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FINAL REPORT
(2015)

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2. PROJECT INFORMATION

Research Organisation Project number	US ENT 11-A2
Project title	Development of a habitat management plan to promote conservation biological control in vineyards.
Short title	Habitat management plan in vineyards

Fruit kind(s)	Vines		
Start date (mm/yyyy)	01/2012	**End date** (mm/yyyy)	03/2014

Key words	Vine mealybug, parasitoids, conservation biological control

Approved by Research Organisation Programme leader (tick box) ☐

THIS REPORT MUST INCLUDE INFORMATION FROM THE **ENTIRE** PROJECT

3. EXECUTIVE SUMMARY

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. Signficantly 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 indivdual 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℃ on *Planococcus citri* (Risso), as initial experiments utilizing *P. ficus* had failed. In constrast 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 ($R_o=159.5$; $T=27.602$; $r_m=0.511$) were different to those determined by Walton (2003) ($R_o=69.94$; $T=29.5$; $r_m=0.149$) except for T which was similar, although the latter study was conducted on *P. ficus*. 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*.

4. PROBLEM IDENTIFICATION AND OBJECTIVES

There is an increasing trend for grape farmers to make use of augmentative releases of parasitic wasps to control vine mealybug. The objectives of this project are to 1) assess various flowering plants for improving parasitism of vine mealybug and longevity and fecundity of adult parasitoids in vineyards; and to 2) assess larval parasitoid survival and parasitization ability on different vine mealybug developmental stages.
The ultimate aim of this study is therefore to improve biological control against vine mealybug through basic biological studies and the initial development of a habitat management plan for vineyards, which could benefit more pests.

5. WORKPLAN (MATERIALS AND METHODS)

1) Food source for adult parasitoids (assessment of plant species):

a) SURVEY:

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. 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. Both *Coccidoxenoides perminutus* and *Cryptolaemus montrouzieri* were released at Plaisir de Merle during the trial period. Mealybug stock cultures were reared on butternuts (*Cucurbita moschata*) in cages (750mm x 500mm x 300mm) in an insectary at a temperature of 25℃ and a 12:12 (light:dark) photoperiod. 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. 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. All statistical analyses were conducted in Statistica, version 12 (Statsoft Inc., 2013).

b) PARASITOID RESPONSES 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
Tulbaghia violacea Harv.	Alliaceae	Wild garlic
Coleonema pulchellum I. Williams	Rutaceae	Confetti bush
Felicia bergeriana (Spreng.) O.	Asteraceae	Kingfisher daisy

Hoffm.		
Gnidia pinifolia L.	Thymelaeaceae	White Gnidia
Euryops abrotanifolia (L.) DC.	Asteraceae	Lace-leaf Euryops
Erica gracillus J.C. Wendl.	Ericaceae	Cape Heath

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 ± 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℃ and a 12:12 hour light:dark photoperiod. 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. 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 was placed. Only the air drawn through the bucket was filtered so as to ensure pure odour from the plants. 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. The plant, in its black plastic bag with soil, was placed inside the experimental arena, on the lid. The olfactometer 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, 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.

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

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.

2) Food source for larval parasitoids (assessment of vine mealybug development stages):

a) Life history of *Coccidoxenoides perminutus* on *Planococcus citri*.

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℃ 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℃ and 7:17 L:D photoperiod. This trial was therefore abandoned and life table parameters were determined using *P. citri* only.

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

No-choice test. A piece of butternut (1cm x 1cm 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.

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℃ and average humidity of 79.63%.

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.

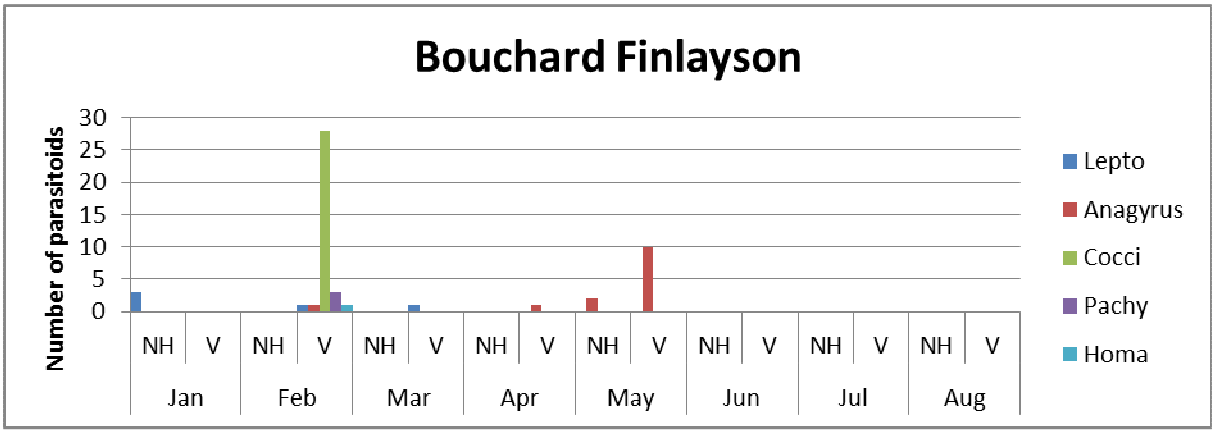
The data for both the instar-specific preference trials and the longevity trial were tested for homogeneity and normality before being analysed using a one-way ANOVA. The analyses were done in Statistica, version 12 (Statsoft Inc., 2013).

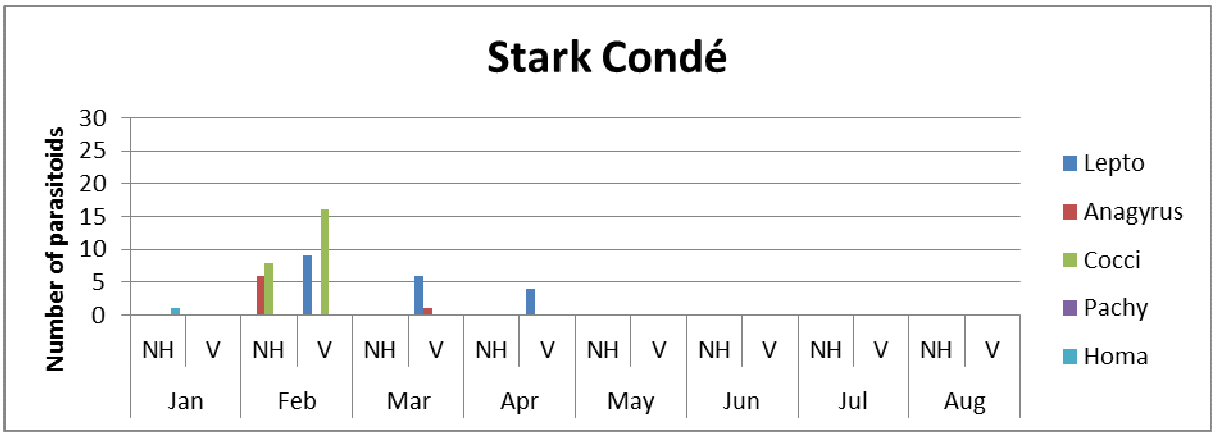
With the age-specific fecundity trial, adult parasitoid age (x), age-specific survival rates (l_x) and number of offspring produced per female per day (m_x) were determined. With this information other life-table parameters, like gross reproductive rate (GRR = $\sum m_x$), net reproductive rate ($R_o = \sum l_x m_x$), mean generation time ($GT = \sum(l_x m_x x)/\sum(l_x m_x)$), the initial estimate of the intrinsic rate of increase ($r_m = Log_e R_o / T$) and doubling time ($DT = \ln(2)/r_m$) were calculated.

RESULTS AND DISCUSSION

1) Food source for adult parasitoids (assessment of plant species):

a) SURVEY: Parasitoids were recovered from the vineyards and surrounding natural habitat from January 2012 to May 2012 (Figure 1). No further parasitoids were reared from the butternuts from June 2012 until October 2013, despite additional sampling efforts. 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.





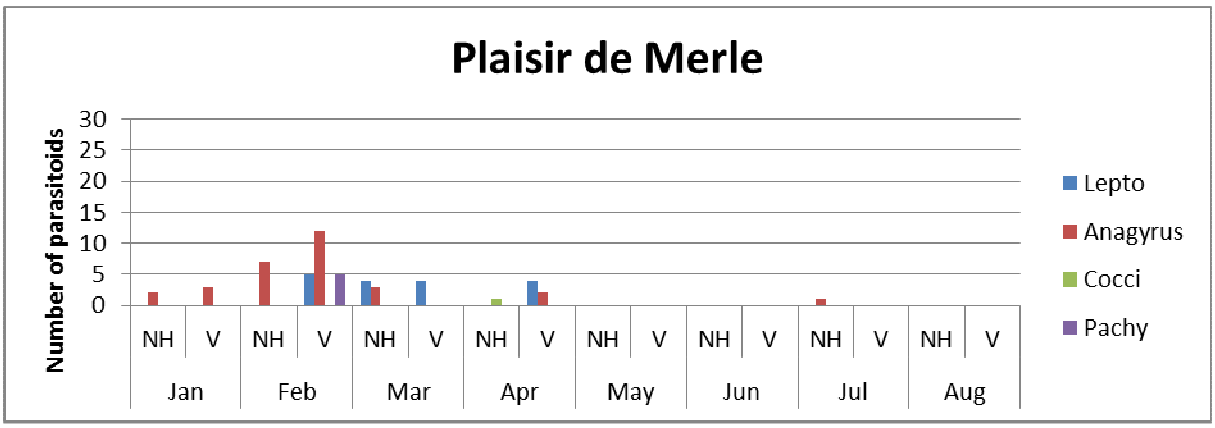


Fig. 1. Abundance of parasitoids Bouchard Finlayson in Hermanus (a), Stark Condé in Stellenbosch (b) and Plaisir de Merle in Simondium (c) from January 2012 to August 2012. Total number of each species of parasitoid species caught in the Natural Habitat (NH) or Vineyard (V) was plotted against the month 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).

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 1). No significant difference was found between farms, $F_{(2, 594)}=0.0006$, $p=0.99$, or between the interaction between farm and habitat type, $F_{(2, 594)}=0.2834$, $p=0.75$. 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.

Table 1. 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
Anagyrus sp. near *pseudococci* (Females)	14	17	2	11	9	5	3	1
Anagyrus sp. near *pseudococci* (Males)	7	13		1	4	12	3	
Pachyneuron spp.	0	8		3	5			
Leptomastix dactylopii	7	34	3	2	4	13		19
Coccidoxenoides perminutus	9	44		28	1		8	16
Homalotylus spp.	1	1		1			1	
Totals	38	117	5	46	23	30	15	36

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).

b) PARASITOID RESPONSES TO FLOWERING PLANTS:

Of the six plants tested, only *Euryops abrotanifolia* 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 (Table 2).

Table 2. Comparison of the attractiveness of six indigenous plants to *Coccidoxenoides perminutus*.

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

*denotes statistically significant effect

One vineyard and 27 flowering plants were surveyed and a total of 20 wasps from 16 different species were sampled (Table 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. 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)	*Trifolia alba* *Brassica* spp. *Anagallis arvensis* *Leptospermum scoparium* *Brunia nodiflora* *Leucospermum* spp.	1	Pteromalidae spp 1
Backsberg (Paarl)	*Plectranthus neochilus* *Drosanthemum speciosum* *Felicia* spp. *Taraxacum officinale*	1	Pteromalidae spp 2
Delheim (Stellenbosch)	*Senecia* spp. *Pelargonium graveolens* *Spartium junceum* *Dombeya wallichii* *Euryops* spp.	2 1	Aphelinidae, near *Aphytis* Bracomidae, near *Apanteles*
Lourensford (Somerset West)	*Ipomoea indica* *Verbena bonariensis* *Taraxacum officinale* *Leucanthemum vulgare*	1	Unknown, near *Aphelinidae*
Waterkloof (Somerset West)	*Lampranthus* spp. *Podylaria* spp. *Euryops* spp. *Erica verticillata* *Scabiosa* spp.		
Vergelegen	*Lampranthus* spp.		

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

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 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, *Acyrthosiphon 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.

2) Food source for larval parasitoids (assessment of vine mealybug development stages):

a) Life history of *Coccidoxenoides perminutus* on *Planococcus citri*.

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. 2).

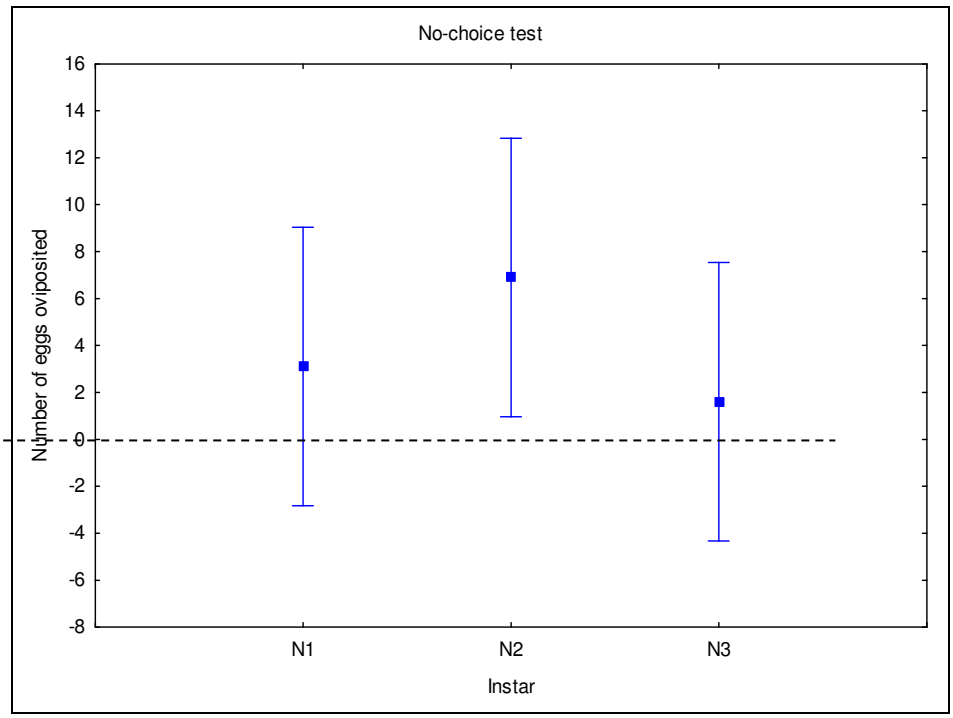


Fig. 2. Mean number of eggs oviposited (inferred from number of mummies) for no-choice test of *Coccidoxenoides perminutus* on its host *Planococcus citri*. Error bars denote 95% confidence intervals.

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. Mean number of *Coccidoxenoides perminutus* mummies recovered from *Planococcus citri* mealybugs in choice tests were, with N1 vs N2 ($F_{1, 19}$=.41320, p=.52803), N2 vs N3 ($F_{1; 19}$=5.4480, p=.03072) and N1 vs N3 ($F_{1, 19}$=4.1655, p=.05539). Ceballo 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. 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). The slight decrease in life expectancy could be due to the exertion of energy in laying eggs, or a need to lay eggs rather than feed. 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).
In this experiment the average lifetime production of progeny (estimated fecundity) by the parasitoids were 135±22.34 eggs.

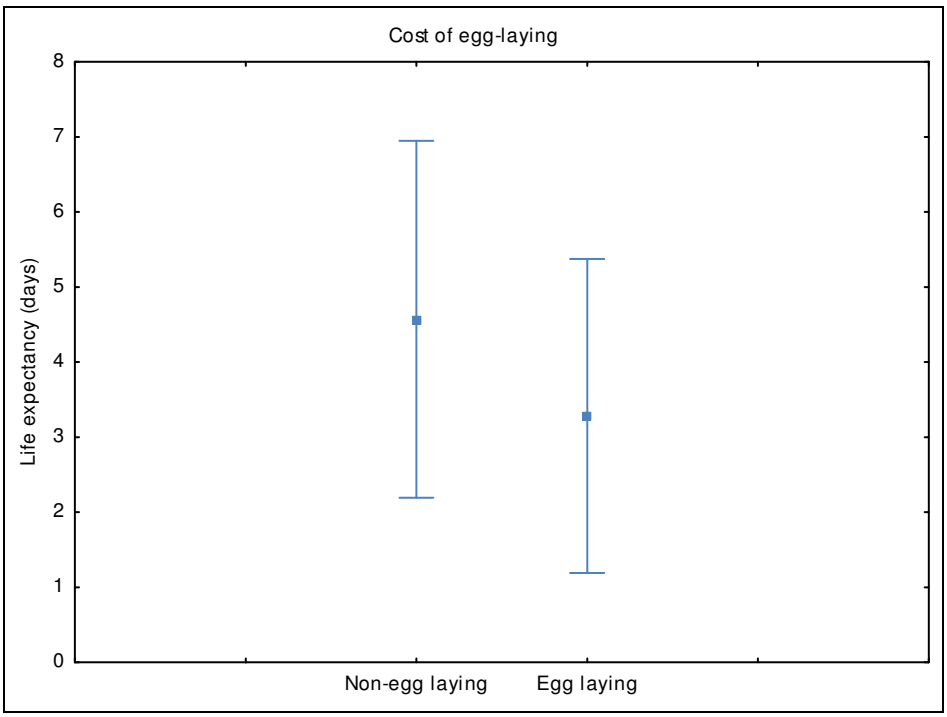


Fig. 4. A comparison of life expectancy of *Coccidoxenoides perminutus* (in days) of egg laying and non-egg laying parasitoids. Error bars denote 95% confidence intervals.

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_o); 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.
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_o) is half of this study's and their intrinsic rate of increase (r_m) a third (Table 4). This difference in R_o 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. 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_o)	159.5
Intrinsic rate of increase (r_m)	0.51
Doubling time (DT)	1.36 days

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 is a summary of all life table parameters published on *C. perminutus* and its main economic hosts *P. ficus* and *P. citri*.

Table 5. 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	*P. citri*	*P. citri*	*P. ficus*
Temperature of study	25.21 ˚C & 25.72˚C	28˚C	25˚C
R_o (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	-	-

6. COMPLETE THE FOLLOWING TABLE

Milestone	Target Date	Extension Date	Date completed	Achievement
1. Field sampling for parasitoids	October 2013		March 2014	Completed
2. Screening of flowering plants	July 2014		July 2014	Completed
3. Choice/no choice tests	October 2014		October 2014	Completed
4. Greenhouse trials	July 2014	July 2015	Not completed	Unable to complete due to host incompatibility
5. Journal publication(s) – final milestone	December 2015			

7. CONCLUSIONS

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.

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.

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.

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.

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8. ACCUMULATED OUTPUTS

a) TECHNOLOGY DEVELOPED, PRODUCTS AND PATENTS

Recommendations for development of a habitat management plan in vineyards, to improve the biological control of vine mealybug.

b) SUGGESTIONS FOR TECHNOLOGY TRANSFER

Winelands publication in preparation
Feedback was provided at four conferences, two at specialized enology conferences and two at specialized entomological conferences.

c) HUMAN RESOURCES DEVELOPMENT/TRAINING

Student Name and Surname	Student Nationality	Degree (e.g. MSc Agric, MComm)	Level of studies in final year of project	Graduation date	Total cost to industry throughout the project
Honours students					

Masters Students					
Sariana Faure	South Africa	MSc	Final year Masters	Dec 2014	R190 000
PhD students					
Postdocs					
Support Personnel					

d) PUBLICATIONS (POPULAR, PRESS RELEASES, SEMI-SCIENTIFIC, SCIENTIFIC)

None to date

e) PRESENTATIONS/PAPERS DELIVERED

Faure S, Addison P, Wohlfarter, M. (2011) The use of a combination of two parasitoids for controlling *Planococcus ficus* (Signoret). Poster, presented at Entomological Society of southern African congress, 2 – 6 July 2011, Bloemfontein.

Faure S, Addison P, Veldtman R. (2012) Improving biological control of the vine mealy bug (VMB) (*Planococcus ficus*) in vineyards. Poster, presented at South African Society for Enology and Viticulture conference, 14-16 November 2012, Allée Bleue.

Faure S, Addison P, Veldtman R. (2013) Improving biological control of the vine mealy bug (VMB) (*Planococcus ficus*) in vineyards. Presentation, at Department of Conservation Ecology and Entomology Research Day, 24 May 2013, Stellenbosch.

Faure S, Addison P, Veldtman R. (2013) Improving biological control of the vine mealy bug (VMB) (*Planococcus ficus*) in vineyards. Presentation, at Entomological Society of southern African congress, 30 June – 3 July 2013, Potchefstroom.

Faure S, Addison P, Veldtman R. (2013) Improving biological control of the vine mealy bug (VMB) (*Planococcus ficus*) in vineyards. Presentation, at South African Society for Enology and Viticulture conference, 14 November 2013, Lord Charles Hotel, Somerset West.

Faure S, Addison P, Veldtman R. (2014) Developing a habitat management plan for the vine mealybug (Planococcus ficus) in vineyards. Thesis defence presentation Department of Conservation Ecology and Entomology Department, Stellenbosch.

9. BUDGET

a) TOTAL COST SUMMARY OF THE PROJECT

YEAR	CFPA	DFTS	Deciduous	SATI	Winetech	THRIP	OTHER	TOTAL
YEAR 1			66840		66840	33400		167080
YEAR 2			75500		75500	30000		181000
YEAR 3					80000	30000		110000
YEAR 4					20000	5000		25000
YEAR 5					0	0		
TOTAL			**142340**		**242 340**	**98400**		**483080**

b) FINAL BUDGET/FINANCIALS OF PROJECT

Project duration	**Proposed budget**	**Actual cost incurred**	**Variance**	**Notes**
TOTAL INCOME				
Industry Funding	384680	384680	0	
PHI Funding	-	-	-	
Other Funding (THRIP)	98 400	98 400	0	
TOTAL EXPENDITURE				
Running Expenses				
General operating costs (printing, communication, etc.)	20000	20000	0	
Local Travel	28000	28000	0	
Publication costs				
Lab Analysis				
Lab Consumables	50000	50000	0	
Other (local conferences)	12000	12000	0	
Running expenses SUB-TOTAL	110000	110000	0	
HR Administration and Project Management				

Project duration	Proposed budget	Actual cost incurred	Variance	Notes
HR Technical	40000	40000	0	
HR Research				
Student Bursaries	190000	190000	0	
HR SUB-TOTAL	230000	230000	0	
OTHER EXPENSES				
12% SU levy	24680	24680	0	
SURPLUS / **DEFICIT**	364682	364682	0	