessing the extent of damage caused by *S. partita* in citrus orchards becomes challenging. Hence, it is crucial to reassess the methodology for damage evaluations, incorporating techniques like night sampling with torches. Night sampling has previously been employed by other researchers (Whitehead & Rust 1972, Fay & Halfpapp 2006). Though its labour-intensive nature, it offers a more precise assessment. Additionally, it aids in attributing damage to the specific fruit-piercing moth species involved. Damage assessments will help to provide more practical guidance to citrus growers, regarding the timing of control measures. Establishing the economic injury level and economic thresholds for this moth is also imperative. This will help determine if extensive research towards control is warranted or if we can just live with it as suggested by Myburgh (1963b). Furthermore, the study also demonstrated that the moth has a strong bias towards Satsuma Mandarins. Citrus growers in outbreak areas can avoid further planting of susceptible cultivars.

Additionally, there is a critical need to expand monitoring efforts by including not only orchards but also the surrounding vegetation and other potential breeding sites. Leong & Kueh (2011) conducted monitoring of fruit-piercing moths in both citrus orchards and adjacent vegetation, revealing variations between the two environments. Adult trapping and larval monitoring techniques in breeding sites can serve as a valuable early warning system for potential outbreaks, aiding proactive pest management strategies.

7.4.2 Natural enemies

The role played by natural enemies, including predators, parasitoids, and pathogens, in the occurrence of *S. partita* remains shrouded in uncertainty. The current study potentially falls short in identifying all parasitoids, thus emphasizing the need for continuous investigation. Myburgh (1963b) noted two tachinid fly species associated with *S. partita*, and Taylor (1965b) mentioned observations by other researchers of parasitoids and predators linked to fruit-feeding moths in the Eastern Cape. In other regions, comprehensive studies have been conducted into the natural enemy complex of fruit-piercing moths (Cochereau 1974, Sands et al. 1993). These studies have revealed the presence of egg parasitoids, such as *Telenomus* sp., *Ooencyrtus* sp., and *Trichogramma* sp. (Cochereau 1977, Waterhouse & Norris 1987, Sands & Liebregts 2005). Furthermore, larval parasitoids, such as *Euplectrus platyhypenae* (Howard), *Echthromorpha agrestoria* (Swederus), *Lissopimpla pacifica* (Morley), and the tachinid fly *Winthemia caledoniae* (Mesnil), have been reported in New Caledonia (Cochereau 1977, Leroy et al. 2021).

These findings suggest the presence of both egg and larval parasitoids that can potentially impact *S. partita* and offer prospects for its management. Additionally, exploring the host range and initiating mass rearing of the tachinid fly is needed for release in *S. partita* breeding sites early in the season. Similar attempts have been made in Fiji, the Cook Islands, and Samoa, involving both larval and egg parasitoids, albeit with varying levels of success (Waterhouse & Norris 1987, Sands & Liebregts 2005). In summary, the precise role of natural enemies in the

dynamics of *S. partita* remains unclear. The need for further research into the host range and mass rearing of the tachinid fly recorded, presents a promising avenue for controlling this pest. Due to complications in establishing *S. partita* egg parasitoids, a trapping approach using yellow traps can be adopted. Trapping can be done in outbreak areas and potential egg parasitoids gut contents can then be sequenced to establish larval host as described by (Mutanen et al. 2020).

In addition to considering the influence of predators and parasitoids, it is imperative to investigate the role played by the identified baculovirus in governing *S. partita* outbreaks and its potential for control through bioassays and subsequent formulation. Baculoviruses have a track record of regulating insect outbreaks, as observed in the Gypsy moth *L. dispar* (Ruiu 2015, Ruiu et al. 2021). It is essential to assess the prevalence of the *S. partita* virus, identified as a nucleopolyhedrovirus (NPV) and named *Serrodes partita* nucelopolyhedrovirus (SepaNPV) (Tahnee Bennett, unpublished data), in field conditions and examine its interactions with other natural enemies. Baculoviruses are promising candidates for larval suppression, especially when targeting larval hosts near orchards. Baculoviruses can be formulated for aerial and ground applications, offering flexibility in control strategies (Wilson et al. 2020, Quiroga-Cubides et al. 2022). Drone technology can be employed to dispense baculoviruses, especially in areas where host plants occur close to citrus orchards. Drone technology, combined with GPS, is increasingly utilized for farm monitoring and pesticide application (Borikar et al. 2022, Hafeez et al. 2023). To enhance spraying precision, this system could integrate species identification technology to pinpoint host plants for targeted treatment. A successful application of this approach has been used in South Africa, where it was employed to monitor invasive macrophytes' coverage at Hartbeespoort Dam (Coetzee et al., 2022). Moreover, it holds promise for tracking larval host flushes, aiding in outbreak prediction.

Additionally, exploring the potential of other pathogens, such as entomopathogenic fungi, which might also impact *S. partita*, is crucial. Therefore, further bioprospecting, particularly for entomopathogens, is warranted. Notably, *S. partita* larvae tend to aggregate beneath their

host trees (Taylor 1951), making entomopathogens particularly suitable for control, due to their ability to exploit this behaviour for effective transmission. Bioprospecting for entomopathogens against other agricultural pests has been conducted in South Africa by several researchers (Malan et al. 2011, Coombes et al. 2017, Albertyn et al. 2021, Mushore et al. 2023).

7.4.3 Mass trapping

Mass trapping shows promise for managing fruit-piercing moths (Fay 2004). Nevertheless, continuous refinement of existing trap designs is essential. The current study highlights the effectiveness of the funnel trap for monitoring and managing *S. partita*, but also exposes its limitations. The research demonstrates that incorporating an electric zapper can enhance the trap's performance for improved moth capture. While the electric zapper addresses the shortcomings of the funnel trap, it introduces added complexity, including the need for a power supply, consequently increasing costs. Therefore, there is a pressing need for further trap refinement to balance effectiveness with cost efficiency.

The standard funnel trap and the electronic version rely on dichlorvos as a fumigant to eliminate captured moths. However, concerns have emerged regarding the toxicity of this pesticide, leading to its ban in some European countries (Okoroiwu & Iwara 2018). Notably, Europe and the UK, which represent significant markets for South African citrus, have been progressively reducing the number of approved pesticides for use in agriculture, including citrus (CGA 2022). Hence, exploring alternative pesticides or enhancing the trap's design is imperative to reduce reliance on chemical pesticides for moth control. Mass trapping can potentially manage *S. partita*, but more work is still needed to determine the optimum trap density. Fay (2002) recommended using 20 traps/ha; however, the efficacy is yet to be tested. Due to variations in population density, as observed in the monitoring in this study, various trap densities might also be required for different moth densities. Moreover, there is a need to determine the efficacy of mass trapping especially under extensive outbreaks.

7.4: Synthetic lure development

Both proprietary Australian lures tested (Fay & Halfpapp 2003) failed to attract *S. partita* effectively. Banana was the most attractive lure for *S. partita*, as demonstrated in various other studies (Reddy et al. 2007, Fullard et al. 2012, Goddard 2016). The identification of specific banana-derived odours, responsible for attracting *S. partita*, is a critical next step. This can be achieved through headspace analysis banana volatiles using Gas Chromatography-Mass Spectrometry (GC-MS), coupled with Electroantennographic Detection (EAD). The identified compounds in odours can then be formulated and developed into synthetic lures, tailored for *S. partita*. Furthermore, the identification of sex pheromones for *S. partita*, in conjunction with fruit volatiles, can enhance control strategies.

Ohmasa et al. (1991) identified two male pheromones, epoxides derived from long-chained unsaturated hydrocarbons in *Oraesia excavata* Butler as major and minor compounds: cis-9,10-epoxy-(Z)-6-heneicosene and cis-9,10-epoxy-(Z,Z)-3,6-heneicosadiene, with a ratio of 86:14. Meanwhile, Mallikarjun et al. (2019) isolated sex pheromone components of *E. materna,* and combinined them with the pomegranate fruit volatile, 2-ethyl hexanal, and managed to attract the moth. Alternatively, differential olfactory assays on larval host plants can be conducted to determine *S. partita* electrophysiological responses, as highlighted by Mallikarjun & Thippaiah (2019). Mas et al. (2023) focused on host shift in *E. phalonia* from vines of the family Menspermaceae to *Erythrina* spp., identifying similar volatile organic compounds that elicited electroantennographic responses in *E. phalonia*. They concluded that these compounds hold the potential for developing generic lures (kairomones) for managing fruit-piercing moths. This approach could also potentially be adapted for *S. partita*.

7.4.5 Biology and laboratory rearing of *Serrodes partita*

Establishing a laboratory-rearing system for *S. partita* is imperative to facilitate further research especially considering that field collected populations cannot be relied on. In this study, *S. partita* could not thrive on artificial and modified artificial diets. However, successful rearing

was achieved when the moths were placed on their natural host plant. This aligns with the common practice of rearing fruit-piercing moths on their respective natural hosts, as reported in prior studies (Muniappan et al. 1994, Reddy et al. 2005, Bhumannavar & Viraktamath 2012, Roland et al. 2012). High mortality in early instars has been documented, and is often often linked to the larval host plant (Cochereau 1977, Zalucki et al. 2002). Therefore, exploring methods to mitigate neonate mortality, such as utilizing fresh foliage to reduce mortality rates or developing a cost-effective strategy to extract quebrachitol (a feeding stimulant) (Hewitt et al. 1969), is crucial.

Another critical aspect of *S. partita*'s biology, deserving further investigation is the determination of its thermal limits and tolerance. Sands et al. (1991) evaluated the impact of temperature on fruit-piercing moth biology by examining degree days at each life stage. Establishing the thermal limits for *S. partita* will enhance our comprehension of the temperature-related factors influencing its biology and contribute to more accurate outbreak forecasts. While this study established the effect of cumulative rainfall over four months, it is also essential to investigate the impact of rainfall events, as highlighted by Lecoq & Sukirno (1999). This additional research will expand our knowledge of the influence of rainfall events on *S. partita*'s biology.

7.5 Conclusion

In conclusion, the intricate challenge of forecasting and managing *S. partita* outbreaks demands continuous monitoring, extensive data collection, and the development of predictive models, all while considering the cyclic nature of these events. These measures can enhance our capacity to predict, mitigate, and manage the impact of *S. partita* outbreaks in citrus orchards. Weather parameters, particularly rainfall, temperature and humidity, are significant in governing the timing and magnitude of *S. partita* outbreaks. These factors are vital for effectively forecasting outbreaks.

Management of *S. partita* is a multifaceted endeavour that holds promise for more effective pest control strategies. The efficacy of mass trapping in fruit-piercing moth management is evident, yet it necessitates a continuous process of trap design improvement. When strategically deployed, the funnel traps offer potential solutions for managing *S. partita* in citrus orchards. The research highlights the potential of the funnel trap and its limitations, with the incorporation of an electric zapper offering a promising solution. Striking a balance between performance and cost-effectiveness remains a critical objective. Moreover, addressing concerns over pesticide toxicity and adapting to changing regulations in key markets is crucial for the sustainable and safe management of *S. partita* populations in citrus orchards. In addition, adopting a targeted sacrificial fruit strategy aligned with the moth's feeding habits offers an effective and practical means of managing *S. partita* in orchards.

The challenges in attracting *S. partita* using proprietary Australian lures emphasise the need to identify and utilize specific banana-derived odours responsible for moth attraction. Advanced techniques like GC-MS coupled with EAD can facilitate this process. Additionally, the incorporation of sex pheromones and the use of host plant volatiles offer promising avenues for improved control strategies. The successful precedent set by previous studies further highlights the potential for innovative approaches in managing *S. partita* populations in citrus orchards.

Managing fruit-piercing moths presents complex challenges, especially in areas where larval host occur near orchards and cannot be removed. Adopting alternative strategies, such as releasing natural enemies or using baculovirus sprays, appears to be a more pragmatic approach, recognizing that complete eradication may not be achievable. It is crucial to assess the suitability of these alternative methods while considering the specific circumstances of each affected citrus-growing region. This can be achieved by understanding and harnessing the role of parasitoids and pathogens, including the identified baculovirus. The continual investigation, broadening our knowledge base, and exploring potential solutions are essential in the ongoing quest to mitigate the impact of *S. partita* outbreaks in citrus orchards.

CHAPTER 8

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