

DEVELOPMENT OF A HABITAT MANAGEMENT PLAN FOR
IMPROVING THE BIOLOGICAL CONTROL OF THE VINE MEALYBUG
PLANOCOCCUS FICUS (SIGNORET) IN VINEYARDS

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DECLARATION

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ABSTRACT

The vine mealybug, *Planococcus ficus* (Signoret) is a major, cosmopolitan pest in all regions where grapes are grown. Vine mealybug has a direct injurious effect on vines through feeding, produces honeydew, on which sooty mould develops and has been shown to be a vector of the grapevine leafroll virus and associated closteroviruses. This project entailed research on the parasitoids of *P. ficus*, mainly *Coccidoxenoides perminutus* (Timberlake). The aim of this work was to contribute basic biological information for the establishment of a habitat management plan in vineyards to improve biocontrol of *P. ficus*. Two surveys were conducted to determine, firstly, the occurrence of mealybug parasitoids in the vineyards and their associated natural habitats, and secondly the association between flowering plants and parasitoids close to vineyards. Olfactometer screenings were conducted to determine attractiveness of six plants as food sources for adult *C. perminutus*. A comprehensive life history experiment was initiated to be compared with previous findings.

In the first survey, to assess the biodiversity of mealybug parasitoids in vineyards and their associated natural habitats, *C. perminutus*, *Anagyrus* sp. near *pseudococci* (Girault) and *Leptomastix dactylopii* (Howard) were the predominant parasitoids found between January and May, with a peak in abundance during February. Significantly more parasitoids were found in vineyards compared to associated natural habitats ($p=0.049$). The survey further indicated that these parasitoids, being density-independent and therefore not in need of high pest populations to sustain numbers, could contribute to integrated pest management, and with effective habitat modifications, their numbers could be naturally boosted to lend a valuable eco-system service.

In the second survey, to determine whether parasitoids occur in the field in flowering plants associated with vineyards, a total of 20 individual parasitoids from 16 species were found. This is a promising indication that, although their impact on *P. ficus* was not measured during this study, the correct flowering plants interplanted in vineyards or on the edges could have a positive effect on the necessary occurrence of mealybug parasitoids as well as other natural enemies and pests in vineyards.

Attractiveness of plants for *C. perminutus* was determined through the screening of a variety of flowering plants with a four-armed Pettersson olfactometer. Of the six plants

tested, only *Euryops abrotanifolia* (L.) DC had a significant attractant effect ($p=0.003926$) on *C. perminutus*. The population of the parasitoid could possibly be increased by planting this plant in or around vineyards to provide a food source, and it is recommended that this plant be further investigated as a parasitoid attractant in the field. Furthermore, more plants need to be tested for inclusion in habitat management, as it is likely that a combination of plants will be more effective for biological control.

To determine life table parameters of *C. perminutus*, including adult fitness and larval host preferences, laboratory experiments were conducted at 25°C on *Planococcus citri* (Risso), as initial experiments utilizing *P. ficus* had failed. In contrast with previous studies where the second and third nymphal instars were parasitised, all nymphal instars were attacked in this study, with no significant difference between them ($p=0.057$). Cost of life when laying eggs or not also came to no significant difference ($p=0.46252$). Lifetable parameters ($R_0=159.5$; $T=27.602$; $r_m=0.511$) were different to those determined by Walton (2003) ($R_0=69.94$; $T=29.5$; $r_m=0.149$) except for T which was similar, although the latter study was conducted on *P. ficus*. These differences could also be attributed to the use of mummies instead of hatched parasitoids, when collecting progeny for the determination of the preferences and parameters.

Information obtained through these above mentioned experiments should be of use to rearing facilities and contribute to the establishment of a habitat management plan in vineyards to improve the control of *P. ficus*.

UITTREKSEL

Die wingerdwitluis, *Planococcus ficus* (Signoret) is 'n ernstige, wêreldwye pes in alle areas waar druiwe verbou word. Wingerd witluis het 'n direkte, skadelike effek op wingerd deur hul voeding, die produksie van heuningdou, waarop roetskimmel groei, en is ook 'n vektor van wingerd rolblaarvirus. Navorsing vir hierdie projek het gefokus op die parasitoïede van *P. ficus*, hoofsaaklik *Coccidoxenoides perminutus* (Timberlake). Die doel van hierdie studie was om basiese biologiese inligting by te dra vir die vestiging van 'n habitat bestuursplan in wingerde om die biologiese beheer van *P. ficus* te verbeter. Twee opnames is gedoen om, eerstens, die voorkoms van witluis parasitoïede in die wingerd en omliggende natuurlike habitat, en tweedens, die verbintenis tussen blomplante en parasitoïede naby wingerde te bepaal. Olfaktometer toetse is gedoen om aantreklikheid van ses inheemse plante vir *C. perminutus* te bepaal en 'n volledige ontwikkelingstudie is gedoen wat met vorige bevindinge vergelyk is.

In die eerste opname, om die biodiversiteit van witluis parasitoïede in wingerd en, meer belangrik, die nabyliggende natuurlike habitat, te evalueer, was *C. perminutus*, *Anagyrus* sp. near *pseudococci* (Girault) en *Leptomastix dactylopii* (Howard) die oorheersende parasitoïede tussen Januarie en Mei, met 'n piek in getalle in Februarie. Daar is beduidend meer parasitoïede in wingerde gevind as die natuurlike habitate ($p=0.049$). Die opname het ook aangedui dat hierdie parasitoïede, wat onafhanklik is van digtheid en dus nie hoë pes populasies nodig het om hul getalle te handhaaf nie, 'n bydrae sal kan lewer tot geïntegreerde plaagbestuur, en met die regte habitat veranderinge, sal hul getalle natuurlik vermeerder kan word sodat hulle 'n waardevolle diens aan die ekosisteem te kan lewer.

In die tweede opname, om te bepaal of parasitoïede wat in die veld voorkom 'n verbintenis met die blomme rondom wingerde het, is 'n totaal van 20 individuele parasitoïede van 16 spesies gevind. Dit is 'n belowende aanduiding dat, alhoewel hul impak op *P. ficus* nie in hierdie studie bepaal is nie, die regte blomplante tussen of om die wingerde geplant 'n positiewe effek kan hê op die nodige voorkoms van witluis parasitoïede, asook ander natuurlike vyande en pests in wingerd.

Die aantreklikheid van verskeie blomplante vir *C. perminutus* is getoets met 'n vier-arm Petterson olfaktometer. Van die ses plante wat getoets is, het slegs *Euryops abrotanifolia*

(L.) DC 'n beduidende aantrekkende effek ($p=0.003926$) op *C. perminutus* gehad. Die populasie van die parasitoïed kan moontlik vermeerder word deur hierdie plant tussen of om wingerde te plant om te dien as 'n voedselbron, en daar word voorgestel dat hierdie plant verder ondersoek word as 'n lokmiddel vir parasitoïede in die veld. Meer plante moet ook getoets word vir insluiting in 'n habitat bestuursplan aangesien 'n kombinasie van plante meer effektief sal wees vir biologiese beheer.

Om die parameters vir die lewenstabelle van *C. perminutus* te bepaal, insluitende fiksheid van volwassenes en voorkeure vir larwala stadia van gashere, is laboratorium eksperimente gedoen teen 25°C op *Planococcus citri* (Risso), aangesien aanvanklike eksperimente op *P. ficus* nie suksesvol was nie. In teenstelling met vorige eksperimente waar die tweede en derde nimfale instars geparasiteer is, is alle nimfale instars in hierdie eksperimente geparasiteer, met geen beduidende verskille ($p=0.057$) nie. Daar is ook geen beduidende verskille gekry vir lewenskoste wanneer die parasitoïed eiers lê of nie ($p=0.46252$).

Parameters vir die lewenstabelle ($R_0=159.5$; $T=27.602$; $r_m=0.511$) het verskil van dié bepaal deur Walton (2003) ($R_0=69.94$; $T=29.5$; $r_m=0.149$), behalwe vir T wat eenders was, alhoewel Walton se studie op *P. ficus* was. Hierdie verskille kan toegeskryf word aan die gebruik van mummies in plaas van parasitoïede wat reeds uitgebroei is, met die insameling van nageslagte vir die bepaling van voorkeure en parameters.

Inligting uit hierdie studie kan van nut wees vir telingsfasiliteite en kan help met die vestiging van 'n habitat bestuursplan in wingerde om biologiese beheer van *P. ficus* te verbeter.

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CHAPTER ONE:

INTRODUCTION AND LITERATURE REVIEW

1.1 PLANOCOCCUS FICUS

The vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) is a major pest in all grape-producing areas, including the Mediterranean region, South Africa, Pakistan and Argentina (Ben-Dov, 1994; Walton, 2003). The vine mealybug has been shown to be a vector of the grapevine leafroll virus and associated closteroviruses (Engelbrecht & Kasdorf, 1990). These viruses cause redness and rolling of the leaves, a decline in yield and sugar accumulation, and delayed ripening of the fruit (Engelbrecht & Kasdorf, 1990; Joyce et al., 2001; Monis & Bestwick, 1997; Rosciglione & Gugerli, 1989), which has direct implications for the production of wine as it reduces the quality of the wine. It has a direct injurious effect on vines caused by the sucking of sap which reduces the vitality of the vines. Honeydew excretion, on which sooty mould develops, and the presence of egg sacs, also has an indirect injurious effect on table grapes (Myburgh, 1951) by rendering fruit unsuitable for export.

1.1.1 Control strategies

Effective control of the vine mealybug is complicated by a lack of monitoring (Geiger & Daane, 2001), inconsistent control of mealybugs with insecticides, an absence of accurate identification and/or establishment of reliable, consistent control (Malakar-Kuenen et al., 2001; Millar et al., 2002). Monitoring can predict the development of infestations and control strategies can be implemented before economic injury levels are reached (Walton, 2001). The poor establishment of natural enemies could be due to a lack of food sources in the surrounding areas.

1.1.1.1 Monitoring

Following the months of vine dormancy vine mealybugs first infest the cordon, followed by infestation of the bunches a few months after that. Cordon infestation can serve as an early warning sign of mealybug infestation. Leaf and shoot infestation cannot be used as indicators as they do not precede bunch infestation. Monitoring procedures include

biweekly trap inspections (yellow delta trap with racemic lavandulyl senecioate, the synthetic *P. ficus* sex pheromone; Walton et al., 2004) from October onwards. When the threshold of 65 males per trap is exceeded, biweekly cordon inspections of female and immature populations in the vine canopy should commence. Two months after cordon inspections started biweekly bunch inspections should commence (De Villiers & Pringle, 2007; Walton, 2003).

1.1.1.2 Chemical control

Despite chemical control still being the most commonly used control method against vine mealybug, control with insecticides is often unsatisfactory because of the protective waxy covering produced by the insect and additionally because of their cryptic nature and distribution patterns (Berlinger, 1977; Bodenheimer, 1951; Franco et al., 2009). In the past control of mealybugs relied on delayed applications of organophosphates and carbamates during grapevine dormancy (Daane et al., 2006; Walton et al., 2004), but these chemicals were not particularly selective and were often detrimental to natural enemies or induced insecticide resistance after prolonged use (Daane et al., 2006; Franco et al., 2009; Holm, 2008; Walton & Pringle, 1999). Due to human toxicity and low selectivity, many chemical products are now found to be unacceptable (Franco et al., 2009).

1.1.1.3 Cultural control

Methods of cultural control are designed to hinder the spread of infections to adjacent or uninfested vineyards (Kriegler, 1954; Daane et al., 2003). The most common precautions are the sterilization of pruning and harvesting equipment and the use of heat-treated nursery stock. However, even though heat-treatments of nursery stock are relatively effective in eliminating viruses, it cannot be regarded as an effective cure for infested material (Haviland et al., 2005; Holm, 2008) and is not practised for mealybug control in South Africa.

The use of suitable cover crops has many benefits (Fourie, 2010). It limits dust, which is disadvantageous to natural enemies, while simultaneously providing alternative food sources and shelter for them. Cover crops – specifically triticale, rye/faba bean mixture, or a biennial rotation of triticale and vetch – used together with a chemical treatment, was found to help with the control of weeds and can increase the soil quality (Walton, 2001; Fourie, 2010).

Addison & Samways (2006), however, found that there was no lasting effect on mealybug or ant population levels when comparing the use of triticale, vetch and fescue with a control plot as cover crops in vineyards, but the numbers of parasitoids in the control plot was higher, possibly due to the larger variety of weeds available.

1.1.1.4 Biological control

Predators and parasitoids are currently the most sustainable method of control of the vine mealybug. The most prevalent enemies of the vine mealybug in South African vineyards are predators like coccinellid beetles (mostly *Nephus* spp.) and parasitoids like *Anagyrus* sp. near *pseudococci* (Girault)(Hymenoptera: Encyrtidae), *Coccidoxenoides perminutus* (Timberlake)(Hymenoptera: Encyrtidae) and *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae) (Walton & Pringle, 2004). Natural enemies are mass-reared and released early in the season to enhance naturally occurring populations to combat the mealybug populations in the vines (Holm, 2008).

Anagyrus sp. near *pseudococci* is the common parasitoid occurring in the vine mealybug's Mediterranean native range and was introduced in California in the 1940s to control the citrus mealybug (Sforza et al., 2005). The effectiveness of parasitoids, however, are hampered by various factors. During winter months vine mealybugs are protected from predators by hiding beneath the vine's bark or underground (Daane et al., 2006; De Villiers, 2006; Holm, 2008). Ants obtain important resources from mealybugs, including the sugary excreta, honeydew, on which they feed. In return ants provide vital services to the mealybugs and interfere with parasitoid activity by providing protection from predators, sanitation by removal of honeydew, and transport to new feeding sites (Mgocheki & Addison, 2009a).

Table 1. Compatibility of some pesticides used in vineyards against *Planococcus ficus* and associated insects with IPM programs (Walton & Pringle, 1999; Mgocheki & Addison, 2009b).

Pesticide Active ingredient	Chemical class/Application*	Comments
α -cypermethrin	Synthetic pyrethrin Contact/stomach poison Foliar application	Not compatible to many IPM programs due to long residual activity
Borax and citrus oil	Biorational contact pesticide Foliar application	Compatible with many IPM programs
Chlorpyrifos	Organophosphate Contact/stomach/respiratory action Soil application as a drench	Not compatible to many IPM programs
Cypermethrin	Synthetic pyrethrin Contact/stomach action Foliar application	Not compatible to many IPM programs
Endosulfan (banned since 2012)	Chlorinated hydrocarbon insecticide Foliar application	Not compatible to many IPM programs
Imidacloprid	Chloro-nicotinyl Systemic Soil application as a drench	Affects beneficials that feed on nectar

*(Anonymous, 2007)

Walton and Pringle (1999) did work to confirm the effects of selected pesticides on the survival of natural enemies in South Africa (Table 1). They found Chlorpyrifos, Endosulfan, which is now banned, and Cypermethrin, three insecticides used in vineyards, to be very toxic to *C. perminutus*. It is important to take care not to disrupt the biological control efforts by limiting the use of chemical sprays to the bare necessity (Walton & Pringle, 2004). During a survey done in South African vineyards, it was found that parasitoids play an important role in the biological control of *P. ficus* (Walton & Pringle, 2002) but the levels of control was not enough to keep infestations below economically important levels. Hattingh *et al.* (1999) found that *Planococcus citri* (Risso)(Hemiptera: Pseudococcidae) was successfully controlled by mass releases of *C. perminutus* but Walton & Pringle (2002) found that successful control of *P. ficus* was only achieved when infestation levels were low. They advised the control of ants by chemical stem barrier treatments, and the use of dormant

season chemical treatments to suppress high populations of *P. ficus* to ensure a better overall control. Control by *C. perminutus*, however, was considered to be at least as effective as chemical control (Walton & Pringle, 2002) and is therefore worth enhancing by conducting more research to increase the level of control.

Coccidoxenoides perminutus

Coccidoxenoides perminutus is an asexual endoparasitoid of *Planococcus citri*, of Australian origin (Ceballo & Walter, 2004; Davies et al., 2004) that was first described in 1919 in Hawaii by Timberlake, and classified as *Pauridia peregrina* (Searle, 1965). According to Searle (1965) it was introduced into California from Honolulu in 1916 and was first recorded in South Africa in 1943. Joyce et al (2001) described it as solitary, thelytokous and pro-ovigenic.

C. perminutus has a lifecycle of about 4 weeks (Joyce et al., 2001). It has a high reproductive potential – 10-20 eggs are laid within 2 days of emergence and thereafter 80-150 eggs are laid until the parasitoid dies after about 8 days (Ceballo & Walter, 2004). Counts made by Searle (1965) over a period of six months yielded 99.5% female parasitoids.

Oviposition does not occur at night and even though up to four or five eggs may be oviposited into a host, only one parasitoid ever emerges (Ceballo & Walter, 2004). It is thought that encapsulation occurs when eggs are oviposited into adult mealybugs as a defence mechanism (Ceballo & Walter, 2004). Eggs only reach maturity when oviposited into immature mealybugs. Joyce et al. (2001) concluded that there were no preferences for specific nymphal instars of *P. ficus*, but it was later discovered that most eggs were deposited into second instar nymphs and that the highest success rate in development also occurred with this instar (Ceballo & Walter, 2004).

An advantage for the mass rearing of the parasitoid is that no mating is required (Ceballo & Walter, 2004). However, this potential for a quick increase in numbers has not been observed in the field. This may be due to a possible sensitivity to low relative humidity (Gol'berg 1982; Davies et al., 2004) and a susceptibility to hot, dry conditions (Davies et al., 2004). These conditions could also be exacerbated through a lack of suitable food sources (Ceballo & Walter, 2004). The lifespan and fecundity of the adult parasitoid is highest when

Alpinia nectar or honey is supplied as a food source in laboratory cultures (Davies et al., 2004).

Anagyrus sp. near pseudococci

Anagyrus pseudococci was originally described from Sicily, Italy, by Girault in 1913 (Girault, 1915) as a polyphagous parasitoid of a wide range of mealybugs. It was accepted to be found all over the world, including in South Africa (Urban & Greeff, 1985; Walton, 2003) but, following a publication by Triapitsyn et al. (2007), its taxonomic identity came under dispute. Morphologically, female *A. pseudococci* can only be distinguished from females of *A. sp. near pseudococci* and *A. dactylopii* (Howard) by the first funicle segment of the antennae. Males of these three species are completely indistinguishable (Triapitsyn et al. 2007; Karamaouna et al., 2011). *Anagyrus sp. near pseudococci* is a solitary, koinobiont endoparasitoid (Islam & Copland, 1997; Franco et al., 2008) that is considered to be of Mediterranean origin (Islam & Copland, 2000).

Daane et al. (2003) found that encyrtiform larvae hatch from eggs after two days. The parasitoid reaches its fifth instar about 6-8 days after oviposition and then forms a pupa for about four days. Adult emergence from the mealybug occurs about 12 days after oviposition and takes two days. Daane et al. (2008) found that the parasitoid can overwinter in a diapause stage, and then hatch as soon as temperatures rise in spring. The parasitoid completes 7-8 generations during the active period which translates into two generations of *A. sp. near pseudococci* for every generation of vine mealybug. The emergence of *A. sp. near pseudococci* is synchronised with the appearance of vine mealybug in the field through seasonal cues. Blumberg et al. (1995) found that a low rate of encapsulation occurs in vine mealybug. Tingle & Copland (1988) found that the developmental rate of *A. sp. near pseudococci* increases as temperature rises, peaking at about 35°C.

Studies have shown that *A. sp. near pseudococci* parasitizes all stages of *P. ficus*, but prefers older stages. The parasitoid is able to discriminate between parasitized and unparasitized hosts, which means superparasitism rarely occurs (Islam & Copland, 1997; Islam & Copland, 2000; Daane et al., 2003). The adult parasitoid does not host feed at all but instead feeds on flower nectar (Daane et al., 2003).

Anagyrus sp. near *pseudococci* rarely attacks vine mealybug in hidden locations, like beneath the bark, which means that control early in the growing season can be compromised (Daane et al., 2003). A comparative summary of various population parameters of *C. perminutus* and *A. sp. near pseudococci* is given in Table 2. Data has shown that *A. sp. near pseudococci* responds better to higher temperatures than *C. perminutus*. This means that earlier in the season, when it is cooler, use of *C. perminutus* is advised and later during the season *A. sp. near pseudococci* should be used as complementary control agents of the vine mealybug (Wohlfarter & Addison, 2014). However, not much of the information in Table 2 is obtained from local research in South Africa, and it is preferable to assess these parameters locally, primarily due to the taxonomic uncertainties still remaining regarding *A. sp. near pseudococci* in particular.

Table 2. Comparative summary of population parameters of *Coccidoxenoides perminutus* and *Anagyrus* sp. near *pseudococci*.

Species	<i>Coccidoxenoides perminutus</i>	<i>Anagyrus</i> sp. near <i>pseudococci</i>
Mode of reproduction	Parthenogenic	Sexual
Temperature tolerance	8-30°C, with an optimum of 21°C (Walton, 2003)	14-34°C (Daane et al., 2004)
Instar preference	Second instar mealybugs (Ceballo & Walter, 2004)	Third instar to adult mealybugs (Daane et al., 2004)
Origin	Australia (Girault, 1915)	Mediterranean (Girault, 1915)
Hosts (*found in South Africa)	* <i>Planococcus citri</i> , * <i>Planococcus ficus</i> , <i>Planococcus minor</i> , <i>Planococcus vovae</i> , (Walton, 2003; Ceballo & Walter, 2004; Kaydan et al., 2006; Francis et al., 2012)	* <i>Planococcus citri</i> , * <i>Planococcus ficus</i> , <i>Planococcus vovae</i> , (<i>Pseudococcus viburni</i> , * <i>Pseudococcus calceolariae</i> , <i>Phenacoccus peruvianus</i> (Kaydan et al., 2006; Bugila et al., 2014)

1.1.1.5 Mating disruption for mealybugs

Hinkens *et al.* (2001) identified the sex pheromone emitted by the vine mealybug females to attract the winged, adult males. It was synthesized and successfully used in monitoring programs (Millar et al., 2002; Walton et al., 2004). In 2003, Daane et al. (2006) conducted studies for the pheromone to be used as a mating disruption method but found that

currently it might not be the most effective method of lowering populations. Mealybug densities did not differ significantly between control and experimental plots and it is thought that the inundation of the vineyards with pheromone could have confused both the parasitoids and the male mealybugs. Another problem was the need to apply lures numerous times per season as the pheromone was depleted after 21 days. Currently Checkmate® VMB-XL from Spectrum Research Services is registered in South Africa and is effective as a mating disruption product for vine mealybugs in vineyards for up to 150 days.

1.1.1.6 Ant control

The presence of ants in vineyards is a reliable indicator that mealybugs can be found (Walton, 2001). Ants hinder parasitoids from foraging for mealybugs by tending the mealybugs for their honeydew (Way, 1963). Ants can be controlled through insecticides that are even more toxic than those used for mealybug control (Addison, 2002). Using baits in combination with insecticides can target foraging ants and their nest mates and so control the whole colony with a lower dosage of chemicals (Daane et al., 2006; Nyamukondiwa, 2008). In South Africa, however, it was found that currently the most effective method of dealing with various species of ants is the use of chemical stem barriers (Addison, 2002) that can keep the ants out of the canopy and so allow effective biological control to take place (Mgocheki & Addison, 2009a).

During experiments by Mgocheki & Addison (2009a) the effects of three species of ants (*Linepithema humile* (Mayr), *Crematogaster peringueyi* Emery and *Anoplolepis steingroeveri*) on two parasitoids (*C. perminutus* and *A. sp. near pseudococci*) were analysed. They found that *C. perminutus* effected significantly more parasitism than *A. sp. near pseudococci* but parasitism by both parasitoids showed a significant decline when mealybugs were tended by ants. They also found a significant increase in mortality of both parasitoids in the presence of ants, with the highest mortality from *C. peringueyi* (about 65 % higher than *A. steingroeveri*). This finding was corroborated with the highest number of parasitoids found foraging in the presence on *A. steingroeveri* and the lowest with *C. peringueyi*. These foraging numbers were still significantly lower than when no ants were present, which indicated that all three ant species are capable of preventing parasitoids from getting near the vine mealybug.

1.1.2 History

Planococcus ficus was first discovered in South Africa in 1914 by De Charmoy but was confused with *P. citri* and wrongfully named *Pseudococcus vitis* by Niedecki in 1870 (De Lotto, 1975). These misunderstandings were clarified by Ezzat and McConnell (1956), a final classification was done by De Lotto (1975) and Ben-Dov (1994) most recently reviewed the classification. The vine mealybug is classified under the Order Hemiptera, Suborder Sternorrhyncha, Superfamily Coccoidea and Family Pseudococcidae.

The vine mealybug was first reported on vines in the Western Cape in 1930 by Joubert (1943). It spread to the Hex River Valley by 1935 and in the following 30 years infestations increased due to careless use of insecticides, which led to the suppression of natural enemies (Myburgh, 1951; Kriegler, 1954; Whitehead, 1957, Walton & Pringle, 2004).

According to Joubert (1943) the vine mealybug was brought into South Africa with rootstocks that were imported to bring Phylloxera (*Daktulspaira vitifoliae*) under control (Kriegler, 1954). It is now a ubiquitous pest that is found throughout most of the Western to Eastern Mediterranean region, largely in Italy, France, Spain, Egypt and Israel (Sforza et al., 2005). It was also introduced, and is considered a pest, in several countries in South America and states in the United States like California.

1.1.3 Biology

Vine mealybugs are sexually dimorphic. Eggs are a light straw-colour. Female mealybugs are ovate, yellowish to slate-coloured and covered in a white powdery wax that has a distinct median line where the waxy layer is thinner. Eighteen pairs of thick uniform filaments protrude from the edges of the female's body. Females undergo incomplete metamorphosis and pass through three nymphal stages. Males undergo a more complete metamorphosis with the penultimate stage a pseudopupa. Males are less than 1mm in size and have no mouthparts, a single pair of wings on the metathorax, and two long filamentous anal setae. The vine mealybug does not diapause and optimal progress through developmental stages is achieved around 25-27°C (Kriegler, 1954). One female can deposit an average of 300 eggs, or up to a maximum of 700, into an ovisac (Sforza et al., 2005).

The vine mealybug has up to six generations per year in North America (Millar et al., 2002) and South Africa (Kriegler, 1954), with generations overlapping, which enables populations to grow fairly quickly. During winter all life stages can be found under the bark. In spring and summer the mealybugs can be found all over the vine but mostly on leaves and bunches. After harvest, depending on food supply, the majority can be found on the leaves before moving back to the stem and, to a depth of 30 cm, on the roots for the winter period (Kriegler, 1954; Whitehead, 1957; Walton & Pringle, 2004).

Due to the sticky nature of the honeydew the mealybugs secrete, infested plant material can be moved by animals, people or equipment in the field. Cross contamination from infected to healthy vineyards can thus readily occur in practice, resulting in a broad distribution of this pest in South African viticulture. Infested nursery stock material can also be overlooked as the mealybugs often hide under the vine's bark (Daane et al., 2006).

1.1.4 Hosts

Vine mealybugs have a wide host range in terms of crops, including grapes, figs, apples, citrus, mangoes, bananas, avocados and dates (Cox, 1989; Hinkens et al., 2001; Millar et al., 2002) as well as some common weeds, such as malva (family Malvaceae), burclover (*Medicago polymorpha*, Fabaceae), black nightshade (*Solanum nigrum*, Solanaceae), sowthistle (genus *Sonchus*, Asteraceae) and lambsquarter (*Chenopodium album*, Chenopodiaceae) (Sforza et al., 2005). Ben-Dov (1994) included Vitaceae, Moraceae, Salicaceae, Rosaceae and Punicaceae in its host plant range.

1.2 HABITAT MANAGEMENT

Habitat management forms a part of conservation biological control and can be described as the manipulation of a landscape by intentionally providing certain plants or plant communities as resources for natural enemies to increase their effectiveness. This is usually done by choosing plants based on the resource they provide, like pollen, nectar or shelter, and then establishing the selected plants within a managed landscape (Fiedler et al. 2008; Pickett & Bugg, 1998; Landis et al. 2000). This manipulation can take many forms, but the technique used most often is field margin manipulation, which consists of non-crop buffer

strips, wildflower strips, restoration of the adjacent natural areas, or a combination of all three (Decourtye et al. 2010; Haaland et al. 2011, Wratten et al. 2012).

Insect pests, plant pathogens and weeds cause up to 40% loss in food production and synthetic chemicals to control these problems are becoming ineffective and unsustainable due to factors like pesticide resistance and suppression of natural enemies (Gurr et al., 1988). Because of these complications, much attention has been given to understanding the role plant-provided resources can play in the biology and ecology of natural enemies and how this can enhance the suppression of pest populations. Attention has been given to which ecosystem services, like nutrient cycling, pollination, biological control (Gurr et al., 2005), and overall biodiversity effects, like protecting soil and water quality by decreasing runoff and protecting against soil erosion (Wratten et al. 2012) can be provided.

Anagyrus sp near *pseudococci* and *C. perminutus*, like many adult parasitoids, require non-host food such as nectar (Landis et al. 2000). Resources provided by floral vegetation can provide adult parasitic wasps with the nutrients and energy needed to increase longevity, fecundity, egg load, and flight ability (Jervis et al. 1993; Dyer & Landis 1996; Wheeler 1996; Heimpel et al. 1997; Jervis et al. 1996; Tooker & Hanks, 2000; Jacob & Evans, 2000; Dib et al., 2012) and can lead to the reduction of pest populations in the field (Irvin et al. 2000; Patt et al. 1997). The critical step in proving the value of floral resources is to show the effect flowering plants have on the effectiveness of a parasitoid in reducing pest populations (Wratten et al. 2000; Berndt et al., 2005). Fiedler et al. (2008) found that a small number of plants that were proven effective in helping biological control have been tested repeatedly, often in areas they are not endemic to, with very few studies aimed at finding new or native species for use in habitat management. Baggen and Gurr (1998) found that the parasitism rate of the potato moth (*Phthorimaea operculella*) by *Copidosoma koehleri* Blanchard was greater when supplied with a strip of borage flowers. In turn, Pimbert & Srivastava (1989) found that when a border of coriander flowers were planted around chickpea crops, parasitism of the gram pod borer, commonly known as the bollworm (*Helicoverpa armigera*) by *Campoletis chloridae*, was four times greater than without the border, which confirmed that the presence of flowers can increase the rate of parasitism (Berndt et al., 2005).

Resource subsidies can therefore be provided to these parasitoids by planting flowers within

an agro-ecosystem, and thus improve biological control of insect pests (Gurr et al. 2004; Kean et al. 2003; Tylianakis et al. 2004; Berndt et al., 2005).

Any manipulations to the field have to be considered in context of the particular agro-ecosystem. The desirable approach is a more self-sustained, energy-efficient agricultural system (Altieri et al., 1983). Any habitat management techniques that clash with practical farming methods will never be realised, as the main objective in modern agriculture is to achieve maximum yields (Gurr et al., 2005).

Anoplolepis custodiens (F. Smith), the pugnacious ant, is very aggressive and can easily out-compete other indigenous ant species when honeydew is available (Samways, 1999). By feeding on the honeydew from mealybugs, it reduces the efficacy of mealybug parasitoids (Kriegler & Whitehead, 1962). Gaigher et al. (2013) found that primary parasitoid abundance increased when *Pheidole megacephala* ant colonies were baited but, unexpectedly, Addison and Samways (2006) found that even when *A. custodiens* population levels decrease, parasitoids levels didn't increase significantly. Research still needs to be done to confirm the belief that once the mutualistic relationship of the ant and mealybug has started, the parasitoids probably wouldn't be able to control it.

1.3 AIM AND OBJECTIVES

It is as yet unknown which food sources parasitoids of *P. ficus* depend on to fuel their parasitisation of the mealybugs. Alternate resources needed to increase survival and parasitisation of mealybugs needs to be investigated in the laboratory to quantify these potential food sources, in terms of their possible value to establishment of habitat management plans and conservation biological control. This information can be used for further field studies.

The aim of this thesis is therefore to contribute basic biological information for the establishment of a habitat management plan in vineyards to improve the biological control of *P. ficus*. The thesis is written as separate research papers, and some repetition may therefore occur.

The objectives are:

- To assess the biodiversity of mealybug parasitoids in Western Cape vineyards and associated natural habitats, which will determine whether any potential ecosystem services, in the form of natural biological control, are provided by natural vegetation in the Western Cape agro-ecosystem.
- To determine attraction of adult parasitoids to flowering plants by assessing a variety of plants in a controlled laboratory environment. This is to lay the foundation for field research in adult parasitoid food preferences, which would create a suitable environment for the parasitoids to colonize and as such have a naturally occurring population in or around the vineyards throughout the year.
- To determine larval-stage host preference of major mealybug parasitoids by testing different instars of *P. ficus* for parasitoid susceptibility both in choice and no choice tests. This will assist in planning augmentative releases more accurately when a combination of two parasitoids would be used to ensure they do not compete for the same mealybug resources.

1.4 REFERENCES

Addison, P., 2002. Chemical stem barriers for the control of ants (Hymenoptera: Formicidae) in vineyards. *South African Journal of Entology and Viticulture* 23: 1-8.

Addison, P. & Samways, M.J., 2006. Surrogate habitats demonstrate the invasion potential of the African pugnacious ant. *Biodiversity & Conservation* 15(1): 411-428.

Altieri, M.A., Letourneau, D.K. & Davis, J.R., 1983. Developing sustainable agroecosystems. *BioScience* 33(1): 45-49.

Anonymous, 2007. *A guide for the control of plant pests* (40th edition). Directorate. Food safety and quality assurance. National Department of Agriculture, Government Printer, Pretoria, South Africa.

Baggen, L.R. & Gurr, G.M., 1998. The influence of food on *Copidosoma koehleri* (Hymenoptera: Encyrtidae), and the use of flowering plants as a habitat management tool to enhance biological control of potato moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae). *Biological control* 11: 9-17.

Ben-Dov, Y., 1994. *A systematic catalogue of the mealybugs of the world (Insecta: Homoptera: Coccoidea: Pseudococcidae and Putoidea) with data on geographical distribution, host plants, biology and economic importance*. Intercept Limited, Andover, UK.

Berlinger, M.J., 1977. The Mediterranean vine mealybug and its natural enemies in southern Israel. *Phytoparasitica* 5(1): 3-14.

Berndt, L.A., Wratten, S.D. & Scarratt, P.G., 2005. Effects of buckwheat flowers on leafroller (Lepidoptera: Tortricidae) parasitoids in a New Zealand vineyard. *Agricultural and Forest Entomology* 4: 39-45.

Blumberg, D., Klein, M. & Mendel, Z., 1995. Response by encapsulation of four mealybug species (Homoptera: Pseudococcidae) to parasitisation by *Anagyrus pseudocci*. *Phytoparasitica* 23(2): 157-163.

Bodenheimer, F.S., 1951. Description of some new genera of Coccoidea. *Entomologische Berichten* 13: 328-331.

Bugila, A.A.A., Branco, M., Silva, E.B.D. & Franco, J.C., 2014. Host selection behaviour and specificity of the solitary parasitoid of mealybugs *Anagyrus* sp. nr. *pseudococci* (Girault) (Hymenoptera, Encyrtidae). *Biocontrol Science and Technology* 24(1): 22-38.

Ceballo, F.A. & Walter, G.H., 2004. Why is *Coccidoxenoides perminutus*, a mealybug parasitoid, ineffective as a biocontrol agent – Inaccurate measures of parasitism of low adult survival? *Biological Control* 33: 260-268.

Cox, J.M., 1989. The mealybug genus *Planococcus* (Homoptera: Pseudococcidae). *Bulletin of the British Museum (Natural History), Entomology* 58(1): 1-78.

Daane, K.M., Bentley, W.J., Macmillan, C. & Walton, V.M., 2003. New control options for the vine mealybug. *Wine Business Monthly* (www.winebusiness.com).

Daane, K.M., Malakar-Kuenen, R.D. & Walton, V.M., 2004. Temperature-dependent development of *Anagyrus pseudococci* (Hymenoptera: Encyrtidae) as a parasitoid of the vine mealybug, *Planococcus ficus* (Homoptera: Pseudococcidae). *Biological Control* 31(2): 123-132.

Daane, K.M., Bentley, W.J., Walton, V.M., Malakar-Kuenen R., Millar, J.G., Ingels, C.A., Weber, E.A. & Gispert, C., 2006. New controls investigated for vine mealybug. *California Agriculture* 60(1): 31-38.

Daane, K.M., Cooper, M.L., Triapitsyn, S.V., Walton, V.M., Yokota, G.Y., Haviland, D.R., Bentley, W.J., Godfrey, K.E. & Wunderlich, L.R., 2008. Vineyard managers and researchers seek sustainable solutions for mealybugs, a changing pest complex. *California Agriculture* 62(4).

Davies, A.P., Ceballo, F.A. & Walter, G.H., 2004. Is the potential of *Coccidoxenoides perminutus*, a mealybug parasitoid, limited by climatic or nutritional factors? *Biological control* 31: 181-188.

Decourtye, A., Mader, E. & Desneux, N., 2010. Landscape enhancement of floral resources for honey bees in agro-ecosystems. *Apidologie* 41(3): 264-277.

De Lotto, G., 1975. Notes on the vine mealybug (Homoptera: Coccoidea: Pseudococcidae). *Journal of the Entomological Society of Southern Africa* 38: 125-130.

De Villiers, M., 2006. *Development of a pest management system for table grapes in the Hex River Valley*. PhD dissertation, Department of Conservation Ecology and Entomology, Stellenbosch University, Private Bag X1, 7620 Matieland (Stellenbosch), South Africa.

De Villiers, M. & Pringle, K.L., 2007. Seasonal occurrence of vine pests in commercially treated vineyards in the Hex River Valley in the Western Cape Province, South Africa. *African Entomology* 15(2): 241-260.

Dib, H., Libourel, G. & Warlop, F., 2012. Entomological and functional role of floral strips in an organic apple orchard: Hymenopteran parasitoids as a case study. *Journal of Insect Conservation* 16: 315-318.

Dyer, L.E. & Landis, D.A., 1996. Effects of habitat, temperature, and sugar availability on longevity of *Eriborus terebrans* (Hymenoptera: Ichneumonidae). *Environmental Entomology* 25(5): 1192-1201.

Engelbrecht, D.J. & Kasdorf, G.G.F., 1990. Transmission of grapevine leafroll disease and associated closteroviruses by the vine mealybug, *Planococcus ficus*. *Phytophylactica* 22: 341-346.

Ezzat, Y.M. & McConnell, H.S., 1956. *Classification of the mealybug tribe Planococcini. (Pseudococcidae, Homoptera)* (No. 84). University of Maryland, Agricultural Experiment Station.

Fiedler, A.K., Landis, D.A. & Wratten, S.D., 2008. Maximizing ecosystem services from conservation biological control: The role of habitat management. *Biological Control* 45: 254-271.

Fourie, J.C., 2010. Soil management in the Breede River Valley wine grape region, South Africa 1. Cover crop performance and weed control. *South African Journal of Enology and Viticulture* 31(1).

- Francis, A.W., Kairo, M.T., Roda, A.L., Liburd, O.E. & Polar, P., 2012. The passionvine mealybug, *Planococcus minor* (Maskell) (Hemiptera: Pseudococcidae), and its natural enemies in the cocoa agroecosystem in Trinidad. *Biological Control* 60(3): 290-296.
- Franco, J.C., Silva, E.B., Cortegano, E., Campos, L., Branco, M., Zada, A. & Mendel, Z., 2008. Kairomonal response of the parasitoid *Anagyrus* spec. nov. near *pseudococci* to the sex pheromone of the vine mealybug. *Entomologia Experimentalis et Applicata* 126: 122-130.
- Franco, J.C., Zada, A. & Mendel, Z., 2009. Novel approaches for the management of mealybug pests. In: Ishaaya, I. & Horowitz, A.R. (eds.). *Biorational control of arthropod pests. Application and resistance management*. Springer Science, New York, p.233-278.
- Gaigher, R., Samways, M.J. & Van Noort, S., 2013. Saving a tropical ecosystem from a destructive ant-scale (*Pheidole megacephala*, *Pulvinaria urbicola*) mutualism with support from a diverse natural enemy assemblage. *Biological Invasions* 15(9): 2115-2125.
- Geiger, C.S. & Daane, K.M., 2001. Seasonal movement and distribution of the grape mealybug (Homoptera: Pseudococcidae): Developing a sampling program for San Joaquin Valley Vineyards. *Journal of Economic Entomology* 94(1): 291-301.
- Girault, A.A., 1915. Australian Hymenoptera Chalcidoidea-VII. The family Encyrtidae with descriptions of new genera and species. *Memoirs of Queensland Museum* 4, pp. 1–184.
- Gol'Berg, A.M., 1982. Influence of temperature and relative humidity on survival and fecundity of *Pauridia peregrina*, a parasite of mealybugs and its interactions with *Planococcus citri*. *Entomologia Experimentalis et Applicata* 32(1): 86-90.
- Gurr, G.M., Van Emden, H.F. & Wratten, S.D., 1998. Habitat manipulation and natural enemy efficiency: implications for the control of pests. *Conservation biological control* 155: 183.
- Gurr, G., Wratten, S.D. & Altieri, M.A. (Eds.), 2004. *Ecological engineering for pest management: advances in habitat manipulation for arthropods*. CSIRO Publishing, Canberra, Australia.

- Gurr, G.M., Wratten, S.D., Kehrli, P. & Scarratt, S., 2005. Cultural manipulations to enhance biological control in Australia and New Zealand: Progress and prospects. *Second International Symposium on Biological Control of Arthropods*, pp. 144-166.
- Haaland, C., Naisbit, R.E. & Bersier, L.F., 2011. Sown wildflower strips for insect conservation: a review. *Insect Conservation and Diversity* 4(1): 60-80.
- Hattingh, V., Moore, S. & Tate, B., 1999. Evaluating efficacy of augmentative releases of arthropod biocontrol agents with mealybug on citrus in Southern Africa as an example. p. 35 *In Anon. Abstracts of the XIVth International Plant Protection Congress (IPPC)*, Jerusalem, Israel, July 25-30, 1999.
- Haviland, D. R., Bentley, W.J. & Daane, K.M., 2005. Hot-water treatments for control of *Planococcus ficus* (Homoptera: Pseudococcidae) on dormant grape cuttings. *Journal of Economic Entomology* 98(4): 1109-1115.
- Heimpel, G.E., Rosenheim, J.A. & Kattari, D., 1997. Adult feeding and lifetime reproductive success in the parasitoid *Aphytis melinus*. *Entomologia Experimentalis et Applicata* 83(3): 305-315.
- Hinkens, D.M., McElfresh, J.S. & Millar, J.G., 2001. Identification and synthesis of the sex pheromone of the vine mealybug, *Planococcus ficus*. *Tetrahedron Letters* 42: 1619-1621.
- Holm, K., 2008. *Construction of a cDNA library for the vine mealybug, Planococcus ficus (Signoret)*. PhD dissertation, Department of Conservation Ecology and Entomology, Stellenbosch University, Private Bag X1, 7620 Matieland (Stellenbosch), South Africa.
- Irvin, N.A., Wratten, S.D., Frampton, C.M., Chapman, R.B. & Tylanakis, J.M., 2000. Understorey management for the enhancement of the leafroller parasitoid *Dolichogenidea tasmanica* (Cameron) in orchards at Canterbury, New Zealand. *Hymenoptera: Evolution, Biodiversity and Biological Control*, pp. 396-403.
- Islam, K.S. & Copland, M.J.W., 1997. Host preference and progeny sex ratio in a solitary koinobiont mealybug endoparasitoid, *Anagyrus pseudococci* (Girault), in response to its host stage. *Biocontrol Science and Technology* 7(3): 449-456.

- Islam, K.S. & Copland, M.J.W., 2000. Influence of egg load and oviposition time interval on the host discrimination and offspring survival of *Anagyrus pseudococci* (Hymenoptera: Encyrtidae), a solitary endoparasitoid of citrus mealybug, *Planococcus citri* (Hemiptera: Pseudococcidae). *Bulletin of entomological research* 90: 69-75.
- Jacob, H.S. & Evans, E.W., 2000. Influence of carbohydrate foods and mating on longevity of the parasitoid *Bathyleptes curculionis* (Hymenoptera: Ichneumonidae). *Environmental Entomology* 29(5): 1088-1095.
- Jervis, M.A., Kidd, N.A.C., Fitton, M.G., Huddleston, T. & Dawah, H.A., 1993. Flower-visiting by hymenopteran parasitoids. *Journal of natural history* 27(1): 67-105.
- Jervis, M.A., Kidd, N.A.C., & Heimpel, G.E., 1996. Parasitoid adult feeding behaviour and biological control - A review. *Biocontrol News and Information*.
- Joubert, C.J., 1943. *Introduction into the Union of South Africa of some natural enemies of mealy bugs*. Western Province Fruit Research Institute, Department of Agriculture and Forestry, South Africa.
- Joyce, A.L., Hoddle, M.S., Bellows, T.S. & Gonzalez, D., 2001. Oviposition behavior of *Coccidoxenoides peregrinus*, a parasitoid of *Planococcus ficus*. *Entomologia Experimentalis et applicata* 98(1): 49-57.
- Karamaouna, F., Menounou, G., Stathas, G.J. & Avtzis, D.N., 2011. First record and molecular identification of the parasitoid *Anagyrus* sp. near *pseudococci* Girault (Hymenoptera: Encyrtidae) in Greece – Host size preference for the vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae). *Hellenic Plant Protection Journal* 4: 45-52.
- Kaydan, M.B., Kilincer, N., Uygun, N., Japoshvilli, G. & Gaimari, S., 2006. Parasitoids and predators of pseudococcidae (Hemiptera: Coccoidea) in Ankara, Turkey. *Phytoparasitica* 34(4): 331-337.
- Kean, J., Wratten, S., Tylianakis, J. & Barlow, N., 2003. The population consequences of natural enemy enhancement, and implications for conservation biological control. *Ecology Letters* 6(7): 604-612.

Kriegler, P.J., 1954. *'n Bydrae tot die kennis van Planococcus citri (Risso) (Homoptera: Pseudococcidae)* (in Afrikaans). PhD dissertation, Department of Conservation Ecology and Entomology, Stellenbosch University, Private Bag X1, 7620 Matieland (Stellenbosch), South Africa.

Kriegler, P.J. & Whitehead, V.B., 1962. Notes on the biology and control of *Crematogaster peringueyi* var. *angustior* Arnold on grape vines (Hymenoptera: Formicidae). *Journal of the Entomological Society of southern Africa* 25: 287-290.

Landis, D.A., Wratten, S.D. & Gurr, G.M., 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology* 45: 175-201.

Malakar-Kuenen, R., Daane, K.M., Bentley, W.J., Yokota, G.Y., Martin, L., Godfrey, K. & Ball, J., 2001. Population dynamics of the vine mealybug and its natural enemies in the Coachella and San Joaquin Valleys. *University of California, Kearney Plant Protection Group, Plant Protection Quarterly* 11(2): 1-5.

Mgocheki, N. & Addison, P., 2009a. Interference of ants (Hymenoptera: Formicidae) with biological control of the vine mealybug *Planococcus ficus* (Signoret) (Homoptera: Pseudococcidae). *Biological Control* 49: 180-185.

Mgocheki, N. & Addison, P., 2009b. Effect of contact pesticides on vine mealybug parasitoids, *Anagyrus* sp. near *pseudococci* (Girault) and *Coccidoxenoides perminutus* (Timberlake) (Hymenoptera: Encyrtidae). *South African Journal of Entology and Viticulture* 30(2): 110-116.

Millar, J.G., Daane, K.M., McElfresh, J.S., Moreira, J.A., Malakar-Kuenen, R., Guillén, M. & Bentley, W.J., 2002. Development and optimization of methods for using sex pheromone for monitoring the mealybug *Planococcus ficus* (Homoptera: Pseudococcidae) in California Vineyards. *Journal of Economic Entomology* 95(4): 706-714.

Monis, J. & Bestwick, R.K., 1997. Serological detection of grapevine associated closteroviruses in infected grapevine cultivars. *Plant Disease* 81(7): 802-808.

Myburgh, A.C., 1951. Mealybug control on table grapes. *Farming in South Africa* 26: 14-16.

Nyamukondiwa, C., 2008. *Assessment of toxic baits for the control of ants (Hymenoptera: Formicidae) in South African vineyards*. PhD dissertation, Department of Conservation Ecology and Entomology, Stellenbosch University, Private Bag X1, 7620 Matieland (Stellenbosch), South Africa.

Patt, J.M., Hamilton, G.C. & Lashomb, J.H., 1997. Foraging success of parasitoid wasps on flowers: interplay of insect morphology, floral architecture and searching behavior. *Entomologia Experimentalis et Applicata* 83(1): 21-30.

Pickett, C.H. & Bugg, R.L. (Eds.), 1998. *Enhancing biological control: habitat management to promote natural enemies of agricultural pests*. Univ of California Press.

Pimbert, M.P. & Srivastava, C.P., 1989. Vegetation management and the biological control of *Helicoverpa armigera* in chickpea. *International Chickpea Newsletter* 21: 16-19.

Rosciglione, B. & Gugerli, P., 1989. Transmission of grapevine leafroll disease and an associated closterovirus to healthy grapevine by the mealybug *Planococcus ficus*. *Phytoparasitica* 17: 63.

Samways, M.J., 1999. Landscape triage for conserving insect diversity. *The Other* 99: 269-273.

Searle, C.M., 1965. The susceptibility of *Pauridia peregrina* Timb. (Hymenoptera: Encyrtidae) to some pesticide formulations. *Journal of the Entomological Society of South Africa* 27(2): 239-249.

Sforza, A., Kirk, A. & Jones, W.A., 2005. Results of foreign exploration for natural enemies of *Planococcus* (Homoptera: Pseudococcidae), a new invasive mealybug in California vineyards. *AFPP 7^{eme} conference internationale sur les ravageurs en agriculture, Montpellier, 26-27 October 2005*.

Tingle, C.C.D. & Copland, M.J.W., 1988. Effects of temperature and host-plant on regulation of glasshouse mealybug (Hemiptera: Pseudococcidae) populations by introduced parasitoids (Hymenoptera: Encyrtidae). *Bulletin of entomological research* 78(1): 135-142.

Tooker, J.F. & Hanks, L.M., 2000. Flowering plants hosts of adult Hymenopteran parasitoids of Central Illinois. *Annals of the Entomological Society of America* 93(3): 580-588.

Triapitsyn, S.V., González, D., Vickerman, D.B., Noyes, J.S. & White, E.B., 2007. Morphological, biological, and molecular comparisons among the different geographical populations of *Anagyrus pseudococci* (Hymenoptera: Encyrtidae), parasitoids of *Planococcus* spp. (Hemiptera: Pseudococcidae), with notes on *Anagyrus dactylopii*. *Biological control* 41: 14-24.

Tylianakis, J.M., Didham, R.K. & Wratten, S.D., 2004. Improved fitness of aphid parasitoids receiving resource subsidies. *Ecology* 85(3): 658-666.

Urban, A.J. & Greeff, F.C., 1985. The integrated control of the vine mealybug *Planococcus ficus* (Signoret), on vines. Final Report. *Plant Protection Research Institute*.

Walton, V., 2001. Wingerdwitluis: Biologie en beheerstrategie. (In Afrikaans). *Wynboer Tegnies* 140: 75-78.

Walton, V.M., 2003. *Development of an integrated pest management system for vine mealybug, Planococcus ficus (Signoret), in vineyards in the Western Cape Province, South Africa*. PhD dissertation, Department of Conservation Ecology and Entomology, Stellenbosch University, Private Bag X1, 7620 Matieland (Stellenbosch), South Africa.

Walton, V.M. & Pringle, K.L., 1999. Effects of pesticides used on table grapes on the mealybug parasitoid *Coccidoxenoides peregrinus* (Timberlake) (Hymenoptera: Encyrtidae). *South African Journal of Enology and Viticulture* 20 (1).

Walton, V.M. & Pringle, K.L., 2002. Evaluating effectiveness of mass releases of the vine mealybug (*Planococcus ficus*) parasitoid *Coccidoxenoides peregrinus* in Western Cape province vineyards, South Africa. In *Proceedings of the 1st International Symposium on Biological Control of Arthropods, Honolulu, Hawaii*, pp. 14-18.

Walton, V.M. & Pringle, K.L., 2004. A survey of mealybugs and associated natural enemies in vineyards in the Western Cape provinces. *South African Journal of Enology and Viticulture* 25 (1).

Walton, V.M., Daane, K.M. & Pringle, K.L., 2004. Monitoring *Planococcus ficus* in South African vineyards with sex pheromone-baited traps. *Crop Protection* 23: 1089-1096.

Way, M.J., 1963. Mutualism between ants and honeydew-producing Homoptera. *Annual review of entomology* 8(1): 307-344.

Wheeler, D., 1996. The role of nourishment in oogenesis. *Annual review of entomology* 41(1): 407-431.

Whitehead, V. B. (1957). *A study of the predators and parasites of Planococcus citri (Risso)(Homoptera: Pseudococcidae) on vines in the Western Cape Province, South Africa.* PhD dissertation, Rhodes University, Grahamstown.

Wohlfarter, M. & Addison, P., 2014. A life table study of *Anagyrus* sp. near *pseudococci* (Girault) (Hymenoptera: Encyrtidae) on its host, *Planococcus ficus* (Signoret) (Homoptera: Pseudococcidae). *African Entomology* 22(2): 250-256.

Wratten, S.D., Gurr, G.M., Landis, D., Irvin, N.A., Berndt, L.A. & Hoddle, M.S., 2000. Conservation biological control of pests: multi-trophic-level effects. In *California Conference on Biological Control II, The Historic Mission Inn Riverside, California, USA, 11-12 July, 2000* at the Center for Biological Control, College of Natural Resources, University of California, pp. 73-80.

Wratten, S.D., Gillespie, M., Decourtye, A., Mader, E. & Desneux, N., 2012. Pollinator habitat enhancement: Benefits to other ecosystem services. *Agriculture, Ecosystems and Environment* 159: 112-122.

CHAPTER TWO:

DIVERSITY OF PARASITOIDS OF THE VINE MEALYBUG, *PLANOCOCCUS FICUS* (HEMIPTERA: PSEUDOCOCCIDAE), IN VINEYARDS AND ADJOINING NATURAL HABITATS OF THE WESTERN CAPE, SOUTH AFRICA

2.1 INTRODUCTION

Over the past 100 years the vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) has become a serious pest in vineyards all over the world (Engelbrecht & Kasdorf, 1990; Joyce et al., 2001, Ben-Dov, 1994; Walton & Pringle, 2004b). The mealybug feeds on the plant's phloem, using needle-like mouthparts, and excretes a sugary substance called honeydew. The presence of honeydew and sooty mould which develop on it make grapes unmarketable. Honeydew also lures ants that hamper biological control of mealybugs (Kriegler, 1954; Whitehead, 1957; Berlinger, 1977; Charles, 1982; Walton, 2003). The vine mealybug is also a vector of leafroll virus, which causes crop loss and is of severe economic and phytosanitary importance (Daane et al., 2006; De Villiers & Pringle, 2007; Daane et al., 2008).

Walton and Pringle (2004a) conducted a survey to determine the distribution and assemblage structure of natural enemies of vine mealybug in vineyards in the Western Cape Province. They found predatory beetles, encyrtid parasitoids and *Chrysopa* spp. (Walton & Pringle, 2004a). The predatory beetles included *Cryptolaemus montrouzieri* Mulsant, *Nephus angustus* (Casey), *N. quadrivittatus* (Mulsant), *N. binaevatus* (Mulsant), *Nephus* sp., *Hyperaspis felixi* (Mulsant), *Cydonia lunata* F., a *Rhizobiellus* sp. and a *Hippodamia* sp. They also found *Scymnus nubilis* Mulsant, which had not been recorded before.

Encyrtid parasitoids recovered from the vineyards included *Anagyrus* sp., *Leptomastix dactylopii* (Howard), *Coccidoxenoides perminutus* Girault, and only recovered twice was a fourth encyrtid, *Chrysoplatecyrus splendens* Howard (Walton & Pringle, 2004a). *Chartocerus* spp. (Hymenoptera: Signiphoridae), *Cheiloneurus* spp. (Hymenoptera: Encyrtidae) and *Pachyneuron* spp. (Hymenoptera: Pteromalidae) were all possible hyperparasitoids reared from the vine mealybugs (Walton & Pringle, 2004a). They found that the range of natural

enemies in the vineyards were very similar to studies done previously by Whitehead (1957) and Urban and Greeff (1985).

Of the natural enemy species, Hymenoptera parasitoid species are the most important group to focus biological control strategies on as parasitoids often play a big role in limiting pest populations (LaSalle & Gauld, 1991; Hawkins & Gross, 1992; LaSalle & Gauld, 1993). According to Kruess & Tschardt (1994) biocontrol based on a rare species is expected to be unsuccessful but parasitic Hymenoptera, however rare, can still have a great regulatory effect on pest populations (Greiler et al., 1992; Stork, 1988).

Some studies have found that the diversity, abundance and possible impact of natural enemies are greatly influenced by the increase in areas of non-cultivated, diverse landscapes adjacent to crop fields (Tschardt et al., 2005; Rand et al., 2006). This has been found true for both coccinellid beetles (Elliot et al., 1999, 2002 a, b) as well as some specialist parasitoids (Cronin & Reeve, 2005).

Increased natural enemy abundance can usually be attributed to nearby alternative resources. These resources include overwintering sites, alternative host species, or alternative sources of energy that can be critical in sustaining a population (Landis et al., 2000; Tylmanakis et al., 2004; Rand et al., 2006). The Western Cape is home to a world biodiversity hotspot, the Cape Floral Kingdom (Myers et al. 2000), which could prove valuable in conserving natural enemies of pest insects, as agriculture is interspersed amongst conservation areas.

The aim of this chapter was to determine the abundance and species richness of parasitoids found in vineyards and their associated natural habitats. A key objective was to determine if known mealybug parasitoids can survive in the natural vegetation. A further goal was to determine if any unknown mealybug parasitoids not previously recorded are found in natural areas. These could be explored for further development in augmentative release programmes. This area of research is novel and as yet underexplored in South African vineyard agro-ecosystems, in particular the natural areas that are so intricately associated with agricultural areas.

2.2 MATERIALS AND METHODS

2.2.1 Sites

One vineyard on each of three farms situated in mountainous areas were selected for the survey based on their proximity to natural vegetation and previous history of *P. ficus* infestation (Figure 2.1). All three vineyards were located in valleys adjoining natural vegetation. Bouchard Finlayson, located in the Hemel-and-Aarde Valley near Hermanus, is surrounded by the Fernkloof Nature Reserve and several conservancies. The terrain ranges in altitude from sea level to 842m and the climate is warm rather than hot in summer, with mild, frost-free winters. Stark-Condé adjoins the Jonkershoek Nature Reserve near Stellenbosch at an altitude of between 150 and 600m, with a cooler summer climate and high winter rainfall. Plaisir de Merle is located at the foot of the Simonsberg Mountain, which forms the Greater Simonsberg Conservancy in Stellenbosch, at an altitude between 180 and 500m, and with moderate summers and wet winters. Vineyard blocks surveyed are described in Table 2.1. Both *Coccidoxenoides perminutus* and *Cryptolaemus montrouzieri* were released at Plaisir de Merle during the trial period.



Fig. 2.1. Map indicating sites surveyed for vine mealybug parasitoids associated with vineyards and adjoining natural vegetation.

Table 2.1. Site description of vineyards and surrounding vegetation surveyed for natural enemies of *Planococcus ficus* from January 2012 to October 2013.

Farm	GPS co-ordinates	Altitude	Cultivar	Soil type	Insecticide use	Vegetation Type*
Bouchard	-34.379443,	165m	Pinot noir	Bokkeveld	No	Western
Finlayson	19.249666			Shale		Coastal Shale Band Vegetation
Plaisir de Merle	-33.861257, 18.932527	325m	Chardonnay	Hutton	No	Boland Granite Fynbos
Stark- Condé	-33.951957, 18.913475	244m	Cabernet franc	Hutton	No	Boland Granite Fynbos

*Mucina & Rutherford, 2006

2.2.2 Source colonies and sampling

Mealybug stock cultures were reared on butternuts (*Cucurbita moschata*) in cages (750mm x 500mm x 300mm) in an insectary at a temperature of 25°C and a 12:12 (light:dark) photoperiod. The cages had glass panels on the sides and a fine mesh lid, to allow air in whilst preventing infestation by parasitoids. Spoiled butternuts were removed and replaced with fresh butternuts, surface sterilized with Sporekill® (100ml/100l), to which crawlers could move. Cultures were supplemented with mealybugs from the insectary at ARC Infruitec-Nietvoorbij.

Sampling took place from January 2012 until October 2013. Throughout the year, every two weeks, whole mealybug-infested butternuts were put in polystyrene fast-food containers (8cm x 14cm x 24cm) and placed in the field. On each farm one butternut was placed in a vineyard block (approximately one ha in size) by attaching it to the main cordon of a vine close to the centre of the block, and one butternut in the surrounding natural vegetation, attached to a small tree or shrub. At Stark-Condé and Plaisir de Merle the butternuts in the natural habitat were about five meters from the vineyard, as these blocks are adjacent to a ravine, but at Bouchard Finlayson, the butternut in the natural habitat was placed about a hundred meters away from the vineyard.

After two weeks, these butternuts were collected from the field and replaced with freshly infested butternuts. The collected butternuts were placed in 2ℓ plastic bottles that had been cut open at the bottom and reassembled when the butternut was placed inside, and covered in black plastic. The bottle top was replaced with a vial to collect emerging parasitoids. The butternuts were left in these bottles for about six weeks, after which all natural enemies that had emerged over this period were collected and placed in 90% ethanol to be identified. Parasitoids were sorted to morphospecies and a reference collection sent to Dr Gerhard Prinsloo at the ARC Biosystematics Division in Pretoria for species identifications.

2.2.3 Statistical analyses

Data was tested for homogeneity and normality before being subjected to a factorial analysis of variance (ANOVA) with number of parasitoid species as the dependant variable; and farm and habitat type as the main effects. A correspondence analysis was performed using five parasitoid species as row variables and the six sites as column variables to assess any associations between farms. All statistical analyses were conducted in Statistica, version 12 (Statsoft Inc., 2013).

2.3 RESULTS AND DISCUSSION

Parasitoids were recovered from the vineyards and surrounding natural habitat from January 2012 to May 2012 (Figure 2.2). No further parasitoids were reared from the butternuts from June 2012 until October 2013, despite additional sampling efforts. Parasitoid numbers peaked in February 2012 but other than that numbers were fairly low. The parasitoid catches on Plaisir de Merle in July 2012 can be attributed to augmentative releases on the farm. Walton and Pringle (2004b) found a peak in parasitoids during November which led to good control of most mealybug colonies by February or March (Walton, 2003). Monitoring data from Plaisir de Merle showed that after the trial period only one vine in a 2ha block was infested with mealybug and similar infestation levels were found on Bouchard Finlayson and Stark Condé (personal communication with farmers). This could explain the low numbers of parasitoids in 2012, as well as not finding any parasitoids

in 2013. On Plaisir de Merle parasitoids and predators were released by producers, but on Bouchard Finlayson and Stark Condé no chemical or biological control was used. The reason for the decline in mealybug numbers from previous years is therefore unclear, although actual mealybug monitoring data from previous years was not available.

Walton and Pringle (2004a) found three primary parasitoids, *Anagyrus* spp, *L. dactylopii* and *C. perminutus*, which were also found in earlier studies by Whitehead (1957) and Urban and Greeff (1985). These parasitoids were still the dominant parasitoids in this study (Table 2.2), with *C. perminutus* the most prolific, whereas *Anagyrus* spp. were the most prolific in the 2004 survey (Walton & Pringle, 2004a).

It was found that there was a significant difference between habitat types, $F_{(1, 594)}=3.8676$, $p=0.049$, with significantly more parasitoids (combined) occurring in vineyards than in adjoining natural habitats (Table 2.2, Fig. 2.3). No significant difference was found between farms, $F_{(2, 594)}=0.0006$, $p=0.99$, or between the interaction between farm and habitat type, $F_{(2, 594)}=0.2834$, $p=0.75$.

According to recommendations from Du Roi IPM and Vital Bugs, releases on farms range from 2 releases of 10 000 parasitoids every 3 weeks, to 4 releases of 60 000 every 2 weeks during the growing season. Walton (2003) worked with 20 000 parasitoids every four weeks. Numbers reared from mealybugs in the field from the current study indicate that the field population may not be high enough to afford good biological control. This is perhaps not an unexpected result, as host numbers would supposedly not be as high in natural areas compared to cultivated areas. To increase parasitoid numbers it might therefore be necessary to determine what other host plants for *P.ficus* can be found in the natural habitats surrounding vineyards. It is important to ensure that other host plants will serve as trap crops for mealybugs and not as a source for new populations to migrate towards vineyards.

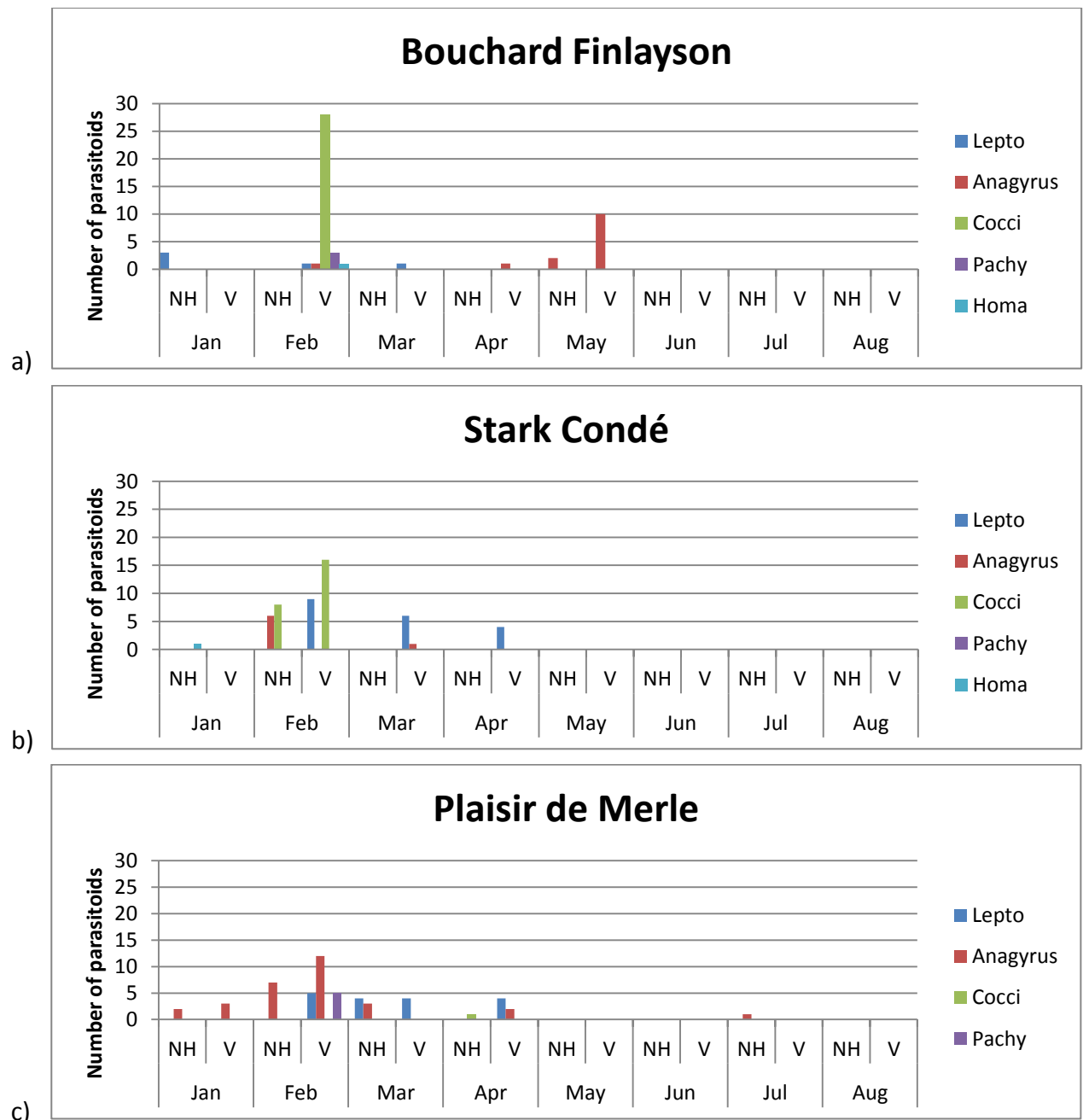


Fig. 2.2. Abundance of parasitoids Bouchard Finlayson in Hermanus (a), Stark Condé in Stellenbosch (b) and Plaisir de Merle in Simondium (c) from January 2012 to August 2012. Total number of each species of parasitoid species caught in the Natural Habitat (NH) or Vineyard (V) was plotted against the month in which the butternut was removed from the field. Abbreviations: *Anagyrus* sp. near *pseudococci* (*Anagyrus*), *Coccidoxenoides perminutus* (*Cocci*), *Leptomastix dactylopii* (*Lepto*), *Pachyneuron* spp. (*Pachy*) and *Homalotylus* spp. (*Homa*).

Table 2.2. Total number of parasitoids reared from vine mealybugs (*Planococcus ficus*) in three different locations from natural habitats (NH) and adjoining vineyards (V) from January 2012 to August 2012.

Species	Total nr. caught		Bouchard Finlayson		Stark Condé		Plaisir de Merle	
	NH	V	NH	V	NH	V	NH	V
<i>Anagyrus</i> sp. near <i>pseudococci</i> (Females)	14	17	2	11	9	5	3	1
<i>Anagyrus</i> sp. near <i>pseudococci</i> (Males)	7	13		1	4	12	3	
<i>Pachyneuron</i> spp.	0	8		3	5			
<i>Leptomastix dactylopii</i>	7	34	3	2	4	13		19
<i>Coccidoxenoides perminutus</i>	9	44		28	1		8	16
<i>Homalotylus</i> spp.	1	1		1			1	
Totals	38	117	5	46	23	30	15	36

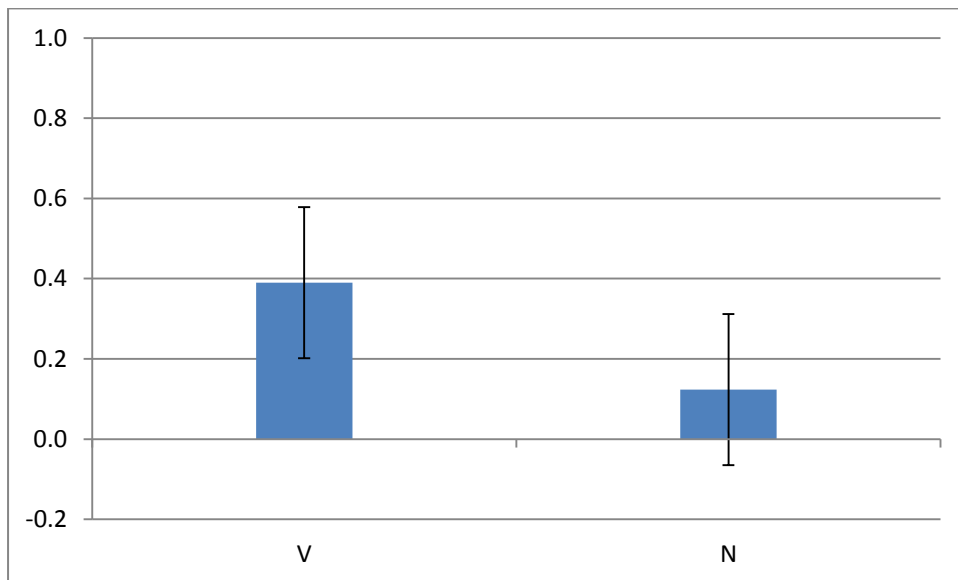


Fig. 2.3. Comparison of the mean number of parasitoids reared from mealybugs in the Vineyards (V) versus in the Natural Habitat (N) from January 2012 to August 2012 at three sites in the Western Cape Province. Error bars denote 95% confidence intervals.

Multivariate-data analysis was used to determine the association between six sites on three farms and the five species of parasitoid found on these sites (Figure 2.4). Dimension 1 accounted for 61.86% of the variation in the graph with an Eigenvalue of 0.328, which represents 100% of the variance. *C. perminutus* was strongly associated with the vineyards on Bouchard Finlayson, as well as both the vineyards and natural habitat on Stark Condé. *A. sp. near pseudococci* was strongly associated with both the natural habitat and vineyards on Plaisir de Merle, as well as the vineyards on Bouchard Finlayson and the natural habitat on Stark Condé. *L. dactylopii* had the strongest association with the vineyards on Stark Condé, but none at all with the natural habitat in that same area. Table 2.2 gives a more detailed breakdown of the numbers of parasitoids found on the farms.

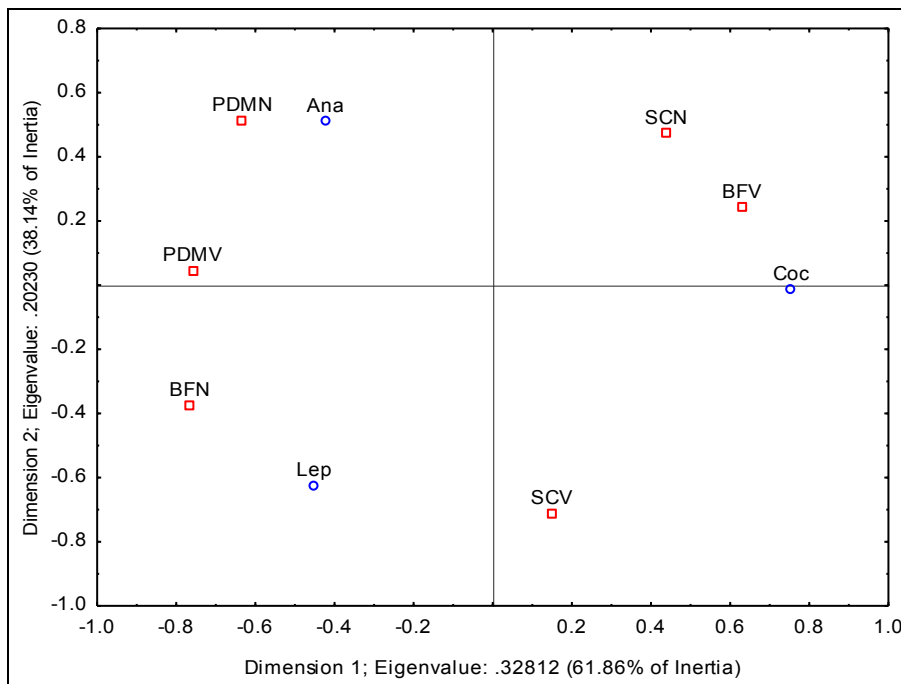


Fig. 2.4. Correspondence analysis illustrating the association between the six sites on three farms and the five parasitoid species found on these sites. Abbreviations: *Anagyrus sp. near pseudococci* (Ana), *Coccidoxenoides perminutus* (Coc), *Leptomastix dactylopii* (Lep), Stark-Condé Vineyard (SCV), Stark-Condé Natural Habitat (SCN), Bouchard Finlayson Vineyard (BFV), Bouchard Finlayson Natural Habitat (BFN), Plaisir de Merle Vineyard (PDMV) and Plaisir de Merle Natural Habitat (PDMN).

Various factors affect parasitoid levels in cultivated areas and adjacent natural habitats, including landscape compositions and temperature fluctuations. Previous surveys to determine the presence and abundance of pest parasitoids, yielded promising results (Ölmez & Ulusoy, 2003; Aslan et al., 2004). Between 1998 and 2000 a survey of aphid parasitoids in Turkey resulted in 16 species of aphid parasitoids found, most of which were found on fruit trees, ornamental trees and weeds in the natural ecosystem. In the pesticide-sprayed areas the species and density of the parasitoids were less, which showed that aphids in the natural areas are being controlled by their parasitoids (Ölmez & Ulusoy, 2003). In a subsequent survey in Turkey, 19 taxa of aphid parasitoids were found, with certain species attacking both economic and non-economic aphids (Aslan et al., 2004).

A recommendation would therefore be to find alternative hosts for the parasitoids that attack *P. ficus* to enhance their numbers, as well as to determine if any other parasitoids attack the mealybug. Early season mortality would affect the pest population more than later mortality and make the fields and its surroundings more favourable for parasitoids (Sigsgaard, 2002).

Despite potential services from wild occurring natural enemies such as Hymenoptera parasitoid species, there are several factors that can affect or diminish the effects of parasitoid biological control. There is thus a large gap between potential natural enemy biological control and realised biological control. Geiger et al. (2010) found that organic farming and other similar schemes that aim to diminish the negative effects of intensive farming improved the diversity of wild insect and plant species but not the diversity of breeding birds. This shows that the negative effects of harmful pesticides banned decades ago in Europe, still persist and reduce the opportunity for effective biological control. They advised a continent-wide shift toward farming with minimal pesticide-use.

A host population can be provided with refuge from parasitoid attack through environmental heterogeneity or patchiness (Crawley, 1992) thereby diminishing the effectiveness of biological control efforts. The main causes of this patchiness are host plant effects, host-induced effects or parasitoid effects. These effects include parasitoid deterrents such as leaf hairs (Woets & Van Lenteren, 1976), the production of large batches of eggs that exceed the ovipositional capacity of a parasitoid (Braune, 1982) and parasitoids

switching between hosts (Murdoch & Oaten, 1975; Mills & Getz, 1996). Intraspecific competition between parasitoids can also have consequences on the host-parasitoid interaction. For example, competition between adult parasitoids may affect the sex ratio of parasitoid larvae (Hassell et al., 1983; Comins & Wellings, 1985). However, by biasing the sex ratio to female progeny, as is the case of *C. perminutus*, a parasitoid population will increase its competitiveness and could ultimately exclude its competitors and even destroy its host population (Kaitala & Getz, 1992; Mills & Getz, 1996).

A parasitoid's impact on a host population depends on its ability to find and parasitise the host, as well as the ability to increase its offspring when needed (Mackauer, 1983; Waage & Hassell, 1982; He, 2008). This is determined by the functional response to host density and density-dependent sex ratio of the parasitoid (Hassel & Waage, 1984; He, 2008). Franco et al., (2004) found that the parasitoids only existed in significant numbers when a high density of mealybugs were found, but according to Van Lenteren (2012) hymenopterans are generally released at high numbers over large areas, which means they are able to find hosts even at lower densities. Walton (2003) determined a 2% stem infestation to be the economic threshold as stem infestation precedes bunch infestation. He also found that when plotting parasitoid numbers on their host numbers, a density dependent relationship could be seen.

The interaction between a parasitoid and its host is thought to be strongly influenced by density dependence (Summy et al., 1986). However, density dependence is not easily detected in the field, which suggests that it is not as important as previously thought (Walter & Zalucki, 1999; Davies et al., 2011a). This could also be ascribed to the statistical methods used when quantifying density dependence as having a big influence in the results obtained and positive density dependence may be far more common than previously suggested (Veldtman & McGeogh, 2004).

A differential rate of parasitism was concluded to be a result of non-host foraging, rather than a response to host numbers (Simmons et al., 1975; Baggen & Gurr, 1998), as an inverse density-dependent relationship was observed for *Copidosoma koehleri* Blanchard (Hymenoptera: Encyrtidae), a parasitoid of the potato moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) in the laboratory (Horne & Horne, 1991), and parasitism

rates did not significantly increase in field trials, even though a greater number of hosts were present (Baggen & Gurr, 1998). According to Walton & Pringle (2002), biological control in South Africa with *C. perminutus* was found to be more effective if *P.ficus* populations were low.

Activity of the parasitoids can be limited by environmental constraints such as chemical applications (Campbell et al., 1991; Scholz, 1994; Hassan et al., 1998), unsuitable climatic conditions (Orr et al., 1997) or habitat characteristics (Davies et al., 2011a, 2011b), even when a large number of hosts are present. A parasitoid population is therefore not completely dependent on host abundance, but also on environmental characteristics (Walter & Hengeveld, 2000; Davies et al., 2011b).

Host preference is another factor to consider, as many parasitoid species are polyphagous (Grabenweger & Lethmayer, 1999) and if a preferred host is abundant and available somewhere else, parasitoids may ignore an abundant non-preferred pest species within a crop. Similarly, if the pest species is a preferred host, parasitism levels may be very high (Davies et al., 2011b). *C. perminutus* does attack other mealybugs, including *Planococcus citri*, *Planococcus minor* and *Planococcus vovae* (Walton, 2003; Ceballo & Walter, 2004; Kaydan et al., 2006; Francis et al., 2012), but it is unknown which of these are the preferred species.

Host-parasitoid interactions are therefore rather outlined in terms of environmental constraints than density-dependence (Davies et al., 2011b).

2.4 CONCLUSION

The three sites chosen were all farms which do not use pesticides, with a history of mealybug infestations. The results indicate that mealybug parasitoids do persist where conditions are favourable. They are found outside of the crop habitat, which could make them valuable as an ecosystem service, although this study did not aim to quantify this. If sufficient nutrient and shelter resources can be put in the field for the parasitoids to be able to survive winter months, better control could be attained with less pesticide use. These results show that natural habitats typically found in the Western Cape agro-ecosystem could

possibly play a role in attracting mealybug parasitoids and maintaining populations in the field if they are planted close to vineyards and not necessarily within vineyards, which may be a more practical method of habitat management for producers. This indicates that these parasitoids, being density-independent (Walter & Zalucki, 1999; Davies et al., 2011a) and therefore not in need of high pest populations to sustain numbers, are truly valuable for integrated pest management and that with the correct habitat modifications, their numbers could be naturally boosted to lend a valuable eco-system service.

2.5 REFERENCES

- Aslan, M.M., Uygun, N. & Starý, P., 2004. A survey of aphid parasitoids in Kahramanmaras, Turkey (Hymenoptera: Braconidae, Aphidiinae; and Hymenoptera: Aphelinidae). *Phytoparasitica* 32(3): 255-263.
- Baggen, L.R. & Gurr, G.M., 1998. The influence of food on *Copidosoma koehleri* (Hymenoptera: Encyrtidae), and the use of flowering plants as a habitat management tool to enhance biological control of potato moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae). *Biological Control* 11: 9-17.
- Ben-Dov, Y., 1994. *A systematic catalogue of the mealybugs of the world (Insecta: Homoptera: Coccoidea: Pseudococcidae and Putoidae) with data on geographical distribution, host plants, biology and economic importance*. Intercept Limited, Andover, UK.
- Berlinger, M.J., 1977. The Mediterranean vine mealybug and its natural enemies in southern Israel. *Phytoparasitica* 5: 3-14.
- Braune, H.J., 1982. Effect of the structure of the host egg-mass on the effectiveness of an egg parasite of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). *Drosera* 1: 7-16.
- Campbell, C.D., Walgenbach, J.F. & Kennedy, G.G., 1991. Effect of parasitoids on lepidopterous pests in insecticide-treated and untreated tomatoes in western North Carolina. *Journal of economic entomology* 84(6): 1662-1667.
- Ceballo, F.A. & Walter, G.H., 2004. Why is *Coccidoxenoides perminutus*, a mealybug parasitoid, ineffective as a biocontrol agent – Inaccurate measures of parasitism or low adult survival? *Biological Control* 33: 260-268.
- Charles, J.G., 1982. Economic damage and preliminary thresholds for mealybugs in Auckland vineyards. *New Zealand Journal of Agricultural Research* 25: 415-420.
- Comins, H.N. & Wellings, P.W., 1985. Density-related parasitoid sex-ratio: influence of host-parasitoid dynamics. *Journal of Animal Ecology* 54: 583-594.
- Crawley, M. J., 1992. Population dynamics of natural enemies and their prey. *Natural enemies: the population biology of predators, parasites and diseases*, pp. 40-89.

Cronin, J.T. & Reeve, J.D., 2005. Host-parasitoid spatial ecology: a plea for a landscape-level synthesis. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 272: 2225–2235.

Daane, K.M., Bentley, W.J., Walton, V.M., Malakar-Kuenen R., Millar, J.G., Ingels, C.A., Weber, E.A. & Gispert, C., 2006. New controls investigated for vine mealybug. *California Agriculture* 60(1): 31-38.

Daane, K.M., Cooper, M.L., Triapitsyn, S.V., Walton, V.M., Yokota, G.Y., Haviland, D.R., Bentley, W.J., Godfrey, K.E. & Wunderlich, L.R., 2008. Vineyard managers and researchers seek sustainable solutions for mealybugs, a changing pest complex. *California Agriculture* 62(4): 167-176.

Davies, A.P., Pufke, U.S. & Zalucki, M.P., 2011a. Spatio-temporal variation in *Helicoverpa* egg parasitism by *Trichogramma* in a tropical *Bt*-transgenic cotton landscape. *Agricultural and Forest Entomology* 13(3): 247-258.

Davies, A.P., Carr, C.M., Scholz, B.C.G. & Zalucki, M.P., 2011b. Using *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) for insect pest biological control in cotton crops: an Australian perspective. *Australian Journal of Entomology* 50: 424-440.

De Villiers, M. & Pringle, K.L., 2007. Seasonal occurrence of vine pests in commercially treated vineyards in the Hex River Valley in the Western Cape Province, South Africa. *African Entomology* 15(2): 241-260.

Elliott, N.C., Kieckhefer, R.W., Lee, J.H. & French, B.W., 1999. Influence of within-field and landscape factors on aphid predator populations in wheat. *Landscape Ecology* 14: 239–252.

Elliott, N.C., Kieckhefer, R.W. & Beck, D.A., 2002a. Effect of aphids and the surrounding landscape on the abundance of Coccinellidae in cornfields. *Biological Control* 24: 214–220.

Elliott, N.C., Kieckhefer, R.W., Michels, G.J. & Giles, K.L., 2002b. Predator abundance in alfalfa fields in relation to aphids, within-field vegetation, and landscape matrix. *Environmental Entomology* 31: 253–260.

Engelbrecht, D.J. & Kasdorf, G.G.F., 1990. Transmission of grapevine leafroll disease and associated closteroviruses by the vine mealybug, *Planococcus ficus*. *Phytophylactica* 22: 341-346.

Francis, A.W., Kairo, M.T., Roda, A.L., Liburd, O.E. & Polar, P., 2012. The passionvine mealybug, *Planococcus minor* (Maskell) (Hemiptera: Pseudococcidae), and its natural enemies in the cocoa agroecosystem in Trinidad. *Biological Control* 60(3): 290-296.

Franco, J.C., Suma, P., da Silva, E.B., Blumberg, D. & Mendel, Z., 2004. Management strategies of mealybug pests of citrus in Mediterranean countries. *Phytoparasitica* 32(5): 507-522.

Geiger, F., Bengtsson, J., Berendse, F., Weisser, W.W., Emmerson, M., Morales, M.B., Ceryngier, P., Liira, J., Tscharntke, T., Winqvist, C., Eggers, S., Bommarco, R., Pärt, T., Bretagnolle, V., Plantegenest, M., Clement, L.W., Dennis, C., Palmer, C., Oñate, J.J., Guerrero, I., Hawro, V., Aavik, T., Thies, C., Flohre, A., Hänke, S., Fischer, C., Goedhart, P.W. & Inchausti, P., 2010. Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland. *Basic and Applied Ecology* 11(2): 97-105.

Grabenweger, G. & Lethmayer, C., 1999. Occurrence and phenology of parasitic Chalcidoidea on the horse chestnut leafminer, *Cameraria ohridella* Deschka & Dimic (Lep., Gracillariidae). *Journal of Applied Entomology* 123(5): 257-260.

Greiler, H.J., Vidal, S. & Tscharntke, T., 1992. Abundance and species richness of Chalcidoidea (Hymenoptera) in fallows and cultivated fields (malaise-trap samples). In *Proceedings of the 4th European Congress of Entomology Budapest*, pp. 299-302.

Hassan S.A., Hafes, B., Degrande, P.E. & Herai, K., 1998. The side-effects of pesticides on the egg parasitoid *Trichogramma cacoeciae* Marchal (Hym., Trichogrammatidae), acute dose-response and persistence tests. *Journal of Applied Entomology* 122: 569-573.

Hassell, M.P. & Waage, J.K., 1984. Host-parasitoid population interactions. *Annual Review of Entomology* 29(1): 89-114.

Hassell, M.P., Waage, J.K. & May, R.M., 1983. Variable parasitoid sex ratios and their effect on host-parasitoid dynamics. *Journal of Animal Ecology* 52: 889-904.

- Hawkins, B.A. & Gross, P., 1992. Species richness and population limitation in insect parasitoid-host systems. *American Naturalist* pp. 417-423.
- He, X.Z., 2008. *Reproductive behaviour of Aphidius ervi Haliday (Hymenoptera: Aphidiidae)*. PhD dissertation, Palmerston North, New Zealand: Massey University.
- Horne, P.A. & Horne, J.A., 1991. The effects of temperature and host density on the development and survival of *Copidosoma koehleri*. *Entomologia Experimentalis et applicata* 59: 289–292.
- Joyce, A.L., Hoddle, M.S., Bellows, T.S. & Gonzalez, D., 2001. Oviposition behavior of *Coccidoxenoides peregrinus*, a parasitoid of *Planococcus ficus*. *Entomologia Experimentalis et applicata* 98(1): 49-57.
- Kaitala, V. & Getz, W.M., 1992. Sex ratio genetics and the competitiveness of parasitic wasps. *Bulletin of Mathematical Biology* 54: 295-311.
- Kaydan, M. B., Kilincer, N., Uygun, N., Japoshvilli, G. & Gaimari, S., 2006. Parasitoids and predators of pseudococcidae (Hemiptera: Coccoidea) in Ankara, Turkey. *Phytoparasitica* 34(4): 331-337.
- Kriegler, P.J., 1954. *'n Bydrae tot die kennis van Planococcus citri (Risso) (Homoptera: Pseudococcidae)* (in Afrikaans). PhD dissertation, Department of Conservation Ecology and Entomology, Stellenbosch University, Private Bag X1, 7620 Matieland (Stellenbosch), South Africa.
- Kruess, A. & Tscharntke, T., 1994. Habitat fragmentation, species loss, and biological control. *Science (Washington)* 264(5165): 1581-1584.
- Landis, D.A., Wratten, S.D. & Gurr, G.M., 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology* 45: 175–201.
- LaSalle, J. & Gauld, I.D., 1991. Parasitic Hymenoptera and the biodiversity crisis. *Redia* 74(3): 315-334.
- LaSalle, J. & Gauld, I.D., 1993. *Hymenoptera and biodiversity*. CAB International, pp. 197-215.

- Mackauer, M., 1983. Quantitative assessment of *Aphidius smithi* (Hymenoptera: Aphidiidae): fecundity, intrinsic rate of increase, and functional response. *The Canadian Entomologist* 115(4): 399-415.
- Mills, N.J. & Getz, W.M., 1996. Modelling the biological control of insect pests: a review of host-parasitoid models. *Ecological Modelling* 92: 121-143.
- Mucina, L. & Rutherford, M.C., 2006. *The vegetation of South Africa, Lesotho and Swaziland*. South African National Biodiversity Institute.
- Murdoch, W.W. & Oaten, A., 1975. Predation and population stability. *Advances in Ecological Research* 9: 1-131.
- Myers, N., Mittermeier, R.A., Mittermeier, C.G., Da Fonseca, G.A. & Kent, J., 2000. Biodiversity hotspots for conservation priorities. *Nature* 403(6772): 853-858.
- Ölmez, S. & Ulusoy, M.R., 2003. A survey of aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae) in Diyarbakır, Turkey. *Phytoparasitica* 31(5): 524-528.
- Orr D.B., Landis, D.A., Mutch, D.R., Manley, G.V., Stuby, S.A. & King, R.L., 1997. Ground cover influence on microclimate and *Trichogramma* (Hymenoptera: Trichogrammatidae) augmentation in sweet corn. *Environmental Entomology* 26: 433-438.
- Rand, T.A., Tylianakis, J.M. & Tscharntke, T., 2006. Spillover edge effects: the dispersal of agriculturally subsidized insect natural enemies into adjacent natural habitats. *Ecology Letters* 9(5): 603-614.
- Scholz, B.C.G., 1994. The effect of insecticides on the survival of *Heliothis* egg parasitoids. In: *Proceedings of the 7th Australian Cotton Conference*, pp. 69-73. 10-12 August, Broadbeach, Queensland, Australia.
- Sigsgaard, L., 2002. A survey of aphids and aphid parasitoids in cereal fields in Denmark, and the parasitoids' role in biological control. *Journal of Applied Entomology* 126(2-3): 101-107.
- Simmons, G.A., Leonard, D.E. & Chen, C.W., 1975. Influence of tree species density and composition on parasitism of the spruce budworm, *Choristoneura fumiferana* (Clem.). *Environmental Entomology* 4: 832-836.

Stork, N.E., 1988. Insect diversity: facts, fiction and speculation. *Biological journal of the Linnean Society* 35(4): 321-337.

Summy, K.R., French, J.V. & Hart, W.G., 1986. Citrus mealybug (Homoptera: Pseudococcidae) on greenhouse citrus: density-dependent regulation by an encyrtid parasite complex. *Journal of Economic Entomology* 79: 891-895.

Tscharntke, T., Klein, A.M., Kruess, A., Steffan-Dewenter, I. & Thies, C., 2005. Landscape perspectives on agricultural intensification and biodiversity – ecosystem service management. *Ecology Letters* 8: 857-874.

Tylianakis, J.M., Didham, R.K. & Wratten, S.D., 2004. Improved fitness of aphid parasitoids receiving resource subsidies. *Ecology* 85: 658-666.

Urban, A.J. & Greeff, F.C., 1985. The integrated control of the vine mealybug *Planococcus ficus* (Signoret), on vines. Final Report. *Plant Protection Research Institute*.

Van Lenteren, J.C., 2012. The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. *BioControl* 57(1): 1-20.

Veldtman, R. & McGeoch, M.A., 2004. Spatially explicit analyses unveil density dependence. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 271(1556): 2439-2444.

Waage, J.K. & Hassell, M.P., 1982. Parasitoids as biological control agents – a fundamental approach. *Parasitology* 84(4): 241-268.

Walter, G.H. & Hengeveld, R., 2000. The structure of two ecological paradigms. *Acta Biotheoretica* 48: 15-46.

Walter, G.H. & Zalucki, M.P., 1999. Rare butterflies and theories of evolution and ecology. In: *Biology of Australian Butterflies* (eds R.E. Jones & N.E. Pierce), pp. 349-368. CSIRO Publishing, Canberra, Australia.

Walton, V.M., 2003. *Development of an integrated pest management system for vine mealybug, Planococcus ficus (Signoret), in vineyards in the Western Cape Province, South*

Africa. PhD dissertation, Department of Conservation Ecology and Entomology, Stellenbosch University, Private Bag X1, 7620 Matieland (Stellenbosch), South Africa.

Walton, V.M. & Pringle, K.L., 2002. Evaluating effectiveness of mass releases of the vine mealybug (*Planococcus ficus*) parasitoid *Coccidoxenoides peregrinus* in Western Cape province vineyards, South Africa. In *Proceedings of the 1st International Symposium on Biological Control of Arthropods, Honolulu, Hawaii*, pp. 14-18.

Walton, V.M. & Pringle, K.L., 2004a. A survey of mealybugs and associated natural enemies in vineyards in the Western Cape provinces. *South African Journal of Enology and Viticulture* 25(1).

Walton, V.M. & Pringle, K.L., 2004b. Vine mealybug, *Planococcus ficus*, (Signoret) (Hemiptera: Pseudococcidae), a key pest in South African vineyards. A Review. *South African Journal of Enology and Viticulture* 25(2).

Whitehead, V. B. (1957). *A study of the predators and parasites of Planococcus citri (Risso)(Homoptera: Pseudococcidae) on vines in the Western Cape Province, South Africa.* PhD dissertation, Rhodes University, Grahamstown.

Woets, J. & Van Lenteren, J.C., 1976. The parasite-host relationship between *Encarsia formosa* (Hymenoptera: Aphelinidae) and *Trialeurodes uaporariorum* (Homoptera: Aleyrodidae). VI. The influence of the host-plant on the greenhouse whitefly and its parasite *Encarsia formosa*. *Proc. 3rd Conf. Biol. Control Greenhouses*. OILB/SROP, 76: 125-137.

CHAPTER THREE:**RESPONSES OF *COCCIDOXENOIDES PERMINUTUS* (TIMBERLAKE) (HYMENOPTERA: ENCYRTIDAE) TO OLFACTORY CUES FROM FLOWERING PLANTS****3.1 INTRODUCTION**

Biological control using augmentative releases of predators and parasitoids is the major alternate method used for managing pest populations of the vine mealybug, *Planococcus ficus* (Signoret)(Hemiptera: Pseudococcidae) in South Africa (Walton & Pringle, 2004).

According to recommendations from Du Roi IPM and Vital Bugs, releases per hectare range from 2 releases of 10 000 parasitoids every 3 weeks, to 4 releases of 60 000 parasitoids every 2 weeks during the growing season, which amounts to between R472.00 and R5,664.00 per growing season. Viticultural areas in South Africa are largely concentrated in the Western Cape and fall in a biodiversity hotspot, the Cape Floristic Region (Goldblatt & Manning, 2012). With vineyards often integrated within conservation areas, conservation biological control (i.e. manipulation of the agricultural environment to improve establishment of parasitoids) seems plausible, if the manipulation could include indigenous vegetation already pre-adapted to the environment. Habitat manipulation can be done by lessening harmful conditions or increasing favourable ones. It can be done in a number of ways, including by providing supplementary resources and manipulating host plants to benefit natural enemies (Van den Bosch & Telford, 1964; Rabb et al., 1976; Landis et al, 2000). Factors that have been identified as harmful for natural enemies in agricultural systems include pesticides, lack of non-host food for adults and lack of alternative hosts (Rabb et al., 1976; Powell, 1986; Dutcher, 1993; Landis et al., 2000).

Various on-farm management practices, like the planting of non-crop habitats adjacent to crop fields (Landis et al, 2000), have been implemented to increase the diversity and abundance of natural enemies. These non-crop habitats can influence natural enemies by providing shelter from crop disturbances or unavoidable pesticide application (Landis et al., 2000), refuges for overwintering, alternative hosts or prey, and additional sources of non-host food (Thies et al, 2003; Bianchi et al, 2006). In agricultural systems this is a practice referred to as habitat management (Woltz et al., 2012). Some adult parasitoid species can obtain all their required resources from their hosts (Jervis & Kidd, 1986), but many other

adult parasitoid species consume non-host food. These parasitoids get nutrients from honeydew, nectar or pollen (van Emden, 1963; Powell, 1986; Jervis et al., 1993; Jervis et al, 1996; Landis et al, 2000) and in a lot of these species, these nutrients can enhance fecundity, longevity (Leius, 1961; Idris and Grafius, 1995; Irvin et al, 1999) and efficiency of parasitism (Powell, 1986; Stapel et al, 1997). Parasitoids are capable of finding these non-host food sources through visual (Wackers, 1994) or olfactory cues (Takasu & Lewis, 1996). Most modern agricultural systems, like vineyards or orchards, are monocultures and therefore lack flowers that could provide these resources (Landis et al, 2000). This means that effectiveness of the parasitoids could be significantly reduced. When broad-spectrum pesticides are used, this does not really matter, but as the current trend is to move away from inorganic methods of pest control, the availability of floral resources could enhance parasitism rates (Berndt et al, 2002).

Previous studies have tested several flowering plant species to enhance the efficiency of parasitoids (Bowie et al, 1995; Idris & Grafius, 1995), with important characteristics for selection being accessible nectar, time of flowering in synchronization with occurrence of the pest and parasitoid, and an ease of cultivation appropriate to on-farm conditions (Landis et al, 2000; Berndt et al, 2002). It is also important to ensure that the selected plant should not become invasive in future (Landis et al, 2000). Another significant factor to bear in mind when choosing flowers as nectar sources is the shape and form of the flower, as this affects accessibility (Idris & Grafius, 1995; Orr & Pleasants, 1996). Irvin (1999) tested suitable species for enhancing leafroller parasitoids in apple orchards in New Zealand, and found buckwheat, *Fagopyrum esculentum* Moench, and alyssum, *Lobularia maritime* (L) Desv., to be the best at enhancing parasitoid abundance and leafroller parasitism. Nicholls et al. (2000) also found that buckwheat enhanced parasitoid populations in a vineyard in California (Berndt et al, 2002). So theoretically the presence of floral resources should increase hymenopteran parasitoid abundance. The need for added nutrients combined with the ability to find these resources should result in a greater abundance of parasitoids when mealybug hosts are present. This has been found in a number of studies on parasitic Hymenoptera (van Emden, 1963; Chaney, 1998; Stephens et al, 1998; Platt et al, 1999; Berndt et al, 2002). However, studies using floral strips have had different rates of success, with some studies showing no effect on parasitoid abundance (Bigger & Chaney, 1998; Koji

et al, 2007) and others leading to increased abundance of parasitoids and decreases in pest populations (Hickman & Wratten, 1996; Langer & Hance, 2004, Lee & Heimpel, 2005; Woltz et al., 2012).

Studies on *Coccidoxenoides perminutus* (Timberlake) (Hymenoptera: Encyrtidae), an endoparasitic, parthenogenic parasitoid of *P. ficus* (Signoret) (Ceballo & Walter, 2004), have shown that fecundity and lifespan of the adult parasitoid in laboratory trials are highest when *Alpinia* nectar or honey is supplied as a food source (Davies et al., 2004). With few studies of this nature having been conducted in South African vineyards, the aim of this study was to screen suitable indigenous flowering plants for their attractiveness to *C. perminutus* under controlled laboratory conditions. The key criteria used for selecting the plants were that they should: 1) flower in late winter/early spring, to provide an abundance of food sources for the parasitoids early in the season. This way, mealybug populations will be managed when their own populations are still low, as parasitoids are more able to cope with low density pest populations (Walton & Pringle, 2002); 2) have small flowers, as previous studies found short or no corolla tubes to be ideal for parasitoid feeding (Idris & Grafius, 1995; Orr & Pleasants, 1996); and 3) be water-wise and indigenous. A secondary survey was done to determine on which flowering plants parasitoids could be found in the field. This was done to determine the abundance and diversity of parasitoids occurring in the vineyards and natural habitats that could have value for biological control of vineyard pests, in particular vine mealybug.

3.2 MATERIALS AND METHODS

3.2.1 Attractance or repellence of parasitoids to flowering plants.

Six plants were chosen for this trial (Table 3.1) with the idea that the various plants have overlapping flowering times, in order to provide resources for the parasitoids for as long a period as possible, and further follow the criteria set out in the introduction above.

Table 3.1. Six indigenous flowering plants chosen for the experiments.

Latin name	Family name	Common name
<i>Tulbaghia violacea</i> Harv.	Alliaceae	Wild garlic
<i>Coleonema pulchellum</i> I. Williams	Rutaceae	Confetti bush
<i>Felicia bergeriana</i> (Spreng.) O. Hoffm.	Asteraceae	Kingfisher daisy
<i>Gnidia pinifolia</i> L.	Thymelaeaceae	White Gnidia
<i>Euryops abrotanifolia</i> (L.) DC.	Asteraceae	Lace-leaf Euryops
<i>Erica gracillus</i> J.C. Wendl.	Ericaceae	Cape Heath



Fig. 3.1. Images depicting the different flowering plants used in the experiments, (a) *Tulbaghia violacea*, (b) *Coleonema pulchellum*, (c) *Felicia bergeriana*, (d) *Gnidia pinifolia*, (e) *Euryops abrotanifolia* and (f) *Erica gracillus*.

3.2.2 Parasitoid colony

The *Coccidoxenoides perminutus* mother colony has been maintained on mealybug-infested (*P. citri*) butternuts at Du Roi IPM in Letsitele, Limpopo, South Africa for 14 years at 26 ± 2 °C, an average RH of $54 \pm 10\%$ and a 9:15 L:D photoperiod. Parasitoids were obtained in pupal form from Du Roi IPM and a colony was maintained on mealybug-infested (*P. ficus*) butternuts from ARC-Infruitec Nietvoorbij in Stellenbosch, Western Cape, South Africa. In Stellenbosch the colony was kept in a temperature-controlled room at a temperature of 25°C and a 12:12 hour light:dark photoperiod.

3.2.3 Experimental set-up and procedure

Trials were conducted in the laboratory. A clear, 70ℓ odourless plastic bucket with a lid was turned upside down and two holes made on opposite sides of the bucket (Fig. 3.2).

In the one hole a small bag with about 6g of activated charcoal was placed, to purify the air coming into the experiment. On the other end the tube leading towards the olfactometer (Fig. 3.3) was placed. Only the air drawn through the bucket was filtered so as to ensure pure odour from the plants.



Fig. 3.2. Experimental set-up for *C. perminutus* olfactory experiments. An upside-down, clear 70ℓ bucket with two holes on opposite ends houses the host plant. In one hole a bag with about 6g of activated charcoal, to purify the air coming into the experiment and the other hole connected via a glass tube to the olfactometer.

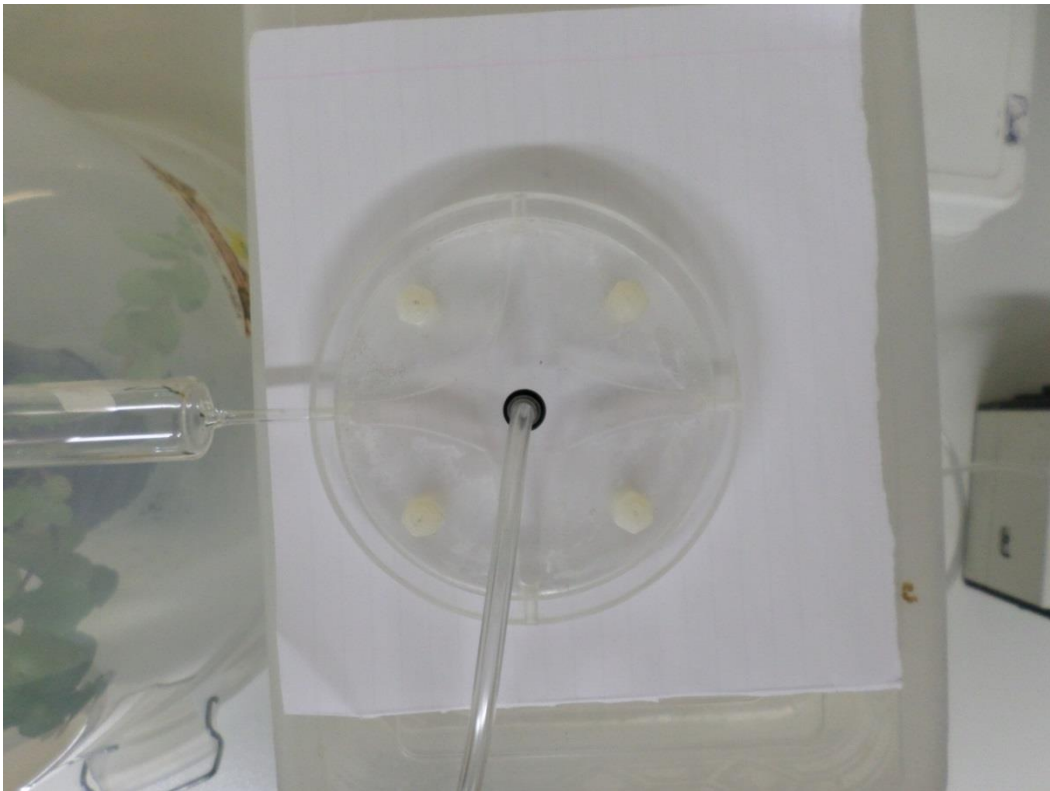


Fig.3.3. Olfactometer seen from above.

The olfactometer created four distinct odour fields, enabling the determination of an attractive or repellent odour by observing the insect. In this experiment three of the odour fields were left empty as a control and the fourth was attached to the flowering plant, as described by Pettersson et al. (1998) and Birkett et al. (2000). To minimise any plant reactions or volatiles being released and altering the outcome of the experiment, whole plants were used instead of just leaves or flowers. The plant, in its black plastic bag with soil, was placed inside the experimental arena, on the lid. The olfactometer used has been previously described by Pettersson et al (1998). It is made up of three transparent Perspex layers screwed together with the middle layer having an exposure chamber with four arms cut out. The four arms each had a gauze-covered inlet, one of which was attached to the odour source. The air flowed through the inlets, drawing the odour or control air into the chamber and out through the arena. The airflow was set at 300ml/s, through the activated charcoal (Vet et al., 1983), which was drawn over the plant or through the control inlets and then pulled into the olfactometer's exposure chamber. A single, newly hatched female parasitoid, that had not been allowed to feed, was placed in the centre of the exposure chamber. As soon as the airflow was switched on, the parasitoid's movements between the different odour fields in the exposure chamber was recorded, for 5 minutes. If a parasitoid didn't move much, it was classified a "sitter" and replaced by another parasitoid. As three arms were used as a control, the time spent there was compensated for by dividing the total time by three before further calculations were made.

This experiment was repeated five times per plant, with a different parasitoid individual for each repetition. Five plants of each species were used, which resulted in a total of 25 repetitions per plant species. After each repetition the olfactometer's exposure chamber was washed out with 70% alcohol and the arms rotated. With every change of plant, the bucket was also wiped down with alcohol to ensure an unbiased result.

The averages of visits in arms with the same odour sources were compared through paired t-tests, as described by Pettersson (1970) and Pettersson et al. (1998) and assumed an even distribution of visits. All statistical analyses were conducted using Statistica, version 12 (Statsoft Inc., 2013).

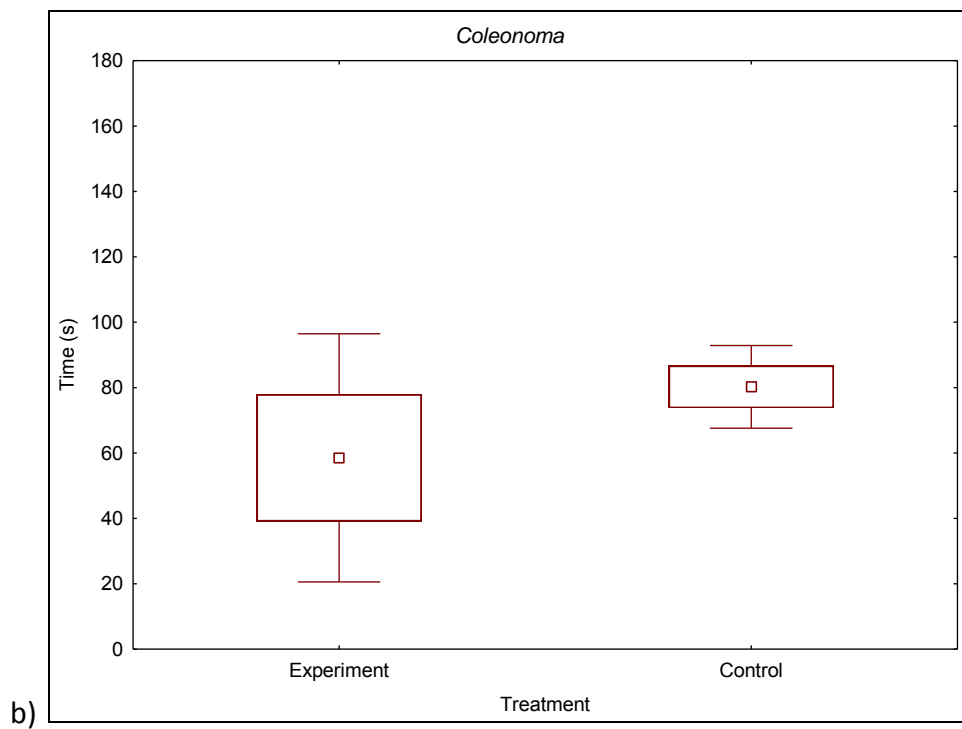
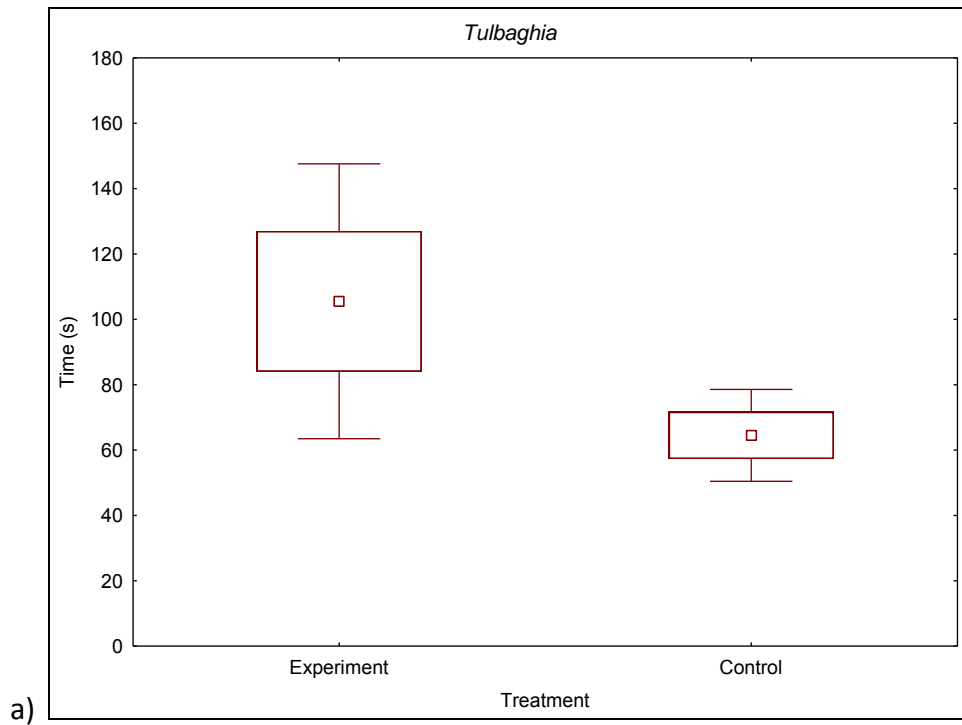
3.2.5 Survey of parasitoids in the field

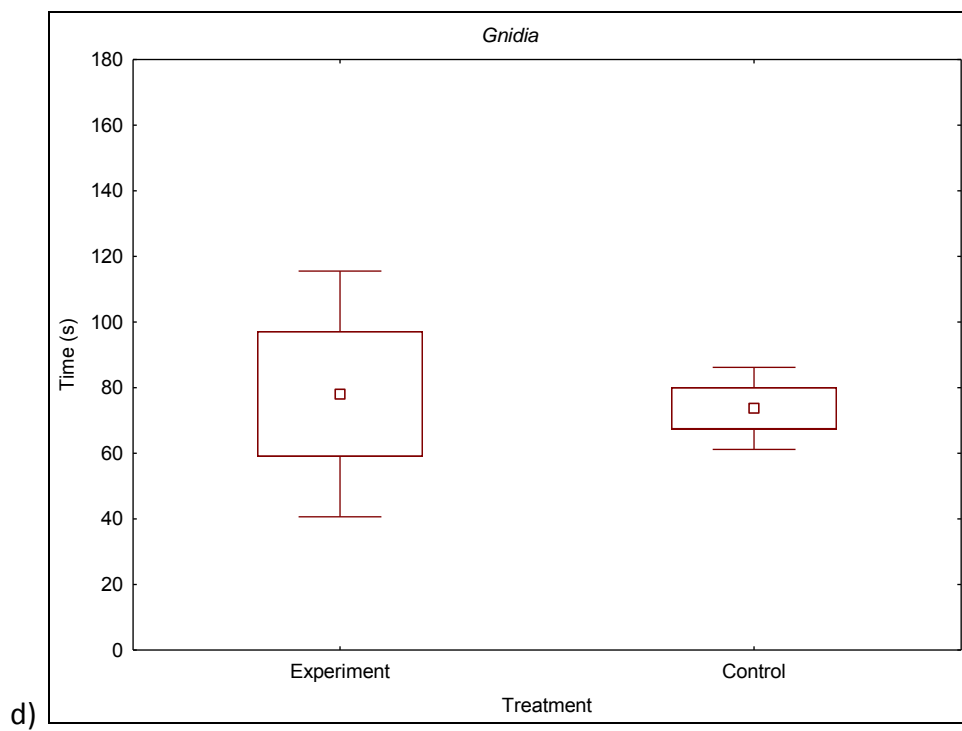
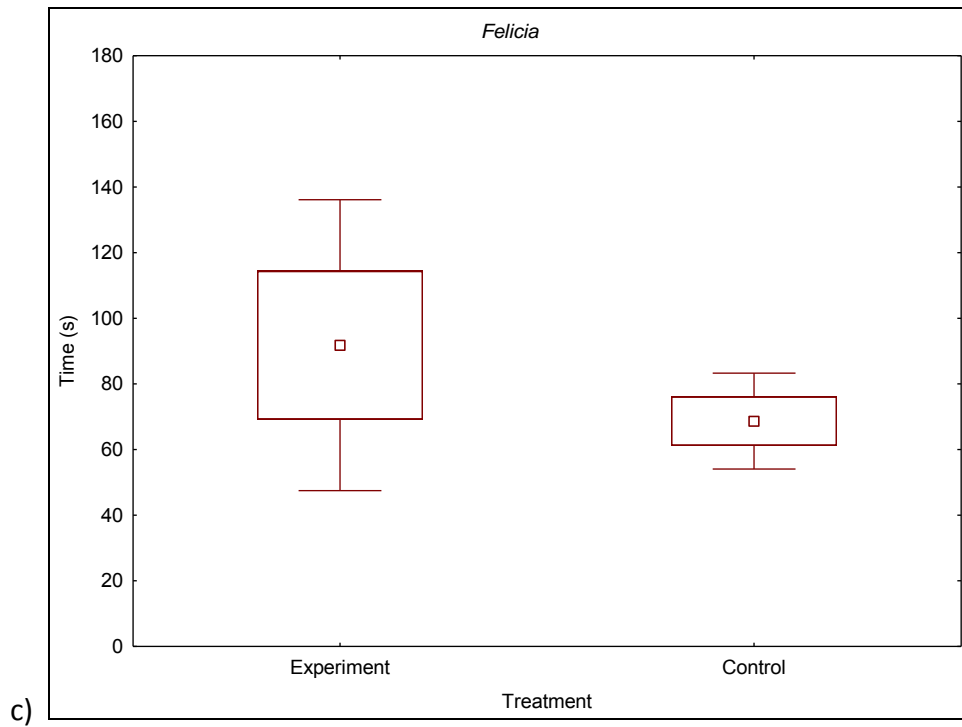
Six farms (Table 3.3) that had substantial areas of undisturbed natural vegetation that is being actively conserved were chosen and visited between mid-October 2014 and mid-November 2014 on clear days with minimal wind to ensure insect activity. This period was chosen due to the abundance of flowering plants available at this time of year in the Western Cape. On each farm flowers from any abundant flowering plants, whether indigenous or not, were picked. Five flowers per species were carefully picked, to minimize disturbance, and placed in a Ziploc® bag. Ten of these bags were collected per species of flower per farm (i.e. 50 samples per species). To compare species caught on the flowers and in the vineyard, vacuum-net samples were taken with a D-Vac vacuum sampler on 14 November 2014 on Stark-Condé. Eight vines were vacuumed for one repetition and placed in a Ziploc® bag, and five repetitions were done. The bags were frozen to ensure the death of the insects after which flowers were sifted through and parasitoids and wasps extracted and identified. The farms did not necessarily have a previous history of mealybug outbreaks, as suitable farms with abundant flowering plants are rare. Typical vine mealybug parasitoids (*C. perminutus*, *Leptomastix dactylopii* and *A. sp* near *pseudococci*) have been found on other mealybug hosts (Prinsloo, 1984; Ivars & Sánchez, 2012)

3.3 RESULTS

3.3.1 Attractance or repellence of plants.

Of the six plants tested, only *Euryops abrotanifolia* (Fig. 3.4e) had a significant positive effect and proved to be attractive to *C. perminutus*. None of the other plants tested had a significant positive or negative effect.





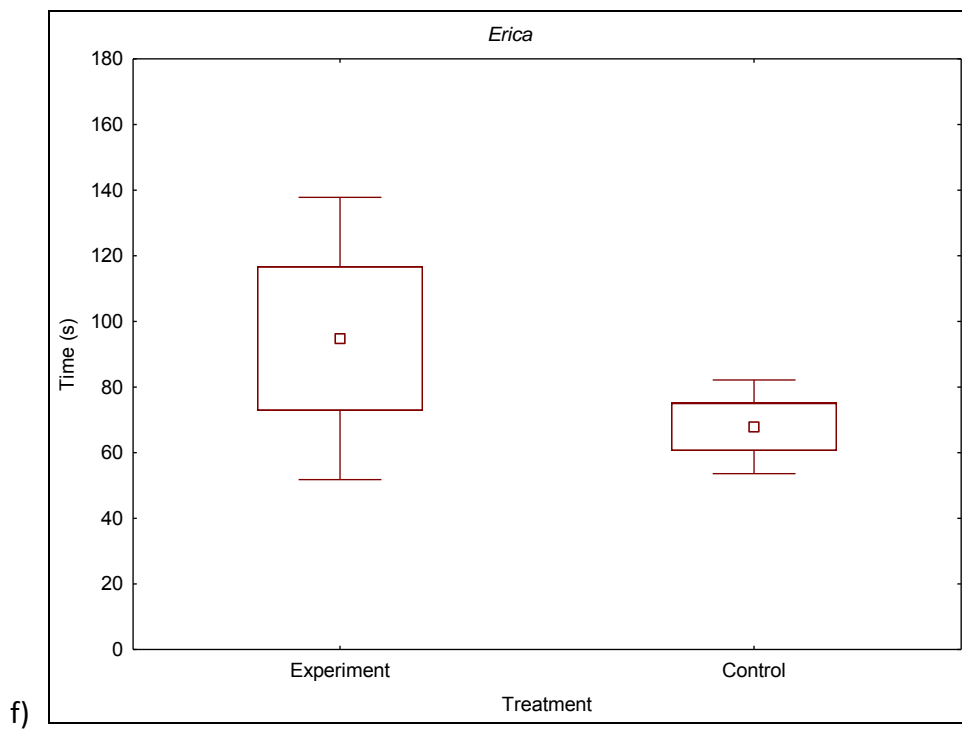
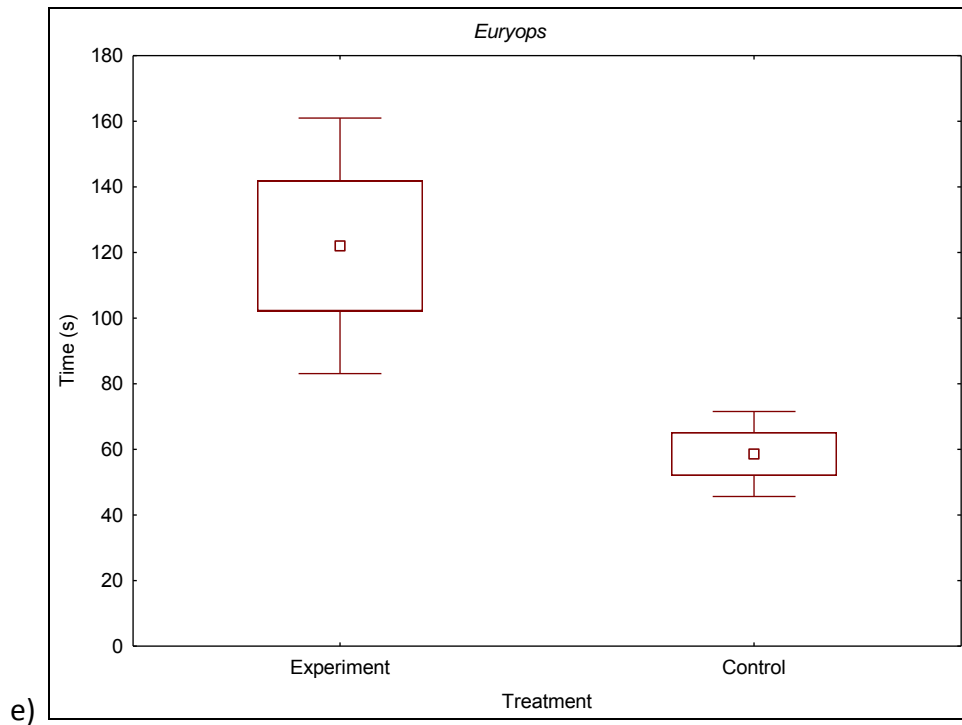


Fig. 3.4. Average time a female *Coccidoxenoides perminutus* spent in each of the following treatments: (a) *Tulbaghia violacea*, (b) *Coleonoma pulchellum*, (c) *Felicia bergeriana*, (d) *Gnidia pinifolia*, (e) *Euryops abrotanifolia* and (f) *Erica gracillus*. Error bars denote a 95% confidence interval.

Table 3.2. Comparison of the attractiveness of six indigenous plants to *Coccidoxenoides perminutus*.

Plants	t-value	df	p-value
<i>Coleonoma pulchellum</i>	-1.06415	48	0.293
<i>Erica gracillus</i>	1.163189	48	0.251
* <i>Euryops abrotanifolia</i>	3.030483	48	0.004
<i>Felicia bergeriana</i>	0.970869	48	0.336
<i>Gnidia pinifolia</i>	0.218495	48	0.828
<i>Tulbaghia violacea</i>	1.812149	48	0.076

3.3.2. Survey of parasitoids in the field

One vineyard and 27 flowering plants were surveyed and a total of 20 wasps from 16 different species were sampled (Table 3.3). None of the known *P. ficus* parasitoids were sampled during this survey. Field sampling of parasitoids further indicates that D-vac (13 individuals caught) is a more suitable method of sampling parasitoids in the field than hand collecting of floral resources (8 individuals caught). It should be noted that the D-vac sampling took place on only one day, while hand collecting took place over several days. While it is not possible to directly compare naturally growing flowering species with the vineyard habitat due to the different sampling methods used, it may be that vineyards are a good habitat for parasitoids due to the higher abundance of pest species found there.

Table 3.3. Parasitoids caught during a survey of flowering plants on six Biodiversity and Wine Initiative-champion farms using flower sampling and in the vineyard on Stark-Condé using D-vac.

FARM	PLANT	NUMBER	SPECIES
Hermanuspietersfontein (Stanford)	<i>Trifolia alba</i>		
	<i>Brassica</i> spp.		
	<i>Anagallis arvensis</i>		
	<i>Leptospermum scoparium</i>	1	Pteromalidae spp 1
	<i>Brunia nodiflora</i>		
	<i>Leucospermum</i> spp.		
Backsberg (Paarl)	<i>Plectranthus neochilus</i>		
	<i>Drosanthemum speciosum</i>		
	<i>Felicia</i> spp.		
	<i>Taraxacum officinale</i>	1	Pteromalidae spp 2
Delheim (Stellenbosch)	<i>Senecia</i> spp.		
	<i>Pelargonium graveolens</i>		
	<i>Spartium junceum</i>	2	Aphelinidae, near <i>Aphytis</i>
	<i>Dombeya wallichii</i>	1	Bracomidae, near <i>Apanteles</i>
	<i>Euryops</i> spp.		
Lourensford (Somerset West)	<i>Ipomoea indica</i>		
	<i>Verbena bonariensis</i>		
	<i>Taraxacum officinale</i>	1	Unknown, near <i>Aphelinidae</i>
	<i>Leucanthemum vulgare</i>		
Waterkloof (Somerset West)	<i>Lampranthus</i> spp.		
	<i>Podylaria</i> spp.		
	<i>Euryops</i> spp.		
	<i>Erica verticillata</i>		
	<i>Scabiosa</i> spp.		

Table 3.3 (continued). Parasitoids caught during a survey of flowering plants on six Biodiversity and Wine Initiative-champion farms using flower sampling and in the vineyard on Stark-Condé using D-vac.

Vergelegen	<i>Lampranthus</i> spp.		
(Somerset West)	<i>Athanasia crithmifolia</i>	2	Encyrtidae spp 1 Eulophidae spp
	<i>Senecio ilicifolius</i>		
Stark-Condé	<i>Vitis vinifera</i>	1	Encyrtidae spp 2
(Stellenbosch)		1	Eupelmidae spp
		1	Eurytomidae spp
		1	Ormyridae near <i>Ormyrus</i> (male)
		1	Ormyridae near <i>Ormyrus</i> (female)
		1	Perilampidae near <i>Perilampus</i>
		3	Pteromalidae spp. 3
		1	Pteromalidae spp. 4
		1	Pteromalidae near <i>Pteromalus</i>
		1	Torymidae near <i>Podagrion</i>

3.4 DISCUSSION

In this experiment, out of six plants, *C. perminutus* was only attracted to the evergreen, hardy *Euryops abrotanifolia*, of the Asteraceae family. It flowers in winter and spring, and requires well-drained, sandy soils. It needs full sun and is fast-growing and quick to exploit disturbed or open ground (Trinder-Smith, 2006). Species in this genus also are known to be tolerant of drought, wind and frost, making them a good candidate for incorporating into a habitat management plan.

A number of experiments have been done to determine the impact of flowering plants on the effectivity and longevity of pest parasitoids (Leius, 1961; Altieri et al., 1977; Treacy et al., 1987; Baggen & Gurr, 1998; Chaney, 1998; Irvin et al., 1999; Berndt et al., 2002) but not

many have been done to determine specific plant species that could be utilized in a specific agro-ecosystem (Bowie et al., 1995; Idris & Grafius, 1995; Hickman & Wratten, 1996; Irvin et al., 1999; Luna et al., 2000; Nicholls et al., 2000; Berndt et al., 2002) or specifically for *C. perminutus* in vineyards.

Floral nectar is an important source of nutrition for most adult Hymenoptera (House, 1977) and affects longevity, fecundity (Idris & Grafius, 1995) and percentage parasitism (Treacy et al., 1987; Baggen & Gurr, 1998). In lettuce (Chaney, 1998) and potato (Baggen & Gurr, 1998) parasitism rate and parasitoid abundance increased when there was a presence of floral resources (Berndt et al., 2002). In experimental apple orchards in Canada (Leius, 1961) and New Zealand (Irvin et al., 1999) flower resources increased parasitism rates, and aphid populations were lower in wheat fields bordered by flowering *Phacelia tanacetifolia* (Benth.) (Hickman & Wratten, 1996; Berndt et al., 2002).

Some crops provide nectar or pollen when in flower, but this is usually for a very limited period of time, which means parasitoids have to expend a great deal of energy and time to search for alternative food sources. This, in turn, affects the distribution of parasitoids in the crop (Baggen & Gurr, 1998). Usually the only source of nectar or pollen for beneficial insects is in the form of flowering weeds, within or around the crop areas (Altieri et al., 1977).

Indeed, in a study conducted in Bonnievale, South Africa, vineyards with various cover crops were compared to weedy plots. Numbers of mealybug parasitoids in weedy plots were higher, in some cases significantly so, than in plots with cover crops (Addison & Samways, 2006). Weeds, however, could enhance arthropod, nematode, and pathogen pests (Van Emden, 1965; Norris, 1986; Baggen & Gurr, 1998). In the field, parasitoids spending more time searching for food would possibly spend less time searching for hosts, and therefore parasitize fewer eggs than data suggests. There is, therefore, value in providing non-host foods for parasitoids by including flowering plants in the management plan. The biggest challenge, however, is in finding plants that increase the beneficial population while not encouraging the pest (Khan et al., 1997, Baggen & Gurr, 1998).

Of the six plants screened in this experiment, only one had a significant effect on the parasitoids. Future research more plants should be screened to get a clearer idea of what attracts the pest parasitoids. It should also be noted that even though odourless plastic was

used in this study, to get the most accurate results from olfactometer screenings, all apparatus should be glass.

A promising indication for the use of flowers in vineyards was the discovery of a wide range of wasps in the field with the survey of flowers. The low total number of parasitoids could be attributed to the early-season sampling but the variety of wasps found is a promising indication of the biodiversity that is naturally found on farms. None of the known mealybug parasitoids were collected during the survey, which could be the result of low hosts available, although two unknown encyrtid species and two unknown aphelinid species were collected. Both Encyrtidae and Aphelinidae are known parasitoids of Hemiptera, with Aphelinidae attacking, among others, the pea aphid, *Acyrtosiphon pisum* (Harris) (Hemiptera, Aphididae) (Mackauer & Finlayson, 1967) and the potato aphid, *Macrosiphum euphorbiae* (Hemiptera, Aphididae) (Azzouz et al., 2005). Other parasitoids found were Pteromalidae which are used as biocontrol agents against the lesser grain beetle *Rhyzopertha dominica* (Fab.) in Saudi Arabia (Ahmed, 1996), as well as the weed, *Acacia longifolia*, in South Africa (Dennill et al., 1993). Eupelmidae are parasitoids of spiders like *Gasteracantha cancriformis* (Arachnidae: Araneidae) (Muma & Stone, 1971), xylophagous beetles (Lotfalizadeh, 2012) and stinkbugs (Basnet, 2014). Perilampidae was reported as a parasitoid of the grape berry moth, *Lobesia botrana* (Dennis & Schiffermüller) (Lepidoptera: Tortricidae) (Lotfalizadeh et al., 2012). Habitat management should be viewed within the greater scope of pest management in vineyards, so as to target more than one pest species. Obtaining a diversity of parasitoids that could affect other pests make the method more likely to be adopted by growers.

Selecting plant species for the olfactometer experiment using flora species information from the survey could significantly ease the process of choosing plant species, as would determining which of the volatiles released by flowering plants are attractive or repellent to the pest parasitoids as a next step in improving parasitism rates of vine mealybug (and potentially other pests) in vineyards.

3.5 REFERENCES

- Addison, P. & Samways, M.J., 2006. Surrogate habitats demonstrate the invasion potential of the African pugnacious ant. *Biodiversity & Conservation* 15(1): 411-428.
- Ahmed, K.S., 1996. Studies on the ectoparasitoid, *Anisopteromalus calandrae* How. (Hymenoptera: Pteromalidae) as a biocontrol agent against the lesser grain borer, *Rhyzopertha dominica* (Fab.) in Saudi Arabia. *Journal of Stored Products Research* 32(2): 137-140.
- Altieri, M.A., Van Schoonhoven, A. & Doll, J., 1977. The ecological role of weeds in insect pest management systems: a review illustrated by bean (*Phaseolus vulgaris*) cropping systems. *Pans* 23(2): 195-205.
- Azzouz, H., Cherqui, A., Campan, E.D.M., Rahbé, Y., Duport, G., Jouanin, L., Kaiser, L. & Giordanengo, P., 2005. Effects of plant protease inhibitors, oryzacystatin I and soybean Bowman–Birk inhibitor, on the aphid *Macrosiphum euphorbiae* (Homoptera, Aphididae) and its parasitoid *Aphelinus abdominalis* (Hymenoptera, Aphelinidae). *Journal of insect physiology* 51(1), 75-86.
- Baggen, L.R. & Gurr, G.M., 1998. The Influence of Food on *Copidosoma koehleri* (Hymenoptera: Encyrtidae), and the Use of Flowering Plants as a Habitat Management Tool to Enhance Biological Control of Potato Moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae). *Biological Control* 11(1): 9-17.
- Basnet, S., 2014. *Biology and pest status of brown marmorated stink bug (Hemiptera: Pentatomidae) in Virginia vineyards and raspberry plantings*. PhD dissertation, Virginia Polytechnic Institute and State University.
- Berndt, L.A., Wratten, S.D. & Hassan, P.G., 2002. Effects of buckwheat flowers on leafroller (Lepidoptera: Tortricidae) parasitoids in a New Zealand vineyard. *Agricultural and Forest Entomology* 4: 39-45.
- Bianchi, F.J.J.A., Booij, C.J.H. & Tscharntke, T., 2006. Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. *Proceedings of the Royal Society B: Biological Sciences* 273(1595): 1715-1727.

- Bigger, D.S. & Chaney, W.E., 1998. Effects of *Iberis umbellata* (Brassicaceae) on insect pests of cabbage and on potential biological control agents. *Environmental entomology* 27(1): 161-167.
- Birkett, M.A., Campbell, C.A., Chamberlain, K., Guerrieri, E., Hick, A.J., Martin, J.L., Matthes, M., Napier, J.A., Pettersson, J., Pickett, J.A., Poppy, G.M., Pow, E.M., Pye, B.J., Smart, L.E., Wadhams, G.H., Wadhams, L.J. & Woodcock, C.M., 2000. New roles for cis-jasmone as an insect semiochemical and in plant defense. *Proceedings of the National Academy of Sciences* 97(16): 9329-9334.
- Bowie, M.H., Wratten, S.D. & White, A.J., 1995. Agronomy and phenology of 'companion plants' of potential for enhancement of insect biological control. *New Zealand Journal of Crop and Horticultural Science* 23: 423-427.
- Ceballo, F.A. & Walter, G.H., 2004. Why is *Coccidoxenoides perminutus*, a mealybug parasitoid, ineffective as a biocontrol agent – Inaccurate measures of parasitism or low adult survival? *Biological Control* 33: 260-268.
- Chaney, W.E., 1998. Biological control of aphids in lettuce using in-field insectaries. In *Enhancing Biological Control: Habitat Management to Promote Natural Enemies of Agricultural Pests* (ed. by C.H. Pickett and R.L. Bugg), pp. 73-83. University of California Press, Berkeley.
- Davies, A.P., Ceballo, F.A. & Walter, G.H., 2004. Is the potential of *Coccidoxenoides perminutus*, a mealybug parasitoid, limited by climatic or nutritional factors? *Biological control* 31: 181-188.
- Dennill, G.B., Donnelly, D. & Chown, S.L., 1993. Expansion of host-plant range of a biocontrol agent *Trichilogaster acaciaelongifoliae* (Pteromalidae) released against the weed *Acacia longifolia* in South Africa. *Agriculture, ecosystems & environment* 43(1): 1-10.
- Dutcher, J.D., 1993. Recent examples of conservation of arthropod natural enemies in agriculture. *Pest management: biologically based technologies*. Washington: American Chemical Society, pp. 101-108.

Goldblatt, P. & Manning, P., 2012. Plants of the Greater Cape Floristic Region. 1: The Core Cape flora. *Strelitzia* 29. South African National Biodiversity Institute, Private Bag X101, 0001 Pretoria, South Africa.

Hickman, J.M. & Wratten, S.D., 1996. Use of *Phacelia tanacetifolia* strips to enhance biological control of aphids by hoverfly larvae in cereal fields. *Journal of Economic Entomology* 89(4): 832-840.

House, H. L., 1977. Nutrition of natural enemies. In *Biological control by augmentation of natural enemies*. Springer, US, pp. 151-182.

Idris, A.B. & Grafius, E., 1995. Wildflowers as nectar sources for *Diadegma insulare* (Hymenoptera: Ichneumonidae), a parasitoid of diamondback moth (Lepidoptera: Yponomeutidae). *Environmental Entomology* 24: 1727-1735.

Irvin, N.A., 1999. *Understorey management for the enhancement of populations of a leafroller (Lepidoptera: Tortricidae) parasitoid (Dolichogenidea tasmanica (Cameron)) in Canterbury, New Zealand apple orchards*. PhD dissertation. Lincoln University, Canterbury, New Zealand.

Irvin, N.A., Wratten, S.D., Chapman, R.B. & Frampton, C.M., 1999. Effects of floral resources on fitness of the leafroller parasitoid (*Dolichogenidea tasmanica*) in apples. *Proceedings of the New Zealand Plant Protection Conference* 52: 84-88.

Ivars, A.B. & Sánchez, A.S., 2012. *Pseudocóccidos de importancia agrícola y ornamental en España*. Editorial Universitat Politècnica de València.

Jervis, M.A. & Kidd, N.A.C., 1986. Host-feeding strategies in hymenopteran parasitoids. *Biological Reviews* 61(4): 395-434.

Jervis, M.A., Kidd, N.A.C., Fitton, M.G., Huddleston, T. & Dawah, H.A., 1993. Flower-visiting by hymenopteran parasitoids. *Journal of natural history* 27(1): 67-105.

Jervis, M.A., Kidd, N.A.C. & Heimpel, G.E., 1996. Parasitoid adult feeding behaviour and biocontrol – a review. *Biocontrol news and Information* 17: 11-26.

Khan, Z.R., Ampong-Nyarko, K., Chiliswa, P., Hassanali, A., Kimani, S., Lwande, W., Overholt, W.A., Pickett, J.A., Smart, L.E., Wadhams, L.J. & Woodcock, C.M., 1997. Intercropping increases parasitism of pests. *Nature* 388(6643): 631-632.

Koji, S., Khan, Z.R. & Midega, C.A.O., 2007. Field boundaries of *Panicum maximum* as a reservoir for predators and a sink for *Chilo partellus*. *Journal of Applied Entomology* 131(3): 186-196.

Landis, D.A., Gurr, G.M. & Wratten, S.D., 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology* 45: 175-201.

Langer, A. & Hance, T., 2004. Enhancing parasitism of wheat aphids through apparent competition: a tool for biological control. *Agriculture, ecosystems & environment* 102(2): 205-212.

Lee, J.C. & Heimpel, G.E., 2005. Impact of flowering buckwheat on Lepidopteran cabbage pests and their parasitoids at two spatial scales. *Biological Control* 34(3): 290-301.

Leius, K., 1961. Influence of food on fecundity and longevity of adults of *Itopectis conquisitor* (Say) (Hymenoptera: Ichneumonidae). *Canadian Entomologist* 91: 771-780.

Lotfalizadeh, H., 2012. Review of chalcidoid parasitoids (Hymenoptera: Chalcidoidea) of xylophagous beetles. *Munis of Entomology and Zoology* 7(1): 309-333.

Lotfalizadeh, H., Masnadi-Yazdinejad, A. & Saber, M., 2011. New records of the grape berry moth Hymenopterous parasitoids in Iran. *Munis Entomology & Zoology* 7(1), 284-291.

Luna, J., Colley, M. & Staben, M., 2000. Enhancing biological control with beneficial insectary plants. *1PM in Oregon: Achievements and Future Directions* 126.

Mackauer, M. & Finlayson, T., 1967. The hymenopterous parasites (Hymenoptera: Aphidiidae et Aphelinidae) of the pea aphid in eastern North America. *The Canadian Entomologist* 99(10): 1051-1082.

Muma, M.H. & Stone, K.J., 1971. Predation of *Gasteracantha cancriformis* (Arachnidae: Araneidae) Eggs in Florida Citrus Groves by *Phalacrotophora epeirae* (Insecta: Phoridae) and *Arachnophaga ferruginea* (Insecta: Eupelmidae). *Florida Entomologist* 54(4): 305-310.

Nicholls, C.I., Parrella, M.P. & Altieri, M.A., 2000. Reducing the abundance of leafhoppers and thrips in a northern California organic vineyard through maintenance of full season floral diversity with summer cover crops. *Agricultural and Forest Entomology* 2(2): 107-113.

Norris, R. F., 1986. Weeds and integrated pest management systems. *HortScience* 21(3): 402-410.

Orr, D.B. & Pleasants, J.M., 1996. The potential of native prairie plant species to enhance the effectiveness of the *Ostrinia nubilalis* parasitoid *Macrocentrus grandii*. *Journal of the Kansas Entomological Society*, pp. 133-143.

Pettersson, J., 1970. Studies on *Rhopalosiphum padi* (L.). I. Laboratory studies on olfactometric responses to the winter host *Prunus padus* L. *Uppsala Lantbrukshogskolans Annaler* 36.

Pettersson, J., Karunaratne, S., Ahmed, E., & Kumar, V., 1998. The cowpea aphid, *Aphis craccivora*, host plant odours and pheromones. *Entomologia Experimentalis et Applicata* 88(2): 177-184.

Platt, J.O., Caldwell, J.S. & Kok, L.T., 1999. Effect of buckwheat as a flowering border on populations of cucumber beetles and their natural enemies in cucumber and squash. *Crop Protection* 18: 305-313.

Powell, W., 1986. Enhancing parasitoid activity in crops. In *Insect Parasitoids* (ed. by J. Waage and D. Greathead), pp. 319-340. Academic Press, London.

Rabb, R.L., Stinner, R.E. & van den Bosch, R., 1976. *Conservation and augmentation of natural enemies*. (Vol. 233). Academic Press, New York.

Stapel, J.O., Cortesero, A.M., De Moraes, C.M., Tumlinson, J.H. & Lewis, W.J., 1997. Extrafloral nectar, honeydew, and sucrose effects on searching behaviour and efficiency of *Microplitis croceipes* (Hymenoptera: Braconidae) in cotton. *Environmental Entomology* 26: 617-623.

- Stephens, M.J., France, C.M., Wratten, S.D. & Frampton, C., 1998. Enhancing biological control of leafrollers (Lepidoptera: Tortricidae) by sowing buckwheat (*Fagopyrum esculentum*) in an orchard. *Biocontrol Science and Technology* 8: 547-558.
- Takasu, K. & Lewis, W.J., 1996. The role of learning in adult food location by the larval parasitoid, *Microplitis croceipes* (Hymenoptera: Braconidae). *Journal of Insect Behaviour* 9: 265-281.
- Thies, C., Steffan-Dewenter, I. & Tschardt, T., 2003. Effects of landscape context on herbivory and parasitism at different spatial scales. *Oikos* 101(1): 18-25.
- Treacy, M.F., Benedict, J.H., Walmsley, M.H., Lopez, J.D. & Morrison, R.K., 1987. Parasitism of bollworm (Lepidoptera: Noctuidae) eggs on nectaried and nectariless cotton. *Environmental Entomology* 16(2): 420-423.
- Trinder-Smith, T.H., Kidd, M.M. & Anderson, F., 2006. *Wild Flowers of the Table Mountain National Park*. (Vol. 12). Botanical Society of South Africa.
- Van den Bosch, R. & Telford, A.D., 1964. Environmental modification and biological control. *DeBach, Paul Biological Control of Insect Pests and Weeds*.
- Van Emden, H.F., 1963. Observations of the effects of flowers on the activity of parasitic Hymenoptera. *Entomologists Monthly Magazine* 98: 236-270.
- Van Emden, H.F., 1965. The role of uncultivated land in the biology of crop pests and beneficial insects. *Scientific Horticulture* 17: 121-136.
- Vet, L.E., Van Lenteren, J.C., Heymans, M. & Meelis, E., 1983. An airflow olfactometer for measuring olfactory responses of hymenopterous parasitoids and other small insects. *Physiological Entomology* 8(1): 97-106.
- Wackers, F.L., 1994. The effect of food deprivation on the innate visual and olfactory preferences in the parasitoid *Cotesia rubecula*. *Journal of Insect Physiology* 40: 641-649.
- Walton, V.M. & Pringle, K.L., 2004. A survey of mealybugs and associated natural enemies in vineyards in the Western Cape provinces. *South African Journal of Enology and Viticulture* 25 (1).

Woltz, J.M., Isaacs, R., & Landis, D.A., 2012. Landscape structure and habitat management differentially influence insect natural enemies in an agricultural landscape. *Agriculture, Ecosystems & Environment* 152: 40-49.

CHAPTER FOUR:

LIFE HISTORY PARAMETERS OF *COCCIDOXENOIDES PERMINUTUS* (TIMBERLAKE) ON *PLANOCOCCUS CITRI* (RISSO).

4.1 INTRODUCTION

The life history parameters of a parasitoid are used to determine optimal release intervals. In most taxa a correlation can be found between preadult life span, body size and adult life span (Stearns & Crandall, 1981; Charnov & Berrigan, 1990; Purvis & Harvey, 1995) but Blackburn (1991) found no such correlations in Hymenoptera. Reasons for this could be the host size that may constrain the growth rate of the parasitoid, or else an adaptive rate of growth depending on the conditions within and outside the host (Eijs & Van Alphen, 1999). It is therefore necessary to determine the specific parameters for each parasitoid species, as there is no way of predicting the fecundity through any measurements.

Coccidoxenoides perminutus (Timberlake) (Hymenoptera: Encyrtidae) is an endoparasitic, parthenogenic parasitoid of *Planococcus ficus* (Signoret) and *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) (Ceballo & Walter, 2004). Only one male of *C. perminutus* had ever been reported (Timberlake, 1919) until Ceballo & Walter (2004) found about 1.2% males in their sample of 1250 individuals. First described in Hawaii by Timberlake, *C. perminutus* was first classified as *Pauridia peregrina* by Searle (1965). It was introduced to California in 1916 and first recorded in South Africa in 1943 (Searle, 1965). It was described as solitary, thelytokous and pro-ovigenic by Joyce et al (2001). A survey by Urban & Greeff (1985), found that three parasitoids, *C. perminutus*, *Anagyrus* species near *pseudococci* (Girault)(Hymenoptera: Encyrtidae), *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae) and predatory beetles of the *Nephus* spp. were the most dominant natural enemies of *P. ficus* (Walton & Pringle, 2002). Most successful biological control programs for mealybugs rely primarily on encyrtid parasitoids that are mealybug specialists (Noyes & Hayat, 1994; Daane et al., 2012).

Coccidoxenoides perminutus has a high reproductive potential, with 10 – 20 eggs laid within two days of emergence and thereafter 80 – 150 eggs laid until the parasitoid dies after about 8 days (Ceballo & Walter, 2004). According to Joyce et al. (2001) the whole lifecycle

takes about four weeks to complete on *P. ficus*. The lifespan and fecundity of the adult parasitoid is highest when supplied with a food source such as honey or nectar (Davies et al, 2004).

It was found that eggs only reached maturity when oviposited into immature mealybugs. At first it was thought there were no preferences for a specific nymphal instar (Joyce et al, 2001) but it was later found that the highest rate of development was achieved in second instar mealybugs (Ceballo & Walter, 2004). It is thought that encapsulation occurs when eggs are oviposited into adult mealybugs (Ceballo & Walter, 2004). Encapsulation of foreign agents like parasitoid eggs or larvae is a common mode of defence used by insects (Ratcliffe, 1993). It involves surrounding the foreign object with cells (Ratcliffe, 1993) or fibers (Boman and Hultmark, 1987), which results in the partitioning of the object from the surrounding tissues of the host (Reed et al., 2007).

Walton & Pringle (2005) determined the life history parameters of *C. perminutus* on *P. ficus* at five different temperatures. As *C. perminutus* is reared on *P. citri* at the commercial rearing facility, it was decided to find out more about the life history parameters and fecundity of *C. perminutus* when parasitizing *P. citri*, and comparing those results with those obtained on *P. ficus*. This would give an indication of how *C. perminutus* develops on different hosts and could provide valuable information for biological control programmes.

The objectives of this study were to (i) determine the parasitoid's age-specific fecundity, (ii) the longevity of the parasitoid when it is allowed to oviposit versus when it is not, (iii) which instar of mealybug it prefers for oviposition, and (iv) to compare development of *C. perminutus* on *P. ficus* and *P. citri*. This information will be important for rearing protocols as well as determining the intervals between releases that will give the best control of *P. ficus* and *P. citri* in the field.

4.2 MATERIALS AND METHODS

4.2.1 Mealybug and parasitoid colonies

All experiments were done at the Du Roi IPM commercial rearing facility in Letsitele (Limpopo, South Africa), where uniformly aged *P. citri* instars on butternuts were readily available. Butternuts (*Cucurbita moschata*) were infested with eggs from the existing *P. citri* mother colony to establish mealybugs of a uniform age on each butternut. Preliminary experiments were conducted whereby parasitoid pupae were transported from DuRoi to Stellenbosch University for the comparative experiments of developmental rates on *P. ficus* and *P. citri*.

Planococcus ficus stock cultures were reared on butternuts in cages (750mm x 500mm x 300mm) in an insectary at a temperature of 25°C and a 12:12 (light:dark) photoperiod. Mealybugs were supplemented from the insectary at ARC Infruitec-Nietvoorbij and fresh butternuts, surface sterilized with Sporekill® (100ml/100l), were added to the cage as needed.

These experiments were unsuccessful, as no parasitization took place. Reasons for this could have been problems with parasitoid quality due to the transportation process, or that parasitoids had adapted to the host *P. citri* which had been reared on butternuts at Du Roi IPM for 14 years at 26-28°C and 7:17 L:D photoperiod. This trial was therefore abandoned and life table parameters were determined using *P. citri* only.

4.2.2 Instar preference trial

To determine which host instars are preferred by the parasitoids an instar-specific test was performed, adapted from methods described in Ceballo & Walter (2004) and Amarasekare et al. (2010). Experiments were all carried out at 25.21°C and 79.63% humidity.

No-choice test. A piece of butternut (1cm x 1cm x 1 cm) with ±100 mealybugs of a specific nymphal stage (N1, N2 or N3) was placed in a 30cm x 15 cm x 10 cm clear plastic arena along with a single, newly emerged parasitoid. To ensure uniform emergence dates the parasitoid was removed after 24 hours. As the cut piece of butternut started drying out after a few days it was placed in a similar arena with a fresh butternut for the mealybugs to

move to. After 15 days the pupae were harvested to estimate percentage parasitism. Sixteen replicates were done.

Choice test. The method was similar to the no-choice test, except that 100 mealybugs each of two of the three nymphal stages were placed together, each on their own piece of butternut, in each arena with the parasitoid for 24 hours. After removal of the parasitoid the two pieces of butternut were placed in separate arenas with a fresh butternut. After 15 days pupae were harvested and percentage parasitism estimated.

Ceballo & Walter (2004) found evidence of encapsulation and subsequent destruction of *C. perminutus* eggs in the haemolymph of adult mealybugs, therefore adult mealybugs were not included in these experiments.

4.2.3 *C. perminutus* life table parameters (longevity and fecundity)

The longevity trial was done to determine if the energy expended during egg-laying leads to an earlier death than when a parasitoid does not lay eggs. 16 replicates of each test was done to account for statistical accuracy.

No eggs laid. A single one-day-old parasitoid was placed in a closed Petri dish with a damp cotton swab and a paper strip with a mixture of honey and yeast. The arenas were checked daily to determine if the parasitoids were still alive.

Eggs laid. A 5ℓ-ice cream container with the lid cut out and replaced with fine muslin was used. A single one-day-old parasitoid was placed inside the container, together with a butternut infested with second instar mealybugs and a damp cotton swab and a paper strip with a mixture of honey and yeast. The containers were checked daily to determine if the parasitoids were still alive. After parasitoid death the butternut was placed in a rearing cage at an average temperature of 25.21°C and average humidity of 79.63%. As described by Powell & Hartley (1987) and Murai & Loomans (2001) pupae were harvested after 15 days and counted to estimate total fecundity.

To determine fecundity per day a butternut infested with four-day-old mealybugs was cut up into 1cm² pieces. A piece was put in a glass vial of 12 cm height and 1.6 cm width, next to a paper strip with a mixture of honey and yeast. A single one-day-old parasitoid was added

and the opening covered with fine muslin. This arena was kept at an average temperature of 25.72°C, an average RH of 85.98% and a 9:15 L:D photoperiod. The piece of butternut was replaced daily with a fresh piece covered with 100 of the specified instar of mealybugs until the parasitoid died. Each day the butternut pieces were removed from the arena, they were placed in separate rearing cages along with a fresh butternut so that mealybugs were able to move to a fresh food source. After 15 days the mummies (developing parasitoid pupae) were harvested and counted to estimate fecundity per day. *C. perminutus* can oviposit more than one egg per mealybug host, but only one wasp has ever been found to emerge (Ceballo & Walter, 2004), therefore mummies were used here as an indicator of number of progeny.

4.2.4 Statistical analysis

The data for both the instar-specific preference trials and the longevity trial were tested for homogeneity and normality before being analysed using a one-way ANOVA. The analyses were done in Statistica, version 12 (Statsoft Inc., 2013).

With the age-specific fecundity trial, adult parasitoid age (x), age-specific survival rates (l_x) and number of offspring produced per female per day (m_x) were determined. With this information other life-table parameters, like gross reproductive rate ($GRR = \sum m_x$), net reproductive rate ($R_0 = \sum l_x m_x$), mean generation time ($GT = \sum (l_x m_x x) / \sum (l_x m_x)$), the initial estimate of the intrinsic rate of increase ($r_m = \text{Log}_e R_0 / T$) and doubling time ($DT = \ln(2) / r_m$) were calculated. The initial estimate of the intrinsic rate of increase was used in the first iteration to solve the equation:

$$\sum_{x=1}^t (e^{-r_m x}) L_x M_x = 1, x = 1, 2, 3, \dots, t \text{ days.}$$

The iterations were continued until the two sides of the equation were within 0.0001 of each other (Watson 1964; Price 1984). The influence of different environmental factors on parasitoid development can be determined with the intrinsic rate of increase (r_m) and the doubling time (DT) (Maia et al., 2000).

4.3 RESULTS AND DISCUSSION

4.3.1 Instar preference

No-choice test. The second nymphal instar (N2) yielded the highest number of mummies (6.90 ± 2.653 S.E.), with a much lower yield from N1 (3.10 ± 2.653 S.E.) and N3 (1.60 ± 2.653 S.E.). However, there was no statistically significant difference in the number of mummies between the instars ($F_{3,27}=2.8314$; $p=0.05713$) (Fig. 4.1).

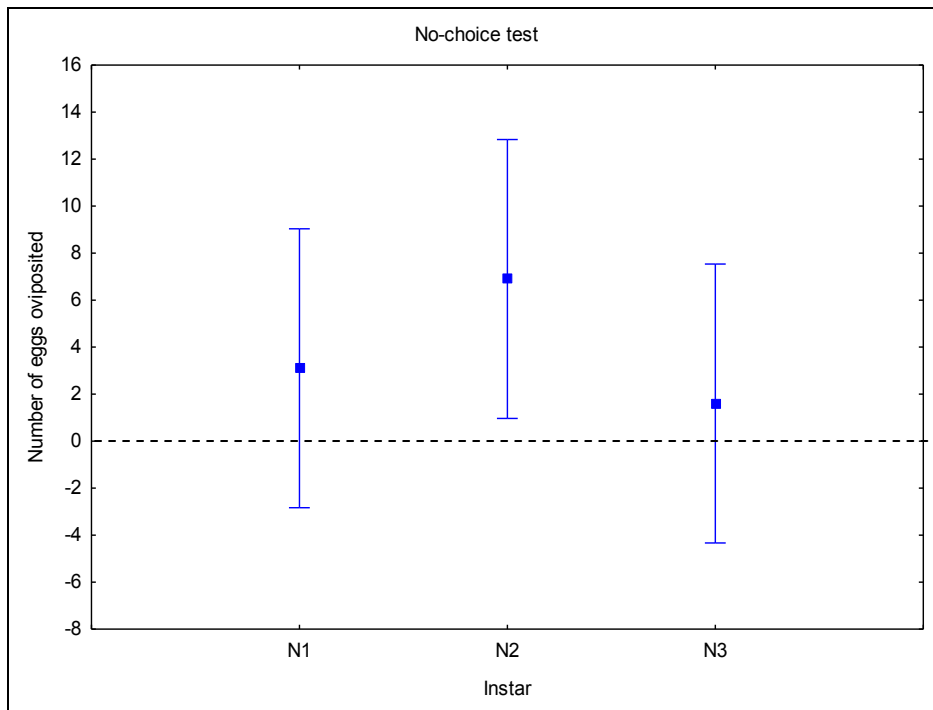


Fig. 4.1. Mean number of eggs oviposited (inferred from number of mummies) for no-choice test of *Coccidoxenoides perminutus* on its host *Planococcus citri*. Error bars denote 95% confidence intervals.

Choice test. When given a choice to oviposit in first or second instar mealybugs, there was no significant difference between the numbers of mummies recovered. When given the choice between third instar and first or second instar mealybugs, there was a preference for the third instar mealybugs, but not significantly so (Fig. 4.2). Ceballos and Walter (2004), on the other hand, found that *C. perminutus* prefers second and third instar *P. citri* in choice tests and second and third instars in no choice tests.

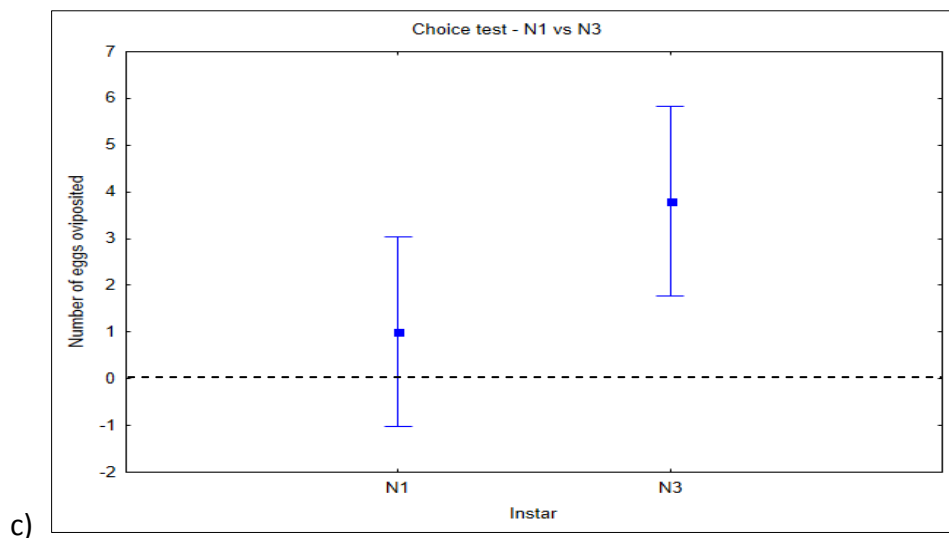
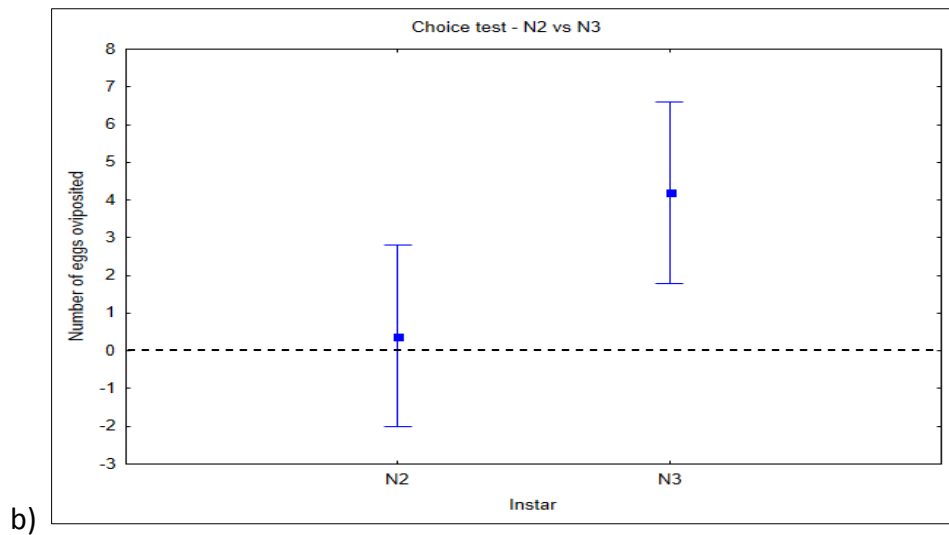
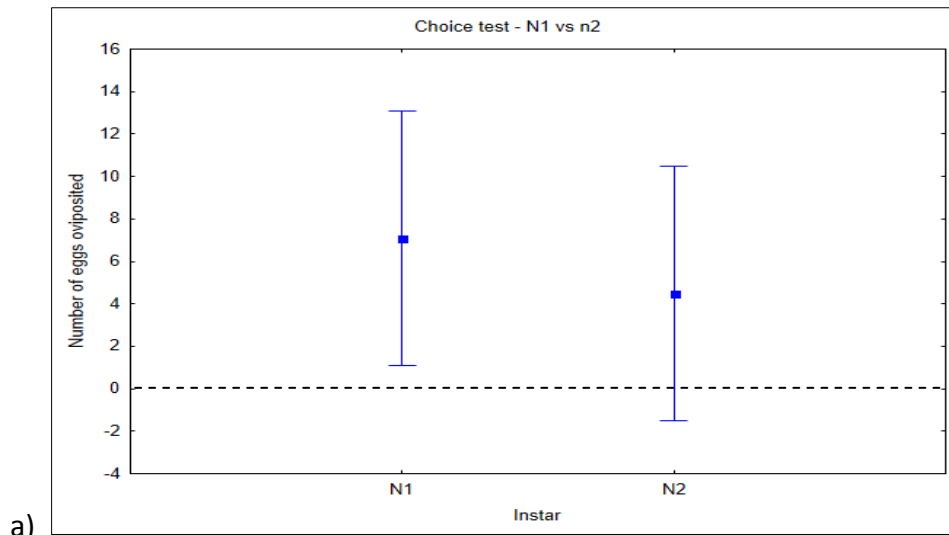


Fig. 4.2. Mean number of *Coccidoxenoides perminutus* mummies recovered from *Planococcus citri* mealybugs in choice tests, with (a) N1 vs N2 ($F_{1,19}=0.41320$, $p=0.52803$), (b) N2 vs N3 ($F_{1,19}=5.4480$, $p=0.03072$) and (c) N1 vs N3 ($F_{1,19}=4.1655$, $p=0.05539$). Error bars denote 95% confidence intervals.

4.3.2 Life table parameters

Although parasitoids not allowed to lay eggs lived an average of 4.571 ± 1.22 days and parasitoids allowed to lay eggs only 3.286 ± 1.22 days, the difference was not statistically significant ($F_{1,27}=0.55550$, $p=0.46252$) (Fig. 4.3). The slight decrease in life expectancy could be due to the exertion of energy in laying eggs (Wheeler, 1996; Rivero & West, 2002), or a need to lay eggs rather than feed (Charnov and Skinner, 1984, 1985; Sirot and Bernstein, 1996; Rivero & West, 2002). A similar change in oviposition behaviour was found by Ahmad (1936) in *Venturia canescens* (Grav.) when parasitoids fed with honey solution lived 36 days, compared to parasitoids given only water which lived four days. However, the lifetime production of *V. canescens* progeny was still identical, proving an alteration in oviposition behaviour when a change in life expectancy occurs (Fletcher et al, 1994).

Joyce et al. (2001) found only one egg per mealybug and for this reason each mummy was counted once. In this experiment the average lifetime production of progeny (estimated fecundity) by the parasitoids were 135 ± 22.34 eggs.

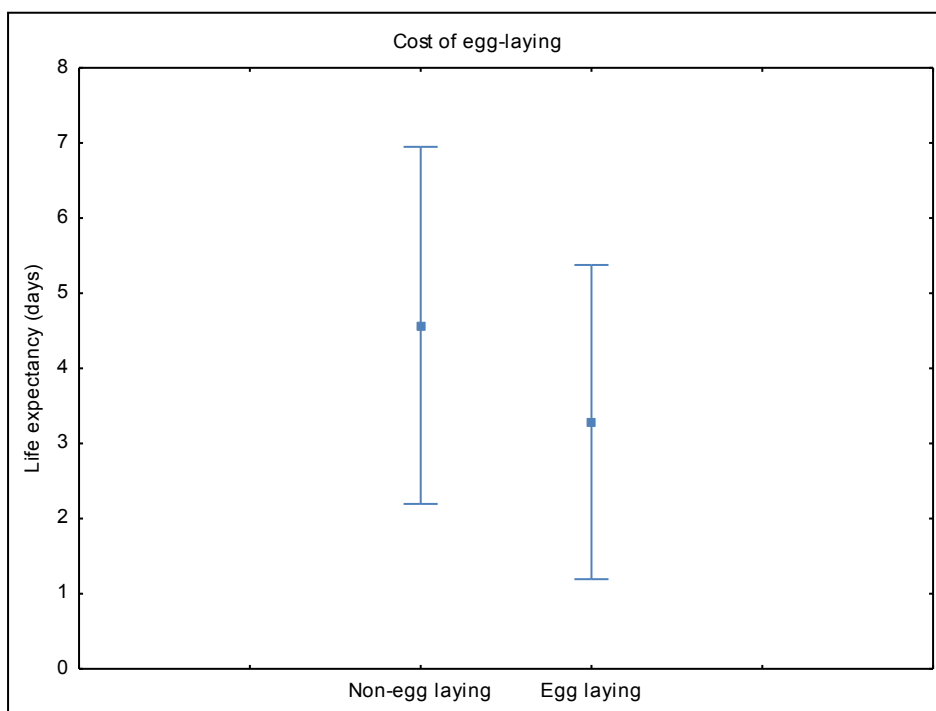


Fig. 4.3. A comparison of life expectancy of *Coccidoxenoides perminutus* (in days) of egg laying and non-egg laying parasitoids. Error bars denote 95% confidence intervals.

Daily fecundity showed a peak in the first three days, after which survivorship went down to 0.56%. The total number of offspring produced by the average female (GRR), with all offspring born after (T) days; the average number of offspring produced during its entire lifetime (R_0); the theoretical maximum rate of increase of the population per individual per day (r_m) and the time, in days, for the entire population to double in numbers (DT) are presented in Table 4.1.

Walton & Pringle (2005) did a life table study with *C. perminutus* on *P. ficus* and although the mean generation time (T) is almost identical, their net reproductive rate (R_0) is half of this study's and their intrinsic rate of increase (r_m) a third (Table 4). This difference in R_0 and r_m could probably be attributed to the methods used. Walton and Pringle (2005) waited for parasitoids to hatch and then counted progeny, whereas in this trial mummies were harvested from mealybugs after 15 days and then counted, therefore an estimate of progeny was made.

Table 4.1. Life table parameters for *Coccidoxenoides perminutus* at a temperature of 25.72°C, an average RH of 85.98% and a 9:15 L:D photoperiod.

Parameter	Value
Gross reproductive rate (GRR)	125.1
Mean generation time (T)	27.6 days
Net reproductive rate (R_0)	159.5
Intrinsic rate of increase (r_m)	0.51
Doubling time (DT)	1.36 days

4.4 CONCLUSIONS

Although there was a slight decrease in life expectancy for parasitoids allowed to lay eggs, it was not significantly different from the life expectancy for parasitoids not allowed to lay eggs. It would be beneficial to find out if there are any differences in behaviour between parasitoids allowed to lay eggs or not.

The difference in instar preference between this study, which found a non-significant preference for third instar mealybugs, and that of Ceballo & Walter (2004), which found a preference for second and third instar mealybugs, requires further investigation, as it has an

impact on *C. perminutus* production and releases. Knowing what instar mealybug is attacked by *C. perminutus* will aid in determining the best intervals for releasing parasitoids.

Mummies are part of the developmental phase in the lifecycle of the parasitoid, but it is not clear how many pupae survive to the adult stage. So it might be beneficial to determine the percentage of mummies that produce wasps, as this could have a large impact on how rearing facilities collect the insects for releases. The current method used by rearing facilities to determine numbers of insects for release programmes may overestimate progeny and a quantification of pupae that fail to emerge as wasps would therefore contribute to increased success of biological control programmes. It would also be beneficial to determine the differences in *C. perminutus* preference between *P. ficus* and *P. citri*, especially by wasps reared for some time on one host only, as host conditioning could currently be occurring at the commercial rearing facilities. As they export wasps to vineyards in the Western Cape, it would be essential to establish whether host conditioning or decreased ability to parasitize through poor transportation procedures could explain the lack of parasitism on the *P. ficus* colony at Stellenbosch University. This could unfortunately not be determined in the present study due to logistic constraints.

4.5. REFERENCES

- Ahmad, T., 1936. The influence of ecological factors on the Mediterranean flour moth, *Ephestia kihniella* and its parasite, *Nemeritis canescens*. *Journal of Animal Ecology* 5: 67-93.
- Amarasekare, K.G., Mannion, C.M. & Epsky, N.D., 2010. Host instar susceptibility and selection and interspecific competition of three introduced parasitoids of the mealybug *Paracoccus marginatus* (Hemiptera: Pseudococcidae). *Environmental entomology* 39(5): 1506-1512.
- Blackburn, T.M., 1991. A comparative examination of life-span and fecundity in parasitoid Hymenoptera. *The Journal of Animal Ecology* 60: 151-164.
- Boman, H.G. & Hultmark, D., 1987. Cell-free immunity in insects. *Annual Reviews in Microbiology* 41(1): 103-126.
- Ceballo, F.A. & Walter, G.H., 2004. Why is *Coccidoxenoides perminutus*, a mealybug parasitoid, ineffective as a biocontrol agent – Inaccurate measures of parasitism of low adult survival? *Biological Control* 33: 260-268.
- Charnov, E.L. & Berrigan, D., 1990. Dimensionless numbers and life history evolution: age of maturity versus the adult lifespan. *Evolutionary Ecology* 4(3): 273-275.
- Charnov, E.L. & Skinner, S.W., 1984. Evolution of host selection and clutch size in parasitoid wasps. *Florida Entomologist* 67: 5–21.
- Charnov, E.L. & Skinner, S.W., 1985. Complementary approaches to the understanding of parasitoid oviposition decisions. *Environmental Entomology* 14: 383–391.
- Daane, K.M., Almeida, R.P.P., Bell, V.A., Walker, J.T.S., Botton, M., Fallahzadeh, M., Mani, M., Miano, J.L., Sforza, R., Walton, V.M. & Zaviezo, T., 2012. Biology and management of mealybugs in vineyards. In *Arthropod Management in Vineyards*, pp. 271-307. Springer Netherlands.
- Davies, A.P., Ceballo, F.A. & Walter, G.H., 2004. Is the potential of *Coccidoxenoides perminutus*, a mealybug parasitoid, limited by climatic or nutritional factors? *Biological control* 31: 181-188.

- Eijs, I.E.M. & Van Alphen, J.J.M., 1999. Life history correlations: why are hymenopteran parasitoids an exception? *Ecology Letters* 2(1): 27-35.
- Fletcher, J.P., Hughes, J.P. & Harvey, I.F., 1994. Life expectancy and egg load affect oviposition decisions of a solitary parasitoid. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 258(1352): 163-167.
- Joyce, A.L., Hoddle, M.S., Bellows, T.S. & Gonzalez, D., 2001. Oviposition behavior of *Coccidoxenoides peregrinus*, a parasitoid of *Planococcus ficus*. *Entomologia Experimentalis et applicata* 98(1): 49-57.
- Maia, A.D.H., Luiz, A.J. & Campanhola, C., 2000. Statistical inference on associated fertility life parameters using jackknife technique: computational aspects. *Journal of Economic Entomology* 93(2): 511-518.
- Murai, T. & Loomans, A.J., 2001. Evaluation of an improved method for mass-rearing of thrips and a thrips parasitoid. *Entomologia experimentalis et applicata* 101(3): 281-289.
- Noyes J.S. & Hayat M.S., 1994. *Oriental mealybug parasitoids of the Anagyrini (Hymenoptera: Encyrtidae)*. CAB International Press, Wallingford.
- Powell, J.E. & Hartley, G.G., 1987. Rearing *Microplitis croceipes* (Hymenoptera: Braconidae) and other parasitoids of Noctuidae with multicellular host-rearing trays. *Journal of economic entomology* 80(4): 968-971.
- Price, P.W., 1984. *Insect Ecology* (2nd Edition). JohnWiley, New York.
- Purvis, A. & Harvey, P.H., 1995. Mammal life-history evolution: a comparative test of Charnov's model. *Journal of Zoology* 237(2): 259-283.
- Ratcliffe, N.A., 1993. Cellular defense responses of insects: unresolved problems. *Parasites and pathogens of insects* 1: 267-304.
- Reed, D.A., Luhring, K.A., Stafford, C.A., Hansen, A.K., Millar, J.G., Hanks, L.M. & Paine, T.D., 2007. Host defensive response against an egg parasitoid involves cellular encapsulation and melanization. *Biological Control* 41(2): 214-222.

- Rivero, A. & West, S.A., 2002. The physiological costs of being small in a parasitic wasp. *Evolutionary Ecology Research* 4(3): 407-420.
- Searle, C.M.St.L., 1965. The susceptibility of *Pauridia peregrine* Timb. (Hymenoptera: Encyrtidae) to some pesticide formulations. *Journal of the Entomological Society of Southern Africa* 27(2): 239-249.
- Sirot, E. & Bernstein, C., 1996. Time sharing between host searching and food searching in parasitoids: state-dependent optimal strategies. *Behavioural Ecology* 7: 189–194.
- Stearns, S.C. & Crandall, R.E., 1981. Quantitative predictions of delayed maturity. *Evolution* 35: 455-463.
- Timberlake, P.H., 1919. Description of new genera and species of Hawaiian Encyrtidae (Hymenoptera). *Proceedings of the Hawaiian Entomological Society* 4, 197–232.
- Urban, A.J. & Greeff, F.C., 1985. The integrated control of the vine mealybug *Planococcus ficus* (Signoret), on vines. Final Report. *Plant Protection Research Institute*.
- Walton, V.M. & Pringle, K.L., 2002. Evaluating effectiveness of mass releases of the vine mealybug (*Planococcus ficus*) parasitoid *Coccidoxenoides peregrinus* in Western Cape province vineyards, South Africa. In *Proceedings of the 1st International Symposium on Biological Control of Arthropods, Honolulu, Hawaii*, pp. 14-18.
- Walton, V.M. & Pringle, K.L., 2005. Developmental biology of vine mealybug, *Planococcus ficus* (Signoret) (Homoptera: Pseudococcidae), and its parasitoid *Coccidoxenoides perminutus* (Timberlake) (Hymenoptera: Encyrtidae). *African Entomology* 13(1).
- Watson, T.F., 1964. Influence of host plant condition on population increase of *Tetranychus telarius* (Linnaeus) (Acarina: Tetranychidae). *Hilgardia* 35: 237–322.
- Wheeler, D., 1996. The role of nourishment in oogenesis. *Annual Review of Entomology* 41: 407–431.

CHAPTER FIVE:

GENERAL DISCUSSION

Planococcus ficus (Signoret)(Hemiptera: Pseudococcidae) is a major pest in all grape growing areas (Ben-Dov, 1994; Walton, 2003) and a vector for grapevine leafroll virus (Engelbrecht & Kasdorf, 1990), which causes redness and rolling of the leaves, a decline in yield and sugar accumulation, and delayed ripening of the fruit (Engelbrecht & Kasdorf, 1990; Joyce et al., 2001; Monis & Bestwick, 1997; Rosciglione & Gugerli, 1989).

This study aimed to contribute information towards a sustainable management plan of *P. ficus* by creating a suitable habitat for mealybug parasitoids. This was done by conducting a survey of the mealybug parasitoids occurring in the vineyards and the surrounding natural habitat which is discussed in Chapter 2. An olfactometer experiment was conducted to determine which selected indigenous flowering plants *Coccidoxenoides perminutus* (Timberlake)(Hymenoptera: Encyrtidae) is attracted to, with a subsequent survey to determine the parasitoid diversity on flowering plants adjacent to vineyards during spring (Chapter 3). Finally, life history tables were completed for *C. perminutus* on *Planococcus citri* (Risso)(Hemiptera: Pseudococcidae) which are discussed in Chapter 4.

During the survey of the natural enemies of *P. ficus* in three vineyards in the Western Cape Province, South Africa, parasitoids were caught from January to May, with a peak in February. Significantly more parasitoids were found in the vineyards than in the surrounding natural habitat ($F_{(1, 594)}=3.8676$, $p=0.049$), but still at relatively low numbers. This could indicate a lack of food and host resources inside and outside the vineyard (Walton & Pringle, 2004). With predators, a critical number of individuals are needed to be able to procreate and hunt for food. However, with parasitoids, survival is higher at lower densities, as the competition for hosts and food sources is less (Courchamp et al., 1999). In field populations of parasitoids direct density dependence, inverse density dependence and density independence are all observed (Walde & Murdoch, 1988; Mills & Getz, 1996). Murdoch et al., (1985) found that an essential characteristic for a successful biocontrol agent is the need for the control agent to aggregate either independently of pest distribution or even better, in patches of high pest density, but there seems to be little field evidence of parasitoids aggregating at high host densities (Morrison & Strong, 1980; Murdoch et al., 1984). It is

uncertain if spatial aggregation of parasitoids is truly desired. By aggregating in areas of high host density the control agent's efficiency would increase, but it could also lead to pest refuges if the control agent does not leave the patches where pest populations have already been wiped out (Murdoch et al., 1985). These interactions will need to be considered when testing and implementing habitat management strategies in vineyards.

Three parasitoid species were found to be dominant in the current study: *Coccidoxenoides perminutus* (Timberlake), *Anagyrus* sp. near *pseudococci* (Girault) and *Leptomastix dactylopii* (Howard) (Hymenoptera: Encyrtidae). These findings confirm those of previous studies (Whitehead, 1957; Walton & Pringle, 2004). For future purposes of conservation biological control and the longterm establishment of parasitoids, the provision of refuge and food resources during months when mealybugs are less or harder to find could help to keep the parasitoid population going and lessen the need for pesticides. Future studies should assess whether mealybug parasitoids overwinter within hosts or die off in Western Cape vineyards, as this has major implications for the implementation of a habitat management plan. Sasa (2011) found a close link between the protea-pest *Delottococcus* spp. and the parasitoid *Anagyrus* spp. He also found that parasitoid abundances were highest in the months when hosts for parasitoids were highest. It is possible, therefore, that protea mealybugs can provide alternate hosts for *Anagyrus* sp. near *pseudococci* and possibly other *P. ficus* parasitoids, providing a valuable and sustainable eco-system service. It should, however, be noted that protea mealybugs were found predominantly in commercial protea cultivations (Sasa, 2011). Passarinho et al. (2006) determined *Anagyrus pseudococci* to be a good candidate for biological control of mealybugs in Proteaceae. Natural, indigenous vegetation found in the Western Cape agro-ecosystem could potentially play a role in attracting natural enemies and maintaining populations.

Adult *C. perminutus* need pollen or nectar to survive, as they do not host feed. In the current study, out of six plants only *Euryops abrotanifolia* (L.) DC proved to be significantly attractive to *C. perminutus*. This plant is ideal for planting in vineyards, as it is a fast-growing, water-wise plant that flourishes in full sun and flowers in winter and spring. Experiments should be continued to determine a complementary array of flowering plants with different flowering periods, to ensure the continuous availability of food and shelter for parasitoids to enhance populations in or around the vineyards (Doutt & Nakata, 1973). As

agricultural systems are monocultures and generally lack alternate resources, management practices, like the planting of non-crop habitats adjacent to crop fields (Landis et al., 2000), have been implemented. Many previous studies have been done to test flowering plants for the enhancement of parasitoids' habitat (Bowie et al., 1995; Idris & Grafius, 1995) with some success. Irvin (1999) found buckwheat, *Fagopyrum esculentum* Moench, and alyssum (*Lobularia maritime* (L) Desv.) to enhance leafroller parasitism in New Zealand apple orchards. In California, buckwheat also enhanced parasitoid populations in vineyards (Nicholls et al., 2000; Berndt et al., 2002). Davies et al., (2004) found that *Alpinia* nectar or honey supplied as a food source increased parasitoid life span in laboratory studies. Using flowering *Phacelia tanacetifolia* (Benth) as a border increased parasitism rates in wheat fields and apple orchards in New Zealand and Canada (Leius, 1961; Irvin et al., 1999; Hickman & Wratten, 1996; Berndt et al., 2002). However, to ensure that a plant does not become invasive and to keep within the conservation ideal, indigenous plants should be considered before any other.

In the survey of parasitoids on flowering plants in the field, a large variety of species were found, of which both Encyrtidae and Aphelinidae are parasitoids of Hemiptera. This indicates that parasitoids do occur in nature, but the abundance and exact host choices are as yet unclear. However, in this study no known parasitoids of *P. ficus* were found. Whether the parasitoids found will be able to provide a valuable ecosystem service without additional releases and improvements in the field is also unknown and warrants further investigation with a view to biological control.

Preliminary tests to determine the instar specific preferences of *C. perminutus* on *P. ficus*, were unsuccessful. This could be because of quality issues due to transportation, or an acclimatization of the parasitoids to the host they were reared on, namely *Planococcus citri*. When testing the effects of shipping and handling on two species of braconid parasitoids in Hawaii, Messing et al., (1993) found adult parasitoid emergence in the field to be significantly reduced from the numbers emerging in the insectary. For *Diachasmimorpha longicaudata* emergence was 26% below that in the insectary and as such just less than 50% of the puparia released in the field emerged as adults. For *Diachasmimorpha tryoni* only 14.8% of parasitized puparia emerged in the field. It was also found that the vessel used for release had an impact on the survival of the parasitoids, as many parasitoids died within the

vessel itself. Shipping problems can also occur due to lack of attention to the proper delivery of the product, which is then in transit for too long a period of time or in conditions that might be unfavourable (Smith, 1996).

The tests to determine the instar-specific preference of *C. perminutus* on *P. citri* (Risso) showed that although there seemed to be a preference to oviposit in second and third nymphal instars when given the choice, it wasn't significant and all nymphal instars were attacked. There was also no real cost to egg-laying, as indicated by experiments to compare parasitoids allowed to lay eggs with those that were not. The non-significant decrease in life expectancy when a parasitoid lays eggs could possibly be attributed to energy expended in the laying of eggs or the need to lay eggs rather than feed. The difference is negligible, however, so it is not something that needs to be taken into consideration when planning the intervals of parasitoid releases. Table 5.1 is a summary of all life table parameters published on *C. perminutus* and its main economic hosts *P. ficus* and *P. citri*.

Table 5.1. Summary of various population parameters of *Coccidoxenoides perminutus* with its hosts *Planococcus ficus* and *P. citri*, affecting the potential success of biological control.

Parameter	Current study	Ceballo & Walter, 2004	Walton & Pringle, 2005
Host	<i>P. citri</i>	<i>P. citri</i>	<i>P. ficus</i>
Temperature of study	25.21 °C & 25.72 °C	28 °C	25 °C
R ₀ (net replacement rate)	159.5	-	69.9
T (generation time)	27.6	-	29.5
r _m (intrinsic rate of increase)	0.51	-	0.15
Longevity (days)	4.57±1.22 SE	5.4±0.55 SE	1.1±0.02 SE
Fecundity (eggs/lifetime)	135±22.34 SE	239.2±34.3 SE	104.0±1.5 SE
Instar preference	First, second and third instar	Second and third instar	-
Doubling time	1.36	-	-

From these comparisons it can be seen that *C. perminutus* had a slightly lower T and a much higher R_0 on *P. citri* than on *P. ficus*. This can also be seen in the r_m , which is almost three times higher on *P. citri*. The temperature at which the studies were conducted could attribute to the differences, although for the *P. ficus* study, 25°C was in fact the temperature with the higher development rates, together with 21°C. The lifetable parameters were similar to those determined by Walton (2003), except for the net reproductive rate and intrinsic rate of increase. This could however be credited to the methods used in collecting progeny, when comparing these parameters to the current study. It seems when comparing the experiments done for this thesis with experiments done by Ceballo & Walter (2004) and Walton & Pringle (2005) that the biggest difference in parameters determined was due to the use of collected mummies in these experiments instead of live progeny as previously done. The use of mummies could give a false estimate of total progeny. This is very important to rearing facilities as they should take this into consideration when sending the product to farms for release. If the actual number of parasitoids released is only half of the estimated number, not enough parasitoids may be released to give the necessary control over a mealybug population. The biggest issue to resolve for the commercial side of parasitoid release is the method of collecting specimens for shipping to farms. It would be advisable to determine what percentage of mummies survive until adulthood, as well as if this percentage is in any way changed by the method of collection.

Recommendations and future research

It would be advisable to test more plants for parasitoid attraction as in this study only one species was found to have a positive effect in laboratory tests. Field sampling indicated that *Athanasia crithmifolia* (endemic Asteraceae), found to host Encyrtidae would be a good candidate to investigate further, while *Spartium junceum* (Fabaceae) and *Taraxacum officinale* (Asteraceae) are both invasive weeds and although they hosted aphelinid wasps, they would not be suitable candidates for incorporation into a habitat management plan. Further field sampling is needed, as this study was limited in its scope. It is therefore recommended that the abundance and diversity of *P. ficus* parasitoids in the field throughout the year be determined to be able to decide what level of intervention is needed for optimum biological control. Assessing *Euryops abrotanifolia* further as a candidate for habitat management and determining if mealybug parasitoids exist for a

longer time period in the presence of this plant would be necessary to see if it makes a difference in the field.

A very necessary experiment is to determine if rearing the parasitoids on one species of mealybug and then releasing them to parasitise another is affecting the parasitoid's ability to control the pest. Rearing the parasitoid on one mealybug species might make it less effective against other mealybug species.

Understanding the habitat requirements (Kromp, 1999; Landis et al., 2000) and clarifying the chemical ecology (Hardie & Minks, 1999; Pettersson et al, 2005; Pickett & Glinwood, 2007) of predators and parasitoids can lead to the development of strategies to manipulate and improve their abundance and distribution and so improve biocontrol. Biological control often fails to be as effective as studies have shown, which could be because parasitoids do not come into the field early enough to prevent the exponential increase in mealybug populations. In a push-pull capacity the use of sex pheromones is very useful. For parasitic wasps, it was found that they can be trained to look for certain chemical cues of their hosts, and semiochemicals emitted by plants attacked by herbivores, and that the wasps may be conditioned to search for these cues before they are released (Tumlinson et al., 1993). These Herbivore-Induced Plant Volatiles (HIPVs) have been synthesized and used in vineyards to attract parasitoids in order to deter herbivores from attacking plants (Khan et al., 2008). This shows great potential for use in vineyards to improve conservation biological control and requires further research in South Africa. For future management of *P. ficus* within a habitat management scenario, with suitable *P. ficus* parasitoid refuges (i.e. well researched indigenous flowering plants, established within vineyard agroecosystems at the most effective time of year), these HIPVs could be utilized to attract parasitoids into areas where even small populations of *P. ficus* occur.

The most important findings can be summarized as follows:

- *Anagyrus* sp. near *pseudococci* and *Coccidoxenoides perminutus* are still the most abundant parasitoids of mealybugs. Higher numbers of parasitoids were found in vineyards than in the surrounding natural habitat, possibly due to the presence of mealybugs in the vineyards.
- As only a single plant (*Euryops abrotanifolia*) was found to be attractive to *C. perminutus*, screening of more plants needs to be done to determine an array of flowering plants attractive to mealybug parasitoids. These plants also need to be investigated to determine if it serves as a suitable food source for mealybug parasitoids in the field.
- Life table results were similar to previous studies. Differences might be ascribed to the method of collecting progeny. In this study, as in commercial rearing facilities, mummies are counted to determine number of progeny and this may overestimate the numbers released in vineyards. Determining the percentage of successful adult emergence after shipping is therefore necessary.
- Currently parasitoids are reared on *Planococcus citri* and released as a control measure for *Planococcus ficus* in vineyards. Control may be affected due to issues with adaptation from one host to the other.
- For habitat management to truly have an effect one needs to determine if other suitable mealybug hosts occur in the natural habitat to sustain parasitoid populations when *P. ficus* populations are low.

5.1 REFERENCES

- Ben-Dov, Y., 1994. *A systematic catalogue of the mealybugs of the world (Insecta: Homoptera: Coccoidea: Pseudococcidae and Putoidae) with data on geographical distribution, host plants, biology and economic importance*. Intercept Limited, Andover, UK.
- Berndt, L.A., Wratten, S.D. & Hassan, P.G., 2002. Effects of buckwheat flowers on leafroller (Lepidoptera: Tortricidae) parasitoids in a New Zealand vineyard. *Agricultural and Forest Entomology* 4: 39-45.
- Bowie, M.H., Wratten, S.D. & White, A.J., 1995. Agronomy and phenology of 'companion plants' of potential for enhancement of insect biological control. *New Zealand Journal of Crop and Horticultural Science* 23: 423-427.
- Ceballo, F.A. & Walter, G.H., 2004. Why is *Coccidoxenoides perminutus*, a mealybug parasitoid, ineffective as a biocontrol agent – Inaccurate measures of parasitism of low adult survival? *Biological Control* 33: 260-268.
- Cook, S.M., Khan, Z.R. & Pickett, J.A., 2006. The use of push-pull strategies in integrated pest management. *Annual review of entomology* 52(1): 375.
- Courchamp, F., Clutton-Brock, T. & Grenfell, B., 1999. Inverse density dependence and the Allee effect. *Trends in ecology & evolution* 14(10): 405-410.
- Davies, A.P., Ceballo, F.A. & Walter, G.H., 2004. Is the potential of *Coccidoxenoides perminutus*, a mealybug parasitoid, limited by climatic or nutritional factors? *Biological control* 31: 181-188.
- Doutt, R.L. & Nakata, J., 1973. The Rubus leafhopper and its egg parasitoid: an endemic biotic system useful in grape-pest management. *Environmental Entomology* 2(3): 381-386.
- Engelbrecht, D.J. & Kasdorf, G.G.F., 1990. Transmission of grapevine leafroll disease and associated closteroviruses by the vine mealybug, *Planococcus ficus*. *Phytophylactica* 22: 341-346.
- Hardie, J. & Minks, A.K., eds., 1999. *Pheromones of Non-Lepidopteran Insects Associated with Agricultural Plants*. Wallingford, Oxon, UK: CABI.

- Hickman, J.M. & Wratten, S.D., 1996. Use of *Phacelia tanacetifolia* strips to enhance biological control of aphids by hoverfly larvae in cereal fields. *Journal of Economic Entomology* 89(4): 832-840.
- Idris, A.B. & Grafius, E., 1995. Wildflowers as nectar sources for *Diadegma insulare* (Hymenoptera: Ichneumonidae), a parasitoid of diamondback moth (Lepidoptera: Yponomeutidae). *Environmental Entomology* 24: 1727-1735.
- Irvin, N.A., 1999. *Understorey management for the enhancement of populations of a leafroller (Lepidoptera: Tortricidae) parasitoid (Dolichogenidea tasmanica (Cameron)) in Canterbury, New Zealand apple orchards*. PhD dissertation. Lincoln University, Canterbury, New Zealand.
- Irvin, N.A., Wratten, S.D., Chapman, R.B. & Frampton, C.M., 1999. Effects of floral resources on fitness of the leafroller parasitoid (*Dolichogenidea tasmanica*) in apples. *Proceedings of the New Zealand Plant Protection Conference* 52: 84-88.
- Joyce, A.L., Hoddle, M.S., Bellows, T.S. & Gonzalez, D., 2001. Oviposition behavior of *Coccidoxenoides peregrinus*, a parasitoid of *Planococcus ficus*. *Entomologia Experimentalis et applicata* 98(1): 49-57.
- Khan, Z.R., James, D.G., Midega, C.A. & Pickett, J.A., 2008. Chemical ecology and conservation biological control. *Biological control* 45(2): 210-224.
- Kromp, B., 1999. Carabid beetles in sustainable agriculture: a review on pest control efficacy, cultivation impacts and enhancement. *Agriculture, Ecosystems & Environment* 74:187-228
- Landis, D.A., Wratten, S.D. & Gurr, G.M., 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology* 45:175-201
- Leius, K., 1961. Influence of food on fecundity and longevity of adults of *Itopectis conquisitor* (Say) (Hymenoptera: Ichneumonidae). *Canadian Entomologist* 91: 771-780.

Messing, R.H., Klungness, L.M., Purcell, M. & Wong, T.T.Y., 1993. Quality control parameters of mass-reared opiine parasitoids used in augmentative biological control of tephritid fruit flies in Hawaii. *Biological Control* 3(2): 140-147.

Mills, N.J. & Getz, W.M., 1996. Modelling the biological control of insect pests: a review of host-parasitoid models. *Ecological modelling* 92(2): 121-143.

Monis, J. & Bestwick, R. K., 1997. Serological detection of grapevine associated closteroviruses in infected grapevine cultivars. *Plant Disease* 81(7): 802-808.

Morrison, G. & Strong, D.R., 1980. Spatial variations in host density and the intensity of parasitism: some empirical examples. *Environmental Entomology* 9(2): 149-152.

Murdoch, W.W., Scott, M.A. & Ebsworth, P., 1984. Effects of the general predator, *Notonecta* (Hemiptera) upon a freshwater community. *The Journal of Animal Ecology* 791-808.

Murdoch, W.W., Chesson, J. & Chesson, P.L., 1985. Biological control in theory and practice. *American Naturalist* 344-366.

Nakashima, Y., Birkett, M.A., Pye, B.J., Pickett, J.A. & Powell, W., 2004. The role of semiochemicals in the avoidance of the seven-spot ladybird, *Coccinella septempunctata*, by the aphid parasitoid, *Aphidius ervi*. *Journal of Chemical Ecology* 30:1103–16

Nicholls, C.I., Parrella, M.P. & Altieri, M.A., 2000. Reducing the abundance of leafhoppers and thrips in a northern California organic vineyard through maintenance of full season floral diversity with summer cover crops. *Agricultural and Forest Entomology* 2(2): 107-113.

Passarinho, A.M., Leandro, M.J., Oliveira, M., Figueiredo, E., Franco, J.C., Neves-Martins, J. & Mexia, A., 2006. Parasitism of mealybugs by *Anagyrus pseudococci* (Girault) in Proteaceae. *Boletín de Sanidad Vegetal, Plagas* 32(2): 215-221.

Pettersson, J., Ninkovic, V., Glinwood, R., Birkett, M.A. & Pickett, J.A., 2005. Foraging in a complex environment—semiochemicals support searching behaviour of the seven spot ladybird. *European Journal of Entomology* 102:365–70

Pickett, J.A. & Glinwood, R., 2007. Chemical ecology. In *Aphids as Crop Pests*, ed. H.F. van Emden, R. Harrington. CABI, UK.

Powell, W. & Pickett, J.A., 2003. Manipulation of parasitoids for aphid pest management: progress and prospects. *Pest Management Sciences* 59:149–55

Rosciglione, B. & Gugerli, P., 1989. Transmission of grapevine leafroll disease and an associated closterovirus to healthy grapevine by the mealybug *Planococcus ficus*. *Phytoparasitica* 17: 63.

Sasa, A., 2011. *Arthropods associated with commercial Proteaceae in the Western Cape Province, South Africa*. PhD dissertation, Department of Conservation Ecology and Entomology, Stellenbosch University, Private Bag X1, 7620 Matieland (Stellenbosch), South Africa.

Smith, S.M., 1996. Biological control with *Trichogramma*: advances, successes, and potential of their use. *Annual review of entomology* 41(1): 375-406.

Tumlinson, J.H., Turlings, T.C. & Lewis, W.J., 1993. Semiochemically mediated foraging behavior in beneficial parasitic insects. *Archives of Insect Biochemistry and Physiology* 22(3-4): 385-391.

Walde, S.J. & Murdoch, W.W., 1988. Spatial density dependence in parasitoids. *Annual review of entomology* 33(1): 441-466.

Walton, V. M., 2003. *Development of an integrated pest management system for vine mealybug, Planococcus ficus (Signoret), in vineyards in the Western Cape Province, South Africa*. PhD dissertation, Department of Conservation Ecology and Entomology, Stellenbosch University, Private Bag X1, 7620 Matieland (Stellenbosch), South Africa.

Walton, V.M. & Pringle, K.L., 2004. A survey of mealybugs and associated natural enemies in vineyards in the Western Cape provinces. *South African Journal of Entology and Viticulture* 25(1).

Walton, V.M. & Pringle, K.L., 2005. Developmental biology of vine mealybug, *Planococcus ficus* (Signoret) (Homoptera: Pseudococcidae), and its parasitoid *Coccidoxenoides perminutus* (Timberlake) (Hymenoptera: Encyrtidae). *African Entomology* 13(1).

Whitehead, V. B. (1957). *A study of the predators and parasites of Planococcus citri (Risso)(Homoptera: Pseudococcidae) on vines in the Western Cape Province, South Africa.* PhD dissertation, Rhodes University, Grahamstown.