

**THE RELATIONSHIP BETWEEN ANTS (HYMENOPTERA: FORMICIDAE),
VINE MEALYBUG (HEMIPTERA: PSEUDOCOCCIDAE) AND PARASITIDS
IN VINEYARDS OF THE WESTERN CAPE PROVINCE, SOUTH AFRICA**

by

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**Dissertation submitted in partial fulfillment of the requirements for the degree of
Doctor of Philosophy at Stellenbosch University.**

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December 2008

DECLARATION

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Date: 15 October 2008

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ABSTRACT

The mutual association between honeydew foraging ants and vine mealybugs in vineyards is detrimental to the biological control of the vine mealybug *Planococcus ficus* (Signoret). This study investigated the relationship between ants, vine mealybugs and their parasitoids to improve biological control of the vine mealybug. The investigation was carried out during two consecutive growing seasons (2005-2006 and 2006-2007) in two major wine grape growing areas of the Western Cape Province, Simondium (two farms) and Breede River Valley (one farm). A presence – absence cluster sampling system was used on a biweekly basis throughout both growing seasons from October to February on each of the farms. Additionally, yellow delta traps with vine mealybug pheromone, were used to catch parasitoids in vineyards. Prior to harvest, economic damage to grape bunches was assessed using a 0-3 damage rating index. Three ant species, *Anoplolepis steingroeveri* (Forel), *Crematogaster peringueyi* Emery and *Linepithema humile* (Mayr) foraged mainly on vine stems during both seasons, relative to other plant parts. Vine mealybugs of all stages were found on all sampling dates. Three species of primary parasitoids attacked the vine mealybug, including *Anagyrus* sp. near *pseudococci* (Girault), *Coccidoxenoides perminutus* (Timberlake) and *Leptomastix dactylopii* Howard. Bunch damage was significantly different between seasons in the Breede River Valley but not in Simondium. There was a negative linear relationship between ants and parasitoid activity in all vineyards. Spatial Analysis by Distance IndicEs (SADIE) was used to analyse spatial distribution of ants and parasitoids and ArcView, with its extension, Spatial Analyst, were used to map the gap, patch and local association indices where significant association and disassociation occurred. A significant association was found between *C. peringueyi* and parasitoids and *L. humile* and parasitoids. There was a significant disassociation between *L. humile* and *C. peringueyi* and between *A. steingroeveri* and *C. peringueyi* indicating interspecific hostility. With a known level of error, an economic threshold (ET) of 20% was determined for the first time for pest ants in vineyards. A laboratory study quantified that *C. peringueyi* and *L. humile* significantly reduced parasitism of the vine mealybug more than *A. steingroeveri*. *C. perminutus* were more ant tolerant and caused significantly higher parasitism than *A. sp. near pseudococci* in the presence of all ant species. Twenty-four hour pesticide bioassays with parasitoids revealed that Fipronil and α -cypermethrin residues were the most toxic pesticides of those tested on *A. sp. near pseudococci* and *C. perminutus*. Mortality of *C. perminutus* was significantly

higher than that of *A. sp. near pseudococci*. The pesticide bioassays demonstrated that the adult stage of parasitoids is more vulnerable to pesticide residues than the protected juvenile stage. Both *A. sp. near pseudococci* and *C. perminutus* were equally susceptible to the systemic pesticide, imidachlopid. Frequent and thorough monitoring of both pests and natural enemies provides essential information in space and time, on the species present, their interactions and stage of development. This information is important in decision making in pest management regarding action thresholds, timing and method of application and choice of control strategies.

OPSOMMING

Die assosiasie tussen heuningdou-voedende miere en witluse in wingerde is skadelik vir biologiese beheer van die wingerd witluis *Planococcus ficus* (Signoret). Hierdie studie het die verwantskap tussen miere, wingerd witluis en hul parasitoëide bestudeer, om die biologiese beheer van wingerd witluis te kan verbeter. Gedurende twee opeenvolgende seisoene (2005-2006 en 2006-2007) is 'n opname in twee hoof wyndruif produksie streke van die Weskaap, Simondium (twee plase) en die Breëriver Vallei (een plaas), gedoen. Elke tweede week gedurende die seisoen vanaf Oktober tot Februarie is stokke vir die teenwoordigheid van miere en witluse geïnspekteer. Geel delta valletjies met feromoon lokmiddels is verdermeer gebruik om mannetjie witluse en parasitoïdes in die wingerde te vang. Die ekonomiese skade van witluis besmetting is kort voor oestyd bepaal, deur 'n skade index van 0-3 te gebruik. Drie mier spesies, *Anoplolepis steingroeveri* (Forel), *Crematogaster peringueyi* Emery and *Linepithema humile* (Mayr) het gedurende beide seisoene hoofsaaklik op stamme, relatief tot die ander plantdele, voorgekom. Witluse van alle stadia is tydens alle datums van monitering gevind. Drie primêre witluis parasitoïdes nl; *Anagyrus* sp. near *pseudococci* (Girault), *Coccidoxenoides perminutus* Timberlake en *Leptomastix dactylopii* Howard is gevind. Trosskade was beduidend verskillend tussen seisoene by Ashton (Breërivier Vallei), maar nie by Backsberg of Plasir de Merle (Simondium) nie. Daar was 'n negatiewe liniêre korrelasie tussen miere en parasitoïdes in alle wingerde. Spatial Distance IndicEs (SADIE) en ArcView met sy uitbreiding, Spatial Analyst, is gebruik om ruimtelike verspreidingspatrone van die insekte ter sprake te bepaal. 'n Beduidende assosiasie is tussen *C. peringueyi* en parasitoïdes, en *L. humile* en parasitoïdes gevind. Daar was 'n betekenisvolle disassosiasie tussen *L. humile* en *C. peringueyi* en tussen *A. steingroeveri* en *C. peringueyi*, wat dui op interspesifieke vyandelikheid. Met kennis van 'n steekproefnemingsfout, kon 'n ekonomiese drempelwaarde (ET) van 20% vir die eerste keer vir skadelike miere in wingerde bepaal word. 'n Laboratorium studie het bepaal dat *C. peringueyi* en *L. humile* betekenisvol meer parasitisme van wingerd witluis verminder het as *A. steingroeveri*. *C. perminutus* was meer mierbestand en het 'n beduidend hoër parasitisme as *A. sp. near pseudococci* tot gevolg gehad. 'n Vier-en-twintig uur plaagdoder bio-assei het getoon dat Fipronil en α -sipermetrien hoogs toksies vir *A. sp. near pseudococci* en *C. perminutus* parasitoïdes is. Mortaliteit van *C. perminutus* was beduidend hoër as die van *A. sp. near pseudococci*. Hierdie plaagdoder

bioassei het verdermeer gedemonstreer dat volwasse parasitoïedes meer sensitief as beskermde onvolwasse stadia vir plaagdoder residue is. Beide *A. sp. near pseudococci* en *C. perminutus* was ewe vatbaar vir die sistemiese middle, imidachloprid. Deeglike monitering van beide plae en natuurlike vyande voorsien belangrike inligting in tyd en ruimte, en dui op spesies wat voorkom, hul interaksies en stadia van ontwikkeling. Hierdie inligting is belangrik om besluite te neem in plaagbestuur, rakende aksie drempelwaardes, tyd van toediening van plaagbeheermaatreëls en keuse van beheer metode.

ACKNOWLEDGEMENTS

This thesis would not have been possible without the generous help from the landowners and farm managers of Ashton Farm, Mr. P. Bruwer, Backsberg Estate, Mr. C. Trent and Plaisir de Merle Estate, Mr. F. Le Roux, who provided vineyards for the investigations. I wish to express my sincere gratitude to all research assistants who helped with data collection. Guys! Thank you very much for your dedication.

Many thanks go to my supervisor, Dr P Addison who painstakingly revised this work and for her steadfast faith in me from the start that this project could and would be completed.

I am also indebted to Drs K.L. Pringle and J. Terblanche and Professors M. Kidd and D Nel of Stellenbosch University for their untiring assistance with statistics and Drs G.L. Prinsloo and J. Kelly of ARC, Pretoria for identifying the parasitoids.

Dr K. Achiano, ARC, Stellenbosch, thank you for your invaluable generosity with mealybug colonies and advice. You did not mind my incessant requests for these precious creatures.

My sincere thanks are due to my sponsors DFPT, THRIP and WINETECH.

I would like to thank all members of staff and colleagues whose moral support meant so much to me.

TABLE OF CONTENTS

	Page
DECLARATION.....	i
ABSTRACT.....	ii
OPSOMMING.....	iv
ACKNOWLEDGEMENTS.....	vi
CHAPTER 1	
GENERAL INTRODUCTION.....	1
Ant management in vineyards.....	3
Mealybug management in vineyards.....	4
Impact of VMB on wine quality.....	5
Impact of pesticides on parasitoids.....	5
The role of ants in the biological control of arthropods.....	6
Association of ants with Hemiptera.....	8
The Argentine ant <i>Linepithema humile</i> (Mayr).....	8
The pugnacious ants (<i>Anoplolepis</i> spp.).....	9
The Cocktail ants (<i>Crematogaster</i> spp.).....	10
Benefits derived by Hemiptera from the ant-hemipteran mutualism...	11
Ant behaviour towards natural enemies of the vine mealybug.....	12
<i>Planococcus ficus</i> parasitoids in Western Cape Province (WCP), South Africa.....	13
<i>Anagyrus</i> species near <i>pseudococci</i> (Girault) (Hymenoptera: Encyrtidae)..	15
<i>Coccidoxenoides perminutus</i> (Timberlake) (Hymenoptera: Encyrtidae)...	16
Practices assisting with the establishment and conservation of parasitoids in vineyards.....	16
OBJECTIVES	17
REFERENCES	18
CHAPTER 2	
INTERACTIONS INVOLVING ANTS, VINE MEALYBUG AND VINE MEALYBUG PARASITIDS	
ABSTRACT.....	29

INTRODUCTION.....	30
MATERIAL AND METHODS.....	31
Study sites.....	31
Sampling Methods.....	32
1. <i>Presence-absence cluster sampling</i>	32
2. <i>Pheromone traps</i>	32
Damage assessment.....	33
Data analysis.....	33
RESULTS	33
Foraging ant fauna.....	33
Seasonal ant movement within the vine.....	34
Seasonal vine mealybug movement within the vine.....	36
Impact of ant infestation on VMB infestation.....	38
Impact of ant infestation on VMB parasitism rate.....	38
Damage rating.....	40
Parasitoid complex.....	43
<i>Parasitoids reared from VMB</i>	43
<i>Parasitoids caught in VMB pheromone traps</i>	44
DISCUSSION.....	45
CONCLUSION.....	49
REFERENCES.....	48

CHAPTER 3

SPATIAL DISTRIBUTION OF ANTS (FORMICIDAE) AND PARASITOIDS IN VINEYARDS

ABSTRACT.....	53
INTRODUCTION.....	53
MATERIAL AND METHODS.....	56
Data Analysis.....	56
RESULTS.....	58
Ashton.....	58
Backsberg.....	58
Plaisir de Merle.....	58
DISCUSSION	66

CONCLUSION.....	67
REFERENCES.....	68

CHAPTER 4

DEVELOPMENT OF AN ECONOMIC THRESHOLD FOR ANTS (HYMENOPTERA: FORMICIDAE) IN VINEYARDS

ABSTRACT.....	72
INTRODUCTION.....	72
MATERIAL AND METHODS.....	74
Study sites.....	74
Sampling method.....	74
Dummy variable regression models.....	75
Sampling errors.....	77
Operating Characteristic (OC) curves.....	78
RESULTS AND DISCUSSION.....	78
Dummy variable regression models.....	81
Sampling error.....	86
Operating Characteristic (OC) for ant infestation.....	89
CONCLUSION.....	90
REFERENCES.....	90

CHAPTER 5

IMPACT OF ANTS (HYMENOPTERA: FORMICIDAE) ON VINE MEALYBUG PARASITIDS *ANAGYRUS* SP. NEAR *PSEUDOCOCCI* (GIRAULT) AND *COCCIDOXENOIDES PERMINUTUS* (TIMBERLAKE) (HYMENOPTERA: ENCYRTIDAE) UNDER LABORATORY CONDITIONS

ABSTRACT.....	92
INTRODUCTION.....	93
MATERIAL AND METHODS.....	94
Insect colonies.....	94
<i>Vine mealybug colonies</i>	94
<i>Ant colonies</i>	95
<i>Parasitoid colonies</i>	95
<i>I. Anagyrus</i> sp. near <i>pseudococci</i>	95

2. <i>Coccidoxenoides perminutus</i>	96
Quantitative Observations.....	96
Data analysis.....	96
RESULTS.....	97
Ant behaviour in the presence of parasitoids.....	97
Parasitoid behaviour in the absence and presence of ants.....	97
Effects of ants on parasitoid mortality and mealybug parasitism...	98
Qualitative observations.....	100
1. Parasitoids.....	100
2. Ants in the presence of parasitoids.....	100
DISCUSSION.....	100
CONCLUSION.....	102
REFERENCES.....	103

CHAPTER 6

EFFECT OF CONTACT PESTICIDES ON THE VINE MEALYBUG PARASITIDS, *ANAGYRUS* SP. NEAR *PSEUDOCOCCI* (GIRAULT) AND *COCCIDOXENOIDES* *PERMINUTUS* (TIMBERLAKE) (HYMENOPTERA: ENCARTIDAE)

ABSTRACT.....	107
INTRODUCTION.....	107
MATERIAL AND METHODS.....	111
Continuous exposure on residues.....	111
Topical bioassays of field rate pesticides on parasitoid pupae.	114
RESULTS.....	114
Continuous exposure on residues.....	114
Topical application of field rate pesticides on parasitoid pupae.	118
DISCUSSION AND CONCLUSION.....	119
REFERENCES.....	121

CHAPTER 7

BIOASSAYS TO DETERMINE THE SUBLETHAL EFFECTS OF A SYSTEMIC INSECTICIDE (IMIDACHLOPRID) ON *ANAGYRUS* SP. NEAR *PSEUDOCOCCI* (GIRAULT) AND *COCCIDOXENOIDES PERMINUTUS* (TIMBERLAKE) (HYMENOPTERA: ENCARTIDAE)

ABSTRACT.....	125
INTRODUCTION.....	125
MATERIAL AND METHODS.....	127
Data analysis.....	127
RESULTS.....	128
DISCUSSION	131
CONCLUSION.....	132
REFERENCES.....	132

CHAPTER 8

GENERAL DISCUSSION	135
FUTURE RESEARCH.....	137
CONCLUSION.....	138
REFERENCES.....	139

CHAPTER 1

GENERAL INTRODUCTION

Ants (Hymenoptera: Formicidae) are ubiquitous, very diverse and the most abundant insect taxon, constituting up to 80% of total animal biomass in tropical ecosystems (Philpott & Armbrrecht 2006). They display great species richness, fascinating community dynamics, inter- and intraspecific interactions, mutualistic associations and invasions. Ants play a major role in the ecological structure of many terrestrial ecosystems performing major functions such as aerating the soil, directing energy and nutrient recycling (Alonso 2000). Ants dominate most of the terrestrial insect fauna by acting as predators and engaging in mutualistic relationships with Hemiptera from which they harvest honeydew and protect these Hemiptera from small predators and parasitoids (Way 1963). They have been known to move hemipterans to better and safer feeding sites or improve conditions for hemipteran offspring (Nixon 1951; Way 1963; Buckley 1987). They act as bioindicators - the presence or absence of plant species in an area can frequently be determined by ant activity such as harvesting, consuming and burying seeds (myrmecochory) (Andersen et al. 2002). Ants also pollinate some plant species. However, some ant species often present problems to farmers: i) they destroy large areas of crop, for example, leaf cutter ants (*Atta sexdens* Linnaeus & *A. cephalotes* L) destroying billions of dollars worth of crops in the USA and South America (Wilson & Hölldobler 1994); ii) ants interfere with biological control efforts promoting hemipteran populations that cause crop loss (Way 1963); iii) many hemipterans are vectors of viral diseases (Way & Khoo 1992), for example, the vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), vectors the grapevine leaf roll-associated virus 3 (GLRaV-3) (Engelbrecht & Kasdorf 1990; Cabaleiro & Segura 1997; Golino et al. 2002; de Borbon et al. 2004).

In South Africa, it is not known at what level of infestation ants pose a threat to biological control of *Planococcus ficus*. To establish this level, the relationship between ants, *P. ficus* and *P. ficus* natural enemies must be clearly defined in the field since this interaction influences the ants' injuriousness and therefore the need to control ants. Most ant species found in vineyards are beneficial and hence the need to protect them from pesticides. Currently, an action threshold of 25% ant stem infestation is used to

warrant chemical control in vineyards (<http://www.ipw.co.za>). However, this figure has not been backed by scientific data and is used as a guideline only. The development of an action threshold for ants will prevent the unnecessary application of pesticides, thereby saving costs and the environment, as well as provide producers with a more accurate guideline for deciding when to implement chemical control against pest ants.

The interactions taking place in this mutualistic relationship have not yet been quantified in South Africa. Different ant species exhibit various degrees of aggression towards mealybug parasitoids (Mansfield et al. 2003) and this would therefore, among other factors, influence the degree of disturbance of biological control of *P. ficus*. It is already known that ants disrupt the biological of *P. ficus* in South Africa (Kriegler & Whitehead 1962). However the role that South African honeydew seeking ants play in this mutualistic relationship with is not yet fully assessed. Nixon (1951) reported that the effect of ants on biological control is dependant on i) the extent to which agricultural crops are infested with hemipterans, ii) the susceptibility of natural enemies to different ant species, and iii) the distribution and density of the ant populations. Work done in California has mainly centred on the invasive Argentine ant (Human & Gordon 1999; Holway 1995; Daane et al. 2004a.; 2007). However there are other dominant ant species tending *P. ficus* in South Africa whose interactions with the local *P. ficus* parasitoids have not been documented to date. Evaluating these interactions will lead us to a better understanding of how to improve *P. ficus* biological control with regard to timing, quantity and extent of augmentative releases of parasitoids in the field.

Over-reliance on synthetic pesticides in global crop protection programmes has resulted in disturbances in the environment, pest outbreaks and resurgence, resistance to pesticides and lethal and sublethal impacts on non-target organisms, particularly natural enemies. With this in mind, the scheme for Integrated Production of Wine (IPW) in South Africa encourages producers to reduce dependence on chemical pesticides in favour of ecosystem manipulations (<http://www.ipw.co.za>). Organophosphates (OPs) and carbamates are mostly used for ant and mealybug control, the former group being toxic to natural enemies due to high contact and long residual toxicity (Wakgari & Giliomee 2003; Prabhaker et al. 2007). Presently, it is not practical for producers to rely on biological control of *P. ficus* only because ants are still a problem and because of the low economic threshold for *P. ficus* infestation, particularly in virus infested vineyards and nurseries. Integrated pest management (IPM) in vineyards, is important to improve natural enemy species richness and abundance and to sustain vineyard viability. IPM

involves the use of preferentially chosen pesticides that are inherently selective, or use of the pesticide in a selective manner, together with natural enemies. Ecological selectivity, in which pest control operations are manipulated in such a way to minimise contact of a susceptible natural enemy with the pesticide, integrates effectively into IPM programmes. This is done through timing, placement and formulation of pesticides.

Ant management in vineyards

All honeydew seeking ants are well known for disturbing biological control in agricultural systems where they associate with Hemiptera. It is therefore imperative to control ants to enhance the effectiveness of natural enemies. In South Africa, ant control is achieved by chemical and physical control methods. Pesticides registered for ant control are chlorpyrifos and α -cypermethrin applied as ring sprays (chemical stem barriers) (Addison 2002) to keep ants out of vines but still allow them to predate on fruit and moth larvae, pupae and even eggs (Mansfield et al. 2003). Despite reducing the impact on the natural enemies, ring sprays are labour intensive, and due to that, low toxic baits are being investigated as a more practical method of chemical ant control. These could be more effective than ring sprays because of the low concentration of pesticide (Nelson & Daane 2007; Daane et al. 2008; Tollerup et al. 2004). A bait is shared among nest mates and queens (trophylaxis) while sprays are only targeted at the foraging workers who, upon identifying the chemical, release an alarm pheromone to nest mates and cease sharing and recruitment. Baits are slow acting and therefore do not prevent sharing and recruiting (Rust et al. 2000). Additionally, low toxic baits also target all ant pests including arboreal ants like *Crematogaster* species.

Non-toxic sticky barriers, applied around all possible pathways leading into the vine canopy, are used to keep ants out of the vines. They are made of polybutene-based glue that does not wash off. They trap ascending and descending ants but become ineffective after some time out in the field as the surface becomes covered in soil and plant material.

Vineyard hygiene is important, particularly weed control, as these provide alternative pathways into the vine canopy or include volunteer plants that harbour *P. ficus* (Walton 2003).

Cover cropping was not found to influence high populations of the common pugnacious ant *Anoplolepis custodiens*, but could be more effective on lowering ant infestations as

this ant favours bare compact ground with high insolation (Addison & Samways 2006; Steyn 1954)

Mealybug management in vineyards

Planococcus ficus causes direct damage by exuding honeydew onto foliage and fruit bunches. Apart from blocking stomata on the foliage, honeydew is a substrate for sooty mold growth which makes bunches unsalvageable. The presence of ovisacs and *P. ficus* themselves reduce the quality of bunches too. Of great concern is the ability of *P. ficus* to transmit GLRaV-3 (Engelbrecht & Kasdorf 1990). Douglas and Krüger (2008) found that the crawlers are the main transmitters of viral diseases since they are able to move faster than any other stage of *P. ficus*. Only one viruliferous *P. ficus* is needed to transmit GLRaV-3. Due to this, farmers use chemicals aggressively to prevent *P. ficus* infestations in their vineyards, particularly mother blocks. A mother block, also called an increase block, is where certified vines, free of viruses are planted for subsequent propagation purposes.

Foliar applications of chlorpyrifos (organo-phosphate), buprofezin (Insect Growth Regulator) and a systemic chloro-nicotinyl, imidachloprid, are effective chemicals against mealybugs. However, *P. ficus* is difficult to control with insecticides as it hides in crevices in the bark, occurs on the roots and secretes thick layers of protective hydrophobic wax (Meyerdick et al. 1981; <http://www.avenuevine.com>; Walton et al. 2004). Chlorpyrifos use in vineyards is becoming a great concern due to its environmental and health hazards as a broad spectrum organo-phosphate.

Mating disruption using female pheromones is now an alternative way to deal with sheltering *P. ficus*. Mating disruption is compatible with sustainable integrated pest management programmes because there is no environmental toxicity, low populations of pests can be effectively controlled and there are no residual effects (Millar et al. 2002; Daane et al. 2004a).

Spot treatments, application of pesticides to limited areas where pests are likely to occur, prevent spread of pests over large areas. In vineyards, spot treatments are aimed at reducing pesticide impacts on beneficials because only the infested plot or block will be treated to conserve natural enemies in uninfested areas (Homan & Claussen 1998).

Planococcus ficus has several natural enemies that are capable of maintaining its population below economic injury levels, for example, *Coccidoxenoides perminutus* (Timberlake) reduced *P. ficus* infestation levels in South Africa to the same levels that

insecticides did (Walton 2003) and *Anagyrus pseudococci* (Girault) was effective in California (Daane et al. 2004b). The control of ants and *P. ficus* is important to maintain virus free vineyards through IPM and as such, the movement of viruliferous *P. ficus* from nearby vineyards should be prevented. Future research could look at entomopathogenic nematodes (EPNs) to manage subterranean *P. ficus* as with soil pupating larvae of fruit fly and codling moth larvae. Stuart et al. (1997) successfully tested the susceptibility of the mealybug *Dysmicoccus vaccinii* (Miller & Polavarapu) to various species and strains of EPNs (Heterorhabditidae and Steinernematidae) in the laboratory and De Waal et al. (2007) demonstrated high susceptibility of *P. ficus* to *H. zealandica* in the laboratory.

Cultural methods of managing *P. ficus* in South Africa include use of certified planting material to prevent incidence and spread of GLRaV-3 and other associated viral diseases that destroy vineyards. The dispersal of *P. ficus* to clean vineyards can be avoided by exercising good vineyard hygiene, for example, use of clean equipment and avoiding movement from infested to uninfested vineyards by workers (van der Westhuizen 2000). Weeds that harbour *P. ficus* should be removed from vineyards and destroyed (Walton & Pringle 2004).

Impact of VMB on wine quality

Only a small number of studies have so far assessed the effects of leaf roll virus on wine quality. Based on spectrographic analyses (Over de Linden & Chamberlain 1970) and sensory evaluations (Schoefling 1980; Ueno et al. 1985; Mannini et al. 1998), all studies found that the wine quality from leaf roll infected vines was reduced compared with that from healthy vines. Mannini et al. (1998) argued that wine produced from healthy Nebbiolo vines was found to have a more complex bouquet and flavour as well as better colour intensity compared with wine from GLRaV-3 and Grapevine vitivirus A infected vines.

Impact of pesticides on parasitoids

Natural enemies get in contact with pesticides through direct exposure to chemicals, contact with pesticide residue or through the food chain. They may encounter toxic residues while on plants, soil surface or while flying. Some may even ingest the toxic substances while feeding on plant material (flower nectar) to obtain nutrients or water, through predation, host feeding by adult parasitoids or immature parasitoids consuming

the host (Desneux et al. 2007). Natural enemies provide an excellent regulation mechanism of pests so that they do not reach pest status. The effects of pesticides on parasitoids can be the following:

- a) Acute toxicity which results from direct exposure to the pesticide, either topical (during spray) or residual (walking over treated surfaces, e.g. leaves, bark)
- b) Sublethal which implies a number of effects other than death which result in reduced capacity of a natural enemy (parasitoid) to control the target pest. Examples include reduced ability of the parasitoid to find or parasitise prey, reduced longevity, reduced prey consumption by developing parasitoids and repellency; reduced fecundity of females (lay fewer eggs over their life time) and egg sterility (eggs do not hatch) (Desneux et al. 2007). Pesticides can also reduce host availability and quality subsequently affecting the local population size and fecundity of endo-parasitoids (Gao et al. 2008). These negative impacts of pesticides on natural enemies are often shown by target pest resurgence when natural enemies are destroyed. Pesticides often kill a greater proportion of natural enemies than the intended pest(s) so that after application the pest can build up populations again (Hajek 2004). They also cause secondary pest outbreaks which can occur when pesticides kill the natural enemies that have been controlling a species that has not previously been a pest.

The role of ants in the biological control of arthropods

Predatory ants can be utilised to control arthropod crop pests especially those found in or on the ground, for example, soil pupating Diptera and Lepidoptera. Ants were the first insects to be used as biological control agents and are still in use today for this purpose. In citrus orchards, they control Mediterranean fruit flies as well as false codling moth in the soil (Steyn 1954; Samways 1982). Mansfield et al. (2003) gives a recent report on the use of ants as egg predators of the cotton bollworm *Helicoverpa armigera* Hübner in Australian cotton crops. Fifty species of predacious ants were utilised by farmers for the control of insect pests and include *Oecophylla*, *Dolichoderus*, *Anoplolepis*, *Wasmania* and *Azteca* spp. in the tropics, *Solenopsis* spp. in the tropics and sub-tropics and *Formica* spp. in temperate environments (Way & Khoo 1992). Table 1 illustrates some of the uses of ants as biological control agents in certain crop production systems and their relative impact rate on natural enemies.

Table 1: Ants as predators of arthropod pest species in different areas and crops, with their relative association with Hemiptera and rated impact on natural enemies.

<i>Ant species</i>	Country	Crop	Pest(s)	Association with Hemiptera	Impact rate on natural enemies*	Reference(s)
<i>Oecophylla longinoda</i> (Latreille)	Solomon Islands	Coconuts	Lepidoptera and rice rodents. e.g. <i>Coreidae</i> <i>Amblypelta cocophaga</i>	Yes	3	Way & Khoo 1992 Philpott & Armbrrecht 2006. Hölldobler & Wilson 1990.
<i>O. smaragdina</i> (Fabricius)	Malaysia	Cacao				
	Papua New Guinea	Oil palm				
	Ghana	Coffee	Egg predators of <i>Helicoverpa armigera</i> . Mirids	Yes	2	Flanders 1951 Steyn 1954 Way & Khoo 1992
<i>Dolichoderus thoracicus</i> F.Smith.	Indonesia	Cacao				
<i>Linepithema humile</i> (Mayr)	Australia	Cotton				
<i>Formica lugubris</i> B.	Germany	Forest	Lepidoptera e.g. <i>Panolis flammea</i>	Yes	1	Way & Khoo 1992
<i>F. rufa</i> L.						
<i>F. polyctena</i> Förs.	Italy					
<i>Anoplolepis custodiens</i> (Smith)	South Africa	Citrus vines	Medfly (<i>Ceratitis capitata</i>)	Yes	3	Steyn 1954 Way 1963 Whitehead 1957 Samways 1982 Leston 1973
<i>A. steingroveri</i> (Forel)	Ghana	Cacao	Boll worm (<i>Helicoverpa armigera</i>)			
			False codling moth (<i>Thaumatotibia leucotreta</i>)			
<i>Azteca instabilis</i> (F)	Trinidad	Citrus	Protect citrus against leaf cutter ants (<i>Atta</i> spp.)	Yes	2	Way & Khoo 1992 Philpott & Armbrrecht 2006
	Mexico	Coffee	Coffee borer			
<i>Solenopsis invicta</i> Buren	USA	Sugar cane	Egg predator of <i>Eldana saccharina</i> in sugar cane	Yes	2	Way 1963 Philpott & Armbrrecht 2006.
		Cotton	<i>H. armigera</i> in cotton			
<i>Pheidole bicarinata longula</i> Emery	USA	Cotton	Egg predator of	N/A	1	Way & Khoo 1992 Kuhlmann & van der Burgt 1998 Mansfield et al. 2003.
<i>P. megacephala</i> (F)	Australia		<i>Diabrotica</i> species in soil e.g. <i>Alabama argillacea</i>			
			<i>H. armigera</i> in cotton.			
<i>Wasmania auropunctata</i> Roger	Solomon Islands	Cacao	Cacao mirids <i>A. cocophaga</i> . Displacing pest ants <i>Iridomyrmex cordatus</i> and <i>P. megacephala</i>	N/A	1	Way & Khoo 1992

*1-least negative (limited) impact; 2- mild negative impact and 3-severe negative impact on natural enemies.

Association of ants with Hemiptera

A number of ants have developed a mutualistic association with honeydew excreting Hemiptera which Wheeler (1925) divided into two groups, namely piercing and sucking pests, as well as biting and chewing pests. The aphids (Aphididae), scale insects (Coccidae), mealybugs (Pseudococcidae), tree-hoppers (Membracidae), whiteflies (Aleyrodidae) and jumping plant lice (Psyllidae) belong to the former group while the second group comprises caterpillars of the Lycaenid butterflies. The association of ants with these taxa is facilitated mostly by the sedentary and gregarious nature of the Hemiptera, enabling the ants to collect a large amount of food without having to forage over large areas or long distances, wasting energy and time (Stadler & Dixon 2005).

Unchecked presence of ants in vineyards can result in injurious levels of scale insects and vine mealybugs. There are four dominant ant species associated with high vine mealybug infestation levels in South Africa, namely the Argentine ant *Linepithema humile* (Mayr), cocktail ant *Crematogaster peringueyi* Emery, and the two pugnacious ants, *Anoplolepis custodiens* (Smith) and *A. steingroveri* (Forel) (Whitehead 1957; Myburgh 1986; Addison & Samways 2000). Leston (1973) defined a dominant ant species as one that is numerically superior and excludes other dominants, i.e., ant species that could otherwise be numerically superior elsewhere. The above ant species are briefly described below, to give an insight into the extent of success of penetrating a habitat and the degree of management that can be rendered to each of the species.

The Argentine ant *Linepithema humile* (Mayr)

Linepithema humile invaded South Africa in 1901 (Flanders 1951; Luruli 2007) and could have possibly been introduced through human dispersal by cargo ships. Apart from their native Argentina and Brazil, *L. humile* also occur in Australia and parts of the United States of America, namely, California and New Orleans and Southern Europe, with distribution blamed on human activities (Giraud et al. 2001).

The workers are monomorphic (same size, 2-3mm long) making them successful invaders. *L. humile* are able to establish huge ant colonies (super colonies) once in a suitable environment because there is little or no intraspecific hostility between members (Markin 1968; Vasquez & Silverman 2008). Communication is through pheromones allowing worker ants to follow trails when foraging. Each super colony can have more than one queen (polygyny), whose role is to lay eggs and establish new colonies (Heller 2004; Holway et al. 2002; Holway 1995). Colonies are unicolonial,

another factor that makes *L. humile* excellent invaders. The colonies grow by budding. A queen can start a new colony with very few workers. Distribution of *L. humile* is concentrated along the humid/moist coastal areas, or further inland in damp microhabitats including households (Suarez et al. 2001; Majer 1993; Cole et al. 1992; Ward 1987; Skaife 1961).

Linepithema humile mutually associates with a number of honeydew excreting Hemiptera in different cropping systems. In South African vineyards, *L. humile* aggressively tends *P. ficus* in vineyards (Myburgh 1986; Whitehead 1957) where they promote high infestation of this pseudococcid due to their consumption of honeydew, a main component of their diet. Flanders (1943) reported severe economic crop losses due to *L. humile* association with the banana mealybug *Pseudococcus elisae* Borchsenius in the Canary islands, sugar cane mealybug *Saccharicoccus sacchari* (Cockerel), *Dysmicoccus boninsis* (Kuwana) (Hemiptera: Pseudococcidae) in sugar plantations in Louisiana and the aphid *Cerataphis latania* (Boisduval), in Belgian greenhouses. Since *L. humile* require and consume large amounts of sugar, they also tend scale insects, soft brown scale, *Coccus hesperidum* (Linnaeus) and subsequently disturb citrus red scale *Aonidiella aurantii* (Maskell) natural enemies (Smit & Bishop 1934; Samways et al. 1982). Due to their invasive nature, *L. humile* displace native ant species, subsequently upsetting the myrmecological functions as is evident in the South African Fynbos due to severe decrease in the abundance and diversity of native arthropod and plant fauna (De Kock & Giliomee 1989; Christian 2001; Heller 2004; Holway et al. 2002).

Linepithema humile build protective structures or temporary nests along their foraging trails. Horton (1918) observed that these shelters served as protective shelters for the ants from sunlight and high temperature while they rest along the foraging trails. The shelters are normally constructed when the food source is far from the nest. Under some shelters, a few mealybugs can be covered. The ants solicit honeydew from the mealybug while the shelters serve their usual protective purpose. Protection of mealybugs from natural enemies may not be the purpose of the shelters because even parasitised mealybugs were obtained from the shelters.

The pugnacious ants (*Anoplolepis* spp.)

The pugnacious ants found in South Africa are the common pugnacious ant, *Anoplolepis custodiens* and the black pugnacious ant, *A. steingroeveri* (Way & Khoo 1992; Steyn 1954, Hölldobler & Wilson 1990). Steyn (1954) outlined the distribution of

pugnacious ants, in particular, *A. custodiens*, in South Africa and quoted their occurrence in Tanzania, implying that they are potential invaders in arid habitats. Pugnacious ants have a wide distribution in South Africa where they are considered an indirect pest in vineyards and citrus orchards as they tend citrus scale, citrus mealybugs and vine mealybugs (Myers 1957; Samways 1982; Smit & Bishop 1934; Steyn 1954; Way & Khoo 1992). Because of their predacious behaviour, pugnacious ants can be allowed to forage on orchard floors where they prey on soil pupating pests like the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) and false codling moth *Thaumatotibia leucotreta* (Meyr) (Samways 1982).

The cocktail ant (*Crematogaster* spp.)

A detailed description of the cocktail ant, *Crematogaster peringueyi* Emery, var. *anguistor* Arnold, is given by Kriegler and Whitehead (1962). They are arboreal ants nesting in the vine canopy, where they tend mealybugs and occasionally block irrigation pipes and irritate workers during harvest.

Crematogaster peringueyi distribution in the Western Cape is not uniform. Kriegler and Whitehead (1962) and Addison and Samways (2000) noted that infestation by this ant tends to be localised and confined to old or neglected vineyards containing dead wood on vines. Philpott and Armbrrecht (2006) noted that arboreal ants obtain little protein through scavenging/predation and behave largely as exudate foragers (cryptic herbivores) leading to a conclusion that canopy ants like *Crematogaster* spp. defend their territories aggressively causing high Hemiptera infestations. Since *Crematogaster* colonies build up within wood crevices, infestations could go unnoticed (Whitehead & Kriegler 1962). Like *L. humile*, *C. peringueyi* build protective carton tents to offer protection to the Hemiptera they tend, creating a biological barrier difficult for natural enemies to penetrate. They consume large amounts of honeydew and move Hemiptera from vulnerable positions to 'refuges' like carton tents or crevices in the vine canes. *C. peringueyi* have been observed holding onto mealybugs during field collections and sometimes even devouring the mealybug (personal observation). Predators like coccinellids have often been seen leaving refuges with a swarm of these ants pursuing them (Whitehead 1957).

Benefits derived by Hemiptera from the ant-hemipteran mutualism

Flanders (1943) described ants as providing ‘military’ and ‘sanitary’ service to Hemiptera in return for food. Additionally ants provide transport for the more sedentary Hemiptera from vulnerable locations to more concealed and safe ones although this is dependant on the ant species concerned.

The military service is rendered when ants reduce the effectiveness of natural enemies while sanitary service is provided by removing the waste product (honeydew) that would otherwise swamp the crawlers. Van der Goot (1916) found that the white cocoa mealybug *Pseudococcus crotonis* (Green), died out under experimental conditions when not tended by ants but flourished in their presence. Kirkpatrick (1927) demonstrated that *Pseudococcus Kenyae* le Pelley was able to increase slowly without ants but multiplied three times as fast when tended by them. He also observed that ant tended mealybugs were less mealy than those not tended by ants probably due to continuous drumming by ants soliciting for honeydew. Strickland (1947) working with *Pseudococcus njalensis* Laing in West Africa, maintained that the accumulation of honeydew caused the growth of fungi, some of which were parasitic on the mealybug. Hanna et al. (1956) confirmed this observation citing that the growth of mould, following cessation of ant tendance was detrimental to the mealybug colony.

Ants provide extra protection by covering coccids and pseudococcids in shelters. For example in vineyards, *C. peringueyi* and *L. humile* covered mealybugs in little tents (carton nests) on stems, shoots, leaves and fruits and construction of these shelters coincided with periods of low mealybug infestations (Whitehead 1957). Strickland (1947) observed the same phenomena with *Ps. njalensis* enclosed in carton shelters on cacao in West Africa. Hanna et al. (1956) described the construction of carton nests by *Crematogaster* species around *Ps. njalensis*, on cacao in the Gold coast. The tents were architected to exclude parasitoids and predators but to allow *Crematogaster* to enter and exit and adult mealybugs would not escape. Whitehead (1957) working in Western Cape vineyards found mealybugs in vines covered by *L. humile* throughout the year. The material used to construct carton shelters was drawn from the underside of leaves, bits of bark and soil particles loosely stuck together. Kriegler (1954) found that *P. citri* (Risso) was covered by *L. humile* in spring, early summer and autumn when mealybug populations were low while Whitehead (1957) found covered mealybugs in midsummer where the vineyard had been partially freed of ants in autumn and most mealybugs

destroyed by natural enemies. *Anoplolepis* spp. do not build any protective structures but rather visit vineyards that are infested with mealybug (personal observation).

While it is almost certain that carton nests are built in response to dwindling mealybug numbers, their obvious purpose or benefit is not as certain. They cannot be nests for *L. humile* because only workers were found in them but probably for *Crematogaster* spp. as there were many cases where the carton tents had mealybugs, ant larvae and eggs. Their role in excluding natural enemies is still not certain because Whitehead (1957) collected coccinellid larvae as well as parasitised mealybug from them.

Ant behaviour towards natural enemies of the vine mealybug

The aggressive behaviour of ants depends upon a number of factors, for example, Flanders (1943) observed that *L. humile* grew more aggressive at low aphid populations, probably due to a limited amount of sugar, while at higher aphid populations ants ignored the attacking natural enemies. The temperament of the ant plays an important role; aggressive ants are more hostile to natural enemies than those of a milder temper. Some ants are disturbed by fast moving bodies but not slow moving ones, for example *L. humile* were not disturbed by slow moving coccinellid larvae (Whitehead 1957). The size, colour and shape of the natural enemy also influences the level at which it suffers the aggression of ants, for example, Way (1963) pointed out that larger ants do not easily recognise small natural enemies, thus minimising the ants' negative impact on parasitoids.

Ants exhibit a number of forms of aggression, for example, chasing away natural enemies by actually pursuing them or releasing chemicals such as formic acid that drive the natural enemy away (Buckley & Gullan 1991). In many instances ants maim or kill natural enemies (Majerus et al. 2007).

The impact of ant aggression on natural enemies is dependant upon a number of factors, most of which have been extensively investigated by a number of researchers. Predators are normally larger or have morphological properties that provide protection against ant attacks, for example adult coccinellidae have hard elytra and highly chitinised appendages that ant bites or stings may have little maiming impact on. However this is enough to drive away the coccinellids. Some coccinellids also release chemicals that repel ants should they be attacked (Itioka & Inoue 1996; Majerus et al. 2007). The juvenile coccinellids either resemble their prey as is the case with the mealybug destroyer *Cryptolaemus montrouzieri* (Mulsant), *Hyperaspis*, *Nephus* and *Scymnus*

species thereby reducing their vulnerability while other species like *Exochomus flavipes* (Thunberg), have spiky hairs that prevent ant attacks, besides their camouflaging colour. Despite these attributes predators are still prone to ant aggression as demonstrated by Kaplan and Eubanks (2002) with the Red imported fire ants *Solenopsis invicta* Buren, attacking coccinellid predators of the cotton aphid, *Aphis gossypii* Glover.

The behaviour of a parasitoid in the presence of ants is determined largely by its own excitability and the kind of response its presence evokes in the ant (Nixon 1951).

Different parasitoids have varying degrees of ant tolerance (i.e. how timid they are in the presence of ants) and subsequent efficiency in parasitising the vine mealybug. Martinez-Ferrer et al. (2003) demonstrated that the oviposition behaviour of primary parasitoids brings about differences in efficacy as biocontrol agents. Parasitoids requiring more time to deposit an egg are more prone to ant disturbance than those requiring less time. Parasitoids are normally disturbed by ants during oviposition when they cannot readily abandon this activity to escape.

***Planococcus ficus* parasitoids in the Western Cape Province (WCP), South Africa.**

A number of parasitoids attack *P. ficus* (Table 2), at various stages of development thereby avoiding inter-specific competition and allowing the parasitoids to complement each other in biological control programmes. *Anagyrus* sp. near *pseudococci* (Girault), *Coccidoxenoides perminutus* (Timberlake) (formerly *C. peregrinus* (Timb.)) and *Leptomastix dactylopii* Howard are present in vineyards as well as hyperparasitoids (those that attack the primary parasitoids). Prinsloo (1983) gives detailed descriptions of these parasitoids.

Table 2: Parasitic Hymenoptera associated with *Planococcus ficus* in South Africa.

All species were identified from parasitised mealybugs collected from Western Cape Province vineyards.

Family	Species	Reference	Comments
Encyrtidae	<i>Anagyrus</i> sp. near <i>pseudococci</i> (Girault)	Prinsloo 1983 Whitehead 1957 Walton 2003	Primary parasitoid
Encyrtidae	<i>Coccidoxenoides perminutus</i> (Timberlake)	Prinsloo 1983 Whitehead 1957 Walton 2003	Primary parasitoid. Also known as <i>C. peregrinus</i> or <i>Pauridia peregrina</i>
Encyrtidae	<i>Leptomastix dactylopii</i> Howard	Prinsloo 1983 Whitehead 1957 Walton 2003	primary parasitoid
Encyrtidae	<i>Cheiloneurus</i> sp. 1	Prinsloo 1983 Whitehead 1957 Wakgari & Giliomee 2003	Possible hyperparasitoid through <i>A.</i> sp. near <i>pseudococci</i> and <i>L. dactylopii</i>
Encyrtidae	<i>Cheiloneurus</i> sp. 2	Prinsloo 1983 Whitehead 1957	Possible hyperparasitoid through <i>A.</i> sp. near <i>pseudococci</i> and <i>L. dactylopii</i>
Encyrtidae	<i>Procheiloneurus pulchellus</i> Silvestri	Prinsloo 1983 Whitehead 1957	Possible parasitoid of <i>P. ficus</i> and <i>P. citri</i> . Hyperparasites of <i>A.</i> sp. near <i>pseudococci</i>
Encyrtidae	<i>Procheiloneurus</i> sp. 1	Prinsloo 1983 Whitehead 1957	Possible parasitoid of <i>H. africanus</i> . Hyperparasites of <i>A.</i> sp. near <i>pseudococci</i>
Encyrtidae	<i>Tropidophryne</i>	Prinsloo 1983	Mealybug parasitoid
Encyrtidae	<i>Chartocerus</i> sp.	Prinsloo 1983 Whitehead 1957	Associate <i>P. ficus</i> parasitoid. Possible hyperparasitoid of <i>Anagyrus</i> sp.
Encyrtidae	<i>Homalotylus africanus</i> Timberlake	Prinsloo 1983 Whitehead 1957	Parasitoid of coccinellids, e.g. <i>Exochomus</i> sp.
Encyrtidae	<i>Homalotylus Flaminius</i> (Dalman)	Prinsloo 1983 Whitehead 1957	Primary parasitoid of <i>P. ficus</i> and coccinellids, e.g. <i>Nephus</i> and <i>Hyperaspis</i> sp.
Encyrtidae	<i>Pseudococcobius dolus</i> Timberlake	Prinsloo 2003 Whitehead 1957	Primary parasitoid of <i>P. ficus</i>
Pteromalidae	<i>Pachyneuron</i> sp	Prinsloo 1983 Whitehead 1957	Associate mealybug parasitoid
Aphenilidae	<i>Marietta connecta</i> Compere	Prinsloo 1983	Hyperparasitoid through <i>L. dactylopii</i>
Aphenilidae	<i>Marietta carnesi</i> Howard	Prinsloo 1983 Whitehead 1957	Hyperparasitoid through <i>L. dactylopii</i>
Aphenilidae	<i>Marietta leopardina</i> Motschulsky	Prinsloo 1983 Whitehead 1957	Hyperparasitoid through <i>L. dactylopii</i>
Aphenilidae	<i>Azotus capensis</i> Howard	Prinsloo 1983 Whitehead 1957	Hyperparasites
Ceraphronoidae Megaspilidae	Unidentified genus and species	Prinsloo personal communication	Possible hyperparasites through <i>A.</i> sp. near <i>pseudococci</i> and <i>L. dactylopii</i> .

* All taxa determined by GL Prinsloo and J Kelly, 2007

This study concentrates on *A. sp. near pseudococci* and *C. perminutus* interactions with the dominant ant species and the subsequent *P. ficus* parasitism rates. These are solitary koinobiont endoparasitoids of the vine mealybug and some related mealybug species such as *Planococcus citri* (Risso), *Pseudococcus comstocki* (Kuwana), *Phenacoccus herreni* Cox and Williams, *Dysmicoccus brevipes* (Cockerell) and *Maconellicoccus hirsutus* (Green) and the grape mealybug *Pseudococcus maritimus* (Ehrhorn) (Daane et al. 2004b; Noyes & Hayat 1994).

***Anagyrus* species near *pseudococci* (Girault) (Hymenoptera: Encyrtidae).**

This parasitoid species is believed to have originated from the Middle East but has become established as an effective biocontrol agent in most countries in the Mediterranean (Noyes & Hayat 1994). A lot of research has been done on this parasitoid's developmental rate and ability to control mealybugs in vineyards, greenhouses and conservatoriums (Walton 2003; Daane et al. 2004a). Due to its wide host range and geographic distribution, *A. sp. near pseudococci* is one of the most commonly commercially reared parasitoid species and has often been used for biological control of pseudococcids in several countries, for example, in Californian vineyards (Daane et al. 2004a), Turkey citrus orchards (Ülgentürk et al. 2006) and in Argentina (Triapitsyn & Triapitsyn 2002). Although it occurs in South Africa, no commercial biological control has been done with *A. sp. near pseudococci*.

Temperatures in South Africa are conducive for the development of *A. sp. near pseudococci* and there are two generations of this parasitoid for every one of *P. ficus* making it a very suitable parasitoid species to suppress mealybug infestations in vineyards (Daane et al. 2004b). *Anagyrus sp. near pseudococci* prefer later mealybug instars resulting in reduced mealybug fecundity (Islam & Copland 1997). There has been a confusing debate as to which species of *A. pseudococci* researchers have been working with in different geographical regions. In South Africa, a (possibly undescribed) species of *A. pseudococci* (with first funicle of the female antennae partially black and partially white) is the most common and was described by Triapitsyn et al. (2007) as *A. sp. near pseudococci* (Girault). However, no differences in developmental rate and host preference were pointed out for the two similar species.

***Coccidoxenoides perminutus* (Timberlake) (Hymenoptera: Encyrtidae)**

Originally from Hawaii, these tiny wasps are now widely distributed throughout the world. They are more effective against the vine and citrus mealybug and are widely used for the biological control of these mealybugs. They favour the first three instars of mealybugs and provide control for low and high infestations and reduce production of new mealybug generations (Joyce et al. 2001; Daane et al. 2008). Because they are well adapted to South African weather conditions, *C. perminutus* are a promising biocontrol agent for *P. ficus* in South Africa (Walton & Pringle 2005) and therefore are now commercially produced as part of a large scale *P. ficus* biocontrol programme. *C. perminutus* have been used previously in biocontrol programmes against *P. citri* in California, Bermuda, Chile and Italy (Bartlett 1977; Bennett 1959; Zinna 1961). The mode of reproduction of *C. perminutus* is almost entirely thelytokous, with males produced sporadically and at low frequency (Davies et al. 2004). The females have both pro-ovigenic and synovigenic traits. The females have a high reproductive potential (10-20 eggs per day) within the first two days (after a 12 h pre-oviposition period), and 80-150 eggs per day thereafter until death at about eight days (Joyce et al. 2001; Ceballo & Walter 2005).

Practices assisting with the establishment and conservation of parasitoids in vineyards

As part of sustainable IPM programmes, producers need to carry out farm management practices that assist conservation and biological control as follows:

- i) reducing wind velocity around vineyards by means of windbreaks
- ii) maintaining optimum humidity while preventing fungal infection through proper timing and mode of irrigation and vine canopy management
- iii) decreasing dust in vineyards through windbreaks and ground cover
- iv) ant control with directed stem barriers and low-toxic baits
- v) proper timing and application of selected pesticides to reduce impacts on parasitoids
- vi) including nectar producing plants in and around vineyards for natural enemy nutrients for example, buckwheat *Fagopyrum esculentum* Moench, alyssum flowers *Loburaria maritima* (L), phacelia *Phacelia tanacetifolia* Bentham and coriander *Coriandrum sativa* (L) (Berndt & Wratten 2005; Berndt et al. 2006; Lavandero et al. 2006; Wratten personal communication).

OBJECTIVES

This study aims to:

- correlate ant infestation with *P. ficus* infestation and parasitism in two main wine grape growing areas of South Africa,
- quantify the spatial distribution of ants and parasitoids in vineyards,
- provide an economic threshold for ants in vineyards which will enable producers to decide when to implement chemical control against ants,
- establish the relative impact of *A. steingroeveri*, *C. peringueyi* and *L. humile* on the biological control effort against *P. ficus*. This will enable producers to make informed decisions pertaining to chemical control of ants in vineyards in a way that conserves natural enemies without compromising crop quality, export market access and ecosystem wellbeing, and
- establish which pesticides pose the least threat to parasitoids in vineyards and therefore can be incorporated in IPM programmes.

Currently there is no documentation on action thresholds for ants as an indirect pest in vineyards. Establishing a threshold backed by scientific data, will prevent needless chemical control of ants without compromising their beneficial role in the agro-ecosystem or underestimating their negative impact on *P. ficus* biocontrol.

While many authors have pointed out that ants disturb biological control efforts against mealybugs, there is no data available on the relative impact of South African ants on *A. sp.* near *pseudococci* and *C. perminutus*. This must therefore be quantified to determine which parasitoids to release and the timing of these releases.

Use of synthetic pesticides to manage ants and mealybugs is still common and is dependant upon their cost and efficacy without giving much attention to the acute and sublethal impact on natural enemies. It is therefore imperative to identify those pesticides that can be included in a sustainable IPM programme to reduce negative impacts on parasitoids.

Information obtained in this investigation will be useful in the planning and implementation of a long term IPM programme for *P. ficus* and its attendant ants *L. humile*, *Crematogaster* spp. and *Anoplolepis* spp.

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CHAPTER 2

INTERACTIONS INVOLVING ANTS, VINE MEALYBUG AND VINE MEALYBUG PARASITOIDS

ABSTRACT

Some ants associate with vine mealybugs which they provide with sanitary services and protection against natural enemies. Presence-absence cluster sampling, together with the vine mealybug pheromone trapping, were performed biweekly in two consecutive growing seasons, 2005-2006 and 2006-2007, in two main wine grape growing areas of the Western Cape Province, South Africa. The seasonal infestations of various species of ants were assessed regarding their impact on vine mealybug infestations, impact of vine mealybug parasitoids and subsequent mealybug damage to grape bunches. Ant and vine mealybug within-vine distributions and infestation levels in vineyards were established during the two growing seasons in Breede River Valley (Ashton farm) and the coastal region, Simondium (Backsberg and Plaisir de Merle farms). Data were analysed using repeated measures ANOVA and bootstrap analysis. The black pugnacious ants *Anoplolepis steingroeveri* (Forel), were only found on Ashton farm together with the arboreal cocktail ant *Crematogaster peringueyi* Emery, which dominated (96%). *C. peringueyi* co-dominated with the Argentine ant *Linepithema humile* (Mayr), on Plaisir de Merle farm (25% and 26.07%, respectively). On Backsberg farm, *L. humile* dominated (37.86%) over *C. peringueyi* (7.86%). Ant infestation differed significantly between parts of the vine across farms and seasons ($F_{(14,77)}=77.47$; $p<0.001$). Stem infestation was the highest throughout both seasons, compared to leaves and bunches. Vine mealybug stem infestation differed significantly between farms ($F_{(28,77)}=16.77$; $p<0.0001$). Ashton farm had more damaged bunches in season 1 (2005-2006) ($F_{(2,18)}=5.41$; $p\leq 0.05$) than Backsberg or Plaisir de Merle. In the 2005-2006 season, there was a weak negative linear correlation ($r=-0.26$) between ant infestation and vine mealybug parasitism rate while in the 2006-2007 season, a strong negative linear correlation ($r=-0.73$) occurred. Knowing the distribution of ants, vine mealybugs and their parasitoids within vines is crucial for implementing control measures against ants and mealybugs with least impact on parasitoids.

Keywords: ants, distribution, infestation, parasitoids, vine mealybugs, parasitism.

INTRODUCTION

Different ant species, especially from the Dolichoderinae, Formicinae and Myrmicinae, associate with honeydew excreting Hemiptera in a mutual interaction (Nixon 1951; Way 1963; Samways et al. 1982; Hölldobler & Wilson 1990). The association of the common pugnacious ant *Anoplolepis custodiens* (Smith) (formerly *Plagiolepis custodiens*) with aphids, scale and mealybugs in citrus groves and vineyards in South Africa has been well documented (Smit & Bishop 1934; Myburgh et al. 1976; Samways et al. 1982; Addison & Samways 2000). The black pugnacious ant *A. steingroeveri* (Forel) (formerly *P. steingroeveri*) is also common in vineyards in the drier Karoo region of the Breede River Valley (Addison & Samways 2000). The cocktail ants *Crematogaster peringueyi* Emery are common in mealybug infested vineyards that are neglected or old where they occur in association with honeydew excreting Hemiptera (Kriegler & Whitehead 1962). The invasive Argentine ants *Linepithema humile* (Mayr) (formerly *Iridomyrmex humilis*) are now abundantly present in most of the coastal vineyards and orchards where they aggressively tend honeydew excreting insects (Buckley 1987; Addison & Samways 2000). This ant species has been blamed for promoting many hemipterous pests in many agricultural systems worldwide (Prins et al. 1990). Many authors have pointed out that ants disturb biological control of the Hemiptera they tend and thus promote the latter's infestations to unacceptable levels. However, no detailed study has been undertaken to quantify the impact of these ant species on the biological control of a hemipteran pest like the vine mealybug (VMB), *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), a key pest in vineyards. Biological control of VMB has been achieved through the use of encyrtid parasitoids, *Anagyrus* sp. near *pseudococci* (Girault), *Coccidoxenoides perminutus* (Timberlake) and *Leptomastix dactylopii* Howard in California and Israel and beetle predators such as the mealybug destroyer *Cryptolaemus montrouzieri* (Mulsant) have been mass released to control mealybugs in California, Australia, and in greenhouses in Europe (Berlinger 1977; Islam & Copland 1997; Daane et al. 2004). *C. perminutus* and *C. montrouzieri* are commercially produced in South Africa for the control of mealybugs.

While most of these VMB natural enemies are resident in vineyards, their activities are hampered by the presence of foraging ants that drive them away (Flanders 1943; Way 1963). Although the geographic distribution of these ants is known, their within-vine distribution has not been established. In this interaction, the relative distributions of ants and mealybugs on the vine are an important factor in implementing biological control of

VMB. Parasitoids successfully attack exposed mealybugs that are not tended by ants. The level of ant infestations on exposed vine sections will influence the efficiency of parasitoids and the subsequent VMB parasitism rate.

This investigation aimed to i) establish the within-vine distribution of mealybug-tending ants to identify those vine sections most utilised by ants and on which to target ant control, ii) determine the parasitism rates of VMB by naturally occurring parasitoids in vineyards infested with these ants and iii) correlate ant infestation with VMB parasitism to determine the degree of aggression ants have on parasitoids.

MATERIAL AND METHODS

Study sites

The investigation was carried out for two consecutive growing seasons (2005-2006 and 2006-2007) in two main wine grape growing areas, Simondium (Backsberg -33.83°S, 18.92°E, 240m and -33.83°S, 18.83°E, 175.2m; Plaisir de Merle -33.83°S, 18.95°E, 193m) and Breede River Valley (Ashton -33.85°S, 20.08°E, 186m). Previous studies on ants found that there is a difference in species of ants foraging in vineyards in these areas (Addison & Samways 2000).

Sampling was done on 21 hectares (ha) of wine grapes with approximately 7 ha on each farm. Each ha block had 20 evenly spaced plots, each consisting of 5 vines. All vineyards were mature (>10 years old) and had a history of ant and VMB infestations. All vineyards consisted of wine grape cultivars susceptible to VMB infestation (Walton 2000). On Ashton farm, the cultivars were Colombar and Steen (Chenin Blanc) while in Simondium the cultivars were Chardonnay and Winery Shiraz. Vineyards were not sprayed with insecticide but only treated with a fungicide during the period of investigation.

In Simondium, ground cover was generally sparse and kept low by mowing throughout the growing seasons. The Plaisir de Merle vineyard was overhead irrigated while the Backsberg vineyard was micro-jet irrigated. On Ashton farm, ground cover was sparse at the beginning of each growing season, but grew denser and up into the vine canopy towards harvest. The vineyards here were micro-jet irrigated.

Sampling Methods

1. Presence-absence cluster sampling

Sampling was done biweekly throughout both growing seasons from October to February on five vines per plot as described by Walton and Pringle (2004) and De Villiers and Pringle (2007). The vine was divided into four sections; stems (cordons, 30 cm on either side of the central stem), shoots (15 cm of the first distal shoot), leaves (1 leaf per vine), and bunches (1 bunch per vine). These sections were inspected during the day, for the presence of foraging ants and mealybugs and were classified as infested or uninfested without regarding the severity of the infestation since only the presence or absence of either pest was used as the monitoring method. This was done in accordance with the standard monitoring system for vineyards (De Villiers & Pringle 2008). All ages of mealybugs were sampled as this impacts on the composition of parasitoids. Samples of foraging ants were taken to the laboratory for identification. The stems and shoots were searched for mealybugs hiding under the bark and crevices. One leaf per vine was inspected on both sides and a bunch preferably touching the wood was also inspected as these were found to be more susceptible to VMB infestation (Geiger & Daane 2001). Depending on availability, five mealybugs per plot were individually collected into gelatin capsules on a bi-weekly basis. These mealybugs were held at room temperature in the laboratory for at least four weeks after which they were examined using a stereo microscope for any emerged parasitoids or mummies. The parasitism rate was calculated using the ratio of parasitised mealybug to the total number of mealybugs collected. Emerged parasitoids were identified and sexed (Prinsloo 1984). Parasitism refers here to any mealybugs that turned into mummies regardless of whether the parasitoid emerged or not.

2. Pheromone traps

Yellow delta traps (normally used to catch male mealybugs) were used to catch parasitoids in the vineyards. These were used because parasitoids are attracted to the mealybug pheromone (host cues). Two traps (peripheral and central) were placed in each block. Each trap consisted of a 36 square grid sticky pad and a species specific VMB pheromone capsule suspended in the centre of the trap (Millar et al. 2002), which were all provided by Chempack, Simondium, South Africa. The pheromone capsule was suspended so that it would not be covered in glue that would otherwise affect its ability to dispense the pheromone. The trap was hung in the vine canopy above the cordons on the trellis wire close to or just above fruit level, where insect activity was high but

avoiding any obstruction from the growing parts of the vine. Each sticky pad was replaced biweekly and the pheromone capsule monthly. Once the sticky pads were removed, they were covered in clear plastic to keep the trapped insects in their positions. The pads were examined under a stereo microscope and counts made for the trapped parasitoids which were identified.

Damage assessment

Prior to harvest, economic damage to grape bunches was rated using a 0-3 damage rating index (Geiger & Daane 2001) as follows: 0 - no VMB damage (clean), 1 - honeydew present but bunch is still salvageable (low damage), 2 - honeydew and VMB present but part of the bunch is still salvageable (moderate damage) and 3 - total loss (severe damage).

One bunch per vine was assessed for VMB damage making a total of 100 bunches per block in each of the 21 blocks. Percentage damage per farm was determined by averaging all plot observations. To determine percentage damage from the proportion of damaged bunches, the following equation was used:

$$\% \text{ Damage} = P_0X_0 + P_1X_1 + P_2X_2 + P_3X_3;$$

where; P = proportion total bunches, X_0 = no damage (clean), X_1 = low damage, X_2 = moderate damage and X_3 = severe damage.

Data analysis

A repeated measures ANOVA (STATISTICA (Stat-soft)) followed by a Boniferroni test was performed for all biweekly data and a bootstrap analysis was performed for bunch damage rating as the data were non-normal.

Simple regression and correlation using an empirical model (Draper & Smith 1998) were used to measure linear association between ant and vine mealybug infestations and ant infestation on the leaf and parasitism rate of the vine mealybug.

RESULTS

Foraging ant fauna

The foraging ant fauna in the vine canopies almost exclusively consisted of three main species; *A. steingroeveri*, *C. peringueyi* and *L. humile* (Figure 1). Occasional species observed in the vineyards included *Camponotus fulvopilosus* (De Geer), (on Ashton farm) and the white footed ant *Technomyrmex albipes* (F. Smith), (on Plaisir de Merle

farm). It could not be established whether *C. fulvopilosus* tended mealybugs or not. *T. albipes* were often associated with clusters of mealybugs on leaves often in plots infested by the *C. peringueyi* but not *L. humile*.

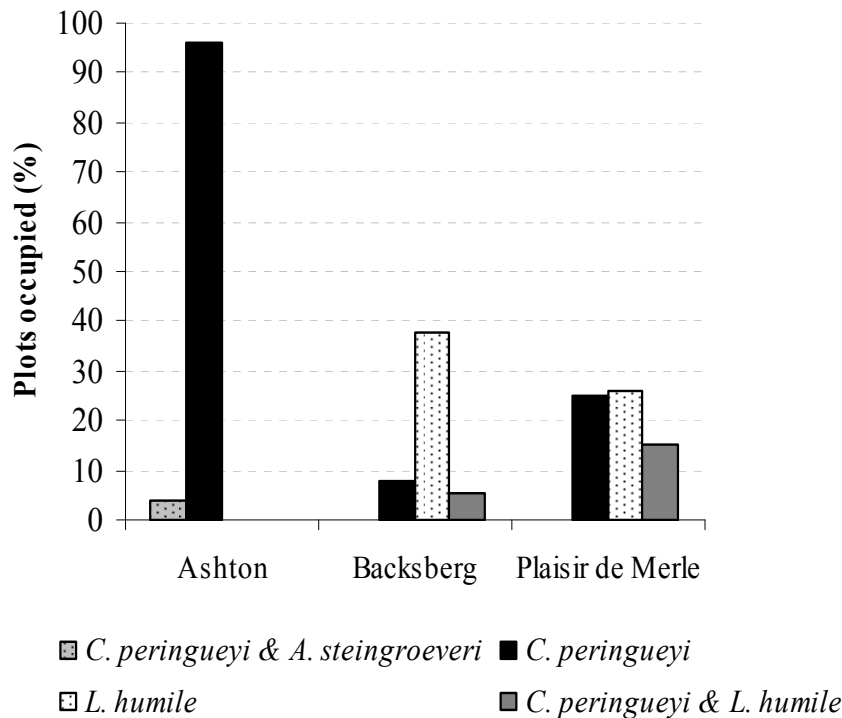


Figure 1: Different ant species foraging on three farms, shown in their percentage occupation of the vineyards sampled in two seasons, 2005-2006 and 2006-2007.

Seasonal ant movement within the vine

Ant infestation differed significantly on parts of the vine across farms and seasons ($F_{(14,77)}=77.47$; $p \leq 0.001$). Stem infestation was the highest throughout both seasons, Figure 2. Ashton had the highest ant infestation and Plaisir de Merle the lowest ant infestation for both season (Figure 3). No significant differences were found between Backsberg and Plaisir de Merle ($p=0.44$). Season long differences in ant infestation were not significant ($p=0.62$).

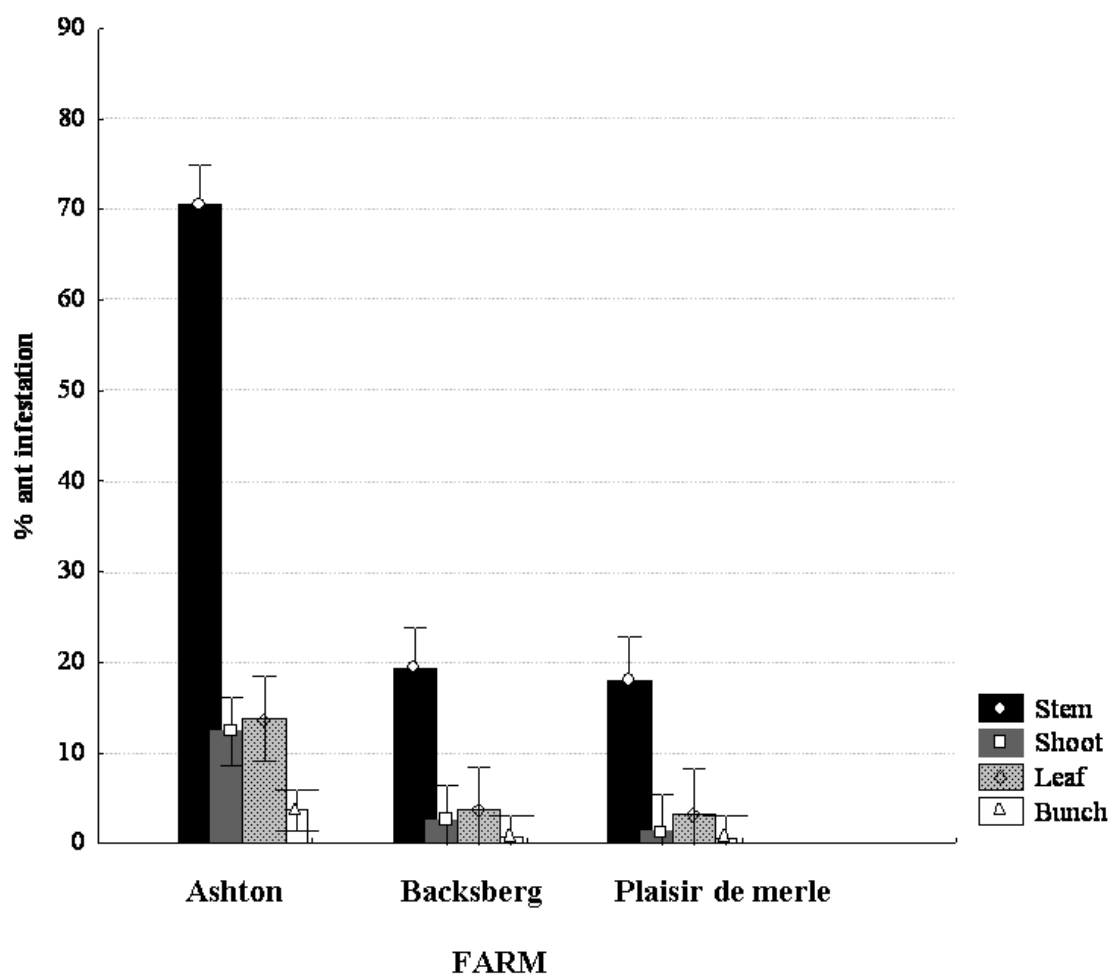


Figure 2: Mean (\pm standard error) seasonal within-vine ant infestation showing proportionate foraging on stems, shoots, leaves and bunches, during two growing seasons (2005-2006 and 2006-2007) from Ashton, Backsberg and Plaisir de Merle farms in the Western Cape Province.

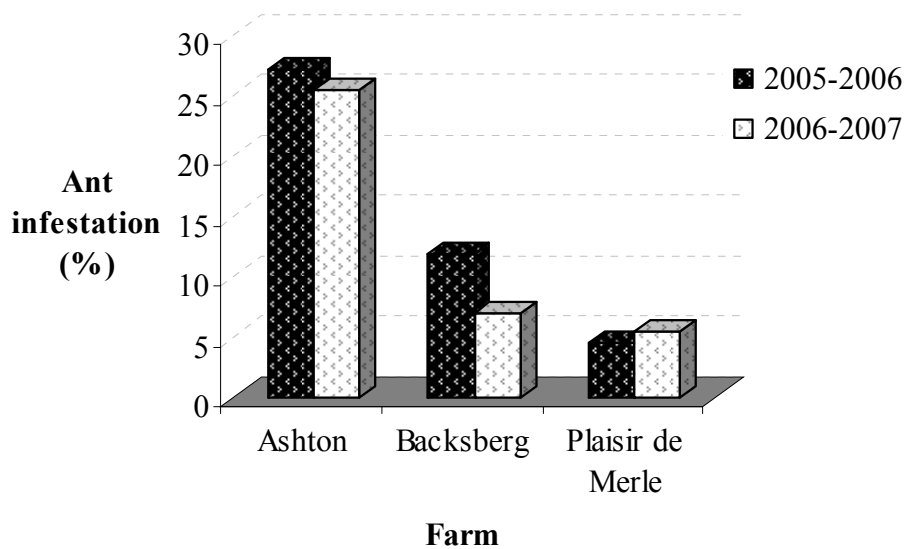


Figure 3: Average ant infestation in vines on three farms (N=700 vines per farm) during two growing seasons, 2005-2006 and 2006-2007.

Seasonal vine mealybug movement within the vine

Vine mealybug infestation decreased on stems while it increased on leaves as the season progressed. Stem infestation differed significantly between farms ($F_{(28,77)}=16.77$; $p<0.0001$) but not between not for shoots, leaves and bunches ($p=0.67$) (Figure 4). Mealybug infestation was highest at Ashton and lowest at Plaisir de Merle (Figure 5).

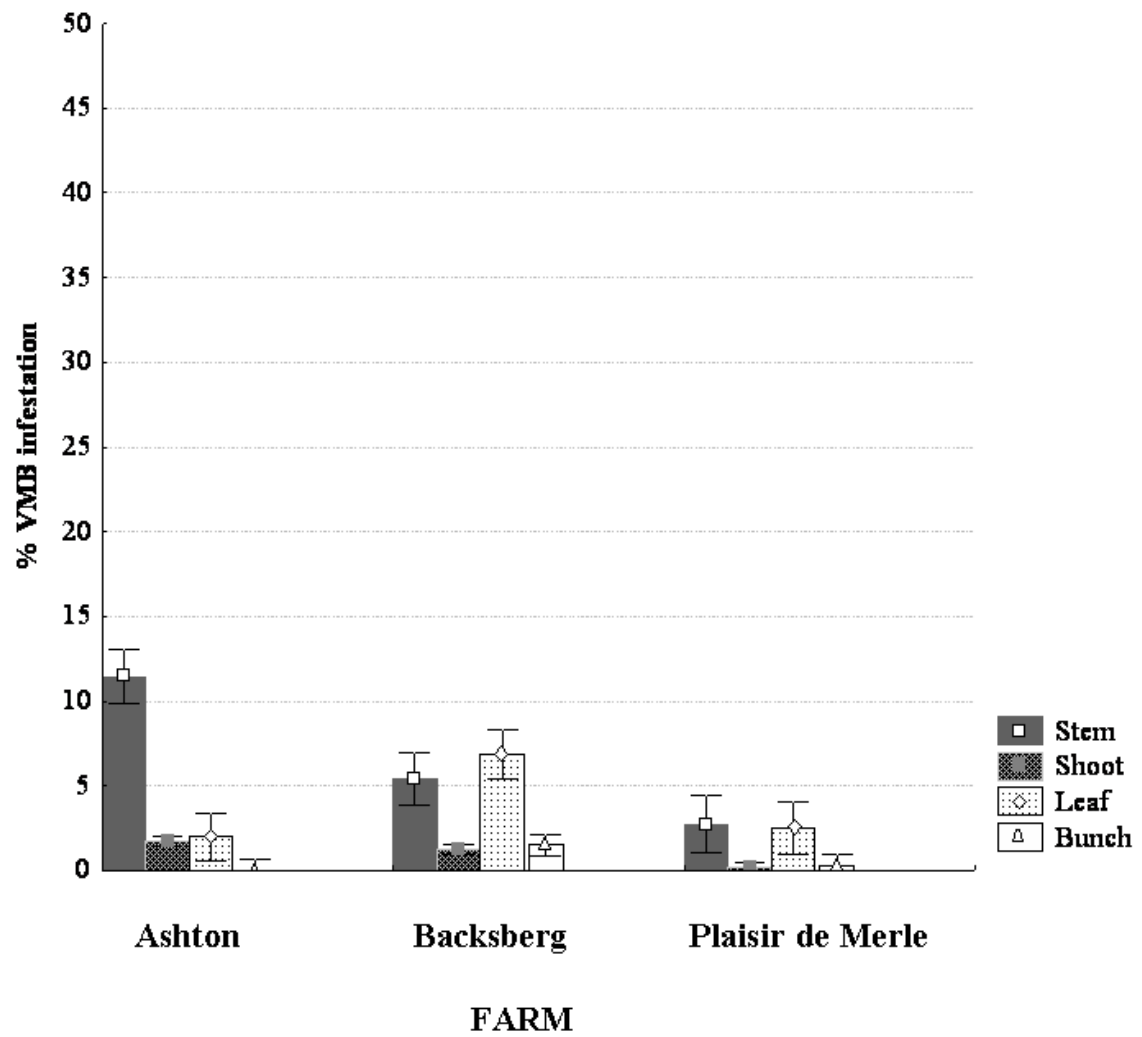


Figure 4: Mean (\pm standard error) seasonal within-vine VMB infestation showing proportionate foraging on stems shoots, leaves and bunches during two growing seasons (2005-2006 and 2006-2007) from Ashton, Backsberg and Plaisir de Merle farms in the Western Cape Province.

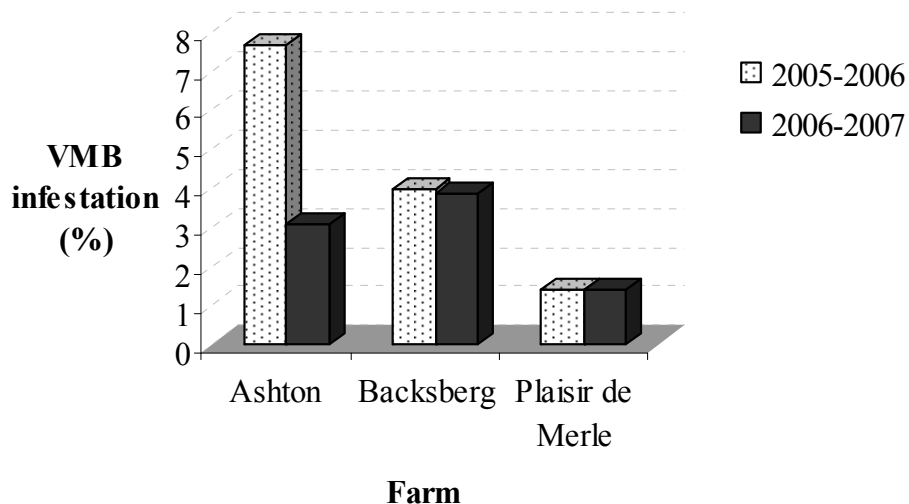


Figure 5: Average VMB infestation in vines on three farms (N=700 vines per farm) during two growing seasons, 2005-2006 and 2006-2007.

Impact of ant infestation on VMB infestation

Ant and VMB infestations had a strong positive linear correlation near to +1 ($r = 0.86$) in season 1 ($y = 0.25x + 0.43$; $p < 0.05$). VMB infestation increased with increasing ant infestation. However in season 2 (2006-2007), a weak positive linear relationship, close to zero ($r = 0.2$) was obtained ($y = 0.05x + 2.36$; $p \leq 0.05$). Figure 6 shows the linear relationship between ant and VMB infestations.

Impact of ant infestation on VMB parasitism rate

In season 1 (2005-2006), a weak negative linear correlation close to 0 ($r = -0.26$) was obtained between ant infestation and VMB parasitism rate ($y = -0.15x + 17.5$; $p < 0.05$). A strong negative linear correlation, close to -1 ($r = -0.73$) was obtained in season 2 (2006-2007) between ant infestation and VMB parasitism rate ($y = -0.76x + 25.87$; $p < 0.05$). Every 1% increase in ant infestation decreased parasitoid activity by 0.76%. Figure 7 shows the negative linear relationship between ant infestation and VMB parasitism rate.

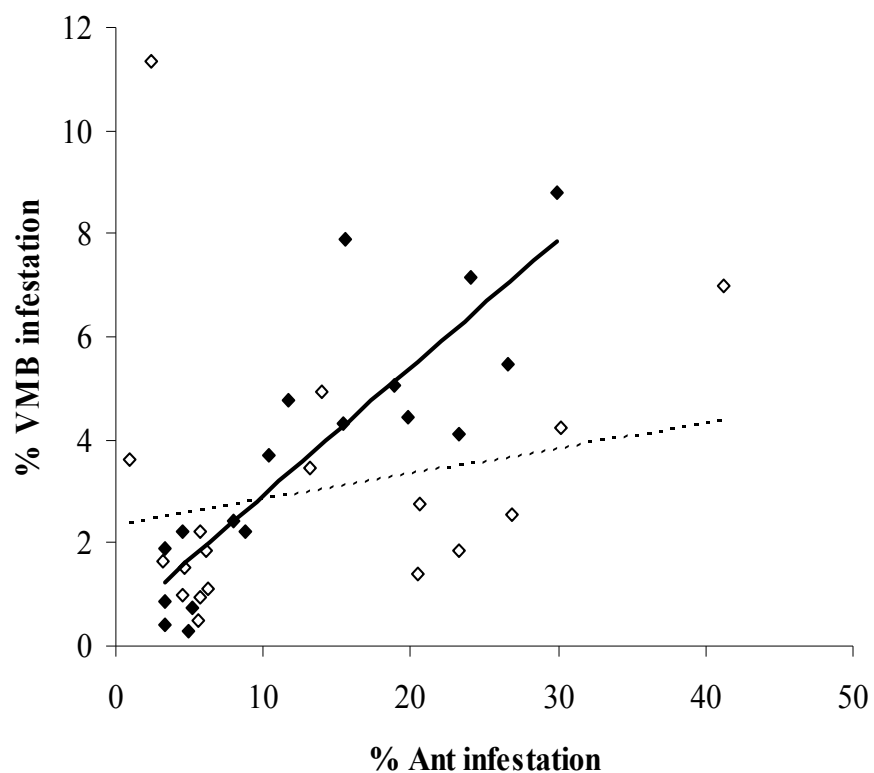


Figure 6: The relationship between ant infestation (%) and VMB infestation (%) for season 1 (—◆—2005-2006) and season 2 (---◇---2006-2007) based on data obtained from Ashton, Backsberg and Plaisir de Merle farms.

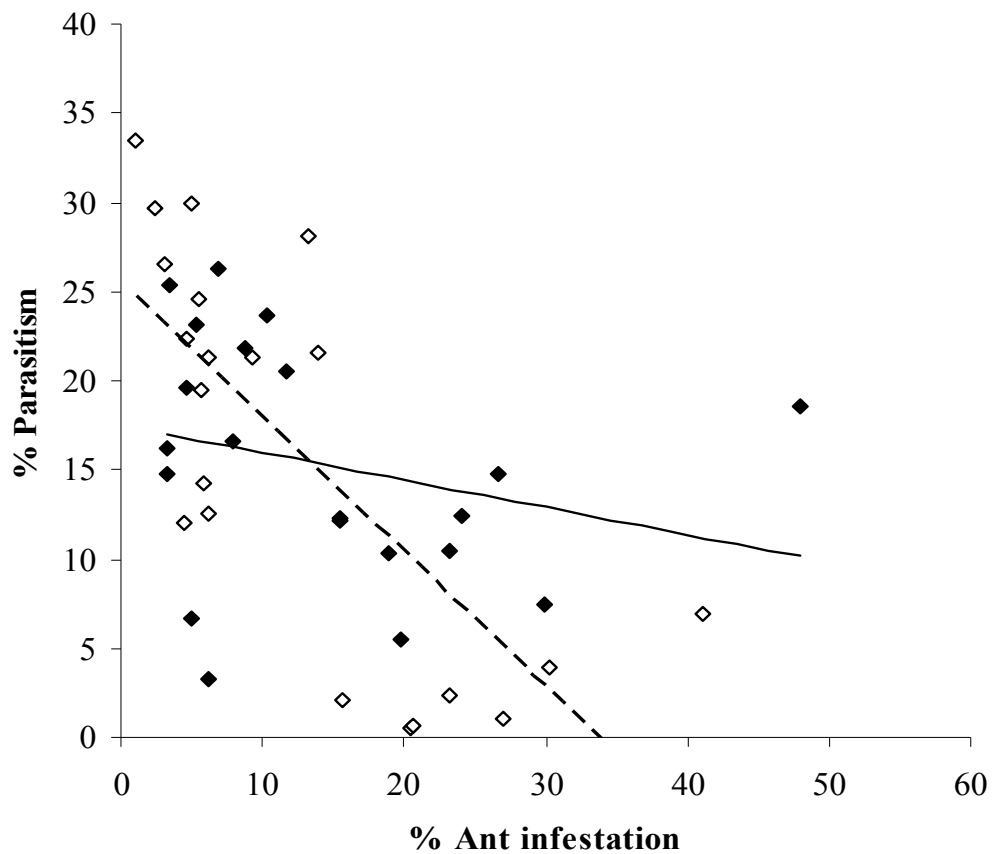


Figure 7: The relationship between ant infestation (%) and parasitism (%) for two seasons (—◆—) 2005-2006 and (---◇---) 2006-2007 based on data obtained from Ashton, Backsberg and Plaisir de Merle farms.

Damage rating

Bunch damage between the three farms (Ashton, Backsberg and Plaisir de Merle) was not significantly different. However, significantly more bunches were damaged in season 1 (2005-2006) than season 2 (2006-2007) on Ashton farm ($F_{(2,18)}=5.41$; $p=0.014$) (Figure 8).

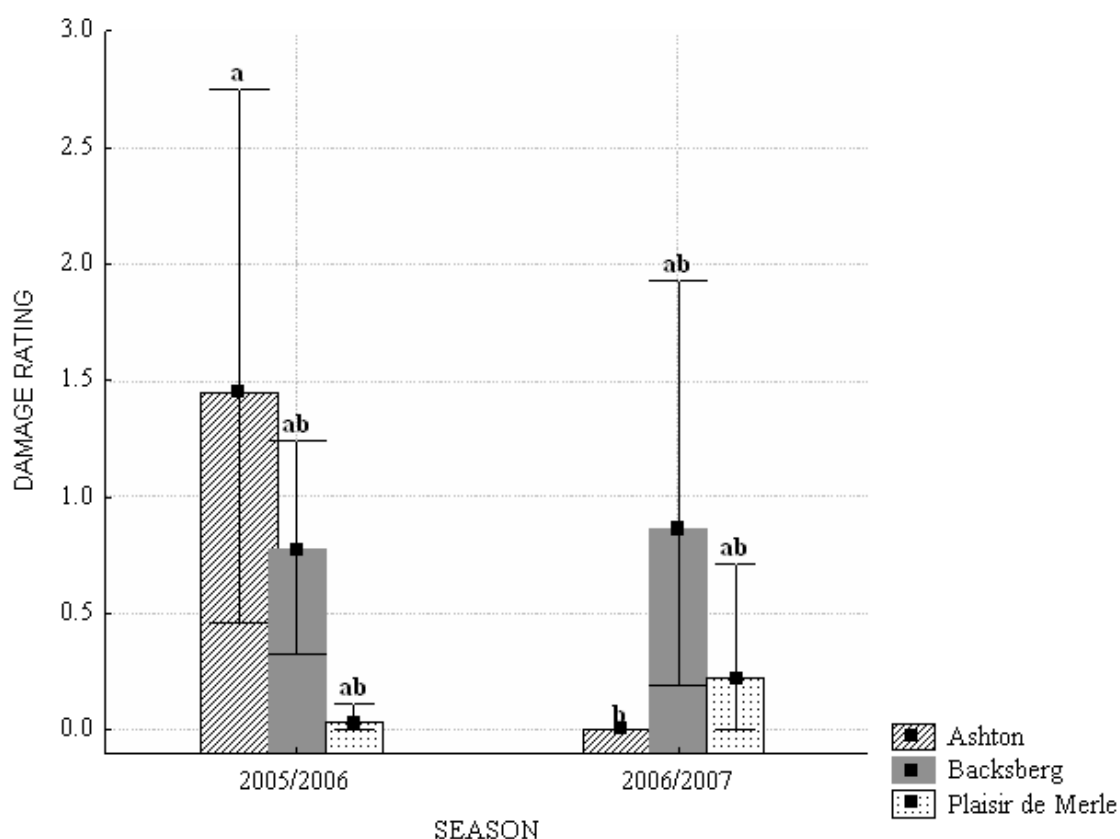


Figure 8: Interaction plot (farm*season) of damage rating of bunches prior to harvest. Damage ratings followed by different letters denote significant differences at $p < 0.05$.

Parasitism was significantly higher in January and February ($F_{(8,22)}=32.98$; $p \leq 0.0001$) than from October to December (Figure 9-10). Total parasitism of the vine mealybug was highest at Backsberg and Plaisir de Merle farms and lowest at Ashton farm ($F_{(4,22)}=6.21$; $p < 0.01$).

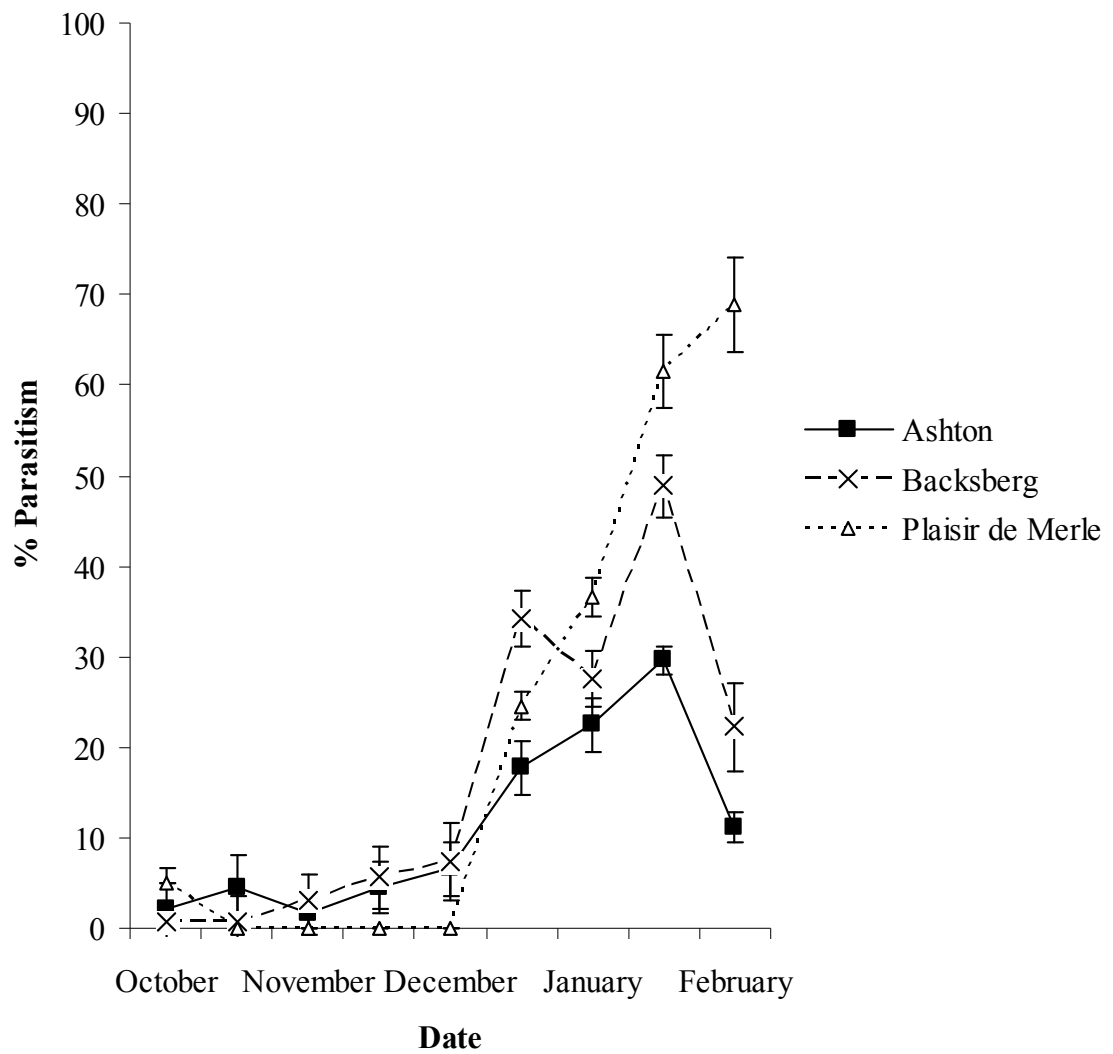


Figure 9: Mean percentage (\pm standard error) parasitism during season 1 (2005-2006) on three farms, Ashton, Backsberg and Plaisir de Merle in the Western Cape Province.

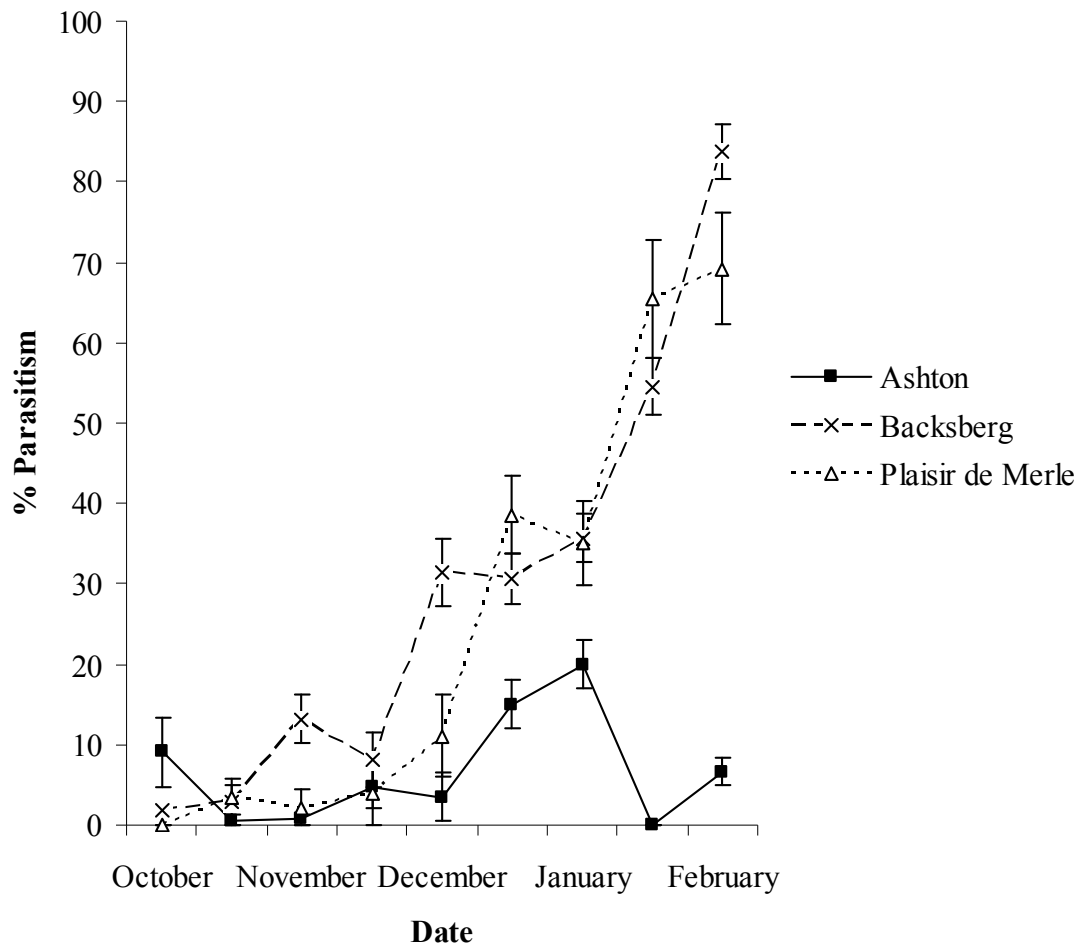


Figure 10: Mean percentage (\pm standard error) parasitism during season 2 (2006-2007) on three farms, Ashton, Backsberg and Plaisir de Merle in the Western Cape Province.

Parasitoid complex

Parasitoids reared from VMB

Three primary parasitoids emerged from field collected VMB at three study sites and included *A. sp. near pseudococci*, *C. perminutus* and *L. dactylopii* (Hymenoptera: Encyrtidae). Other parasitoids reared included mostly hyperparasitoids. Figure 11 shows the relative prevalence of the reared parasitoids across the sampling sites. *Leptomastix dactylopii* were the most commonly reared parasitoid and *C. perminutus* the least. The number of parasitoids reared from mealybugs differed significantly between farms ($F_{(8,666)}=3.358$; $p=0.0005$) but not between seasons. Ashton had the lowest number of *A. sp. near pseudococci* while Backsberg had the highest number of *L. dactylopii*.

Parasitoids caught in VMB pheromone traps

The same species of parasitoids that emerged from mealybugs were also trapped on the yellow delta traps (Figure 12). Significantly more *A. sp. nr pseudococci* were trapped at Plaisir de Merle than either Backsberg or Ashton and *L. dactylopii* numbers differed significantly only between Ashton (highest) and Plaisir de Merle (lowest) ($F_{(8, 582)}=10.159$; $p<0.0001$). No significant differences between farms were obtained for *C. perminutus* and hyperparasitoids.

Significant seasonal differences were found only for trapped hyper parasitoids ($F_{(4,291)}=3.2406$; $p=0.0127$) but not for reared and trapped primary parasitoids. The largest numbers of hyperparasitoids were collected in season 2 (2006-2007).

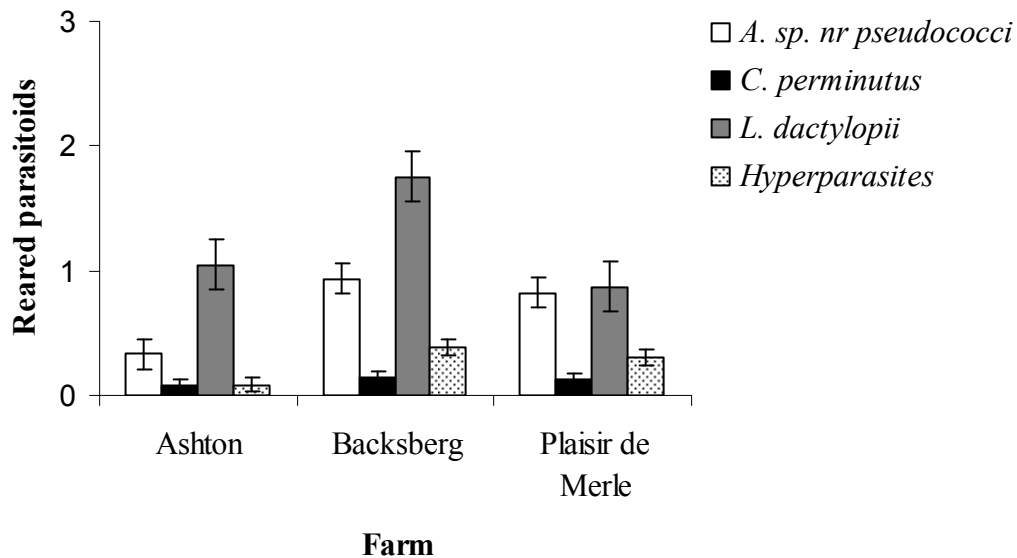


Figure 11: Mean (\pm standard error) weekly number of parasitoids that emerged from vine mealybugs reared in gelatin capsules during two growing seasons (2005-2006 and 2006-2007).

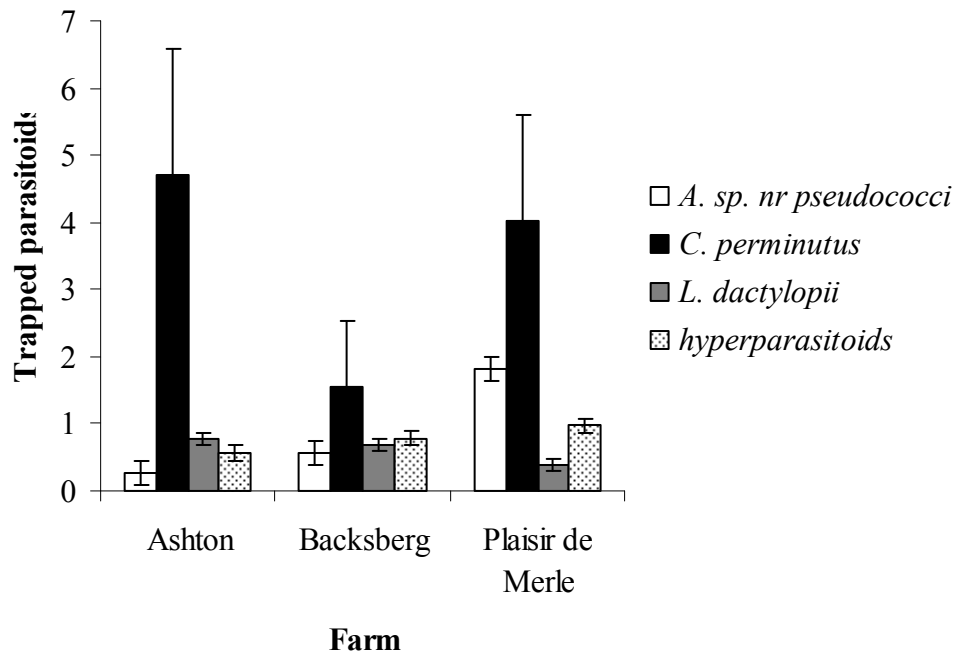


Figure 12: Mean (\pm standard error) weekly number of parasitoids trapped on yellow delta traps with VMB pheromone from vineyards during two growing seasons (2005-2006 and 2006-2007).

DISCUSSION

The foraging ant fauna in the vine canopies almost exclusively consisted of three ant species; *A. steingroeveri*, *C. peringueyi* and *L. humile*. Although this investigation has not shown that inter-specific hostility occurred, this can be deduced from the results as none of the three ant species occupied the same vine although they could be sampled from the same plots. However, this may not interfere with stem treatments.

It appears that ants, regardless of species, paid less attention to exposed mealybugs because although VMB infestations were building up on leaves, ant infestations on the leaves remained low while ant infestations were high on stems where VMB infestations were decreasing. Flanders (1943) commented that honeydew only forms part of the diet for *L. humile*, therefore this ant species would not forage further into the vine canopy if honeydew on the stem was sufficient. Furthermore, this ant species favours moist and warm locations (Suarez et al. 2001; Majer 1993; Cole et al. 1992). Like *L. humile*, *C. peringueyi* are not fond of light and heat and will tend to remain mainly on the stem where they receive protection from the vine canopy (personal observation).

Ant infestations on the three farms showed significant differences in ant species, which possibly indicates differences in microclimates and farm management practices. The vineyards on Ashton farm were the oldest (established in 1983) with a lot of dead wood that provided a suitable habitat for *C. peringueyi* (Whitehead & Kriegler 1962). *A. steingroeveri* made use of the hard compact soil found on the farm roads and frequently visited peripheral plots, which was also found by Addison and Samways (2000). Being coastal, Backsberg and Plaisir de Merle provided favourable damp conditions for *L. humile* and additionally, Plaisir de Merle was overhead irrigated thereby increasing humidity in the vine canopy.

The weak linear association between ants and VMB could have resulted from ants mainly foraging on stems while VMB continued colonising new growth with less attention from ants. Although high ant infestations were coupled with high mealybug infestations, the findings of this investigation are not conclusive as no exclusion experiments were done. However, similar investigations by other researchers have confirmed that high ant infestations promote VMB infestations to unacceptable levels (Daane et al. 2007; Buckley 1987; Flanders 1943). As the VMB population in season 2 was comparatively lower than that in season 1, parasitoid activity was negatively impacted by ants. Ants could have grown more aggressive towards parasitoids at lower mealybug populations (Way 1963; Buckley 1987), as the chances of parasitoids being encountered by ants increased while the parasitoids tried to access their limited hosts.

The increased parasitoid activity from mid season to harvest coincided with increased VMB infestations on leaves. Because ant attendance on exposed vine sections (shoots, leaves and bunches) was relatively low, a great proportion of the VMBs were vulnerable to parasitoid attack which resulted in parasitoids becoming more numerous towards the end of the season confirming the findings of other researchers (Daane et al. 2004; Walton 2000; Whitehead 1957). The three mealybug parasitoids, *A. sp.* near *pseudococci*, *C. perminutus* and *L. dactylopii* have various degrees of ant tolerance due to their morphological and ovipositional behaviour, amongst others (Nixon 1951). Different ant species exhibit different levels of aggression towards parasitoids and protection to Hemiptera. When mealybug populations were low, *L. humile* and *C. peringueyi* built protective structures over VMBs they were attended possibly to limit parasitoid attack. Smit and Bishop (1934) argued that the shelters were of primary benefit for the ant although they also conferred limited benefit to VMB, reducing exposure to natural enemies. This could be true because on many occasions during this

study, parasitised mealybugs were collected from them and even predatory beetle larvae fed on VMB ovisacs underneath these shelters, particularly within fruit bunches. *C. peringueyi* displayed aggressive behaviour towards collectors, often attempting to carry the mealybugs to their concealed nests.

Several authors have already pointed out the negative impact of ants, notably, *L. humile*, *Crematogaster* spp. and *Anoplolepis* spp on mealybug parasitoids (Horton 1918; Kriegler & Whitehead 1962; Smit & Bishop 1934; Steyn 1954; Samways et al. 1982). Joubert (1943) noted that the parasite *Coccophagus gurneyi* Compere was severely hindered by *L. humile* in controlling *P. maritimus* (Ehrhorn) and Compere (1940) found that the incidence of *Saisetia oleae* Olivier in the Cape between 1936 and 1937 had greatly increased due to the presence of *L. humile*. The soft brown citrus scale *Coccus hesperidum* L, a pest that is heavily parasitised in the Western Cape Province, is never of economic importance in the absence of *L. humile* or *Crematogaster* spp. Ants interfered with parasitoids which would thus lay fewer eggs than would probably happen in the absence of ants (Martinez-Ferrer et al. 2003; Bartlett 1961). The greatest negative impact of ants is the limitation of the number of eggs laid by parasitoids although ants could have brought about some parasitoid mortality through physical attack. Ants do not necessarily aim to disturb parasitoids in the field, but rather this disturbance may be incidental. Samways et al. (1982) found that *A. custodiens*, while tending soft brown scale on citrus trees caused incidental increases in the population of red scale *Aonidiella aurantii* (Maskell). Whitehead (1957), while working on natural enemies of *P. citri* (misidentified *P. ficus*), in Western Cape vineyards found 13 different species of parasitoids attacking this mealybug, with *A. pseudococci*, *C. perminutus* and *L. dactylopii* as primary parasitoids while the rest were a complex of hyperparasitoids. In this investigation, one additional unidentified species from the family Encyrtidae and another from the Ceraphronidae Megaspilidae family also emerged. *L. dactylopii* was always the first primary parasitoid to emerge early in the season with only a few *C. perminutus*. This was possibly because of the presence of large ovipositing VMB females and early mealybug instars, respectively, preferred by these parasitoids (Ceballo & Walter 2005; Joyce et al. 2001). *Pachyneuron* spp. was the first hyperparasitoid to emerge. The marked difference in the number of emerged and trapped parasitoids was possibly a result of chemical cues. A synergy of honeydew and natural mealybug pheromone could have been a stronger attractant than the synthetic female VMB pheromone in the traps for *A. sp.* near *pseudococci* and *L. dactylopii* while

C. perminutus could have responded more to the synthetic female VMB pheromone. However, this observation could not be ascertained because low counts from VMBs could have been due to the sampling methods used. Ceballo & Walter (2005) used sticky traps to collect *C. perminutus* parasitised-mealybugs citing inadequacies of the presence-absence sampling system for this data.

CONCLUSION

This investigation showed that honeydew seeking ants mainly foraged on vine stems although foraging on other parts does occur. Complete ant control is not essential; however some reduction in ant activity in the vine canopy is important to achieve optimum biological control of the vine mealybug. There is less ant activity on exposed vine sections, which allows for parasitoids to attack VMB with limited disturbance from ants. This observation can be utilised in the application and timing of chemical control actions against ants in vineyards, for example, timely application of chemical stem barriers to prevent ants from entering the vine canopy.

Three primary parasitoid species mainly attack VMB in Western Cape Province vineyards. *L. dactylopii* were active throughout the season and together with *A. sp* near *pseudococci* they attacked later stages of VMB frequently. Unfortunately, these two sexually dimorphic primary parasitoids have hyperparasitoids that tend to reduce their efficiency in the field. *C. perminutus* target the early VMB instars and are capable of reducing VMB infestations by preventing further reproduction. This species owes its great potential as a biocontrol agent through its lack of hyperparasites, short oviposition period and parthenogenesis. A negative linear relationship between ants and VMB parasitism rate was observed which implies that ants reduce the efficiency of parasitoids and as such preventing their entry into the vine canopy should be emphasized.

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16 June 2007.

CHAPTER 3

SPATIAL DISTRIBUTION OF ANTS (FORMICIDAE) AND PARASITOIDS IN VINEYARDS

ABSTRACT

Ant activity levels play a key role in the survival of their attended Hemiptera and hence the distribution patterns and abundance of these hemipterans and their natural enemies. The spatial distribution patterns of ants and a complex of vine mealybug parasitoids were investigated for two consecutive growing seasons on three wine farms where no ant control was applied. The spatial association between ant species and parasitoids was assessed. Spatial Analysis by Distance IndicEs (SADIE) was used to analyse spatial distribution of insects and ArcView with its extension Spatial Analyst were used to map the gap, patch and local association indices where significant association and disassociation occurred. *Crematogaster peringueyi* (Emery) and *Anoplolepis steingroeveri* (Forel) showed an aggregated pattern during both seasons while *Linepithema humile* (Mayr) was either aggregated or randomly dispersed throughout vineyards. Parasitoids showed an aggregated pattern in season 1 but showed a regular dispersion pattern in season 2. A significant association was found between *C. peringueyi* and parasitoids; and *L. humile* and parasitoids. A significant disassociation was found between *C. peringueyi* and *A. steingroeveri* and between *C. peringueyi* and *L. humile*. This implies that interspecific competition between ant species could play a role, and that ants share mealybugs as a primary resource with parasitoids.

Keywords: Ants, parasitoids, spatial pattern, spatial association, disassociation, distribution, aggregation.

INTRODUCTION

Parasitoids can have a major impact on the control of hemipteran pests of field and orchard crops but the presence of honeydew foraging ants often seriously reduces parasitoid efficiency (Bartlett 1961; Samways et al. 1982; Buckley 1987). Information on spatio-temporal population dynamics of ants, mealybugs and their natural enemies could be used to develop integrated pest management (IPM) strategies that conserve natural enemies such as parasitoids and therefore enhance their role as biological control

agents (Thomson et al. 2007). The effectiveness of parasitoids depends on good synchrony (in time and space) between adult parasitoids and their hosts (Maron & Harrison 1997; Bjørnstad & Bascompte 2001).

In South Africa, the use of parasitoids is being incorporated into large scale IPM strategies to suppress vine mealybugs, *Planococcus ficus* (Signoret), in vineyards using naturally occurring populations of *Anagyrus* sp. near *pseudococci*, *Coccidoxenoides perminutus* and *Leptomastix dactylopii* by temporal targeting of ant control in vineyards. In addition, mass releases of *C. perminutus* are taking place to augment biological control efforts. The exclusion of ants from the vine canopy allows parasitoids ample access to vine mealybugs feeding on exposed locations such as leaves and developing bunches (Daane et al. 2004). Parasitoids engage in intimate interactions with their hosts resulting in a spatial association. The presence of ants in vineyards modifies the host-parasitoid interaction and could result in host-parasitoid spatial patterns different to those that may exist in the absence of ants. Spatial association is the similarity of the spatial pattern of two sets of data. If individuals of different species have a mutualistic relationship, they are likely to be positively associated, whereas if they compete with each other or show aggression to one another, then a negative association (disassociation) can result (Perry et al. 1995; Perry 1998a).

Information on spatial characteristics of pest and natural enemy populations can be used to develop improved sampling techniques and ensure more judicious use of pesticides. Ferguson et al. (1999) argues that spatial patterns for a single species and associations between species have been done by methods that discard information concerning the locations (Murchie et al. 1999). Spatial Analysis by Distance IndiCes (SADIE) (Perry 1995, 1998a, b) allows analysis of the spatial information as counts from geographically referenced points making it possible to identify the exact location of pest hot spots.

This investigation utilised SADIE to characterise the spatial population dynamics of three species of ants, *Anoplolepis steingroeveri* (Forel), *Crematogaster peringueyi* Emery and *Linepithema humile* (Mayr) and a complex of vine mealybug parasitoid species in vineyards. The distributions of ants in relation to other ant species and parasitoids were compared in commercial vineyards where no ant control was applied. Parasitoids were obtained from parasitised mealybugs and as such, vine mealybug distribution and association with ants was assumed to be reflected by that of the parasitoids implying that parasitoid abundance was used as a surrogate for VMB abundance since the former have a direct impact on the abundance of the latter. The

observed distributions are discussed in relation to the implications for the development and implementation of IPM strategies to suppress vine mealybug while conserving parasitoids.

MATERIAL AND METHODS

This investigation was carried out for two consecutive growing seasons (2005-2006 and 2006-2007) in two main wine grape growing areas, the coastal region, Simondium (Backsberg -33.83°S, 18.92°E, 240m and -33.83°S, 18.83°E, 175.2m; Plaisir de Merle -33.83°S, 18.95°E, 193m) and Breede River Valley (Ashton -33.85°S, 20.08°E, 186m). Previous studies on ants found that there is a difference in species of ants foraging in vineyards in these areas (Addison & Samways 2000).

Sampling was done on 21 hectares (ha) of wine grapes with approximately seven ha. on each farm. Each ha block had 20 evenly spaced plots, each consisting of five vines. All vineyards were mature (>10 years old) and had a history of ant and VMB infestations. All wine grape cultivars were susceptible to VMB infestation (Walton 2000). On Ashton farm, the cultivars were Colombard and Steen (Chenin Blanc) while on Backsberg farms the cultivars were Chardonnay and Winery Shiraz and Chardonnay on Plaisir de Merle farm. Vineyards were not sprayed with insecticide but only treated with a fungicide during the period of investigation. A presence-absence cluster sampling system was used for monitoring ant infestations (Binns et al. 2000). Sampling was done at two-weekly intervals from October to February during both growing seasons on five vines per plot (giving a total of 420 plots altogether). Each vine was divided into four sections: the stem (the cordons 30 cm on either side of the central stem), shoots (15 cm of the first distal shoot), leaves (1 leaf per vine) and bunches (1 bunch per vine). All sections of the vine were inspected for foraging ants and classified as infested or uninfested without regarding the severity of the infestation.

Depending on availability, five mealybugs (2nd instar to ovipositing females) per plot were individually collected into gelatin capsules on a bi-weekly basis. The female VMB collected included juveniles (2nd and 3rd instars) and adults. Mealybugs were held at room temperature in the laboratory for at least four weeks after which they were examined using a stereo microscope for any emerged parasitoids. Emerged parasitoid data were pooled for each vineyard. The parasitoid complex included primary vine mealybug parasitoids like *Anagyrus* sp. near *pseudococci*, *Coccidoxenoides perminutus*, *Leptomastix dactylopii* and hyperparasitoids such as *Cheiloneurus* species,

Procheiloneurus, *Marietta* and *Pachyneuron* species, among others. Most of these are believed to be hyperparasites through *A. sp.* near *pseudococci* and *L. dactylopii* (Whitehead 1957).

Data Analysis

To measure the spatial pattern of ants and parasitoids within plots, SADIE was used to calculate cluster indices and to test for significance (Perry et al. 1995). Since parasitoids were obtained from mealybugs, cluster indices were not calculated for the latter. The index of aggregation, I_a , was determined where; $I_a = 1$ indicates random arrangement of counts or no significant spatial pattern; $I_a > 1$ = aggregated arrangement giving clusters of observed counts and $I_a < 1$ = regular arrangement of counts. The probability P_a tests for deviations from random dispersion where $P_a > 0.975$ indicates regular dispersion, $P_a < 0.025$ indicates spatial aggregation and $0.025 < P_a < 0.975$ indicates randomness (Perry 1998a). The index of clustering, v , was also determined for areas with above average density ($v_i > 1.5$) and below average density ($v_j < -1.5$) (Maestre & Cortina 2002; Winder et al. 2001; Perry et al. 1999) where subscripts i and j indicate patches and gaps, respectively. The mean values of clustering indices (\bar{v}_i and \bar{v}_j) were used to test for randomness using a one-tailed test at the 5% level.

Two populations may be spatially positively associated, disassociated or occur randomly with respect to each other (Perry et al. 1998a). The local spatial association was measured using an index χ_k based on similarities between clustering indices of two populations, for example two ant species or ant and parasitoid populations measured at the k th sample unit. Positive values of χ_k (association) were indicated by a coincidence of two patches or two gaps while negative association (disassociation) resulted from a patch coinciding with a gap, in both populations. The overall spatial association X , was calculated as the mean of local values of the two populations (two ant species, or ant and parasitoids) (Winder et al. 2001; Perry & Dixon 2002; Perry et al. 2002). The significance of X was tested by randomisations, with values reassigned among sample units, after a small-scale autocorrelation in cluster indices from either population (Dutilleul 1993). At the 5% level, the statistic $P < 0.025$ indicated significant association and $P > 0.975$ indicated significant disassociation.

Mapping of gap, patch and association indices was done only where significant association or disassociation was detected. These included the values of $v_i > 1.5$

(significant patches), values of $v_j < -1.5$ (significant gaps) and values of $\chi_k > 0.5$ (significant association and $\chi_k < -0.5$ (significant disassociation) (Perry et al. 1999). ArcView and its extension, Spatial Analyst (ESRI.com) were used to interpolate between data points using the inverse distance weighted method.

Backsberg B01 vineyard was destroyed after season 1, therefore, B03 vineyard was used in season 2 and both were mapped separately for the respective seasons. Backsberg B01 vineyard was also mapped separately due to spatial distance from the other Backsberg vineyards. Ashton vineyards (A01 and A02) were continuous and were therefore mapped together. Plaisir de Merle vineyards (P01 and P02) were also mapped separately due to spatial distance between them. Table 1 summarises the vineyards used in the investigation.

Table 1: Vineyards used for the spatial analyses with SADIE for season 1 (2005-2006) and season 2 (2006-2007) indicating cultivar and number of sampling points used during each season.

Farm	Vineyard/Cultivar	No. of sampling points	Season
Ashton	A01 & A02		
	(Colombar & Chenin blanc)	140	1 & 2
Backsberg	B01		
	(Chardonnay)	70	1
	B02		
	(Winery Shiraz)	70	1 & 2
Plaisir de Merle	B03		
	(Chardonnay)	70	2
	P01		
	(Chardonnay)	80	1 & 2
Plaisir de Merle	P02		
	(Chardonnay)	60	1 & 2

RESULTS

Ashton

Crematogaster peringueyi was the dominant ant species in the continuous Ashton vineyard. Both *A. steingroeveri* and *C. peringueyi* showed significant patches and gaps with an aggregated pattern for both seasons (Table 2). Parasitoids were clustered into patches with an aggregated pattern in season 1 while in season 2 they were randomly distributed (Table 2). There was a significant association between *C. peringueyi* and parasitoids ($P < 0.025$) and a significant disassociation between *C. peringueyi* and *A. steingroeveri* ($P > 0.975$) during both seasons. The spatial patterns and associations are mapped in Figure 1.

Backsberg

In the B01 vineyard, overall species (*L. humile* and *C. peringueyi*) distributions were spatially aggregated and gaps in distributions were clear (i.e. significant) whereas patches were weakly (i.e. non-significantly) formed (Table 2). *Linepithema humile* was dominant over *C. peringueyi*. Parasitoids were also clustered into non-significant patches with significant gaps, but were randomly distributed (Table 2). There was a significant disassociation between *L. humile* and *C. peringueyi* ($P > 0.975$) (Table 3).

Linepithema humile, the only ant species present in the B02 vineyard, and parasitoids showed significant patches and gaps forming an aggregated pattern (Table 2) in season 1 but a random pattern in season 2 (Table 2). The association between *L. humile* and parasitoids was significant ($P < 0.025$) in season 2 only (Table 3).

In the B03 vineyard, *L. humile* was randomly distributed while parasitoids were significantly aggregated (Table 2). No significant association was found between *L. humile* and parasitoids in this vineyard (Table 3). The spatial patterns and significant associations for Backsberg are mapped in figures 2 and 3.

Plaisir de Merle

Crematogaster peringueyi and *L. humile* were the main ant species in these vineyards. In the P01 vineyard, *L. humile* showed a random distribution pattern during both seasons ($I_a < 1$, $0.025 < P_a < 0.975$) (Table 2). *C. peringueyi* showed a random pattern in season 1 ($I_a < 1$; $0.025 < P_a < 0.975$) while in season 2 they showed significant aggregation ($I_a > 1$; $P_a < 0.025$) (Table 2). Parasitoids showed a random distribution pattern in both seasons ($I_a < 1$; $0.025 < P_a < 0.975$) (Table 2). There was no significant association

between ants and parasitoids ($P = 0.4369$) and a significant disassociation was found between *L. humile* and *C. peringueyi* ($P > 0.975$) in season 1 only (Table 3).

Linepithema humile showed significant gaps and patches in the P02 vineyard in season 1 (Table 2). Both *C. peringueyi* and *L. humile* formed an aggregated pattern in season 1 while parasitoids were randomly distributed throughout this vineyard during both seasons (Table 2). The association between parasitoids and *L. humile* was significant during season 2 ($P < 0.025$). No spatial association was found between the two ant species in this vineyard. Spatial patterns and significant associations are mapped in figure 4.

Table 2: Spatial patterns of ants (*Linepithema humile*, *Anoplolepis steingroeveri* and *Crematogaster peringueyi*) and vine mealybug parasitoid complex in vineyards on three farms, Ashton, Backsberg and Plaisir de Merle.

Vineyard [†]	Season	<i>L. humile</i>						Parasitoids						<i>C. peringueyi</i>					
		I _a	P _a	\bar{v}_i	\bar{p}_i	\bar{v}_j	\bar{p}_j	I _a	P _a	\bar{v}_i	\bar{p}_i	\bar{v}_j	\bar{p}_j	I _a	P _a	\bar{v}_i	\bar{p}_i	\bar{v}_j	\bar{p}_j
B01	1	1.68	0.014	1.26	0.117	-1.73	0.01	1.19	0.151	1.148	0.192	-1.14	0.2028	1.54	0.025	1.285	0.084	-1.47	0.036
B02	1	2.26	0.001	2.05	0.001	-2.39	0.01	1.91	0.005	1.868	0.007	-1.859	0.0062	-	-	-	-	-	-
	2	1.23	0.13	1.19	0.17	-1.28	0.12	1.13	0.22	1.106	0.229	-1.102	0.2507	-	-	-	-	-	-
B03	2	1.15	0.2	1.11	0.24	-1.17	0.17	1.77	0.007	1.678	0.008	-1.766	0.0059	-	-	-	-	-	-
P01	1	0.69	0.3	0.42	0.88	-0.83	0.23	0.6	0.613	0.741	0.23	-0.284	0.6068	0.93	0.885	0.529	0.748	-0.6	0.585
	2	0.99	0.096	0.57	0.3	-0.36	0.64	1.43	0.079	0.341	0.853	-2.185	0.0541	2.6	0.006	0.651	0.356	-7.37	0.01
P02	1	1.84	0.01	1.55	0.03	-1.87	0.01	0.97	0.446	1.052	0.316	-0.97	0.4428	1.64	0.024	1.726	0.014	-1.59	0.029
	2	0.88	0.64	0.88	0.63	-0.89	0.62	1.06	0.305	1.046	0.331	-1.053	0.3177	1.01	0.391	1.011	0.39	-0.99	0.413
<i>A. steingroeveri</i>																			
A01 &	1	1.69	0.01	1.59	0.01	-1.69	0.01	1.53	0.013	1.35	0.04	-1.535	0.0156	1.57	0.011	1.481	0.017	-1.59	0.008
A02	2	1.5	0.02	1.51	0.02	-1.51	0.03	0.94	0.563	0.976	0.461	-0.964	0.4892	1.66	0.006	1.586	0.006	-1.7	0.002

[†]A01 and A02 vineyards (Ashton farm); B01, B02 and B03 vineyards (Backsberg farm); P01 and P02 vineyards (Plaisir de Merle Farm)

I_a= Index of aggregation, P_a= probability level; \bar{v}_i = cluster (patch), \bar{p}_i = probability level; \bar{v}_j = cluster index (gap), \bar{p}_j = probability level.

Table 3: Spatial associations of ants (*Linepithema humile*, *Anoplolepis steingroeveri* and *Crematogaster peringueyi*) and vine mealybug parasitoid complex in vineyards on three farms during 2 growing seasons 2005/06 (season 1) and 2006/07 (season 2).

Farm (Vineyard)	Season	Association									
		<i>L. humile</i>		<i>C. peringueyi</i>		<i>L. humile</i>		<i>A. steingroeveri</i>		<i>A. steingroeveri</i>	
		/parasitoids		/parasitoids		/C. peringueyi		/parasitoids		/C. peringueyi	
		X	P	X	P	X	P	X	P	X	P
Backsberg (B01)	1	0.1136	0.1829	0.0915	0.2224	-0.4437	>0.9999	-	-	-	-
Backsberg (B02)	1	0.6715	0.0629	-	-	-	-	-	-	-	-
	2	0.3748	0.0173	-	-	-	-	-	-	-	-
Backsberg B03	2	0.168	0.1302	-	-	-	-	-	-	-	-
Plaisir de Merle (P01)	1	0.0284	0.4369	0.0838	0.2123	-0.2287	0.9792	-	-	-	-
	2	0.0287	0.3826	-0.024	0.4023	0.0442	0.241	-	-	-	-
Plaisir de Merle (P02)	1	0.2783	0.0381	-0.032	0.585	-0.1576	0.8418	-	-	-	-
	2	0.3947	0.0109	0.2134	0.054	0.1827	0.1127	-	-	-	-
Ashton (A01 & A02)	1	-	-	0.34	0.0009	-	-	-0.0422	0.677	-0.5283	0.9998
	2	-	-	0.295	0.0019	-	-	-0.0805	0.783	-0.5216	0.9999

X = index of spatial association and P = probability level.

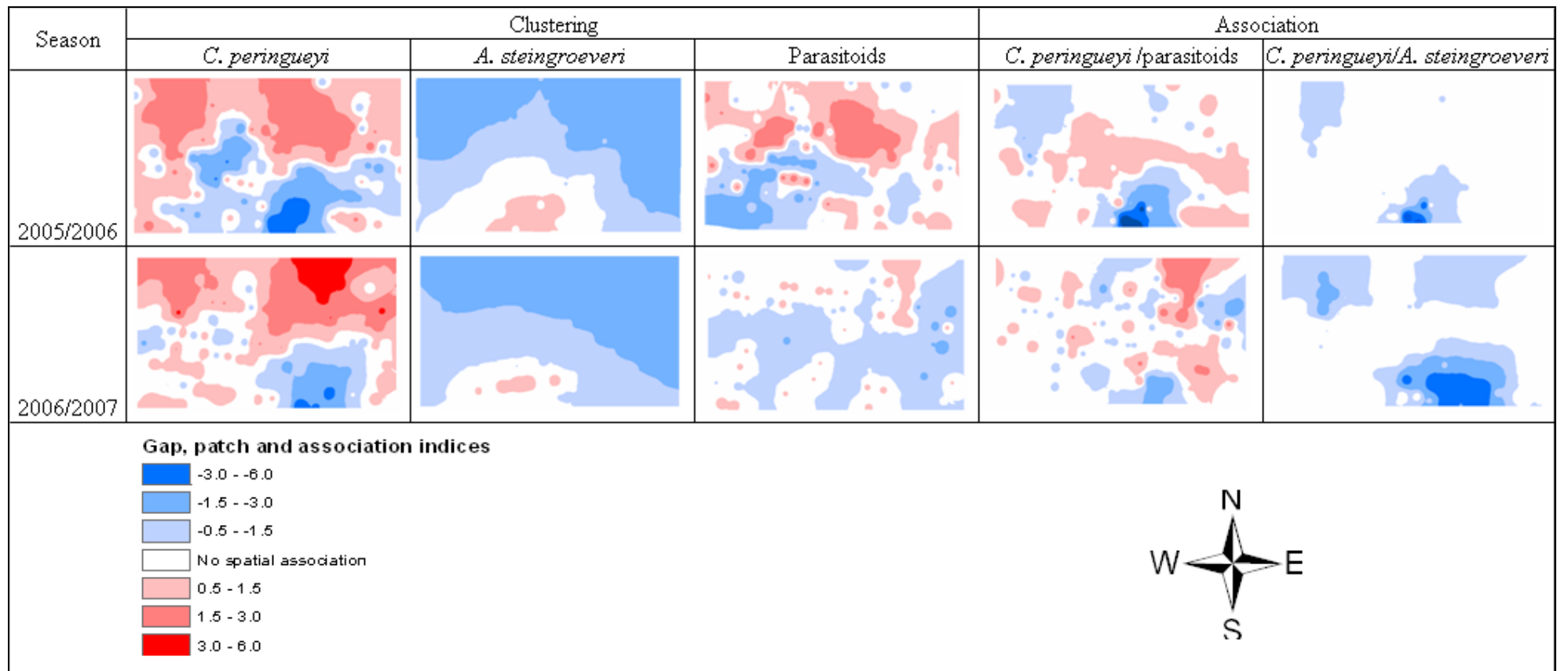


Figure 1. Interpolated spatial clustering and association of *Crematogaster peringueyi*, *Anoplolepis steingroeveri* and vine mealybug parasitoids in the two vineyards at Ashton during 2005-2006 and 2006-2007. Only significant indices were mapped: $v_i > 1.5$ (patches) and $v_j < -1.5$ (gaps) for aggregation; $\chi_k > 0.5$ (positive association) or $\chi_k < -0.5$ (disassociation). Red indicates patches and association. Blue indicates gaps and disassociation.

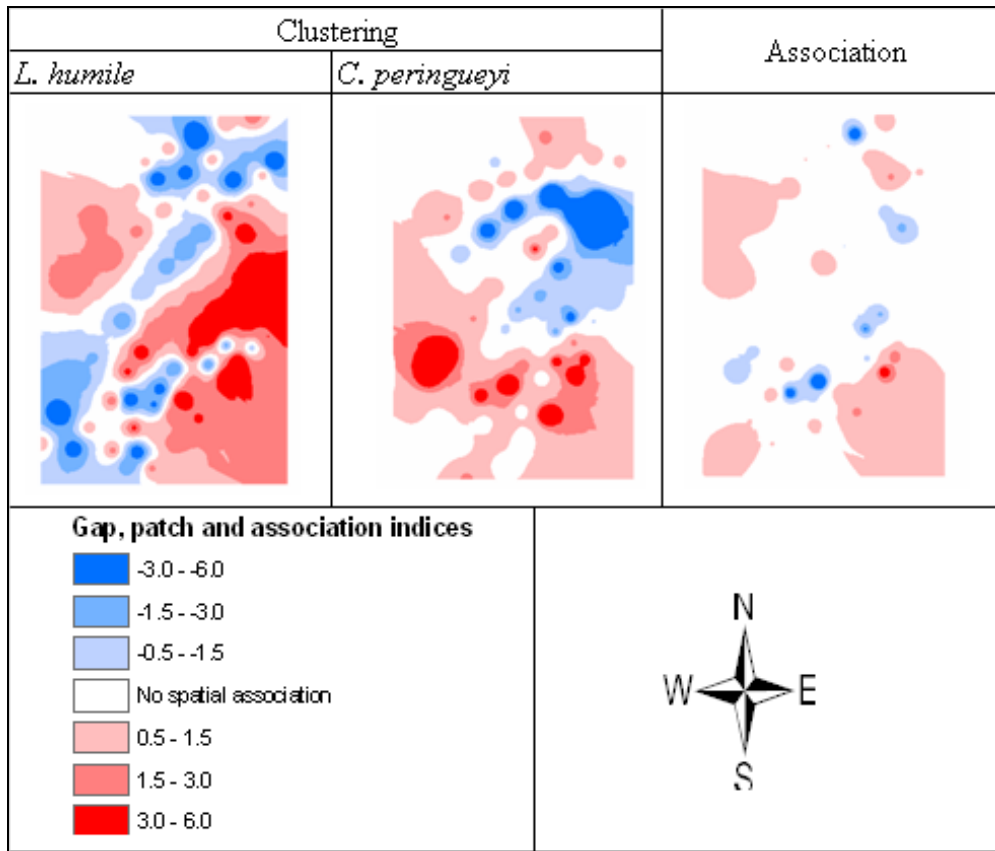


Figure 2: Interpolated spatial clustering and association of *Linepithema humile* and *Crematogaster peringueyi* vine infestation at Backsberg B01 vineyard (Chardonnay) for 2005-2006 season. Only significant indices were mapped ($v_i > 1.5$) (patches) and $v_j < -1.5$ (gaps) for aggregation; $\chi_k > 0.5$ for positive association and $\chi_k < -0.5$ for disassociation. Red indicates patches and association. Blue indicates gaps and disassociation.

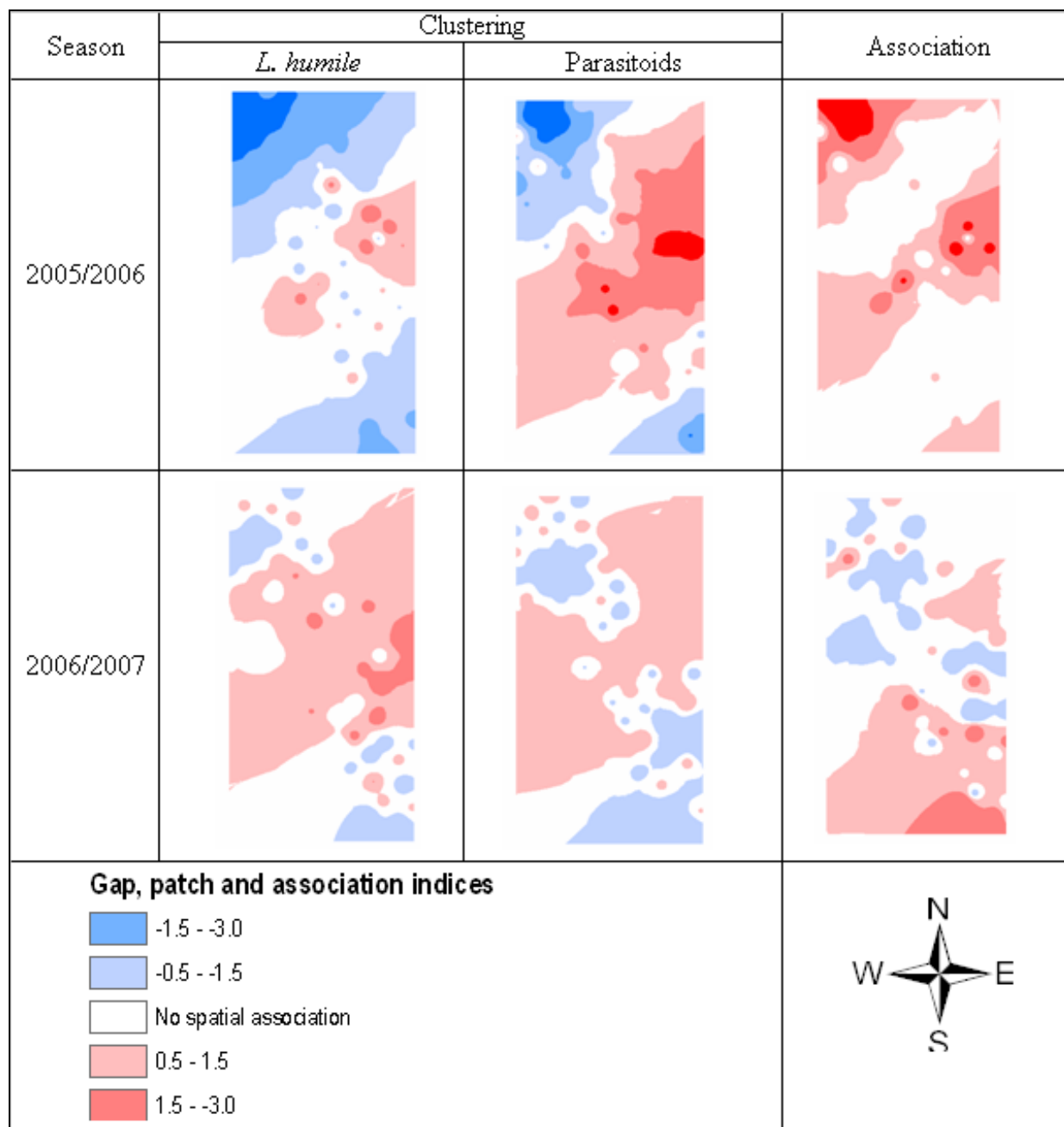


Figure 3: Interpolated spatial clustering and association of *Linepithema humile* and vine mealybug parasitoids in the vine at Backsberg B02 vineyard (Winery Shiraz) for both seasons. Only significant indices were mapped ($v_i > 1.5$) (patches) and $v_j < -1.5$ (gaps) for aggregation; $\chi_k > 0.5$ for positive association and $\chi_k < -0.5$ for disassociation. Red indicates patches and association. Blue indicates gaps and disassociation.

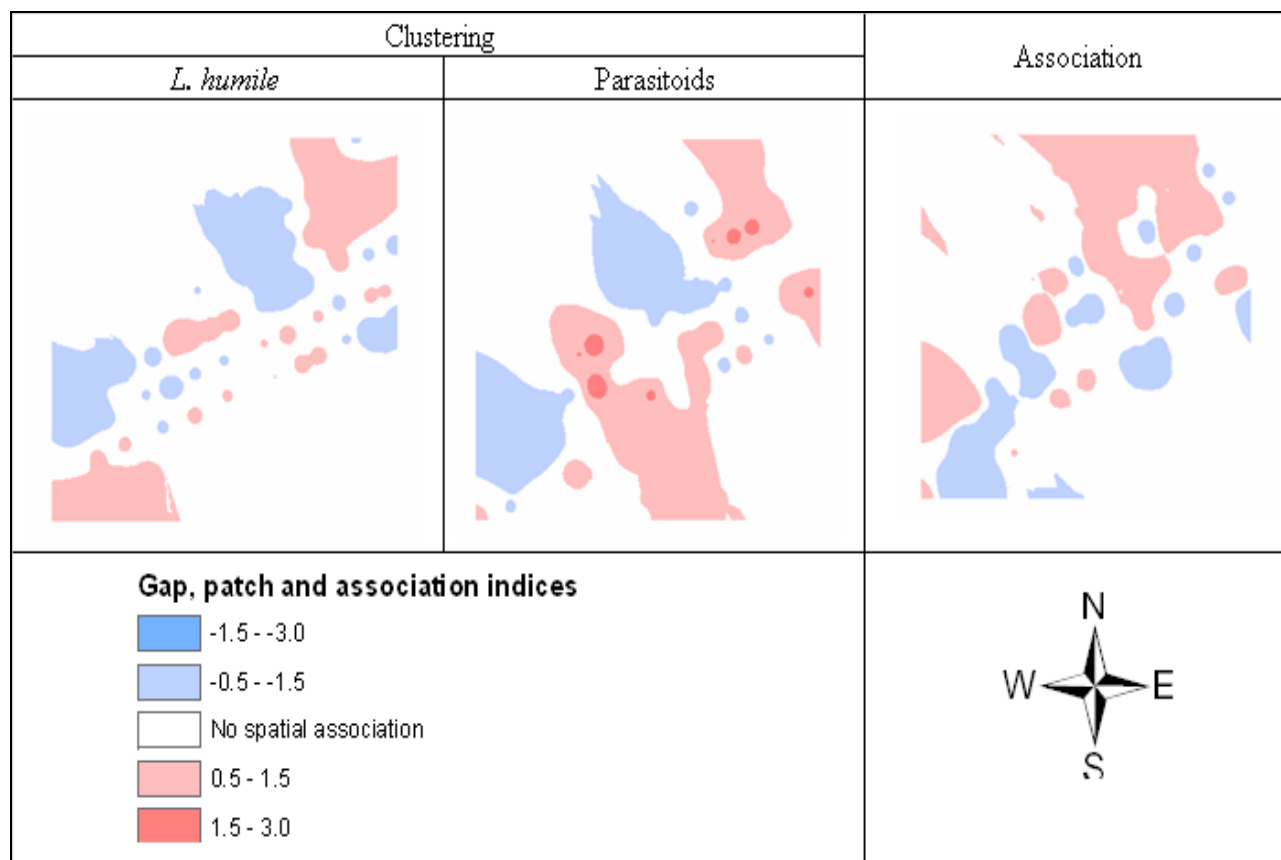


Figure 4: Interpolated spatial clustering and association of *Linepithema humile* and vine mealybug parasitoids at Plaisir de Merle P02 vineyard (Chardonnay), 2006-2007 season. Only significant indices were mapped ($v_i > 1.5$) (patches) and $v_j < -1.5$ (gaps) for aggregation; $\chi_k > 0.5$ for positive association and $\chi_k < -0.5$ for disassociation. Red indicates patches and association. Blue indicates gaps and disassociation.

DISCUSSION

A significant spatial positive association between ants (*C. peringueyi* and *L. humile*) and parasitoid distributions shows that these parasitoids (obtained by collecting parasitised mealybugs) are effective in searching for their vine mealybug hosts that are usually mutually associated with ants. The change from a spatial aggregation pattern in season 1 to a more random pattern in season 2 for parasitoids could be the result of reduced mealybug infestations during season 2 (Chapter 2), which indicates a density dependant relationship between parasitoids and mealybugs. This agrees with the data obtained by Walton (2003), who also worked in Western Cape vineyards. The presence of a parasitoid population may affect the spatial pattern of its host (Perry 1998a). Intuitively, parasitoids and mealybugs are spatially associated. When mealybugs escape parasitoid attack into refuges, they cause a change in the spatial pattern of parasitoid populations which then follow their host in its new aggregations. *C. perminutus* infested mealybugs fall to the ground (Ceballo & Walter 2005), and this could have influenced the distribution pattern as these mealybugs could not be collected during sampling.

While the scarcity of mealybugs could have caused association of ants and parasitoid species, subsequent increased disturbance of parasitoid activity on the limited VMB population could have also occurred. Pure populations of *C. peringueyi* at Ashton and *L. humile* at one Backsberg vineyard could have exhibited increased aggression towards parasitoids than mixed ant populations (Buckley & Gullan 1991; Kaneko 2003). To improve protection of their Hemiptera, these two ant species are known to construct protective structures over dwindling mealybug populations (Whitehead 1957; Holway et al. 2002) thereby reducing parasitoid activity. Again, a random distribution pattern of parasitoids was accompanied by increased negative impact of ants. This random distribution possibly ensued due to low VMB populations causing a dispersed parasitoid distribution, and in search for their scarce hosts, parasitoids could have been encountered by aggressive foraging ant species.

Crematogaster peringueyi is an arboreal species that needs to be controlled before parasitoids become active from mid summer to harvest (Walton 2003; 2000). Continuous sampling after harvest will provide information on spatial distribution of this species so that control can be carried out before the onset of the succeeding growing season to prevent pending infestations. *A. steingroeveri* occupied mostly peripheral plots in a previous survey of ants in vineyards. On Ashton farm, this ant species showed a significant cluster on the periphery of the vineyard, supporting the edge effects found

by Addison and Samways (2000) in the same study area, which was not the case with other ant species. This was possibly due to unfavourable wet habitat due to irrigation, ground cover and disturbed soil in the vineyard (Addison & Samways 2000). Their significant disassociation with *C. peringueyi* indicates interspecific hostility. Management of *Anoplolepis* spp. in vineyards could be aimed at manipulating vineyard floors such as cover cropping to discourage nest building in the vineyard (Addison & Samways 2006). An aggregated pattern of *L. humile* depicted high mealybug infestation and subsequent bunch damage as on Backsberg farm vineyards and Plaisir de Merle P02 vineyard in season 2. Their random pattern was often coupled with low to moderate mealybug infestation levels. *L. humile* are introduced species in South Africa that virtually belong to the same supercolony. As such, there is a possible decline in aggression and intraspecific competition allowing this ant species to invade large areas while displacing native species such as *Crematogaster* and *Anoplolepis* species (Tsutsui et al. 2003; Thomas et al. 2005). Continuous sampling is therefore necessary to prevent spread of this invasive and aggressive species.

Crematogaster peringueyi distribution pattern may not always explain mealybug infestation levels. Significant aggregation in season 1 on Ashton and Plaisir de Merle P02 vineyard was associated with high mealybug infestations and bunch damage but this was not the case in season 2 when mealybug infestation was low with little or no bunch damage in the same vineyards.

Anoplolepis steingroeveri did not have any significant association with parasitoids implying that it was less of a threat to vine mealybug parasitoids in the vineyards studied.

A significant spatial disassociation between *L. humile* and *C. peringueyi* also demonstrates interspecific competition between these two species. *L. humile* can displace the native *C. peringueyi* and *A. steingroeveri* and promote higher mealybug infestations than the latter species, whose distribution in the Western Cape is not as wide as that of *L. humile* (Luruli 2007; Prins et al. 1990).

CONCLUSION

Ants and vine mealybugs on vines in South African vineyards coexist with vine mealybug parasitoids and other natural enemies. It is imperative, therefore, to break the vine mealybug-ant mutualism in vineyards to enable parasitoids to keep mealybugs below injurious levels. Even though ant and parasitoid distributions were spatially

associated, they were not always coincident throughout the vineyards, making spot treatments on stems more appropriate than full cover treatments. Full cover chemical treatments may not only be uneconomic but unnecessary as ants are usually aggregated into patches making spot treatments more effective. Because ants are usually associated with mealybugs under natural conditions, the spatial patterns of mealybugs, hence parasitoids can change dramatically if ant control is applied. The possibility of a change in spatial patterns and distributions of ants and parasitoids due to chemical ant control in vineyards requires further attention and would provide insight into the efficacy of ant control practices.

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CHAPTER 4

DEVELOPMENT OF AN ECONOMIC THRESHOLD FOR ANTS (HYMENOPTERA: FORMICIDAE) IN VINEYARDS

ABSTRACT

Pests need to be controlled once they reach a certain density (action threshold) but before they reach a level that causes unacceptable economic damage (economic injury level). This study presents an approach that can be used to determine the level at which ants should be controlled before they promote mealybug infestations to injurious levels in vineyards. Data on ant infestation were collected in a two season study (2005-2006 and 2006-2007) from two main vine growing areas in the Western Cape Province using a presence –absence sampling system. On each of three farms, seven blocks, each consisting of 20 plots of 5 vines each, were inspected biweekly. A regression analysis of observed versus binomial variance using dummy variables to test differences due to season and location was performed. None of the differential co-efficients in the full model were statistically significant for stems, shoots and leaves. Therefore, the linear regression of the observed versus binomial variance was not affected and a reduced model was used. For bunches, significant differences were found and a full model was applied. The models were used to estimate the sampling error as well as the probability of correctly deciding to take chemical control action against ants on each of the vine sections. A sampling error of 22% was obtained which remained constant even when more than 20 plots were sampled. Taking or initiating control action against ants when 20% of the vines were infested would be a reliable decision in 95% of the cases.

Key words: Ants, sampling, Economic Threshold, Operating Characteristic curves.

INTRODUCTION

Crop loss and downgrading due to ant-tended vine mealybug (VMB) *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) infestations in South Africa can be prevented by timely application of control measures and monitoring regularly. At present, decisions regarding ant control are arbitrary and not based on ecologically-sound principles. Currently an economic threshold (ET) of 25% ant infestation is used before

chemical control of ants in vineyards is applied (<http://www.ipw.co.za>). However, this is not backed by scientific data. In an integrated pest management (IPM) programme the ET and the economic injury level (EIL) need to be determined. Stern et al. (1959) defined the ET as “the population density at which control action should be initiated to prevent an increasing pest population from reaching the EIL, the latter being the lowest population density that will cause economic damage”.

Economic thresholds together with pest monitoring systems, can be used to determine the necessity and timing of control measures to control herbivorous pest arthropods, especially those attacking high value crops (Pringle 2006), such as the VMB. Most pests are tolerated at relatively low levels in vineyards, for example, a 2% stem infestation is used as a threshold for VMB (Walton 2003). To date, no documentation is available on thresholds for pest ants in agriculture. Low ant infestations in vineyards, in most cases, are not sufficient justification to warrant chemical control because the costs and risks of taking such action would far outweigh any benefits. Ants have other important ecological functions, including myrmecochory and predation of other pest arthropods like fruit fly and moth larvae (Samways 1982). In high numbers, honeydew-seeking ants can promote VMB infestations by compromising biological control, resulting in crop loss or even vineyard loss if grapevine leaf roll associated virus (GLRaV-3) vectored by VMB is present (Engelbrecht & Kasdorf 1990). Currently α -cypermethrin and chlorpyrifos are registered for ant control. The average annual cost per ha of controlling ants in wine grape vineyards, including labour, was between R100.00 and R360.00 ha⁻¹ (2006) which would be expected to rise by 4-6% due to inflation in successive years (Wohlfarter pers. comm.). It is important to set the ET for ants at a figure that neither results in crop loss nor upsets the ecological equilibrium. As indirect pests, there can be some degree of tolerance for ants in vineyards.

The objective of this investigation was to develop an economic threshold for key ant pests in commercial vineyards with known levels of error. This will enable producers to correctly decide when to implement chemical control against pest ants, therefore saving costs on unnecessary chemical treatments (when over reacting) and reducing risk of crop loss (when under reacting).

MATERIAL AND METHODS

Study sites

This investigation was carried out during two consecutive growing seasons (2005-2006 and 2006-2007) in two main wine grape growing areas, the coastal region, Simondium (Baksberg, 33.83°S, 18.92°E, 240m and 33.83°S, 18.83°E, 175.2m; Plaisir de Merle, 33.83°S, 18.95°E, 193m) and Breede River Valley (Ashton, 33.85°S, 20.08°E, 186m).

Sampling was done on 21 hectare (ha) of wine grapes with approximately 7ha on each of the three farms. The 7ha on each farm were divided into 1ha blocks, each of which had 20 evenly spaced plots, consisting of 5 vines between two trellis poles. This was in accordance with the standard monitoring systems developed for vineyards (Walton & Pringle 2004; de Villiers & Pringle 2007). All vineyards were mature (>10 years old) and were not sprayed with insecticide but only treated with fungicides during the period of investigation.

Sampling method

A presence-absence cluster sampling system was used for monitoring ant infestations (Madden & Hughes 1999; Binns et al. 2000). Sampling was done at two-weekly intervals from October to February during both growing seasons on five vines per plot (in a total of 420 plots altogether). Each vine was divided into four sections: the stem (the cordons 30 cm on either side of the central stem), leaves (1 leaf per vine), shoots (15 cm of the first distal shoot) and bunches (1 bunch per vine) (De Villiers & Pringle 2008). All sections of the vine were inspected for foraging ants and classified as infested or uninfested without regarding the severity of the infestation. Each plot of five vines was treated as the primary unit while vines within the plot were secondary units. In cluster sampling, the proportion of infested units, p , (stems, shoots, leaves and bunches) was estimated using the expression,

$$p = \frac{\sum_{i=1}^n \sum_{j=1}^N X_{ij}}{nN} \quad (1),$$

(Madden and Hughes 1999; Binns et al. 2000) for N plots (20 in this investigation) and n stems, shoots, leaves or bunches (5 in each plot in this investigation) (for the i th and j th unit).

The binomial variance, S_B^2 , was estimated using the expression,

$$Var(Bin) = S_B^2 = \frac{p(1-p)}{n} \quad (2),$$

(Binns et al.2000), because n was constant for each plot ($n = 5$ vines/plot).

The observed variance, S_O^2 , was estimated using,

$$Var(Obs) = S_O^2 = \frac{\sum_{j=1}^N (p_j - p)^2}{N-1} \quad (3),$$

(Binns et al. 2000).

The regression (Binns et al. 2000),

$$\ln(S_O^2) = \ln(a) + (b) \ln(S_B^2) \quad (4),$$

was fitted . Taking the antilog of (4), an expression relating to the observed variance to the binomial variance was obtained:

$$S_O^2 = a(S_B^2)^b = a \left\{ \frac{p(1-p)}{n} \right\}^b \quad (5),$$

which is similar to Taylor's power law. If infestations were random, then the variance of infested plots will conform to the binomial distribution given in (2) implying that every vine in each plot had an equal chance of being infested by ants.

The linear regression of $\ln(S_O^2)$ on $\ln(S_B^2)$ was estimated to produce the estimates of a and b for all data on stems, shoots, leaves and bunches.

Dummy variable regression models

Dummy variables can be used to test for differences in qualitative factors like season and locality in regression analysis (Gujarati 1970a, 1970b). In this investigation the differences due to season and farms were tested. The dummy variables were assigned as follows:

D₁- 2005-2006 season, Backsberg

D₂- 2005-2006 season, Plaisir de Merle

D₃- 2006-2007 season, Ashton

D₄- 2006-2007 season, Backsberg

D₅- 2006-2007 season, Plaisir de Merle

No dummy variable was assigned to the 2005-2006 season for Ashton as this would result in a singular data matrix that cannot be inverted (Gujarati 1970b).

Therefore, the full regression model was,

$$\ln(S_o^2) = \ln(a) + (b)\ln(\bar{x}) + a_1D_1 + a_2D_2 + a_3D_3 + a_4D_4 + a_5D_5 + (b_1)(D_1)\ln(\bar{x}) + (b_2)(D_2)\ln(\bar{x}) + (b_3)(D_3)\ln(\bar{x}) + (b_4)(D_4)\ln(\bar{x}) + (b_5)(D_5)\ln(\bar{x}) \quad (6).$$

The regression equations for the different combinations of season and farm were,

$$\ln(S_o^2) = \ln(a) + (b)\ln(\bar{x}) \text{ season 1, Ashton}$$

$$\ln(S_o^2) = [(a_1 + \ln(a))] + (b_1 + b)\ln(\bar{x}) \text{ season 1, Backsberg}$$

$$\ln(S_o^2) = [(a_2 + \ln(a))] + (b_2 + b)\ln(\bar{x}) \text{ season1, Plaisir de Merle}$$

$$\ln(S_o^2) = [(a_3 + \ln(a))] + (b_3 + b)\ln(\bar{x}) \text{ season 2, Ashton}$$

$$\ln(S_o^2) = [(a_4 + \ln(a))] + (b_4 + b)\ln(\bar{x}) \text{ season 2, Backsberg}$$

$$\ln(S_o^2) = [(a_5 + \ln(a))] + (b_5 + b)\ln(\bar{x}) \text{ season 2, Plaisir de Merle.}$$

The intercept of the regression for season 1, Ashton, is $\ln(a)$ and the slope is b . For data collected during season 1 from Backsberg, the intercept, $\ln(a)$, is increased by a_1 and the slope, b , by b_1 and so on for other farms and seasons (a_1 - a_5 are differential intercepts and b_1 - b_5 are differential slopes). To investigate the effect of locality (farm) and season on the slope, a reduced regression model was formulated as:

$$\ln(S_o^2) = \ln(a) + (b)\ln(\bar{x}) + a_1D_1 + a_2D_2 + a_3D_3 + a_4D_4 + a_5D_5 \quad (7),$$

in which there was one slope coefficient, b , with 6 intercept coefficients, $\ln(a)$, a_1 , a_2 , a_3 , a_4 and a_5 , implying that the model proposed six parallel lines with the assumption that b_1 , b_2 , b_3 , b_4 and b_5 were equal to zero. This can be tested in the usual way for reduced regression models using extra degrees of freedom (E.d.f) and extra sum of squares (E.SS).

E.d.f = (d.f residual in reduced model)-(d.f residual in full model)

E.SS = (SS residual in reduced model)-(SS residual in full model)

A F-test was then used to determine the significance of the hypothesis that b_1, b_2, b_3, b_4 and b_5 were equal to zero. The null hypothesis would be

$$H_0: b_1=b_2=b_3=b_4=b_5=0$$

and this would be compared with the alternative hypothesis,

$$H_1: b_i \neq 0 \text{ for any } i = 1, 2, 3, 4, 5 \dots$$

The model can be further reduced to one with a common intercept as well as a common slope if the differential intercept and differential slope are statistically insignificant (Gujarati 1970a). In the case of a significant difference between a reduced model and a full model, the reduced model was rejected.

Sampling errors

A general equation for estimating sampling error could be expressed as,

$$D = \frac{\sqrt{S^2 / N}}{p} \quad (8),$$

(Binns et al. 2000), where p was the average ant infestation. Substituting (5) into (8), an estimate of the sampling error (D), can be obtained by any value of the average infestation p .

$$D = \frac{\sqrt{\frac{a}{N} (S_B^2)^b}}{p} \quad (9),$$

or

$$D = \frac{\sqrt{\frac{a}{N} \left\{ \frac{p(1-p)}{n} \right\}^b}}{p} \quad (10),$$

(Binns et al. 2000).

Operating Characteristic (OC) curves

When the infestation estimated by sampling is below a fixed economic threshold (ET), operating characteristic (OC) curves can be drawn. An OC curve can be used to determine the probability that a decision not to intervene is correct at any pest population level estimated from a sample (Binns et al. 2000). Operating characteristic curves can be estimated using a range of values for the average infestation \bar{x} , in,

$$z = \frac{\bar{x} - ET}{\sqrt{S^2/N}} \quad (11),$$

where ET was the fixed economic threshold of 25% (<http://www.ipw.co.za>) and z the cumulative normal probability function. In the case of a binomial distribution, this can be expressed as (Binns et al. 2000),

$$z = \frac{p - ET}{\sqrt{\frac{1}{N}(S_B^2)}} \quad (12),$$

Substituting (5) into (11)
$$z = \frac{p - ET}{\sqrt{\frac{a}{N} \left\{ \frac{p(1-p)}{n} \right\}^b}} \quad (12),$$

gives an expression for estimating the OC function for a fixed value of ET and a range of values for p . The corresponding probability levels of z can be obtained from the right one tailed normal probability tables. This provides estimates for the probability of correctly deciding not to apply control measures at a range of infestation levels estimated by sampling.

RESULTS AND DISCUSSION

A number of ant species were observed tending mealybugs on the three study sites. In Stellenbosch, the Argentine (*Linepithema humile* (Mayr)) and cocktail ants (*Crematogaster peringueyi* Emery) were common. The cocktail ant was the dominant ant in the Breede River Valley with the black pugnacious ant (*Anoplolepis steingroeveri* (Forel)) also present. There was a gradual movement of mealybugs from stems early in the season onto leaves and bunches as the season progressed, which supports the results

obtained by Walton and Pringle (2004) (Figures 1, 3 and 4). Ant infestations followed the same pattern, except that stem infestations remained much higher throughout the season, while leaf and bunch infestations never increased substantially (Figures 1, 3 and 4). Shoot infestations were erratic early in the season, with a slow decline later in the season for both ants and mealybugs (Figure 2). This indicates the necessity for directed control of ants on stems throughout the growing season but particularly early in the season.

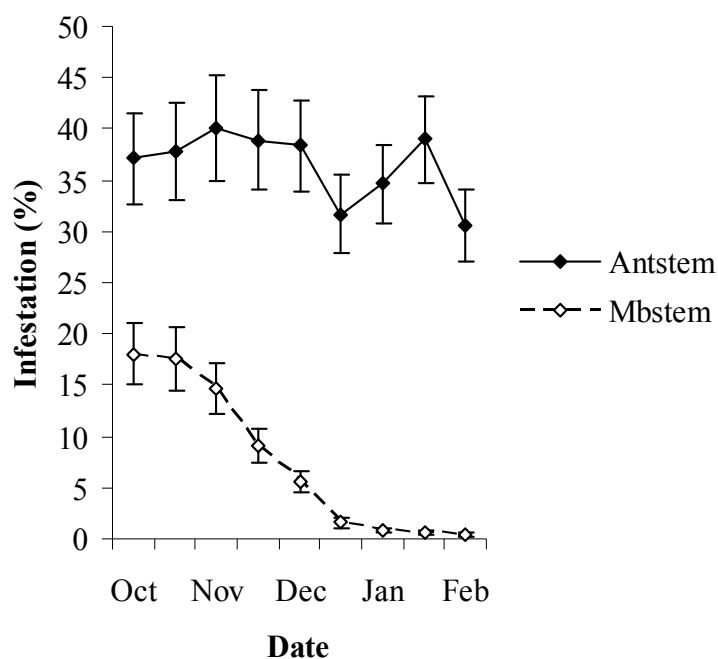


Figure 1: Mean (\pm standard error) ant infestation on stems (Antstem) and vine mealybug infestation on stems (Mbstem) during two seasons (2005-2006 and 2006-2007) for three study sites.

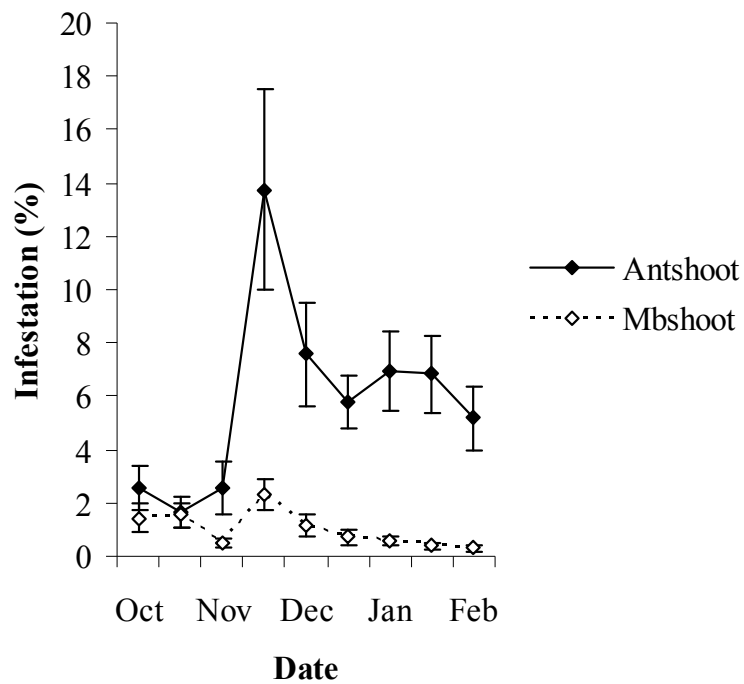


Figure 2: Mean (\pm standard error) ant infestation on shoots (Antshoot) and vine mealybug infestation on shoots (Mbshoot) during two seasons (2005-2006 and 2006-2007) for three study sites.

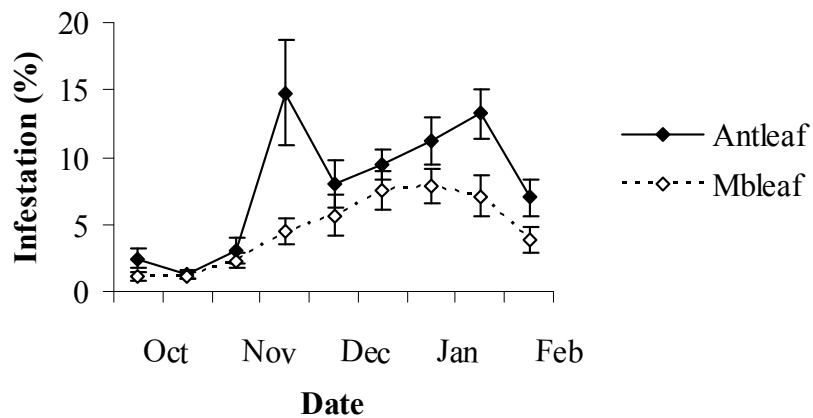


Figure 3: Mean (\pm standard error) ant infestation on leaves (Antleaf) and vine mealybug infestation on leaves (Mbleaf) during two seasons (2005-2006 and 2006-2007) for three study sites.

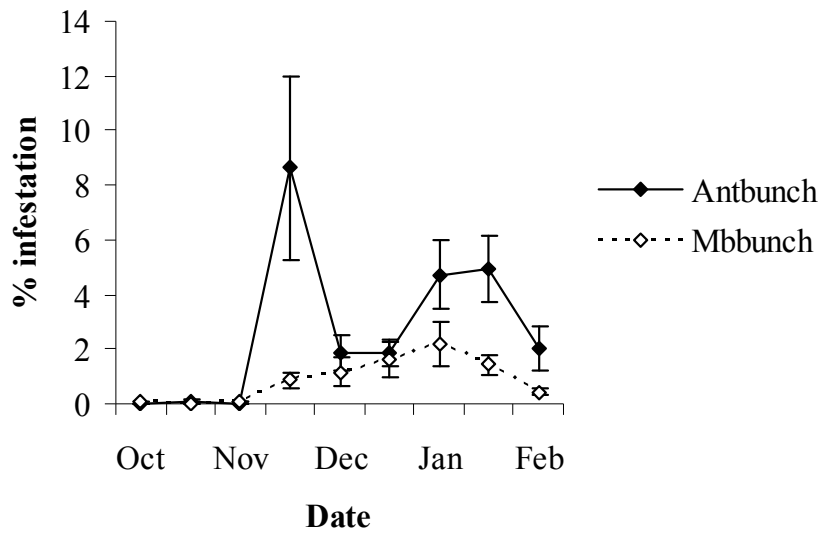


Figure 4: Mean (\pm standard error) ant infestation on bunches (Antbunch) and vine mealybug infestation on bunches (Mbbunch) during two seasons (2005-2006 and 2006-2007) for three study sites.

Dummy variable regression models

None of the differential regression coefficients in the full model (6) were statistically significant ($p > 0.05$) for stems, shoots and leaves (Table 1). Therefore, neither farm nor season had an effect on the regression of $\ln(S_O^2)$ on $\ln(S_B^2)$. The full model (6) could, therefore, be reduced to (7) for stems, shoots and leaves, and one regression could be used for all farms and seasons. These regression co-efficients are given in Table 2. The correlation coefficients of all linear regressions were good and their linear relationships are shown in figures 5 and 6.

In the case of bunches, the differential slopes for season 2 at Backsberg and Plaisir de Merle significantly differed from that of Ashton ($t_{(110)} = -2.4472$; $p < 0.05$ and $t_{(110)} = 2.8850$; $p < 0.01$, respectively), suggesting that infestations for the first season (2005-2006) were different from those of the second season (2006-2007). The intercept of the second season was $5.3273 + (-2.4506)$ and the slope was $1.2615 + (-0.2795)$ for Backsberg. For Plaisir de Merle, the second season intercept was $5.3273 + 3.6893$ and the slope was $1.2615 + 0.4008$. Therefore, the regressions for the individual farms and seasons were:

$$\ln(S_O^2) = \ln(a) + (b)\ln(S_B^2) \text{ for all farms season 1}$$

$$= 5.32730 + 1.26152\ln(S_B^2)$$

$$\ln(S_O^2) = (\ln(a) + a_4) + (b + b_4)\ln(S_B^2)$$

$$= 2.8767 + 0.98209 \ln(S_B^2) \text{ for 2006-2007, Backsberg}$$

$$\ln(S_O^2) = (\ln(a) + a_5) + (b + b_5)\ln(S_B^2)$$

$$= 9.01656 + 1.66231 \ln(S_B^2) \text{ for 2006-2007, Plaisir de Merle.}$$

Table 1: The regression coefficients (RC) with their *t* and *p* values for the full model for ant infestation data on stems, shoots, leaves and bunches.

Season	Vine section	Ashton				Backsberg				Plaisir de Merle				
		d.f.	RC	t	p	RC	t	p	RC	t	p			
Season 1 (2005-2006)	Stem		a	4.1897	8.0198	<0.0001	a	4.0404	-0.1692	0.8657	a	4.1858	-0.0049	0.9961
		360	b	1.0618	13.0110	<0.0001	b	1.0900	0.2034	0.8389	b	1.1246	0.5313	0.5955
	Shoot		a	5.8557	14.4660	<0.0001	a	4.8678	-1.3539	0.1770	a	5.9525	0.0804	0.9360
		248	b	1.3230	23.7048	<0.0001	b	1.2068	-1.2591	0.2092	b	1.3203	-0.0194	0.9845
	Leaf		a	4.9860	13.7639	<0.0001	a	4.1012	-1.4470	0.1490	a	4.4146	-0.8517	0.3951
		277	b	1.2152	23.9459	<0.0001	b	1.1277	-1.0463	0.2963	b	1.1553	-0.7100	0.4783
	Bunch		a	5.7198	12.0091	<0.0001	a	5.9462	0.2238	0.8232	a	5.4526	-0.1704	0.8650
		135	b	1.3229	20.7063	<0.0001	b	1.3257	0.0232	0.9815	b	1.2716	-0.2751	0.7837
Season 2 (2006-2007)	Stem		a	0.5248	0.7388	0.4605	a	1.5399	1.6376	0.1024	a	2.1006	2.0088	0.0453
		360	b	0.0775	0.7016	0.4834	b	0.2622	1.9600	0.0508	b	0.3661	2.4209	0.0160
	Shoot		a	0.5870	0.9180	0.3595	a	-0.1609	-1.0404	0.2991	a	1.0998	0.5529	0.5809
		248	b	0.0789	0.8863	0.3763	b	-0.0082	-0.9521	0.3420	b	0.1253	0.4110	0.6814
	Leaf		a	1.1227	2.0174	0.0446	a	1.3701	0.3881	0.6982	a	0.6973	-0.6280	0.5305
		277	b	0.1413	1.8089	0.0716	b	0.1719	0.3640	0.7162	b	0.1026	-0.4497	0.6533
	Bunch		a	0.8549	1.2091	0.2287	a	2.4767	-2.4986	0.0139	a	9.0166	3.0518	0.0029
		110	b	0.0824	0.8874	0.3764	b	0.9820	-2.4472	0.0160	b	1.6623	2.8850	0.0047

Table 2: The regression coefficients with their F-values and probability levels, p , for the reduced model on ant infestation on stems, shoots, leaves and bunches.

Season	Farm	Vine		a	b	F	p	R ²
		section						
Both	All	Stem	5.0619	1.2282	(1,370)	1666.79	<0.001	0.82
Both	All	Shoot	5.6948	1.2997	(1,258)	13072.81	n.s.	0.92
Both	All	Leaf	5.1666	1.2439	(1,287)	3078.04	<0.001	0.91
2005-2006	All	Bunch	5.6062	1.2898	(1,145)	1596.21	<0.001	0.92
2006-2007	Ashton	Bunch	5.3273	1.2615	(5,110)	290.95	<0.001	0.93
2006-2007	Backsberg Plaisir de	Bunch	2.8767	0.982	(5,110)	290.95	<0.05	0.93
2006-2007	Merle	Bunch	9.0166	1.6623	(5,110)	290.95	<0.01	0.93

† n.s. = not significant at 95% confidence interval

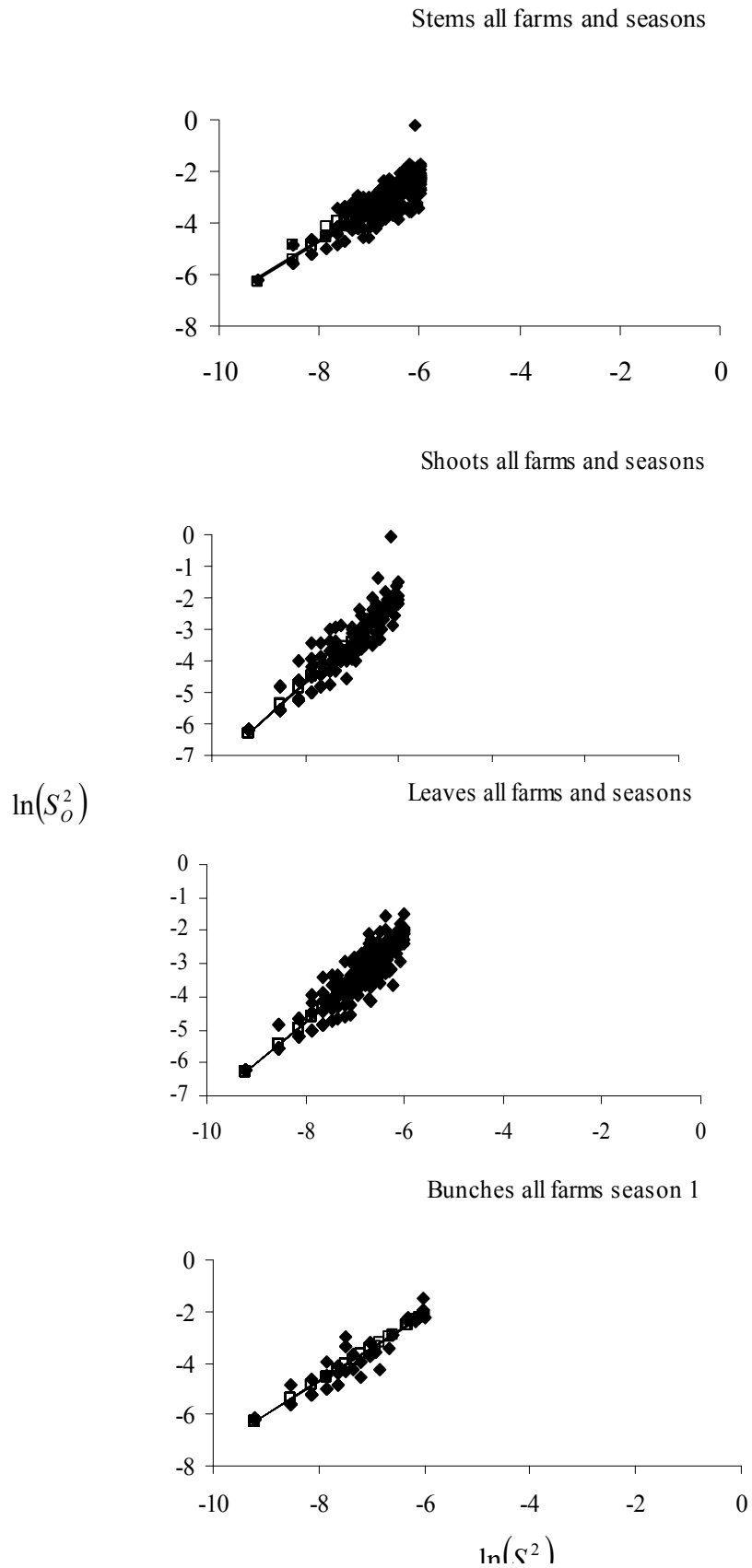


Figure 5: Relationship between the natural log of the observed variance, $\ln(S_o^2)$, and binomial variance, $\ln(S_b^2)$, for ant infestation on stems, shoots and leaves for all seasons and bunches for season 1 (2005-2006) from all three study sites.

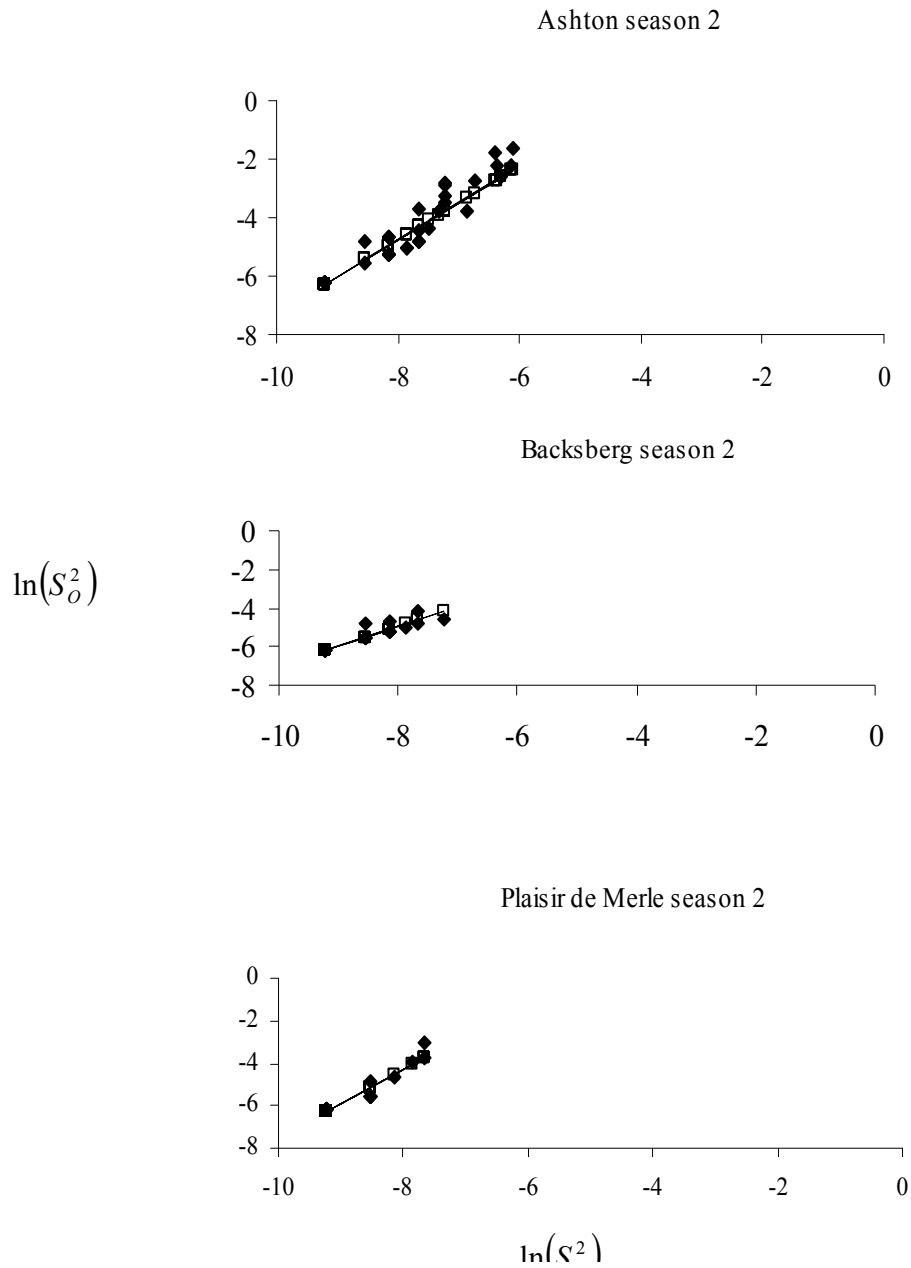


Figure 6: Relationship between the natural log of the observed variance, $\ln(S_o^2)$, and the binomial variance, $\ln(S_B^2)$, for ant infestation on bunches for season 2 (2006-2007) at the three study sites.

Sampling error

The regression data in Table 2 were used in (8) to estimate the sampling error, D , for a range of infestation levels, p , to stems, shoots, leaves and bunches using 20 plots (Figure 7) and a range of number of plots using a 25% infestation level (Figure 8). Because the regression

constants were so similar across the vine sections, this resulted in very similar estimates of the sampling error.

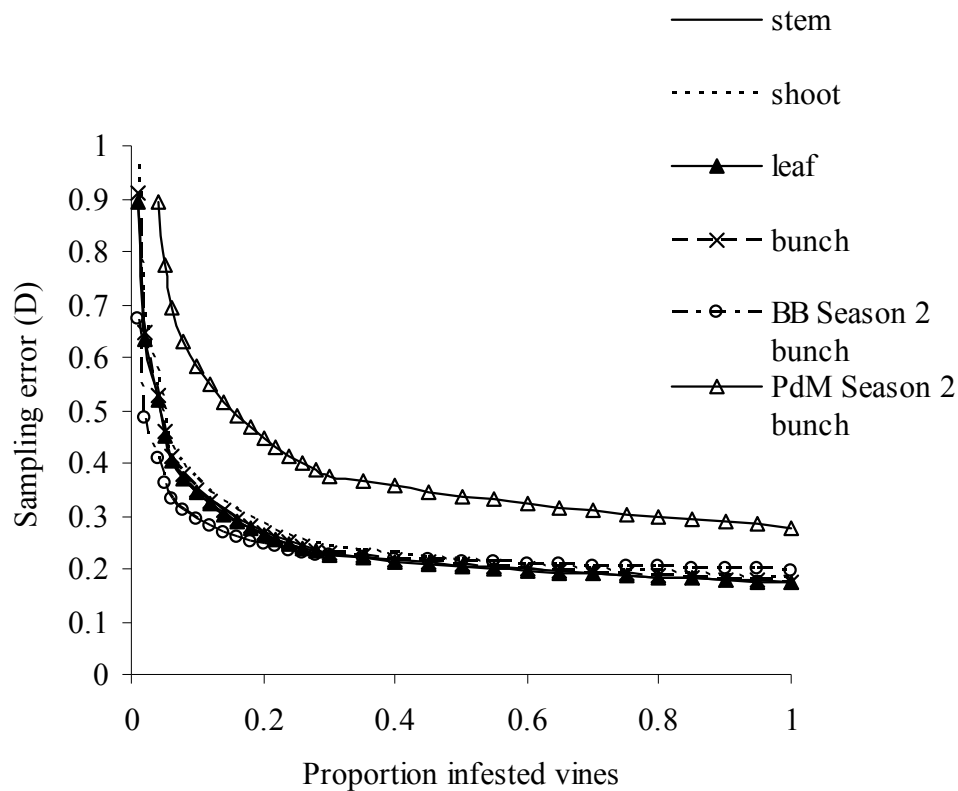


Figure 7: Sampling error, D , plotted against the proportion of stems, shoots, leaves and bunches (for all farms and seasons, Backsberg 2006-2007 (BB season 2) and Plaisir de Merle 2006-2007 (PdM season 2), infested with ants at different infestation levels and constant sampling units, N , ($N=20$ plots). As the proportion, p , of infested vines increased, the sampling error decreased.

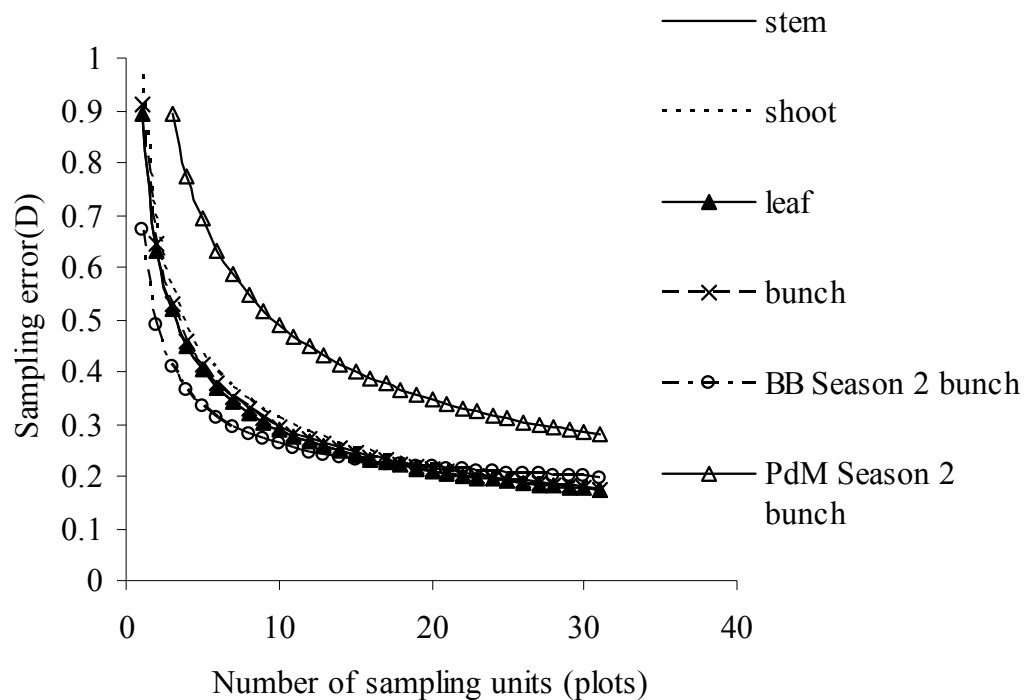


Figure 8: Sampling error, D , plotted against the number of sampling units (plots) infested with ants. As the number of sampling units, N , increased, so the sampling error decreased ($N=20$) using an Economic Threshold of 25% infestation.

There was no marked difference in sampling error between vine sections as the curves almost coincided reflecting the similarity between the regression constants. Sampling error was high at low infestation or pest population levels. For Plaisir de Merle season 2, the regression constant b was higher than that for other vine sections and seasons resulting in higher estimates of the sampling error.

When 20 plots were sampled at an infestation level of 25%, the error was 0.21 for stem, leaf and bunch infestations and 0.22 for shoot infestation (Figure 8). Increasing the sample size improved the sampling precision (decreases sampling error). However, increasing the sampling units beyond 20 plots per block did not lower the sampling error significantly (Pringle 2006).

Operating Characteristic (OC) for ant infestation

OC curves (Figure 9) were produced using the regression coefficients (Table 2) in (15) for all vine sections. With an ET of 25%, the decision not to implement chemical control when 20% of the vines were infested, would not lead to under reacting (exceeding the ET) in 95% of the cases. For vines with ant infestations of between 22% and 24%, the reliability of the decision not to intervene will be reduced to between 88% and 65% of the cases, respectively.

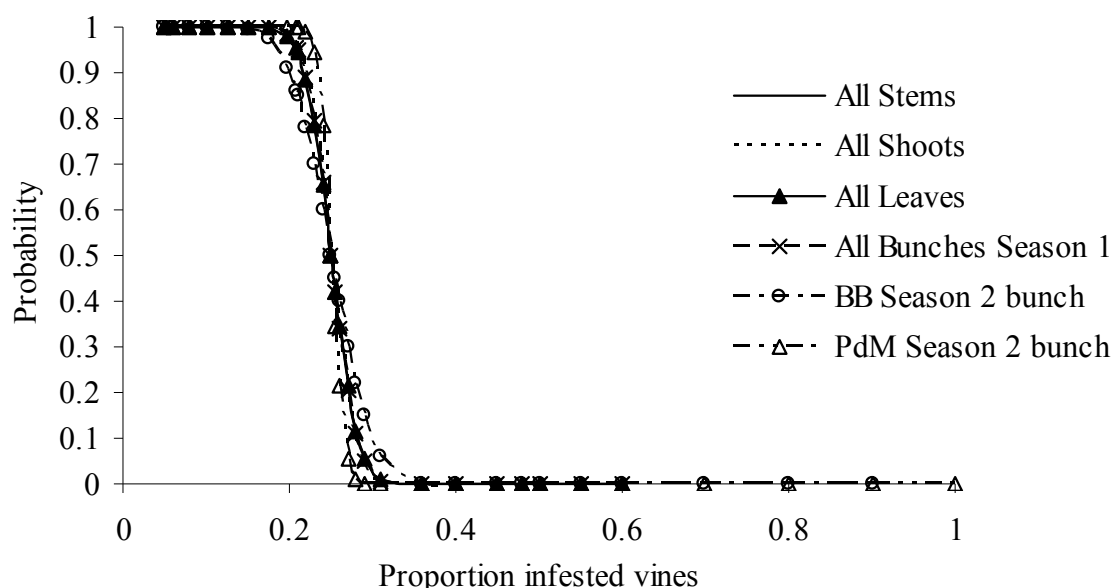


Figure 9: Operating Characteristic (OC) curve for sampling ants on stems, shoots, leaves and bunches and Backsberg and Plaisir de merle (BB season 2 bunch and PdM season 2 bunch) 2006-2007 season bunches using an Economic Threshold (ET) of 25% infestation per block.

From figure 8, when 20 plots were inspected, the sampling error was between 0.21 and 0.22 (21 and 22%. With regular monitoring (every two weeks in this study) a 0.22 error made in the short term will not cost the producers much because ants are an indirect pest. Because no significant differences were found between farms on ant infestations on stems, shoots and leaves (Table 1), this threshold is not specific to any particular honeydew seeking ant and therefore can be applied to all species.

Since all the OC curves were steep and coincided, the decision whether or not to intervene against ants can be made using any of the vine sections. The decision to intervene using vine sections other than the stem is not recommended as parasitoids mainly attack VMB on exposed areas like shoots, leaves and bunches (Malakar-Kuenen 2001). It would make sense to use chemical stem treatments for ant control as such directed sprays would least affect parasitoids. Furthermore, ants were active on stems throughout the season, while ant infestations on shoots, leaves and bunches appeared to mirror mealybug infestations but were more erratic. Deciding to take chemical action against ants on these exposed areas (shoots, leaves, bunches) would i) destroy natural enemies and ii) result in chemical residues on the crop causing marketing problems. Stem infestation is the most reliable as it provides information before damage is done and when chemical action can be applied with least disruption to parasitoids.

CONCLUSION

With the presence-absence cluster sampling system, monitoring for ants in vineyards can be done every second week during the growing season. There is a 95% chance of correctly initiating ant control measures when 20% of the stems are infested with ants. Monitoring for ants on more than 20 plots/ha of 5 vines each is not only time consuming but needless because the sampling error does not significantly fall beyond these sampling units. Control measures taken can include the use of registered synthetic pesticides, applied as direct stem treatments, and physical stem barriers. Routine monitoring of honeydew foraging ants will therefore reduce unnecessary expenditure on pesticides, benefit the environment and improve on biological control.

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05 September 2007.

CHAPTER 5

IMPACT OF ANTS (HYMENOPTERA: FORMICIDAE) ON VINE MEALYBUG PARASITOIDS *ANAGYRUS* SP. NEAR *PSEUDOCOCCI* (GIRAULT) AND *COCCIDOXENOIDES PERMINUTUS* (TIMBERLAKE) (HYMENOPTERA: ENCYRTIDAE) UNDER LABORATORY CONDITIONS

ABSTRACT

Anagyrus species near *pseudococci* and *C. perminutus* are potential parasitoids that can be used for the biological control of the vine mealybug, *Planococcus ficus* (Signoret), a key pest in vineyards. Three ant species, *Anoplolepis steingroeveri* (Forel), *Crematogaster peringueyi* Emery and *Linepithema humile* (Mayr) forage for honeydew from the vine mealybug in vineyards and promote the latter's infestations to unacceptable levels. These ant species exhibit various degrees of aggression towards vine mealybug parasitoids and hence their impact on biological control should be quantified. Ants and parasitoids were allowed to forage on vine mealybug infested butternuts. The number of ants and parasitoids was recorded for a one minute period at ten minute intervals for two hours after which parasitoids were allowed to forage for 24 hours. Parasitoid mortality and vine mealybug parasitism by the two parasitoids was then recorded in the presence and absence of the three mealybug-tending ant species. Data were analysed using a repeated measures generalised linear model (GEEs) approach in SAS. The mean number of ants on the mealybug-infested butternuts differed significantly between ant species, time intervals and parasitoid species ($p < 0.0001$ in all cases). The mean number of parasitoids foraging on the mealybug infested butternut differed significantly over the two hour period between ant species, parasitoid species and time ($p < 0.0001$ in all cases). *C. peringueyi* and *L. humile* caused significantly higher mortality of both parasitoids ($p < 0.001$) than *A. steingroeveri* during the 24-hour exposure period. *C. perminutus* significantly parasitised more vine mealybugs than *A. sp. near pseudococci* for all treatments ($p < 0.0001$). Ant control is essential for the release of parasitoids for optimum biological control of the vine mealybug in South Africa. This study has highlighted potential differences in efficacy between the two parasitoids, which should be borne in mind during potential field releases of these parasitoids.

Key words: aggressive, ant species, biological control, parasitism, parasitoid, mortality,

INTRODUCTION

Parasitic wasps are often confronted with ants that associate with hemipteran hosts. Ants often tend honeydew-excreting insects such as aphids, coccids, lycaenid butterfly larvae, pseudococcids, jumping lice and membracids, which they protect against parasitoids, predators and even competitors (Jiggins et al. 1993; Hölldobler & Wilson 1990; Pierce & Mead 1981; Adenuga 1975; Bradley 1973; Bartlett 1961; Steyn 1954; Buckley 1943). A number of studies have documented that ant attendance reduces the parasitism of honey-dew excreting Hemiptera though attacks and disturbances against ovipositing female parasitoids (Bartlett 1961; Martinez-Ferrer et al. 2003; Itioka & Inoue 1996; Stechmann et al. 1996). Ant foraging in vine canopies reduces natural enemy activity and promotes vine mealybug, *Planococcus ficus* (Signoret), infestations and therefore, biological control of *P. ficus* is compromised by ants (Whitehead 1957; Myburgh 1986). Removal of honeydew from their surroundings is also of sanitary benefit for the mealybugs (Buckley 1987).

The Argentine ant, *Linepithema humile* (Mayr) was found to be disruptive to the black scale, *Saissetia oleae* Olivier, parasitoid *Coccophagus scutellaris* (Dalman) in California (Horton 1918). In South Africa *Metaphycus helvolus* (Compere), a parasitoid of black scale which is effective in the absence of *L. humile* was disturbed (Flanders 1943; Compere 1940). The cocktail ant, *Crematogaster peringueyi* Emery, is also disruptive to natural enemies of soft brown scale, *Coccus hesperidum* L., and vine mealybugs. This ant species provides maximum protection to its hemipteran hosts by constructing carton shelters over the mealybugs (Kriegler & Whitehead 1962). The common pugnacious ant, *Anoplolepis custodiens* (Smith), incidentally disturbed the parasitoids of California red scale, *Aonidiella aurantii* (Maskell), while tending soft brown scale in citrus orchards in South Africa (Samways & Tate 1984; Steyn 1954).

Buckley & Gullan (1991) concluded that the incidence of coccid parasitisation was correlated with the relative inoffensiveness of the attendant ant species in a field study in Australia. They measured low parasitism rates (<10%) of coccids in the presence of *Oecophylla* and *Solenopsis* species and >15% in the presence of the more aggressive *Tapinoma* and *Iridomyrmex* species. In California, *L. humile* reduced parasitism and host mutilation of the California red scale by the parasitoids *Comperiella bifasciata* (Howard) (59.1%) and *Aphytis melinus* De Bach (79.5%), in a laboratory trial, even if

there were no honeydew excreting soft scale (Martinez-Ferrer et al. 2003). Itioka and Inoue (1996) in a comparative field investigation found a 94% decrease of the mealybug *Pseudococcus citriculus* Green by natural enemies in the absence of the attendant ant *Lasius niger* (L.) It is clear that the magnitude of ant protection differs depending on the parasitoid and ant species involved. Some parasitoids have developed escape strategies from ants to improve their efficacy while others are so ant sensitive that after an encounter with ants, they are deterred not only by ants but by any moving object including other parasitoids or the host itself, thereby greatly reducing their potential as biological control agents (Martinez-Ferrer et al. 2003).

Much attention has been given to investigating the impacts of ants on biological control agents, especially parasitic wasps and coccinellid predators, on citrus pests, while limited work has been done in vineyards. *Coccidoxenoides perminutus* (Timberlake) is a primary parasitoid of mealybugs that has been used to control citrus mealybug *Planococcus citri* (Risso), vine mealybug and other related species (Ceballo et al. 1998; Bartlett 1977; Zinna 1961). In South Africa, *C. perminutus* is commercially available and mass releases have been carried out in vineyards resulting in significant decreases of mealybug infestations, provided good ant control is achieved (Walton & Pringle 2005). Other important parasitoids, like *Anagyrus pseudococci* (Girault) and *Leptomastix dactylopii* Howard, occur naturally in vineyards and keep mealybug populations under control where ant infestations are low (Whitehead 1957). In this investigation, another form of *A. pseudococci*, here called *Anagyrus* sp. near *pseudococci* (Girault) (Triapitsyn et al. 2007), was sampled from the field and mass reared.

This investigation used the presence and absence of these three ant species to quantify the relative effectiveness of two vine mealybug parasitoids, *A. sp.* near *pseudococci* and *C. perminutus*, under laboratory conditions. There is great potential for these parasitoids in controlling the vine mealybug and hence the need to incorporate them into a long term integrated management strategy against vine mealybug.

MATERIAL AND METHODS

Insect colonies

Vine mealybug colonies

Colonies of vine mealybugs were maintained on butternuts *Cucurbita moschata*, in the laboratory at $27\pm1^{\circ}\text{C}$ with a 12:12 (L: D) hour photoperiod and $65\pm5\%$ RH. Butternuts

were washed in 5% bleach solution to prevent fungal growth. The butternuts were infested with mealybug crawlers. After the first molt, mealybugs were thinned to approximately 100 individuals that were allowed to develop up to a desired stage before use in experiments. For *A. sp. near pseudococci*, 3rd instar to preovipositing female vine mealybugs were used and for *C. perminutus*, 2nd instar mealybugs were used (Islam & Copland 1997; Joyce et al. 2001).

Ant colonies

Ants (workers and queens) were collected from commercial vineyards. *A. steingroeveri* and *C. peringueyi* were collected from Ashton (-33.85°S, 20.08°E, 186m) in the Breede River Valley (BRV) while *L. humile* were collected from Simondium (-33.83°S, 18.83°E, 175.2 m). The three ant species were maintained in plastic containers (18cm x 18cm x 16cm) in the laboratory containing soil or material from the original nests. Each ant nest was connected to a clear Perspex container (25cm x 25cm x 20cm) with clear plastic tubing (20cm long and 6mm in diameter). A mealybug infested butternut was placed into each Perspex container and ants were allowed to forage freely, mimicking the field situation, on this butternut for honeydew until 48 hours prior to the experiment. All ant colonies were kept at 27±0.5°C, 65±5% RH and a 12:12 (L: D) photoperiod.

Parasitoid colonies

1. Anagyrus sp. near pseudococci

To establish a colony of *A. sp. near pseudococci*, field collected vine mealybugs were incubated individually in gelatin capsules at room temperature. Mealybugs were checked daily for any emerging parasitoids which were identified under a stereo microscope. Only *A. sp. near pseudococci* species was selected. The parasitoids were placed in a cage (66cm x 66cm x 37cm) containing butternuts infested with vine mealybug. Parasitoids were offered a 50% honey: water solution and kept at 27°C, 65±5% RH with a 12:12 (L: D) photoperiod. After 7 days, parasitised mealybugs were moved into another cage for parasitoid emergence. Emerged parasitoids were supplied with an unlimited number of mealybugs. Newly emerged individuals were allowed to feed and mate before they were used. Only mated 2 day old females were used in the experiment. An unlimited number of males were given access to newly emerged females for 24 hours (Tingle & Copland 1988; 1989). Testing was done when sufficient newly emerged parasitoids were available and each individual was used only once. *A.*

sp. near *pseudococci* from the field were regularly added to laboratory colonies to prevent inbreeding of the laboratory colony.

2. *Coccidoxenoides perminutus*

Coccidoxenoides perminutus were obtained from DuRoi Integrated Pest Management (Letsitele, South Africa) as mature pupae. Newly emerged individuals were allowed to feed before use in the experiments. Field collected *C. perminutus* were not used as rearing both parasitoids would have been difficult due to logistic constraints.

Quantitative Observations

Ants foraged on a butternut infested with 100 vine mealybugs in each of the six experimental cages of 21.5cm x 21.5cm x 16cm (three cages, one for each ant species per parasitoid species). An ant free cage was included as a control for each parasitoid species. The ants were allowed to forage for three hours before 20 two-day old fertilised *A. sp.* near *pseudococci* females were introduced. Similarly, 20 one-day old *C. perminutus* were used. Observations were made 10 minutes after the release of parasitoids for each treatment whereby the number of ants and parasitoids on the butternut was recorded during a one minute period at 10 minute intervals for two hours. Parasitoids were then left in the experimental cages for 24 hours after which they were removed and the number of surviving, dead and/or missing parasitoids, if any, recorded. Mortality of the parasitoids was defined as the number of dead + missing parasitoids/total number of parasitoids and expressed as a percentage. All mealybugs were removed and incubated individually in gelatin capsules at 27°C, 65±5% RH with a 12:12 (L: D) photoperiod, for two weeks after which they were examined for parasitism under a stereo microscope. Percentage parasitism of the vine mealybug was defined as the number of parasitised mealybugs/ total number of mealybugs.

The tests were performed on five different dates for each parasitoid species with five replicates per ant colony and their controls (ant free treatment).

Data analysis

Data were analysed using Generalised Estimating Equations (GEEs) (Liang & Zeger 1986) in GENMOD procedure of SAS (SAS Enterprise Guide 3, 2004) using Poisson distribution and an identity link function. Abbott's correction formula (Abbott 1925) was used to correct for control mortality.

RESULTS

Ant behaviour in the presence of parasitoids

The mean number of ants on the mealybug-infested butternuts differed significantly ($p < 0.0001$) between ant species ($\chi^2 = 17520.4$; $df = 2$, time intervals ($\chi^2 = 9.671E7$; $df = 11$) and parasitoid species ($\chi^2 = 200.58$; $df = 1$). *C. peringueyi*, recruited in larger numbers than *L. humile* or *A. steingroeveri* (Figure 1). The number of ants (across species) on the mealybug infested butternut was significantly higher in the presence of *A. sp. nr. pseudococci* than in the presence of *C. perminutus*.

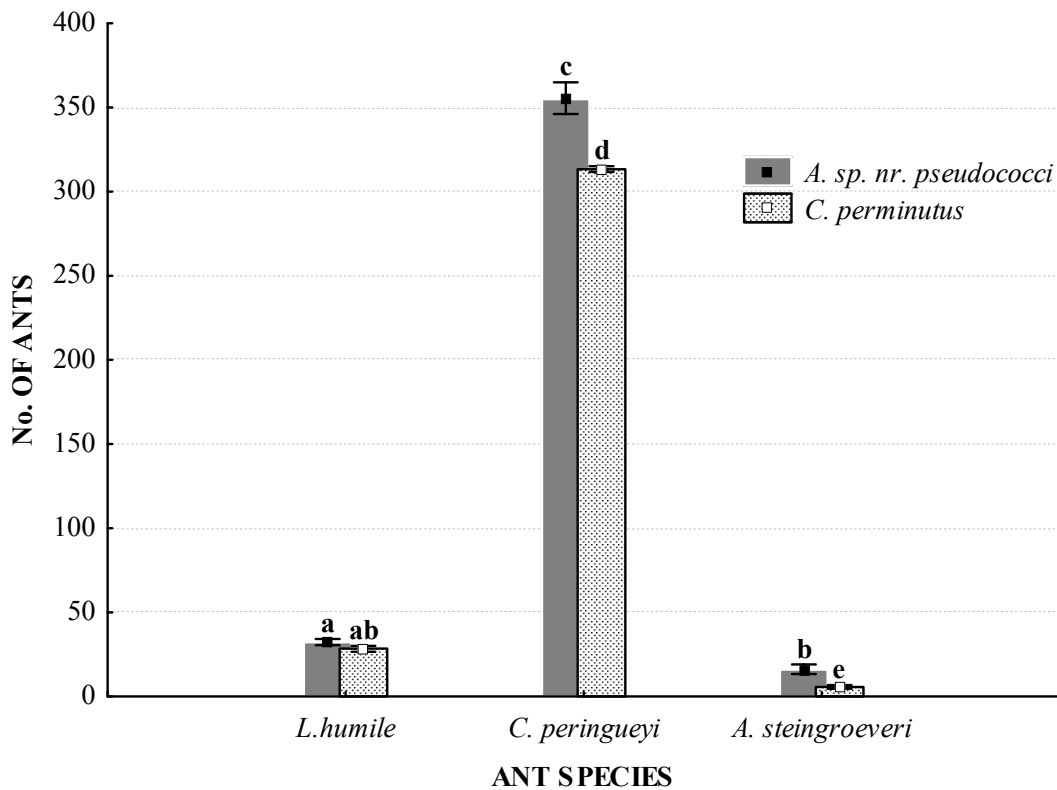


Figure 1: The mean number of ants, *Linepithema humile*, *Crematogaster peringueyi* and *Anoplolepis steingroeveri* on a mealybug-infested butternut for different parasitoid species during a one minute observation period over two hours. Vertical bars denote 0.95 confidence intervals.

Parasitoid behaviour in the absence and presence of ants

The mean number of parasitoids foraging on the mealybug infested butternut differed significantly over the two hour period ($p < 0.0001$), between ant species ($\chi^2 = 7900.14$; $df = 3$), parasitoid species ($\chi^2 = 69.22$; $df = 1$) and time ($\chi^2 = 8886663$; $df = 11$). The interactions; time*parasitoid species*ant species ($\chi^2 = 3.883E7$; $df = 66$), parasitoid species*ant species ($\chi^2 = 322.3$; $df = 3$) and time*parasitoid ($\chi^2 = 322.3$; $df = 3$); were also

highly significant ($p < 0.0001$). *C. perminutus* searched for mealybugs in significantly higher numbers than *A. sp. near pseudococci* (Figure 2).

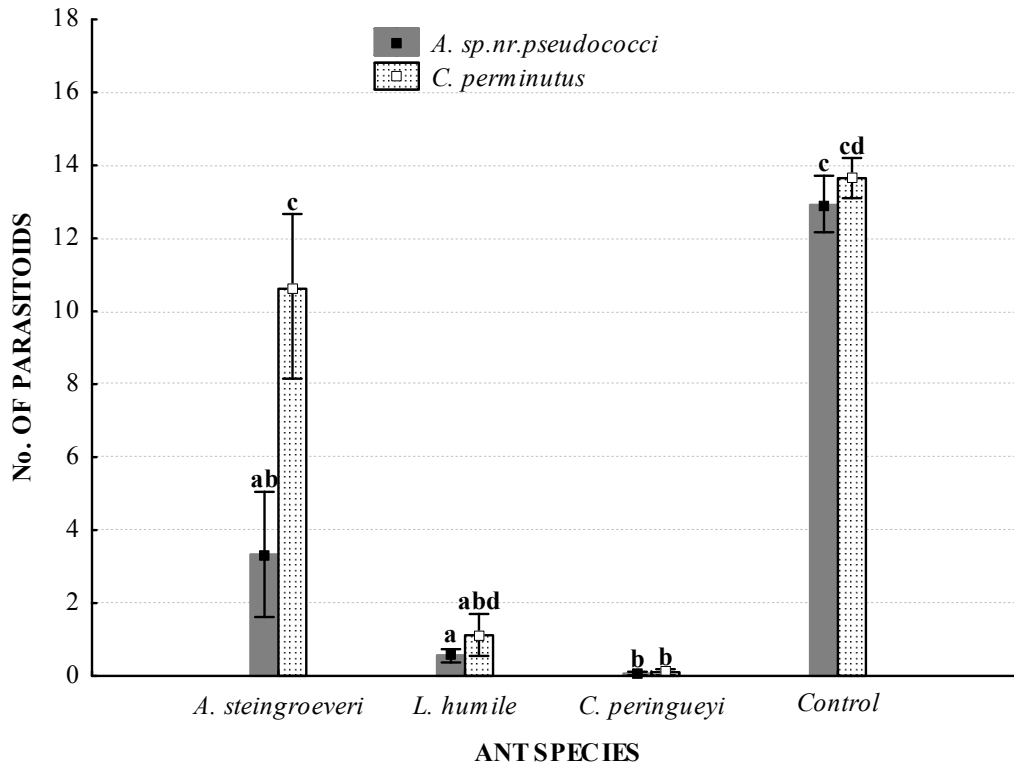


Figure 2: The mean number of *Anagyrus* sp. near *pseudococci* and *Coccidoxenoides perminutus* on the mealybug-infested butternuts for each treatment during one minute observation periods over 2 hours. Means followed by different letters differ significantly for each parasitoid species ($p \leq 0.05$). Vertical bars denote 0.95 confidence intervals.

Effects of ants on parasitoid mortality and mealybug parasitism

Parasitoid mortality was significantly different between parasitoid species ($\chi^2 = 13.47$; $df = 1$; $p < 0.001$) and ant species ($\chi^2 = 2168.53$; $df = 3$; $p < 0.0001$) (Figure 3). *A. steingroeverii* caused the least parasitoid mortality during the 24-hour exposure period. Percentage parasitism differed significantly ($p < 0.0001$) between parasitoid species ($\chi^2 = 38.18$; $df = 1$) and ant species ($\chi^2 = 10351.8$; $df = 3$). *C. perminutus* caused significantly more parasitism than *A. sp. near pseudococci* in the presence of all ant species tested (Figure 4).

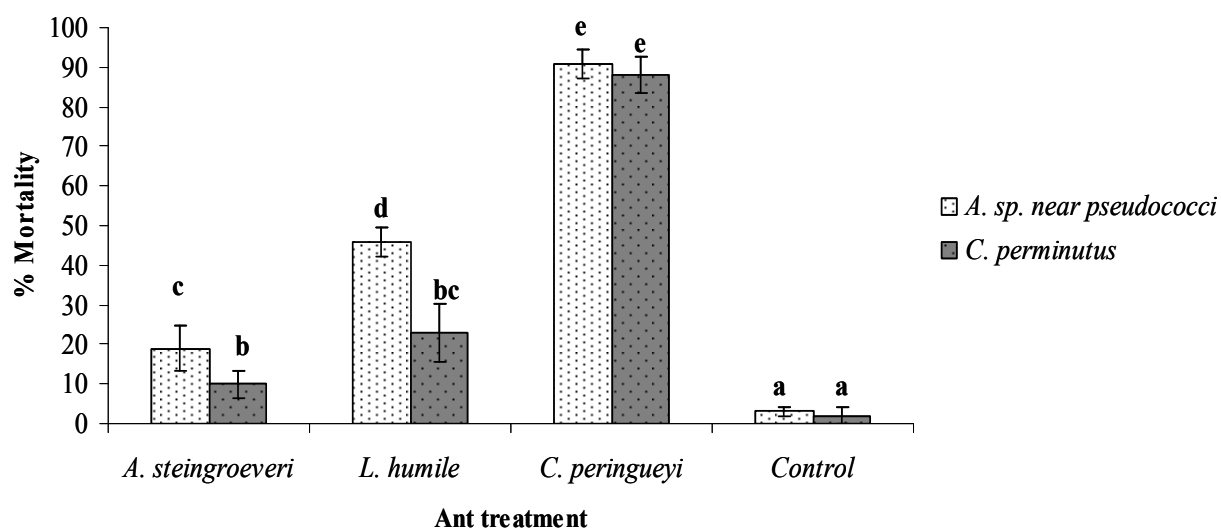


Figure 3: The mean (\pm standard error) percentage mortality of *Anagyrus* sp. near *pseudococci* and *Coccidoxenoides perminutus* after a 24-hour exposure to different ant treatments.

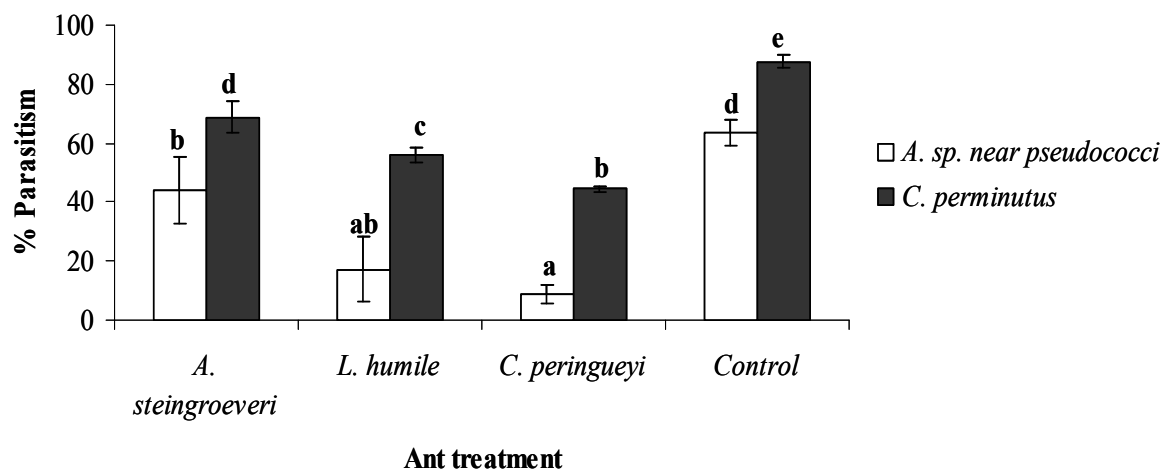


Figure 4: Relative ant aggression (% parasitism \pm standard error) towards *Anagyrus* sp. near *pseudococci* and *Coccidoxenoides perminutus* after a 24 hour exposure period to various ant species.

Qualitative observations

1. Parasitoids

A. sp. near *pseudococci* often got entangled with the mealybug host during oviposition and struggled to pull the ovipositor out during which time they were seized by ants. They also experienced host resistance where the mealybugs flipped up their caudal ends to drive away the wasps which sometimes abandoned the host. Some times *A. sp.* near *pseudococci* pressed body and antennae on mealybug honeydew and remained motionless such that some ants would walk over them. Only when they were confronted would they run away, jump off or fly away. Unlike *A. sp.* near *pseudococci*, *C. perminutus* did not have problems with completing the oviposition process due to host resistance where the vine mealybugs flipped up their caudal filaments to scare away the parasitoids.

2. Ants in the presence of parasitoids

All three ant species were observed ‘guarding’ vine mealybugs and occasionally attacking, killing and carrying dead parasitoids to their nests. The ants also deliberately chased parasitoids and disturbed any activities leading to oviposition. *L. humile* seized the parasitoids frequently but would mostly drive the parasitoids away rather than kill them which was the case on only a few occasions. No ant species differentiated between parasitised and non-parasitised *P. ficus*.

DISCUSSION

Attendance by ants greatly reduced the number of parasitoids on *P. ficus* colonies compared with where ants were excluded. Parasitism occurred less frequently in *A. steingroeveri*-attended *P. ficus* probably because these ants are less aggressive. Martinez-Ferrer et al. (2003) noted that larger ants do not easily recognize small natural enemies. *A. steingroeveri* are mainly predatory, epigaeic ants which tend to attack other insects within their foraging territory, promoting mealybug infestations due to incidental protection from natural enemies (Way 1963; Henschel 1998). However, they pose less of a threat to biological control than other ant species appears to be limited by conditions with high humidity (e.g. heavily irrigated vineyards, cover cropping) (Addison & Samways 2000). In this study their impact on biological control was not as marked as parasitism in their presence was not significantly different from the control.

The presence of *C. peringueyi* negatively affected *P. ficus* parasitism rates. Almost complete protection by this ant species against *Anagyrus* sp. near *pseudococci* and *C. perminutus* is likely to be responsible for the substantially reduced parasitism rates observed here. *C. peringueyi* remained on the butternut in large numbers creating a biological barrier that covered the butternut. A similar situation is created on vine stems in vineyards (personal observation). A large proportion of the mealybugs were therefore immune to parasitoid attack, resulting in low parasitism rates. *C. peringueyi* caused the highest parasitoid mortality. Schatz and Hossaert-McKey (2003) described arboreal ants like *Crematogaster* spp. as predacious on other insects in plant canopies. This allows such ant species to obtain protein to complement their carbohydrate-rich diet (Varon et al. 2007). Although very aggressive, *C. peringueyi* are not widely distributed in Western Cape vineyards (Addison & Samways 2000), but are confined to old neglected vineyards where they utilise old or diseased canes for nest building, presumably providing better protection to mealybugs than would epigeic ant species. Their impact is significant when their infestations are high but at low infestations, this species often confines itself to nests, with small colonies of mealybug and hence parasitoid activity in the vine foliage may not be interfered with (Kriegler & Whitehead 1962).

The presence of *L. humile* is mostly associated with injurious hemipteran infestations in agroecosystems (Buckley 1987; Flanders 1943). They were very aggressive towards parasitoids causing a significant reduction in parasitoid efficiency even if their numbers were comparably lower than those of *C. peringueyi*, possibly due to their rapid movement causing frequent incidental disturbance. Given their invasive nature and wide distribution (Luruli 2007; Carpintero & Reyes-López 2008) *L. humile* present a serious threat to the biological control of hemipteran pests like the vine mealybug, as they are capable of affecting parasitoids over large areas. In vineyards, they make numerous nests in the soil; they also make temporary nests on vine stems, leaves and bunches, making them a serious threat to foraging parasitoids. Their presence in any community should elicit awareness for methods of manipulating their behaviour to the advantage of beneficial insects, for example, chemical control with low toxic baits which allows nest mate sharing of poison during trophallaxis.

The behaviour of a parasitoid in the presence of ants largely determines its own effectiveness as a biological control agent. *A. sp.near pseudococci* and *C. perminutus* evoked different responses in the ants (Nixon 1951). In this study, *A. sp.near pseudococci* often got entangled with the host while ovipositing and were seized more

than *C. perminutus* by ants, while struggling to retract their ovipositor. Parasitoids often abandoned oviposition and kept away from mealybugs to avoid ants, limiting the number of eggs that could be oviposited into the host. While some parasitoids have developed escape strategies from ants to improve their efficacy, others are so ant sensitive that after an encounter with ants, they are deterred not only by ants, but by any moving object including other parasitoids or the host itself, thereby greatly reducing their potential as biological control agents (Martinez-Ferrer et al. 2003). It is apparent that ants not only interfere with percentage parasitism of their adopted Hemiptera, but also reduce parasitoid abundance by causing direct mortality and low reproductive success. Daane et al. (2007) noticed an almost complete absence of parasitoids in vineyards infested with *P. maritimus* attended by *L. humile*. Overall, the temporal ant*parasitoid interactions had a significant effect on the parasitism rate of *P. ficus*. Attendance by ants provided considerable protection for *P. ficus* by providing an enemy-free space over time against *A. sp. near pseudococci* and *C. perminutus*.

Results from this investigation are important to growers who should be aware of the species of pest ants foraging in their vineyards. Because the responses of parasitoids used in this investigation differed between ant species, this affects the choice of biological control agent.

CONCLUSION

Coccidoxenoides perminutus were more tolerant towards ants than *A. sp. near pseudococci* as indicated by the generally higher parasitism rates achieved whilst in the presence of ants. *C. peringueyi* and *L. humile* impacted the most on parasitism while *A. steingroeveri* impacted the least. In vineyards, exclusion of ants, particularly *L. humile*, can reduce vine mealybug populations, which could be partly due to an increase in parasitism as parasitoids get undeterred access to mealybugs. South African environmental conditions are conducive for both *A. sp. near pseudococci* and *C. perminutus*. Early season mass release of *C. perminutus* is done to augment natural populations that target the first generation of mealybugs in the growing season. Due to their sensitivity to ants, augmentative releases of *A. sp. near pseudococci* in ant infested vineyards may not be as effective. Producers can engage in vineyard management practices that improve and conserve natural populations of this parasitoid species. Ant control should be considered when parasitoids are to be used as biocontrol agents of

hemipteran pests as ant presence will not only affect parasitoid abundance but also reproductive success and possibly oviposition strategy of female parasitoids.

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CHAPTER 6

EFFECT OF CONTACT PESTICIDES ON THE VINE MEALYBUG PARASITIDS, *ANAGYRUS* SP. NEAR *PSEUDOCOCCI* (GIRAULT) AND *COCCIDOXENOIDES PERMINUTUS* (TIMBERLAKE) (HYMENOPTERA: ENCYRTIDAE)

ABSTRACT

Natural enemies are often destroyed by pesticides intended for major pests. The effect of many pesticides on natural enemies has not been assessed in vineyards. Pesticide bioassays were carried out in the laboratory on the mealybug parasitoids *Anagyrus* sp. near *pseudococci* (Girault) and *Coccidoxenoides perminutus* (Timberlake) with α -cypermethrin, buprofezin, fipronil, mancozeb and an insecticidal soap. In one bioassay, parasitoids were exposed continuously to pesticide residues over 24 hours and dose response data were analysed with Probit analysis. In a second bioassay, field rate pesticides were applied topically to parasitised mealybugs (mummies). Mortality and longevity of adult parasitoids that emerged from mummies were assessed and data analysed using ANOVA. Fipronil and α -cypermethrin caused significant acute toxicity of both parasitoids. Low mortality was recorded for all these pesticides for parasitoids emerging from mummies. Therefore, the mummy case was an effective barrier to pesticides for parasitoids. Buprofezin, mancozeb and an insecticidal soap were not toxic to parasitoids in both bioassays.

Keywords: bioassay, dose, pesticide, longevity, mortality, parasitised mealybugs, parasitoids, survival.

INTRODUCTION

Use of pesticides in integrated pest management (IPM) depends in part, on knowledge of the effects of pesticides on beneficial insects like natural enemies and pollinators. The knowledge allows the use of strategies that minimise the disruptive effect of pesticides, such as use of selective compounds and reduced rates or proper timing of applications (Hassan et al. 1994; Williams & Price 2004). Direct impacts of pesticides due to direct contact with toxins are manifested as short term mortality or relatively

long term sublethal effects which generally have the greatest impact on natural enemies' life span, fecundity and ability to locate hosts (Desneux et al. 2007).

Anagyrus sp. near *pseudococci* (Girault) and *Coccidoxenoides perminutus* (Timberlake) (Hymenoptera: Encyrtidae) are tiny solitary koinobiont endoparasitoids of the vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) (Islam & Copland 1997). These parasitoids have potential for use in augmentative release programs for suppression of vine mealybug in Western Cape Province vineyards (Whitehead 1957; Walton & Pringle 1999). Effective use of *A.* sp. near *pseudococci* and *C. perminutus* in the augmentative release programs will depend on timing parasitoid releases so that the disruptive effects of pesticides are minimised. Walton and Pringle (1999) noted a negative effect on parasitoid performance of mass released *C. perminutus* in Western Cape Province vineyards due to injudicious application of pesticides during release periods. Because releases of parasitoids are made after pesticide applications, an understanding of the direct effects of pesticide residues on these two parasitoids is critical for the development of appropriate guidelines for timing of releases.

Work has been done on impacts of field weathered pesticides residues on *Aphelinus mali* Haldeman, a parasitoid of woolly apple aphid, *Eriosoma lanigerum* (Hausmann) in apple orchards (Heunis & Pringle 2003) and in citrus orchards on *C. perminutus* (formerly *Pauridia peregrine* Timb) (St L. Searle 1963; Hattingh & Tate 1995). There is limited information on impacts of pesticides on parasitoids in vineyards yet some, like chlorpyrifos, fipronil, α -cypermethrin, among others, are used against vine mealybugs and ants.

Several pesticides were found compatible with natural enemies in apple and citrus orchards in South Africa (Heunis & Pringle 2003; Wakgari & Giliomee 2001). Very little is known about direct effects of pesticides used in vineyards in South Africa on *A.* sp. near *pseudococci* and *C. perminutus*. A better understanding of these impacts could lead to development of strategies that reduce the disruptive effects of the pesticides in commercial vineyards.

There is a growing concern on health and environmental problems caused by heavy reliance on pesticides used against ants and mealybugs. Pesticides are used based on their efficacy and/or cost rather than their potential impacts. The presence of pesticide residues in fruit and wine results in rejection incidences on the international markets

such as the USA and Taiwan (Urquhart 1999; Page 2001) and buyer prerequisites in the UK and Western Europe outlets such as Sainsbury, Tesco, Asda and Marks and Spencer (Page 2001) imposing strict limits on pesticide residues. In South Africa, IPW scheme (<http://www.ipw.co.za>) has set down standard guidelines on the application and timing of pesticides to reduce the health and environmental risks associated with pesticide residues. Table 1 summarises the toxicological properties of some of the pesticides used in vineyards against ants and mealybugs.

This investigation focused on evaluating the effects of direct contact with pesticide residues on leaf tissue since parasitoids mostly come in contact with leaves during their search for mealybug hosts, feeding, mating and resting (Longley & Jepson 1997; Stapel et al. 2000). Topical application of pesticides on mummies containing parasitoid pupae determined the ingestion of pesticide residues upon adult exit from the mummy.

The objective of these bioassays was to assess impact of pesticide residues on acute mortality of *A. sp. near pseudococci* and *C. perminutus*. This would lead to the refinement of timing of parasitoid releases to reduce pesticide-induced mortality of parasitoids in vineyards.

Table 1: Toxicological characteristics of some pesticides used in vineyards and orchards against ants and mealybugs.

Pesticide	Type/Application *	Mode of action	Comment(s)
Buprofezin	Insect growth regulator Contact/ stomach poison Foliar application	Effective against nymph stages of whitefly, scale and mealybug by inhibiting chitin biosynthesis, i.e. kills insect upon molting. Suppresses oviposition of adults and reducing egg viability (Izawa et al. 1985)	Compatible with IPM programmes utilising parasitic wasps, lacewings, mites, spiders and predators except veridalia beetles.
Imidachloprid	Chloro-nicotinyl Systemic Soil application as a drench	Affects the nervous system by blocking the post synaptic acetyl cholinesterase receptors (Stenersen 2004, Buckingham et al. 1997).	Affects beneficials that feed on nectar.
Fipronil	Phenyl pyrazole- chemicals with herbicidal effect. Contact and stomach poison and moderately systemic Foliar application	Disrupts insect central nervous system via the gamma-amino butyric acid (GABA) regulated chloride channel, i.e. binds to the GABA receptor (Stenersen 2004; Jepson 1989)	Affects some beneficials Incompatible with many IPM programs
α -cypermethrin	Synthetic Pyrethrin (pyrethroid) Contact and stomach poison. Racemic mixture of two of the four <i>cis</i> - isomers comprising cypermethrin Foliar application	Highly active broad-spectrum insecticide Affects the nervous system by blocking the sodium pump during nerve transmission (Stenersen 2004)	Not compatible with many IPM programs
Mancozeb	Ethylene bisdithio carbamate (EBDC) protectant fungicide Foliar application as dust or wettable powder.	Enzyme inactivation (Stenersen 2004; Jepson 1989, Krieger et al. 2001).	Compatible with IPM programs
Borax and citrus oil	Pesticide, fungicide, miticide, biorational contact pesticide Foliar application	Biorational contact pesticide with broad spectrum control of foliar pests and diseases Immediate knockdown effect. Kills on contact by physically disrupting the target organisms' lipid membrane rendering the organism susceptible to desiccation by the environment. Effective on various stages of pest (eggs, nymphs, larvae and adults) (Krieger et al. 2001).	Compatible with many IPM programs Can be mixed with pyrethroids as a wetting agent or as a tank adjuvant

* Anonymous 2007.

MATERIAL AND METHODS

Continuous exposure on residues

Parasitoids were exposed to pesticide residues on treated glass plates for 24 hours over a range of doses and replicated five times. Exposure chambers consisted of two pesticide treated glass plates (10cm x 10cm) fitted to a Munger cell (10cm x 10cm x 2cm internal measurements) with six holes (0.8cm diameter) through the side of the walls for ventilation. The holes were covered with fine gauze using a non-toxic adhesive (Universal Silicon, Global sealants South Africa). One hole was left uncovered for introduction of parasitoids. After the introduction of parasitoids the hole was plugged with cotton wool soaked in 50% honey-water solution, a food source for the parasitoids. For each of five replicates, six Munger cells were assembled as described by Hassan (1992) and Hassan et al. (1994) consisting of 5 dose rates and a blank, consisting of distilled water as a control treatment. After trial runs (range finders), α -cypermethrin was tested from 1/32 times to 1/2 times the recommended field dose for both parasitoids, buprofezin 1/4 to 4 times (*C. perminutus*) and 8 to 128 times (*A. sp. near pseudococci*), fipronil 1/8 to double (*C. perminutus*) and 1/4 to 4 times (*A. sp. near pseudococci*) and mancozeb and the insecticidal soap, 8 to 128 times for both parasitoids.

The glass plates were thoroughly cleaned with a detergent, rinsed with distilled water and then air dried. A stock solution of the highest dose was prepared for each pesticide (depending on the range established). Serial dilutions with distilled water were then performed to give doses representing lower doses for each parasitoid species- pesticide combination as shown in Table 2.

The aqueous solutions/suspensions of pesticides were applied onto the glass plates using a standard laboratory Potter's Spray Tower (Burkhard Manufacturing Co., Ltd., Hertfordshire, UK) (Potter 1952) with 2ml of each dose rate at a pressure of approximately 50kPa (7.25lb in⁻²) delivering approximately 0.02ml liquid cm⁻² for each glass slide.

The spray tower was thoroughly cleaned and flushed with acetone and distilled water between treatments. Pesticides were applied in order of increasing dose rate. Each time fresh solutions/suspensions were made, i.e. chemical solutions were not stored.

After application, the glass plates were air dried for 10-15 minutes. The Munger cells were then assembled with treated glass surfaces facing inwards. Twenty parasitoids were carefully released into each cell through the uncovered hole using a special aspirator. One day old *C. perminutus* and one to two-day old *A. sp. near pseudococci*

were used. The munger cells were connected to a manifold which split the air stream to each of the six cells. To minimise pesticide vapour in the cells, the whole system was ventilated with humidified air ($70\pm 5\%$ RH) using a small aquarium pump connected to the main rubber tube. The complete system was maintained in an environment chamber at $25\pm 0.5^{\circ}\text{C}$ with a 12:12 (L: D) photoperiod.

Parasitoids were checked 6, 12, 18 and 24 hours after introduction. They were regarded as dead when they did not move (after 10 seconds) upon disturbance. A magnifying lens (Optivisor- Donegan Optical Co. USA) was used to examine the parasitoids. Dose-mortality data were adjusted for control mortality using Abbott's formula (Abbott 1925) and Probit analyses performed with POLO-PC programme (LeOra Software 1987) to obtain dose-response statistics (Robertson et al. 2007; Finney 1971).

Table 2: Pesticides tested on *Anagyrus* sp. near *pseudococci* and *Coccidoxenoides perminutus* adults with formulations, target pests and range of doses tested.

Pesticide (active ingredient (a. i.))	Formulation		Field rate	Target pest	Dose rates tested (ml/L)
	Trade name*	Grams pure a.i.			
Buprofezin	Applaud SC	400g/L	60ml/100L (0.6ml/L)	<i>Planococcus ficus</i> (Vine mealybug)	0.15; 0.3; 0.6; 1.2; 2.4; 4.8; 9.6; 19.2; 38.4; 76.8
α -cypermethrin	Fastac SC	100g/L	250ml/100L (2.5ml/L)	Formicidae (Ants)	0.0781; 0.1563; 0.3125; 0.625; 1.25
Fipronil	Regent SC	200g/L	10ml/100L (0.1ml/L)	Formicidae (Ants)	0.0125; 0.025; 0.05; 0.1; 0.2; 0.4
Mancozeb	Dithane M45 WP	800g/Kg (80%)	200g/100L (2g/L)	<i>Plasmopara viticola</i> (Downy mildew)	16; 32; 64; 128; 256
Insecticidal soap (borax and orange oil)	Wet-Cit EC	Borax 10g/kg Orange oil 50g/Kg	50ml/100L (0.5ml/L)	<i>Planococcus ficus</i> (Vine mealybug)	4; 8; 16; 32; 64

*SC=soluble concentrate; WP = Wettable power; EC = Emulsifiable concentrate.

Topical bioassays of field rate pesticides on parasitoid pupae

A. sp. near pseudococci and *C. perminutus* mummies were exposed to pesticide residues to measure their susceptibility to pesticides and also to investigate the role of the mummy case as a barrier to pesticides. Vine mealybugs of appropriate developmental stages were exposed to *C. perminutus* adults and fertilised females of *A. sp. near pseudococci*. After mummification, 20 mummies with each type of parasitoid were placed on a sticky tape. The tapes were placed on glass plates which were sprayed with the recommended field dose rate for the pesticides using a standard Potter's spray tower (protocol described above). The sticky tapes were air dried for one hour then sprinkled with fine soil to prevent emerging parasitoids from coming in contact with the pesticide residues and from getting stuck on the adhesive. The tape was placed in ventilated Petri dishes (9.6cm diameter) and kept under controlled conditions ($70\pm 5\%$ RH, $25\pm 0.5^{\circ}\text{C}$ with a 12:12 (L: D) photoperiod) in an environment chamber. This experiment was replicated five times for each pesticide and parasitoid species.

Parasitoid emergence was checked daily between 14:00 and 15:00 hours. Emerged parasitoids were placed in ventilated vials supplied with 50% honey-water solution. Longevity was assessed over one week (*C. perminutus*) and three weeks (*A. sp. near pseudococci*). Abbott's correction formula was used to adjust for control mortality. Repeated measures ANOVA followed by Tukey's HSD test was performed in the computer program STATISTICA v.7 (Stat-Soft, South Africa) on parasitoid emergence data.

RESULTS

Continuous exposure on pesticide residues

All slopes for the chemicals were positive indicating an increase in mortality with an increase in dose rate (Tables 3 and 4). Population responses to pesticides for *A. sp. near pseudococci* and *C. perminutus* were significantly different since none of the 95% fiducial limits overlapped for the two parasitoid species (Tables 3 and 4) (Robertson et al. 2007).

For *A. sp. near pseudococci*, the LD_{50} value for fipronil was 1.5 times larger than the field dose rate. However fipronil is one of the most persistent pesticides making it toxic to parasitoids over a long period of time (Stenersen 2004). α -cypermethrin LD_{50} was 10 times lower than the field dose rate for the same parasitoid.

For *C. perminutus*, the field dose rates were higher than the LD₅₀ values for α -cypermethrin and fipronil by 13 times and 5 times, respectively. These results indicate that these two pesticides were the most toxic of those tested on *A. sp. near pseudococci* and *C. perminutus*. Figure 1 shows the probit regression curves of α -cypermethrin and Fipronil for the two mealybug parasitoids. The hypotheses of equality ($\chi^2_{df=2}=17.4813$ and $\chi^2_{df=2}=365.7$; $p \leq 0$, respectively) and parallelism ($\chi^2_{df=1}=9.3027$; $p=0.002$ and $\chi^2_{df=1}=10.4753$; $p=0.001$, respectively) of probit regression lines for α -cypermethrin and Fipronil were rejected.

Buprofezin, mancozeb and the insecticidal soap were not toxic to parasitoids within their recommended field rates although at high doses *C. perminutus* was more affected by these pesticides compared to *A. sp. near pseudococci* (Figure 2). For buprofezin, mancozeb and the insecticidal soap, the hypothesis that probit regression lines were equal was rejected ($\chi^2_{df=2}=123.6$, 340.28 and 196.28, respectively; $p \leq 0$ in all cases) while that of parallelism was accepted ($\chi^2_{df=1}=0.021$; $p=0.963$, $\chi^2_{df=1}=0.2965$; $p=0.586$ and $\chi^2_{df=1}=3.0392$; $p=0.081$, respectively).

Probit analysis could not establish the 95% fiducial limits from the insecticidal soap and mancozeb for *A. sp. near pseudococci* because parasitoid mortality remained low throughout the 24-hour bioassay period even after raising the dose rate to extreme values and therefore no probit mortality was estimated for this parasitoid.

Table 3: Probit parameters of dose responses of *Anagyrus sp. near pseudococci* to various doses of different pesticide residues during a 24-hour bioassay.

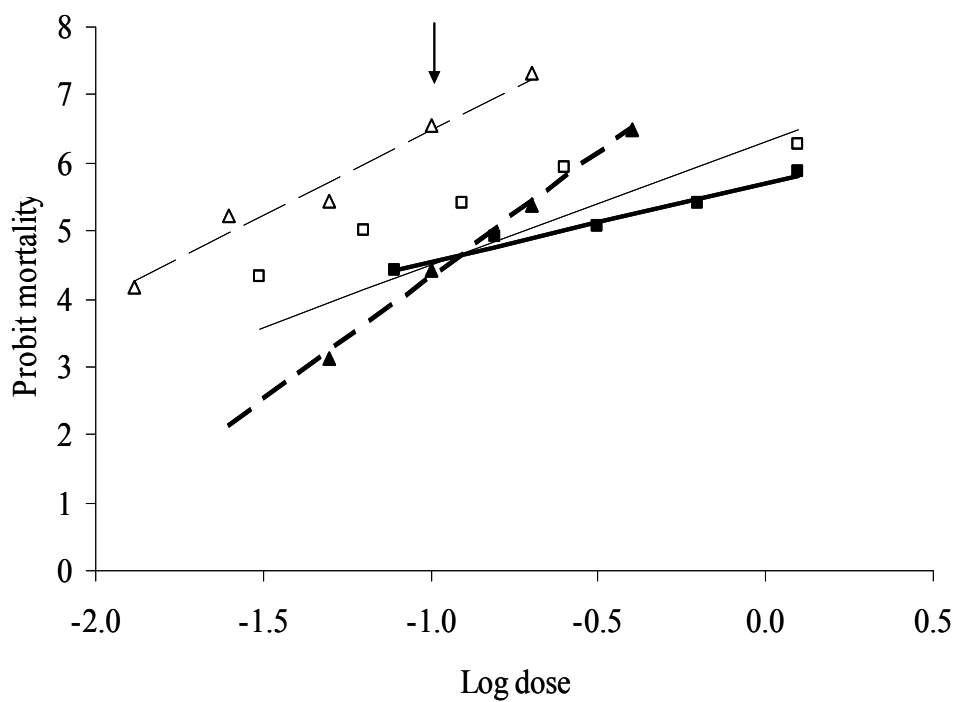
Pesticide	Field dose rate (ml/L)	LD ₅₀ (ml/L)	95% fiducial limits	LD ₉₀ (ml/L)	95% fiducial limits
α -cypermethrin	2.5	0.248	0.187 to 0.317	3.279	1.948 to 7.574
Fipronil	0.1	0.154	0.138 to 0.169	0.344	0.298 to 0.413
Buprofezin	0.6	31.816	19.2932 to 54.164	125.7482	68.1348 to 752.822
Mancozeb	2g	4287.15	-	31091	-
Insecticidal soap	0.5	103.1936	-	196.9378	-

Table 4: Probit parameters of dose responses of *Coccidoxenoides perminutus* to various doses of different pesticides residues during a 24-hour bioassay.

Pesticide	Field dose rate (ml/L)	LD ₅₀ (ml/L)	95% fiducial limits	LD ₉₀ (ml/L)	95% fiducial limits
α -cypermethrin	2.5	0.190	0.154 to 0.227	0.956	0.744 to 1.345
Fipronil	0.1	0.26	0.014 to 0.039	0.083	0.052 to 0.254
Buprofezin	0.6	2.5857	2.0839 to 3.7783	10.1096	5.9151 to 32.921
Mancozeb	2g	86.784	66.1119 to 116.4609	1217.1545	646.8795 to 3492.2308
Insecticidal soap	0.5	29.5714	19.5015 to 44.9682	106.8319	63.2940 to 410.5229

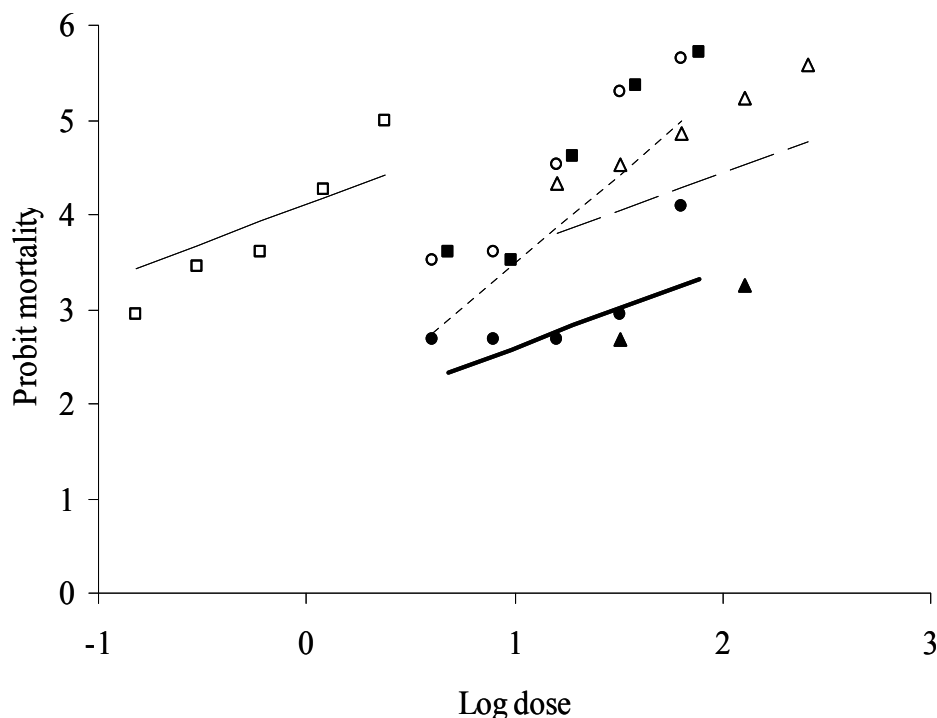
Table 5: Comparison of regression lines for pesticides tested against mealybug parasitoids, *Anagyrus* species near *pseudococci* and *Coccidoxenoides perminutus*

Pesticide	Common intercept (\pm std.err.)	Common slope (\pm std.err.)
α -cypermethrin	5.9611 (0.7343)	1.4312 (0.1094)
Fipronil	6.9564 (0.1334)	1.6234 (0.1077)
Buprofezin	3.7944 (0.746)	0.8225 (0.6502)
Mancozeb	2.7136 (0.2385)	0.8 (0.1214)
Insecticidal soap	1.5606 (0.2884)	1.8614 (0.1877)



A. sp. near pseudococci: — α -cypermethrin; - - - Fipronil
C. perminutus: — α -cypermethrin; Fipronil; - - -

Figure 1: Dose response of *Anagyrus* sp. near *pseudococci* and *Coccidoxenoides perminutus* adults exposed continuously to various doses of fipronil and α -cypermethrin residues, showing probit mortality, in a 24 hour bioassay. α -cypermethrin doses were below the recommended field rate. The arrow shows fipronil field dose rate (0.1ml/L).



A. sp. nr. pseudococci: — Buprofezin
C. perminutus: — Buprofezin; — — Mancozeb; Insecticidal soap — —

Figure 2: Dose responses of *Anagyrus* sp. near *pseudococci* and *Coccidoxenoides perminutus* adults exposed continuously to various doses of buprofezin, mancozeb and insecticidal soap residues, showing probit mortality, in a 24 hour bioassay. All doses were higher than the recommended field rate.

Topical application of field rate pesticides on parasitoid pupae

Days to parasitoid emergence after treatment with pesticides significantly differed between treatments ($F_{(5,24)} = 24.48$; $p \leq 0.001$) with buprofezin causing a significant delay in emergence by almost a week relative to other treatments (Table 6). Days to emergence did not differ significantly between species ($F_{(1,58)} = 1.02$; $p = 0.3167$).

Significantly, more *C. perminutus* emerged than *A. sp. near pseudococci* ($F_{(10,46)} = 6.514$; $p \leq 0.001$). No significant differences were found between treatments for *C. perminutus* ($F_{(5,24)} = 0.6842$; $p = 0.6399$). *A. sp. near pseudococci* mortality due to fipronil and α -cypermethrin was significantly higher than the other treatments ($F_{(5,24)} = 19.604$; $p \leq 0.05$).

Table 6: The mean number of days to emergence and number of emerged parasitoids after topical pesticide treatments of 10 day old mummies (n=20).

Pesticide Treatment	<i>A. sp. near pseudococci</i>		<i>C. perminutus</i>	
	Days to emerge [†]	Emerged	Days to emerge [†]	Emerged
Water	7.1 ^a	14.4 (0.20)	5.2 ^a	16.5 (0.23)
α -cypermethrin	5.5 ^a	12.5 (0.25)	6.0 ^a	15.1 (0.93)
Buprofezin	12.7 ^b	14.1 (0.20)	11.8 ^a	15.7 (0.51)
Fipronil	7.5 ^a	12.5 (0.40)	5.4 ^a	14.5 (0.60)
Mancozeb	7.1 ^a	14.1 (0.4)	6.2 ^a	15.1 (0.25)
Insecticidal soap	6.3 ^a	13.6 (0.33)	7.0 ^a	14.9 (0.44)

[†]Means in columns with different letters denote significant difference at 95% confidence limits.

±SE in parenthesis

DISCUSSION AND CONCLUSION

Mortality rates due to insecticide residues on glass plates in cells provide an indication of impact of pesticide residues on parasitoids. However, the field situation with pesticide residues on vine foliage is most likely lower. Longley & Jepson (1997) indicated a difference in bioavailability due to pesticide residues becoming bound with the epicuticular layers on leaf surfaces, amongst other factors. The toxicity calculations obtained in this investigation pertain to glass plates as substrates and may therefore differ from results obtained using natural substrates such as leaves. Additionally, insects in the field can shelter in places where pesticide residues may not reach them, for example, parasitised vine mealybug can hide under the bark or crevices subsequently protecting the developing parasitoids. Results may also vary due to insect generation, sex, species and size of parasitoids.

Fipronil is used to control ants in vineyards. Control of ants in mealybug infested vineyards allows *A. sp. near pseudococci* access to mealybug that they would not

otherwise access in the presence of ants. Chapter 5 demonstrated that this parasitoid is significantly impacted by the main ant species present in Western Cape vineyards.

α -cypermethrin and fipronil caused high mortality of the parasitoids, therefore, may not be compatible with IPM programs utilising parasitoids for vine mealybug control, unless these pesticides are used in containerised low toxic baits, or applied to an area of the vine not utilised by parasitoids, such as the stem. Walton & Pringle (1999) also found cypermethrin to be very toxic to *C. perminutus* and discouraged full cover application of this pesticide during augmentative release periods.

Observations showed that parasitoids died as they gnawed an exit hole with their mandibles through the dorsal portions of the mummies treated with fipronil and α -cypermethrin. The resulting partial emergence indicated the high degree of toxicity of these two pesticides. Chewing an exit hole presented a risk for parasitoids ingesting the pesticides, which are stomach poisons. Mortality of parasitoids at the time of emergence has been documented for adults of aphid parasitoids (Lingren et al. 1972; Hsieh & Allen 1986; Krespi et al. 1991; Longley & Jepson 1997; Heunis & Pringle 2003). Low mortality of *C. perminutus* when exposed to fipronil and α -cypermethrin could imply a different mechanism of exiting the mummy case other than chewing a hole, but rather pushing to crack open the mummy case. Mortality rates were low across treatments indicating that the mummy case is indeed, an efficient barrier to pesticides. From this investigation, the adult stage of parasitoid was more vulnerable to pesticides than the juvenile stages developing in the mummies.

Timing of insecticide application is very crucial given the continued conventional high volume spraying in commercial vineyards. The use of economic injury levels (EIL) and economic thresholds (ET) for pests pays no regard to the role of natural enemies, therefore some adaptations to population dynamics of important parasitoid species is required. Pesticide treatments can be restricted to periods of low activity of the vulnerable stages of parasitoids (adults), for example, early spring treatments. The limited persistence of active ingredients such as α -cypermethrin may be exploited to achieve selectivity (Elzen 1989). If only the insensitive stages of parasitoids within mummified mealybugs are exposed to treatment, the more sensitive adults maybe protected (Metcalf 1980). Stem application of pesticides in hot spots later in the season minimises risk to parasitoids which by this time will have a large prey population to achieve maximum parasitism rates, and provides areas where parasitoids can shelter.

Parasitoid longevity is crucial for the efficacy of parasitoids as biological control agents (Desneux et al. 2007). A longer life span implies greater chances of searching and successfully attacking a host. Although buprofezin and the insecticidal soap showed little negative impact on parasitoids in the laboratory, in vineyards, these two pesticides can reduce populations of parasitoids indirectly by reducing populations of VMB (Grafton-Cardwell et al. 2006). In South Africa, the use of buprofezin has been warned against especially when utilising coccinellid predators as main natural enemies for mealybugs and cottony cushion scale (Hattingh & Tate 1995). Exposure of mealybug mummies containing parasitoid pupae to buprofezin showed a delayed emergence of adults. This may interfere with the phenological synchrony between mealybugs and their parasitoids resulting in reduced ability of the parasitoids to regulate mealybug populations.

α -cypermethrin and fipronil were very toxic vineyard pesticides to mealybug parasitoids while buprofezin, mancozeb and insecticidal soap did not cause any significant mortality at the recommended field rates. *A. sp. near pseudococci* adults were more robust and resilient to pesticides than *C. perminutus*, possibly due their larger size. Although the insecticidal soap and buprofezin caused no significant parasitoid mortality, they can impact on parasitoids indirectly by reducing the host (mealybugs) population. Timing of pesticide application is very important regarding the vulnerable stages of parasitoids. *C. perminutus* are released as pupae while *A. sp. near pseudococci* are released as adults. This affects the choice of parasitoid and timing of augmentative release regarding breakdown of pesticides on plant surfaces.

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CHAPTER 7

BIOASSAYS TO DETERMINE THE SUBLETHAL EFFECTS OF A SYSTEMIC INSECTICIDE, IMIDACHLOPRID, ON *ANAGYRUS* SP. NEAR *PSEUDOCOCCI* (GIRAULT) AND *COCCIDOXENOIDES PERMINUTUS* (TIMBERLAKE) (HYMENOPTERA: ENCYRTIDAE)

ABSTRACT

The susceptibilities of two vine mealybug endoparasitoids, larval *Anagyrus* sp. near *pseudococci* (Girault) and *Coccidoxenoides perminutus* (Timberlake), to different doses of imidachloprid were investigated in the laboratory. Vine mealybugs were allowed to feed on potted vines treated with different doses of imidachloprid and the parasitoids were allowed to attack the mealybugs after two days of feeding. After a further two days of feeding, mealybugs were individually incubated in vials until parasitoids emerged. The numbers of emerged and unemerged individuals of each parasitoid species were determined. The emerged parasitoids were fed and allowed to mate before parasitising mealybugs which did not come into contact with imidachloprid. The emerging F_1 generation was fed with 50% honey-water solution for one week (*C. perminutus*) and three weeks (*A. sp. near pseudococci*) while their longevity was assessed. Dose responses for the parasitoids were analysed with Probit analysis. Repeated measures ANOVA followed by Tukey's HSD test was used for emergence data and Kaplan-Meier (product-limit) survival analysis for the longevity of the F_1 generation. No significant differences were found in the dose responses of the two parasitoid species. Longevity of F_1 generation was significantly different between the control and treatments for *A. sp. near pseudococci* but not *C. perminutus*.

Keywords: *Anagyrus* sp. near *pseudococci*, bioassay, *Coccidoxenoides perminutus*, mortality, imidachloprid, survival.

INTRODUCTION

Imidachloprid is a systemic insecticide that has soil, foliar and seed uses for the control of sucking pests such as aphids, thrips, whiteflies and mealybugs (Widiarta et al. 2001; Ahmed et al. 2001; Pringle 1998). In vineyards, it is applied as a soil drench against vine mealybugs at budburst to pea berry size and then 21-45 days after first application,

if a split application is required (<http://www.bayercropscience.co.za/products>). Imidachloprid belongs to the chloronicotinyl class of insecticides that act on the nervous system causing a blockage of the post synaptic acetyl cholinesterase receptors (Mukherjee & Gopal 2000; Buckingham et al. 1997). Soil applied imidachloprid is taken up by roots and is translocated acropetally within the xylem and degraded quickly in plants (Sur & Stork 2003). Most imidachloprid is transported through the xylem because there is no active loading of the active substance. However, due to its high dissociation constant ($pK_a = 14$), its distribution between the phloem and xylem is not affected as it is non-ionised (Bromilow & Chamberlain 1989). Once in leaves, imidachloprid is trapped there (with higher content in older leaves) and cannot be re-transported back to stems and roots. Vine mealybugs feeding on leaves ingest the residues, however root feeding mealybugs may not (depending on when they feed), as imidachloprid cannot be transported back to the roots.

Unlike the non-systemic insecticides discussed in Chapter 5, many systemic insecticides as well as their metabolites are regarded as 'safe' for natural enemies and other beneficial insects like bees since direct contact between insecticides and insects requires the latter to feed on plant extra floral nectar, mortality may occur depending on the insecticide's persistence (Stapel et al. 2000; Ozawa et al. 1998).

Most bioassays documented have mainly looked at the acute toxicity due to parasitoids getting into contact with pesticide residues or sublethal effects resulting in altered host searching or foraging ability, fecundity and male: female ratio (Desneux et al. 2007). Juvenile stages of parasitoids are subjected to systemic insecticides when they develop in hosts that have fed on treated plants. Such hosts will have fed off plants with weathered pesticide that is not sufficient to cause death in the host or have developed resistance to the systemic pesticide (Stapel et al. 2000; Desneux et al. 2007).

The management of vine mealybugs with imidachloprid is accepted as one of the most efficient and safe methods because being systemic, this insecticide targets all stages of the vine mealybug including those which are cryptic, except for the eggs and males. However, there is risk of insecticide resistance which is a cause for concern to growers. The use of parasitoids can accommodate this short coming. It is therefore important to identify the risks imidachloprid presents to vine mealybug parasitoids foraging in treated vineyards.

This investigation aimed to establish the detrimental effects on the development of *A. sp. near pseudococci* and *C. perminutus* feeding on imidachloprid-contaminated vine mealybugs as indicated by the subsequent emergence and survival of the F_1 generation.

MATERIAL AND METHODS

Using Confidor 350SC (BayerCropScience, Paarl, South Africa), a stock solution of the highest dose (12ml imidachloprid/1000ml water, \equiv 4 times field recommended rate) was prepared and then serial dilutions made with distilled water to give double, field, $\frac{1}{2}$ and $\frac{1}{4}$ recommended rates.

Vines were pre-watered at least one hour before application of insecticide to ensure adequate wetting. Just before bud break, 166ml imidachloprid was applied as a soil drench around the base of each of 5 potted vines for all application rates. A blank treatment with no imidachloprid (water control) was included as a sixth treatment and the experiment replicated five times. The pesticide was allowed to be translocated for 48 hours and then 150ml clean water applied to each vine to wash the imidachloprid into the soil. Thereafter vines were irrigated with the same amount of water every 3 days until 21 days after treatment. Vines were infested with 100 1st and 2nd instar mealybugs (for *C. perminutus*) and 100 3rd instar to pre-ovipositing female mealybugs (for *A. sp. near pseudococci*). The vines were covered in clear muslin cloth and mealybugs allowed to feed for 2 days. Parasitoids were then released onto the vines to attack mealybugs for 24 hours after which they were removed. Mealybugs were allowed to feed on the vines for a further 2 days after which they were kept in vials at $26 \pm 0.5^\circ\text{C}$, $65 \pm 5\%\text{RH}$ and a 12:12(L:D) photoperiod. They were inspected daily between 12:00 and 15:00 hours for any emerged parasitoids. When no more parasitoids emerged, the percentage of emerged parasitoids was calculated.

The emerged parasitoids were allowed to reproduce and their offspring (F_1 generation) examined for longevity over 21 days (*A. sp. near pseudococci*) and seven days (*C. perminutus*). *A. sp. near pseudococci* females were mated while the parthenogenetic *C. perminutus* were not.

Data analysis

Bioassay data were analysed using Probit analysis (Polo-PC LeOra Software 1987) after correction for control mortality using Abbott's formula (Abbott 1925). Repeated measures ANOVA followed by Tukey's HSD test was performed to compare

differences in emergence rate (or mortality as shown by the percentage of unemerged parasitoids) of the two parasitoid species. Longevity of the F_1 generation females was analysed with the Kaplan-Meier (product limit) survival analysis in STATISTICA v.7 (StatSoft).

RESULTS

Probit regression revealed that fiducial limits for the two parasitoids overlapped (Table 1) and therefore mortality did not differ significantly between the two parasitoid species (Robertson et al. 2007). Both *A. sp. near pseudococci* and *C. perminutus* failed to emerge at high doses of imidachloprid. The probit regression line intercepts and slopes (Table 1) for both *A. sp. near pseudococci* and *C. perminutus* did not differ significantly and therefore the hypothesis of equality of regression lines was accepted ($\chi^2_{df=2}=5.778$; $p=0.055$) as well as that of parallelism ($\chi^2_{df=1}=0.189$; $p=0.664$). This implies that *A. sp. near pseudococci* and *C. perminutus* are equally susceptible to imidacloprid (Figure 1). Cumulative proportion surviving, i.e. the cumulative proportion of F_1 generation of *A. sp. near pseudococci* and *C. perminutus* surviving up to 21 and seven days, respectively, is shown in Figure 2 (*A. sp. near pseudococci*) and Figure 3 (*C. perminutus*). The survival function of *A. sp. near pseudococci* drops off sharply in the first eight days and thereafter declines much less sharply until 17 days. Survival was significantly different between the control and insecticide treatment for *A. sp. near pseudococci* ($\chi^2= 5.0563$; d.f. = 3; $p = 0.1677$), but not for *C. perminutus* ($\chi^2= 23.7975$; d.f. = 3; $p = 0.00003$). Survival could not be compared between the two parasitoid species because of natural differences in life span. *A. sp. near pseudococci* females can survive up to 40 days with weekly feedings (Rinco-Vitova Insectaries, <http://www.rinconvitova.com/parasite.htm>) while *C. perminutus* can live up to 5 days (DuRoi IPM, personal communication).

Table 1: Probit parameters of dose responses of *Anagyrus* sp. near *pseudococci* and *Coccidoxenoides perminutus* to imidachloprid.

Parasitoid	LD ₅₀ (ml/L) (95% fiducial limits)	LD ₉₀ (ml/L) (95% fiducial limits)	Intercept* (±std.err.)	Slope* (±std.err.)
<i>A. sp. near pseudococci</i>	1.1198 (0.57 to 1.67)	11.3572 (7.21 to 25.76)	4.7961 (0.8578)	1.2272 (0.1449)
<i>C. perminutus</i>	1.7608 (0.49 to 3.33)	23.1282 (8.95 to 891.5)		

*Common intercept and slope.

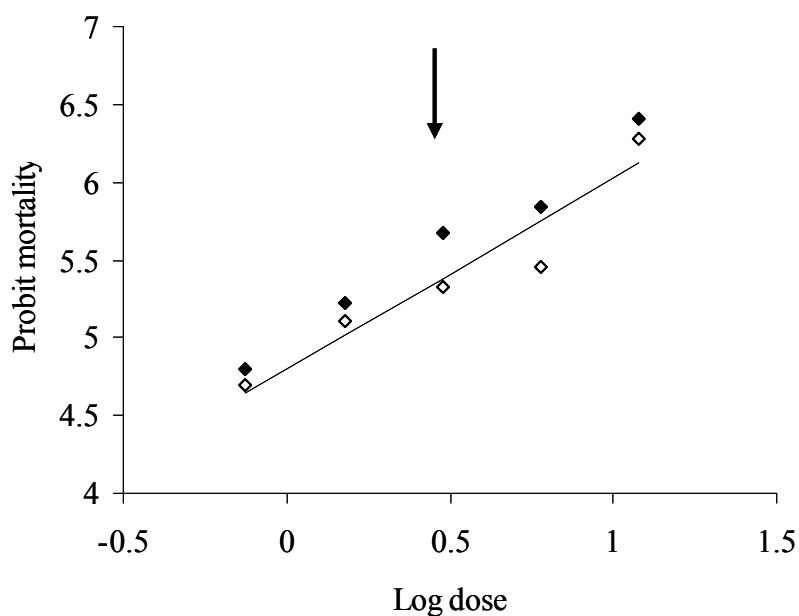


Figure 1: Probit mortality (inability to emerge) of *Anagyrus* sp. near *pseudococci* (◆) and *Coccidoxenoides perminutus* (◇) to various doses due to systemic imidacloprid using vine mealybugs feeding on treated vines. Arrow indicates field dose rate (3ml/L).

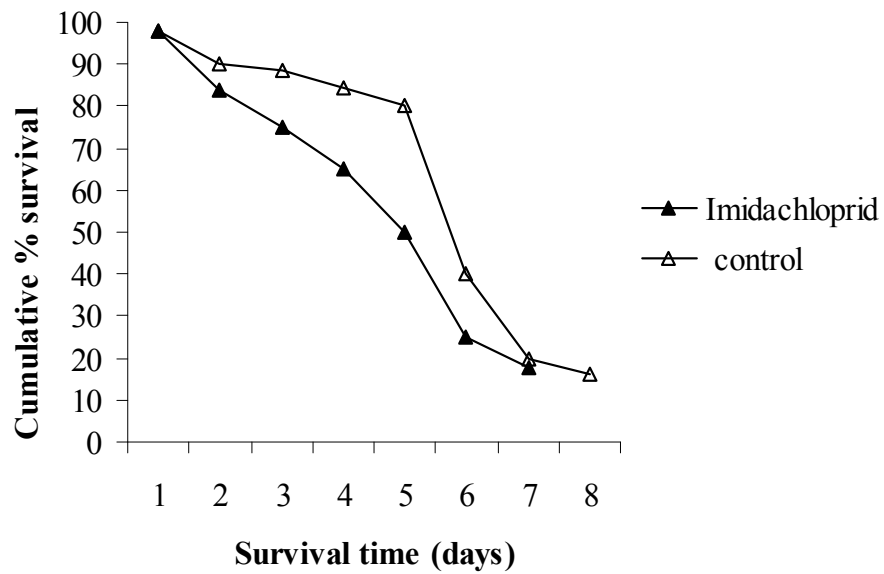


Figure 2: Survival function of *Anagyrus* sp. near *pseudococci* F_1 generation females that emerged from imidachloprid contaminated individuals.

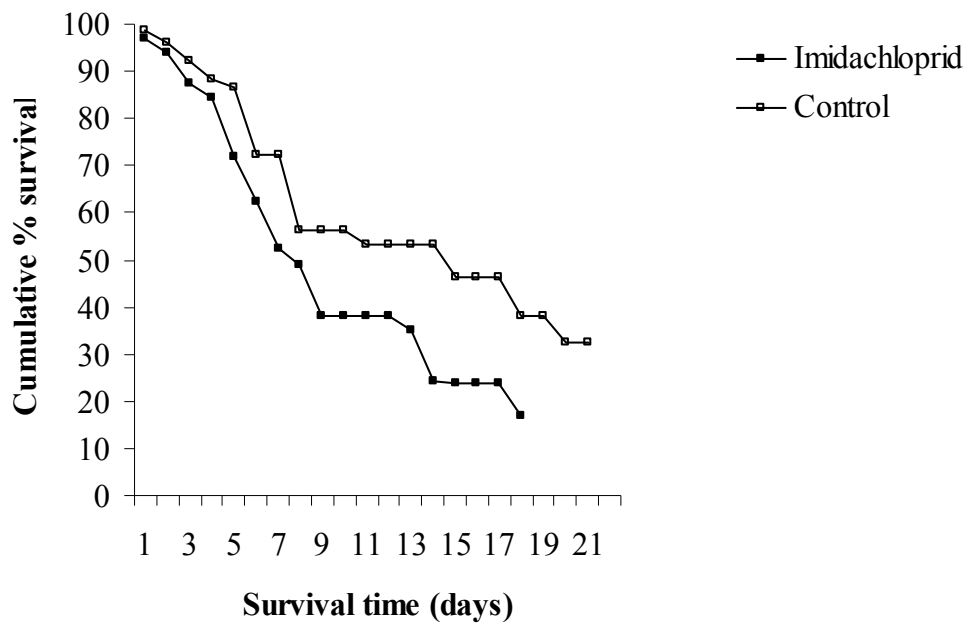


Figure 3: Survival function of *Coccidoxenoides perminutus* F_1 generation that emerged from imidachloprid contaminated individuals.

DISCUSSION

This investigation has demonstrated that when parasitoid larvae develop in imidachloprid-contaminated vine mealybugs, their development and longevity are affected depending on the amount of residue in the host. Mealybugs got contaminated by feeding on imidachloprid treated vines. The developing parasitoid larvae fed on the contaminated vine mealybugs leading to subsequent failure to emerge at higher doses. At low doses ($\frac{1}{4}$ and $\frac{1}{2}$ and full recommended field rate), some parasitoids managed to emerge but longevity of F_1 generation was significantly reduced for *C. perminutus*. Stapel et al. (2000), also reported reduced longevity of the parasitoid *Microplitis croceipes* Cresson (Hymenoptera: Braconidae) after feeding on extra floral nectar from cotton treated with soil-applied imidachloprid. Imidachloprid significantly reduced the survival of *A. sp. near pseudococci*. The efficiency of this parasitoid species was therefore limited due to the reduced life span. In the case of *C. perminutus*, survival of F_1 generation was not affected by the imidachloprid treatments. Rebek and Sadof (2003) found that systemic insecticides like imidachloprid reduce parasitism by endoparasitoids. Imidachloprid is a newly registered pesticide for use in orchards and vineyards in South Africa, and as such there may be uncertainties over the long term regarding its impacts on established IPM programmes. The longevity of the progeny of emerged parasitoids is important in suppressing potentially imidachloprid-resistant mealybug populations as it determines the likelihood of the parasitoid to attack the host before its premature death.

Soil-applied imidachloprid is persistent and can continue to kill pests for more than 30 days (Widiarta et al. 2001). Therefore, soil-applied imidachloprid is particularly detrimental to *C. perminutus* as this parasitoid species should be released early in November to prevent build-up of high mealybug populations, which it can control effectively (Walton 2003).

The release of parasitoids can be carried out approximately 45 days or more after treatment to minimise negative impacts on parasitoids. This is the length of period after which another imidachloprid treatment can be applied or when residues are less of a threat to parasitoids. This period also accommodates the local withholding period of 112 days as prescribed by the National Department of Agriculture, South Africa, regarding maximum residue limits for permissible chemicals (Anonymous 2000). Use of insecticides should be carried out only when needed to prevent detrimental effects on natural enemies. Imidachloprid application prevents clustering of mealybugs while the

use of parasitoids brings about total control late in the season (Daane et al. 2006). This approach allows synergy between the insecticide and parasitoids. Parasitoids will then act on reduced populations of mealybugs, which they would exploit more efficiently resulting in higher percentage parasitism. Use of parasitoids in mealybug integrated pest management helps reduce the incidence of insecticide resistance that often ensues due to frequent use of pesticides.

CONCLUSION

This study has indicated that *C. perminutus* and *A. sp. near pseudococci* are equally susceptible to imidachloprid systemic residues as shown by the emergence rate and/or mortality.

The progeny of imidachloprid-contaminated *A. sp. near pseudococci* and *C. perminutus* can still survive periods long enough to have an impact on mealybugs. This investigation did not, however, establish the impact of this insecticide on important physiological activities like oviposition, searching ability, host recognition, amongst others (Ruberson et al. 1998). Split applications can be substituted by one imidachloprid treatment at budburst to pea berry size, followed by release of parasitoids about 45 days after treatment.

To understand the impacts of a systemic insecticide like imidachloprid, more bioassays are required to investigate the translocation of imidachloprid in grapes and its selectivity on mealybug parasitoids (natural enemies). Since this investigation was laboratory based, it is expected that imidachloprid could be less harmful under field conditions due to less efficient insecticide applications taking place in the field.

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CHAPTER 8

GENERAL DISCUSSION

Three main ant species, *Anoplolepis steingroeveri* (Forel), *Linepithema humile* (Mayr) and *Crematogaster peringueyi* Emery were found foraging in vine mealybug (VMB), *Planococcus ficus* (Signoret), infested vineyards on the three farms in the South Western Cape Province. *C. peringueyi* occurred in both the coastal area, Simondium, and the inland Breede River Valley area while *A. steingroeveri* was found in the Breede River Valley only and *L. humile* was found in Simondium only. An economic threshold (ET) of 20% was determined for stem infestation in this study. Ant infestation was weakly correlated to VMB infestation and therefore bunch damage. Ants promoted mealybug infestations due to their aggressive behaviour towards parasitoids. The construction of carton shelters by *C. peringueyi* and *L. humile* may have some benefits to VMB, allowing small populations to survive parasitoid attack. Since these shelters appear to be constructed in response to low VMB populations, they offer some improved protection from natural enemies, although they can not completely exclude ant tolerant natural enemies, such as mealybug mimicking coccinellid larvae like *Cryptolaemus montrouzieri* (Mulsant), *Nephus* and *Scymnus* spp. that often prey on the protected mealybugs. Parasitised mealybugs were also sampled from these protective shelters in other studies (Whitehead 1957; Smit & Bishop 1934; Horton 1918).

Anagyrus sp. near *pseudococci* (Girault), *Coccidoxenoides perminutus* (Timberlake) and *Leptomastix dactylopii* Howard and a complex of hyperparasitoids were reared from mealybugs and trapped on sticky pads from the studied vineyards. Whitehead (1957) also obtained the same parasitoid species from *Planococcus citri* (Risso) (misidentified *P. ficus*) from the same province. This investigation indicated a difference in the number of species reared from mealybugs and those caught on traps implying various degrees of response to chemical cues by different parasitoid species. An aggregated pattern of parasitoids was associated with a high mealybug infestation level while a random pattern was associated with low mealybug infestation levels, implying a density dependant relationship between parasitoids and their mealybug host. This was also confirmed by Walton (2003). A spatial association between *C. peringueyi* and *L. humile* and parasitoids that were observed for some individual vineyards could

explain the negative linear relationship between ant infestation and parasitoid activity observed at low mealybug populations (Chapter 2). Because of a significant association between ants and parasitoids, ants pose a threat to biological control of mealybugs and should be controlled prior to augmentative parasitoid releases as well as prior to application of management practices to enhance and conserve parasitoids.

Although the decision to take control measures against ants can be made using any of the vine sections, reliable decisions can only be made using stem infestation. Ants infest the stems first before new growth. Therefore, sampling for ants on the stem can be used to anticipate infestation on other vine sections when ants continue foraging for honeydew as the season progresses. This temporal separation is pivotal in planning future control measures such as use of chemical and physical stem barriers and low toxic baits (Addison 2002; Rust et al. 2000).

While *C. perminutus* proved to be more ant tolerant and efficient than *A. sp. near pseudococci*, they were more vulnerable to pesticides. Walton and Pringle (1999) advised against injudicious use of pesticides during release periods of this parasitoid species. Pesticides currently used against ants (fipronil and α -cypermethrin) were the most toxic to both *C. perminutus* and *A. sp. near pseudococci*, making these incompatible with early season parasitoid releases. However, the current recommendation for application of these chemicals is as directed stem sprays only (Nel et al. 1999) which should afford some protection for parasitoids. Both parasitoids were equally susceptible to the systemic mealybug insecticide, imidachloprid. Mass releases of *C. perminutus* and *A. sp. near pseudococci* should be avoided in imidachloprid, fipronil and α -cypermethrin treated vineyards. The more resilient *A. sp. near pseudococci* has the benefit of being selectively pesticide tolerant. Krischik et al. (2007) found that soil applied imidachloprid was only poisonous to nectar feeding *A. pseudococci*. However, broad-spectrum and systemic insecticides are toxic to both parasitoids and releases should be done when there is minimum impact on the parasitoids. Currently, *C. perminutus* is the only parasitoid being commercially produced for biological control of the VMB in South Africa. However, other ways of improving biological control of VMB are necessary. *A. sp. near pseudococci* and *C. perminutus* can complement each other in a biocontrol programme. *A. sp. near pseudococci* has a longer survival period, has a preference for later VMB stages and adults (Islam & Copland 1997), and is effective in searching for mealybugs at low

populations. It is therefore best used under initial or low mealybug infestations. The mealybug stage should be carefully assessed to ensure the production of female parasitoids from later mealybug instars. *C. perminutus*, with a short survival period and preference for early mealybug instars, would be suitable for dense mealybug populations and hence effective in treating mealybug hot spots where the parasitoids do not need to search over wide areas. The choice of host stage by the two parasitoid species also necessitates their complimentary use. Vine mealybugs of all stages were available on all sampling dates due to overlapping generations. The two parasitoid species can co-exist in vineyards to establish long lasting mealybug control through subsequent establishment of their generations, reducing use of insecticides in the long run.

With the established ET of 20% ant stem infestation, and 2% VMB stem infestation, ant control would be recommended for Ashton farm while VMB and ant control would be recommended for Backsberg farm. This is in accordance with the standard monitoring system developed by Walton and Pringle (1999) and De Villiers and Pringle (2007). No ant control would be necessary for Plaisir de Merle farm since ant and VMB infestations were below their ETs. Using the standard monitoring system, producers can sample many vine pests in a single sampling session resulting in cost effective and time saving monitoring (De Villiers et al. 2006). From the results of this study, this monitoring system can now be updated to include the ET for ants.

Timing of insecticide treatments of ants on stems should coincide with the period of minimum disruption to parasitoids, which is during bud dormancy for the arboreal *C. peringueyi* and mid spring to early summer for the epigeal *L. humile* and *Anoplolepis* spp. when the ants are starting to actively forage on stems with little parasitoid activity.

FUTURE RESEARCH

Previous studies have confirmed that a pure population of *L. humile* is more aggressive than a mixed ant population towards natural enemies promoting injurious levels of mealybugs and other Hemiptera (Flanders 1943; Nixon 1951; Bartlett 1961; Buckley 1987; Hölldobler & Wilson 1990; Daane et al. 2007). Due to the presence of more than one ant species in the study sites, this study could not establish the extent of aggression towards parasitoids by a pure population of *L. humile*, *C. peringueyi* or *A. steingroeveri*, in a field situation. Further research is required to quantify this and to compare

aggression towards parasitoids by a pure population of a given ant species or a mixture of the species, in the same or similar study areas.

Currently, in South Africa, ant control is achieved through chemical and sticky stem barriers (Addison 2002). These are less disruptive to parasitoids but are labour intensive and producers are not prepared to implement them on a large scale. Besides, they only target foraging workers and therefore may not be effective in the long run, especially when the foraging workers release an alarm pheromone to cease recruiting to toxic areas or relocate to insecticide free areas. Further research is already underway, developing low toxic baits for ants. Baits are slow acting and therefore do not prevent sharing and recruiting among nest mates (Rust et al. 2000). Additionally, low toxic baits also target the whole colony, including the queen and the brood.

This study mainly focused on the extent to which ants disrupt VMB primary parasitoids. It should be noted that this is a multi-trophic level interaction involving four levels, i.e. the vine → VMB → primary parasitoids → hyperparasitoids. The impact of ants on the fourth trophic level needs further research to establish whether or not there is any benefit conferred to the third trophic level. Furthermore, we need to determine how to make the environment more suitable for the conservation of natural enemies in vineyards after augmentative releases.

CONCLUSION

From the investigations in this study, it can be concluded that:

- Frequent monitoring of pests and natural enemies provides accurate information on the species present, their location, stage of development and spatial patterns and relationships.
- The different ant species, *A. steingroeveri*, *C. peringueyi* and *L. humile* were significantly disassociated from each other while ants and parasitoids were significantly associated.
- A large sample size improves the sampling precision; however, increasing the number of sampling units beyond 20 plots per hectare block would not significantly decrease the sampling error of 22% at an ET of 25%.
- If ET = 25%, there would be a 95% chance of not under reacting if no chemical intervention was applied or initiated when 20% of the stems were infested with ants.

- *L. humile* and *C. peringueyi* were more aggressive towards parasitoids than *A. steingroeveri*.
- *C. perminutus* were more ant tolerant than *A. sp. near pseudococci*.
- Adult parasitoids are more vulnerable to pesticides than their juvenile stage.
- α - cypermethrin and fipronil were the most toxic of the pesticides of those tested on *A. sp. near pseudococci* and *C. perminutus*.
- Survival of *C. perminutus* F_1 generation was significantly reduced by imidachloprid while that of *A. sp. near pseudococci* was not significantly reduced.
- *C. perminutus* are a more efficient biocontrol agent in vineyards than *A. sp. near pseudococci*. However, the latter are more robust and resilient. Ant control is a prerequisite before parasitoids can be mass released in vineyards.

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