The development of a management strategy for the control of the Cape grapevine leafminer, *Holocacista capensis* (Lepidoptera: Heliozelidae), in South African table grape vineyards

by
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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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SUMMARY

The Cape grapevine leafminer, *Holocacista capensis* Van Nieukerken & Geertsema (Lepidoptera: Heliozelidae) has become a common pest on table grapes and wine grapes in the Western Cape province of South Africa, since it was first reported in 2012. The presence of cocoon casings on grape bunches intended for export makes them a pest of economic importance, although its recognised pest status does not reflect the severity of some of the infestations that have occurred in the Berg River region. To date, control strategies have consisted of insecticide applications or manual, labour intensive post-harvest removal of rooted cocoon casings from table grape bunches during the packing process. To aid in the development of an integrated pest management (IPM) strategy, this study focused on understanding aspects of cultural, chemical and biological control strategies, whilst considering genetic diversity and environmental variables that influence *H. capensis* populations. In agreement with other studies conducted on problematic leafminers, field trials indicated that ambient light intensity, climatic conditions and plant nutrient composition affected *H. capensis* populations in commercial vineyards. Correlations derived from the evaluation of temporal satellite imagery to determine the normalized difference vegetation index (NDVI), indicated the potential for the use of this technology for monitoring leafminer invasions in the future. A preliminary study on the genetics of the pest involved the extraction of DNA from 52 male moths collected from commercial vineyards and natural forests (using baited Delta traps) in and around the Western Cape. The study was able to confirm species identity and synonymy of the insects collected from field-placed traps. An insecticide screening trial, conducted in the laboratory using varying doses of a variety of commercially available insecticides, identified spinetoram (spinosyn), dichlorvos (organophosphate) and cypermethrin (pyrethoid) as good candidates for inclusion in an IPM strategy. High mortality (> 87%) was recorded at the lowest doses (a quarter of the recommended field dose). Entomopathogenic nematodes (EPNs) were screened in the laboratory as an alternative to a management strategy focused solely on the use of chemical applications. Using a 200 infective juvenile (IJ)/50 µl of distilled water solution, EPNs were able to penetrate leaf galleries (mines) and cause larval mortality. Three EPNs, *Heterorhabditis baujardi* Phan, Subbotin, Nyugen & Moens, *Heterorhabditis indica* Poinar, Karunakar & David and *Heterorhabditis noenieputensis* Malan, Knoetze & Tiedt, were able to cause > 86% mortality of leaf-mining larvae and have the potential to be adopted in an IPM strategy against *H. capensis*. The use of bunch covers as a physical control strategy was tested in the field, for cases where leafminer infestations are unavoidable and maximum residue limits (MRLs) have been reached, to preclude insecticide treatments. All covers tested proved to successfully reduce the presence of rooted cocoon casings on bunches. This study has provided a positive forecast for the success of future chemical and biological applications and has provided the groundwork for the development of an IPM strategy against *H. capensis* on grapevines.

OPSOMMING

Die Kaapse wingerdbladmyner, *Holocacista capensis* Van Nieukerken & Geertsema (Lepidoptera: Heliozelidae), het 'n algemene plaag op tafel- en wyndruiwe in die Wes-Kaap provinsie van Suid-Afrika geword, sedert dit in 2012 gerapporteer is. Die teenwoordigheid van kokonne op druiwetrosse bestem vir uitvoer maak dit 'n plaag van ekonomiese belang, hoewel die erkende plaagstatus nie die erns van sommige van die besmettings, wat in die Bergrivierstreek voorkom, weerspieël nie. Tans bestaan die beheerstrategieë uit insekdoder toedienings of arbeidsintensiewe na-oes verwydering van gehegte kokonne op tafeldruiwe met die hand, tydens verpakking. Om te help met die ontwikkeling van 'n geïntegreerde plaagbestuurstrategi (IPM), het hierdie studie gefokus op aspekte van kulturele, chemiese en biologiese beheerstrategieë, terwyl die genetiese diversiteit en omgewingsveranderlikes wat *H. capensis* populasies beïnvloed, oorweeg is. Veldproewe het gevind het dat omringende ligintensiteit, klimaatstoestande en plantvoedingstof samestelling die bevolkings van *H. capensis* in kommersiële wingerde beïnvloed, wat ooreenstem met vorige studies wat op ander problematiese bladmyners uitgevoer is. Korrelasies wat afgelei is van die evaluering van temporale satellietbeelde om die genormaliseerde verskil-plantegroei-indeks (NDVI) te bepaal, het aangedui dat daar potensiaal is vir die gebruik van hierdie tegnologie vir die monitering van bladmyner voorkoms in die toekoms. In 'n voorlopige studie oor die genetika van die plaag, is DNA onttrek van vanuit 52 mannetjie-motte, wat versamel is met behulp van Delta-lokvalle in kommersiële wingerde en natuurlike woude in en om die Wes-Kaap. Die studie kon die identiteit van die spesies bevestig, asook dat die mannetjies wat gevang is en dat almal wel *H. capensis* is. 'n Insekdoder-proef, wat in die laboratorium uitgevoer is, het verskeie dosisse van verskillende kommersieel beskikbare insekdoders getoets. Spinetoram (spinosyn), dichlorvos (organofosfaat) en sipermetrien (piretoid) is geïdentifiseer as goeie kandidate om ingesluit te word by 'n IPM-strategie. Hoë mortaliteit (> 87%) is aangeteken teen die laagste dosisse ('n kwart van die aanbevole veld dosis). As 'n alternatief vir ’n bestuurstrategie wat uitsluitlik op die gebruik van chemiese middels gefokus is, is entomopatogeniese nematodes (EPNs) in die laboratorium getoets. Teen 'n konsentrasie van 200 infektiewe larwes (IJs)/50 µl gedistilleerde water, was EPNs in staat die blaargalerye (myne) binne te dring en larvale mortaliteit te veroorsaak. Drie EPNs, *Heterorhabditis baujardi* Phan, Subbotin, Nyugen & Moens, *Heterorhabditis indica* Poinar, Karunakar & David en *Heterorhabditis noenieputensis* Malan, Knoetze & Tiedt het > 86% mortaliteit by bladmynerlarwes veroorsaak en het dus die potensiaal om deel te word in 'n IPM-strategie teen *H. capensis*. Die gebruik van trosbedekkings as 'n fisiese beheerstrategie is in die veld getoets. Al die trosbedekkings was suksesvol om die kokonne op trosse te verminder en is dus ’n goeie opsie in gevalle waar besmettings onvermydelik is en maksimum toelaatbare residuperke (MRL's) klaar bereik is. Hierdie studie dui op 'n positiewe vooruitsig vir die sukses van toekomstige chemiese en biologiese beheermaatreëls, sowel as ’n basis vir die ontwikkeling van 'n IPM-strategie teen *H. capensis*.

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Chapter 1

General Introduction

Leaf-mining insects and control options for their management, with special reference to *Holocacista capensis* (Lepidoptera: Heliozelidae) in South Africa's table grape vineyards

Introduction

Within the southern hemisphere, Chile (56% - market share), South Africa (21%) and Peru (21%) are the largest contributors to the table grape industry (SATI Statistics Booklet 2018). Commercially produced table grapes and dried grapes account for approximately 32% of the 79 912 hectares of land planted to deciduous fruit trees in South Africa, followed by apples (30%) and pears (15%) (Key Deciduous Fruit Statistics 2017). As a result, the table grape and dried grape industry supports the highest on-farm employment rates (49 500 seasonal and 9 750 permanent employees), when compared to all other deciduous fruit industries (Key Deciduous Fruit Statistics 2017). In South Africa, grapevines are host to more than 35 insect pests (the key pest orders include Hemiptera, Coleoptera and Lepidoptera) (Allsopp *et al*. 2015), which pose a considerable threat to the industry.

The Western Cape is home to three of the five primary table grape producing regions in South Africa, namely the Olifants River, Berg River and Hex River regions (SATI Statistics Booklet 2018). Together they account for 58% of the table grapes produced nationally (5%, 21% and 32% respectively) (Fig. 1.1).

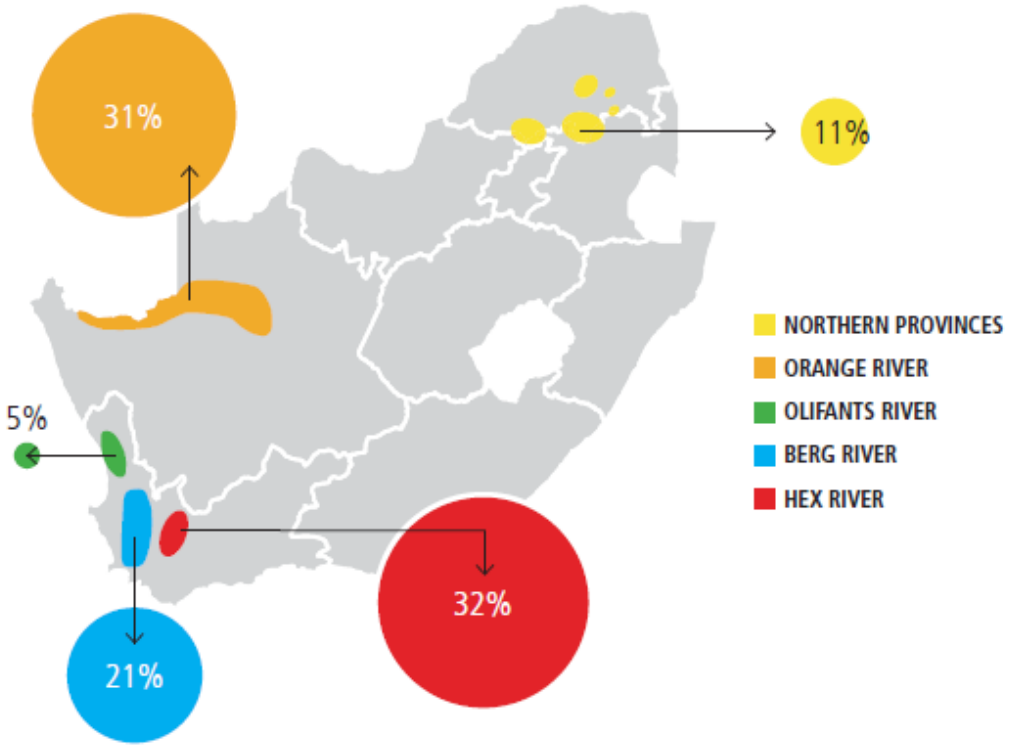

Figure 1.1: A representation of the percentage of table grapes produced (total intake) within each of the table grape producing regions of South Africa in the 2017/2018 growing season. Taken from SATI Statistics Booklet (2018).

In 2012, an unknown leaf-mining heliozelid was reported infesting a table grape vineyard in the Western Cape province, South Africa. At the time, the known heliozelid fauna from Africa was limited to three species described from South Africa (Van Nieukerken & Geertsema 2015). Subsequent field visits indicated high larval/leaf mine abundances and cocoon casings were detected on foliage, stems, trellises and grape bunches in vineyards. The presence of cocoon casings on bunches make them surface contaminants of significant quarantine importance, especially on grapes destined for export. In 2015, the leafminer was described by Van Nieukerken & Geertsema (2015) as *Holocacista capensis* Van Nieukerken & Geertsema (Lepidoptera: Heliozelidae). Since the discovery of *H. capensis* in 2012, a concomitant study by Wang *et al*. (2015) using gas chromatography-mass spectrometry has identified the sex pheromone (more accurately, an attractant) of *H. capensis*. In 2016, Torrance conducted baseline studies to better understand the bio-ecology of *H. capensis*, in the Western Cape.

The sustainable, effective control of the Cape grapevine leafminer is pertinent for the vine-growing industry in the Western Cape to avoid the development of resistance against commonly used insecticides. This review consolidates the available literature regarding the leaf-mining habit,

lepidopteran miners as pests, and the effect of the environment on their infestation levels. Management options for leafminers with regard to chemical control, the use of entomopathogenic nematodes (EPNs), parasitoids and other control measures were considered in the light of possible future control options for *H. capensis* on grapevines in South Africa.

Leaf-mining insects

Globally, little is known of leaf-mining insects (Vári 1961; Auerbach *et al*. 1995; Lees *et al*. 2014). Leaf-mining insects are a taxonomically diverse group of endophagous insects and the larvae of leaf-mining taxa are, in most cases, concealed within the plant tissue of their hosts during larval development or, at least, part thereof (Hering 1951; Kirichenko *et al*. 2018). The duration of the leaf-mining stage varies between species and is not always only associated with the developing larval instars, but can also cover the development of pupae and the emergence of adult insects (Connor & Taverner 1997).

Despite the fact that the leaf-mining habit is ancient, it continues to be lost and acquired by a number of phytophagous insect lineages (Connor & Taverner 1997) and has evolved independently numerous times (Auerbach *et al*. 1995). The leaf-mining habit is known to occur in at least 57 families within four insect orders, accounting for more than 10 000 leaf-mining species (Connor & Taverner 1997). The mines originating from the respective orders are classified into specific groups, namely lepidopteronome (Lepidoptera), dipteronome (Diptera), coleopteronome (Coleoptera) and hymenopteronome (Hymenoptera).

The geographical distribution of endophagous insects, like leafminers, is inevitably dependent on the distribution of their larval host plants. In most cases, however, the distribution of a leafminer is less extensive than that of its host plant (Hering 1951). Amongst the herbivorous insects, many leafminers pose a threat to a variety of forest and urban plant species, whilst other are regarded as important pests of agricultural crops and are considered an economically important group globally (Spencer 1973; Nielsen & Common 1991; Digweed *et al*. 2009).

Over the last decade, an increase in incidents of leaf-mining insect records has attracted the attention of plant-related industries, due to their presence in commercial forests, agricultural landscapes and on ornamental plant varieties of high value (Van Nieukerken & Geertsema 2015; Kirichenko *et al*. 2018).

The leaf-mining habit

In the past, the concealed feeding environment of endophagous insects was speculated to provide a competitive advantage when compared to their exophagous counterparts (Hering 1951; Nielson & Common 1991). The concealed feeding strategy was thought to protect feeding larvae from natural enemies (Hering 1951; Price *et al*. 1987). It also provides a buffer against the physical environment (Connor & Taverner 1997), and enables the feeding larvae to avoid plant defences (Feeny 1970) and thus facilitates selective consumption of more nutritious leaf tissue (Cornell 1989). Price *et al*. (1987) and Connor & Taverner (1997) reviewed some of these hypotheses amongst various endophagous feeders and arrived at similar conclusions. Connor & Taverner (1997) suggested that the selective advantages inherent to the leaf-mining habit are to facilitate: 1) increased feeding efficiencies, which supports some hypotheses and findings of Cornell (1989); 2) the avoidance of negative effects associated with disease, should it be present within a population or species, by internally feeding larvae; 3) the protection of larvae from the direct and indirect effects of photochemical changes in plant chemistry, for example due to UV radiation, and 4) the reduction of water loss and lessening the risk of desiccation by the presence of a buffered micro-environment within the feeding gallery.

Connor & Taverner (1997) also highlighted the disadvantages of the leaf-mining habit. These include: 1) the loss of mobility and thus larvae are unable to escape parasitoids and predators, supported by statements made by Nielsen & Common (1991); 2) decreased species richness within leaf-mining lineages, when compared to that of exophagous insects; 3) mortality associated with plant senescence, herbivory and premature abscission of leaves, and 4) reduced fecundity, due to small size of individuals.

From an evolutionary perspective, the disadvantages of the leaf-mining habit outweigh the advantages. The persistence of leaf-mining guilds in various insect orders and environmental niches in the present day, however, proves that for some taxa, the leaf-mining habit is a feasible means of survival under certain circumstances (Connor & Taverner 1997).

Lepidopteran leaf mines

Apodal lepidopteran leaf-mining larvae (or "serpentine larvae") consume mesophyll between the upper and lower epidermal layers of a leaf (Stehr 1992; Bernardo *et al*. 2015), creating small blotch mines or galleries within the parenchymal tissues of host plants (Hering 1951). These feeding channels, or cavities, both serve as living and feeding quarters for leaf-mining larvae (Hering 1951).

The shape of a leaf mine and the presence of voluminous frass often depicts a unique feeding pattern within an infested leaf, which can be used as a diagnostic tool for species-specific identification (Hering 1951; Kirichenko *et al*. 2018). Mines produced by any leaf-mining insect can be used to determine the order, family and in many cases, the particular genus (Hering 1951; Vári 1961). Lepidopteran hyponomology often provides a clear and more accurate indication of species identity than comparing fine differences in larval and adult morphology.

Lepidopteran leaf-mining pests

Lepidoptera account for the majority of leaf-mining insects (Kirichenko *et al*. 2018). As a result of this, and due to the destructive qualities of the larval life stages of some of the leaf-mining species, the Lepidoptera are considered to be of great economic importance (Nielsen & Common 1991). At least 40 lepidopteran families exhibit leaf-mining habits, which can vary considerably between species. These lepidopteran leafminers account for approximately 70% of all known insect families associated with leaf-mining activities (Connor & Taverner 1997; Kirichenko *et al*. 2018). Within the Lepidoptera the three families of economic importance, due to their leaf-mining habits, include the Gelechiidae, major pests in the forestry and agricultural industries (Lee *et al*. 2009); the Gracillariidae, notorious as invasive leaf-mining pests of woody plants (Kirichenko *et al*. 2018); and the Heliozelidae, predominantly pests on trees and vines (Davis 1998). A list of lepidopteran leaf-mining agricultural pests is presented in Table 1.1.

Table 1.1: A (non-exhaustive) summary of agriculturally important leaf-mining lepidopteran pests.

Family	Leaf-mining species	Common name	Commercial host	Native range	Region of invasion	Source
Bedelliidae	*Bedellia somnulentella* (Zeller)	Sweet potato leafminer	Sweet potato	Eurasia	Cosmopolitan	Visser (2015a); Santos *et al*. (2018)
Heliozelidae	*Holocacista capensis* Van Nieukerken & Geertsema	Cape grapevine leafminer	*Vitis vinifera*	Southern Africa	Southern Africa	Van Nieukerken & Geertsema (2015)
	Holocacista rivillei Stainton	European grapevine leafminer	*V. vinifera*	Europe	Southern Europe, Western Asia	Van Nieukerken *et al*. (2012)
	Antispila oinophylla Van Nieukerken & Wagner	Grapevine leafminer	*V. vinifera*	Eastern North America	Northern Italy	Van Nieukerken *et al*. 2012)
	Antispila uenoi Kuroko	Grapevine leafminer	*V. vinifera*	Japan	Japan	Van Nieukerken *et al*. 2012)
	Antispila nysaefoliella Clemens	Tupelo leafminer	Black gum	Southeastern United States	United States	Low (2012)
	Coptodisca splendoriferella Clemens	Resplendent shield borer	Apples, cranberries	Unknown	North America	Boush & Anderson (1967)
Incurvariidae	*Protaephagus capensis* Scoble	Blotch leafminer	Protea, *Leucadendron* sp.	Southwestern Cape (South Africa)	Southern Africa	Wright (2015)
Gelechiidae	*Aproaerema modicella* (Deventer)	Groundnut leafminer	Ground nut, soybean	South, South-East Asia	South, South-East Asia	Shanower *et al*. (1993)
	Aproaerma simplexella (Walker)	Groundnut leafminer	Groundnut, soybean, possibly lucerne	Africa or Australia	Africa, Australia	Buthelezi *et al*. (2012)
	Bilobata subsecivella (Zeller)	Groundnut leafminer	Ground nut, soybean, lucerne	South-East Asia	Africa	Du Plessis (2015)

Table 1.1: continued.

Family	Leaf-mining species	Common name	Commercial host	Native range	Region of invasion	Source
Gelechiidae	*Tuta absoluta* (Meyrick)	South American tomato pinworm	Tomato, potato	Western neotropics	South America, Afro-Eurasia	Siqueira *et al*. (2001); Biondi *et al*. (2018)
	Phthorimaea operculella (Zeller)	Potato tuber moth	Potato, tomato, gooseberry, brinjal, chilli, tobacco	South America	All tropical, subtropical potato-growing regions	Kroschel & Zegarra (2013); Visser (2015b)
	Symmetrischema tangolias (Gyen)	Andean potato tuber moth	Potato and tomato	South America	South America, New Zealand, Australia, United States	Kroschel & Zegarra (2013); Sporleder *et al*. (2017)
Gracillariidae	*Phyllocnistis vitegenella* Clemens	American grape leafminer	*Vitis vinifera*	North America	Europe	Ureche (2016)
	Phyllocnistis citrella Stainton	Citrus leafminer	Citrus	South-east Asia	Worldwide (all citrus producing areas)	Kirichenko *et al*. (2018)
	Acrocercops bifasciata Walsingham	Cotton leafminer	Cotton and okra	Unknown	Southern Africa	Bennett (2015)
	Acrocercops gossypii Vári	Cotton leafminer	Cotton	Unknown	Southern Africa	Bennett (2015)
	Spulerina sp.	Mango twig miner	Mango	Unknown	Southern Africa	Grové *et al*. (2015)
	Phyllocnistis sp.	Thin line leafminer	Protea	Unknown	Southern Africa	Wright (2015)
Lyonetiidae	*Leucoptera caffeina* Washburn and *Leucoptera meyricki* Ghesquière	Coffee leafminer	Coffee	Central, East, Southern Africa	Africa	Fragoso *et al*. (2002); Schoeman (2015)
	Leucoptera coffeella (Guérin-Méneville & Perrottet)	Coffee leafminer	Coffee	Africa	Neotropics, Mexico	Fragoso *et al*. (2002); Lomelí-Flores (2010)

Heliozelidae (Lepidoptera: Adeloidea) - the "shield bearers"

The Heliozelidae is a group of widely distributed, cosmopolitan, minute, diurnal micro-lepidoptera (Davis 1998; Powell 2003, Van Nieukerken *et al*. 2011; Regier *et al*. 2015; Milla *et al*. 2018), present in all major faunal realms, with no representatives in New Zealand and Antarctica. One hundred and twenty five described species comprise the Heliozelidae, placed in 12 genera (Van Nieukerken *et al*. 2011; 2012; Van Nieukerken & Geertsema 2015). The family is taxonomically poorly studied, although, taxonomic revisions associated with heliozelids have been conducted by Van Nieukerken *et al*. (2011), Van Nieukerken & Geertsema (2015), Regier *et al*. (2015) and Milla *et al*. (2018) (Fig. 1.2) in recent years.

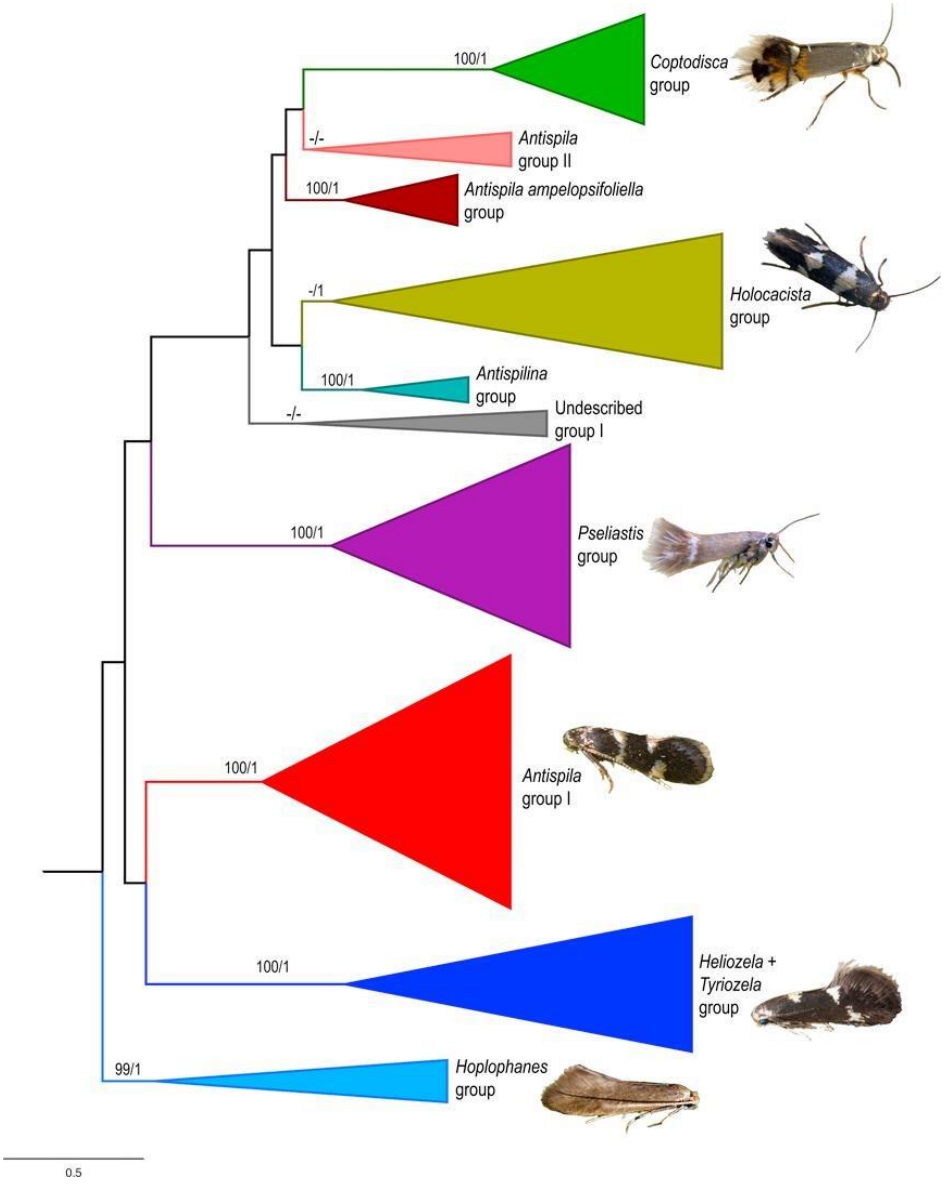

Figure 1.2: The Maximum Likelihood tree compiled and adapted by Milla *et al*. (2018) that represents the major genera within the Heliozelidae family.

Heliozelid moths are typically small, with their forewings ranging between 1.7 to 7.0 mm in length (Regier *et al*. 2015). Due to their small size, most heliozelids are rarely seen or collected, even when population abundances are high (Powell 2003; Regier *et al*. 2015). Most adult moths within the Heliozelidae possess fundamentally dark wing colouration with iridescent scaling (Scoble 1992; Powell 2003).

Larval instars are obligate leafminers, with the exception of the final instar (Stehr 1992; Regier *et al*. 2015). A flat, lenticular case is constructed by this last instar from the epidermal layers of a mined leaf, lined and bound with silk to form a firm, cocoon-type covering (Holloway *et al*. 1987; Stehr 1992; Regier *et al*. 2015). The vernacular name "shield bearers" refers to the oval, lenticular shape of the crafted casing (Scoble 1992; Davis 1998). The casing is either suspended, by means of a silken thread, carried or dragged from the infested leaf by the encased larvae (Scoble 1992; Regier *et al*. 2015). The larvae will anchor themselves by means of weaving a silken mat to objects that they come into contact with.

Detailed accounts of the morphology of all the life stages of the Heliozelidae are documented by Bourgogne (1951), Hering (1951), Holloway *et al*. (1987), Scoble (1992), Davis (1998), Powell (2003) and Patočka & Turčáni (2005). Keys in Mey (2011) and Patočka & Turčáni (2005) enable the identification of some genera and species within the Heliozelidae.

Almost all individual heliozelid species are hostplant-specific, confined to genus level or, at least, at the plant family level (Regier *et al*. 2015), which may lead to gregarious behaviour, depending on local plant assemblages. Within the agricultural context, a number of heliozelids are considered to be of economic importance (Table 1.1). Over the last three decades, three heliozelids have been unexpectedly encountered on commercial grapevines. These include *Antispila oinophylla* Van Nieukerken & Wagner (reported in Northern Italy with North American origins), *Antispila uenoi* Kuroko (a pest native to Japan, recently reported on commercial vineyards) and *H. capensis* (a pest thought to be a native species, presently reported on commercial vineyards in South Africa) (Van Nieukerken & Geertsema 2015).

Holocacista capensis

Holocacista capensis, the Cape grapevine leafminer or "bladmyner/wingerdblaarmyner" in Afrikaans - as it is locally known amongst growers - is a multivoltine pest present throughout a grapevine growing season (Van Nieukerken & Geertsema 2015; Torrance 2016).

Morphology and known biology

The adults are small, diurnal moths with a wingspan of ca. 3.9 – 4.9 mm (Van Nieukerken & Geertsema 2015). The black/dark coloured wings are characterised by white spots (a conglomeration of silvery fasciae). The head and face are covered by silvery-white (metallic), appressed scales. Male and female moths can be differentiated based on the colour of the posterior abdominal segments (lead-coloured in males, jet black in females) and the markings on their forewings (in females the first costal and dorsal spots are joined to form a contiguous band) (Fig. 1.3). The adults of *H. capensis* closely resemble *Holocacista salutans* (Meyrick) and *Holocacista varii* (Meyrick). Eggs are laid singly in leaves by females after mating (Van Nieukerken & Geertsema 2015).

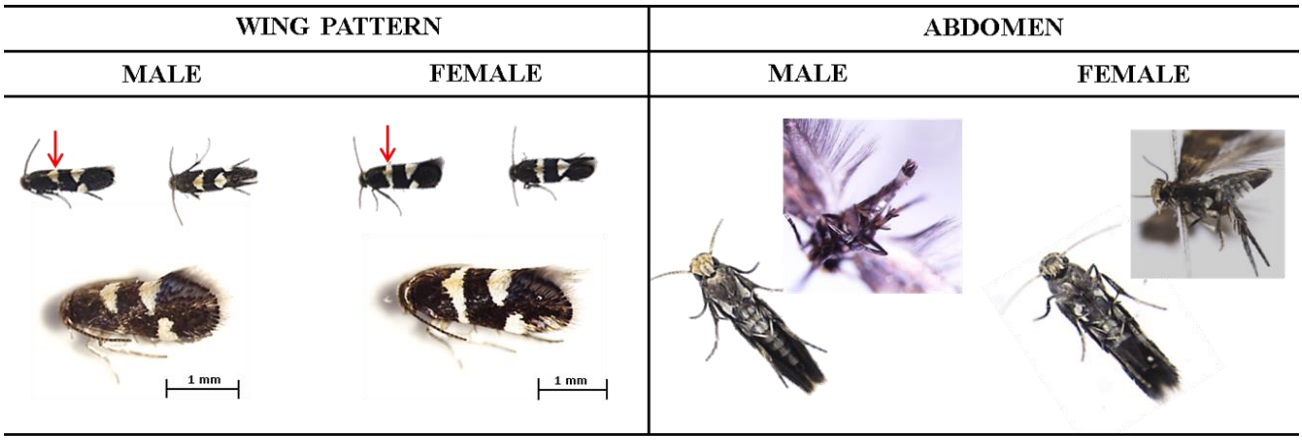

Figure 1.3: The difference in wing (indicated by red arrows) and abdominal patterns between male and female *Holocacista capensis* adults. Adapted from Torrance (2016).

The larvae develop through four feeding instars (Van Nieukerken & Geertsema 2015). These larvae are unable to move to other leaves, should the natal leaf or mine be drastically disturbed or destroyed (Torrance 2016). The heads of feeding larvae are usually characterised by dark, prognathous head capsules. Their bodies are yellow or whitish (Van Nieukerken & Geertsema 2015) (Fig. 1.4). The larvae feed on leaves only (Torrance 2016) and completed mines reach 12 – 15 mm in length (Van Nieukerken & Geertsema 2015). The fifth, final instar, is non-feeding and constructs the cocoon casing in which it will pupate.

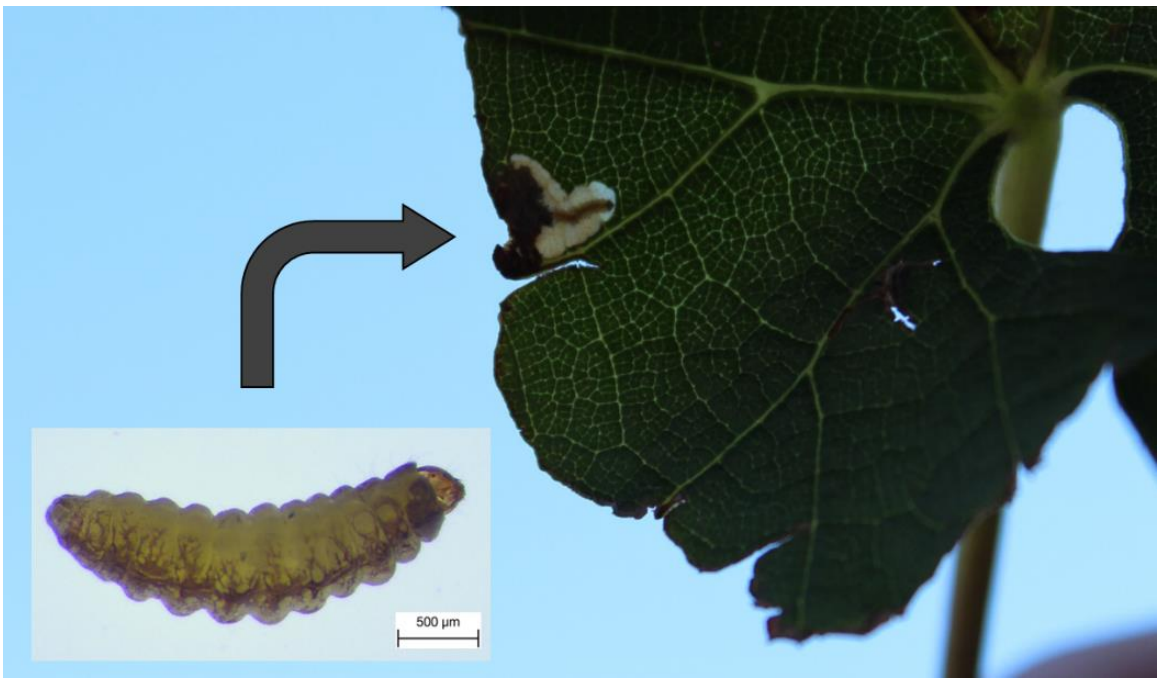

Figure 1.4: *Holocacista capensis* larva feeding within a grapevine leaf and a corresponding image of a larva extracted from a leaf mine viewed under a microscope.

Damage symptoms

Holocacista capensis larvae mine the leaves and thus cause physical damage to infested leaves (Fig. 1.5). The effect of the mines, which are predominantly found along the leaf margin, on the photosynthetic ability of a grapevine is not yet known, although it appears to be limited (Nieukerken & Geertsema 2015). High infestations are recorded later in a growing season, usually after harvest, or when leafminer populations have been left unmanaged [Fig. 1.5 (3)]. In these instances the photosynthetic ability of a plant may, potentially be affected.

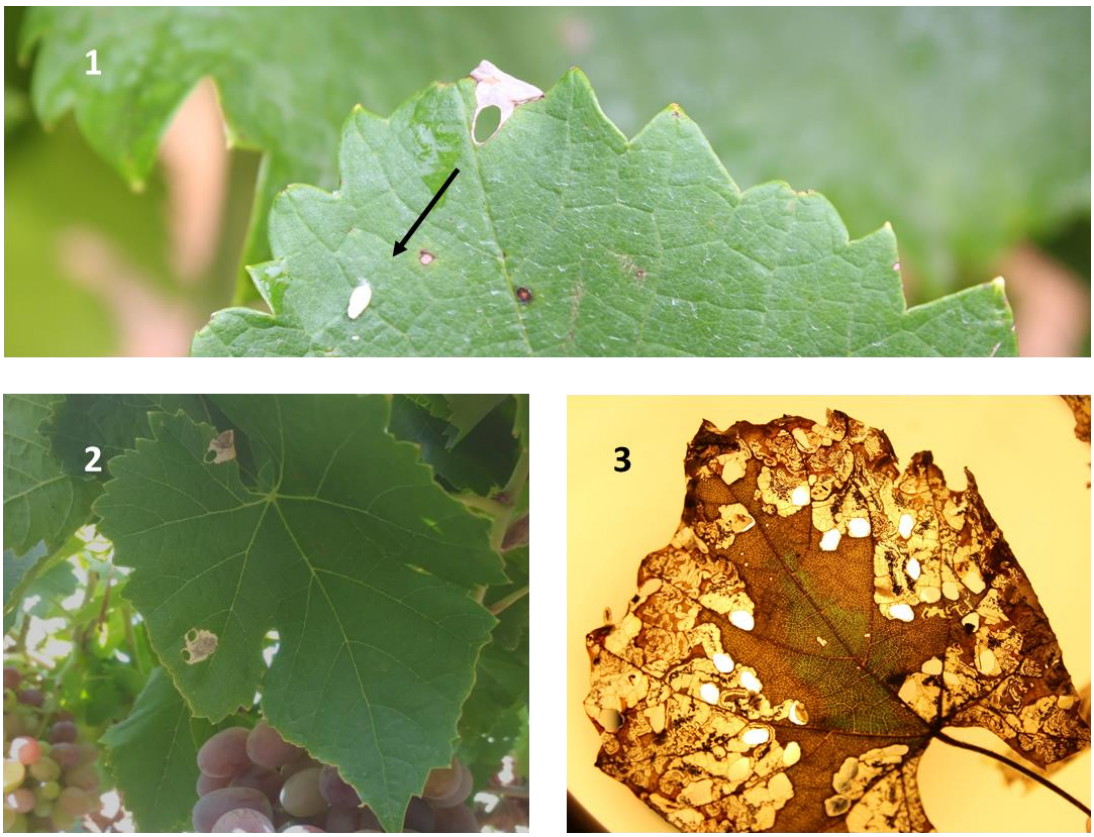

Figure 1.5: Leaf damage and varying degrees of damage caused by *Holocacista capensis* in infested table grape vineyards. 1, fully matured leaf mine (in this case the larva within the cocoon settled close to the native mine); 2, three fully matured mines, indicating medium to low vineyard infestation; 3, a leaf indicating high vineyard infestation (many mines, matured mines and cocoons visible).

The final instar descends from the leaf in a cocoon casing by means of a silken thread (similar to most other leaf-mining heliozelids) (Torrance 2016). Upon landing on an object in its surroundings (e.g. leaf, trellis post or grape bunch) the larva will crawl to an appropriate location and firmly attach itself to the object (Nieukerken & Geertsema 2015; Torrance 2016). It is undesirable when the cocoon casings are present on fruit intended for export (Fig. 1.6) as they are considered a phytosanitary risk. Any form of the insect present on export fruit can, therefore, potentially lead to the rejection of fruit from international markets.

Figure 1.6: Two examples of the occurrence of *Holocacista capensis* cocoon casings rooted to table grape bunches.

Bio-ecology

Larval and adult abundance tends to increase throughout a season, with increasing temperatures (Torrance 2016). The months of February and March mark the peak in adult and larval abundance (Van Nieukerken & Geertsema 2015; Torrance 2016). According to Torrance (2016), temperature was shown to play a vital role in leafminer population abundances. Other variables (including trellis angle and block aspect) affecting leafminer infestation were also investigated, but definite conclusions regarding their effect on population numbers could not be drawn (Torrance 2016).

It is estimated that the life cycle of the moth takes at least seven weeks to complete and a minimum of four generations can be present within a growing season (Torrance 2016). The leafminer overwinters in the larval or pupal life stage within the cocoon casing that is sheltered from the elements (e.g. under the bark of a grapevine stem, in leaf litter or in crevices of trellising posts) (Torrance 2016). These individuals will eclose in the ensuing growing season and will produce the first generation in the new season (Van Nieukerken & Geertsema 2015; Torrance 2016).

The Cape grapevine leafminer is widely distributed throughout the Western Cape, South Africa, and has established itself in relatively high abundances in two of the major table grape producing regions in southern Africa, namely the Berg River and the Hex River regions (Torrance 2016). The leafminer has also been reported from other grape producing regions in the country (Fig. 1.7). Synonymy amongst populations (molecular identifications) has, as yet, not been confirmed.

Figure 1.7: The known distribution of *Holocacista capensis* (successful trap catch marked in black) in South Africa (Van Nieukerken & Geertsema 2015; Torrance 2016).

Variables affecting leafminer infestation

Auerbach *et al*. (1995) states that the dominant cause of mortality or absence of leafminer populations in suitable habitats can be attributed to vertical (interactions between miners, host plants and natural enemies) and horizontal interactions (include inter- and intraspecific interactions between miners and herbivores). This does not, however, account for environmental and abiotic factors affecting leafminer infestation.

Little is known of the direct effects of abiotic factors or variables on leafminer abundance and survival (Auerbach *et al*. 1995). Pereira *et al*. (2007) identified rainfall as an important factor affecting mortality of *Leucoptera coffeella* (Guérin-Méneville & Perrottet) (Lepidoptera: Lyonetiidae) and also considered that weather conditions could have an effect on egg mortality. However, their study concentrated on the environmental factors operative between the two seasons (rainy vs. dry) and not necessarily the factors influencing population abundances within a particular season. Potter (1992) excluded shade as an important factor affecting the abundance of *Phytomyza ilicicola* Loew (Diptera: Agromyzidae). An interesting study by Johns & Hughes (2002) identified a negative association between the emergence success and adult weight of *Dialectica scalariella* Zeller (Lepidoptera: Gracillariidae) in Paterson's Curse, *Echium plantagineum* (Boraginaceae) and elevated CO_2, as a

result of reduced foliar quality of *E. plantagineum*. The invasion ecology of the horse chestnut leafminer, *Cameraria ohridella* Deschka & Dimić (Lepidoptera, Gracillariidae), on the other hand, has been found to be affected by long-distance dispersal and increased human population densities (increasing the probability of accidental transport of leafminers as a result) (Gilbert *et al*. 2004).

In the case of *H. capensis*, the average male adult abundance has been strongly correlated with the average minimum humidity (and thus also to the average maximum temperature) (Torrance 2016). Edge effects, the difference between externally located plots and internally located plots, did not affect leafminer abundance. Spatial distribution and abundance in grapevine blocks have not, however, been assessed and require further investigation. Human-mediated means of dispersal have also been speculated (Torrance 2016).

Pest management

On a global scale, most commercial vineyards are protected against leaf-mining pests (as with a number of other pests) by the use of insecticides (Maier 2001). Various other control strategies have, however, also been used to control pest populations. A summary of these strategies and their respective leaf-mining insect targets is given in Table 1.2.

Table 1.2: A (non-exhaustive) summary of the various control strategies that have been used against leaf-mining pests.

Control method/ Order	Pest family	Common name	Scientific name	Strain/Active agent/Species	Success	Source
Insecticides:						
Diptera	Agromyzidae	Celery leafminer	*Liriomyza trifolii* (Burgess)	Abamectin	Yes	Hara *et al*. (1993)
Diptera	Agromyzidae	Celery leafminer	*L. trifolii*	Abamectin, cyromazine	Yes - resistance reported	Trumble (1985); Ferguson (2004)
Diptera	Agromyzidae	Celery leafminer	*L. trifolii*	Spinosad	Yes - resistance reported	Ferguson (2004)
Diptera	Agromyzidae	Celery leafminer	*L. trifolii*	Methomyl	No	Trumble (1985)
Diptera	Agromyzidae	Pea leafminer	*Liriomyza huidobrensis* (Blanchard)	Abamectin, cyromazine	Yes - negative effects on parasitoids	Weintraub & Horowitz (1998)
Diptera	Agromyzidae	Pea leafminer	*Phtomyza atricornis* Goureau	Acetamiprid, methamidophos, imidacloprid, biopesticide	Yes	Khan *et al*. (2015)
Lepidoptera	Gelechiidae	Tomato leafminer	*Tuta absoluta* (Meyrick)	Abamectin, chlorantraniliprole	Yes	Pereira *et al*. (2014)
Lepidoptera	Gelechiidae	Tomato leafminer	*T. absoluta*	Chlorpyrifos	Yes - resistance reported	Haddi *et al*. (2017)
Lepidoptera	Gelechiidae	Tomato leafminer	*T. absoluta*	Diamide	Yes - resistance reported	Roditakis *et al*. (2017)
Lepidoptera	Gelechiidae	Tomato leafminer	*T. absoluta*	Indoxacarb, spinosad	Yes - resistance reported	Pereira *et al*. (2014); Roditakis *et al*. (2018)

Table 1.2: continued.

Control method/ Order	Pest family	Common name	Scientific name	Strain/Active agent/Species	Success	Source
Lepidoptera	Gelechiidae	Tomato leafminer	*T. absoluta*	Methamidophos, phenthoate, cartap hydrochloride, chlorfenapyr	Yes - effects on natural enemies	Pereira *et al*. (2014)
Lepidoptera	Gracillariidae	Citrus leafminer	*Phyllocnistis citrella* Stainton	Permethrin, methidathion, fenoxycarb	Yes	Beattie *et al*. (1995b)
Lepidoptera	Gracillariidae	Citrus leafminer	*P. citrella*	Petroleum spray oil	Yes	Beattie *et al*. (1995a)
Lepidoptera	Gracillariidae	Citrus leafminer	*P. citrella*	Polysaccharides	No	Beattie *et al*. (1995a)
Lepidoptera	Gracillariidae	Horse chestnut leafminer	*Cameraria ohridella* Deschka & Dimić	Harpin protein, potassium phosphite, salicylic acid derivative	Yes	Percival & Holmes (2016)
Lepidoptera	Gracillariidae	Horse chestnut leafminer	*C. ohridella*	Benzothiadiazole, probanazole, deltamethrin	No	Percival & Holmes (2016)
Lepidoptera	Lyonetiidae	Coffee leafminer	*Perileucoptera coffeella* (Guérin-Méneville)	Chlorpyrifos, disulfoton, ethion, methyl parathion	Yes - resistance reported	Fragoso *et al*. (2002)
Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae):						
Diptera	Agromyzidae	Celery leafminer	*Liriomyza trifolii* (Burgess)	*Steinernema bicornutum* Tallosi, Peters & Ehlers; *Heterorhabditis indica* Poinar, Karunakar & David	Yes	Jacob & Mathew (2016)
Diptera	Agromyzidae	Celery leafminer	*L. trifolii*	*Steinernema carpocapsae* (Weiser, 1955) Wouts, Mráček, Gerdin & Bedding	Yes	LeBeck *et al*. (1993); Jacob & Mathew (2016)

Table 1.2: continued.

Control method/ Order	Pest family	Common name	Scientific name	Strain/Active agent/Species	Success	Source
Diptera	Agromyzidae	Celery leafminer	*L. trifolii*	*Steinernema feltiae* (Filipjev) Wouts, Mráček, Gerdin & Bedding	Yes - dependent on relative humidity	Hara *et al*. (1993)
Diptera	Agromyzidae	Pea leafminer	*L. huidobrensis*	*S. feltiae*	Yes	Williams & Walters (2000)
Diptera	Agromyzidae	Tomato leafminer	*Liriomyza bryoniae* Kaltenbach	*S. feltiae*	Yes	Williams & Walters (2000)
Diptera	Agromyzidae	Chrysanthemum leafminer	*Chromatomyia syngenesiae* (Hardy)	*S. feltiae*	Yes	Williams & Walters (2000)
Hymenoptera	Tenthredinidae	Amber-marked birch leafminer	*Profenusa thomsoni* (Konow)	*S. carpocapsae*	No	Progar *et al*. (2015)
Lepidoptera	Gelechiidae	Tomato leafminer	*T. absoluta*	*Heterorhabditis bacteriophora* Poinar	Yes	Batalla-Carrera *et al*. (2010); Gözel & Kasap (2015); Van Damme *et al*. (2015); Kamali *et al*. (2017)
Lepidoptera	Gelechiidae	Tomato leafminer	*T. absoluta*	*Steinernema affine* Wouts, Mráček, Gerdin & Bedding	Yes	Gözel & Kasap (2015)
Lepidoptera	Gelechiidae	Tomato leafminer	*T. absoluta*	*S. carpocapsae*	Yes	Batalla-Carrera *et al*. (2010); Gözel & Kasap (2015); Van Damme *et al*. (2015); Kamali *et al*. (2017)
Lepidoptera	Gelechiidae	Tomato leafminer	*T. absoluta*	*S. feltiae*	Yes	Batalla-Carrera *et al*. (2010); Gözel & Kasap (2015); Van Damme *et al*. (2015)

Table 1.2: continued.

Control method/ Order	Pest family	Common name	Scientific name	Strain/Active agent/Species	Success	Source
Lepidoptera	Gelechiidae	Tomato leafminer	*T. absoluta*	*Steinernema karii* Waturu, Hunt & Reid, *Heterorhabditis* sp.	Yes	Mutegi *et al*. (2017)
Lepidoptera	Gracillariidae	Citrus leafminer	*P. citrella*	*S. carpocapsae*	Yes	Beattie *et al*. (1995b)
Insecticides combined with entomopathogenic nematodes:						
Diptera	Agromyzidae	Pea leafminer	*L. huidobrensis*	*S. feltiae* added independently to: abamectin, deltamethrin, heptenophos	Yes	Head *et al*. (2000)
Diptera	Agromyzidae	Pea leafminer	*L. huidobrensis*	*S. feltiae* added independently to: trichlorfon, dimethoate	No	Head *et al*. (2000)
Parasitoids:						
Diptera	Agromyzidae	Celery leafminer	*L. trifolii*	*Chrysocharis flacilla* (Walker) (Eulophidae)	Yes	Muchemi *et al*. (2018)
Diptera	Agromyzidae	Celery leafminer	*L. trifolii*	*Diglyphus isaea* (Walker) (Eulophidae)	Yes	Minkenberg & Van Lenteren (1986)
Diptera	Agromyzidae	Potato leafminer	*L. huidobrensis*	*C. flacilla*	Yes	Muchemi *et al*. (2018)
Diptera	Agromyzidae	Potato leafminer	*L. huidobrensis*	*D. isaea*	Yes	Maharjan *et al*. (2017)

Table 1.2: continued.

Control method/ Order	Pest family	Common name	Scientific name	Strain/Active agent/Species	Success	Source
Diptera	Agromyzidae	Potato leafminer	*L. huidobrensis*	*Opius dissitus* Muesebeck (Braconidae)	Yes	Wei & Kang (2006)
Diptera	Agromyzidae	Vegetable leafminer	*Liriomyza sativae* Blanchard	*O. dissitus*	Yes	Wei & Kang (2006)
Diptera	Agromyzidae	Vegetable leafminer	*L. sativae*	*C. flacilla*	Yes	Muchemi *et al*. (2018)
Diptera	Agromyzidae	Holly leafminer	*Phytomyza ilicis (Curtis)*	*Chrysocharis gemma* (Walker) (Eulophidae)	Yes	Heads & Lawton (1983)
Diptera	Agromyzidae	Holly leafminer	*P. ilicis*	*Opius ilicis* (Nixon) (Braconidae)	Yes	Kirichenko *et al*. (2018)
Hymenoptera	Tenthredinidae	Amber-marked birch leafminer	*Profenusa thomsoni* (Konow)	*Lathrolestes thomsoni* Reshchikov (Ichneumonidae)	Yes	Soper *et al*. (2015)
Hymenoptera	Tenthredinidae	Birch leafminer	*Fenusa pumila* Leach	*Lathrolestes nigricollis* (Thomson) (Ichneumonidae), *Grypocentrus albipes* Ruthe (Ichneumonidae)	Yes	Langor *et al*. (2000)
Lepidoptera	Gelechiidae	Tomato leafminer	*T. absoluta*	*Trichogramma euproctidis* Girault, *Trichogramma achaeae* Nagaraja & Nagarkatti (Trichogrammatidae)	Yes	El-Arnaouty *et al*. (2014)
Lepidoptera	Gelechiidae	Tomato leafminer	*T. absoluta*	*Trichogramma pretiosum* Riley (Trichogrammatidae)	Yes	Parra & Zucchi (2004)
Lepidoptera	Gracillariidae	Citrus leafminer	*P. citrella*	*Ageniaspis citricola* Logvinovskaya (Encyrtidae)	Yes	Hoy *et al*. (2007)
Lepidoptera	Gracillariidae	Citrus leafminer	*P. citrella*	*Citrostichus phyllocnistoides* (Narayanan) (Eulophidae)	Yes	Garcia-Marí *et al*. (2004)

Chemical control

Chemical control can be achieved through the use of synthetic chemical insecticides or botanical insecticides (Isman 2006). In terms of environment-friendly pest management, botanical insecticides pose an attractive alternative to the use of synthetic insecticides, due to the fact that they can be of less threat to human health or the environment. Synthetic chemical pesticides, on the other hand, have been shown to exhibit some adverse effects. These include: acute and chronic poisoning of farmworkers (especially individuals involved in their application) and consumers (residue issues); the demise of wildlife (including bees, beneficial insects, fish and birds); disruption of established biological control mechanisms and pollination; contamination of groundwater (with far-reaching threats to human and environmental health); and the development of pesticide resistance in pest populations (Isman 2006).

To date, no chemical insecticides are registered for the control of *H. capensis*, although short-term (seasonal) control has been achieved by the use of dichlorvos and spinosad in vineyards of the Western Cape (Torrance 2016). A considerable amount of research has been conducted on insecticide use and corresponding insecticide resistance of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Biondi *et al*. 2018), raising concern for long term control strategies for other leaf-mining pests with similar generation times, including *H. capensis*.

Entomopathogenic nematodes

Of the various beneficial, parasitic groups within the nematode complex, entomopathogenic nematodes (EPNs) are used to control insect pests (Stock & Hunt 2005; Stock 2015). The genera within this group include members of the genera *Steinernema* Travassos (Steinernematidae: Rhabditida) and *Heterorhabditis* Poinar (Heterorhabditidae: Rhabditida) (Kaya *et al*. 1993). Together with their associated pathogenic bacteria (from the genus *Xenorhabdus* and *Photorhabdus* for steinernematids and heterorhabditids, respectively), EPNs kill their hosts within a few days (Fig. 1.8) (Dillman *et al*. 2012, Lewis *et al*. 2015).

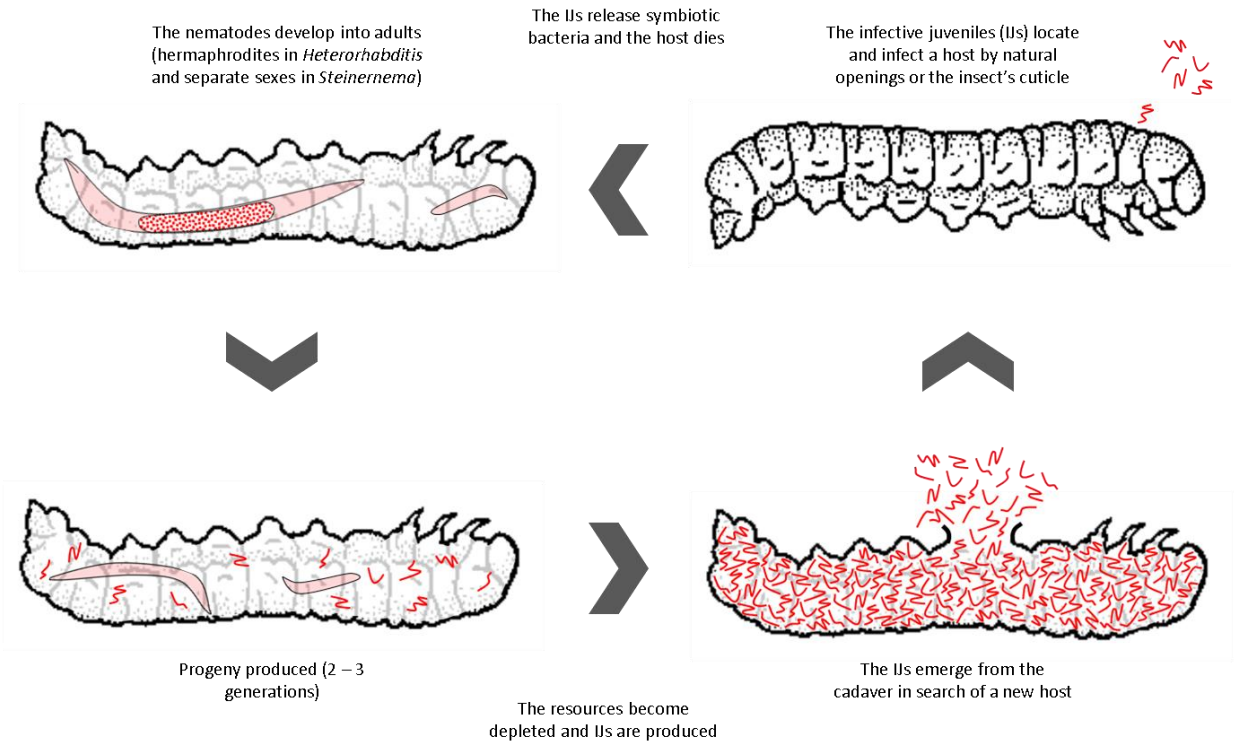

Figure 1.8: The life cycle of entomopathogenic nematodes in an insect host. Adapted from Griffin *et al*. (2005) and Dillman *et al*. (2012).

For all EPNs there is a free-living, non-feeding stage known as the infective juvenile (IJ) or dauer (Griffin *et al*. 2005). When an appropriate host is located, an IJ will enter through any natural opening (i.e. mouth and anus), the cuticle or spiracles in search of the nutrient-rich haemolymph. Here, the IJs will release their symbiotic bacteria from their intestines, which reproduce and release toxins. The death of the infected insect usually occurs within 48 h. Within the cadaver the IJs feed on the bioconverted host tissues (and bacteria), and are able to grow and develop into adults. As the food source becomes scant within the cadaver, the nematodes develop in crowded conditions and become arrested as IJs. The new IJs, with their specific symbiotic bacteria, will emerge from the cadaver in search of a new host (Griffin *et al*. 2005).

A variety of EPNs have been used to successfully control certain leaf-mining pest populations (Table 1.2). In the case of *T. absoluta*, leaf bioassays conducted on leaves infested with larvae, using 1 000 IJs/ml concentrations (equivalent to a 60 IJs/cm^2 dose) of *S. carpocapsae*, *S. feltiae* and *H. bacteriophora*, proved to cause significantly high levels of mortality (88.6%, 92% and 76.3%, respectively) after 72 h of exposure to the respective EPNs (Batalla-Carrera *et al*. 2010). These results revealed that the EPNs were able to find and kill larvae, despite their relative position on or within a leaf (i.e. outside of or within leaf galleries). Field trials conducted by Gözel & Kasap (2015) with the same EPNs on netted plants, using a conventional airblast-sprayer at an application rate of 50 IJs/cm^2,

confirmed these results (ca. 46%, 92% and 82% total mortality, respectively). Similar results were also obtained by Van Damme *et al*. (2015), who applied a concentration of 27.3 IJs/cm^2 of each of the three EPN species to infested leaves by means of an automated spray boom. Beattie *et al*. (1995b) tested *S. carpocapsae* against the larvae of *Phyllocnists citrella* Stainton (Lepidoptera: Gracillariidae) at concentrations of 5 x 10^6, 10 x 10^6 and 30 x 10^6 IJs/l water. A significant increase in mortality was only obtained at the highest dose, resulting in 35% mortality. To date, no EPNs have been tested against *H. capensis*.

Cover cropping

Ingels & Klonsky (1998) described a cover crop as a crop (or secondary plants) of little to no economic significance that is grown in intra- and inter-rows of vineyards, the presence of which, however, provides numerous potential other benefits. Parolin *et al*. (2012) provided an extended definition for barrier plants as: "…a plant which is used within or bordering a primary crop for the purpose of disease suppression and/or interception of pests and/or pathogens". In terms of their potential to harbour pests and pathogens, the effect of barrier plants or cover crops on population numbers of most leaf-mining pests is not known and should be investigated, as the use of different cover crops in vineyards are seen as the way forward.

Parasitoids

In contradiction to the inferences made by Ayabe & Hijii (2016) regarding the study by Connor & Taverner (1997), the leaf-mining habit does not allow leafminers to escape predation. According to Connor & Taverner (1997), the loss of mobility, and thus escape strategies, in leaf-mining insects has led to higher mortality rates associated with hymenopteran parasitoids than in exophagous insects. This has led to the evolution of more species of associated parasitoids than in any other insect feeding guild. In the case of *H. capensis*, several parasitoids have been found to attack the larval and pupal life stages, although these parasitoids have not yet been identified. The use of parasitoids against leaf-mining insects is a popular alternative to the use of insecticides.

A few case studies with promising results have been listed in Table 1.2. Trichogrammatidae, Encyrtidae and Eulophidae (all of which belong to the superfamily Chalcidoidea) have been found to parasitise lepidopteran leaf-mining pests (Table 1.2). The species within the Chalcidoidea are generally smaller than 3 mm in length, making it extremely difficult to collect and study individuals (Noyes 2003).

Other means of pest management

Entomopathogenic fungi (EPF) have been used successfully in a variety of integrated pest management (IPM) strategies against many pests of economic importance (Shah & Pell 2003). Various strains of *Metarhizium anisopliae* (Metschnikoff) (Sorokin) (Hypocreales: Clavicipitaceae) and *Beauveria bassiana* (Balsamo) (Vuillemin) (Hypocreales: Cordycipitaceae), have been used to control the pea leafminer, *Liriomyza huidobrensis* (Blanchard) (Diptera: Agromyzidae) (Migiro *et al*. 2010; 2011); and the tomato leafminer, *T. absoluta* (Rodríguez *et al*. 2006; Allegrucci *et al*. 2017).

Mating disruption implies the use of a formulated female pheromone to disrupt or regulate the mating habits of a target pest species (Cardé & Minks 1995). Amongst the leaf-mining Lepidoptera, mating disruption has only been explored and successfully achieved against *P. citrella* (Stelinski *et al*. 2008; Stelinski *et al*. 2010; Willett *et al*. 2015). Mating disruption studies on *T. absoluta* have only proved successful under greenhouse conditions (Vacas *et al*. 2011; Cocco *et al*. 2013).

The practice of bagging grapes (as a physical measure of control) using a bunch cover/bag dates back to 1919 (Signes *et al*. 2007). After ripening, bunches are typically covered with a cover/bag which is only removed during harvest. Bagging has been used to promote the uniform colour development within a bunch; reduce the incidence of blemished fruit; reduce incidence of disease; delay the ripening process (ideally when harvest needs to be delayed for increased market access); increase hygiene (reduced contact with pesticides sprays and other contaminants); protect grape bunches against adverse environmental variables (e.g. moisture, hail, sunburn and cracking/burst of fruit); and to provide protection against the attack of birds and insects (Signes *et al*. 2007; Sharma *et al*. 2014). Pre-harvest fruit bagging has been used to avoid insect infestation in a variety of crops (Sharma *et al*. 2014).

The use of netting (overhead netting, vineyard layover netting and zone netting) in vineyards has become widespread in recent years (Suvočarev *et al*. 2013). Netting is used to reduce the number of pests (reduced immigrant invasion from surrounds) leading to a reduction in the number of pesticide applications; reduce radiation exposure of plants during hot summer months; and minimise hail and bird damage (Suvočarev *et al*. 2013). Neither bagging of grapes nor netting, however, have been tested to exclude leaf-mining pests.

Conclusion

The discovery of a novel pest in an industry that contributes to a country's economy requires novel and baseline studies to understand the pest's ecology and distribution to adequately control pest populations. When studying the various control options, it is important to consider the restrictions

imposed on growers regarding the use of harmful chemical insecticides and the effect of insecticides on the evolution of insecticide resistance. The investigation of alternative control strategies is, therefore, pertinent in enhancing IPM strategies. This study focused on the control of the larval life stage of *H. capensis* considering various environmental and abiotic variables that affect the leafminer's population abundance. The research will increase the current knowledge of *H. capensis* and the use of chemical and biological control options, which could potentially be used as a reference for studies focused on other emerging leaf-mining pests, such as *T. absoluta*, in South Africa.

Aim and objectives

The overall aim of the research was to identify viable management strategies to control *Holocacista capensis* in heavily infested, commercial vineyards in Western Cape grape-growing regions.

In order to accomplish this, a survey was conducted to collect samples in table and wine grape producing regions to confirm synonymous identification of known populations of *H. capensis*. Monitoring was also conducted in natural forests to provide information on their hypothesized native range.

Various conventional and biological control strategies were investigated in the laboratory and in the field to identify the appropriate means of control to be introduced into an integrated pest management (IPM) strategy for *H. capensis*. An investigation into the environmental and abiotic factors affecting leafminer infestation needed to be conducted to identify non-obtrusive adaptations of present farming practises to reduce leafminer infestation to optimise and supplement potential management protocols.

The study consists of four chapters, each addressing specific objectives/research questions. The chapters are as follows:

i. determining the abiotic and environmental variables affecting leafminer infestation and population densities in the field;
ii. a survey of the pest heliozelid populations in and around three of the largest table grape producing regions of South Africa;
iii. a laboratory and field study of the potential chemical and physical management strategies that could be used against *H. capensis*; and
iv. the use of entomopathogenic nematodes against the larval life stage of *H. capensis* as a viable means of control

These chapters are structured as individual publications. Some repetition in information content is thus unavoidable.

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Chapter 2

Variables Responsible for Infestation

A field study of the abiotic and environmental variables influencing the presence and abundance of *Holocacista capensis* (Lepidoptera: Heliozelidae) in table grape vineyards

Introduction

South Africa is one of the largest producers of table grapes in the southern hemisphere (SATI Statistics Booklet 2018) and approximately 32% of the land planted to deciduous fruit is occupied by table grapes and dried grapes, the most planted fruit crops within South Africa. Some of the major pests on locally produced table grapes have been documented by Allsopp *et al*. (2015), as a part of the latest compendium of Insects of Cultivated Plants and Natural Pastures by Prinsloo & Uys (2015), and include a variety of leafhoppers, scale insects, mealybugs, weevils, fruit flies and moths. Absent from this list is a native leaf-mining lepidopteran, *Holocacista capensis* Van Nieukerken & Geertsema (Heliozelidae), reported for the first time on table grapes in 2012 in the Paarl region, within the Western Cape province, South Africa. Direct damage caused by leaf-mining larvae is not considered to be of economic importance, the presence of cocoons on table grape bunches, however, poses a phytosanitary risk which could potentially lead to the rejection of consignments for export.

There are three sources of mortality relevant to leaf-mining insects; vertical, horizontal and abiotic sources (Auerbach *et al*. 1995). Vertical sources refer to the interactions between leafminers and their natural enemies and host plants. Horizontal sources include inter- and intraspecific interactions between miners and herbivores. The last of these sources of mortality is attributed to abiotic factors, which include meteorological fluctuations and environmental variables (i.e. wind, rain, moisture and extreme temperatures) (Auerbach *et al*. 1995).

It is widely known that temperature directly impacts various aspects of insect development, range/distribution, abundance and survival (Bale *et al*. 2002). Pereira *et al*. (2007) recognised the effect of temperature and varying weather conditions on *Leucoptera coffeella* (Guérin-Méneville & Perrottet) (Lepidoptera: Lyonetiidae), whilst Lomelí-Flores *et al*. (2010) also explored shade cover and elevation as factors affecting *L. coffeella* population densities, although these differences were fundamentally caused by changes in temperature under varying shaded conditions and elevations. Potter (1992) found contradictory results pertaining to the relevance of shade in the case of a dipteran

leafminer, *Phytomyza ilicicola* Loew (Agromyzidae). Generally, warmer climates are also known to facilitate a greater number of generations (Tauber *et al*. 1982). A preliminary study by Torrance (2016), which ran over two grapevine growing seasons, identified relative humidity and temperature as important determinants of male moth abundance.

Through various technological advancements, temporal phenomena can be monitored with the analysis of multi-date imagery acquired by satellite-based, multi-spectral sensors such as those acquired by Sentinel-2 (Coppin *et al*. 2004; Martimort 2007). Satellite-derived vegetation reflectance data, of varying spatial resolutions, have been used to establish the extent of defoliation caused by various insects in forested vegetation types (Jepsen *et al*. 2009). That said, satellite-based monitoring of the extent of insect damage has not yet been incorporated into operational pest monitoring and management programmes as the sensor temporal resolution needs to be appropriately matched to the temporal manifestation of the host plant damage. In addition, the satellite derived measure of damage must be verified as a valid proxy for insect pest population density fluctuations in the field (Jepsen *et al*. 2009).

Plant-insect interactions are mediated, in part, by host plant chemistry (Tasin *et al*. 2011a). Sensory cues such as olfaction, vision and contact chemoreception are used by females to locate and select suitable host plants for oviposition (Chapman, 2003; Tasin *et al*. 2011b). Tasin *et al*. (2011a) showed that volatiles are significantly correlated with the quality of host plants [done by analysing the preferences of ovipositing *Lobesia botrana* (Denis and Schiffermueller) (Lepidoptera: Tortricidae)]. Host plant quality describes the predominant components of the host plant (Awmack & Leather 2002). It is often linked to soil fertility management which, in many cases, dictates the susceptibility of plants to insect pests and subsequent levels of herbivore damage (Altieri & Nicholls 2003). To a certain extent, reduced plant quality can lead to herbivore mortality (Stiling *et al*. 1999). The interaction between the infection potential of leaf-mining pests and plant host quality, however, is not well known. Various studies, such as those by Gaston *et al*. (2004) on *Phytomyza ilicis* Curtis (Diptera, Agromyzidae) and Pereyra & Sánchez (2006) on *Tuta absoluta* (Meyrick) (Gelechiidae) (another leaf-mining pest on tomato plants that has become of economic importance in South Africa), have failed to correlate plant quality to leafminer abundance. Stiling *et al*. (1999), however, studied the effects of elevated atmospheric CO_2 conditions on three leaf-mining insects (*Stigmella*, *Cameraria* and *Stilbosis* species) and recorded a decrease in leafminer abundance, higher mortality of the leaf-mining insects, increased leaf consumption (per capita) and also recorded a decrease in plant nitrogen concentrations. Interestingly, an increase in atmospheric CO_2 also indirectly altered leafminer feeding behavior and elicited greater top-down pressure from natural enemies.

The presence of crop cover vegetation in agricultural systems can increase the abundance of natural enemy populations through the provisioning of resources (e.g. alternative hosts) and shelter (e.g. crop-applied insecticides) (Landis *et al*. 2000). A variety of studies have been able to establish the success of controlling lepidopteran pest populations by the use of cover crops (e.g. Irvin *et al*. 2006; Prasifka *et al*. 2006; Danne *et al*. 2010). Relevant in the case of the Western Cape, Bone *et al*. (2009), however, warned against the reliance on fortuitous control by cover crops and suggested site-specific investigations within local deciduous fruit growing crops, as the beneficial effects of cover crops may be limited in commercial settings that experience limited annual rainfall.

The aim of the current study was to gain a greater understanding of the abiotic conditions, as described by Auerbach *et al*. (1995), that dictate the severity of *H. capensis* infestations in commercial table grape vineyards. It involved the investigation of a variety of environmental and biological aspects within the leafminers' immediate field environments during the 2017/2018 grapevine growing season. More specifically, this study involved the use of two response variables (factors indicative of total leafminer infestation), namely the number of male moths and the proportion of infested bunches (presence of rooted cocoon casings) within each block to explain the variation (if any) associated with the trellising system, cultivar/grape colour, climatic conditions, light intensity, leaf composition (plant quality), plant greenness and ground cover composition within table grape vineyards.

Materials and methods

Study sites

The current study was conducted on three infested farms in the Western Cape province, South Africa, from January to February 2017. The farms were situated in Halfmanshof (33°08'48.7"S 18°59'18.0"E), Paarl (33°41'11.2"S 18°57'10.6"E) and Wellington (33°35'52.7"S 18°58'46.0"E) situated in the Berg River table grape producing region. A total of 28 blocks were sampled on one occasion in January/February, 2017 (Table 2.1).

Table 2.1: Detailed information regarding the biological and physical aspects of each of the blocks sampled in the 2017 grapevine growing season.

Area	Cultivar	Berry colour	Trellis type		Block status
			Y	T	
Halfmanshof	Alison	Red	x		Ripening
	Alison	Red	x		Ripening
	Autumn Royal	Black	x		Ripening
	Crimson Seedless	Red	x		Ripening
	Crimson Seedless	Red	x		Ripening
	Crimson Seedless	Red	x		Ripening
	Crimson Seedles	Red		x	Ripening
	Dan-ben-Hannah	Black	x		Ripening
	Melody	Black	x		Ripening
	Red Globe	Red	x		Ripening
	Red Globe	Red	x		Ripening
	Regal Seedless	White	x		Ripening
	Sugraone	White		x	Ripening
	Tawny Seedless	Red	x		Ripening
	Victoria	White	x		Ripening
Paarl	Crimson Seedless	Red		x	Ripening
	Crimson Seedless	Red		x	Ripening
	Sugrasixteen	Black		x	Harvested
	Sugrasixteen	Black		x	Harvested
	Sugrasixteen	Black		x	Harvested
	Sugrasixteen	Black		x	Harvested
	Victoria	White		x	Harvested
	Victoria	White		x	Harvested
Wellington	Autumn Royal	Black	x		Ripening
	Crimson Seedless	Red		x	Ripening
	Melody	Black	x		Ripening
	Sugranineteen	Red	x		Ripening
	Sundance	White	x		Ripening

Environmental and abiotic variables recorded

Moth abundance and bunch infestation

Male moth population abundances within canopies were recorded between the 26th of January and the 21st of February, 2017, by placing a single baited yellow Delta Trap lined with a sticky pad (Chempac, Pty Ltd., Paarl) in each of the sampled blocks. The sex attractant was synthesised at Lund University, Sweden (Wang *et al*. 2015). Dispensers, impregnated with the attractant, were used to bait lined Delta traps. Dispensers were stored at -20°C once imported, to avoid a reduction in pheromone/attractant efficacy, until field experiments were conducted.

As some baited traps were not placed and selected on the same day (due to logistics), the number of male moths caught per trap per day was calculated by:

$$MM/Day = \frac{Total\ MM}{Total\ Days}$$

where MM is the number of male moths caught on a sticky pad.

Adapted from the sampling methods of De Villiers & Pringle (2008), 100 grape bunches were randomly selected within each block and inspected for the presence of rooted cocoon casings.

Cultivar and trellis type vs. moth abundance

Red-, black- or white-berried cultivar varieties, in each of the blocks sampled, were recorded to determine possible relationships between the plant composition and phenols presented by each of the colour types (also refered to as cultivar colour type) and the total moth abundance/bunch infestation. Similarly, the trellising system (T- or Y-shaped trellising systems; Fig. 2.1) was recorded to determine possible relationships between the shade and temperature differences experienced under each of the trellising systems and the corresponding moth abundance/bunch infestation. Effort was made to obtain the highest sample sizes possible. As a result, at least 11 of each of the two trellising systems and at least six of each of the cultivar colour types were sampled, monitored and analysed.

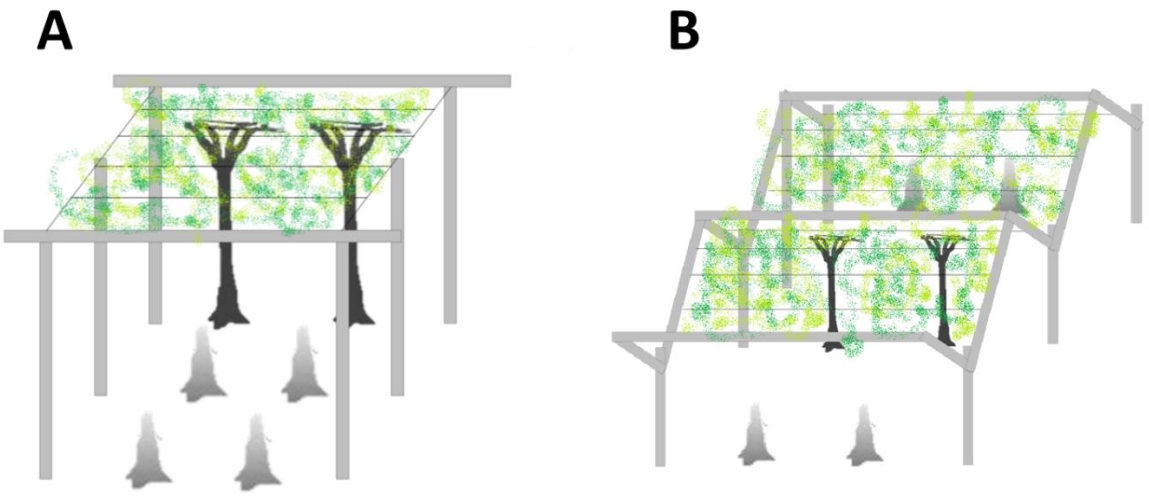

Figure 2.1: The T-shaped (A) and Y-shaped (B) trellising systems adopted for optimal growth of table grapes in the Western Cape.

Ground cover composition and light intensity

To determine whether or not a relationship exists between moth abundance and ground cover, three 1 m x 1 m quadrats were established within the centre row of each sampled block to determine the composition of ground cover (Fig. 2.2). Natural weed cover on the vineyard floor dominated all blocks at the time of sampling. The percentage vegetation cover, soil/sand cover, leaf litter and rock cover were qualitatively estimated within each quadrat. A Brannan Professional Light Meter (S. Brannan & Sons, England) was used to record the light intensity (foot candles) experienced at different layers of the plant profile (i.e. soil surface, understorey, canopy and ambient surroundings) (Fig. 2.2) to establish a possible relationship between microclimate and moth abundance.

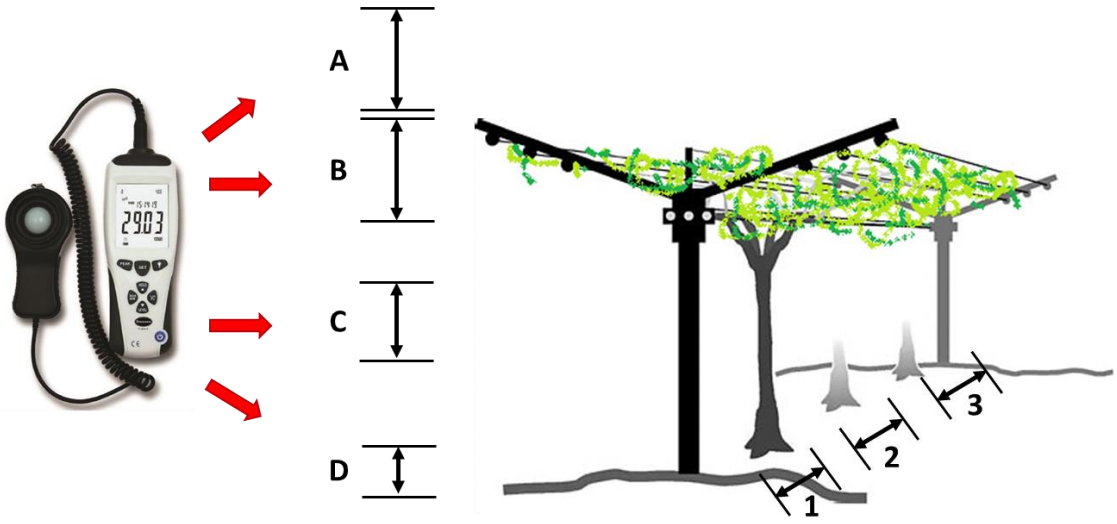

Figure 2.2: The layout of sampling layers for a light intensity and ground cover survey. A: sampling area above canopy (used to standardise light intensity - control); B: sampling area within the canopy; C: sampling area within the understorey; D: leaf/litter and soil surface sampling layer; 1: first ground cover quadrat; 2: second ground cover quadrat; and 3: third ground cover quadrat.

Weather station data and microclimate

Weather data were obtained from the Agricultural Research Council (ARC) (Agrometeorology Staff. 2006. ARC-ISCW Climate Information System. ARC-Institute for Soil, Climate and Water, Pretoria). Three temperature recording data loggers (iButtons, DS1921, Maxim Integrated Products Inc., USA) (accuracy of ± 0.5°C) were placed in the canopy along the center row of each block (Fig. 2.3). Temperature readings were recorded every 15 min.

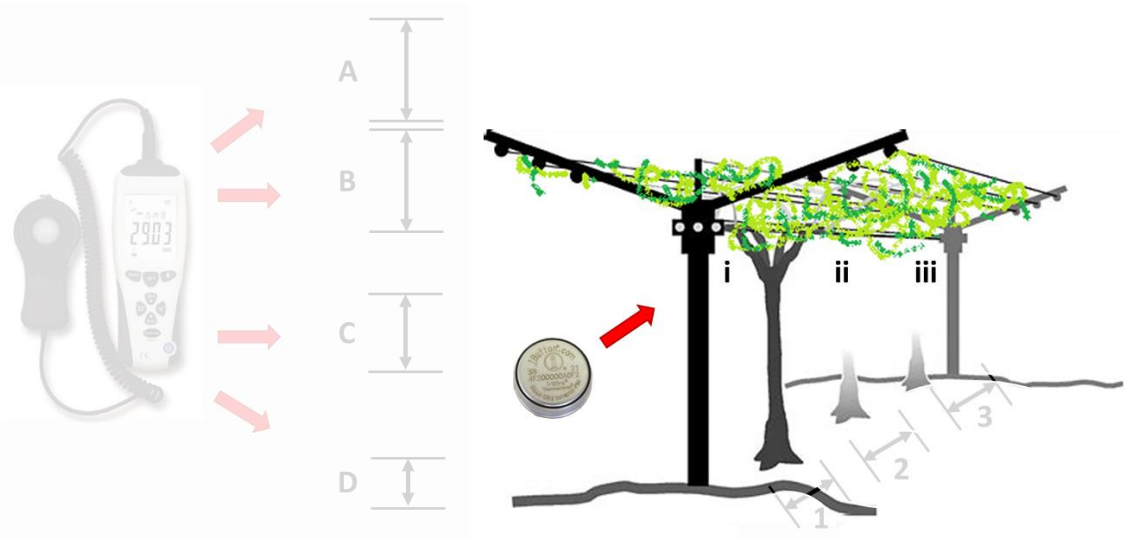

Figure 2.3: The positions (i, ii and iii) of iButton temperature data loggers in the center row of the sampled table grape blocks in relation to other sampling efforts.

Leaf composition

One hundred leaves were collected at random from each of the sampled blocks. The leaves were immediately placed in cold storage in the field and transported to Bemlab (Bemlab, Somerset West) for a standard profile analysis (determination of N, P, K, Ca, Mg, Na, Mn, Fe, Zn, Cu, B) of leaf composition.

Normalized Difference Vegetation Index

The Normalized Difference Vegetation Index (NDVI) (Rouse *et al*. 1974) is the most well-known and commonly used vegetation index (VI) and is a simple but effective VI for quantifying green vegetation (Myneni *et al*. 1995). It is calculated by the assessment and measurement of a reflected surface (in this case a table grape block) by satellite sensors. Essentially, the VI normalizes green leaf (mesophyll leaf structure) distribution/spread in the near-infrared wavelength and chlorophyll absorption in the red wavelength, linking NDVI to vegetation productivity (Pettorelli *et al*. 2005). Negative NDVI values (values approaching -1) correspond to water bodies (i.e. the absence of vegetation). Values close to zero (-0.1 to 0.1) generally correspond to clusters of rock, sand or snow (barren areas). Low positive values represent shrub and grassland (approximately 0.2 to 0.4), whilst higher values indicate temperate and tropical rainforests (values approaching 1) (Pettorelli *et al*. 2005).

The relevant bands (Band 4: red wavelength and Band 8: near infrared wavelength) from the satellite images were obtained from the Sentinel-2 Multispectral Instrument (MSI) satellite (EU Copernicus Programme, European Space Agency, France) and imported into ArcGIS 10.6.1 (Esri, USA). In ArcGIS, the raster calculator was used to establish the NDVI image. Thereafter, the NDVI values were calculated and obtained through the use of zonal statistics within the spatial analysis toolbox as described below (statistical analyses).

The mean NDVI values were calculated using:

$$NDVI = \frac{(NIR - Red)}{(NIR + Red)}$$

where *NIR* and *Red* (can be also be represented by *VIS*) stand for the spectral reflectance measurements acquired in the red (visible) and near-infrared regions, respectively.

Two satellite images were acquired for each of the blocks throughout the January/February sampling period. As a result, the NDVI values obtained from each of the images, for each of the blocks, were averaged.

Statistical analyses

The integrity of the physical number of male moth abundance (caught per block) was questioned due to the fact that male moths could have been attracted from surrounding blocks (the efficacy of the attractant/pheromone attraction has not yet been tested in the field). As a result, a correlation analyses was conducted on moth abundance and bunch infestation data.

The susceptibility of white-, red- and black-berried cultivars, as well as that of T- and Y-shaped trellising systems, to leafminer infestations (adult abundance and bunch infestation), was determined by carrying out non-parametric Kruskal-Wallis ANOVA (as residuals of the ANOVA were not normally distributed) and analysing the relevant multiple comparison *p*-value tests.

Microclimate data were calculated relative to the hourly weather station data as the temperature loggers were placed in the field at varying times and in different locations (towns).The three sets of recorded temperatures (a temperature recording was taken every 15 minutes) were averaged per block for the respective sampling periods. The data were correlated with all forms of leafminer infestation (adult abundance and bunch infestation) using Spearman Rank Order Correlations. The other variables (ARC weather station data, ground cover, mean NDVI, light intensity and leaf composition) were correlated in a similar manner.

A Best Subsets Regression (an exploratory model building regression analysis), using Mallows C_p (Mallows 1973) as the selection criterion, was carried out in order to establish the best covariates as predictors of adult infestation and bunch infestation once collinear variables were removed from the analysis [if covariates had absolute correlation larger than 0.5 ($R > 0.5$) with other covariates]. The response variables were normalised using a square root transformation. Weather station data was removed from the analysis due to the high degree of multicollinearity. Four covariates were selected for moth abundance, whilst eight covariates were selected for bunch infestation.

Unless otherwise stated, all analyses were performed using STATISTICA 13.0 (Dell Inc., Headquarters in Round Rock, Texas, USA).

Results

Moth abundance and bunch infestation

The analysis indicated a strong correlation between the number of male moths caught per day and the number of cocoon-infested bunches ($r = 0.70$; $n = 22$; $p < 0.001$) (Fig. 2.4). The male moth abundance and bunch infestation data were thus used as the dependent variables.

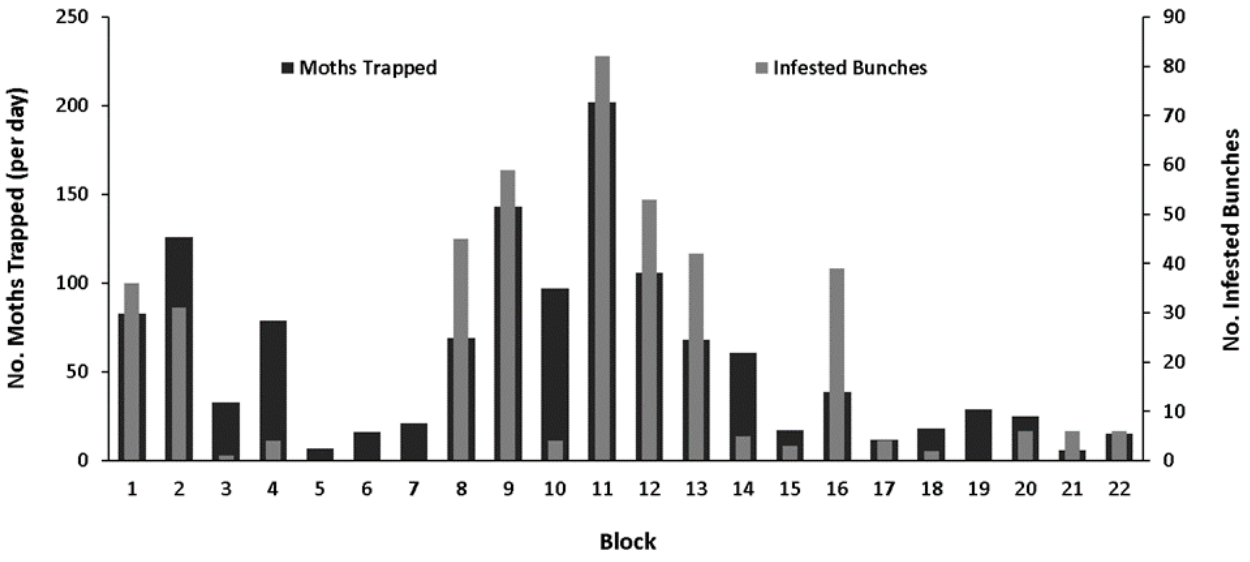

Figure 2.4: The number of *Holocacista capensis* male moths caught per block per day (primary vertical axis) and the number of infested bunches (out of 100 bunches inspected) (secondary vertical axis) recorded in the 2017/2018 grapevine growing season in each of the 22 blocks (y-axis) used in the correlation analyses. Harvested blocks were removed from analysis (see Table 2.1).

Cultivar colour type vs. leafminer abundance

An investigation into the effect of cultivar colour type on male moth abundance indicated no significant differences ($p = 1$) between the three main colour types [red (65.92 ± 15.40), black (47.00 ± 14.17) and white (38.33 ± 12.29)] ($H_{2, 28} = 0.84$; $p = 0.69$) [Fig. 2.5 (I)]. The Kruskal-Wallis test based on the effect of cultivar colour on the number of cocoon casings on bunches mirrored these results ($H_{2, 22} = 5.94$; $p = 0.05$) [Fig. 2.5 (II)]. The number of cocoon casings on white-berried cultivar grape bunches (1.5 ± 0.96) did not significantly differ from that of red- (27 ± 7.04) ($p = 0.05$) and black-berried (14.2 ± 11.26) ($p = 0.62$) cultivar grape bunches. The number of cocoon casings on red- and black-berried cultivar grape bunches did not differ significantly from each other ($p = 0.97$).

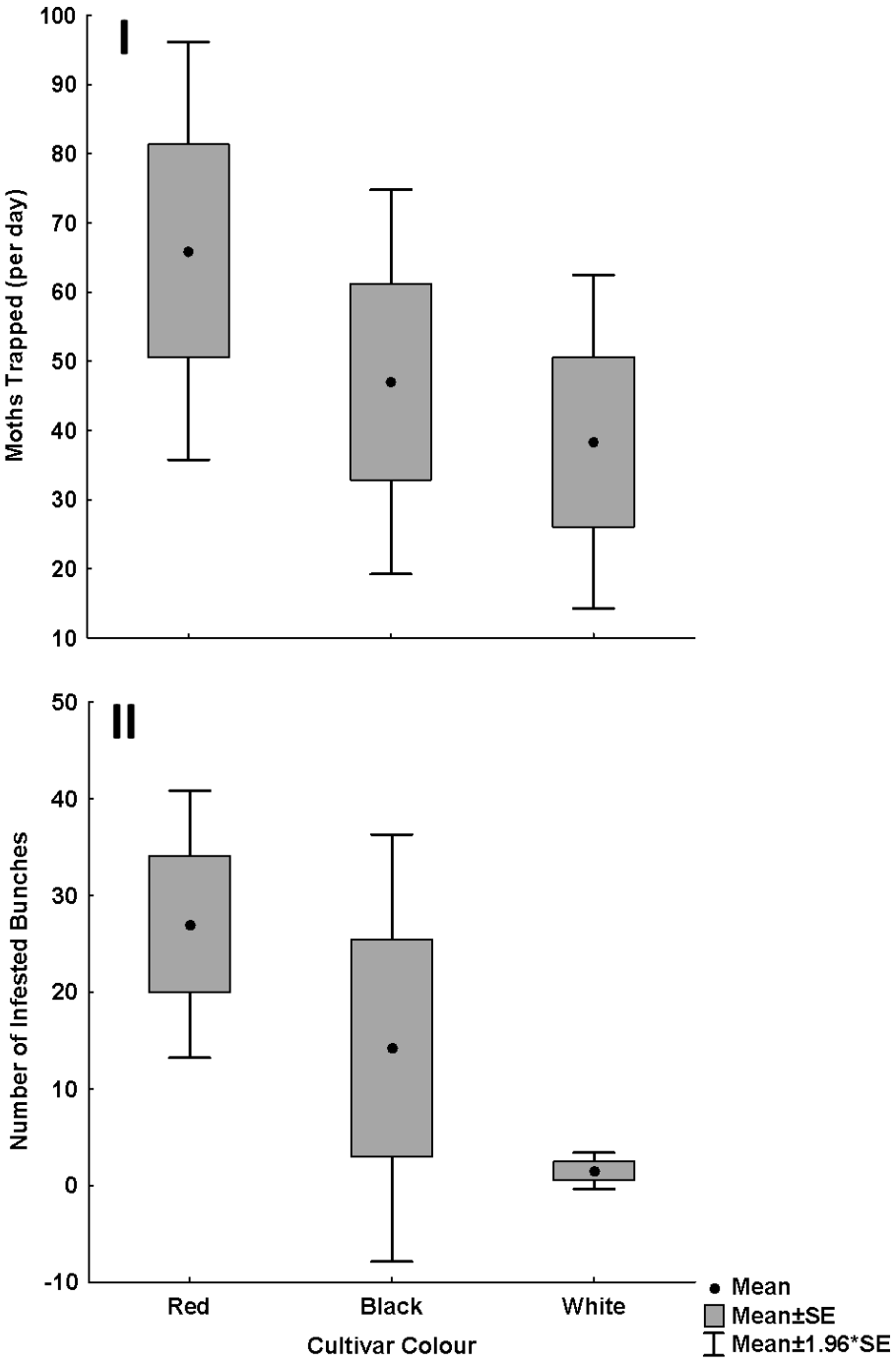

Figure 2.5: The effect of cultivar/berry colour type ("Red", "Black" and "White") on *Holocacista capensis* male moth abundance (moths caught per trap per day) (I) and the number of cocoon infested bunches (out of 100 bunches sampled) (II). Vertical lines denote 0.95 confidence intervals.

Trellis type vs. moth abundance

For both male moth abundance (Fig. 2.6) and bunch infestation, no significant differences ($p > 0.659$) were recorded between trellising system types in the field ($F_{1, 26} = 0.197$; $p = 0.661$ and $F_{1, 20} = 0.111$; $p = 0.743$).

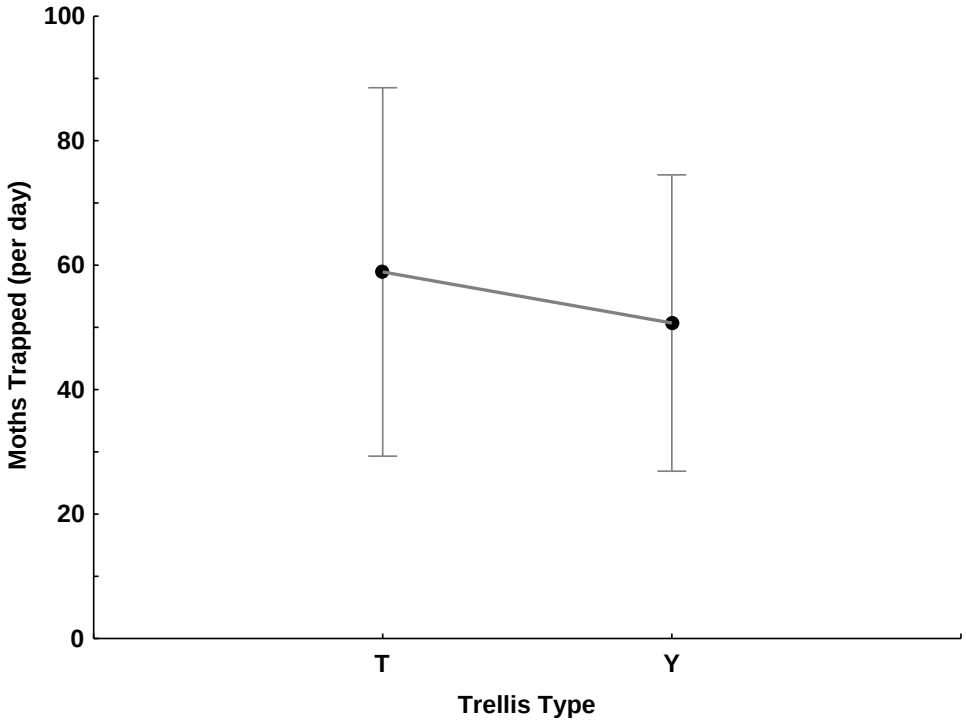

Figure 2.6: The effect of trellis type/system (roof/T-shaped –"T" and Y-shaped – "Y") on *Holocacista capensis* male moth abundance (moths caught per trap per day). Vertical lines denote 0.95 confidence intervals.

Correlation analyses

In general, there were more significant correlations recorded between the number of infested bunches and the abiotic/environmental variables recorded (Table 2.2). A significant positive correlation exists between the mean NDVI and male moth abundance ($r = 0.654$; $p < 0.001$), whereas significant negative correlations were recorded for male moth abundance and ambient light intensity ($r = -0.395$; $p = 0.037$) and the percentage plant/weed cover ($r = -0.406$; $p = 0.032$). In the case of the number of infested bunches, significant positive correlations were recorded with the mean NDVI values, average temperature, average wind speed, average rainfall, copper content within collected leaves and with the percentage sand/soil cover. Significant negative correlations, on the other hand, were recorded between bunch infestation and the prevalent wind direction, average relative humidity, average cold units, ambient light intensity and the percentage plant/weed cover (Table 2.2).

No significant correlations were recorded between leafminer infestation (moth abundance and bunch infestation) and the average radiation, average evapotranspiration, average microclimate temperature (iButton data), rock and leaf ground cover percentages, all crop related (canopy, understorey and ground level) light intensity readings and most elements associated with plant/leaf composition (with the exception of copper) (Table 2.2).

The recorded correlations do not, however, account for covariance and correlated abiotic and environmental variables.

Table 2.2: The Spearman Rank Order Correlations between leafminer infestation (adult abundance and infested bunches) and the various abiotic and environmental variables collected and recorded. Numbers in bold are indicative of significant values.

Variable category	Abiotic/environmental variable	Moths trapped (per day)		Number of infested bunches (out of 100)	
		Spearman R	***p*-value**	**Spearman R**	***p*-value**
NDVI	NDVI values (mean)	**0.6540**	**0.0002**	**0.4433**	**0.0388**
ARC weather station data	Temperature	0.2887	0.1362	**0.6075**	**0.0027**
	Radiation	0.0511	0.7964	-0.3282	0.1359
	Wind speed (m/s)	0.3227	0.0940	**0.6075**	**0.0027**
	Wind direction	-0.0379	0.8482	**-0.4823**	**0.0230**
	Rainfall (mm)	0.0177	0.9286	**0.4329**	**0.0442**
	Relative humidity	-0.1104	0.5761	**-0.4823**	**0.0230**
	Evapotranspiration	0.0768	0.6976	-0.3654	0.0945
	Cold units	-0.3227	0.0940	**-0.6075**	**0.0027**
Microclimate	Relative microclimate	-0.0892	0.6516	0.3632	0.0966
Light intensity (foot candles)	Ambient	**-0.3950**	**0.0375**	**-0.6039**	**0.0029**
	Canopy	-0.2332	0.2323	-0.1044	0.6437
	Understorey	-0.2932	0.1300	-0.1816	0.4186
	Ground level	-0.0151	0.9394	-0.0585	0.7961
Leaf composition (Bemlab)	Nitrogen	-0.2287	0.2418	-0.3729	0.0874
	Phosphorus	-0.2725	0.1606	-0.1259	0.5767
	Potassium	0.1209	0.5400	0.1070	0.6354
	Calcium	0.0233	0.9064	0.0616	0.7852
	Magnesium	-0.0271	0.8913	-0.1325	0.5566
	Sodium	0.2618	0.1784	0.0693	0.7593
	Manganese	0.1705	0.3858	-0.3344	0.1283
	Iron	0.1426	0.4691	0.2202	0.3248
	Copper	0.3643	0.0567	**0.4850**	**0.0221**
	Zinc	0.1619	0.4104	-0.2205	0.3241
	Boron	0.0387	0.8452	-0.0338	0.8811
Ground cover (%)	Rock	-0.1789	0.3623	-0.0628	0.7814
	Sand/soil	0.3413	0.0755	**0.4905**	**0.0205**
	Leaf litter	-0.0791	0.6893	-0.0165	0.9419
	Plant/weed cover	**-0.4058**	**0.0322**	**-0.4601**	**0.0312**
	Abiotic (rock + sand/soil)	0.2552	0.1899	0.4090	0.0587
	Biotic (leaf litter + plant/weed)	-0.2531	0.1938	-0.4102	0.0579

Best subsets regression analyses

The best subsets regression analyses for the two indicators of leafminer infestation (male moth abundance and bunch infestation on their respective predictors) were similar (Table 2.3). The regression analysis conducted on the male moth abundance, using the best four predictors (covariates) gave R^2 = 0.443 and *Adjusted* R^2 = 0.346, which included relative percentage soil/sand cover, magnesium (leaf composition), sodium and boron. The regression analysis conducted on the number of infested bunches using the best eight predictors (covariates) gave R^2 = 0.911 and *Adjusted* R^2 = 0.856, and included mean NDVI values, percentage sand/soil cover, leaf litter cover, potassium (leaf composition), magnesium, sodium, copper and boron.

In the case of bunch infestation (the response variable assumed to be the most appropriate indication of infestation within a grapevine block), an increase in leafminer infestation was proportional to an increase in percentage sand/soil cover, sodium (leaf composition) and copper. A decrease in bunch infestation was, however, inversely proportional to an increase in the mean NDVI values (i.e. a decrease in leaf greenness resulted in an increase in the number of infested bunches or, in this case, it may be that an increase in infested bunches resulted in a decrease in leaf greenness), potassium (leaf composition), magnesium and boron. When moth abundance was considered, an increase in leafminer infestation was proportional to an increase in the percentage sand/soil cover and sodium (leaf composition). A decrease in male moth abundance was inversely proportional to an increase in magnesium (leaf composition) (i.e. as magnesium within leaves increased, moth abundance decreased) and boron. For both bunch infestation and moth abundance, infestation was affected most by the percentage sand/soil cover as well as sodium (as indicated by the most significant values). In both cases, as the percentage of soil/sand cover increased and as the sodium content within leaves increased, the leafminer infestation increased.

Table 2.3: The regression summary for the response variables (male moth abundance and bunch infestation) on their selected predictors.

Variables relevant to best subsets models (*n* = 28/22)	Regression coefficient (*b*)		*p*-value	
	Moth abundance	Infested bunches	Moth abundance	Infested bunches
Constant (intercept)	8.3936	16.6587	0.0166	0.0001
NDVI (mean)	Excluded	-11.2305	Excluded	0.0059
Sand/soil cover (%)	0.0972	0.1381	0.0006	0.0000
Leaf litter (%)	Excluded	0.0429	Excluded	0.0049
Potassium	Excluded	-5.2076	Excluded	0.0021
Magnesium	-16.0196	-20.3523	0.0955	0.0042
Sodium	0.0147	0.0198	0.0047	0.0000
Copper	Excluded	0.0975	Excluded	0.0001
Boron	-0.0935	-0.1573	0.1679	0.0016

Discussion

A key aspect of devising sound IPM strategies is to understand the interactions within the relevant agroecosystems and how the interactions affect pest populations on host plants (essentially implementing appropriate control strategies based on educated decisions) (Binns & Nyrop 1992). Few studies have, however, explored the variables that affect leafminer infestations. More often than not, leafminer pests that become of economic importance are sporadic and, as a result, are addressed by studies aimed at the identification and control of the pest. Once appropriate control strategies are established, studies generally cease. *Holocacista capensis* is a sporadic pest on table grapes in South Africa and the current study identified aspects of leaf greenness, temperature, light intensity, leaf composition and ground cover as important determinants of the severity of the leafminers' infestation in local table grape vineyards.

Despite the fact that male moth abundance (the number of moths caught per trap per day) was reasonably correlated with bunch infestation (the number of bunches infested with cocoons), it is argued, in light of the findings, that bunch infestation was a better indication of *H. capensis* infestation within a given block. More significant correlations were recorded between bunch infestation and the variables tested, suggesting the presence of confounding factors that influence the adult abundances (e.g. the ability of moths to move from a grapevine block or source populations to other locations). This is a convenient inference as the male moth attractant is not yet commercially available in South Africa and bunch infestation is relatively easy to monitor, following the standard monitoring protocol for other table grape pests (De Villiers & Pringle 2008), which is currently adopted by growers. A drawback of this approach, however, is that populations cannot be monitored post-harvest. Ideally,

monitoring would involve the sampling of bunches pre-harvest and shift to monitoring of baited traps post-harvest, necessitating the commercialization of the male attractant to determine whether management practices have been effective.

Torrance (2016) identified a possible relationship between the adopted trellising system and the corresponding leafminer infestation as a result of varying shaded conditions (although small sample sizes were acknowledged). The findings of the current study refute the previous findings based on bunch infestation and moth abundance recorded under the respective trellising systems. This finding is also supported by the correlation analyses, which indicated no specific preferences of the leafminer for varying light intensities within and below the canopy. They are, however, affected by the ambient light intensity.

The current study found that as ambient light intensity increases, leafminer abundance decreases in support of the carbon/nutrient (C/N) balance hypothesis. The C/N balance hypothesis proposes that the relative availability of carbon and nutrient resources, within a given crop, significantly influences the carbon-based allelochemical ratios found within plant tissues by the adjustment of the plants' carbohydrate stores (Bryant *et al*. 1983). The hypothesis states that in low-nutrient or light intense environments, plants accumulate large carbohydrate reserves (Chapin 1980), allowing the development of tannins and carbon-based phenolics once optimum growth levels have been reached (Fajer *et al*. 1992). Any increase in nutrient availability, in nutrient-poor or light intense environments would, therefore, be accompanied by a decline in carbon-based allelochemicals (Dudt & Shure 1994).

The investigation into the effect of cultivar colour type on leafminer infestation yielded interesting results. Although no significant relationships were recorded, an overall trend of white-berried cultivars being less susceptible to infestation was noted for both of the response variables (moth abundance and more so for bunch infestation). This suggests that differences may lie between specific cultivars rather than the predominant cultivar colour types as a result of taxonomic and evolutionary deviations between *Vitis vinifera* L. varieties (including native, wine and ornamental varieties in addition to table grape cultivars) as established by Straw & Tilbury (2006), Fermaud (1998) and Every *et al*. (1998) on other crop and grain pests. Additional factors associated with plant quality, such as female preferences for suitable oviposition sites and plant volatiles, are likely to play a role in host plant selection.

When each of the variables recorded in this study were correlated independently to the dependent/response variables, strong significant correlations were recorded. This did not, however, account for collinearity among the variables or predictors. As a result, the regression analysis was able to establish collinearity between the variables and select the most valuable predictors in terms of the severity of *H. capensis* infestations, regardless of causation. The regression analysis identified the

percentage sand/soil cover as well as the sodium content of leaves as the most important variables affecting both moth abundance and bunch infestation.

In line with a review on habitat management by Landis *et al*. (2000) and Ratnadass *et al*. (2012), it is theorized that an increase in the percentage of sand/soil (i.e. ground) cover decreases the availability of environments that would otherwise be used as a refuge for parasitoid wasps of leafminer larvae and pupae (e.g. mulched and crop covered inter-rows). Thus, as a result of decreased biodiversity, it is likely that in the case of *H. capensis*, the assemblages of beneficial insects become fewer, allowing leafminer populations to thrive. This will, however, require further investigation into parasitoid assemblages in relation to *H. capensis* infestations in vineyards and corresponding cover crop management.

According to Maathuis (2013), low levels of sodium can be beneficial to plants, especially when potassium is deficient, although the presence of sodium is not essential for most plants. Despite the presence of salt-tolerant grapevine varieties (also dependent on rootstocks used), high concentrations of sodium (Na^+) and the presence of chloride (Cl^-) (concentrations of which are often associated with irrigation water used in water deficit and/or adverse conditions experienced in a grapevine season) can, however, cause sodium stress (soil salinity), which inevitably decreases plant growth (Storey *et al*. 2003; Paranychianakis & Angelakis 2008; Maathuis 2013). A study by Paranychianakis & Angelakis (2008) concluded that salt content within leaves alone cannot be used as an indication of salt-tolerant grapevine varieties and that salinity-induced leaf damage was linked directly with prevailing environmental conditions experienced within a growing season. From the current study it can only be speculated that infestation was affected by sodium content (and thus, perhaps as salt stress in plants increased, their susceptibility to leafminer invasion increased). The exact mechanisms behind this phenomenon are not yet known and the presence of leafminers in infested vineyards could be coincidental to confounding factors such as drought stress (experienced in the Western Cape in prevailing years) or altered insecticide spray programmes.

Interestingly, insect infestations can be monitored through the computation of NDVI values, as in the case of the mountain pine beetle (Goodwin *et al*. 2008; Coops *et al*. 2010), gypsy moth (Townsend *et al*. 2004), jack pine budworm (Radeloff *et al*. 1999) and Siberian silk moth (Kharuk *et al*. 2007). Van Nieukerken & Geertsema (2015) speculated that the effect of the presence of leaf mines does not affect the photosynthetic ability of grapevines, although significant variation in leaf greenness (as a result of leafminer infestation) suggests otherwise and studies should be focused on this aspect in the future. The only downfall of using satellite imagery is that monitoring is dependent on the quality of the image (dependent on cloud cover and presence of fire at the time the image is recorded) and the frequency at which fine resolution can be recorded (every 10 days using Sentinel-2 satellites). The

time and costs associated with obtaining, processing and calibrating individual fine resolution images can also be excessive. In addition, this monitoring approach is only relevant to table grape farming practices and cannot be used to monitor leafminer infestations on wine producing grapevines due to planting strategies used in the field (i.e. the parallel trellising systems). As the uses of this technology are becoming increasingly adopted (Wulder *et al*. 2012), however, the constraints on its use could potentially be minimized through further development.

It is evident that a variety of abiotic factors and environmental variables are responsible for fluctuations in *H. capensis* leafminer infestations. To pinpoint these variables as definite indicators of infestation, or the severity thereof, is challenging due to the myriad of varying conditions in each table grape producing block. Other factors, such as human mediated means of dispersal and the influence of surrounding source populations, may also be important aspects to consider. In an agricultural system where conditions are never static, it is apparent that confounding variables are at play and more rigorous studies are necessary to assess the presence, absence and fluctuations in leafminer abundance (not only that of *H. capensis*) in the agricultural context. The current study was able to reveal the importance of ground cover and leaf composition in the abundance of leaf-mining insects. The manipulation of these variables in an IPM strategy may prove to reduce leafminer infestation levels. The results suggest that actively encouraging diversity of ground cover within the inter-rows of infested vineyards may benefit management strategies, although the exact mechanisms at play are not presently clear and require further study. In terms of leaf composition, increasing sodium levels (potentially associated with salt stress within vines), yielded higher leafminer infestations. This indicates that appropriate soil management could alter leafminer abundances. This too, however, requires a more detailed research focus. Future studies should be therefore be focused on understanding the underlying mechanisms controlling these associations.

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Chapter 3

Molecular Study of *Holocacista capensis*

A survey of *Holocacista capensis* (Lepidoptera: Heliozelidae) in vineyards and natural forests within the Western Cape and surrounding provinces in the Western and Eastern regions of southern Africa.

Introduction

The 12 monotrysian Heliozelidae genera (Lepidoptera: Adeloidea) are found on most continents, comprising of 125 described species (Van Nieukerken *et al*. 2011). The family is characterised by small, drab, diurnal moths, most of which facilitate a leaf-mining larval stage (Van Nieukerken *et al*. 2011; 2012; Regier 2015; Van Nieukerken & Geertsema 2015; Milla *et al*. 2018). A study by Milla *et al*. (2018) presented the first global phylogenetic framework of the Heliozelidae. Using two mitochondrial (COI and COII) and two nuclear (H3 and 28S) genes they identified five major monophyletic clades (*Coptodisca*, *Holocacista*, *Antispilina*, *Pseliastis* and *Hoplophanes*) and a polyphyletic group (*Antispila*) within the Heliozelidae. The relationships between the clades, however, remain unresolved as these relationships lacked significant statistical support. To resolve relationships between clades that diverged in the Late Cretaceous, it would be necessary to increase the number of nuclear genes in order to resolve older nodes by providing more accurate phylogenetic information. Interestingly, they suggest that the family is of southern hemisphere origin as the majority of undescribed diversity (at the genus and species level) occurred in the southern hemisphere.

The African Heliozelidae fauna is restricted to four known species described from South Africa. The species include *Antispila argyrozona* Meyrick, 1918, *Holocacista salutans* Meyrick, 1921, *Antispilina varii* Mey, 2011 and *Holocacista capensis* Van Nieukerken & Geertsema (2015). Unlike the former, *H. capensis* is a multivoltine, leaf-mining pest of economic concern occurring on table grapes (Van Nieukerken & Geertsema 2015). The leaf-mining larvae of *H. capensis* feed between the epidermal layers, predominantly along the leaf margin of an infested leaf (Van Nieukerken & Geertsema 2015). A final instar larva will descend from the blotch mine/gallery to attach its cocoon casing (constructed from the epidermal layers of the mined gallery) to any object below the infested leaf, including berry bunches. Due to phytosanitary concerns, the presence of cocoon casings on bunches can lead to the rejection of consignments for export, making the leafminer a pest of economic importance on table grapes.

The pest was reported for the first time in 2012 on commercial and ornamental varieties of *Vitis vinifera* L. (Vitaceae) in the surroundings of Paarl in the Western Cape province, South Africa (Van Nieukerken & Geertsema 2015). The Cape grapevine leafminer, as it is otherwise known, is thought to have undergone a host-plant switch from the native Vitaceae (for example, *Rhoicissus* Planch. and *Cissus* L. species) to commercial and ornamental varieties of *Vitis vinifera* L. (Vitaceae) (Van Nieukerken & Geertsema 2015). Van Nieukerken & Geertsema (2015) also reported the leafminer on *Rhoicissis* in the vicinity of Wilderness in the Western Cape. Torrance (2016) identified several *H. capensis* populations in three table grape producing regions within the Western Cape. High population numbers were detected in the Berg River, the Hex River and the Olifants River regions. However, no wild populations were detected in the study. In addition, the identities amongst the collected individuals were not confirmed as being solely *H. capensis*. As a result, the populations of different origins have not yet been synonymised.

The aim of the current work was to confirm the identity of *H. capensis* amongst collected moth specimens and to gain a preliminary understanding of the genetic relationships and diversity that exist between leafminer populations from diverse locations. An exploratory study of the genetic diversity within *H. capensis* populations was conducted, using two mitochondrial and two nuclear genes (Milla *et al*. 2018), to gain a clearer idea of the true origin of the pest and the genetic variation currently present within pest populations. If genetically distinct pest populations are present the management strategies adopted in the future may be affected. As a result, the findings of this preliminary study will aid in decisions associated with the development of control strategies.

Materials and methods

Surveying natural forests and commercial vineyards

Holocacista capensis individuals collected in a previous study by Torrance (2016) were used in conjunction with individuals collected as part of a survey of the natural forests in and around the Western Cape of South Africa between December 2017 and May 2018. At least one baited yellow Delta Trap lined with a sticky pad (Chempac, Pty Ltd., Paarl) was placed in each of the sampled areas (Table 3.1). The bait/attractant dispensers (Wang *et al*. 2015) were synthesised and supplied by Lund University, Sweden. Ad-hoc sampling was also conducted in Halfmanshof, Riebeeck Kasteel, Robertson, George and a variety of grape producing areas within the Northern Cape province (Table 3.1).

Table 3.1: A list of trapping locations to detect *Holocacista capensis* male moths since the pest was first reported in 2012. Specimens from locations in bold were included in the molecular analysis.

Province	Region/area	Area/Town	Traps	Vicinity	> 2 moths
Western Cape	Berg River	George	1	33°49'09.1"S 22°21'41.2"E	No
		Halfmanshof	**> 5**	**33°08'44.8"S 18°59'10.3"E**	**Yes**
		Piketberg	**2**	**32°58'44.8"S 18°44'55.9"E**	**Yes**
		Porterville	**1**	**33°00'38.6"S 19°00'47.6"E**	**Yes**
		Riebeeck Kasteel	**3**	**33°23'00.3"S 18°54'36.5"E**	**Yes**
		Tulbagh	**2**	**33°17'30.8"S 19°05'18.6"E**	**Yes**
		Wellington	**2**	**33°35'48.2"S 18°58'33.4"E**	**Yes**
		Wolseley	**1**	**33°24'47.7"S 19°14'06.7"E**	**Yes**
	Hex River	Ashton	1	33°49'23.2"S 19°58'41.0"E	No
		Bonnievale	2	33°51'46.1"S 19°59'12.8"E	No
		De Doorns	> 5	33°30'12.1"S 19°35'59.6"E	No
		McGregor	**1**	**33°54'11.6"S 19°52'30.7"E**	**Yes**
		Robertson	**2**	**33°50'19.0"S 19°54'52.3"E**	**Yes**
	Olifants River	Klawer	2	31°45'26.7"S 18°33'49.5"E	No
		Trawal	1	31°53'13.2"S 18°37'47.3"E	No
		Vredendal	**1**	**31°41'21.2"S 18°30'20.3"E**	**Yes**
Northern Cape	Orange River	Augrabies	3	28°42'4.58"S 20°27'25.90"E	No
		Blouputs	3	28°28'55.62"S 20° 7'13.08"E	No
		Friersdale	1	28°43'49.29"S 20°44'43.82"E	No
		Kakamas	3	28°45'42.46"S 20°33'52.29"E	No
		Kanon Eiland	1	28°39'18.45"S 21° 6'55.86"E	No
		Upington	2	28°24'24.18"S 21°19'34.60"E	No
Western Cape	Natural forests	Heidelberg	1	33°59'20.2"S 20°49'24.4"E	No
		Harkerville	1	34°03'08.2"S 23°14'08.6"E	No
		Knysna	1	33°59'30.0"S 23°07'25.0"E	No
		Natures Valley	2	33°58'20.1"S 23°33'02.2"E	No
		Rheenendal	2	33°54'31.5"S 22°57'49.9"E	No
		Riviersonderend	5	34°04'41.9"S 19°49'47.1"E	No
		Sedgefield	2	34°01'51.4"S 22°50'18.1"E	No
		Stellenbosch	**2**	**33°59'24.8"S 18°56'16.7"E**	**Yes**
		Suurbraak	1	34°00'07.4"S 20°37'46.6"E	No
		Wilderness	1	33°59'46.4"S 22°33'42.1"E	No
Eastern Cape		Storms River	3	33°59'51.7"S 23°57'21.7"E	No

Retrieval of male moths from sticky pads

Male moths were manually extracted from the sticky pads. A small square of the sticky pad, containing the specimen, was cut out of the trap/pad and placed in a small pool of eucalyptus oil (Miller *et al*. 1993). A small paintbrush (Prime Art *Bianco* R 000) was used to ease the specimen from the sticky trap and care was taken to remove as much of the sticky trap adhesive as possible without the loss of antennae, legs or wing scales. The processed samples were stored in absolute ethanol (96% - 99%) before DNA extraction. DNA was obtained and analysed for each specimen.

Molecular identification

Genomic DNA extraction was performed using a Quick-DNA Miniprep Plus Kit (Zymo Research) according to the manufacturer's instructions. The amount of DNA (ng/µl) in the final product was measured for each specimen using a Spectrophotometer ND-1000 (NanoDrop Technologies) to confirm successful DNA extraction.

The amplification of four genes (COII, COI, H3 and 28S) was carried out using four different primer pairs for the molecular identification of the collected male specimens (Table 3.2). The final PCR reaction mixtures contained 10 µl OneTaq 2x master mix (NEB) with standard buffer, 1 µl of the extracted genomic DNA (10 – 30 ng/µl), 1 µl forward primer (10 µM), 1 µl reverse primer (10 µM) and 7 µl nuclease free water (NEB). PCR protocol conditions for each of the primer pairs (COI, COII, 28S and H3) included an initial denaturation of 94°C for 30 s; then 35 cycles of 94°C for 30 s, annealing at 50°C for 30 s, extension at 68°C for 1 min; a final elongation of 68°C for 10 min and a hold at 4°C. For each PCR run, a positive (known specimen) and negative (deionized water) control was included, to ensure that there was no contamination or error made during the amplification.

Table 3.2: The gene amplification primers used for the PCR analysis adapted from Milla *et al*. (2018).

Gene	Primer name	Direction	Primer sequence (5'-3')	Reference
COI	LepF1	Forward	ATTCAACCAATCATAAAGATATTGG	Herbert *et al*. (2003)
	LepR1	Reverse	TAAACTTCTGGATGTCCAAAAAATCA	Herbert *et al*. (2003)
COII	COIIF	Forward	GGAGCATCTCCTTTAATAGAACA	Sperling *et al*. (1995)
	COIIR	Reverse	GAGACCATTACTTGCTTTCGATCATCT	Caterino & Sperling (1999)
28S	28SF	Forward	GAGAGTTMAASAGTACGTGAAAC	Downton & Austin (1998)
	28SR	Reverse	TCGGARGGAACCAGCTACTA	Whiting *et al*. (1997)
H3	H3HEXAF	Forward	ATGGCTCGTACCAAGCAGACGGC	Ogden & Whiting (2003)
	H3HEXAR	Reverse	ATATCCTTGGGCATGATGGTGAC	Ogden & Whiting (2003)

PCR amplicons were run on a 1% agarose gel (CSL-AG500, Cleaver Scientific Ltd) stained with EZ-vision® Bluelight DNA Dye (Amresco) to confirm successful amplification. Post-PCR products were enzymatically purified using the ExoSAP master mix [prepared by combining 50 µl Exonuclease I (NEB) 20 U/µl and 200 µl Shrimp Alkaline Phosphatase (NEB) 1 U/µl]. The reaction mixture was prepared by combining 10 µl of the amplified PCR product and 2.5 µl ExoSAP master mix, mixed and incubated at 37°C for 30 min and then at 95°C for 5 min. The purified fragments were sequenced in the forward direction (BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000, Nimagen) and purified (ZR-96 DNA Sequencing Clean-up Kit™, Zymo Research). The purified fragments were analyzed on the ABI 3500xl Genetic Analyzer (Applied Biosystems, Thermo Scientific) with a 50 cm array, using POP7 Polymer (Applied Biosystems). Thereafter, sequence chromatogram analysis was performed using FinchTV analysis software (Geospiza). All molecular analyses were conducted at inqaba biotechnical Industries (Pty) Ltd., Pretoria, South Africa.

DNA analysis

The DNA sequences were aligned and edited in CLC Main Workbench v8.0.1 (QIAGEN Bioinformatics, Denmark). Sequences were subjected to a BLAST search (Altschul *et al*. 1997), performed in GenBank® nucleic acid sequence database via the National Center for Biotechnology Information (NCBI) (U.S. National Library of Medicine, Rockville Pike, USA), to determine the closest sequence match. To indicate the phylogenetic position of the male moths collected during the survey, 80 sequences were compared to other Heliozelidae sequences from NCBI (Table 3.3). Phylogenetic and molecular analyses were conducted based on maximum likelihood (ML) and maximum parsimony (MP) using MEGA7 (Kumar *et al*. 2016).

Maximum likelihood phylogenetic trees, based on the Jukes-Cantor model (Jukes & Cantor 1969), inferred from each of the genes was generated. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances, estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. For the COI, COII, H3 and 28S genes the analysis involved 44, 23, 55 and 24 nucleotide sequences, respectively, with all positions containing gaps and missing data eliminated. There were 133, 373, 201 and 331 positions in the final dataset, for each of the respective gene sequence datasets.

A single outgroup was selected for the purposes of this study, from the Nepticulidae family, based on outgroup selections made by Milla *et al*. (2016). The number of haplotypes (the probability of two haplotypes from the same sample being different when randomly selected), gene diversity (the gene diversity within each sample) and nucleotide diversity (the level of polymorphism within the population) were calculated using ARLEQUIN v3.5.2.2 (Excoffier & Lischer 2010).

Results

Survey and identification

The DNA of 52 male moths was extracted and used for sequencing (Table 3.3). Optimisation in the laboratory ensured the acquisition of 32 COI gene sequences, three COII gene sequences, 44 H3 gene sequences and a single 28S gene sequence representing an individual from Robertson.

Table 3.3: *Holocacista capensis* sequences generated from forested habitats and table grape producing regions in the surroundings of the Western Cape, South Africa, for four mitochondrial and nuclear genes (COI, COII, H3 and 28S). Where no sequence could be obtained for a specimen, it is indicated by '-'. Some COI sequences did not positively identify with *H. capensis* in the BLAST search and are referred to as "Sequencing Error" here. Reference sequences and outgroups, used to compile phylogenetic trees (downloaded from Genbank®) are presented last.

Specimen Code	Origin/Species	GENBANK® ACCESSION NUMBERS			
		COI	COII	H3	28S
4	Riebeeck Kasteel	Genbank pending	Genbank pending	Genbank pending	-
5	Riebeeck Kasteel	Genbank pending	Genbank pending	Genbank pending	-
14	Riebeeck Kasteel	Genbank pending	-	Genbank pending	-
15	Riebeeck Kasteel	Sequencing Error	-	-	-
16	Riebeeck Kasteel	-	-	Genbank pending	-
17	Riebeeck Kasteel	-	-	Genbank pending	-
25	Piketberg	Sequencing Error	-	-	-
38	Piketberg	Genbank pending	-	Genbank pending	-
40	Piketberg	Sequencing Error	-	-	-
41	Tulbagh	Genbank pending	-	-	-
45	Tulbagh	Genbank pending	-	Genbank pending	-
46	Tulbagh	Sequencing Error	-	Genbank pending	-
48	Tulbagh	-	-	Genbank pending	-
53	Tulbagh	Sequencing Error	-	Genbank pending	-
63	Porterville	Genbank pending	-	Genbank pending	-
67	Porterville	Sequencing Error	-	-	-
70	Porterville	Genbank pending	-	Genbank pending	-
74	Porterville	Genbank pending	-	Genbank pending	-
75	Porterville	Genbank pending	-	Genbank pending	-
85	Vredendal	Genbank pending	-	Genbank pending	-
86	Vredendal	Genbank pending	-	Genbank pending	-
91	Vredendal	Genbank pending	-	Genbank pending	-
93	Vredendal	Genbank pending	-	Genbank pending	-
94	Vredendal	Genbank pending	-	Genbank pending	-
95	Vredendal	-	-	Genbank pending	-
97	McGregor	Genbank pending	-	Genbank pending	-
99	McGregor	Genbank pending	-	Genbank pending	-
101	McGregor	Genbank pending	-	Genbank pending	-
102	McGregor	-	-	Genbank pending	-
103	McGregor	Sequencing Error	-	Genbank pending	-
105	Robertson	-	-	Genbank pending	-
112	Robertson	Genbank pending	-	Genbank pending	-
113	Robertson	Genbank pending	-	Genbank pending	MK213721
117	Robertson	Genbank pending	-	Genbank pending	-
120	Robertson	Genbank pending	-	Genbank pending	-
121	Robertson	Genbank pending	-	Genbank pending	-
132	Halfmanshof	Genbank pending	-	Genbank pending	-
133	Halfmanshof	-	-	Genbank pending	-
134	Halfmanshof	Genbank pending	-	Genbank pending	-
140	Halfmanshof	Genbank pending	-	Genbank pending	-
144	Halfmanshof	Genbank pending	-	Genbank pending	-

Table 3.3: continued.

Specimen Code	Origin/Species	GENBANK® ACCESSION NUMBERS			
		COI	**COII**	**H3**	**28S**
145	Wolseley	Genbank pending	-	Genbank pending	-
147	Wolseley	-	-	Genbank pending	-
148	Wolseley	-	-	Genbank pending	-
150	Wolseley	-	-	Genbank pending	-
155	Jonkershoek	Genbank pending	-	Genbank pending	-
156	Jonkershoek	Genbank pending	-	Genbank pending	-
160	Jonkershoek	Genbank pending	-	Genbank pending	-
161	Paarl	Genbank pending	Genbank pending	Genbank pending	-
Reference Sequences	*Holocacista capensis*	MF118292	MF118386	MF118505	MF118208
	Holocacista capensis	MF118321	MF118411	MF118477	MF118236
	Holocacista rivillei	MF118323	MF118413	MF118507	MF118238
	Holocacista varii	MF118340	MF118429	MF118524	MF118254
	Antispila ampelopsia	-	-	MF118536	-
	Antispila ampelopsifoliella	-	MF118397	-	MF118221
	Antispila argentifera	-	MF118374	-	MF118193
	Antispila cleyerella	MH094884	MF118407	MF118500	MF118231
	Antispila cornifoliella	-	MF118391	-	MF118215
	Antispila distyliella	-	-	-	MF118233
	Antispila hydrangaeella	-	MF118370	-	MF118188
	Antispila metallella	-	MF118395	-	-
	Antispila oinophylla	-	-	-	MF118200
	Antispila uenoi	MH094881	MF118420	MF118515	MF118246
	Antispila viticordifoliella	-	MF118385	-	MF118207
	Antispila voraginella	-	-	-	MF118199
	Antispilina varii	KP697806	-	-	-
	Heliozela aesella	MG363374	-	-	-
	Heliozela eucarpa	MF118313	MF118405	MF118498	-
	Heliozela resplendella	-	-	-	MF118210
	Heliozela sericiella	MF118293	-	MF118478	MF118209
	Coptodisca juglandiella	MG361333	-	MF118452	MF118183
	Coptodisca lucifluella	-	MF118393	-	MF118217
	Coptodisca ostryaefoliella	-	MF118368	-	MF118186
	Coptodisca quercicolella	-	MF118418	-	MF118244
	Coptodisca splendoriferella	-	MF118376	-	MF118195
	Pseliastis spectropa	-	MF062320	-	-
	Pseliastis xanthodisca	-	-	-	MF062411
	Tyriozela porphyrogona	-	MF171067	-	-
Outgroup	*Ectoedemia olvina*	KM077659	KM078269	KM078570	KM078456

Positive *H. capensis* BLAST results were obtained for 45 of the individuals sequenced (Table 3.3). Seven specimens ["*H. capensis*" 15 (Riebeeck Kasteel), "*H. capensis*" 25 (Piketberg), "*H. capensis*" 40 (Piketberg), "*H. capensis*" 46 (Tulbagh), "*H. capensis*" 53 (Tulbagh), "*H. capensis*" 67 (Porterville) and "*H. capensis*" 103 (McGregor) – all noted as "*Sequencing Error*" in Table 3.3] did not return as *H. capensis* when blasted using the COI gene alone. These individuals identity rather returned the closest match as *Amblyomma pattoni* with 85% BLAST identity (Table 3.4). Interestingly, however, sequences from H3 for three of the specimens (15, 46, 103), resulted in a positive match to *H. capensis* through NCBI using the same extracted DNA products used for the COI gene sequencing analysis.

Table 3.4: The sequences that displayed a "sequencing error" on COI gene sequences.

Specimen Code	Origin	COI sequence	Closest BLAST result	BLAST coverage	BLAST identity
15	Riebeeck Kasteel	GGTTTTGGTAATTGGCTAGTTCCTATTATACTTGGAGC TCCAGATATGGCTTTTCCTCGTATAAATAATATAAGAT TTTGGTTACTCCCACCCTCTTTATGTCTTTTAATTAATT CATCATTAGTAGAATCAGGAGCAGGAACTGGATGAAC AGTTTATCCTCCTTTATCATCAAATTTATCTCATTATGG ACCTTCTGTAGATATAGCTATCTTTTCCCTTCATTTAGC AGGTGCATCATCAATTCTAGGTTCAATTAATTTTATTA CTACTATTATTAACATACGATCAATTGGCATAACTATA GAACGCGTTCCCTTATTCGTTTGATCAGTTTTAACAAC CACAATTCTTTTACTTCTTTCGCTACCAGTATTAGCAGG GAGCAATTACAATGCTATTAACTGATCGTAATTTTAAT ACTTCATTTTTTGATCCTTCAGGAGGTGGAGATCCTAT TTTATATCAACATTTATTTTGATTTTTTGGAC	*Amblyomma pattoni* HM193876.1 (Chelicerata, Ixodidae)	99%	85%
25	Piketberg	GGTTTTGGTAATTGGCTAGTTCCTATTATACTTGGAGC TCCAGATATGGCTTTTCCTCGTATAAATAATATAAGAT TTTGGTTACTCCCACCCTCTTTATGTCTTTTAATTAATT CATCATTAGTAGAATCAGGAGCAGGAACTGGATGAAC AGTTTATCCTCCTTTATCATCAAATTTATCTCATTATGG ACCTTCTGTAGATATAGCTATCTTTTCCCTTCATTTAGC AGGTGCATCATCAATTCTAGGTTCAATTAATTTTATTA CTACTATTATTAACATACGATCAATTGGCATAACTATA GAACGCGTTCCCTTATTCGTTTGATCAGTTTTAACAAC CACAATTCTTTTACTTCTTTCGCTACCAGTATTAGCAGG AGCAATTACAATGCTATTAACTGATCGTAATTTTAATA CTTCATTTTTTGATCCTTCAGGAGGTGGAGATCCTATTT TATATCAACATTTATTTTGATTTTTTGGAC			
40					
46	Tulbagh				
53					
67	Porterville				
103	McGregor				

Mitochondrial and nuclear gene analysis

The phylogenetic analyses of the mitochondrial COI and COII genes (Fig. 3.1 and Fig. 3.2, respectively) indicate that the specimens collected in each of the table grape producing areas fall within a well-supported clade and can be regarded as the same species. Three individuals, noticeable in the COI phylogeny (Fig. 3.1), however, formed a sub-group within the phylogenetic tree. The individuals are not from the same sampling site and there are representative individuals from the same areas that do not fall within this sub-group. This finding is not supported by the phylogenetic tree created using the nuclear H3 gene, which confirms equal synonymy between all of the specimens sampled and contains no sub-groups (Fig. 3.3).

The deviations noted when analysing the COI gene were not recorded in any of the other phylogenetic analyses, but the general trend indicates that the individuals collected from each of the respective regions (including the individuals collected from natural forests) fall within a well-supported clade. Although fewer sequences were available for the mitochondrial COII gene (Fig. 3.2) and the nuclear 28S gene (Fig. 3.4), it is clear that the sequences confer with the more populated COI and H3 phylogenies, denying differences between geographic origin or habitat, and can be synonymised with the existing *H. capensis* samples recorded on NCBI.

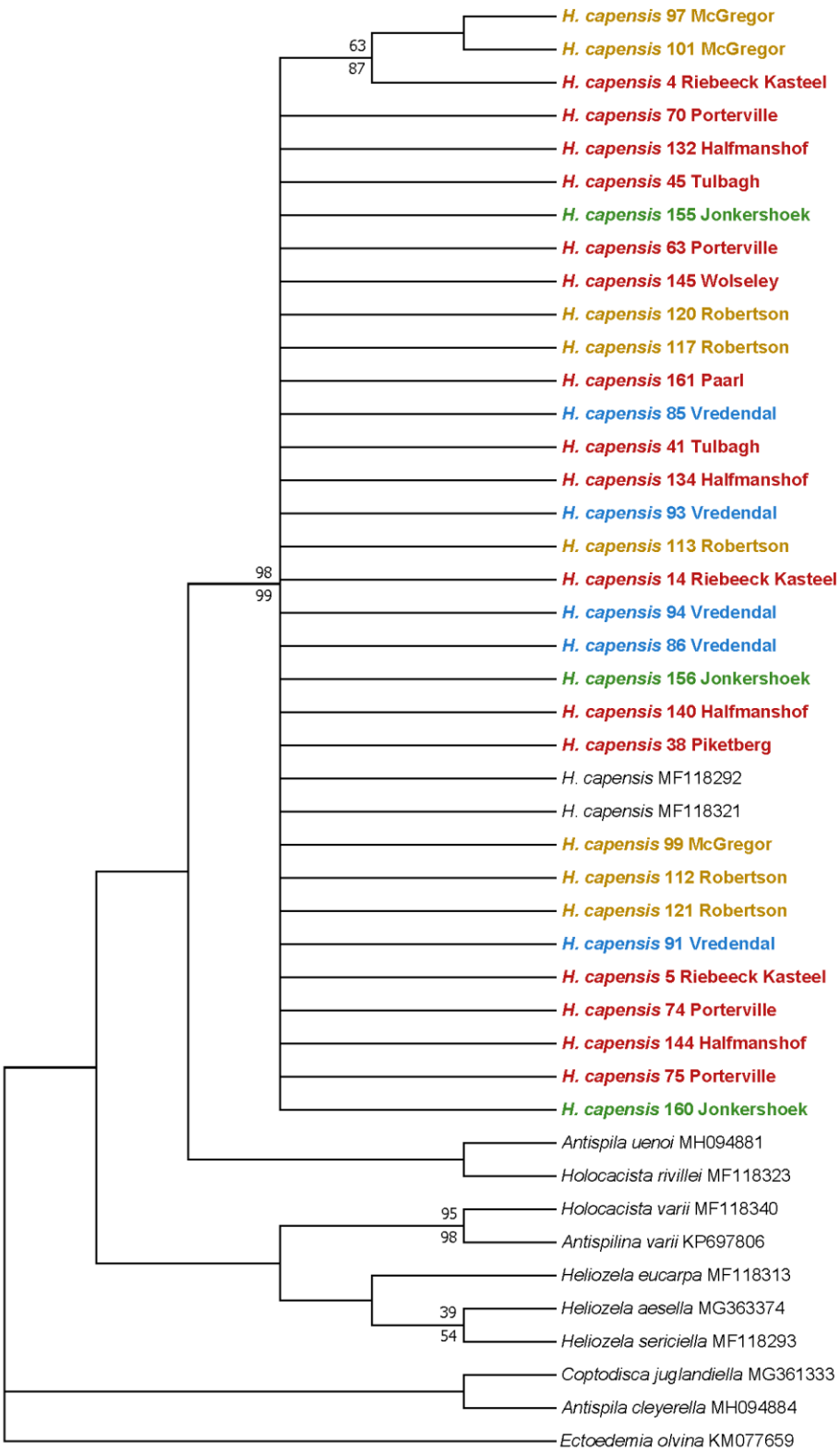

Figure 3.1: A Maximum Likelihood (ML) phylogenetic tree (highest log likelihood = -798.89) showing the relationship of *Holocacista capensis* with other species selected from Genbank® for the mitochondrial COI gene. Numbers at the nodes represent bootstrap proportions (50% or more, 1000 replicates) for ML (top) and Maximum Parsimony (bottom) (Felsenstein 1985). Specimens collected from the table grape producing regions, Berg River (red), Hex River (orange), Olifants River (blue) and natural forests (green) are represented in the tree. *Ectoedemia olvina* was selected as an outgroup.

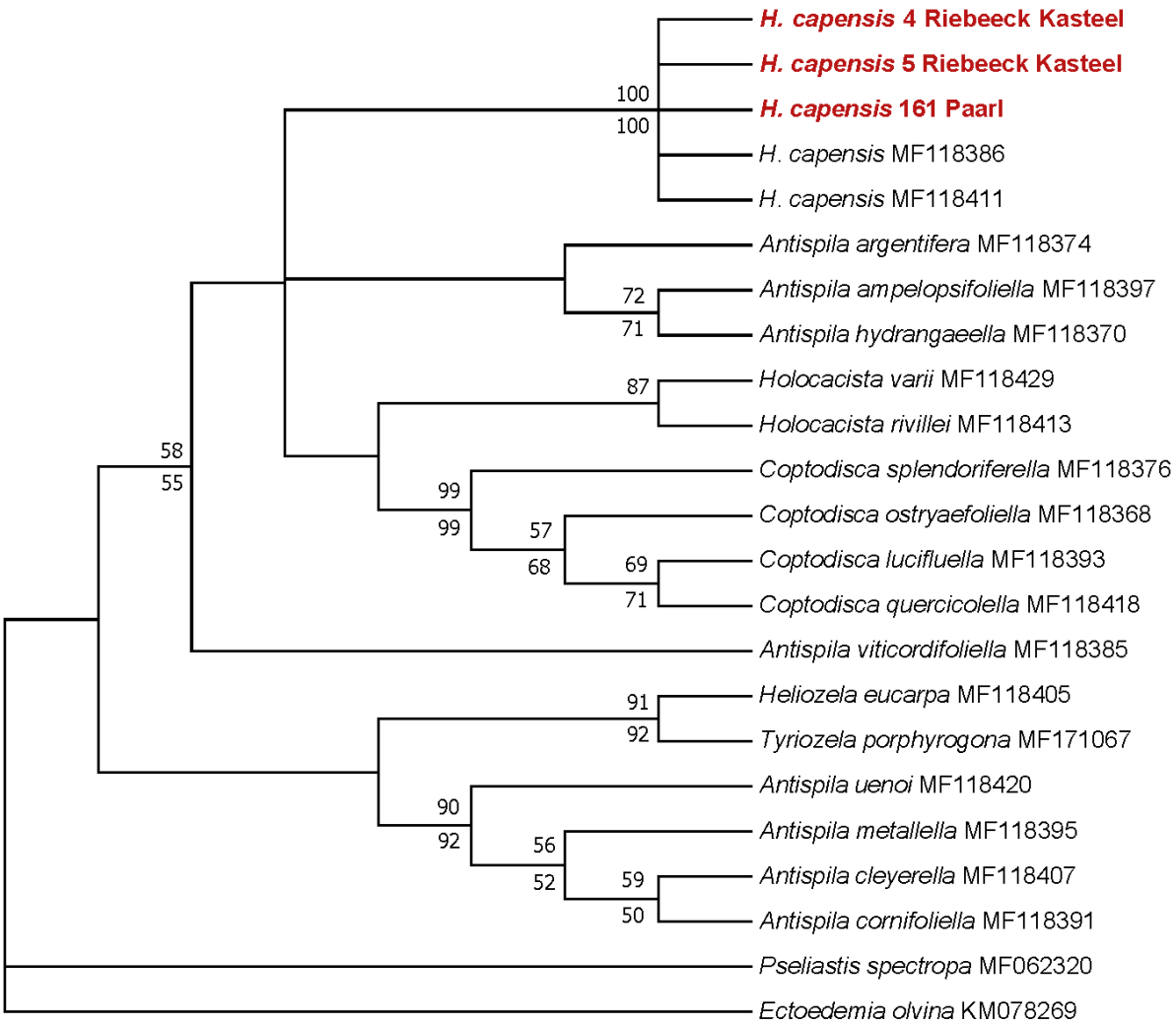

Figure 3.2: A Maximum Likelihood (ML) phylogenetic tree (highest log likelihood = -3640.60) showing the relationship of *Holocacista capensis* with other species selected from Genbank® for the mitochondrial COII gene. Numbers at the nodes represent bootstrap proportions (50% or more, 1000 replicates) for ML (top) and Maximum Parsimony (bottom) (Felsenstein 1985). Specimens collected from the Berg River table grape producing region (red) are represented in the tree. *Ectoedemia olvina* was selected as an outgroup.

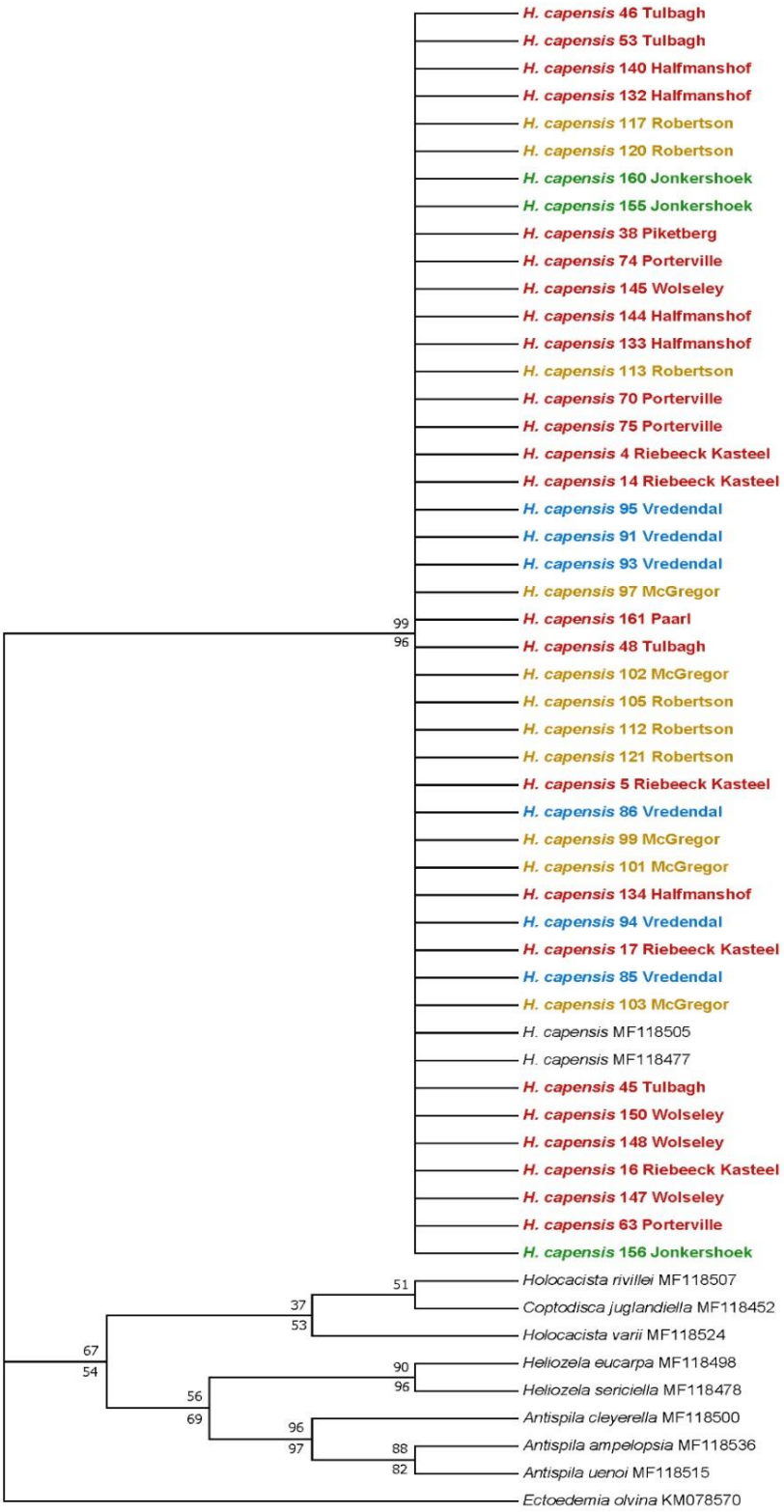

Figure 3.3: A Maximum Likelihood (ML) phylogenetic tree (highest log likelihood = -773.86) showing the relationship of *Holocacista capensis* with other species selected from Genbank® for the nuclear H3 gene. Numbers at the nodes represent bootstrap proportions (50% or more, 1000 replicates) for ML (top) and Maximum Parsimony (bottom) (Felsenstein 1985). Specimens collected from the table grape producing regions, Berg River (red), Hex River (orange), Olifants River (blue) and natural forests (green) are represented in the tree. *Ectoedemia olvina* was selected as an outgroup.

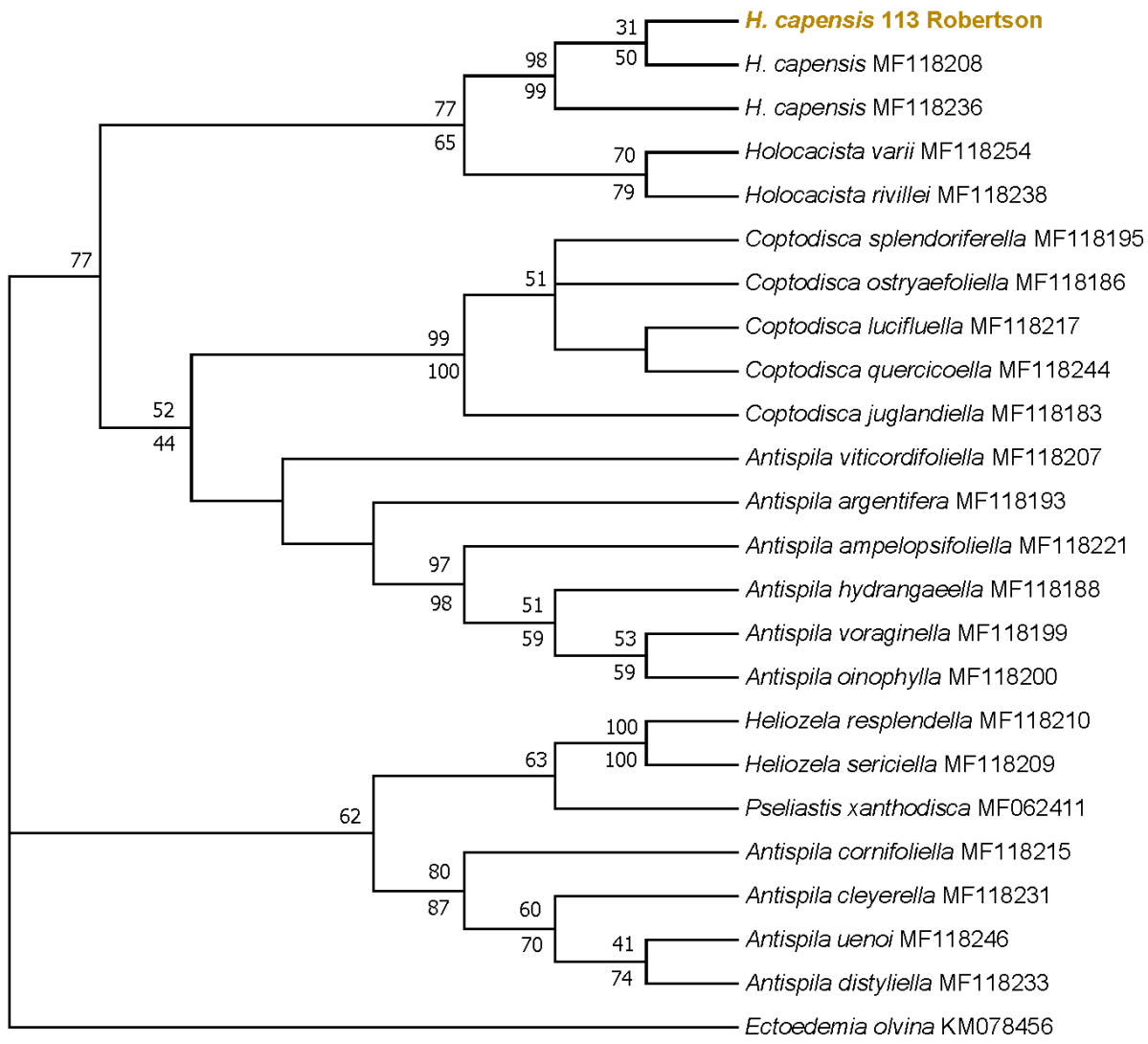

Figure 3.4: A Maximum Likelihood (ML) phylogenetic tree (highest log likelihood = -1798.45) showing the relationship of *Holocacista capensis* with other species selected from Genbank® for the nuclear 28S gene. Numbers at the nodes represent bootstrap proportions (50% or more, 1000 replicates) for Maximum Likelihood (top) and Maximum Parsimony (bottom) (Felsenstein 1985). Specimens collected from the Hex River table grape producing region (orange) are represented in the tree. *Ectoedemia olvina* was selected as an outgroup.

COI genetic diversity

Based on COI sequences for 28 *H. capensis* specimens the gene (0.262 ± 0.102) and nucleotide (0.002 ± 0.001) diversity was low. Eight polymorphic loci and three haplotypes were recorded (Table 3.5). Haplotype 1 (H1) was the most frequently encountered and accounted for approximately 87.5% of all of the specimens used in the COI phylogenetic assessment. Haplotype 2 (H2) was recorded from McGregor and Riebeeck Kasteel in addition to H1, whilst haplotype 3 (H3) occurred only in Vredendal in addition to H1. All other areas (Piketberg, Tulbagh, Porterville, Robertson, Halfmanshof, Wolseley, Jonkershoek and Paarl) were limited to H1.

Table 3.5: The eight polymorphic sites of the three COI haplotypes of *Holocacista capensis*.

Haplotype	**Site**							
	2	**8**	**81**	**89**	**290**	**329**	**549**	**562**
H1	C	G	G	T	G	G	A	C
H2	T	A	.	C	A	A	G	T
H3	.	.	A	.	.	.	.	.

Discussion

The identity of *H. capensis* in all of the detected populations within each of the table grape producing regions and natural environments has been confirmed by this study. This confirmation permitted the preliminary investigation of the genetic relationships and diversity between existing *H. capensis* leaf-mining populations within these environments.

The low genetic diversity, based on gene/nucleotide diversity and the presence of a few, and seemingly closely related, haplotypes amongst the collected specimens poses a conflicting result, considering the fact that *H. capensis* is thought to be a native pest. Although one can only speculate (due to the scale of the sampling efforts adopted in this study), this finding is in contrast with expectations of a theoretically native lepidopteran that would not have been exposed to typical bottleneck effects of alien and invasive insect pests (Nei *et al*. 1975; Roderick & Navajas 2003), as seen in the case of *Cameraria ohridella* Deschka & Dimić (Gracillariidae) (Lees *et al*. 2011). Unless, of course, a recent change in host-plant preferences has led to the establishment of a new pest colony and the native populations remain undetected. To gain more clarity on this particular issue, more rigorous sampling efforts, in more diverse habitats, need to be adopted in future studies.

Ball & Armstrong (2006) concluded that in the case of lymantriid lepidopteran species, DNA barcoding using COI is promising, especially for taxa that are well defined at species level. It is also true, however, that COI is a maternally inherited mitochondrial gene, and thus cannot be used as a detection tool for discerning hybridization events. In this case, sequencing the nuclear H3 gene could not validate the presence of hybridised individuals and does not reflect the same sub-grouping phenomenon noted in the COI phylogeny. The H3 gene did, however, confirm the correct *H. capensis* identification of three specimens classified as *A. pattoni* (Chelicerata, Ixodidae) using the COI gene sequences. Again, there is too little information to make sweeping statements regarding the mechanisms at play. However, it is possible that nuclear mitochondrial pseudogenes (numts) (which are essentially non-functional copies of mtDNA within the nucleus, which become specifically problematic when a short fragment of the mitochondrial COI gene is amplified) are responsible for the phenomenon noted in this study (Song *et al*. 2008).

A survey of the natural forests of the Cape yielded disappointing results. *Holocacista capensis* males were only collected from one of the natural forests in Jonkershoek (Stellenbosch) and it is possible, although improbable, that the moths were present in higher numbers in the surrounds of the potentially infested vineyards (and thus present in Jonkershoek) on the foothills of Stellenbosch Mountain. Equally puzzling, if the theory on the origin of the pest holds true, is that no genetic differences were recorded between this "native" population and individuals collected from commercial vineyards.

Interestingly, the genetic variation in *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) from its native range (South America) and the invaded countries of the Mediterranean, mirrors that of *H. capensis* in the current study and exhibited high genetic homogeneity (Cifuentes *et al*. 2011). Cifuentes *et al*. (2011) attributed this to the founder effects invading populations of *T. absoluta* underwent as an invasive species. Assefa *et al*. (2013) recorded similar findings for *Eldana saccharina* Walker (Lepidoptera: Pyralidae) within invaded regions, although considerably high genetic diversity was recorded between populations in the regions of West Africa, Ethiopia and South Africa. These studies raise burning questions regarding the true origin of *H. capensis*, which can only be answered through more rigorous sampling efforts, the identification (and collection) of native populations on natural hosts and the analysis of other genetic markers.

Despite the fact that conclusions based on the genetic relationships and diversity of *H. capensis* cannot be made, the current study has been able to identify limited variation in infested, commercial landscapes and a lack of regional genetic variation accommodates unanimous control efforts, where chemical intervention is necessary. It is evident, however, that more thorough investigations are required to acquire a more representative data set in order to establish a firm understanding of the

intricate population genetics encountered amongst native and invasive (within commercial vineyards) *H. capensis* leafminer populations. Future research efforts should include more rigorous sampling in natural and invaded landscapes and would ideally include a survey of the infested vineyards in the Gauteng province, South Africa, where the leafminer has been detected in the past. In the case of *H. capensis* specifically, it is clear that the amplification of nuclear genes is imperative to confirm mitochondrial gene phylogenies. The current study can be used as a starting point for future studies focused on establishing the relationships between *H. capensis* in their native hosts and the hosts that they have switched to.

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Chapter 4

Conventional Control Methods

A laboratory and field study of chemical and physical management strategies against *Holocacista capensis* (Lepidoptera: Heliozelidae)

Introduction

Over the last thirty years, four lepidopteran leaf-mining insects have, unexpectedly, become pests on grapevines. Two are leafminers native to North America, *Phyllocnistis vitegenella* Clemens (Gracillariidae) and *Antispila oinophylla* Nieukerken & Wagner (Heliozelidae), which have crossed continents and invaded Northern Italy and other parts of Europe (Van Nieukerken *et al*. 2012). The other two cases are unique in that they are both believed to be native pests that have undergone a change in host-plant preferences and have become pests on cultivated grapevines. One of these is *Antispila uenoi* Kuroko (Heliozelidae), native to Japan (Van Nieukerken *et al*. 2012), and another is *Holocacista capensis* Van Nieukerken & Geertsema (Heliozelidae).

In South Africa, the leaf-mining larvae of *H. capensis*, or the Cape grapevine leafminer, occurs on *Vitis vinifera* L. and has become a pest of economic importance in the Western Cape's table grape industry. The adult is a small, diurnal moth native to South Africa and was reported for the first time in vineyards in 2012 from a table grape vineyard close to Paarl, Western Cape (Van Nieukerken & Geertsema 2015; Torrance 2016). The last instar larvae construct lenticular cases from the epidermal layers of a leaf that remain after mining activity (Regier *et al*. 2015). The larvae descend from infested leaves in their crafted cocoon casings, by means of a silken thread (Regier *et al*. 2015). The cocoon casings can be found attached to any objects in their surrounds, including berry bunches, which poses a phytosanitary risk to export markets (Van Nieukerken & Geertsema 2015). When bunch infestation is severe it is necessary to manually remove cocoon casings from berry bunches after harvest, which is labour intensive and requires fine motor skills, due to their small size. The leafminer has since been found in high abundances on multiple varieties of *Vitis vinifera* L. (Vitaceae), including commercial wine producing vineyards and ornamental vines (Van Nieukerken & Geertsema 2015; Torrance 2016).

The main defence against economically important leafminers and other insects in most commercial agro-ecosystems is synthetic chemical insecticides (Maier 2001), the excessive use of which can cause a myriad of problems associated with the health of a vineyard and its surrounds (Lim 1990;

Alavanja *et al*. 2004). Some of these problems include the development of insecticide resistance; the loss of beneficial insects and the destruction of non-target organisms; the resurgence and establishment of primary and secondary pests; the presence of chemical residues on produce; and, most importantly, the exposure of humans and the environment to harmful chemical compounds (Stein & Parrella 1985; Lim 1990). In the case of *H. capensis*, seasonal control has been reported after the application of dichlorvos and spinosad (which both target the feeding larval stage within the plant foliage) (Torrance 2016) when targeting other table grape pests. No insecticides have, however, been registered for the control of *H. capensis* (Agri-Intel 2018a).

A variety of chemicals have been used to control lepidopteran leaf-mining pest populations in agricultural landscapes (Beattie *et al*. 1995a; Beattie *et al*. 1995b; Head *et al*. 2000; Pereira *et al*. 2014; Percival & Holmes 2016). A great deal of the current knowledge on the use of insecticides against leaf-mining lepidopteran pests is limited to studies conducted on *Tuta absoluta* (Meyrick) (Gelechiidae), the South American tomato pinworm or the tomato leafminer, a cosmopolitan pest of economic concern on tomatoes. The widespread use of insecticides that coincided with its rapid spread throughout Afro-Eurasia, from the western Neotropics, led to the development of insecticide resistance and has created undesirable consequences for beneficial arthropods in infested agricultural systems (Biondi *et al*. 2018). Insecticide resistance in *T. absoluta* populations has been detected when applying abamectin, cartap hydrochloride and organophosphates in South America and pyrethroids, indoxacarb, diamide and spinosad in both South America and Europe (Salazar & Araya 2001; Siqueira *et al*. 2001a; Siqueira *et al*. 2001b; Silva *et al*. 2011; Guedes & Siqueira 2012; Gontijo *et al*. 2013; Campos *et al*. 2014, 2015; Roditakis *et al*. 2015). This prompted the use of a wider selection of insecticides, disrupting integrated pest management (IPM) programs on tomato (Guedes & Picanço 2012; Guedes & Siqueira 2012), which consequently led to even more incidents of insecticide resistance (Guedes & Siqueira 2012; Gontijo *et al*. 2013). The phenomenon caused panic, due to the possibility of control failure and sparked the need for *T. absoluta*-resistant tomato cultivars and the development of cost-effective, robust and successful biological control options for the sustainable control of *T. absoluta* (Biondi *et al*. 2018).

Mid- to late-season chemical applications are restricted by withholding periods related to the use of insecticides on all fruit intended for export, as stipulated by the Fertilizers, farm feeds, agricultural remedies and stock remedies act, 1947 (Act No. 36 of 1947) and the Foodstuffs, cosmetics and disinfectants act, 1972 (Act No. 54 of 1972) [similar to that set out by Agri-Intel (2018b) and the APVMA (2014)], which is used to ensure that chemical residues on export fruit do not exceed maximum residue limits. In addition, Van Nieukerken & Geertsema (2015) and Torrance (2016) have indicated that the control of *H. capensis* may be troublesome, due to its multivoltine behaviour and

the gradual increase in population abundance experienced throughout a grapevine growing season. The above-mentioned phenomena and restrictions on the use of insecticides emphasise the need for an alternative to chemical control, as well as to determine the initial efficacy of chemical insecticides. With the exception of biological control, the solution may lie in the use of physical control strategies.

The aim of the current study was to assess the efficacy of a variety of commercially available insecticides against the leaf-mining larvae of *H. capensis*. To supplement or replace (in the case of organic vineyards) insecticide applications, various bunch covers (currently available as a bird protection mechanism) were applied to bunches in the field, to assess the feasibility of a physical control strategy to reduce the number of cocoon casings present on bunches intended for export. Combined chemical and physical control strategies could be used to supplement an IPM strategy aimed at the control of *H. capensis* in South African vineyards.

Materials and methods

Source of insects

Larvae of *H. capensis* used for insecticide bioassays, were collected from infested vineyards from farms on the outskirts of Halfmanshof (33°08'48.7"S 18°59'18.0"E), Klapmuts (33°49'30.3"S 18°55'36.8"E) and Paarl (33°40'20.4"S 18°56'25.0"E) in the Western Cape, South Africa. Approximately 120 infested leaves were collected from the field for each treatment and placed in cold storage during transport back to the laboratory.

Study sites

The bunch cover field trial was conducted on two infested farms in the Western Cape, in February 2018. These farms were situated in Halfmanshof (Onverwags: 33°08'48.7"S 18°59'18.0"E) and Wellington (St Malo: 33°35'52.7"S 18°58'46.0"E), in the Berg River region. Farms throughout the area were regularly monitored. These particular farms were selected due to the fact that bunch infestation was present on late season cultivars very early on in the grapevine growing season (November 2017).

Insecticide laboratory trial

A total of 1 920 infested leaves (containing live/feeding larvae) were collected from the field for all dip test trials. In the laboratory, 100 live individuals (active/feeding leaf-mining larvae of mixed instars) were exposed to five insecticide treatments. Delegate 250 WG and Dichlorvos 1000 EC were selected based on their history of successful control in previous years. The other insecticides were selected based on availability and suggestions made by various growers and their field advisors. Effort was made to include a wide range of products including biological insecticides. Doses ranged from a quarter of the recommended field dose (RFD) (recommended for the control of lepidopteran pests or for the treatment of grapevines, unless otherwise stated) to four times the RFD (Fig. 4.1). In most cases the RFD was selected based on the quantity recommended for table grapes. If the product was not registered on table grape crops then the selected RFD was based on the predominant recommendation for lepidopteran pests listed on the product labels (Table 4.1). When laboratory trials began the RFD of Delegate 250 WG for false codling moth (20 g/100 l water) had not yet been released. For more information on each of the respective insecticides used in the laboratory trial, refer to Table 4.1.

The leaves were dipped (suspended until the whole leaf was covered) in each of the respective treatments and set aside to dry. For each treatment, 20 individuals were dipped in a 500 ml treatment solution (water and treatment dose) (Table 4.2). Once dry, leaves were placed and stored in 2 l plastic containers. After 48 h, the leaves were inspected using a microscope and larval mortality was recorded.

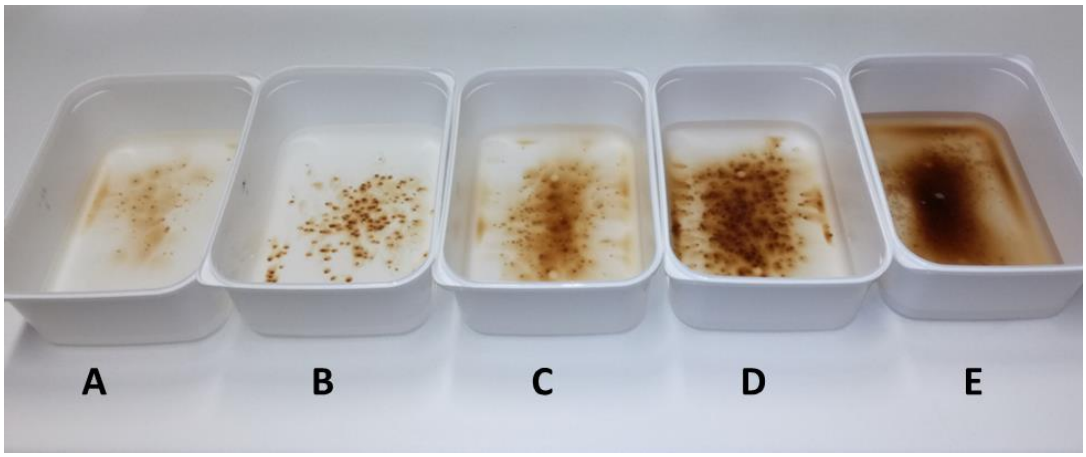

Figure 4.1: An example of a laboratory trial using Indoxacarb (Steward WG) (not yet mixed) against *Holocacista capensis* larvae. "A" = a quarter of the recommended field dose; "B" = a half of the recommended field dose; "C" = the recommended field dose; "D" = two times the recommended field does; and "E" = four times the recommended field dose.

Table 4.1: Additional information on the insecticides tested against *Holocacista capensis* larvae in the current study (Agri-Intel 2019a).

Active ingredient	**Product**	**Class**	**Targets (Lepidoptera on table grapes &/or other leaf-mining pests)**	**Produced/Supplied by**	**Recommended Field Dose (RFD)**	**Table grape withholding period**
Bacillus thuringiensis, var. *kurstaki*	DiPel DF	Bacterium	African bollworm, pear leaf roller	Philagro South Africa (Pty) Ltd.	500 g/ha (equivalent to 50 g/100 l water)	None
Azadirachtin	Bio-Neem	Liminoid	Pea leafminer	Agro-Organics (Pty) Ltd.	500 ml/100 l water	
Cypermethrin (grapevines not registered)	Knox Worm	Pyrethroid	Africa bollworm, lesser bollworm, codling moth, false codling moth, diamond back moth	PE-BEE Agri (Pty) Ltd.	10 ml/100 l water (various crops)	4 – 14 days (dependent on crop)
Spinosad	Tracer 480 SC	Spinosyn	African bollworm, potato tuber moth Celery leafminer, pea leafminer, tomato leafminer, potato leafminer	Dow AgroSciences Southern Africa (Pty) Ltd.	20 ml/100 l water	28 days
Spinetoram	Delegate 250 WG	Spinosyn	False codling moth Pea leafminer, tomato leafminer, potato leafminer		10 g/100 l water	3 days
Chlorantraniliprole	Coragen	Anthranilic diamide			17.5 ml/100 l water	
Indoxacarb	Steward	Oxadiazine	African bollworm, false codling moth Pea leafminer, tomato leafminer, potato leafminer	DuPont De Nemours South Africa (Pty) Ltd.	20 g/100 l water	14 days
Dichlorvos	Dichlorvos 1000 EC	Organophosphate	Blotch leafminer, canal leafminer	Villa Crop Protection (Pty) Ltd.	75 ml/100 l water	7 days

Table 4.2: The doses/treatments used in the insecticide dip test trials against *Holocacista capensis* larvae. Infested leaves (containing live/feeding larvae) were exposed to a 500 ml insecticide solution. "RFD" refers to the recommended field dose.

TREATMENT	DOSE (g/ml)/500 ml WATER							
	Bacillus thuringiensis (Biological)	Azadirachtin (Biological)	Cypermethrin	Spinosad	Spinetoram	Chlorantraniliprole	Indoxacarb	Dichlorvos
Control (water)	0 g	0 ml	0 ml	0 ml	0 g	0 ml	0 g	0 ml
A (quarter RFD)	0.0625 g	0.625 ml	0.0125 ml	0.025 ml	0.0125 g	0.021875 ml	0.025 g	0.09375 ml
B (half RFD)	0.125 g	1.25 ml	0.025 ml	0.05 ml	0.025 g	0.04375 ml	0.05 g	0.1875 ml
C (RFD)	0.25 g	2.5 ml	0.05 ml	0.1 ml	0.05 g	0.0875 ml	0.1 g	0.375 ml
D (double RFD)	0.5 g	5 ml	0.1 ml	0.2 ml	0.1 g	0.175 ml	0.2 g	0.75 ml
E (four times RFD)	1 g	10 ml	0.2 ml	0.4 ml	0.2 g	0.35 ml	0.4 g	1.5 ml

Bunch cover field trial

Bunch cover trials were conducted in order to determine whether or not bunch covers deter the attachment of cocoon casings, on bunches intended for export, by final larval instars descending from mined leaves. In a single block, a total of 240 bunches were assessed, 40 of which constituted the control group (no bunch cover). Within a table grape producing block, eight rows were monitored. Within each row, five plots, containing each of the bunch cover types, were established at random (Fig. 4.2). Five covered bunches within each plot were assessed weekly, for the presence (i.e. the number of infested bunches) and number of attached cocoon casings, before harvest (from 1 February 2018 – 22 February 2018). Within each control plot, five bunches were marked using twist ties and monitored weekly. Four bunch cover types/designs were used in the current study (Table 4.3). The attractiveness of each bunch cover was tested to assess whether *H. capensis* larvae selected specific designs more to attach their cocoons to.

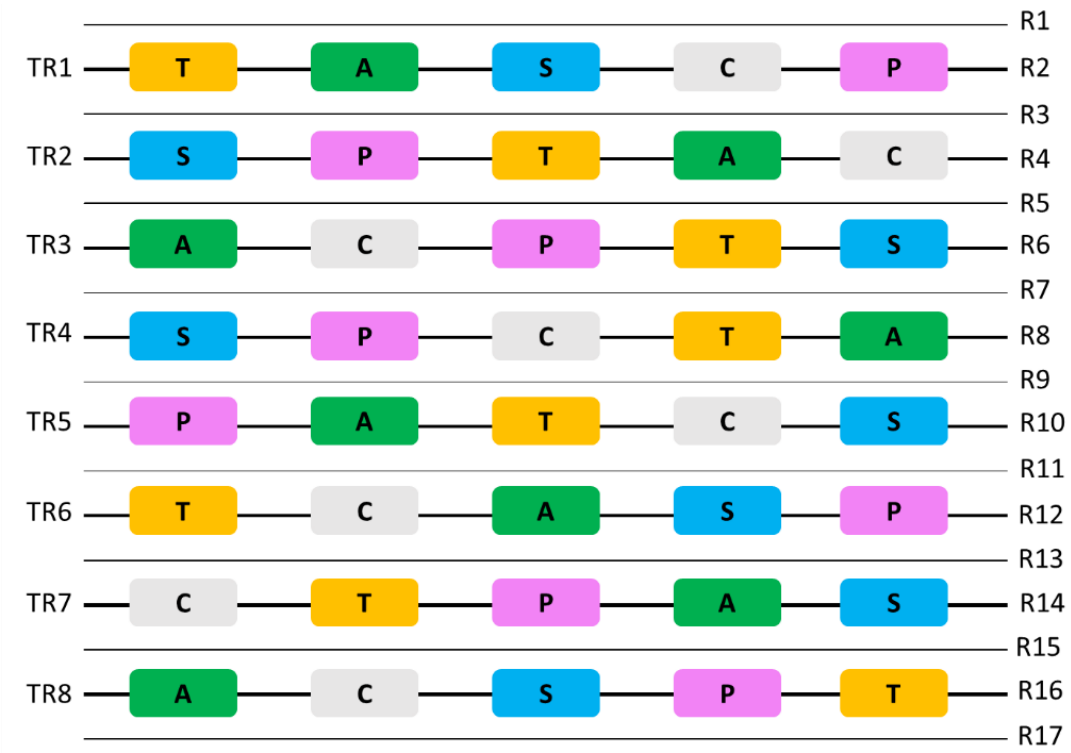

Figure 4.2: An example of the bunch cover trial layout of randomly distributed bunch cover plots within a single table grape producing block. "R" = rows and "TR" = trial/experimental rows. Colour coded plots: "T" = Tetra Pak cover; "A" = altered polypropylene bag (also referred to as "material" covers) cover; "S" = birdspun sleeve (also referred to as "sleeve" covers); "C" = control plot (devoid of bunch covers); and "P" = paper bag cover. Five bunches were treated per plot.

Table 4.3: A summary of the bagging methods/bunch cover types used in the current study.

Bagging Method	Description	In-field application	Price per Bunch	Source
Control	No cover Bunch tagged using 100 mm twist ties, "Twisties" (paper coated twist wire ties)		2c per twisty	"Twisties" sourced from Agrimark (Kaap Agri Corporate) Website: https://myagrimark.co.za/
Bird protection, Tetra Pak grape cover	Can be set to cover a radius of 180 mm, 170 mm or 160mm, depending on the size of the bunch		45c (rounded up to the nearest R0.05)	GD Packaging Website: http://gdpack.co.za/
UV stabilized polypropylene bags (altered) used in the cut flower industry	165 mm x 265 mm, stitching removed and cut to the centre of the folded line, edges stapled in the field		65c (rounded up to the nearest R0.05)	7Trees Fabrics Contact: raven@7trees.co.za
Birdspun (polypropylene, spunbonded, non-woven) bird protection sleeves	250 mm x 250 mm (length cut to size - dependent on the expected size of the bunch)		45c (rounded up to the nearest R0.05, incl. a single twist tie per bunch)	Cape Agricultural Products (Pty) Ltd Website: http://www.great-pruning-products.com/
Aralar paper bags	210 mm x 330 mm		30c (rounded up to the nearest R0.05, incl. a single twist tie per bunch)	Azapak Website: http://www.azapak.co.za/

Statistical analyses

All analyses were performed using STATISTICA 13.0 (Dell Inc., Headquarters in Round Rock, Texas, USA). A Kruskal-Wallis analysis of variance (ANOVA) was used to determine significant differences between insecticides and significance between the bunch cover types. Thereafter, a factorial ANOVA and a Bonferroni post-hoc test was conducted to compare the effect of dose amongst the different insecticides tested. The factorial ANOVA was analysed per group with a Fischer LSD post-hoc test to determine differences amongst doses within a single insecticide treatment. To confirm differences in mortality amongst doses for each treatment, a one-way ANOVA and a Fischer LSD post-hoc test, by group (insecticide), was conducted. A Kruskal-Wallis ANOVA was conducted to determine significant differences between the numbers of attached cocoon casings on each of the bunch cover types. To determine whether or not the size or the shape of a bunch cover (altered surface area) affects the attractiveness of bunches, a Kruskal-Wallis ANOVA was performed.

Results

Insecticide laboratory trial

The use of insecticides significantly increased mortality of *H. capensis* leaf-mining larvae ($H_{8, 90}$ = 74.079; $p < 0.001$) (Fig. 4.3). Dichlorvos treatments caused 100% larval mortality (at every dose) but did not, however, differ significantly from the cypermethrin (96% ± 2.08%; $p = 1.0$) and spinetoram treatments (94.50% ± 2.27%; $p = 1.0$). The azadirachtin (bio-neem) treatments caused the lowest recorded larval mortality (1.50% ± 1.50%) and did not differ significantly from the *Bacillus thuringiensis* (DiPel DF) (10.00% ± 4.28%; $p = 1.0$), indoxacarb (27.00% ± 9.64%; $p = 1.0$), spinosad (27.50% ± 8.44%; $p = 1.0$) and control treatments (5.00% ± 1.97%; $p = 1.0$). The chlorantraniliprole treatments (66.50% ± 4.89%), on the other hand, only differed significantly from the azadirachtin treatment ($p = 0.049$).

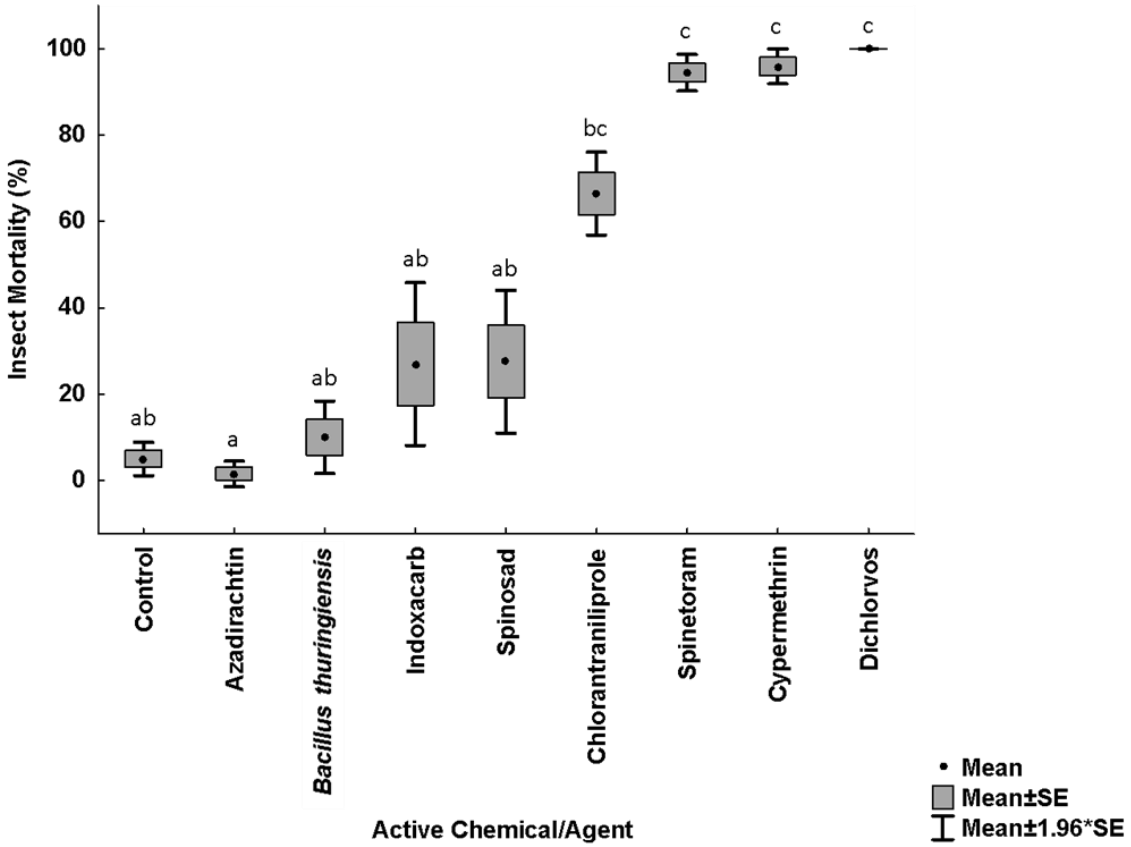

Figure 4.3: The percentage insect mortality of *Holocacista capensis* larvae 48 h after treatment with eight commercially available insecticides, at a range of doses (mortality averaged). Deviations in lettering above the boxplots indicate significant differences between treatments ($p < 0.05$).

No significant dose effects were recorded between insecticides and their respective doses ($F_{32, 45}$ = 0.101; p = 1.0). An increase in dose for any of the treatments did not cause a significant increase in insect mortality (Fig. 4.4). This was confirmed when no significant differences were recorded between doses when analysed per group (active chemical/agent) ($p > 0.05$). A decrease in mortality was recorded with an increase in dose from two times the recommended field dose to four times the recommended field dose for the azadirachtin, *Bacillus thuringiensis*, indoxacarb, spinosad, chlorantraniliprole and cypermethrin treatments, but these decreases were not significant ($p > 0.05$).

It is important to note that in the case of dichlorvos, cypermethrin and spinetoram, very high larval mortality was achieved at a quarter of the recommended field dose. As a result, probit analyses were not performed.

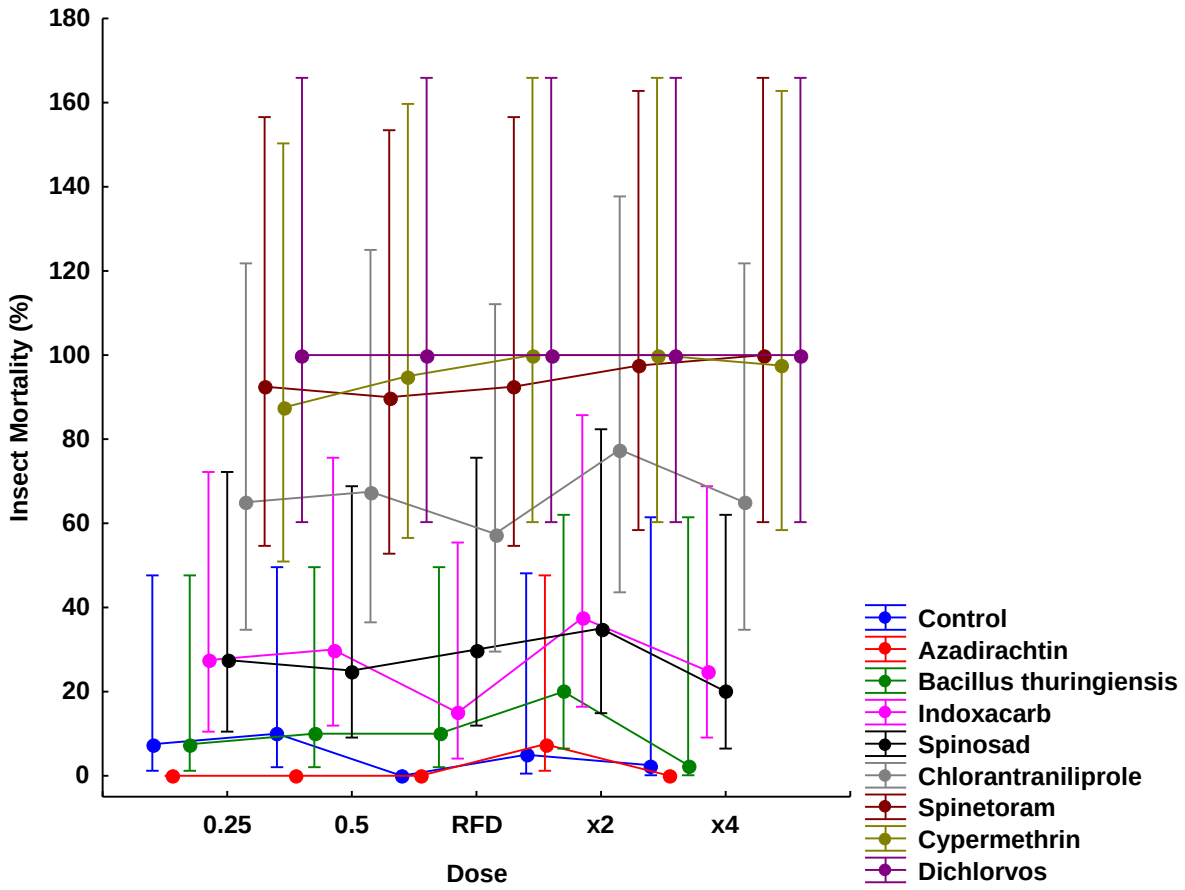

Figure 4.4: The effect of dose of various insecticides on the mortality of *Holocacista capensis* larvae. "0.25" = a quarter of the recommended field dose; "0.5" = half of the recommended field dose; "RFD" = recommended field dose; "x 2" = two times/double the recommended field dose; and "x 4" = four times the recommended field dose. Vertical lines denote 0.95 confidence intervals.

Bunch cover field trial

The use of bunch covers in infested vineyards proved to significantly reduce the number of infested bunches in infested table grape vineyards ($H_{4, 30} = 20.195$; $p < 0.01$) (Fig. 4.5). The aralar paper bags and the birdspun polypropylene sleeves prevented bunch infestation completely. Despite the fact that cocoons were recorded on bunches, the number of infested bunches did not differ significantly from the previously mentioned bunch cover types or the control treatment (3.67 ± 1.31), when the Tetra Pak (0.33 ± 0.21) and the altered polypropylene covers (0.33 ± 0.33) (in all cases $p = 1.0$) were used on bunches.

It should be noted that the aralar paper bags restricted the growth of bunches in the field, and berry damage was noted on several occasions as a result (when covers were removed shortly before harvest). In addition, grape bunches within birdspun polypropylene sleeves and aralar paper bags had not ripened to the extent of control treatment bunches at the time of harvest.

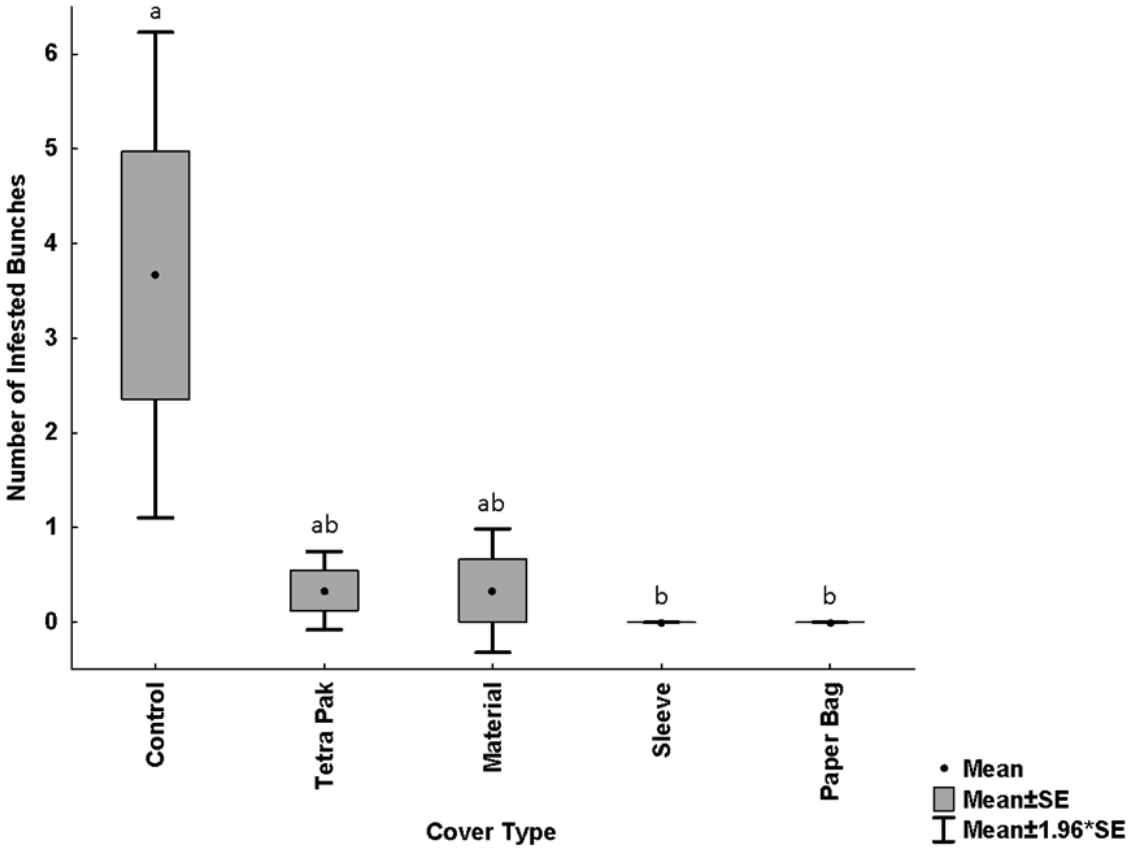

Figure 4.5: The number of table grape bunches infested with *Holocacista capensis* cocoon casings after a variety of bunch cover types were applied to an experimental block. "Control" = no bunch cover (bunch marked using a "twistie" for monitoring; "Tetra Pak" = Tetra Pack grape cover (bird protection); "Material" = altered polypropylene bags; "Sleeve" = birdspun polypropylene bird protection sleeves; and "Paper Bags" = aralar paper bags. Deviations in lettering above the boxplots indicate significant differences between treatments ($p < 0.05$).

When the effect of the shape/size of the bunch cover on the attractiveness of a covered bunch was analysed, it was found that the number of cocoons attached to the bunch cover itself did not significantly differ between bunch cover types ($H_{3, 24} = 1.266$, $p = 0.737$) (Fig. 4.6).

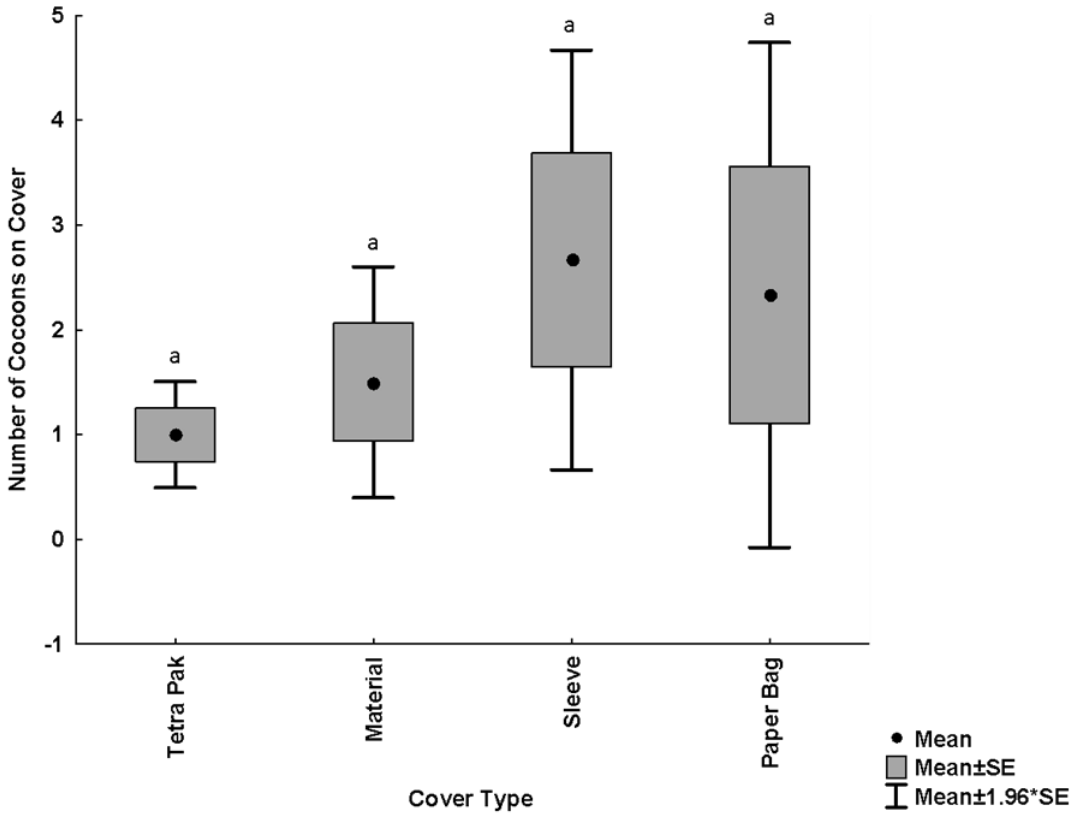

Figure 4.6: The number of *Holocacista capensis* cocoon casings physically attached to each of the bunch cover types in the field. "Tetra Pak" = Tetra Pack grape cover (bird protection); "Material" = altered polypropylene bags; "Sleeve" = birdspun polypropylene bird protection sleeves; and "Paper Bags" = aralar paper bags. Deviations in lettering above the boxplots indicate significant differences between treatments ($p < 0.05$).

Discussion

The use of organophosphates, spinosad/spinosyns and pyrethroids have been associated with the control of three economically important leaf-mining insects, namely *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) (Ferguson 2004), *Phytomyza atricornis* Goureau (Diptera: Agromyzidae) (Khan *et al*. 2015), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) (Beattie *et al*. 1995a) and *T. absoluta* (Salazar & Araya 2001; Siqueira *et al*. 2001b; Silva *et al*. 2011; Guedes & Picanço 2012; Gontijo *et al*. 2013; Pereira *et al*. 2014; Campos *et al*. 2015; Roditakis *et al*. 2018). The results of the present study confirmed efficacy of these products for the control of *H. capensis* larvae. It has been shown that dichlorvos (organophosphate) and spinetoram (spinosyn) caused high levels of mortality of *H. capensis* larvae in laboratory bioassays. These results support anecdotal reports that these products control leaf-mining *H. capensis* larvae. Larval mortality associated with cypermethrin treatments did not differ significantly from dichlorvos and spinetoram treatments and should,

therefore, also be considered in future registration trials against *H. capensis*. As many products have been registered for the control of other leaf-mining pests on a variety of other fruit and vegetables in South Africa (Agri-Intel 2019b), future studies should focus on testing other active agents registered for use on table grapes (Agri-Intel 2019c), against *H. capensis*, to avoid the development of insecticide resistance.

Insecticide resistance has been recorded in Brazilian populations of *Perileucoptera coffeella* (Guérin-Méneville) (Gracillariidae), the coffee leafminer, likely caused as a result of cross-selection of insecticides (mainly between organophosphates) (Fragoso *et al*. 2002). Reports of insecticide resistance in dipteran leaf-mining pests have also been of concern for the long-term control of pests (Trumble 1985; Ferguson 2004). When considering *T. absoluta* and *P. coffeella* as case studies for the control of *H. capensis*, the possibility of the development of insecticide resistance should be understood. The development of insecticide resistance is linked to the extensive, repeated use of a small selection of insecticides (Sparks & Nauen 2015). It is, therefore, important to consider that the use of organophosphates, spinetoram/spinosyns and pyrethroids, which have been associated with insecticide resistance of other leaf-mining lepidopterans (Silva *et al*. 2011; Guedes & Picanço 2012; Gontijo *et al*. 2013; Campos *et al*. 2015), for the control of *H. capensis*, should be integrated into an effective insecticide resistance management (IRM) strategy, essential for the preservation of the success of current and future insecticide applications (Sparks & Nauen 2015). Thus minimising the risk of the development of insecticide resistance in *H. capensis* is a priority in the development of a suitable IPM strategy.

An IRM strategy aimed at the effective control of *L. trifolii*, the celery leafminer, by Gao *et al*. (2017) has been successful in Florida celery and other vegetable crops for many years. It provides recommendations on cultural controls, avoiding the use of broad-spectrum insecticides (to reduce negative effects on natural enemies) and regular monitoring to avoid excessive insecticide use. Insecticide applications are limited to three consecutive applications (preferably only two) of the same product and rotate between the use of cyromazine and abamectin. This application strategy decreases insecticide selection pressure and selection against successive generations with the same chemical product. Bielza (2008) also recommended a similar strategy, for western flower thrips, that is designed to avoid selection of resistance mechanisms.

To meet the ever growing demands of a growing human populace for food, the use of agrochemicals is indispensable (Kumar & Singh 2015). There is scope, however, to incorporate biopesticides (naturally occurring, non-toxic substances/organisms that control pests in an environmentally-friendly manner) into IPM strategies. These strategies focused on environmental safety and reducing the negative effects of harmful insecticides, i.e. insecticide resistance, secondary

pest outbreaks, and the presence of residues in/on the produce and within ecological systems (Kumar & Singh 2015). With the exception of spinetoram (spinosyn – derived from fermented bacteria), it is unfortunate that high levels of *H. capensis* larval mortality were not obtained with the biological insecticides tested (azadirachtin, *Bacillus thuringiensis* var. *kurstaki* and spinosad). The use of biopesticides, in general, is gaining interest because of the advantages associated with target-specificity and efficacy, environmental safety, biodegradability and suitability in IPM programs (Kumar & Singh 2015), and they should, therefore, be studied in a greater capacity for the control of *H. capensis*.

In the current study, significant levels of larval mortality were recorded at the lowest RFD (a quarter of the recommended field dose). This is a good indication of the potential success rate of future in-field registration trials. The excessive use of chemicals on pest populations does, however, facilitate the persistence of resistant genotypes and results in reduced pesticide efficiency (Roditakis *et al*. 2018), although it is important to note that the development of insecticide resistance is potentially unique to each pest and is ultimately dependent on a variety of factors including genetic, biological (biotic and behavioural) and operational (chemical and mode of application) factors (Georghiou & Taylor 1977). In light of this, it is essential that control efforts be supplemented with the implementation of appropriate cultural, physical and/or biological control strategies, for the effective control of *H. capensis*, which prompted the investigation of alternatives to insecticide applications in the current study.

In South Africa, the use of bunch covers is not common but they are used, on occasion, to avoid berry damage associated with birds. In Spain, bunch covers or grape bags are not only used for protection against birds and insects, but have the added benefits of creating uniformity in bunch colouration and causing a delay in the ripening process (ideal for extending sales periods in export markets) (Signes *et al*. 2007). The present study indicated that the application of birdspun polypropylene bunch covers (± R4 500 to cover 10 000 bunches) can be used to supplement the integrated control of *H. capensis*. In cases where 0% bunch infestation is desired, birdspun polypropylene bunch covers were able to completely eliminate the presence of cocoon casings on bunches intended for export. The use of bunch covers, such as these, were observed to delay the ripening process, potentially extending access to export markets, although the effect of the bunch cover on the quality of the grapes is not yet known. Although equally effective, the use of aralar paper bags was associated with bunch damage and thus, unless larger bags can be obtained, should be avoided. The polypropylene material covers and the Tetra Pak covers did not significantly deter the establishment of cocoon casings on bunches when compared to the control treatment, but since they did not differ significantly from the aralar paper bags and the birdspun polypropylene cover

treatments, the application thereof could be adopted depending on the specific needs of the grower. If delayed ripening of bunches is not desired, and covers are adopted to deter bird associated damage, the use of Tetra Pak covers would be ideal, although it must be noted that bunches will still need to be monitored in packing sheds for the presence of rooted cocoon casings.

It can be argued that the cost of bunch cover applications is substantial on a larger scale, but the cost of the physical bunch covers and the additional labour required to apply them can be offset by the cost incurred by the loss of time and increased labour required to manually remove *H. capensis* cocoon casings in the packing shed after harvest. This will, however, depend on the severity of infestation and the scale of harvest quantities and should thus be considered by growers and their needs at the time. The application of bunch covers would be ideal in scenarios where maximum residue levels have been met and leafminer infestation cannot be further suppressed by other means.

Torrance (2016) stated that an economic threshold of 0% bunch infestation can be maintained if control strategies are applied when fewer than 87 *H. capensis* male moths are caught per trap per fornight. In the case of bunch covers and insecticide applications, management options should be considered when the threshold limit has been met to avoid unnecessary expenditure of labour and finances.

The shape and size of any given bunch cover did not affect the number of cocoons present on a particular bunch cover type. This is important as a descending larva (covered in a cocoon casing) is able to move to an appropriate site for pupation (and could potentially crawl under, or into, a bunch cover type), and thus the risk of a larvae establishing itself on a covered berry bunch is decreased.

A control strategy dominated by the use of harsh insecticides is considered to be a short-term solution to insect control although, in practice, the use of chemical control remains the most commonly used means of control, especially in developing countries (Ecobichon 2001). If the use of insecticides is inevitable within an IPM strategy, it is necessary that a wider selection of biopesticides (e.g. neem seed extract) are developed to avoid cross-/multiple-selection within pest populations and other non-target organisms, as well as to avoid the loss of natural enemy populations (Shanower *et a*l. 1993; Maier 2001; Zhao *et al*. 2002, 2006; Lietti *et al*. 2005; Sparks & Nauen 2015). In the unique case of *H. capensis*, it is highly likely that complete eradication in vineyards is unattainable, due to the fact that seasonal infestation is likely replenished by local source populations, overwintering pupae and commercially bought scions. As a result, control strategies should adhere to IPM protocols for the long-term control of the pest. The inclusion of physical and biological control strategies is, therefore, imperative in successful, long-term suppression efforts.

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Chapter 5

Biological Control Methods

A laboratory study of local South African entomopathogenic nematodes to control *Holocacista capensis* (Lepidoptera: Heliozelidae)

Introduction

The Cape grapevine leafminer, *Holocacista capensis* Van Nieukerken & Geertsema (Lepidoptera: Heliozelidae), or 'bladmyner' as it is commonly known, is a sporadic pest of economic importance found in South African table and wine grape producing vineyards (Van Nieukerken & Geertsema; Torrance 2016). The cocoon casings, constructed by final instar leaf-mining larvae, can be found attached to grape bunches, posing a phytosanitary risk for table grape export markets. The multivoltine pest occurs on most ornamental and commercial varieties of *Vitis vinifera* L. The leaf-mining larvae were first reported in 2012 in a table grape vineyard close to Paarl in the Western Cape province, South Africa, later spreading to the surrounding areas as the season progressed (Van Nieukerken & Geertsema 2015).

To date, no insecticides have been registered against the pest. This has been attributed to the fact that *H. capensis* is a sporadic pest, and because the male attractant is not yet commercially available, leafminer population abundance cannot be monitored throughout the season.

To avoid control strategies that rely solely on harmful insecticides, a variety of biological control strategies should be studied. Hagler (2000) described biological control as "the deliberate exploitation of a natural enemy for pest control". A wide range of economically important insect pest populations have been suppressed through the deliberate application of insect-parasitic organisms known as entomopathogens, and include entomopathogenic nematodes (EPNs) of the genera *Heterorhabditis* (Rhabditida: Heterorhabditidae) and *Steinernema* (Rhabditida: Steinernematidae) (Stock & Hunt 2005). In South Africa, a total of 17 *Steinernema* and seven *Heterorhabditis* species have been isolated from soil surveys conducted throughout the country (Malan & Hatting 2015; Malan & Ferreira 2017).

The ability of an EPN and its associated symbiotic bacteria to succeed as a control agent is essentially dependent on four factors; its moisture requirements (or desiccation tolerance, ± 85% relative humidity required for optimum control), favourable temperature range, pathogenicity for the

targeted insect, and its foraging strategy (Lacey & Georgis 2012). EPNs occur naturally in soils throughout the world and are able to control pests from a variety of diverse habitats, including foliar, soil borne, cryptic, and subterranean pests (Lacey & Georgis 2012). They are suitable for including in an integrated pest management (IPM) programme, as no secondary effects of EPNs on non-target organisms are known to exist (Wright *et al*. 2005). The method of cover and placement of EPNs on open surfaces (like foliar habitats) is critical, as humidity and temperature conditions are not buffered, such as those experienced in soil environments (Wright *et al*. 2005). The use of EPNs has been successfully implemented in control strategies for two leaf-mining lepidopteran pests, namely *Tuta absoluta* (Meyrick) (Gelechiidae) (tomato leafminer) (Batalla-Carrera *et al*. 2010; Gözel & Kasap 2015; Van Damme *et al*. 2015; Kamali *et al*. 2017; Mutegi *et al*. 2017) and *Phyllocnistis citrella* Stainton (Gracillariidae) (citrus leafminer) (Beattie *et al*. 1995).

A majority of the studies on leaf-mining Lepidoptera have focused on the use of steinernematids, including *Steinernema affine* Wouts, Mráček, Gerdin & Bedding (Gözel & Kasap 2015), *Steinernema carpocapsae* Wouts, Mráček, Gerdin & Bedding (Beattie *et al*. 1995; Batalla-Carrera *et al*. 2010; Gözel & Kasap 2015; Van Damme *et al*. 2015; Kamali *et al*. 2017), *Steinernema feltiae* (Filipjev) Wouts, Mráček, Gerdin & Bedding (Batalla-Carrera *et al*. 2010; Gözel & Kasap 2015; Van Damme *et al*. 2015) and *Steinernema karii* Waturu, Hunt & Reid (Mutegi *et al*. 2017). *Heterorhabditis bacteriophora* Poinar has also been used in an attempt to control *T. absoluta* pest populations (Batalla-Carrera *et al*. 2010; Gözel & Kasap 2015; Kamali *et al*. 2017).

The use of EPNs to control leaf-mining insects through foliar applications using adjuvants seems promising, regardless of concerns regarding humidity and temperature requirements, as the protection provided by the blotch mine created by the pest insect could potentially reduce the desiccation potential of infective juveniles (IJs) and exposure of these individuals to ultraviolet light (Gözel & Kasap 2015). With the exception of field trials conducted by Beattie *et al*. (1995), using *S. carpocapsae* against *P. citrella*, field trials have not yet been successful in adequately controlling the other major lepidopteran leaf-mining pest, *T. absoluta*. Gözel & Kasap (2015) speculate that the sensitive relationships between the selected EPN and the target pest (regarding virulence, host-seeking strategy, and ecological factors) must be satisfied, before field applications are considered. Thereafter, additional knowledge of the appropriate application strategy (i.e. field dosage, volume, irrigation and appropriate application methods) must be considered.

Preliminary evidence, based on findings associated with other leaf-mining, foliar pests, indicates that EPNs could potentially be used to control *H. capensis* larvae in an IPM strategy. The aim of the current study was to determine whether *H. capensis* larvae were susceptible to EPNs. The main objective was, therefore, to screen different local EPN species and to test their respective

pathogenicity against *H. capensis* larvae under laboratory conditions. Thereafter, concentration assays using IJs were carried out to determine the lethal dose required to cause effective mortality of the lepidopteran leaf-mining larvae.

Materials and methods

Source of insects

Grapevine leaves infested with *H. capensis* larvae were collected from farms on the outskirts of Halfmanshof (33°08'48.7"S 18°59'18.0"E), Robertson (33°50'19.6"S 19°54'52.7"E), Klapmuts (33°49'30.3"S 18°55'36.8"E) and Paarl (33°40'20.4"S 18°56'25.0"E). The larvae could not be stored for more than 24 h after collection (pilot trials indicated high levels of mortality in control treatments when experiments were not carried out on the same day as collection), and were stored at 10°C before the larvae were counted and subsequently used in EPN screening trials.

Source of EPNs

Seven EPN species were tested for their pathogenicity against *H. capensis* larvae and were obtained from the collection housed in the Department of Conservation Ecology and Entomology, Stellenbosch University (Table 5.1). Prior to screening, modified White's traps were used to collect IJs cultured from EPN inoculated *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae (otherwise known as greater wax moth) (Kaya & Stock 1997). More specifically, 10 *G. mellonella* larvae were placed in a 90 mm diam. Petri dish (lined with filter paper) and inoculated with 800 µl solution of an EPN species suspension. The Petri dish was stored in a growth chamber at 25°C. Forty eight hours later, *G. mellonella* cadavers were transferred to clean Petri dishes, lined with moist filter paper. After 7 to 10 days, the Petri dish containing the dead larvae was placed on a modified White's trap to facilitate the collection of emerging IJs (White 1927). The IJ/distilled water solution was harvested and transferred to vented culture flasks. The flasks were then stored at 14°C in a dark growth chamber, before being used for the screening tests.

Table 5.1: *Heterorhabditis* and *Steinernema* species, obtained from the collection housed in the Department of Conservation Ecology and Entomology (Stellenbosch University), tested against *Holocacista capensis* in laboratory bioassay trials.

Family	Species	Isolate	GenBank accession no.	Reference
Heterorhabditidae	*H. bacteriophora*	SF351	FJ4558443	Malan *et al.* (2006)
	H. baujardi	MT19	MF535520	Abate *et al.* (2018)
	H. indica	SGS	KU945293	Unpublished
	H. zealandica	MJ2C	MF370073	James *et al.* (2017)
	H. noenieputensis	SF669	JN620538	Malan *et al.* (2014)
Steinernematidae	*S. jeffreyense*	J194	KP164886	Malan *et al.* (2016)
	S. yirgalemense	157C	EU625295	Malan *et al.* (2011)

Preparation of larvae

Once collected from the field, each leaf was examined with the aid of a stereomicroscope, for the presence of active/feeding *H. capensis* larvae. Occupied mines (containing a single larva) were carefully cut from the collected leaves to avoid any cuts or tears of the epidermal layers of the leaf surrounding the leaf-mining larva. A margin of 2 – 5 mm of unmined leaf tissue was maintained around the entire leaf-mining gallery (Fig 5.1). Approximately 2 340 feeding larvae were manually processed in this manner for the experiments conducted in the present study (excluding mines that were damaged in the cutting process or the number of leaves examined that contained recently deceased larvae).

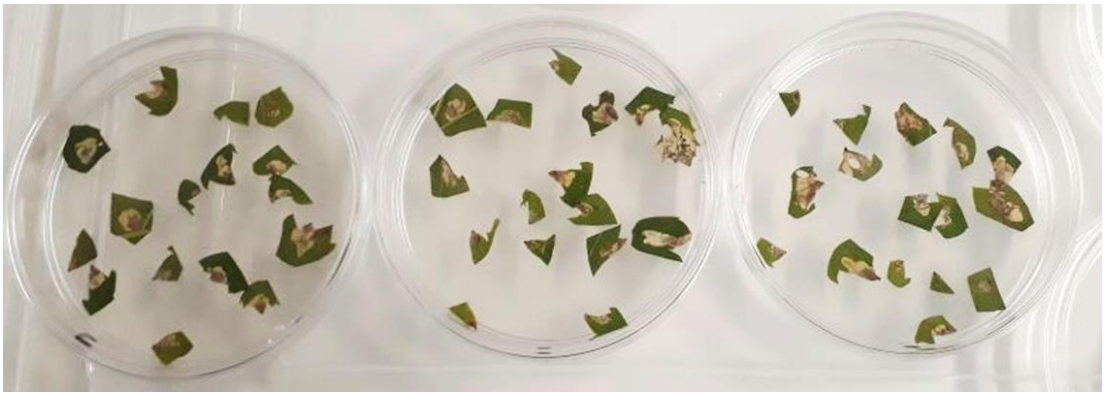

Figure 5.1: An example of the occupied leaf mines cut from infested grapevine leaves containing live/feeding *Holocacista capensis* larvae.

Virulence assays for Holocacista capensis *larvae*

The pathogenicity of each EPN species (Table 5.1) to *H. capensis* larvae was tested at a concentration of 200 IJs/50 µl of distilled water per larva, while the controls were inoculated with 50 µl of distilled water only, using 24-well bioassay plates (Flat bottom, Nunc™, Cat. No. 144530) as the test arena. Ten wells within each bioassay plate were lined with a 13 mm (diam.) circular piece of filter paper, placed in alternate wells, to obtain an even distribution throughout the plate. Each of the wells was inoculated with 200 IJs/50 µl of distilled water using an Eppendorf® micropipette for the different EPN species. An occupied leaf mine (containing a single live/feeding *H. capensis* larvae) was added to each inoculated well (Fig. 5.2). The lid of the bioassay plate was secured in place with a rubber band. Three replicate plates were used for each of the EPN species tested (*n* = 30 occupied leaf mines/ treatment). The plates were stored in 2 l plastic containers lined with moistened tissue paper (to maintain high humidity) and placed in a growth chamber at 25°C for 48 h. Thereafter, *H. capensis* larvae were removed from the leaf mines and mortality was determined. The experiment was repeated using a fresh batch of cultured IJs and leaf-mining larvae on a subsequent date.

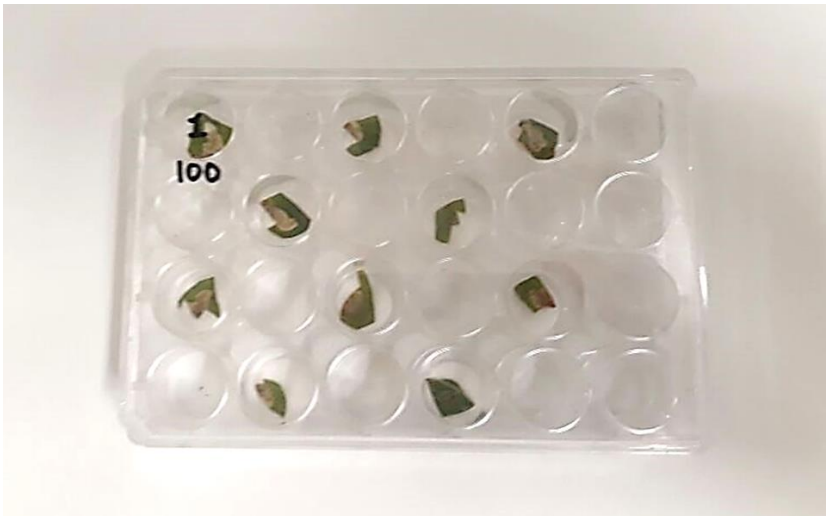

Figure 5.2: An example of the layout of occupied *Holocacista capensis* leaf mines and filter papers in a 24-well bioassay plates.

Penetration analysis

Dead larvae from the virulence assays were placed in a clean Petri dish (90 mm diam.) lined with filter paper. These were placed in the moistened 2 l plastic containers and transferred back to the 25°C growth chamber for a further 24 h. Thereafter, the number of IJs that penetrated each larva was recorded by dissection, with the aid of a stereomicroscope.

Lethal dose

The three most virulent EPN species from the previous experiments, namely *Heterorhabditis baujardi* Phan, Subbotin, Nguyen & Moens, *Heterorhabditis indica* Poinar, Karunakar & David and *Heterorhabditis noenieputensis* Malan, Knoetze & Tiedt, were used to determine their respective lethal concentrations. EPN concentrations of 0, 25, 50, 100, 200 and 400 IJs/larva were tested against *H. capensis*. Again, 24-well plates were used as the test arena with each containing 10 alternatively placed leaf mines containing a single live/feeding *H. capensis* larvae. Three 24-well plates (n = 30) were used for each EPN concentration for each treatment tested.

Similar to the experimental procedure adopted previously, 13 mm filter papers were placed in 10 alternate wells, to which the occupied leaf mines were added. Thereafter, the leaf mines were inoculated with the relevant concentration of IJs in 50 µl of distilled water. Distilled water only was used for control treatments. The plates of each treatment were placed in a 2 l plastic container lined with moist tissue paper (to maintain high humidity), and kept in a growth chamber at 25°C. After 48 h, the larvae were removed from the mines and the percentage mortality by infection of *H. capensis* larvae was determined and recorded.

Statistical analyses

All analyses were performed using STATISTICA 13.0 (Dell Inc., Headquarters in Round Rock, Texas, USA). As no significant test date versus treatment interactions were recorded ($F_{1-46} = 0.152$; p = 0.698) (determined using the two test dates as the main effects in a one-way ANOVA by group/EPN species), the data from the various test dates were pooled and analysed using a one-way ANOVA, in order to identify the most virulent EPN species. A Fisher LSD post-hoc test was performed to determine significance between means. To confirm differences in mortality amongst IJ doses within each species, a one-way ANOVA and a Fisher LSD post-hoc test, by group (nematode species), was conducted. The recorded penetration data of IJs found within *H. capensis* larvae 72 h after inoculation, were analysed with a one-way ANOVA and a Fisher LSD post-hoc test. Correlation analyses, a factorial ANOVA and a Fisher LSD post-hoc test were conducted in order to compare the effect of dose amongst the different EPNs tested. To determine the relevant lethal doses of the three most virulent EPN species, a probit analysis (Finney 1952) was carried out on mortality data, using Polo PC (LeOra Software 1987).

Results

Virulence assays for Holocacista capensis *larvae*

The inspection of larvae 48 h after inoculation with IJs confirmed that the EPNs were able to penetrate undamged leafminer galleries and infest the *H. capensis* larvae within (Fig. 5.3).

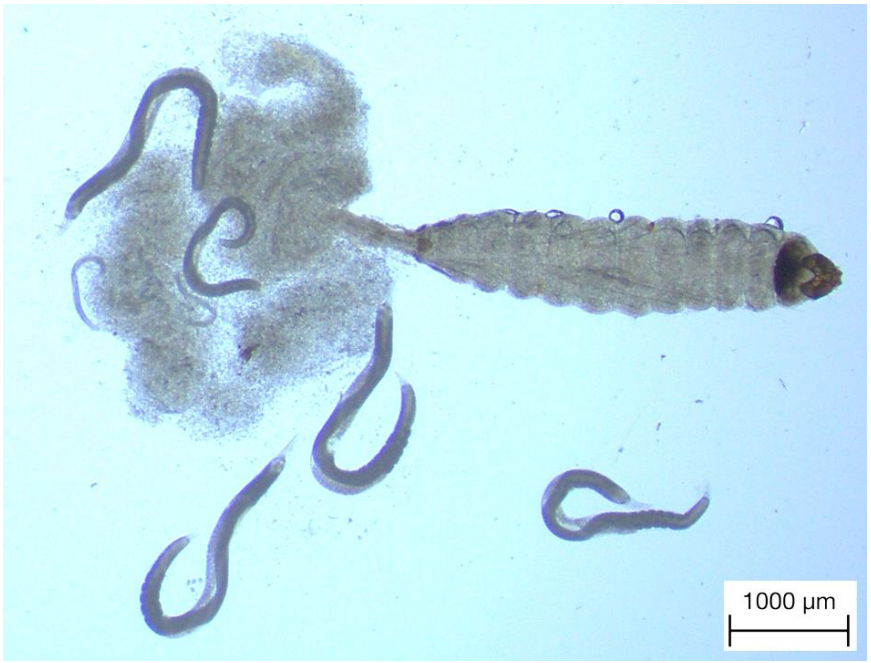

Figure 5.3: *Heterorhabditis baujardi* first generation hermaphrodites pressed from a *Holocacista capensis* larvae, inoculated with a 200 IJ solution 72 h after inoculation.

The analysis of the pooled data and the subsequent multiple comparisons test showed that all of the screened EPN species caused significantly higher mortality of *H. capensis* larvae than the untreated control treatment ($F_{7, 43} = 56.099$; $p < 0.001$). Mortality (infection) associated with *H. bacteriophora* (26.67% ± 4.94%) and *Steinernema jeffreyense* Malan, Knoetze & Tiedt (28.33% ± 4.77%) did not significantly differ ($p = 0.804$), and was the lowest in the screening trials. The natural mortality of the control treatment was 1.11% ± 1.11% (Fig. 5.4). Virulence of *Heterorhabditis zealandica* Poinar (38.33% ± 7.03%) did not differ significantly from that of *H. bacteriophora* ($p = 0.088$), *S. jeffreyense* ($p = 0.142$) or *Steinernema yirgalemense* Nguyen, Tesfamariam, Gozel, Gaugler & Adams (50.00% ± 2.58%) ($p = 0.088$). *Heterorhabditis baujardi* (91.67% ± 3.07%), *H. indica* (83.33% ± 7.60%) and *H. noenieputensis* (85.00% ± 4.28%) caused the highest *H. capensis* larval mortality. The three treatments did not differ significantly from each other ($p > 0.2$).

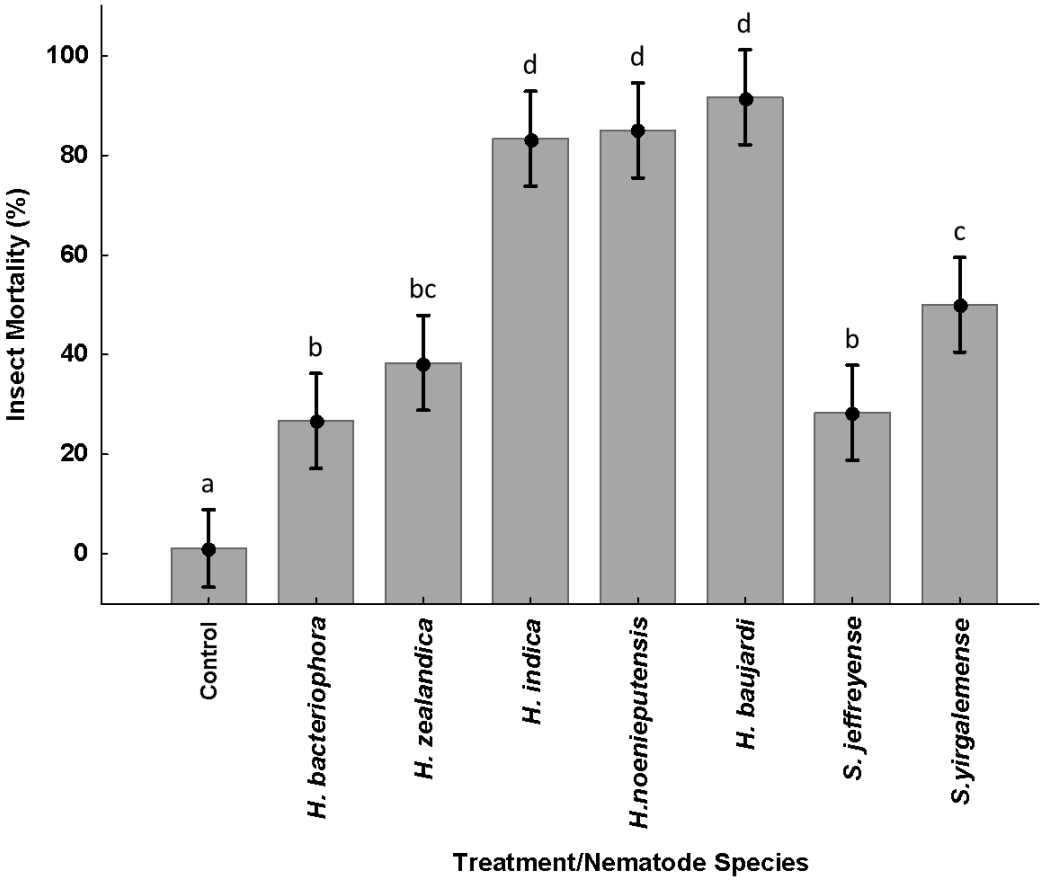

Figure 5.4: The percentage mortality of *Holocacista capensis* larvae (with 0.95 confidence intervals) caused by the seven entomopathogenic nematodes species tested, using a concentration of 200 IJs/50 µl distilled water, and a water only control. Deviations in lettering above the bars indicate significant differences between treatments ($p < 0.05$).

Penetration analysis

The mean number of IJs that penetrated *H. capensis* larvae significantly differed between the screened EPN species ($F_{6, 35} = 13.157$; $p < 0.001$) (Fig. 5.5). The highest mean penetration was achieved by *H. noenieputensis* (34.27 ± 6.94), which differed significantly from *H. baujardi* (18.23 ± 2.90) ($p = 0.002$) and *H. indica* (17.02 ± 3.98) ($p = 0.001$). Therefore, 34 IJs of the 200 IJ *H. noenieputensis* suspension penetrated *H. capensis* larvae. The lowest mean penetration of 1.45 (± 0.33) was achieved by *S. jeffreyense*, which did not significantly differ from that of *H. bacteriophora* (2.72 ± 0.82) ($p = 0.789$), *H. zealandica* (4.13 ± 1.61) ($p = 0.571$) or *S. yirgalemense* (4.97 ± 1.10) ($p = 0.458$).

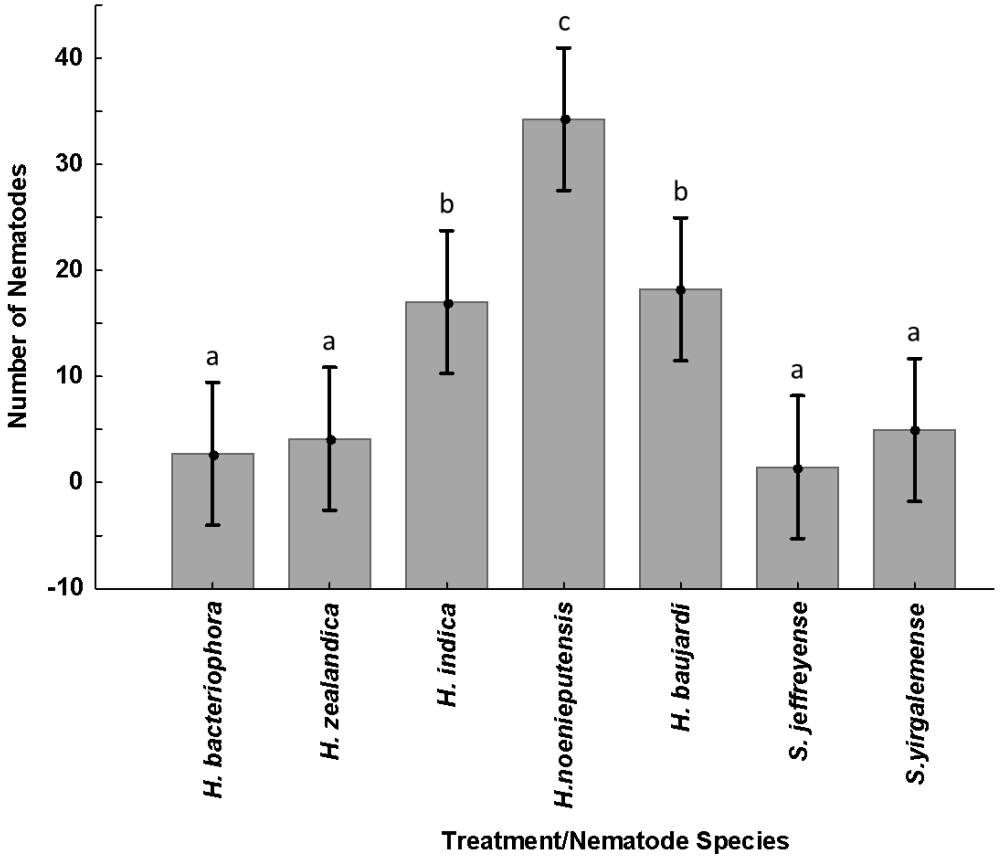

Figure 5.5: The mean number of nematodes able to penetrate *Holocacista capensis* larvae, recorded 72 h after inoculation. Vertical bars denote 0.95 confidence intervals. Deviations in lettering above the bars indicate significant differences between treatments ($p < 0.05$).

Lethal dose

A positive correlation between the dosage and lethal capacity of the EPN species tested was recorded ($r = 0.783$). The natural control mortality (< 6.67% ± < 6.67%, for each of the respective treatments) [significantly different from all other doses ($p < 0.01$), but not significantly different amongst all control treatments ($p > 0.7$)] was removed from the analysis to appropriately gauge differences in infection between the treatments and the respective doses (Fig. 5.6). The 25, 50 and 400 IJ treatments did not differ significantly between the EPN species tested ($p > 0.05$). The 100 IJ and 200 IJ treatments, however, differed between *H. baujardi* and *H. noenieputensis* ($p = 0.016$ and $p = 0.0375$, respectively) (Fig. 5.6). No effect of dose was recorded between the *H. indica* treatments ($p > 0.05$). The 25 IJ (38.33% ± 11.67%) and 400 IJ (73.33% ± 13.33%) *H. noenieputensis* treatments differed significantly ($p = 0.023$). In the case of *H. baujardi*, the highest insecticidal activity reached at 400 IJs (100%) did not significantly differ from the 200 IJ ($p = 0.724$) and 100 IJ ($p = 0.597$) treatments.

For all of the EPN species tested, the highest insecticidal activity was reached at 400 IJ treatments (*H. indica*: 83.12% ± 0.22%) and no significant differences were recorded between the 100, 200 and 400 IJ treatments ($p > 0.5$) for each respective species.

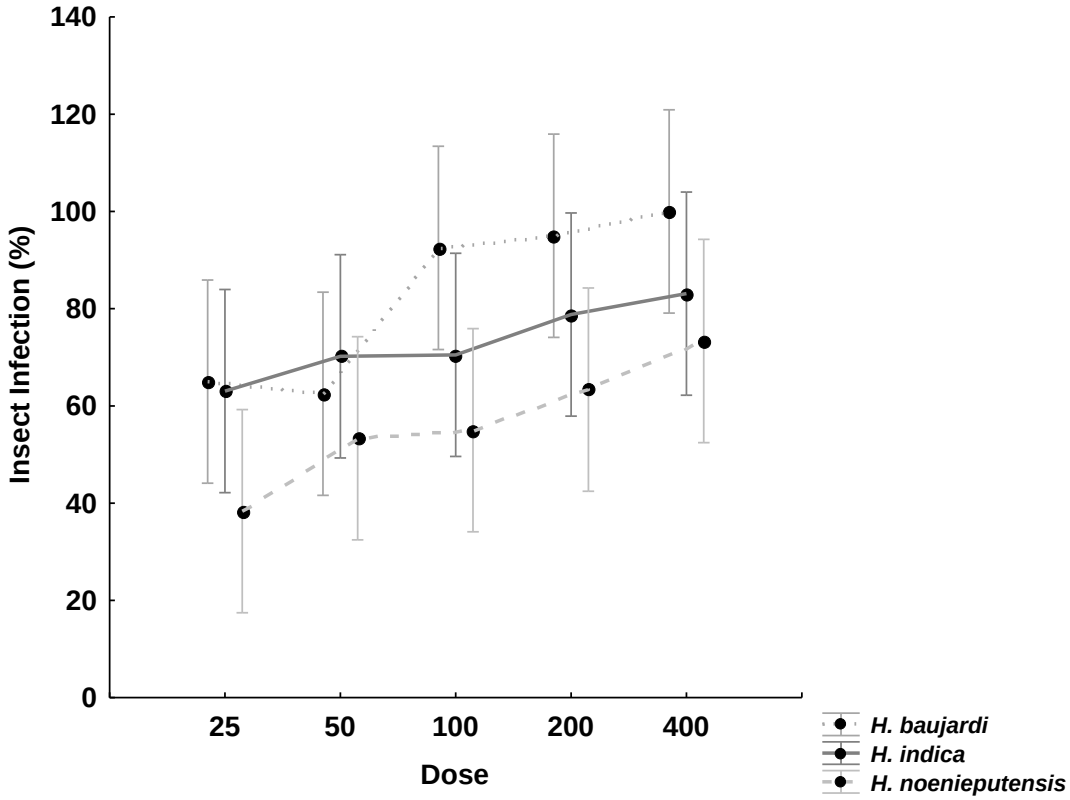

Figure 5.6: The effect of dose [number of infective juveniles (IJs) per larva] of three entomopathogenic nematode species (*Heterorhabditis baujardi*, *H. indica* and *H. noenieputensis*) on the infection of *Holocacista capensis* larvae at concentrations of 25, 50, 100, 200 and 400 IJs per 50 µl distilled water. Vertical lines denote 0.95 confidence intervals.

The probit analysis indicated that slopes and intercepts of the regression lines for the three EPN species, *H. baujardi*, *H. indica* and *H. noenieputensis*, differed significantly ($\chi^2 = 36.835$; $df = 4$; $p < 0.01$). Their slopes were, however, constrained (i.e. parallel) ($\chi^2 = 4.451$; $df = 2$; $p = 0.108$) (Fig. 5.7). Trends in lethal capacity differ slightly for each of the EPN species, as indicated by the probit regression lines for the three heterorhabditids ($y = 1.6957x + 2.7918$, $y = 0.8126x + 3.8453$ and $y = 0.8269x + 3.4043$, respectively) (Fig. 5.7).

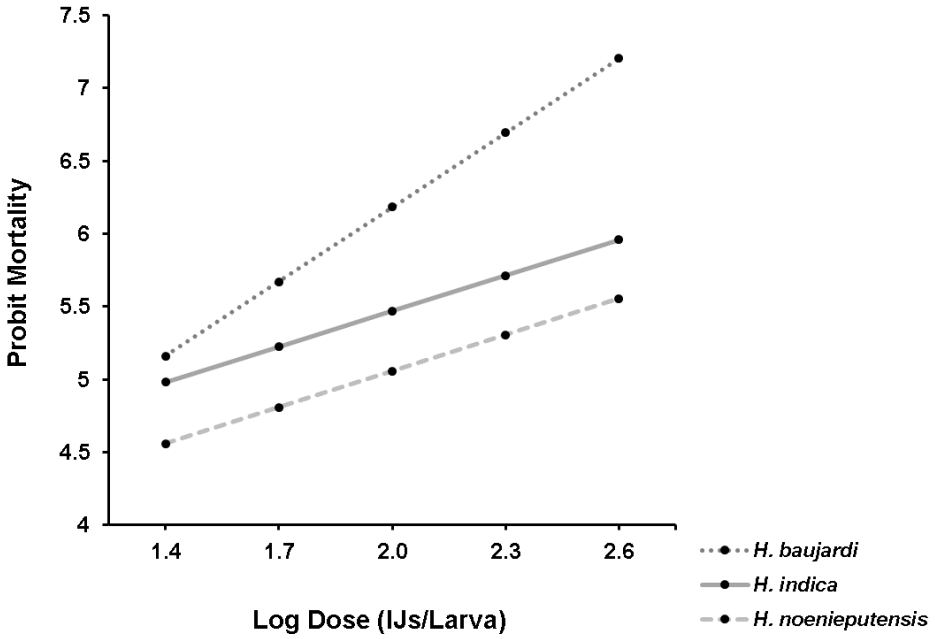

Figure 5.7: Probit mortality obtained at each log concentration tested for *Heterorhabditis baujardi*, *H. indica* and *H. noenieputensis* against *Holocacista capensis* larvae. The regression line formulae for *H. baujardi*, *H. indica* and *H. noenieputensis* were Y = 2.791803 + 1.695689x, Y = 3.845282 + 0.812622x and Y = 3.404347 + 0.826913x, respectively, where x = log (concentration) and Y = probit mortality.

Heterorhabditis baujardi exhibited the lowest LD_{50} and LD_{90} values (9.69 and 94.37 IJs/larva, respectively), whereas *H. noenieputensis* had the highest LD_{50} and LD_{90} values (88.51 and 1517.60 IJs/larva, respectively) (Table 5.2). The relative potency of *H. baujardi* was 3.56 times higher than it was for *H. indica*, whilst that of *H. indica* was 2.57 times higher than *H. noenieputensis* (Table 5.3).

Table 5.2: The lethal dose (LD) of infective juveniles against *Holocacista capensis* larvae inoculated with varying concentrations of *Heterorhabditis baujardi*, *H. indica* and *H. noenieputensis* with lower and upper 95% confidence intervals.

	H. baujardi			***H. indica***			***H. noenieputensis***		
Lethal dose	**Effective dose (IJs/insect)**	**95% confidence limits**		**Effective dose (IJs/insect)**	**95% confidence limits**		**Effective dose (IJs/insect)**	**95% confidence limits**	
		Lower	**Upper**		**Lower**	**Upper**		**Lower**	**Upper**
LD_{50}	9.69	3.00	19.42	34.47	16.15	58.19	88.51	48.35	155.54
LD_{90}	116.17	94.37	363.03	591.056	309.39	1787.91	1517.60	667.56	6630.50
LD_{95}	371.90	193.63	1078.91	1322.80	594.13	5677.70	3396.30	1264.30	21349.00

Table 5.3: The relative potency of three entomopathogenic nematode (EPN) species (*Heterorhabditis baujardi*, *H. indica* and *H. noenieputensis*).

EPN species	Relative to	Potency	95% Limit	
			Lower	Upper
H. baujardi	*H. indica*	3.5568	1.6728	9.6526
H. noenieputensis	*H. indica*	0.3895	0.3895	0.8200

Discussion

EPNs regulate insect populations by using an insect as a host, to reproduce and breed (Griffin *et al*. 2005). The application of different obligate parasitic EPN species, in conjunction with other chemical, cultural and physical control strategies, can be used to control leaf-mining insects. The current study aimed to determine whether or not local EPNs could potentially be used as biological control agents to aid in the control of *H. capensis*, a foliar pest of various commercially grown table grape varieties in South Africa. Appropriate virulence would allow the inclusion of EPN applications (after necessary field trials are conducted) in an integrated approach for the control of this phytosanitary pest. This is the first study that considers biological control strategies for the control of *H. capensis* using locally isolated (South African) EPNs.

All of the local EPN species tested caused a significant increase in the larval mortality of *H. capensis* compared to the control (untreated) treatments, signifying their ability to gain access to protected blotch mines and infest the target pest. Three of the EPN species, namely *H. indica*, *H. noenieputensis* and *H. baujardi*, yielded excellent results (between 83% and 91% larval mortality) pertaining to virulence and the ability to penetrate leaf-mining galleries. It is, therefore, highly recommended to assess these isolates in field trials to confirm virulence in field situations. The other EPN species tested caused between 26% and 50% larval mortality. Virulence did not differ significantly between the EPN species tested at lower doses and changes thereof only become apparent through the application of IJ concentrations of 100 IJs and more. In a similar study by Van Damme *et al*. (2015), *S. feltiae* and *S. carpocapsae* caused the highest mortality of *T. absoluta* larvae, whilst *H. bacteriophora* did cause mortality, but did not perform as well.

Bastidas *et al*. (2014) identified the importance of size of the target insect, as well as that of the EPN species used against the target. In their study it was found that, although all the steinernematids screened were able to invade small insect hosts, the invasion potential decreased as the target insect's size decreased and as the EPN species' size increased. This was confirmed in a study by Katumanyane *et al*. (2018) where large South African EPN species (> 1000 µm) such as *Steinernema khoisanae*

Nguyen, Malan & Gozel, *S. jeffreyense* and *Steinernema litchii* Steyn, Knoetze, Tiedt & Malan caused almost zero control of another micro-insect, *Bradysia impatiens* Johannsen (Diptera: Sciaridae). The effect of the size of the nematodes compared to that of the host was confirmed in a study by Dlamini *et al*. (2019, in press), using *Frankliniella occidentalis* (Thysanoptera: Thripidae) as a host. The high virulence and higher penetration potential recorded for *H. indica*, *H. noenieputensis* and *H. baujardi*, in the present study, may be attributed to the relatively small body size of these EPN species. It is speculated that smaller hosts limit the development of the invading nematodes and that the presence of larger hosts is necessary for the long-term persistence of nematodes (Bastidas *et al*. 2014), raising concern for the adequate control of *H. capensis* under field situations. It may be necessary to synchronise the applications of the EPNs to control other table grape pests that have been shown to be susceptible to EPNs, such as *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) (Le Vieux & Malan 2013), *Phlyctinus callosus* (Schönherr) (Coleoptera: Curculionidae) (Ferreira & Malan 2014), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) (Malan & Moore 2016) and a variety of other thrips and weevils (Allsopp *et al*. 2015). The susceptibility of these pests to the three successful nematodes identified in this study have not, however, been tested as yet and this knowledge gap should be addressed in the future. Furthermore, appropriate mass-rearing techniques need to be developed for the area-wide use of the EPNs in commercial vineyards. Advancements in improving the method of mass-rearing EPN colonies of heterorhabditid species (by improving solid state and liquid cultures), however, will facilitate the use of the successful candidates identified in this study on a commercial scale in the future (Ehlers *et al*. 2000; Ferreira & Malan 2014). As the three most virulent species in this study are heterorhabditids, it is possible that the possession of a dorsal tooth (generally absent in steinernematids) may also have facilitated the penetration potential into the leaf-mining larvae (Griffin *et al*. 2005).

The probit analysis indicated a positive relationship, for all EPN species tested, between total larval mortality and IJ concentration/dose (i.e. larval mortality increased as the IJ dose increased). When considering *H. baujardi*, *H. indica* and *H. noenieputensis* as potential biological control agents, it is important to note that *H. baujardi* proved to be just over three times more potent than *H. indica* and thus the number of IJs required to attain a lethal dose of 90% of larvae was lower than that of *H. indica*. In turn, *H. indica* was just over two times more potent than *H. noenieputensis*. These three species should not only be tested under field conditions for the control of *H. capensis*, but should also be tested against other economically important leaf-mining insects, such as *T. absoluta* and *P. citrella*.

To enable as many IJs as possible to locate a mine entrance of a leaf-mining insect, Wright *et al*. (2005) emphasized the need to maximize the density and distribution of EPNs on leaf surfaces. A nematode's residual infectivity generally lasts only a few hours, therefore, appropriate cover and

placement is critical in foliar applications of EPNs targeted at controlling leaf-mining insects. The importance of sufficient cover and placement is further underscored by the fact that there are limited migration opportunities to compensate for suboptimal placement, made even more acute in the dry, summer conditions of the Cape. The need to apply foliar and aerial EPN treatments for a variety of pests that persist above the soil surface has necessitated the study of various adjuvants to maintain suitable moisture conditions to ensure longevity of IJs on field-treated crops. Positive results have been achieved with the addition of adjuvants to various EPN species solutions for successful pest control against codling moth (De Waal *et al*. 2013), citrus mealybug (Van Niekerk & Malan 2015), wheat stem sawfly (Portman *et al*. 2016) and vine mealybug (Platt *et al*. 2018, 2019).

In conclusion, the novel findings of this study have identified a variety of locally-sourced EPNs as potential biological control agents for *H. capensis* in South Africa and provide a baseline to identify EPN species for further research regarding the targeted control of *H. capensis* larvae. Field trials should aim to determine the frequency of EPN applications required under field conditions, optimal EPN concentrations and appropriate application techniques. The adoption of biological control measures for the integrated control of *H. capensis* would minimize the risk of development of insecticide resistance in pest populations and pose less of a risk to the well-being and longevity of humans.

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Chapter 6

General Discussion

In principal, integrated pest management (IPM) combines biological, physical, cultural and chemical control strategies to provide efficient species-specific management protocols that can be adapted in the varying climates and habitats encountered by the targeted pest (Gentz *et al*. 2010). The use of broad-spectrum insecticides, however, remains the predominant control method for the management of recalcitrant insect pests, due to the high versatility, variety and efficacy of easily obtainable and administrable commercial insecticides (Nauen *et al*. 2008). Mounting pressure from export markets and a realization of the negative far-reaching consequences of the excessive use of insecticides (i.e. danger associated with environmental and human health) have led to the investigation of alternative control strategies (Lim 1990; Alavanja *et al*. 2004). In light of past findings associated with leaf-mining pests of economic importance, the implementation of alternate strategies are pertinent to their adequate, long-term control due to the loss of associated parasitoid assemblages and numerous incidents of insecticide resistance, developed as a result of relentless and monotonous insecticide use.

The Cape grapevine leafminer, *Holocacista capensis* Van Nieukerken & Geertsema (2015) (Lepidoptera: Heliozelidae), occurs on many commercial and ornamental grapevine varieties as well as on natural *Rhoicissus* (Vitaceae) hosts, which allows it to persist in agricultural environments, even after chemical control strategies have been adopted. This study aimed to 1) investigate the various abiotic and environmental variables that affect leafminer populations, 2) conduct an exploratory study on the genetic diversity of the pest in all of the infested regions to aid in decision-making pertaining to control strategies, and finally, 3) to explore potential chemical, physical and biological control options.

Climatic conditions, ambient light intensity, leaf composition and ground cover seem to play an important role in the presence and abundance of the Cape grapevine leafminer in infested vineyards. These associations are supported by findings of others, on similar leaf-mining pests, for example the devastating tomato leafminer, *Tuta absoluta* (Meyrick) (Gelechiidae) (Biondi *et al*. 2018). In addition, the association between moth abundance and average relative humidity recorded by Torrance (2016) was confirmed by the findings of the current study. The underlying mechanisms are, however, not yet understood and should be studied in the future. Regardless of this, the identification of these variables (as described in the best fitting regression models discussed in Chapter 2) could provide crucial information with regards to potential monitoring and detection strategies adopted in

the future. Interestingly, the study was able to identify leaf greenness (mean NDVI values) as a potential indicator of the extent of leafminer damage. Despite the fact that the use of high-resolution satellite imagery is associated with prohibitive costs, as the fruit industry becomes more reliant on precision agriculture (which requires the application of geospatial techniques and sensors to identify field related variations) (Zhang & Kovacs 2012), more emphasis will be placed on optimising a reliable and affordable end product suitable for the growers' individual needs.

The International Plant Protection Convention (IPPC) describes a quarantine pest as "a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled". The National Plant Protection Organization (NPPO) of South Africa released a report in 2016 (via the IPPC), classifying the leafminers' status as "present, not regulated". It is stipulated that it (the larvae) can be found, occasionally, on table grapes where damage symptoms can be detected. The report fails to mention the potential presence of cocoon casings on bunches intended for export. Should this pest become of greater concern to export markets, the inability to meet phytosanitary standards would likely lead to market exclusion and, as the table grape industry supports a large number of employees, socio-economic problems are likely to ensue. To formulate adequate control strategies it is important to investigate population structure, gene flow and the potential for rapid evolution in native and invasive pest populations. In the case of *H. capensis*, the use of the attractant has not been well studied and it is not known if other heliozelids are attracted to the traps placed for monitoring purposes. An understanding of genotypic variability could potentially provide insights into the dynamics of small populations that become successful invaders (Grapputo *et al*. 2005).

The current study was able to confirm synonymy between *H. capensis* populations (from specimens collected from attractant-baited traps) from different grape producing regions. Results from this study, through the analysis of two mitochondrial and genomic gene sequences, indicate little genetic variation between pest populations from each of the table grape producing regions. No genetic variations were identified between moths collected from "native" forested habitats in Stellenbosch and pest populations, indicating no genetic divergence occurring yet. If native populations, originating from source populations adjacent to commercial vineyards, were indeed collected in the survey our findings raise questions regarding the true age and origin of *H. capensis* as a higher degree of genetic variation between populations of a native species (with archaic family origins) is expected. In terms of IPM, however, the lack of regional genetic variation allows consensus for area-wide pest management although, it should be stressed that additional sampling efforts will be necessary to confirm this. Sampling in a variety of habitats would be beneficial to determine the exact niche that *H. capensis* utilizes. This sampling should also be coupled with more extensive surveys in regions

where *H. capensis* has been reported in the past [see Van Nieukerken & Geertsema (2015) and Torrance (2016)].

To date, the suppression of the leafminer in some commercial vineyards has been gained through the application of spinosyns and organophosphates. The laboratory-based chemical bioassays using spinetoram, cypermethrin and dichlorvos proved to cause exceptionally high levels of mortality of leaf-mining larvae. Sole-reliance on these insecticides should, however, be avoided as resistance has been documented in other leaf-mining pests within each of their associated chemical classes (spinosyns, pyrethroids and organophosphates, respectively). Low yield, poor crop quality and the threat of potential market exclusion due to leafminer infestations will necessitate the use of chemical control strategies, at least until alternative control strategies have been explored and tested under field-conditions. Until then, all three chemicals should be used in rotation to avoid the development of insecticide resistance to a particular chemical class once registration trials have been conducted. Ignoring the implementation of alternative control strategies in the near future will likely result in insecticide resistance of one or more chemical classes, which could otherwise be implemented in long-term IPM strategies.

To maximize IPM strategies, the selection of control strategies should include methods that are economically, environmentally and socially favorable (Kogan 1998). Microbial biological control agents, such as entomopathogenic nematodes (EPNs), are ideal candidates to maximize the efficiency of management protocols (Lacey *et al*. 2015) and provide an environmentally-friendly and sustainable alternative to chemical control. EPNs have been used to control a number of leaf-mining pests of economic importance, as shown in previous research (Hara *et al*. 1993; Beattie *et al*. 1995; Williams & Walters 2000; Gözel & Kasap 2015; Progar *et al*. 2015; Jacob & Mathew 2016; Kamali *et al*. 2017; Mutegi *et al*. 2017). All of the local EPN species screened in the present study were pathogenic against the leaf-mining larvae. *Heterorhabditis indica* Poinar, Karunakar & David, *Heterorhabditis noenieputensis* Malan, Knoetze & Tiedt and *Heterorhabditis baujardi* Phan, Subbotin, Nguyen & Moens, three of the smaller EPNs (≈ 500 µm), caused the highest mortality of > 83%. The vision of using EPNs in an IPM strategy to control above ground pests [e.g. mealybugs (Le Vieux & Malan 2013)] and soil borne/associated pests [e.g. false codling moth (Malan & Moore 2016) and banded fruit weevil (Ferreira & Malan 2014)], would provide additional spin-offs for the control of *H. capensis* in commercial vineyards if the same EPN species have the ability to control multiple pests. In practice this will prove to be a challenge until the potential hosts (of any given EPN intended as a control agent) and appropriate commercial culturing methods have been established. While using EPN mixtures may be a suitable solution to controlling multiple pests, this would likely be too expensive. The biggest concern with the application of EPNs in foliar environments is the adoption

of an appropriate application method to optimize humidity and temperature conditions to encourage optimal EPN persistence in harsh and fluctuating field environments (Wright *et al*. 2005). Field trials using complimentary adjuvants need to be carried out before field applications are officially conducted and it is likely that shorter application intervals of applied EPNs will be necessary.

Physical control measures (i.e. bunch covers) were investigated as a means of mitigating the contamination of bunches, particularly when maximum residue levels (MRLs) have been reached and bunch infestation seems inevitable. To reduce the use of chemical applications until appropriate field trials have been conducted, the use of bunch covers may also prove to be a useful tool. Here, the use of bunch covers has been referred to as a "control measure", although, a preventative measure is probably more appropriate. More specifically, the use of bunch covers does not reduce the pest load within a grapevine block and should, therefore, be considered as a supplementary form of control. This is an ideal control method for organic table grape farming, where more effort is placed on finding alternative measures of control. Although no heavily infested organic vineyards have been detected in the Western Cape, it would be interesting to study the plant-pest interactions and parasitoid assemblages in such vineyards.

The investigations laid out over the last few chapters have provided a greater understanding of the pest and, for the first time, provided insights into the development of potential control strategies to manage *H. capensis* pest populations in future. As *H.capensis* is a native pest, the presence of the leafminer in cultivated grapevines is undeniable and control strategies should, therefore, aim to incorporate effective, long term solutions (i.e. avoid control efforts that are detrimental to environmental and human health). Using the biological information gathered by Torrance (2016) various preliminary recommendations can be made in terms of pest management: As moths are present throughout the year control efforts should be adopted at bud break, to avoid the development of the first generation of leaf-mining larvae especially if post-harvest control strategies are not conducted in the previous growing season. Population peaks experienced at the end of a grapevine season warrant extra care when considering the management of late season cultivars. It is, however, apparent that high levels of bunch infestation can occur in early season cultivars if conditions are optimal for development and infestations in the previous season are not managed appropriately.

Registration trials of a wide variety of insecticides, including but not limited to cypermethrin, spinetoram and organophosphates, will reduce the development of insecticide resistance. Future studies should be conducted to identify effective insecticides from a wide range of chemical classes that have been registered against other leaf-mining pests [such as abamectin, cyromazine, deltamethrin, methomyl and oxamyl (Agri-Intel 2019)], to further reduce the development of insecticide resistance. Due to their undeniable presence in agricultural landscapes, integrated control

strategies should aim to suppress pest populations as opposed to outright eradication thereof. Interestingly, this realization parallels the ethos of IPM philosophy (Kogan 1998). The prospect of adequate, long-term control in most grape producing situations seems obtainable if insecticides are used in conjunction with other physical (e.g. bunch covers) and biological control agents (e.g. EPNs). The success of strategies which promote a reprieve on the reliance of insecticidal applications, such as these, however, is dependent on grower perceptions and appropriate adaptations to current management practices. Field trials and corresponding registration trials need to be conducted to confirm efficacy of the successful chemical and biological candidates identified in this study, in order to be able to implement these control strategies in infested vineyards.

The scope for future research is vast and includes assessing other control strategies, such as the use of entomopathogenic fungi (EPF) to suppress adult populations, the introduction of parasitoids, the development of mating disruption and sterile insect technique (SIT), to name but a few. The development of an IPM strategy against *H. capensis* in the future, however, is dependent on the commercialization of the attractant. Through commited monitoring (which necessitates the use of the attractant) and adequately timed control efforts, bunch infestation can be avoided and the financial costs associated with excessive or unnecessary control efforts will be reduced. The current status of the availability of the attractant in South Africa is uncertain and dependent on a commercial entity to engage with the developer in Sweden [see Wang *et al*. (2015)] in order to initiate registration and commercialization.

This study has provided baseline information for the development of a field-appropriate control strategy, focused specifically on the suppression of *H. capensis* populations. It also highlights the need for a tandem approach to the use of chemical insecticides and biological control agents once the necessary cultural aspects are satisfied. In addition, this study can advise other chemical and alternative control strategies of other economically important leaf-mining pests.

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