An investigation into the integrated pest management of the obscure mealybug, *Pseudococcus viburni* (Signoret) (Hemiptera: Pseudococcidae), in pome fruit orchards in the Western Cape Province, South Africa.

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DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the owner of the copyright thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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ABSTRACT

Pseudococcus viburni (Signoret) (Hemiptera: Pseudococcidae) (obscure mealybug), is a common and serious pest of apples and pears in South Africa. Consumer and regulatory pressure to produce commodities under sustainable and ecologically compatible conditions has rendered chemical control options increasingly limited. Information on the seasonal occurrence of pests is but one of the vital components of an effective and sustainable integrated pest management system needed for planning the initiation of monitoring and determining when damage can be expected. It is also important to identify which orchards are at risk of developing mealybug infestations while development of effective and early monitoring tools for mealybug populations will help growers in making decisions with regards to pest management and crop suitability for various markets. It is also essential to determine the presence and efficacy of naturally occurring biological control agents in orchards so as to ascertain the potential of biological control as a viable alternative in orchards. However, under the current integrated pest management protocol, it has been difficult to determine this, due to the sporadic and relatively low incidence of mealybug infestations in some orchards, or by simply relying on naturally occurring field populations of biocontrol agents. Knowledge of the environmental conditions under which *P. viburni* population levels may become destructive is also essential for timing the release of insectary reared natural enemies as well as understanding the population ecology of this pest and its natural enemies. Information was gathered regarding the seasonal phenology of *P. viburni* and its natural enemies in pome fruit orchards in the Western Cape Province during the 2007/08 and 2008/09 growing seasons. Seasonal population studies showed that *P. viburni* has multiple overlapping generations with all life stages present throughout the year. The highest orchard infestations occurred during the summer period until early winter (January to

early June). This was followed by a decrease in population from late June to November, before another increase in December. Presence-absence sampling of mealybugs on the host plant revealed that woody parts of the tree, such as the trunk and old stems were the most preferred sites for mealybug habitation, due to the availability of protected refuge sites. Migration of mealybug populations to newer growth and the upper sections of the tree crown, such as the new stems, leaves and eventually the fruit, was observed from December throughout the summer period until the early winter in June. Fruit colonization in both apples and pears commenced in January, when the fruit had developed a size sufficient for *P. viburni* to penetrate and occupy spaces such as the fruit core, calyx and stem end. There was no evidence of *P. viburni* occurring beneath the soil surface or on the roots of host trees. Two natural enemies of mealybugs, namely *Pseudaphycus maculipennis* (Mercet) and *Coccidoxenoides perminutus* (Girault), were found to be active in apple and pear orchards in the Western Cape. However, the status of *C. perminutus* as a parasite of *P. viburni* still needs to be verified despite evidence of emergence from *P. viburni* mummies, which was not sufficient enough to suggest that it is a useful biological control agent. Seasonal abundance trends of the two natural enemies revealed that their lifecycle is synchronized with that of the host. However, there was no evidence of *P. maculipennis* activity in Ceres. No predators were found during the course of this study. The rate of *P. viburni* parasitism at harvest was 46.52%, with *P. maculipennis* and *C. perminutus* constituting 98.966% and 1.034% of the parasitoids recovered from mealybug mummies, respectively. Studies on the use of pheromone traps as early monitoring tools for *P. viburni* showed that there was a positive and significant relationship between the fruit infestation and number of *P. viburni* adult males caught in pheromone-baited traps (r^2 = 0.454). The action threshold level was estimated to be 2.5 male *P. viburni* caught per trap per fortnight at an economic threshold of 2% fruit infestation. Laboratory studies on the development of *P. viburni*

at a range of temperatures showed that the development time from egg to oviposition, including the pre-oviposition period of adult female mealybugs, decreased from 132.33 days at 18°C to 47.80 days at 25°C. At 27°C, it increased to 68.73 days. The maximum number of eggs oviposited per female was approximately 240 at 25°C. The minimum and maximum threshold temperatures for *P. viburni* development were estimated to be 16.00°C and 27.97°C, respectively, while the optimum temperature for development was estimated to be 24.72°C. The information generated from this study is a useful guideline for further research into the biological control and improvement of the current integrated management protocol for *P. viburni*. A better understanding of the ecology and development of *P. viburni* was gained while a suitable early warning monitoring tool was developed to aid producers in deciding on suitable export markets.

UITTREKSEL

Pseudococcus viburni (Signoret) (Hemiptera: Pseudococcidae) (ligrooswitluis), is ‘n algemene en ernstige plaag van appels en pere in Suid-Afrika. Druk deur verbruikers en regulasies om kommoditeite onder volhoubare en ekologies verenigbare toestande te produseer het chemiese beheeropsies toenemend beperk. Inligting oor die seisoenale voorkoms van plae is een van die essensiële komponente van ‘n effektiewe en volhoubare geïntegreerde plaagbestuurprogram. Dit is in die aanvanklike beplanning van monitering en om te bepaal wanneer skade verwag kan word. Dit is ook belangrik om boorde vroegtydig te identifiseer wat die risiko het om witluisbesmettings te ontwikkel. Die ontwikkeling van effektiewe en vroeë moniteringstegnieke vir witluisbevolkings sal produsente help met besluitneming rakende plaagbestuur en die geskiktheid van gewasse vir verskeie markte. Dit is ook noodsaaklik om die teenwoordigheid en effektiwiteit van biologiese beheer agente wat natuurlik in boorde voorkom te bepaal ten einde die potensiaal van biologiese beheer as ‘n lewensvatbare alternatief vas te stel. Onder die huidige geïntegreerde plaagbestuurprotokol was dit egter moeilik om laasgenoemde te bepaal weens die sporadiese en relatiewe lae voorkoms van witluisbesmettings in sommige boorde of deur bloot staat te maak op die veldpopulasies van biologiese beheer agente wat natuurlik voorkom. Kennis van die omgewingstoestande waaronder *P. viburni* bevolkingsvlakke skadelik raak is ook noodsaaklik vir die beplanning van vrylating van biologiese beheer agente, asook om die bevolkingsekologie van hierdie plaag en sy natuurlike vyande te verstaan. Inligting oor die seisoenale fenologie van *P. viburni* en sy natuurlike vyande in sagtevrugte boorde in die Westelike Kaapprovinsie is gedurende die 2007/08 en 2008/09 groeiseisoene versamel. Seisoenale bevolkingstudies het getoon dat *P. viburni* verskeie oorvleuelende generasies het met alle stadia teenwoordig regdeur die jaar. Die hoogste boordbesmettings het

gedurende die somerperiode tot en met vroeë winter (Januarie tot vroeë Junie) voorgekom. Dit is gevolg deur ‘n afname in bevolking vanaf laat Junie tot November met ‘n toename in Desember. Aanwesigheid-afwesigheid monitering van witluise op die gasheerplant het getoon dat houtagtige dele van die boom, soos die hoofstam en ou systamme, die mees gewenste posisies vir witluisbewoning was, weens die beskikbaarheid van beskermde skuilplekke. Migrasie van witluisbevolkings na nuwer groei en die boonste seksies van die boomtop, soos nuwe stamme, blare en uiteindelik die vrugte is vanaf Desember regdeur die somerperiode tot die vroeë winter in Junie waargeneem. Kolonisasie van vrugte by appels en pere het in Januarie begin wanneer die vrugte ‘n voldoende grootte bereik het vir *P. viburni* om ruimtes soos die vrugkern, kelk en stamend binne te dring en te beset. Daar was geen bewys dat *P. viburni* onder die grondoppervlak of op die wortels van die gasheerbome voorkom nie. Twee natuurlike vyande van witluise, naamlik *Pseudaphycus maculipennis* (Mercet) en *Coccidoxenoides perminutus* (Girault) is aktief in appel- en peerboorde in die Wes-Kaap. Die status van *C. perminutus* as ‘n parasiet van *P. viburni* moet egter bevestig word, ten spyte van die verskyning vanuit *P. viburni* mummies, wat nie voldoende was om voor te stel dat dit ‘n bruikbare biologiese beheer agent is nie. Seisoenale voorkoms van die twee natuurlike vyande het aangedui dat hul lewensiklus met dié van die gasheer gesinkroniseer is. Daar was egter geen bewys van *P. maculipennis* aktiwiteit in Ceres nie. Geen predatore is gedurende die verloop van hierdie studie gevind nie. Die tempo van *P. viburni* parasitisme by oes was ongeveer 46.52% met *P. maculipennis* en *C. perminutus* wat 98.966% en 1.034% van die parasitoïede wat vanuit die witluismummies verkry is onderskeidelik uitgemaak het. Studies in die gebruik van feromoonvalle as vroeë moniteringstegnieke vir *P. viburni* het aangetoon dat daar ‘n positiewe en betekenisvolle verhouding was tussen vrugbesmetting en die aantal *P. viburni* volwassenes wat in die feromoonvalle gevang is (r^2 = 0.454). Die

aksiedrempelwaarde is beraam op 2.5 *P. viburni* mannetjies wat per val per twee weke gevang is teen ‘n ekonomiese drempelwaarde van 2% vrugbesmetting. Laboratoriumstudies op die ontwikkeling van *P. viburni* by ‘n reeks van temperature het getoon dat die ontwikkelingstyd vanaf die eier na eierlegging, insluitende die voor-eierleggingsperiode van volwasse wyfie witluise, vanaf 132.33 dae by 18°C na 47.80 dae by 25°C afgeneem het. By 27°C het dit na 68.73 dae toegeneem. Die maksimum aantal eiers wat per wyfie gelê is was ongeveer 240 by 25°C. Die minimum en maksimum drempel temperature vir *P. viburni* ontwikkeling is beraam om 16.00°C en 27.97°C onderskeidelik te wees, terwyl die optimum temperatuur vir ontwikkeling beraam is op 24.72°C. Die inligting wat uit hierdie studie bekom is is ‘n bruikbare riglyn vir verdere navorsing oor biologiese beheer en die verbetering van die huidige geïntegreerde bestuursprotokol vir *P. viburni*. ‘n Beter begrip van die ekologie en ontwikkeling van *P. viburni* is verkry, terwyl ‘n geskikte vroeë-waarskuwings moniteringstegniek ontwikkel is om produsente te help met besluite oor geskikte uitvoermarkte.

DEDICATION

I dedicate this thesis to my wife Precious and daughter Makanaka Natasha. Your constant support, love and understanding during the course of this work continue to make me the proudest husband and father. To my parents Richard Austin and Raviro Audrey Mudavanhu you have always been instrumental in my life, you nurtured and groomed me well, I know this is what you have always yearned.

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CONTENTS

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 History of the pest in South Africa

Three mealybug species from the genus *Pseudococcus* have been reported in apples and pears fruit in South Africa. These are the citrophilous mealybug, *P. calceolariae* (Maskell), the long-tailed mealybug, *P. longispinus* (Targioni-Tozzetti) and the obscure mealybug, *P. viburni* (Signoret) (Myburgh *et al.,* 1975) (Van Der Merwe, 2000). The latter appears to be the most important mealybug species on pome fruit in South Africa (Swart, 1977; Wakgari & Giliomee, 2004) and is the subject of this study.

The origin of the obscure mealybug is unknown and Daane *et al.* (2008) report that literature can be found that suggests both Australia and South America as possible origins. The history of the obscure mealybug is poorly documented partly due to earlier taxonomic confusion. It is a close relative of the grape mealybug, *P. maritimus* (Ehrhorn) and was often misidentified (Miller *et al.,* 1984). According to Kriegler and Basson (1962), mealybugs were relatively unimportant pests that have always been present on apple trees to a limited extent. Infestations were of such an inconspicuous nature that earlier research workers made no mention of mealybug on pome fruit. This was the assumption until *P. viburni* (formerly *P. obscurus* Essig.) suddenly came to the fore in epidemic proportions in the Elgin District of the Western Cape Province of South Africa (Kriegler & Basson, 1962). Prior to the epidemic of the 1960s ,mealybugs were just well known pests of grape vines as well as a serious pest on pears in the 1930s (Kriegler & Basson, 1962). Since *P. viburni* is an exotic pest with

few natural enemies (Varela *et al.,* 2006), it may have probably been introduced by humans and/or animals via plant material (Schoen & Martin, 1999).

1.2. Taxonomy

The latest classification of the obscure mealybug was by Ben-Dov (1994) who described it as falling under the order Hemiptera, superfamily Coccoidea, family Pseudococcidae, genus *Pseudococcus* and having the specific name *viburni*. The insect was formerly known as *Pseudococcus affinis* (Maskell) and *Pseudococcus obscurus* (Essig) but originally described by Signoret as *Dactylopius viburni* and *Dactylopius indicus* in 1875 (Ben-Dov & Matile-ferrero, 1995; Gimpel & Miller 1996). Both *P. affinis* and *D. viburni* have now been designated by Ben-Dov and Matile-ferrero (1995) as junior and senior synonyms of *P. viburni,* respectively. The full list of *P. viburni* synonyms and the keys for identifying the female of this species are available on ScaleNet (Ben-Dov & Germany, 2002). According to Sandanayaka *et al.* (2009), citing Gimpel and Miller (1996), the geographic origins of *P. viburni* are unknown, although it is placed taxonomically within the predominantly North American *P. maritimus* species-complex. Detailed information on the geographical distribution and spectrum of host plants is given by Ben-Dov (1994).

1.3. Morphometrics

Wakgari & Giliomee (2004) gave a detailed account of the description of the adult and immature female instars of *P. viburni* found on apples in South Africa. This information on age distinction criteria was vital for the developmental biology study of this pest (Chapter 4). The major micromorphological characteristics employed by Wakgari & Giliomee (2004) to identify and distinguish the different instars of *P. viburni* include body size, size of apical setae, number of cerarii around body margin, presence/absence and/or number of oral rim ducts, number of antennal segments

and occasionally presence/absence of auxiliary setae on cerarii. For the purpose of our developmental biology study only certain characteristics were chosen and these are presented in Table 1.1.

Table 1.1. Micromorphological characteristics for distinguishing developmental stages of *Pseudococcus viburni* (Wakgari & Giliomee, 2004) (Wearing *et al.,* 1999)

Stage	Average length (mm)	Average width (mm)	Notes
Egg	---	---	Yellow straw – orange in colour
First instar nymph	0.42	0.22	Oval shaped body, yellow-orange & six-segmented antennae with apical segment longest.
Second instar nymph	0.79	0.44	Body elongate, oval shaped antennae six-segmented with apical segment longest, yellow to orange brown body.
Third instar nymph	1.31	0.66	Body elongate oval, seven segmented antennae.
Adult Female	2.5	1.5	Wingless, light-pinkish & mealy in appearance due to waxy secretion, eight-segmented antennae.

Wakgari & Giliomee (2004) did not give a description of the egg stage of *P. viburni*., and no published information on the micromorphological characteristics of the eggs of *P. viburni* could be found. We therefore assumed that eggs of *P. viburni* are similar to those of *P. ficus,* a closely related mealybug species. Kriegler (1954) estimated the average size of *P. ficus* eggs to be 0.41 mm long and 0.21 mm wide. Several authors have also described the colour of the eggs as yellow straw to orange (Wearing *et al.,* 1999 & Miller *et al.,* 2007). A photograph of the adult female *P. viburni* is given in Figure 1.1.

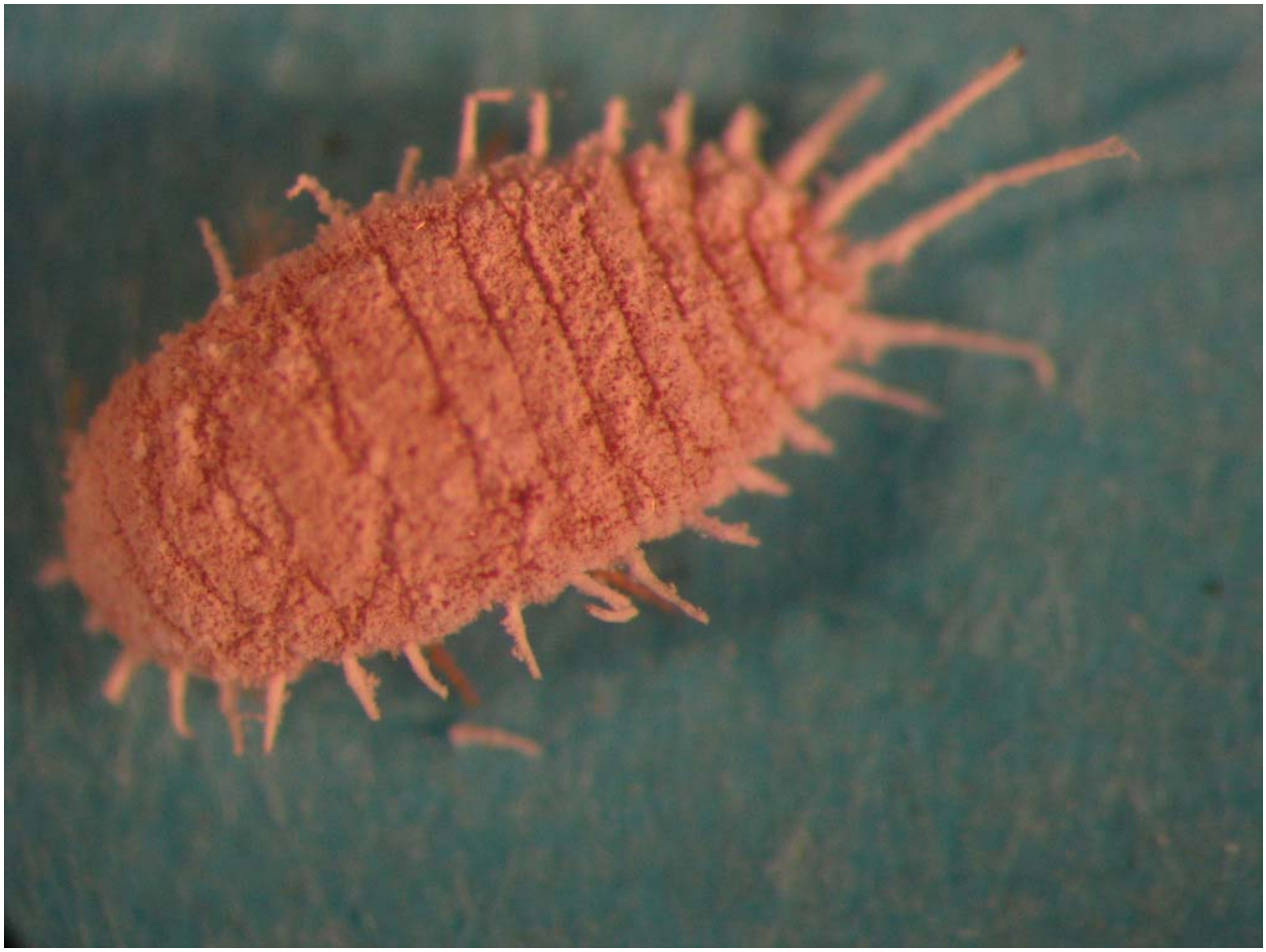

Fig 1.1. Adult female *Pseudococcus viburni*

Wakgari & Giliomee (2004) noted that the identification of *P. viburni* in the field is extremely difficult because of its close morphological resemblances with other mealybug species. A typical example is that of *P. viburni* and *Planococcus citri* which were formerly regarded as synonyms (see Lindinger 1912, cited by Ben-Dov 1994) owing to their morphological similarity. The identification guide developed by Wakgari & Giliomee (2004) is thus an extremely valuable aid for correct identification in view of the fact that some of the morphological structures used for distinguishing metamorphic stages of *P. viburni* and separating species are not easily discernible.

1.4. Lifecycle

Several authors have described the lifecycle and development stages of *P. viburni.* The obscure mealybug does not have a diapausing stage and all life stages are

present throughout the year; this distinguishes it from other mealybug species such the grape mealybug (Varela *et al.,* 2006). Two to three overlapping generations may be observed per year depending on temperature and it overwinters as eggs inside ovisacs and as nymphs in secluded spaces on the host plant such as under the bark or in cracks and crevices.

Daane & Bentley (2002) identified seven stages that can be distinguished during the development of female *P. viburni*. (1)The eggs are deposited by adult females in protective ovi – sacs or egg –sacs covered with wax filaments emanating from the posterior half of their bodies for protection against predators and the environment (Swart, 1977, Daane & Bentley 2002). Eggs are laid all year round and in warm seasons the egg laying period is about 10-14 days (Wearing *et al.,* 1999). A female *P. viburni* is capable of laying up to 500 eggs provided temperatures are mild and food is available (Daane & Bentley, 2002). (2) Eggs hatch into first instar nymphs or crawlers. (3) A first instar crawler develops into a settled first instar nymph which begins to secrete wax that gives the body a whitish appearance. (4) A second and third instar nymphal stage follows at which the insect develops distinctive lateral caudal spines, increases in body size and begins to excrete copious amounts of honeydew. (5) An immature female stage follows. (7) The final stage is a mature adult female which is 2.5 mm long, 1.5 mm wide (Wakgari & Giliomee, 2004). The mature adult female is flat, oval shaped with a white waxy coating and wax filaments sticking out from circumference of the body – these are not part of the insect's body and are lost with each moult (Daane & Bentley, 2002). Except for the eggs and ovipositing adult females all instars are potential crawlers with the third instar larva and female being the most mobile (Panis, 1986) and responsible for dispersal of the mealybug population on the host plant (Wearing *et al.,* 1999).

Swart (1977) and Daane & Bentley (2002) described the development of male *P. viburni* as being similar to that of the female from the egg to the third instar stage. Male *P. viburni* spin a cocoon, enter a non-feeding pupal stage and molt several times within the cocoon. Compared to the female, a male pupa is more slender and elongate. A winged adult male with a single pair of wings and halters eventually emerges from the pupa. A male *P. viburni* is tiny but visible with the naked eye, has a single pair of cerci at the end of the abdomen and flies short distances to mate. The adult male *P. viburni* also secretes wax threads, has no functional mouth parts and therefore does not feed. Their only function is to mate with females (Swart, 1977) and each male lives for an average of only two to three days as an adult. Reproduction in *P. viburni* appears to be sexual and obligatory (Daane & Bentley, 2002), although recent studies suggest some mealybug species reproduce pathogenically after stress is induced (Ravuiwasa *et al.,* 2009).

1.5. Seasonal movement pattern.

According to Myburgh (1962) little or nothing is known of the lifecycle and habits of this mealybug in orchards. However, Swart (1977) gave a general account of the movement pattern in apple and pear orchards of three mealybug species, namely, *P calceolariae, P. longispinus* and *P. viburni.* The author described the three mealybug species as spending their entire life mostly on the woody parts of host trees. The mealybugs are reported to overwinter in colonies, in sheltered places such as underneath loose bark, cracks and crevices of host trees where they breed slowly during the winter months (Fig. 1.2). Crawlers then move considerable distances to shoots, fruits and leaves to feed and breed further during the late spring and summer periods. According to Panis (1986) a number of the mealybugs migrating on the host plant fall to the ground and lie beneath various shelters where they are eventually predated on with few returning to the trunk.

Fig 1.2. Adult *Pseudococcus viburni* occupying spaces underneath the bark of an old stem on a pear tree.

Swart (1977) also stated that infestation of fruit occurs from December or January onwards or even earlier. Mealybugs breed and multiply in the stem and calyx-ends of fruit and are capable of moving into the core or ovary of the fruit via small openings at the calyx-end. Mealybugs then migrate back to the woody parts of the host trees to overwinter and breed. Ben-Dov (1994), Gonzalez *et al.,* (1996) and Walton and Pringle (2004) have reported the occurrence of *P. viburni* on the roots of common vineyard weeds such as *Bidens pilosa* (L) and *Malva neglecta* (Wallroth). This presents serious challenges with regards to the control of this pest given the fact that no below-ground control measures are currently available for this pest. It is also assumed that there is a possibility of *P. viburni* overwintering on the roots beneath the soil surface.

1.6. Ecology and host plants

The population of *P. viburni* is dominated by eggs and first instars (Hamlet, 2005). However population size is limited by availability of refuge sites on trees as overcrowding displaces insects from these sites exposing them to harsh climatic conditions, such as high temperatures and low humidity. Both natural enemies and climate are important in the mortality of older mealybugs and play a key role in population dynamics of *P. viburni* (Wearing *et al.,* 1999) Ants are often found in association with *P. viburni* because they feed on the honeydew – a sugary excrement produced by mealybugs. In fact, the ants will tend *P. viburni* and keep away natural enemies in order to maximize the production of honeydew (Varela *et al.,* 2006). Daane *et al.,* (2008) citing Phillips and Sherk (1991), reported the occurrence of *P. viburni* in coastal vineyards of California, especially in association with the Argentine ant, *Linepithema humile* (Mayr).

P. viburni is a cosmopolitan, polyphagous and bisexual insect pest species with a worldwide distribution (Ben-Dov, 1994). It is recorded from 296 host plant species in 87 families in all zoogeographical regions ranging from evergreen, deciduous, perennial and annual hosts to surrounding shelter-belts or shrubs (Ben-Dov *et al.,* 2002).

In South Africa, *P. viburni* has also been reported in grapes (Kriegler & Basson, 1962; Wakgari & Giliomee, 2004). It is worth noting that during the 1930s and 1960s this mealybug species was often confused and formerly misidentified as the grape mealybug, *P. maritimus* as noted earlier in the chapter and was also even referred to

as the pear or citrus mealybug (Kriegler & Basson, 1962). *P. viburni* has also been recorded on apples and pears (Swart, 1977; Van Der Merwe, 2000; Wakgari & Giliomee, 2004) as well as on roots of weeds in vineyards (Walton & Pringle, 2004). In other parts of the world, *P. viburni* has been observed primarily on ornamental plants (Daane *et al.,* 2008), citrus (Panis, 1986), tea (Abbasipour *et al.,* 2007), pip fruit (Charles *et al.,* 2004) and various other fruit and field crops (Bartlett & Lloyd, 1958; Summy *et al.,* 1986; Williams & Granara de Willink, 1992; Franco *et al.,* 2001).

1.7. Economic Importance and Damage

Mealybugs are pests of universal economic importance infesting various fruit, field and ornamental crops (Franco *et al.,* 2001) throughout the world. They have been widely investigated as potential targets for biological control and integrated pest management (IPM) programmes in different parts of the world owing to their sedentary lifestyle and economic importance (Walton, 2006 citing Wakgari & Giliomee, 2003). In some orchards up to 60% of the crop was unsuitable for both the local and export market (Kriegler & Basson, 1962). More recently, fruit consignments destined for foreign markets have been rejected for phytosanitary reasons because the young instars and adult stages of *P. viburni* could not be identified. This resulted in subsequent loss of revenue and access to key markets for the South African deciduous fruit industry. However, Wakgari & Giliomee (2004) developed a key for proper identification of the life stages of *P. viburni*, which has lead to this pest not being of phytosanitary significance for South Africa's existing export markets.

As earlier noted, Myburgh (1962) stresses the point that the mealybug pest on apples has serious implications in view of the fact that little is known about the lifecycle and habits of the insect pest in fruit orchards in South Africa. Mealybug damage is of a secondary nature in that fruit becomes fouled with the mealybugs themselves, which

infest the stem end of the fruit calyx and even penetrate deeper into the fruit core (Swart, 1977). Mealybug wax secretions, egg sacs, presence of live and dead mealybugs and honeydew on which sooty mould grows are economically damaging in that they render the fruit unmarketable (Hattingh, 1993). Honeydew also results in close association with ants which may extensively disrupt the population regulatory potential of natural enemies (Hattingh, 1993). Heavy mealybug infestations may seriously weaken young or small plants and are reported to cause uneven ripening of the fruit on pear trees (Wearing *et al.,* 1999).

Hattingh (1993) and Hattingh *et al.* (1998) explained how mealybugs have gained notoriety as insect pests of great economic importance. Mealybugs have a broad host range, as phloem feeders they are potential virus vectors while some species are known to inject potent phytotoxins during feeding. The cryptic behavior of mealybugs, which have a tendency to overwinter and occupy cracks and crevices on the entire tree network as well as the fruit calyx and ovary make the pest itself difficult to detect. This behavioral trait protects individuals from their natural enemies so that later in the season these individuals will have matured and developed waxy protective barriers on their body surfaces and become largely impervious to control by insecticides and/or natural enemies (Hattingh, 1993). Gutierrez et al. (2008) gave an account of the importance of ‘refuges’ in mealybug biological control.

1.8. Mealybug Management

1.8.1 Chemical control

The management of this pest in South Africa has been dominated by use of broad – spectrum organophosphates (Fig 1.3) (Swart, 1977) but this has had its own shortcomings: including high cost of pesticides as well as negative impacts on biodiversity, food and water quality, human and animal health and potential

environmental contamination. There is also a possibility of resistance to pesticides. According to reports by Charles *et al.,* (1993) and Walker *et al.,* (1993) resistance to chlorpyriphos in some mealybug strains was confirmed in New Zealand.

Fig 1.3. Farmer spraying an organophosphate insecticide in a pear orchard in Ceres using a tractor drawn mist-blower.

The fact that most of the pesticides are broad-spectrum means that they have adverse non-target effects on beneficial biocontrol agents and can result in potentially disruptive interference with biocontrol agents for other key pests of other crops. The South African deciduous fruit industry exports to discerning international markets which demand commodities produced under sustainable and ecologically compatible conditions (Wakgari & Giliomee, 2004) and therefore sole dependence on broad-spectrum materials is not consistent with modern day integrated pest management strategies. Since the 1980s, success in managing mealybugs has been

due to the implementation of integrated management principles (Van Der Merwe, 2000) but these have often not been implemented. However, Van Der Merwe (2000) and Wakgari & Giliomee (2003) state that a steady increase in mealybug infestation has been taking place in some pome fruit growing areas of the Western Cape and, since the 1996/97 season, these reports have become more frequent. Earlier reports by Kriegler & Basson (1962) and Myburgh *et al*. (1975) state that the obscure mealybug acquired secondary pest status as a result of the introduction of DDT, parathion and azinphos-methyl against codling moth, *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae). Van der Merwe (2000) argues that the actual pest status of pome fruit mealybugs is difficult to determine due to the localized occurrence and sporadic nature of the insect pests. Van Der Merwe (2000) suggests poor ant control, insufficient wetting of trees during pesticide application, use of ineffective pesticides, faulty timing of sprays, absence of follow-up spray applications, application of pesticides in too low concentrations and possible pesticide resistance, as reasons for the increase.

The general recommendation concerning the effective and economic use of pesticides include determining the status of mealybug infestations in orchards and correct timing of sprays in such a way that action must take place during the early part of the season (December) before the fruits are attacked (Kriegler & Basson, 1962). No effective monitoring for mealybugs in pome fruit orchards has been taking place on the farms and this could be one of the major reasons responsible for mealybug outbreaks (Van Der Merwe, 2000).

The full and updated list of insecticides currently being used for control of mealybugs is available in the South African Department of Agriculture publication on registered pesticides and guidelines for control of plant pests (Anonymous, 2007).

1.8.2. Biological control

Much success has been achieved with augmentative releases of parasitic wasps to control vine mealybug, *P. ficus* (Walton, 2006). The parasitic wasp *Coccidoxenoides perminutus* (Encyrtidae) (Girault), a well known parasitoid of *P. ficus* (Walton & Pringle, 2005), *P. calceolariae* and *P. longispinus* (Walton, 2006) are being commercially reared for the control of vine mealybug in South Africa. However, in the case of pome fruit, research is ongoing on the prospects of implementing biological control techniques against mealybugs, as little is known about the efficacy of naturally occurring biocontrol agents (Walton, 2006). According to Wakgari & Giliomee (2004) and Walton (2006), it has been difficult to determine which biological control agents could be used successfully in a biological control programme due to the relatively low incidence of mealybug infestations. Under the current situation it is therefore difficult to ascertain this by just relying on naturally occurring infestations in the field. A survey of the identity and incidence of natural enemies as well as an investigation of the rate of parasitism of mealybugs in pome fruit orchards is necessary to determine the status of potential biocontrol agents.

Predators have an important role in the biological control of mealybugs (Daane *et al.,* 2008) and a more rigorous description of predator densities on *P. viburni* (with and without ants) is given by Daane *et al.* (2007). Walton (2006) also states that currently no predators have been identified for mealybugs on pome fruit in South Africa. However, in other parts of the world, the predatory ladybird beetle (also known as the mealybug destroyer), *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae) has been found feeding on *P. viburni* (Charles, 1993) while Hattingh & Moore (2004) reported this predatory beetle on citrus mealybugs in South Africa. There is therefore still a need to investigate the evidence of predation occurring in pome fruit orchards as well as to ascertain the extent to which predation of *P. viburni* occurs if any.

Recently, Wakgari & Giliomee (2004) conducted a survey of mealybugs and associated natural enemies in the Western Cape Province of South Africa and their findings were similar to those by Whitehead (1957) and Urban (1985). They found no predators from the infested apple fruit but a total of five primary hymenopteran parasitoids were reared from *P. viburni* on apples. *Pseudectroma* sp. was the predominant parasitoid species accounting for 84.3% of the total parasitoids reared ahead of four other species namely *Anagyrus* sp., *Acerophagus* sp*., Pseudaphy*cus *maculipennis* (Mercet) **(**Hymenoptera: Encyrtidae) and *Tetracnemoidea* sp. *P. maculipennis,* which accounted for 6% of the recoveries, is a highly specific parasitoid of *P. viburni* (Sandanayaka *et al.,* 2009). This parasitoid is commercially available in the Netherlands and also the primary biological control agent used against *P. viburni* in New Zealand pip fruit orchards (Charles *et al.,* 2004).

Chemical control options are becoming increasingly limited in view of the strict trade requirements demanded by both the local and export markets regarding pesticide residue regulations. This calls for the South African pome fruit industry to continue responding to the movement of the global economy towards a free market economy and free trade by redesigning its pest management strategies to conform to the strict market regulations and remain globally competitive.

1.9. Monitoring systems

Monitoring systems can improve pest detection making it possible to avoid over and under spraying. They therefore form the backbone of insect pest management (Brown & Pringle, 2006). One of the chief reasons noted by Van Der Merwe (2000) for the recent increase in mealybug populations in apple and pear orchards is the absence of effective pest monitoring systems on farms. Swart (1977) also

recommends the careful determination of the status of mealybug infestations in orchards to manage mealybugs effectively and economically. Swart (1977) standardized a method of sampling and inspection of fruit before they enter packhouses. The method is still recommended to date (Van Der Merwe, 2000) and is described in full in the Fruit and Fruit Technology Research Institute (FFTRI) Manual for Monitoring of Orchard Pests (Barnes, 1992).

A recent, but general, monitoring system for pests on pome fruit was developed by Brown & Pringle (2006). This system is based on scouting, trapping, pre-thinning and pre-harvest damage assessments conducted in ±2ha blocks or orchard subdivisions. Under this system scouting, pre-thinning and pre-harvest damage inspections are conducted on 25 trees per ±2ha block. The authors recommend that scouting be done on the last variety to be harvested while pre-thinning and pre-harvest damage assessments are done on all varieties. The scouting procedure is conducted on a fortnightly basis and is such that insect damage, presence or absence of insect pests and their natural enemies are examined on shoot tips, fruit clusters, leaves, leaf axils and on half of each tree section.

Fruit damage inspections are conducted by counting all fruit in five fruit clusters from each of the same 25 trees as those used during scouting. One fruit per cluster is dissected through the ovary or core to detect presence of insect pests, such as mealybugs and chinch bugs, which penetrate the fruit calyx. These assessments are done twice during the production season and are used to determine damage and infestation levels at harvest. No action thresholds have yet been determined for *P. viburni*.

Nevertheless visual sampling of mealybugs is a laborious and time consuming process while cursory examination of trees in orchards has led to assessments which are inaccurate (Myburgh *et al.,* 1975). Inspection of culled fruit in packhouses has also had its challenges. Not all infestations are spotted during the sampling process which has led to under-estimation of the severity of infestation potential. The symptoms of mealybug infestation are sometimes confused with those of wooly apple aphid *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae) (Van Der Merwe, 2000). Furthermore cull analyses are not a direct indication of mealybugs on a particular farm since the majority of the damaged fruit is eliminated during orchard culling. *P. viburni* has a typical clumped distribution and cryptic lifestyle during much of the year similar to that of a closely related species, the vine mealybug (*Planococcus ficus)* (Signoret) (Walton *et al.,* 2004). This renders visual monitoring methods ineffective especially late in the summer when mealybugs are in higher densities, free moving and residing in exposed locations. Unfortunately, most damage will have already been done by the time this period and these conditions are encountered. An effective monitoring system which is able to provide information early in the season and at low mealybug densities in order to target control actions and appropriately schedule insecticide applications is therefore required (Walton *et al.,* 2004).

A suitable system for monitoring *P. ficus* population levels was successfully developed (Walton, 2003 & Walton *et al.,* 2006). Walton *et al,* (2003, 2004) studied the use of pheromone-baited traps for monitoring *P. ficus* in vineyards in the Western Cape Province, South Africa. Walton *et al.* (2003, 2004, 2006) successfully incorporated their biweekly trap catch information with visual plant inspection data into a system for monitoring and managing *P. ficus* in local vineyards.

1.10. *Study objectives*

The aim of this study was to investigate a sustainable pest management system for *P. viburni* in pome fruit orchards, with the focus on monitoring and biological control. Specific objectives were as follows:

- To determine the seasonal abundance of *P. viburni* and its natural enemies in three pome fruit growing areas of the Western Cape Province.
- To conduct a survey of the identity and incidence of natural enemies.
- To investigate the rate of parasitism of mealybugs in pome fruit orchards for the determination of status of potential biocontrol agents.
- To develop an effective monitoring system based on pheromone-baited traps, which will in future assist producers in obtaining accurate estimation of mealybug infestations early in season and at low mealybug densities.
- To determine the developmental times and estimate the temperature thresholds of *P. viburni* at a range of constant temperatures to optimize future mass rearing and release.

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CHAPTER 2

SEASONAL POPULATION STUDIES OF OBSCURE MEALYBUG, *PSEUDOCOCCUS VIBURNI* (SIGNORET) (HEMIPTERA: PSEUDOCOCCIDAE) AND ITS NATURAL ENEMIES IN POME FRUIT ORCHARDS IN THE WESTERN CAPE PROVINCE, SOUTH AFRICA

2.1 INTRODUCTION

The obscure mealybug, *Pseudococuss viburni* (Signoret) is a well known pest of apples and pears (Van der Merwe, 2000). A steady increase in mealybug infestation has taken place in some pome fruit growing areas of the Western Cape since the 1980s (Van der Merwe, 2000). This pest is difficult to detect hence outbreaks are often observed only after proper control measures were not employed or those that were used did not provide effective control. The absence of effective monitoring on farms has made it difficult to determine the actual pest status of mealybug in pome fruit orchards (Van der Merwe, 2000). Excessive use of insecticides in orchards has also resulted in the destruction of mealybug natural enemies with a subsequent increase in mealybug populations (Kriegler & Basson, 1962; Myburgh *et al.,* 1975). In some situations, natural enemies have been reported to control mealybug populations below economic thresholds but only when their activity is not hampered by the use of broad-spectrum pesticides (Myburgh *et al.,* 1975).

Information on the seasonal occurrence of pests is needed for planning the initiation of monitoring and determining when damage can be expected (de Villiers & Pringle, 2006). Seasonal occurrence can be determined by monitoring pest populations directly on the plant itself as well as determining the number of insects caught in

pheromone-baited traps (de Villiers & Pringle, 2007). Parasitoids of *P. viburni* are also attracted to pheromone-baited traps (Bell *et al.,* 2006).

The major factors affecting population development of obscure mealybug during the growing season in South Africa are, however, still not fully understood. There is also little information on the phenological trends of *P. viburni* and its natural enemies. While it is believed that natural enemies play an important role in biological control of mealybugs, it has been difficult to determine their impact in South African pome fruit due to the relatively low incidence of mealybug infestations, the wide use of broad spectrum insecticides and the lack of rigorous studies of naturally occurring populations of biological control agents in the field (Wakgari & Giliomee 2004, Walton 2006). This chapter therefore addresses these shortfalls and focuses on determining the period when the pest and its natural enemies are active. The relative significance of natural enemies in the mealybug population dynamics was studied. The seasonal occurrence of obscure mealybug in terms of their presence or absence on different locations of the host plant was determined in three pome fruit growing areas in the Western Cape Province using pheromone-baited traps and visual plant inspections.

2.2 MATERIALS AND METHODS

2.2.1 Study sites

2.2.1.1 Seasonal monitoring

Two orchards per site, each approximately 1 ha in area were inspected fortnightly in each of three different pome fruit growing areas in the Western Cape Province during the period November 2007 to June 2009. The three sites included Elgin (34.16S, 19.05E, Elevation: 312 m) (Oak Valley Farm: Granny Smith apples planted in both orchards), Ceres (33.34S, 19.57E, Elevation: 1046 m) (Lakenvlei Farm: Royal Gala apples and Beurre Hardy pears) and Stellenbosch (33.90S, 18.86E, Elevation: 183

m) (Timberlea Farm: Forelle pears and a mixture of Forelle and Packham pears) (Fig 2.1). In each orchard block six evenly spaced rows with six trees per row (36 trees in total per experimental block) were selected for sampling. The same trees were sampled over the duration of this study. The orchard blocks were at least 100 m away from each other in all sites.

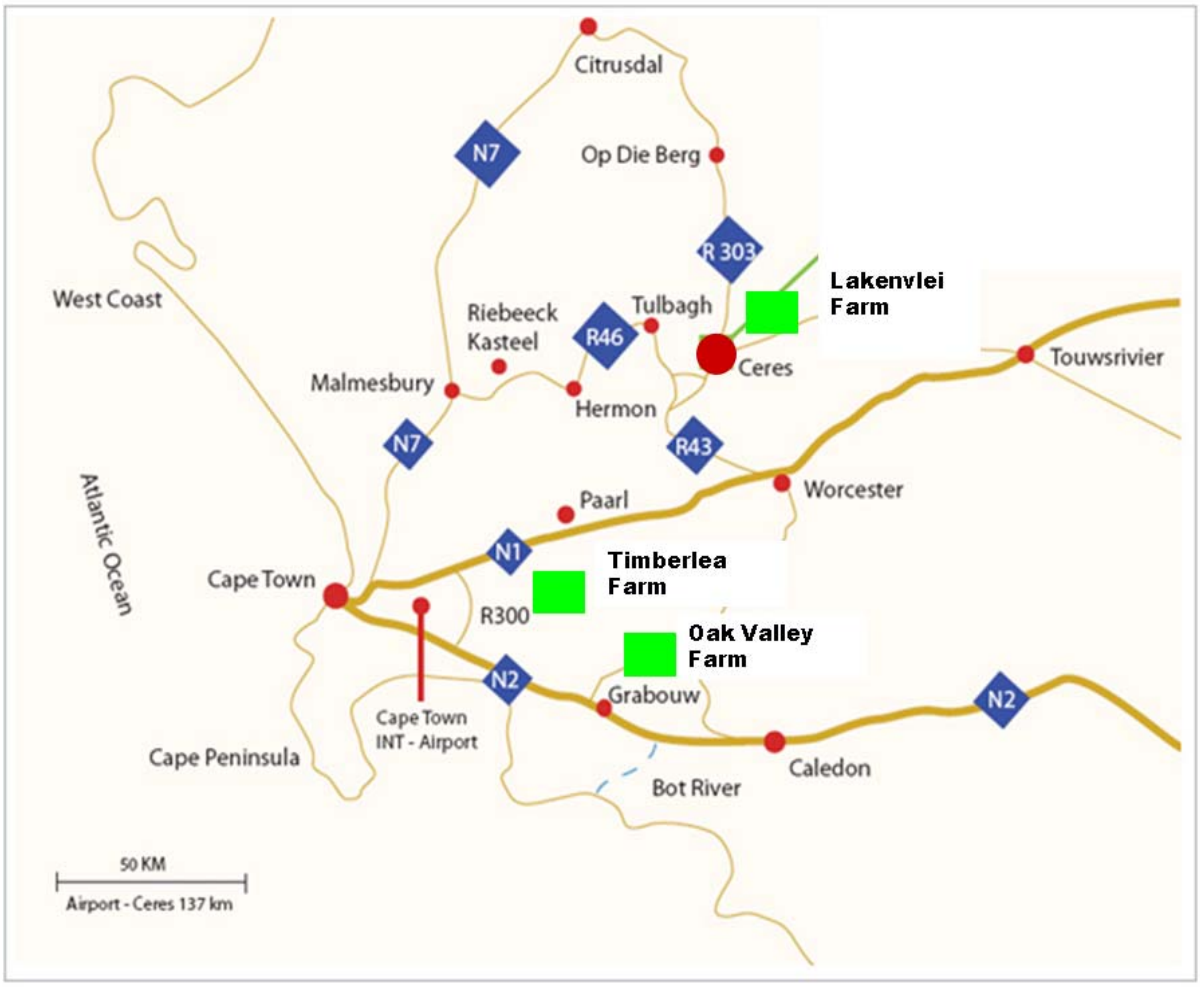


Fig. 2.1. Map showing areas used as study sites for seasonal monitoring of *Pseudococcus viburni* and its natural enemies (Dutoit, 2009).

2.2.1.2 Parasitism and predation rates

Due to relatively low mealybug infestation on the other three farms described above, a commercial orchard in Elgin at Molteno Brothers Farm (34.15S, 19.05E, Altitude: 329 m) with high infestation was selected for this study. The orchard was comprised of a mixture of Starking Red, Granny Smith and Golden Delicious apple cultivars and approximately three hectares in area. The experimental block consisted of six evenly

spaced rows with six evenly spaced trees per row (36 trees in total per experimental block) similar to the experimental blocks described in 2.2.1.1.

2.2.2 Sampling

2.2.2.1. Female Mealybugs

Presence/absence sampling of obscure mealybug was conducted in each of the six experimental blocks. The objective of the seasonal population study was to identify possible trends in their abundance. The data were presented graphically. Each of the 36 trees per orchard was visually searched for signs of female mealybugs on seven positions, namely, the ground and roots (up to 5 cm below soil surface), main trunk, old and new stems (vertical and lateral branches), crutch (fork in trunk), leaves and fruit (Fig 2.2).

Fig. 2.2 Presence – absence sampling of female *Pseudococcus viburni* under corrugated cardboard bands wrapped around the trunk (left) and beneath the bark of tree trunk (right)

Corrugated cardboard bands were wrapped around the trunk, approximately 20 cm above the soil surface, as a monitoring aid to attract female *P. viburni* crawling on the trunk to occupy the spaces and gaps on the cardboard (Fig 2.2). Sampling was conducted fortnightly from November 2007 to June 2009.

2.2.2.2. Natural enemies

The sex pheromone of *P. viburni* has recently been identified and synthesized (Millar *et al*., 2005). Zaviezo *et al.* (2007) described its field use. *P. viburni* parasitoids are attracted to the sex pheromone in the field (Bell *et al.,* 2006; Zaviezo *et al.,* 2007). The flight activity of adult parasitoids and predators was monitored by placing and servicing three, evenly spaced pheromone-baited traps in each of the orchard blocks described above. Yellow delta sticky traps (210mm X 180mm X 100mm) (Chempack®, Simondium, Paarl, South Africa) were used to sample parasitoids and predators (Fig 2.3). The traps were placed at least 50m apart at head height (Fig 2.3) in a diagonal orientation, running across each orchard (two on opposite edges and one in the centre). Pheromone-baited lures made from grey rubber septa loaded with a 0.1mg dose of racemic synthetic pheromone in hexane (Millar *et al.,* 2005) were placed onto white sticky pads inside the traps (Fig. 2.3.)

Fig 2.3. White sticky pad with pheromone lure loaded onto a rubber septum (left) and yellow delta trap hung on tree branch at head height (right).

Sticky pads and pheromone lures were checked and replaced on each field visit every two weeks. The species composition and seasonal abundance of parasitoids was noted during and between seasons.

Live female *P. viburni* and mummies (parasitized mealybugs) were collected during the seasonal monitoring process in all study areas and isolated in individual gelatin capsules. They were then stored in temperature-controlled incubation chambers at 25±1°C and observed daily for emergence of parasitoids. Parasitoids were sent to G.L. Prinsloo (Plant Protection Research Institute, Pretoria) for identification. Some of the parasitic wasps were also positively identified at the University of Stellenbosch Conservation Ecology and Entomology department.

2.2.3. Estimation of parasitism rates at harvest

The role of natural enemies in the mortality of *P. viburni* was investigated by estimating the rate of *P. viburni* parasitism at harvest. This is the period when parasitoids are reportedly most active and abundant (Walton, 2006). A total of 108 infested fruits (three per tree) were picked just before harvest from the 36 evenly spaced trees described in 2.2.1.2 (Fig 2.4).

Fig 2.4. Apple fruit with calyx end infested with *Pseudococcus viburni* (left) and a dissected apple fruit with ovary infested with *Pseudococcus viburni* crawlers and adult females (right).

Fruits were labeled and dissected in the laboratory to expose the calyx and ovary. Only third instar, immature and mature adult stages of female *P. viburni,* including mummies, found on each dissected fruit were collected and isolated individually in gelatin capsules and then held in temperature-controlled incubation chambers at

25±1°C until parasitoid emergence. Some parasitoid species practice host stage discrimination with respect to feeding and oviposition (Kidd & Jervis, 1991; Karamaouna & Copland, 2000). Sandanayaka *et al.* (2009) reported host stage discrimination on *P. viburni,* where parasitoids preferred relatively large instars (third instar or adult females) for oviposition. Therefore, in the current study eggs, first and second instar nymphs of *P. viburni* were not examined. The meaning of "Percent Parasitism" (%PA) in studies of insect parasitoids was described by van Driesche (1983) and calculated as follows:

$$\%PA = \frac{EMP + LP}{EMP + LP + UMH}$$

where EMP = Emerged parasitoids, LP = all live parasitoids and UMH = unparasitized mealybug hosts. To simplify the formula EMP + LP = Total Parasitized Hosts, EMP + LP + UMH = Total Mealybug Hosts.

2.2.4 Weather data and insecticide spray programme

Weather data for the duration of the study period in all study sites were obtained from the ARC Institute for Soil Climate and Water (Agrimet, Stellenbosch). These data included daily average, minimum and maximum temperatures. The detailed insecticide spray schedules for all the experimental blocks used were also obtained from each respective fruit grower. The purpose of these data was to investigate the impact and influence of seasonal temperature changes and pesticide spray applications on the seasonal phenology and population dynamics of *P. viburni* and its natural enemies.

2.2.5. Statistical analysis

The data from the seasonal monitoring of female *P. viburni* in all three growing regions were presented graphically to show the seasonal abundance trend over the two seasons 2007/08 and 2008/09. Presence/absence data of female *P. viburni* on different plant parts were plotted seperately for the two fruit kinds(apple and pear) to show the seasonal movement trends on the entire host plant framework.

The non-parametric Kruskal Wallis ANOVA Test was performed in Statistica (StatSoft, 2008) to test for differences in average seasonal infestation levels of female *P. viburni* between orchards and between regions for the two fruit kinds.

Data from the seasonal monitoring of parasitoids using pheromone traps were analyzed using the non-parametric Kruskal Wallis ANOVA Test in Statistica (StatSoft, 2008). We tested for significant differences in the average seasonal abundance of each parasitoid species between orchards. Data for seasonal monitoring of *P. viburni* natural enemies were plotted to show seasonal abundance trends and flight activity of adult parasitoids and predators over two seasons 2007/08 to 2008/09 for the three respective regions

2. 3 RESULTS AND DISCUSSION

2.3.1 Seasonal monitoring of female mealybugs

In all areas, all *P. viburni* life stages were visible on the host plant throughout the year. This supported the claims that *P. viburni* has multiple overlapping generations with all life stages present throughout the year (Hamlet, 2005). The seasonal mealybug population trends observed in apple and pear orchards are illustrated in Figures 2.5.A & 2.5.B.

There was no significant difference in average mealybug infestation levels between the two pear orchards at Timberlea farm in Stellenbosch ($F_{(2, 123)} = 18.249, P > 0.1$), but there was a significant difference in the seasonal infestation levels between these two orchards and the pear orchard at Lakenvlei farm in Ceres ($F_{(2, 123)} = 18.249; P < 0.01$). There was no significant difference in female *P. viburni* infestation levels in the three apple orchards in Elgin and Ceres ($F_{(2, 123)} = 1.4124\ P > 0.1$).

A similar *P. viburni* seasonal population trend was observed on the two fruit kinds (Fig 2.5A & B). In all three study areas female *P viburni* were more active and visible in higher populations during the warm spring and summer periods until early winter (November to mid-June). A decreasing population trend was then observed during the cold and rainy winter period (late June to October) when mealybugs were overwintering and in sheltered places such as underneath the bark, cracks and crevices on the host tree. However, female *P. viburni* infestation levels in the two Stellenbosch pear orchards were higher than was observed in the Ceres pear block (Fig 2.5.A). Compared to the pear blocks, *P. viburni* infestation levels in apples were lower over the two growing seasons (Fig 2.5B). This observation supports a suggestion by Walton (2006) that pears are more prone to mealybug infestations due to the rougher bark providing better refuge sites.

All orchards monitored were commercial blocks on which different management practices and insecticide spray applications were conducted by each respective grower (See Appendix A: Tables A1 – A4). This may have also accounted for differences in infestation levels observed in the respective individual orchards and fruit kinds. Generally, the orchards in Ceres and Elgin received a wide range of insecticide applications and at more frequent intervals than those in Stellenbosch. Mealybug infestation levels were higher in Stellenbosch compared to Elgin and

Ceres (Fig. 2.5A &B). In Stellenbosch, individual fruits were also left unpicked on trees or on the orchard floor long after harvest. *P. viburni* presence in unpicked fruit was observed and these may have been used as breeding sites resulting in a potential source of mealybug infestation.

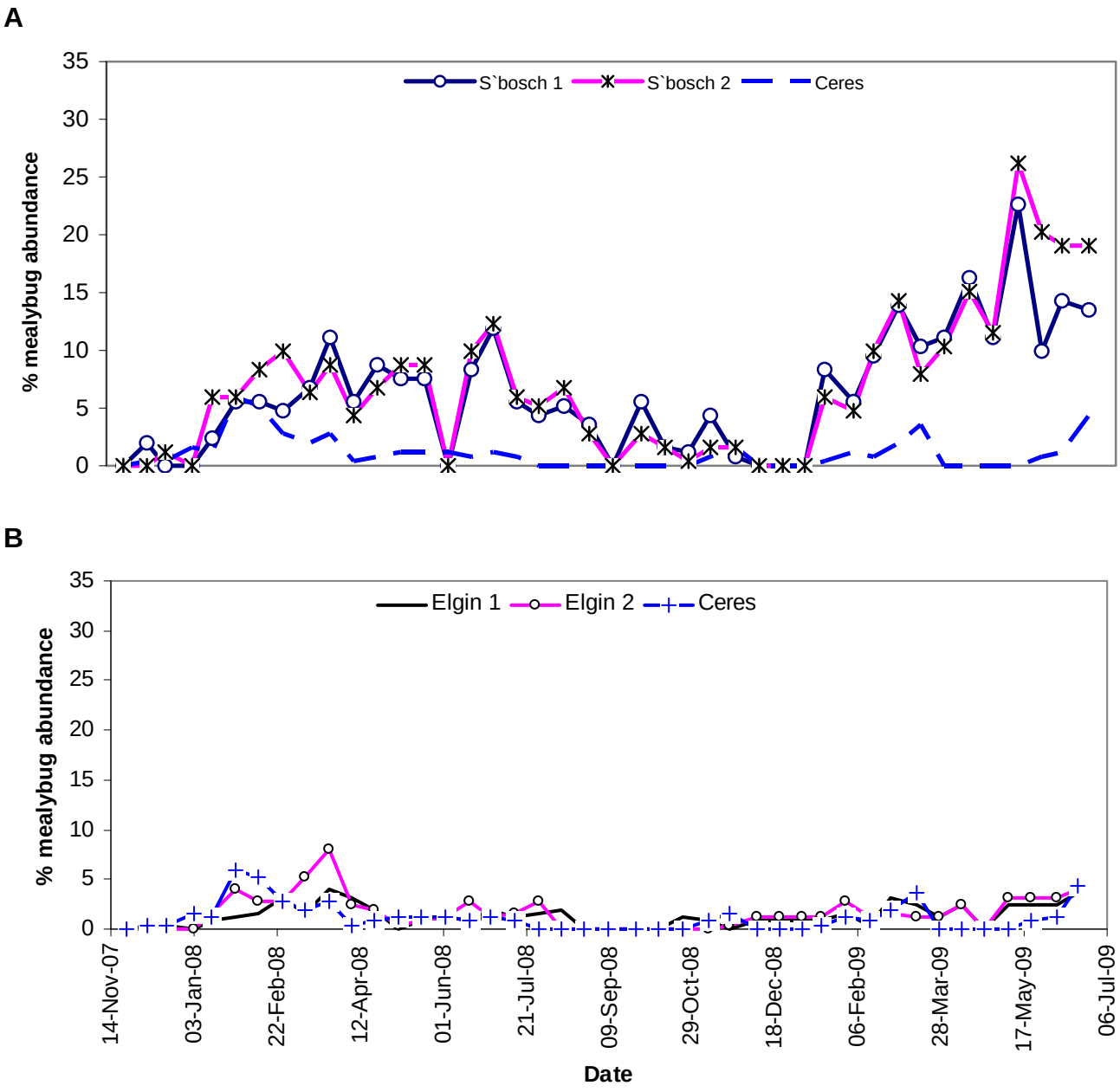


Fig. 2.5 Total % female *Pseudococcus viburni* abundance in (A) Stellenbosch and Ceres pears and in (B) Elgin and Ceres apple orchards during 2007-08 and 2008-09 seasons.

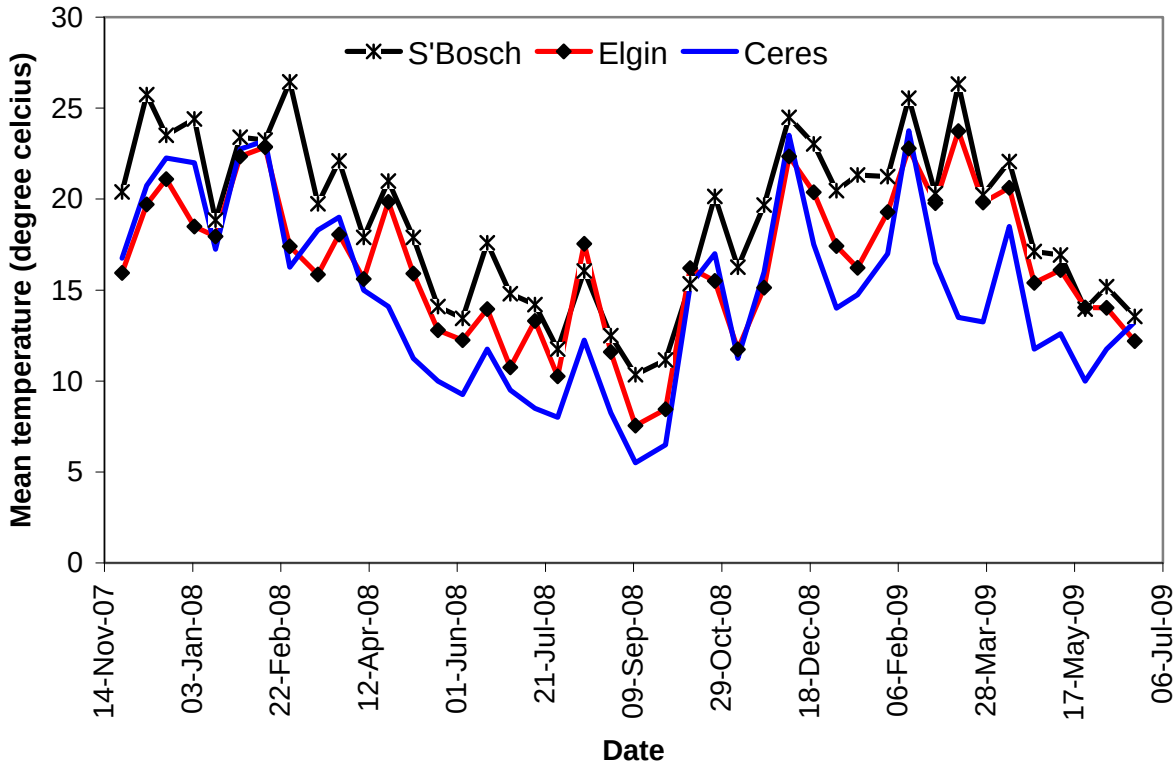


Fig 2.6 Mean monthly temperatures for Stellenbosch, Elgin and Ceres for the 2007-08 and 2008-09 seasons.

Presence/absence data of female *P. viburni* on different plant parts of the host tree showed that there was no evidence of mealybug colonies on the roots or ground throughout the season, contrary to reports by Ben-Dov (1994) and Gonzalez *et al.,* (1996). The trunks and old stems were the most preferred refuge sites due to the availability of protective hiding spaces such as beneath the bark, cracks and crevices. *P. viburni* occurred on these woody plant parts almost throughout the whole year in all three study sites (Figs. 2.7A & B; Figs. 2.8A & B). Similar trends in the seasonal movement and occurrence of mealybugs on trunks, old stems and crutches (fork in the trunk) were observed in all three study regions. The Stellenbosch pear orchards (Fig 2.7A) had the highest infestation levels while both Ceres orchards (Fig 2.7B & Fig 2.8B) recorded the lowest populations. Mealybug colonies were abundant on the trunks and old stems during the warm summer months (November – April) and populations remained high during the early winter period (May – June) despite lowering temperatures.

As the winter progressed (July – October) mealybugs continued to be visible on trunks, crutches and old stems but in lower numbers. Mealybugs were observed to overwinter as eggs, crawlers and adult. The winter period was characterized by extensive insecticide applications (Appendix A: Tables A1 – A4), high rainfall and a drop in mean daily temperatures (Fig. 2.6), and which therefore recorded the lowest *P. viburni* population levels in all regions. The highest percentage trunk and old stem infestations were recorded during May and June in Stellenbosch pears (Fig 2.7A), January to March in Ceres pears (Fig 2.7B), February and March in Elgin apples (Fig 2.8A) and March and June in Ceres apples (Fig 2.8B).

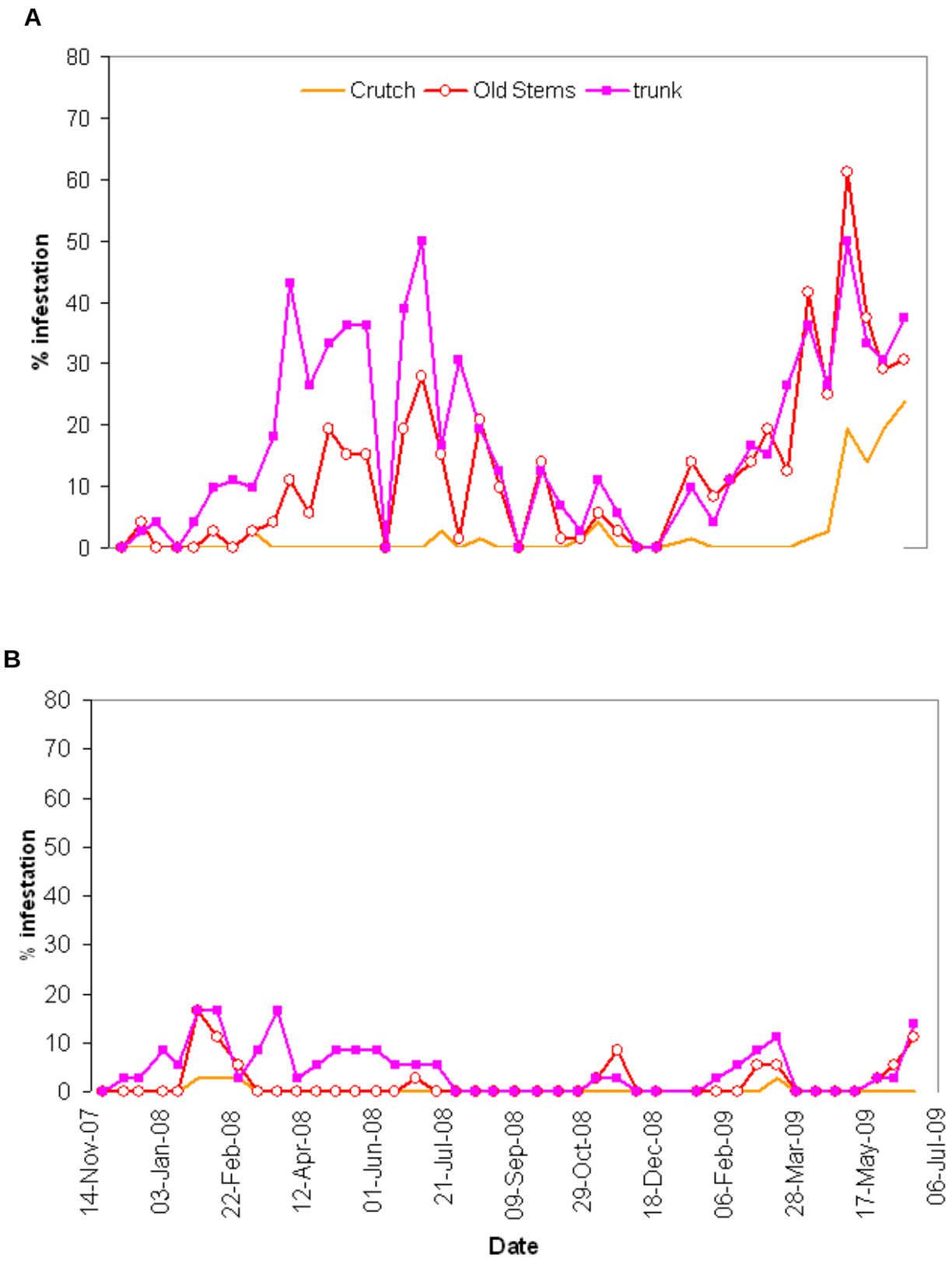


Fig. 2.7 Female *Pseudococcus viburni* infestation levels based on presence/absence field sampling on crutches, old stems and trunks during two seasons in A, Stellenbosch and B, Ceres pear orchards

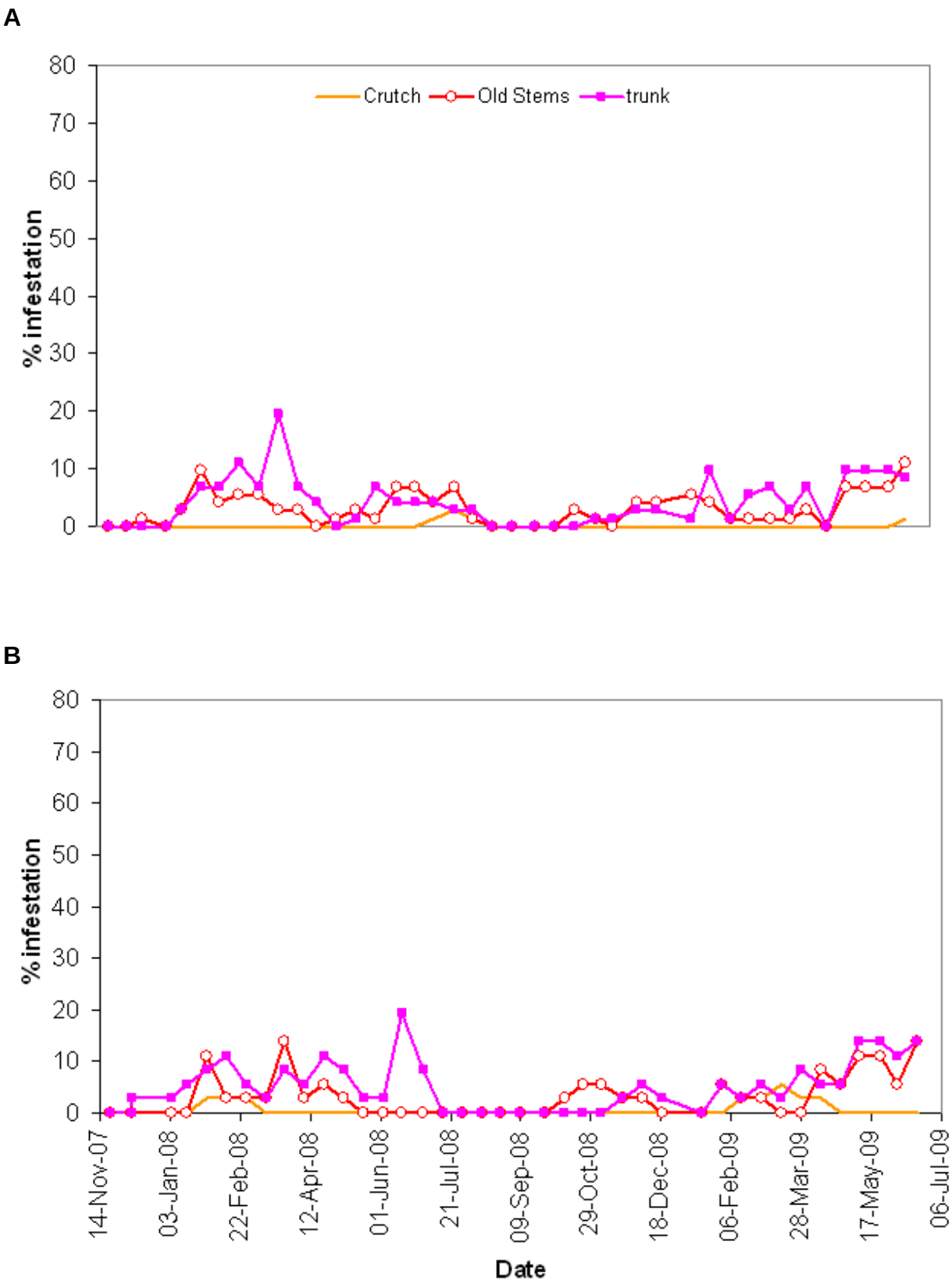


Fig. 2.8. Female *Pseudococcus viburni* infestation levels based on presence/absence field sampling on crutches, old stems and trunks during two seasons in A, Elgin and B, Ceres apple orchards.

Rising temperatures in December coincided with the first visibility of mealybug colonies on new stems and leaves. A portion of *P .viburni* that survived the winter

was observed actively dispersing further up the tree crown towards newer growths, fruit and foliage in response to rising temperatures in spring. This movement trend was similar to observations by Swart (1977) and Panis (1986), who stated that mealybugs migrate from their overwintering sites to the upper locations and new growths of the tree crown for feeding and further reproduction. This overwintering population is responsible for fruit infestation during the summer period. However, a larger proportion of the *P. viburni* population still remained either as eggs, adults or early instars in their secluded overwintering sites. This also supports reports by Swart (1977) who stated that mealybugs spend their entire life mostly on woody parts of host trees due to the availability of refuge sites. Climate may also have had a significant influence on the seasonal movement and population development of *P. viburni* throughout the season.

In the case of fruit, *P. viburni* colonized the fruit during the stage when it had attained a size sufficient enough to penetrate the calyx. Our observations were similar to those by Swart (1977). In Stellenbosch pear orchards, fruit colonization began in January and mealybugs remained present in fruit past the commercial harvest period until July (Fig 2.9A). *P. viburni* were present on unharvested fruit as well as fruit that were unpicked and left to rot on the orchard floor. In Elgin apple orchards fruit infestation became evident in February and mealybug presence in fruit also continued to be visible until April (on un-harvested fruit) (Fig. 2.10A). In Ceres, mealybug colonies appeared on fruit during January until end of February for both fruit kinds (Figs 2.9B & 2.10B).

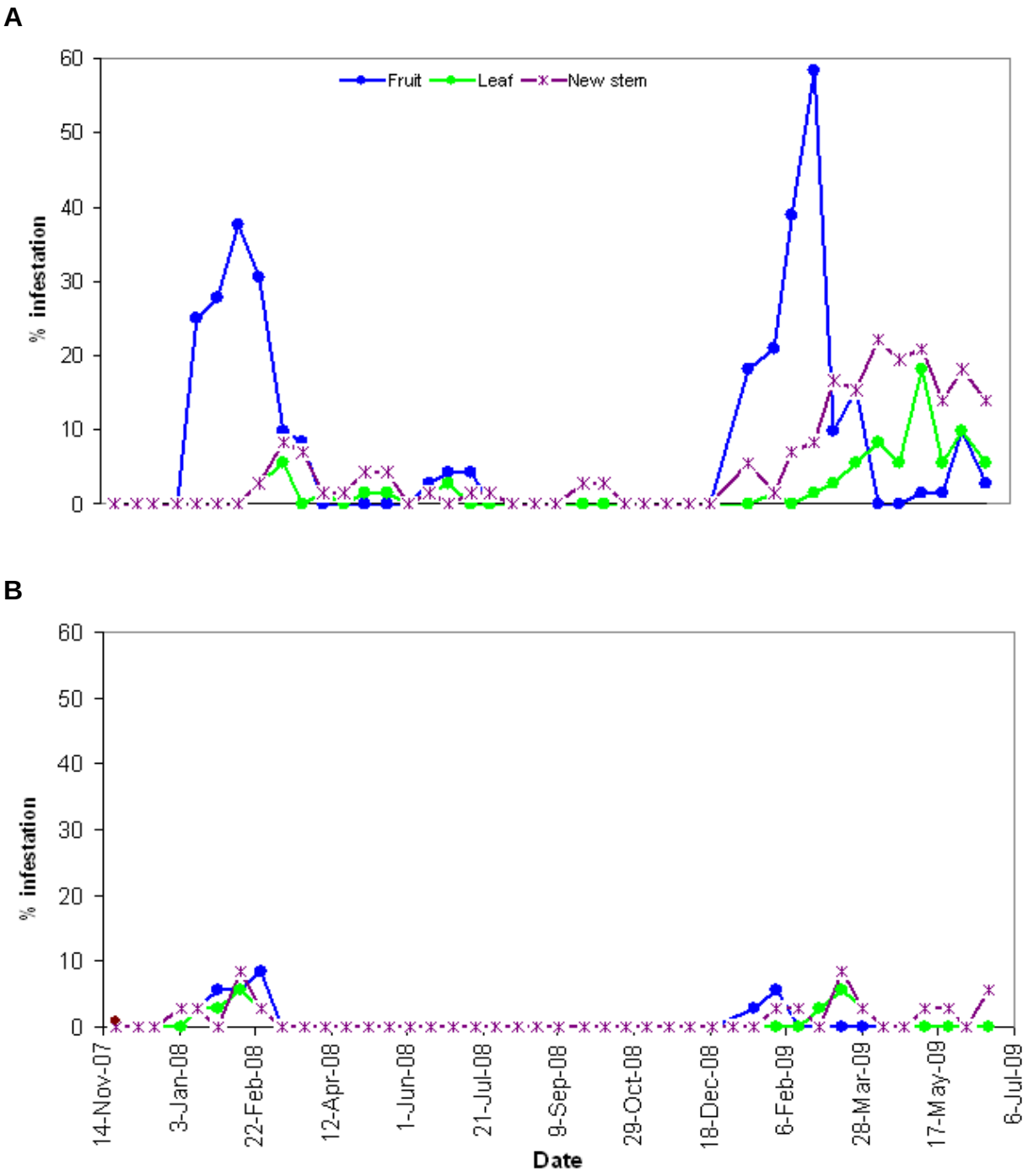
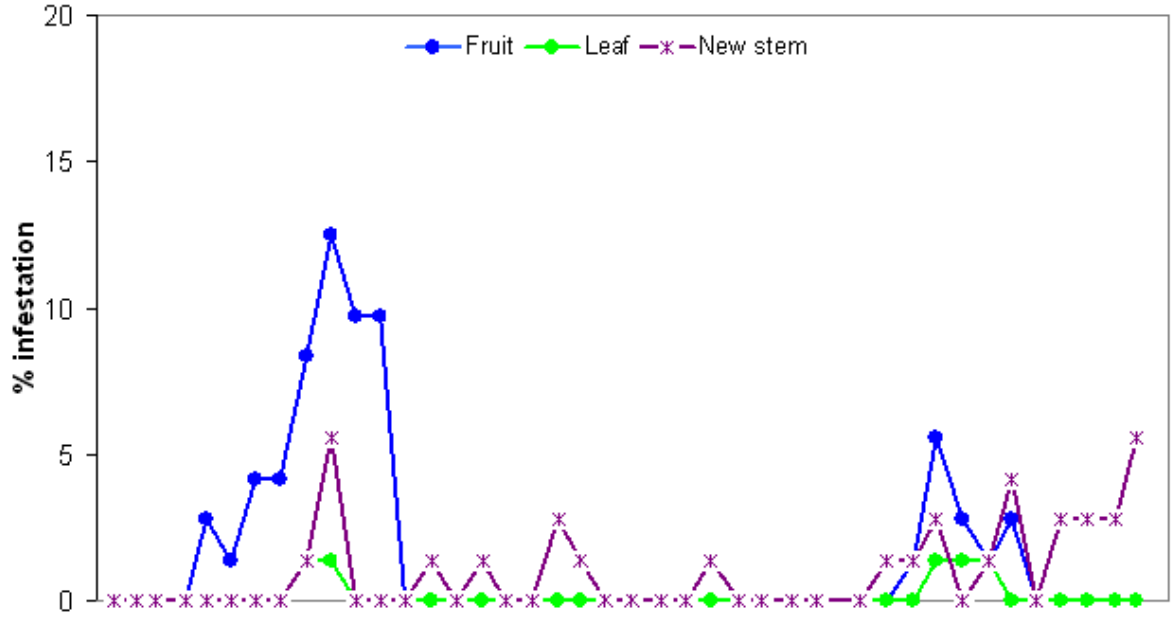


Fig. 2.9. Female *Pseudococcus viburni* infestation levels based on presence/absence field sampling on fruits, new stems and leaves during two seasons in A, Stellenbosch and B, Ceres pear orchards.

A



B

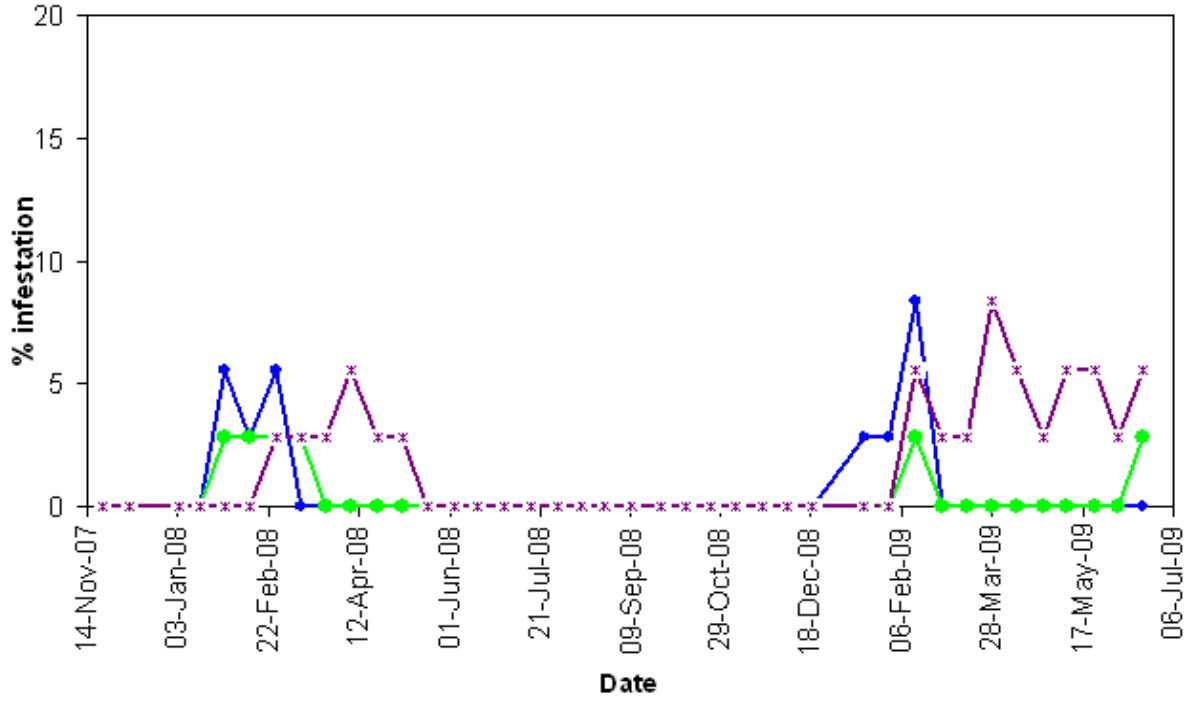


Fig. 2.10 Female *P. viburni* infestation levels based on presence/absence field sampling on fruits, new stems and leaves during two seasons in A, Elgin and B, Ceres apple orchards

2.3.2 Natural enemies

2.3.2.1. Parasitoid complex

No mealybug predators were found in any of the areas surveyed. Three Encyrtid parasitoids were identified as follows:

1) A small yellow wasp with clear wings, which is an undescribed species of *Pseudaphycus*, collected from mealybug mummies,

2) *Pseudaphycus maculipennis* (Mercet). A wasp with a yellowish head, thorax and abdomen, blackish above and pale below, wings darkened with a pale cross – band (Fig 2.11), collected from mealybug mummies.

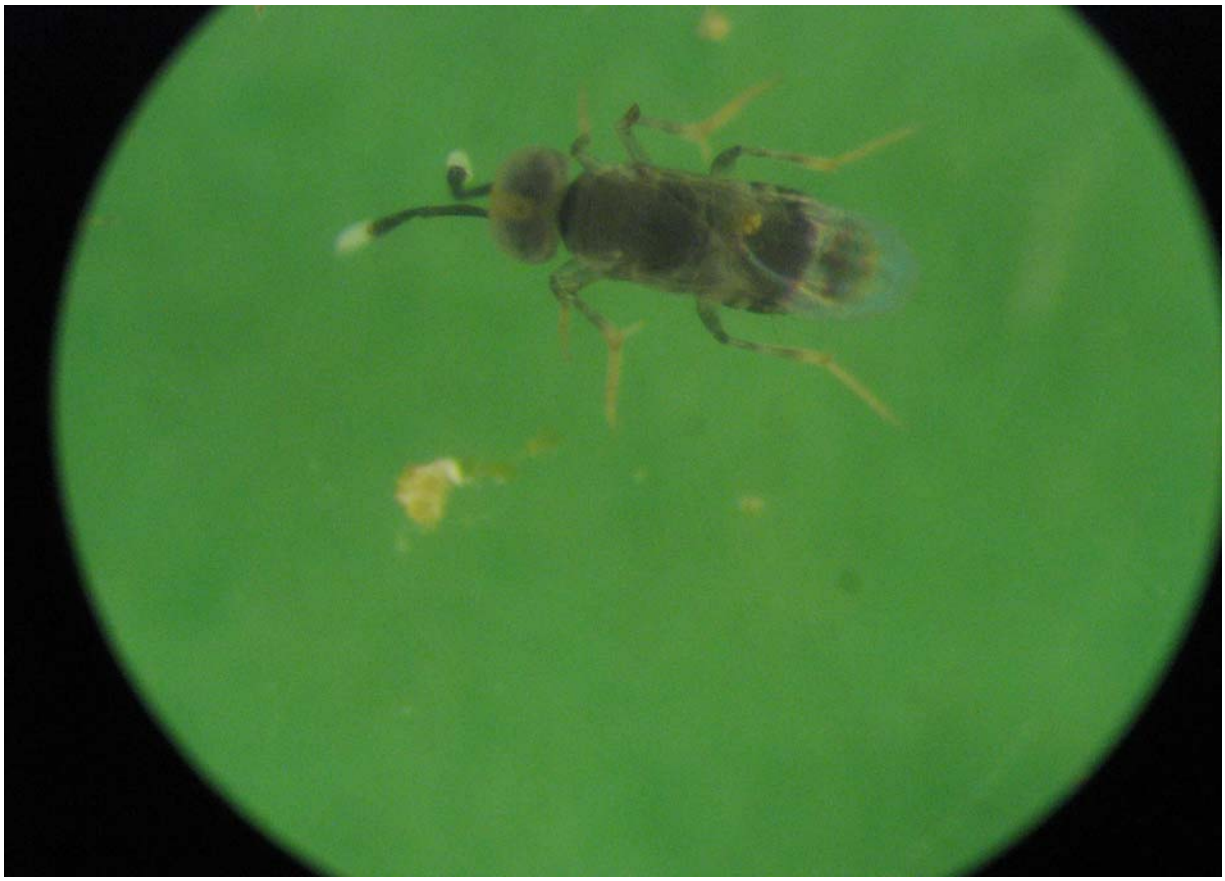


Fig 2.11. *Pseudaphycus maculipennis* (Mercet)

3) *Coccidoxenoides perminutus* (Girault), a small wasp visible with the naked eye, black in colour, with relatively long antennae and with noticeable translucent wings (Fig 2.12), collected primarily from pheromone traps, with a small number also collected from mealybug mummies.

Fig 2.12. *Coccidoxenoides perminutus* (Girault)

The role and efficacy of *C. perminutus* in the biological control of *P. viburni* is still unclear. Reports from the quarantine facility of the University of California, Berkeley, indicate that locally found and commercially available *C. perminutus* attacked *P. viburni* (Walton, 2006). However *C. perminutus* is a primary biological control agent used against the vine mealybug, *Planococcus ficus* (Signoret) (Walton & Pringle, 2005), the citrus mealybug, *Planococcus citri* (Risso) (Ceballo & Walter, 2005), the citrophilous mealybug *Pseudococcus calceolariae* (Maskell) and the long-tailed mealybug, *Pseudococcus longispinus* (Targioni-Tozzetti) (Daane *et al.,* 2008).

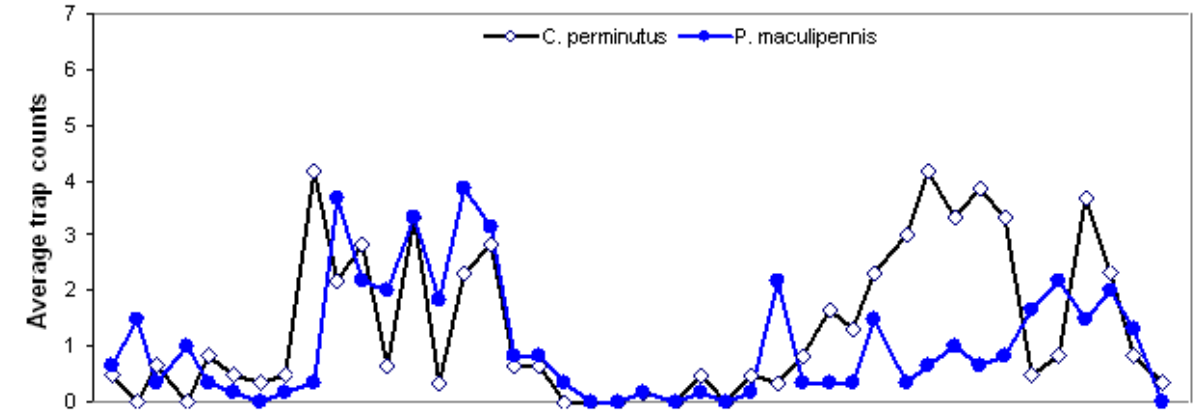
2.3.2.2. Seasonal monitoring of natural enemies

Three parasitic wasps were identified from pheromone-baited traps namely *P. maculipennis, C. perminutus* and *Aphelinus mali* (Haldeman) (Hymenoptera:

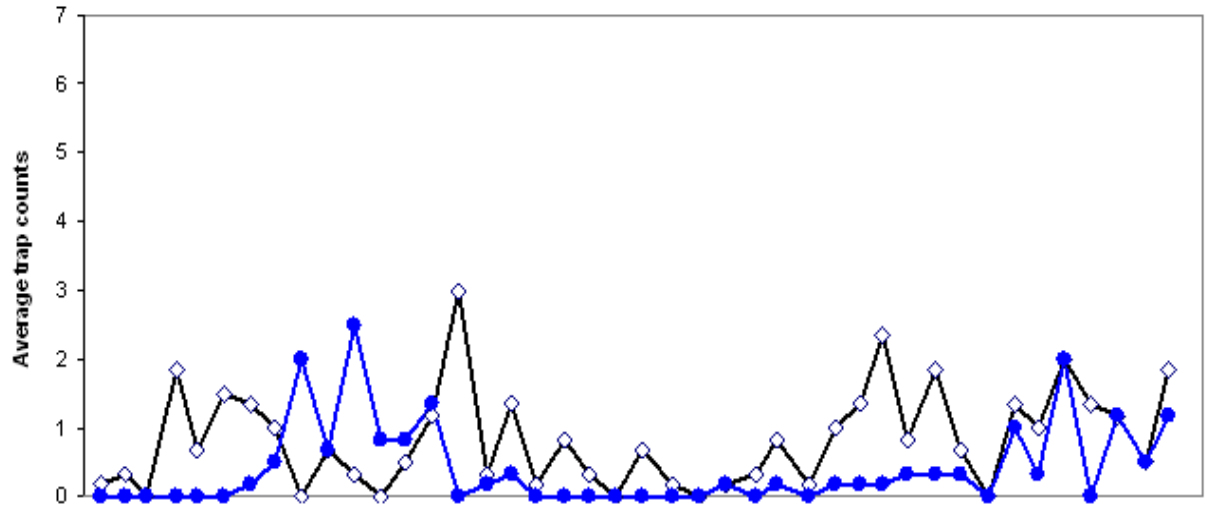
Aphelinidae). However, *A. mali* despite being an established, well known and highly specific biological control agent for the wooly apple aphid, *Eriosoma lanigerum* (Hausmann) (Prinsloo, pers.comm.; DeBach 1964; Heunis & Pringle, 2001) was regularly retrieved from the sticky bottoms of the *P. viburni* pheromone-baited traps in large numbers.

Seasonal abundance of *P. maculipennis* and *C. perminutus* were plotted for the three respective regions (Figs. 2.13A, B & C). No predators were recovered on the sticky bottoms of the pheromone-baited traps. In Ceres, there was no significant difference in the seasonal abundance of *C. perminutus* recorded in apple and pear orchards ($F_{(1.\ 80)} = 0.81981,\ P > 0.05$) hence data from both orchards were combined and analyzed together (Fig. 2.13C). The general seasonal population trends of *P. maculipennis* and *C. perminutus* were similar to those of the host. The peak and dormant periods of both natural enemy species (Figs. 2.13A, B & C) coincided with those of the host (Figs. 2.5A & B). This seems to suggest that the lifecycle of both natural enemies is well synchronized with that of the host.

A



B.



C

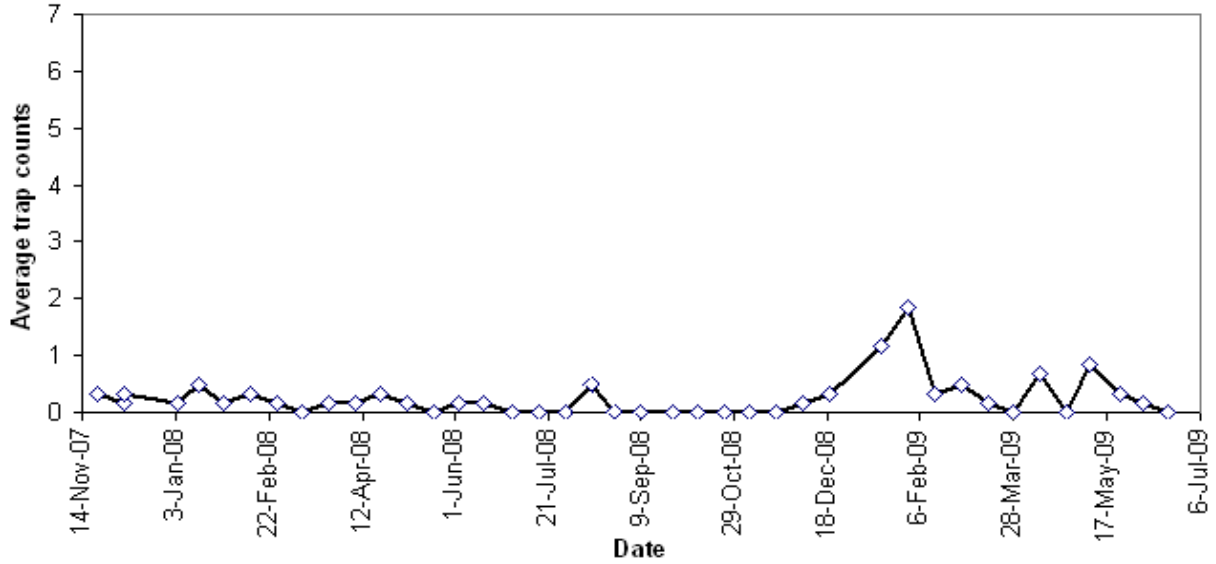


Fig. 2.13. Hymenopteran parasitoids caught on yellow sticky traps in orchards during two seasons in A, Stellenbosch; B, Elgin and C, Ceres.

The peaks and troughs in the graphs seem to indicate that parasitoid abundance was in response to the activity, abundance and availability of hosts during the respective periods. The lowest natural enemy population from both species in all areas was recorded during the cold winter months (July to September in Stellenbosch and Elgin and June to October in Ceres). The highest parasitoid populations occurred during the summer (February to June in Stellenbosch and Elgin and January to February in Ceres) (Fig. 2.13A – C). Data from yellow sticky traps also showed that *P. maculipennis* was only present in Stellenbosch and Elgin but absent in Ceres while *C. perminutus* was the dominant parasitoid and present in all three sites (Fig. 2.13A – C). In addition, *P. maculipennis* was more abundant in Stellenbosch than Elgin. The number and frequency of pesticide applications in Ceres and Elgin was more than those in Stellenbosch (See Appendix A: Tables A1 – A4). Correlating these differences with the spray records on each farm, there is possibility that perhaps *P. maculipennis* is highly susceptible to insecticides hence its absence in Ceres and higher abundance in Stellenbosch.

2.3.3. Parasitism rates

All fruits collected from Molteno Farm were infested with *P. viburni*. Parasitism percentage was estimated to be 46.52% (n = 460). Two parasitoid species emerged from the mummies of the parasitized mealybugs and these were identified as *P. maculipennis* and *C. perminutus* (98.97% and 1.034%, respectively, n = 677).

The status and potential of *C. perminutus* as a biological control agent against *P. viburni* still needs to be verified since the 1. 034% parasitism realized from our fruit sample is not sufficient evidence to suggest that *C. perminutus* is a useful parasitoid of *P. viburni*. Furthermore, low levels of parasitism by *C. perminutus* have been reported

and attributed to negative effects of a harsh environment and short adult life of *C. perminutus* (Ceballo & Walter, 2005). In addition, parasitized mealybugs initially become highly active following exposure to parasitoids, undergo behavioral and physical changes in which their body becomes cylindrical and legs rigid resulting in mealybugs becoming immobile and resembling a typical mummy appearance (Ceballo & Walter, 2005). Consequently, impaired locomotion leads to mealybugs dropping to the ground, resulting in under sampling of this parasitoid using the method employed here because the final site of mummification cannot be established.

2.4 CONCLUSION

Based on the observations on the seasonal phenology of *P. viburni* and its natural enemies, indications are that temperature changes throughout the year are the primary factor limiting seasonal abundance in orchards and movement on the host tree. Temperature changes also affect apple and pear tree phenology. It is possible that movement and abundance of mealybugs is also connected to sugars moving through the trees in the phloem and therefore the food source also affects mealybug population structure (Daane, pers.comm). In summer, temperatures reach optimum levels, conditions that favor population development and mealybug activity while in winter temperatures drop below the threshold levels resulting in mortality and a decrease in the population (Romoser & Stoffolano, 1998; Dent, 2000). Another possible reason for the high mealybug activity in summer is the availability of new foliage, shoots and fruit on which mealybugs feed and breed, while in winter when the tree sheds off its foliage and enters dormancy activity decreases as mealybugs migrate to the woody parts for overwintering (Swart, 1977). Our results also agree with Karamaouna and Copland (2009), who stated that the rate of development of

parasitoids increases with an increase in temperature, hence the abundance in parasitoid population during the warm summer months and a decrease in winter.

The synchrony between the host and parasitoid lifecycles indicates that the development and seasonal abundance of the natural enemy is dependent on the supply and availability of hosts (Jones & Ives, 1979).

Based on the survey of natural enemies occurring in pome fruit orchards in the Western Cape Province, *P. maculipennis* is a potential candidate for biological control of *P. viburni* in South Africa. The natural presence and abundance of this parasitoid in Stellenbosch and Elgin indicate that conditions for its development and establishment are suitable. Furthermore parasitism rates by naturally occurring field populations observed at Molteno Brothers Farm in Elgin indicate that *P. maculipennis* has an important role in the biological control of *P. viburni* and this can be optimized by mass rearing and release in an inundative biological control programme. The use of insecticides in all orchards sampled has been a major limiting factor to the population dynamics of both *P. viburni* and its natural enemies. If biological control is to be implemented in apple and pear orchards in the Western Cape, it is strongly recommended that this only be instituted once alternative control measures are found to control primary pests.

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CHAPTER 3

THE DEVELOPMENT OF EARLY MONITORING TOOLS FOR THE OBSCURE MEALYBUG *PSEUDOCOCCUS VIBURNI* (SIGNORET) (HEMIPTERA: PSEUDOCOCCIDAE) USING PHEROMONE-BAITED TRAPS.

3.1 INTRODUCTION

In South Africa, *Pseudococcus viburni* is being controlled primarily with insecticides (Myburgh *et al.,* 1975; Swart, 1977; Van der Merwe, 2000; Walton, 2006). Despite the huge effort to keep this pest from reaching damaging levels, increases in mealybug infestations have been ascribed to the inadequate, improper and incomplete use of these pesticides. Geiger & Daane (2001) and Walton (2003) argue that the challenges facing chemical control of mealybug range from poor insecticide coverage under dense leaf cover or bark, where this pest often resides, to insecticide run off over the mealybug`s waxy body secretion (Walton *et al.,* 2004). For these reasons, and in line with the principles of sustainable integrated pest management, the use of insecticides must be exercised only when needed and limited to target applications against the most vulnerable life stage.

The importance of timely applications of insecticides necessitates the use of a species-specific monitoring programme that can quickly determine pest presence and density (Walton *et al.,* 2004). Visual sampling of mealybug is a laborious and time consuming process while, examination of trees in orchards has led to assessments which do not accurately reflect its pest status (Myburgh *et al.,* 1975). Inspection of culled fruit in packhouses has also had its challenges. Not all infestations are spotted during the sampling process which has led to under-estimation of the severity of infestation potential to be expected during ensuing seasons. *P. viburni* has a typical clumped distribution and cryptic habit during much

of the year, similar to that of a closely related species, the vine mealybug (*Planococcus ficus)* (Signoret) (Walton *et al.,* 2004). This renders visual monitoring methods ineffective since late in the summer, when mealybugs occur in higher densities, most damage will have already been done.

Walton *et al.* (2004) and Brown & Pringle (2006) stated that an effective monitoring system will provide information early in the season and at low mealybug densities in order to target control actions and appropriately schedule insecticide applications. These actions may subsequently help to prevent infestations of fruit, limit insecticide residues as well as prevent outbreaks in ensuing seasons. According to Pedigo (1999), trapping consists of some of the most vital sampling methods for insect surveys. Monitoring programs based on the use of pheromone-baited traps offer a more convenient monitoring method. This monitoring tool may also be extremely useful to help producers determine whether their crop should be considered for export. The sex pheromone of the obscure mealybug *P. viburni* has recently been identified and synthesized (Millar *et al.* (2007). Zaviezo *et al.* (2007) described its field use. According to Romoser and Stoffolano (1998) pest damage can be related to the number of insects caught in a trap and management decisions can be made accordingly. The abundance of *P. ficus* males caught on sticky pheromone traps was correlated with stem infestations (Walton *et al.,* 2004) making it possible to use trap catch information to predict when stem inspections should commence *(*De Villiers & Pringle, 2007). Rising male mealybug trap counts correlated well with rising crop infestation levels according to findings for *P. ficus* in vineyards in California and South Africa (Walton *et al.,* 2004; Walton 2006).

The objective of this study was to investigate the relationship between *P. viburni* males caught in sticky pheromone-baited traps and fruit infestation levels as recorded by visual sampling methods. We also aim to establish an action threshold based on pheromone-baited trap counts, which corresponds to the 2% fruit infestation economic threshold proposed by Swart (1977), which is based on orchard cull analysis. This will in future help producers make more informed decisions regarding fruit destined for various markets.

3.2 MATERIAL AND METHODS

3.2.1. Sites

The study was conducted in each of the orchards in three pome fruit growing areas described in Chapter 2 (2.2.1.1). It is worth noting that the two orchard blocks used in each site were at least 100 m apart.

3.2.2 Monitoring of male P. viburni using traps

The flight activity of adult male *P. viburni* was monitored by placing and servicing three, evenly spaced pheromone-baited traps in each of the orchards in all three pome fruit growing regions used for the study. Monitoring was done fortnightly from November 2007 to June 2009.

P. viburni pheromone lures consisting of grey rubber septa loaded with 0.1 mg dose of racemic synthetic pheromone in hexane (Millar *et al.,* 2005) were placed onto white sticky pads with a black grid-lined surface (total sticky surface area = 320 cm^2). The lure and sticky pad were placed in yellow delta traps (Chempack®, Simondium Paarl, South Africa) and hung in the tree canopy at head height. The traps were at least 50m apart in a diagonal orientation, running across each orchard (two on

opposite edges and one in the centre). The sticky pads and pheromone lures were checked and replaced on each field visit every two weeks. All adult male *P. viburni* caught on the sticky traps were counted in the laboratory using a stereo microscope (Fig 3.1).

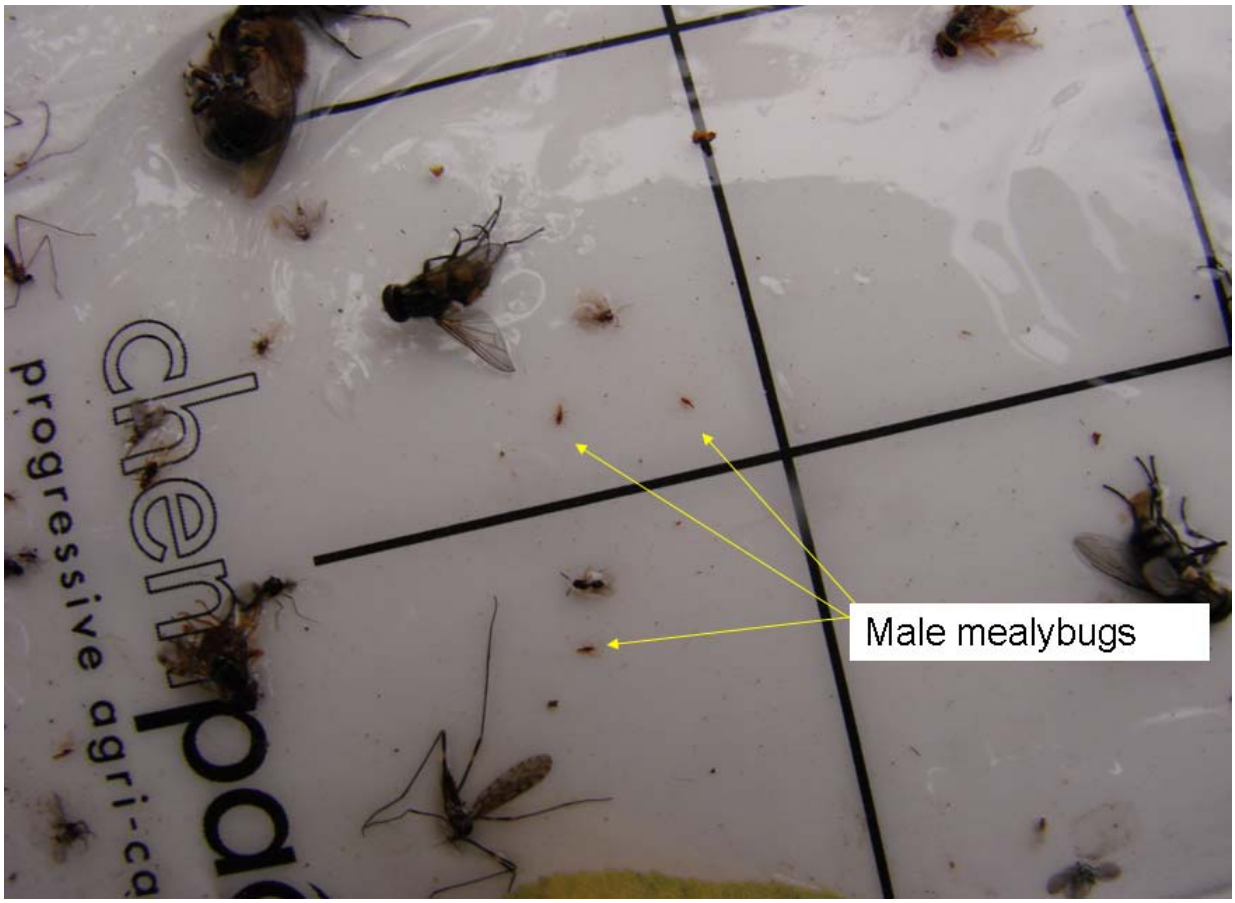


Fig 3.1. Sampling of male *P. viburni* on sticky pad

3.2.3 Correlation between trap counts and fruit infestation

The abundance of male *P. viburni* captured on pheromone-baited traps was correlated with data obtained from fruit infestation assessments, as determined by visual monitoring. The visual monitoring method used was based on methodologies described by Van der Merwe (2000) and Walton (2006). The same orchard blocks in the three areas used for all other experiments were used for this study. Three fruits per tree were randomly picked and labelled from each of 36 trees per trial block every

fortnight from November until harvest. Fruit were taken to the laboratory, dissected to expose the calyx and ovary. Any presence of live or dead mealybugs, ovisacs, wax filaments, sooty mold and/or honeydew on the surface, stem end, calyx or ovary of the fruit was noted as mealybug infestation and absence was noted as un-infested (van der Merwe, 2000).

3.2.4 Data analysis

The pheromone-baited trap data from three traps in each orchard for each sampling date was averaged and transformed to natural log form (LN Counts) while the fruit infestation data for the respective dates were transformed to empirical logistic form (Logit Z) (Cox, 1970). Correlations were done between Ln (counts) and Logit (Z) using weighted regression analysis. Trap and fruit infestation data for each sample date and orchard block were compared and differences tested using dummy variables. The regression analysis was based on data collected during the entire fruit season from early November to March.

$$Logit(z) = a + (b)\ ln\ (n) \qquad (1)$$

Where z is the proportion of infested fruit, n is the average number of male *P. viburni* caught in the three pheromone-baited traps and a and b are the regression constants.

Dummy variable regression (Gujarati, 1970a; Gujarati 1970b; Neter & Wasserman 1974; Draper & Smith, 1998) was used to test for possible differences in the regression constants in (1) between the three sites. The proportion of infested fruit, z, could be estimated for any number, n, of males trapped using Cox (1970),

$$z = \frac{exp\,[a + b(n)]}{1 + exp\,[a + b(n)]}, \qquad (2).$$

Expression (2) was solved for *n* by iteration using *z* = 0.02, or 2% infested fruit, the economic threshold suggested by Swart (1977).

3.3 RESULTS AND DISCUSSION

3.3.1. Correlation between male trap counts and fruit infestation

There was a positive and significant relationship between the fruit infestation and number of *P. viburni* adult males caught in pheromone-baited traps for each orchard block and sample date for the 2007/08 & 2008/09 seasons (F = 311.9545, d.f. = 1, 375, $P < 0.0001$, r^2 = 0.454) (Fig 3.2).

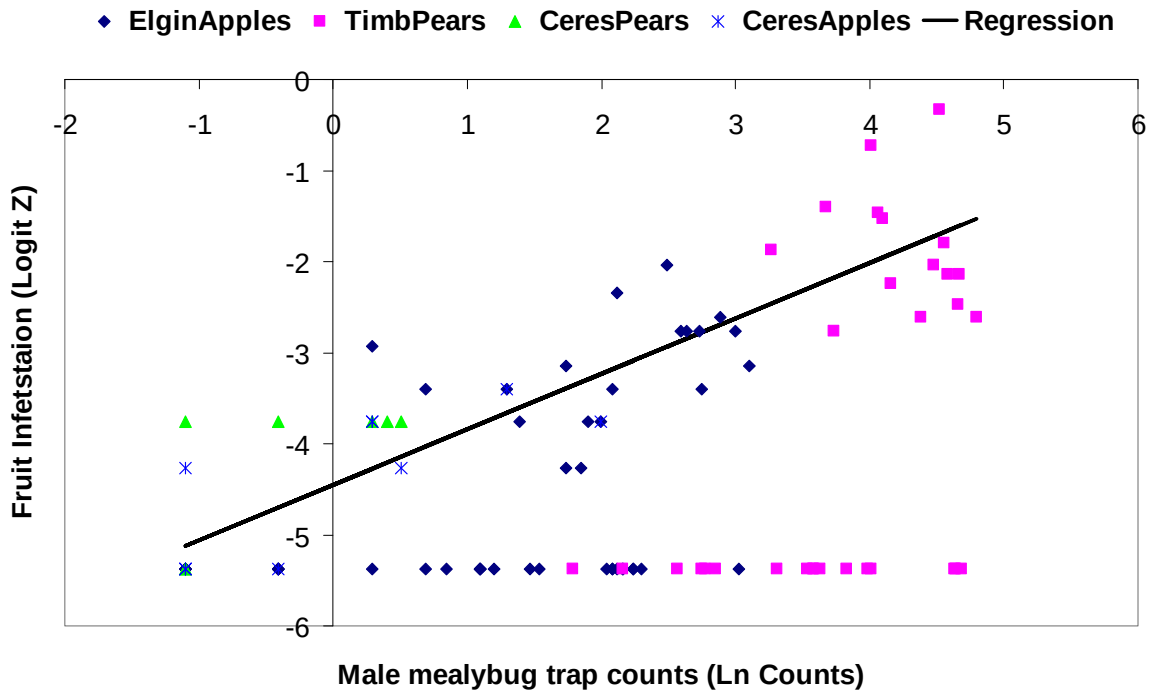


Fig. 3.2. Weighted regression of *Logit (z)* on *Ln (n)* for *z* is the proportion of infested fruit and *n* = number of male *Pseudococcus viburni*/trap/fortnight.

The regression lines from the three areas did not differ significantly (F = 2.049; d.f. = 6, 369, P = 0.059) producing a single regression equation,

Logit (z) = -4.450 + 0.609 (n) (3).

Extrapolating data from the correlation of fruit infestation to pheromone trap counts we were able to estimate crop damage. According to Swart (1977) the status of mealybug in apple and pear orchards is regarded as light when 2% of fruit is infested, medium when 2 – 4% fruit is infested and severe when more than 4% of the fruit is infested. Swart (1977) further stated that under conditions of light infestation, chemical control is justified. Therefore solving expression (2) for *n* at an economic threshold of 2% infestation (*z* = 0.02) gave an action threshold level of 2.5 male *P. viburni* per trap per fortnight.

There were significant between-region differences in pheromone trap counts (*F = 26.94*, d.f = 5, 113, *P< 0.001; F = 17.27*, d.f = 5,127, *P < 0.001*, for the 2007-08 and 2008-09 seasons, respectively). However, there were no significant between region differences in fruit infestation (*F = 2.19*, d.f = 5, 113, *P > 0.05; F = 1.45*, d.f = 5.127, *P > 0.05*, for 2007-08 and 2008-09 seasons, respectively) (Table 3.1).

Table 3.1. Seasonal average (±SEM) of male trap counts and percent fruit infestation for 2007-08 and 2008-09 growing seasons for three pome fruit growing areas (Block numbers in brackets).

Orchard region	**Season 2007-08**		**Season 2008-09**	
	Trap counts	**% Fruit infestation**	**Trap counts**	**% Fruit infestation**
S`bosch 1(TF)	45.7 ± 4.25 a	1.61 ± 0.99 a	45.2 ± 3.86 a	2.86 ± 0.91 a
S`bosch 2 (TF&P)	30.1 ± 4.14 a	3.1 ± 0.97 a	26.15 ± 3.95 b	2.95 ± 0.93 a
Elgin 1 (EW48)	8.5 ± 4.14 b	0.42 ± 0.97 a	6.5 ± 3.95 c	1.09 ± 0.93 a
Elgin 2 (EG9)	10.7 ± 4.14 b	1.81 ± 0.97 a	2.6 ± 3.95 c	0.46 ± 0.93 a
Ceres 1 (CA)	0.9 ± 4.14 b	0.28 ± 0.97 a	0.8 ± 3.95 c	0.21 ± 0.93 a
Ceres 2 (CP)	0.4 ± 4.14 b	0.28 ± 0.97 a	0.2 ± 3.95 c	0.25 ± 0.93 a

*Within each column, means followed by different letters are significantly different (Tukey HSD test, *P < 0.05*).

There were no significant between-season differences (*Bonferroni test P > 0.05*) within each region in pheromone trap counts and fruit infestation. However, there were

significant between-season differences in male trap counts for Elgin 2 (orchard EG9: Granny Smith apples; $F_{(1, 40)} = 12.818\ P < 0.001$) (Table 3.2).

Table 3.2. Between-season differences in male trap counts and percent fruit infestation within three pome fruit growing regions during the 2007-08 and 2008-09 growing seasons.

Orchard region	**Pheromone trap counts (d.f. = 1, 40)**	**% Fruit infestation (d.f. = 1, 40)**
S`bosch 1(TF)	*F = 0.0018, P = 0.966*	*F = 0.3191, P = 0.575*
S`bosch 2 (TF&P)	*F = 0.3803, P = 0.541*	*F = 0.0048, P = 0.945*
Elgin 1 (EW48)	*F = 1.1467, P = 0.291*	*F = 1.3667, P = 0.249*
Elgin 2 (EG9)	** F = 12.818, P = 0.00092*	*F = 3.6042, P = 0.065*
Ceres 1 (CA)	*F = 0.0497, P = 0.825*	*F = 0.1105, P = 0.741*
Ceres 2 (CP)	*F = 1.3160, P = 0.258*	*F = 0.0168, P = 0.898*

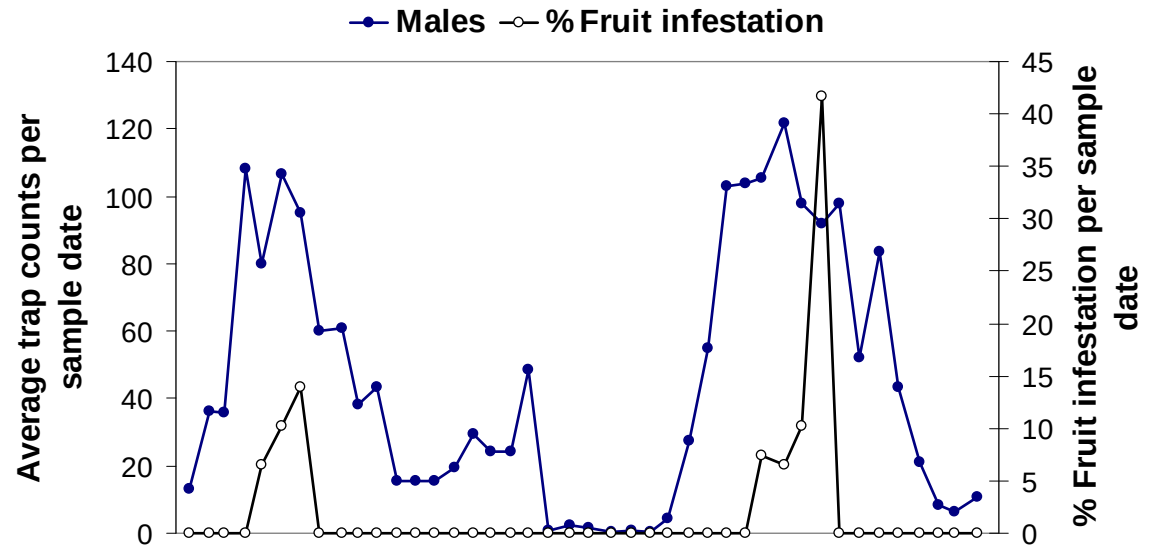
Using dummy variables to test for differences in regression constants (Gujarati 1970a, 1970b; Neter & Wasserman, 1974) we tested if differences in *P. viburni* densities among regions and seasons resulted in different relationships between fruit infestation and trap counts. The results showed that such differences did not impact the relationship between fruit infestation and male *P. viburni* trap counts ($F_{(6,370)} = 2.049$; *Reduced regression model F – test, P* > 0.05).

Dynamic changes corresponding to male flight activity and fruit infestations are evident in the seasonal comparison of fruit infestation and trap counts (Figs. 3.3 – 3.5). In Stellenbosch, *P. viburni* trap densities were significantly higher than in Elgin and Ceres (Table 3.1) hence the different seasonal density patterns shown in figures 3.3 – 3.5. In Stellenbosch and Ceres (Figs. 3.3 & 3.5, respectively) seasonal fruit infestation counts showed that fruit became attractive for mealybug colonization in January and mealybugs remained in the fruit until harvest in March. In Elgin, where both orchards were planted with the late maturing Granny Smith apple cultivar, block Elgin 1 (EW48) (Fig. 3.4A) had a mixture of Golden Delicious and younger Granny

Smiths though only the Granny Smith trees were sampled. The period of infestation was from February to April in block EG9 (Fig. 3.4B) and March to May for EW48 (Fig. 3.4A).

In all areas, trap counts generally showed that individual male flight activity is apparent almost throughout the year except for the one Ceres pear orchard where *P. viburni* density was very low (Fig. 3.5B). In Stellenbosch, Elgin 1 and Ceres apples (Figs. 3.3A & B, 3.4A & 3.5A, respectively) trap counts followed a similar trend of a steady increase to a peak from late October to January through February coinciding with an increase in fruit infestation then temporarily declining from March to May. Trap counts then briefly rose from late May to early July before declining with the progression of the winter and tree dormancy. A different seasonal flight pattern was observed in the granny smith apple orchard EG9 (Elgin 2) (Fig. 3.4B) during both seasons where the peak male mealybug flight period was during the late summer to early winter (March to June), compared to the other orchards.

A.



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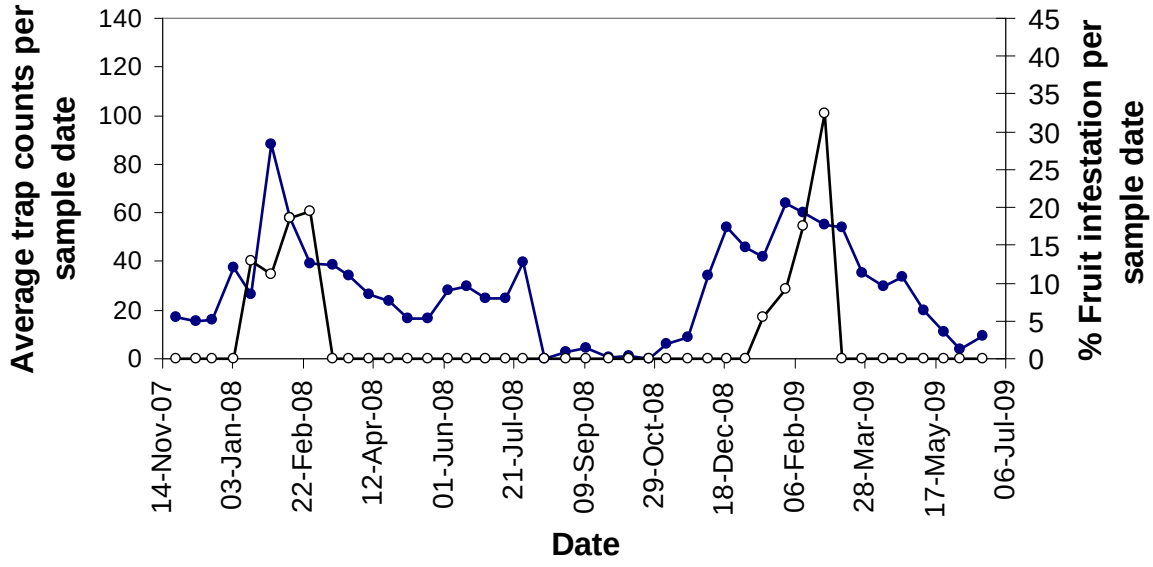
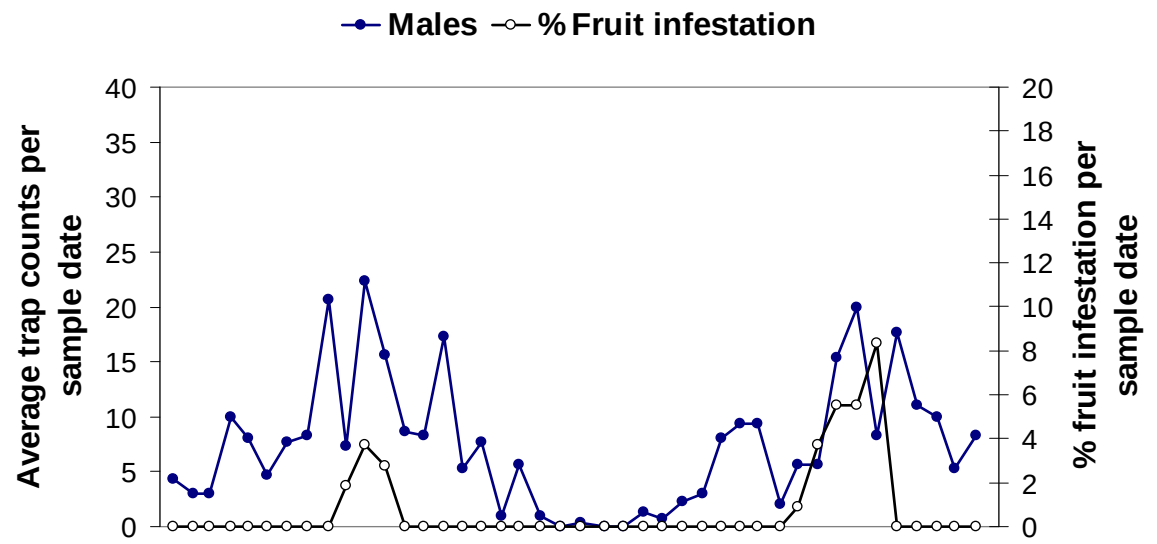


Fig. 3.3. Stellenbosch region adult male *Pseudococcus viburni* trap counts (left – hand scale) and percent fruit infestations (right – hand scale) for each of two pear orchard blocks sampled (A: Stellenbosch 1 & B: Stellenbosch 2)

A.



B.

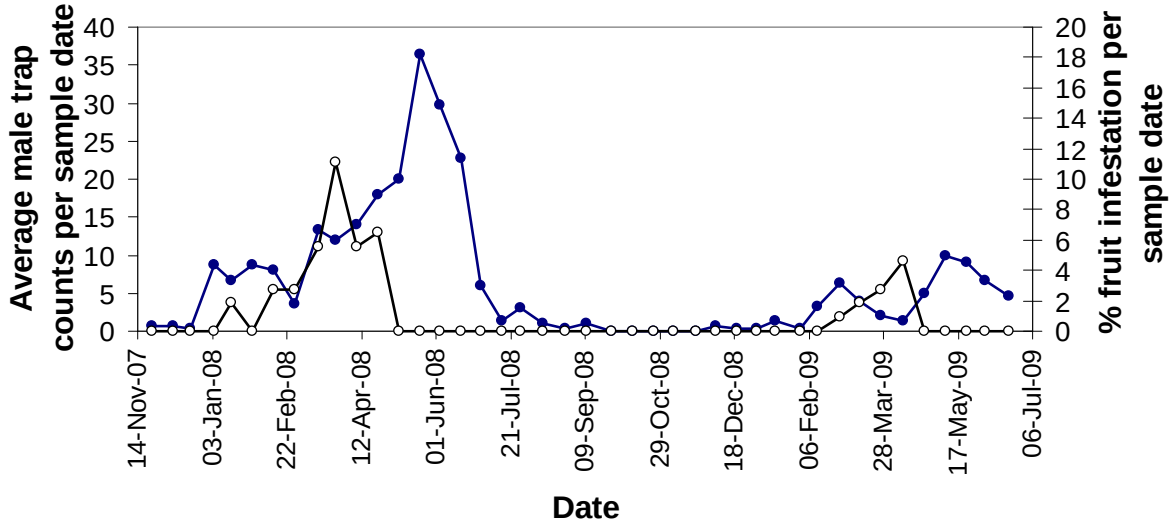
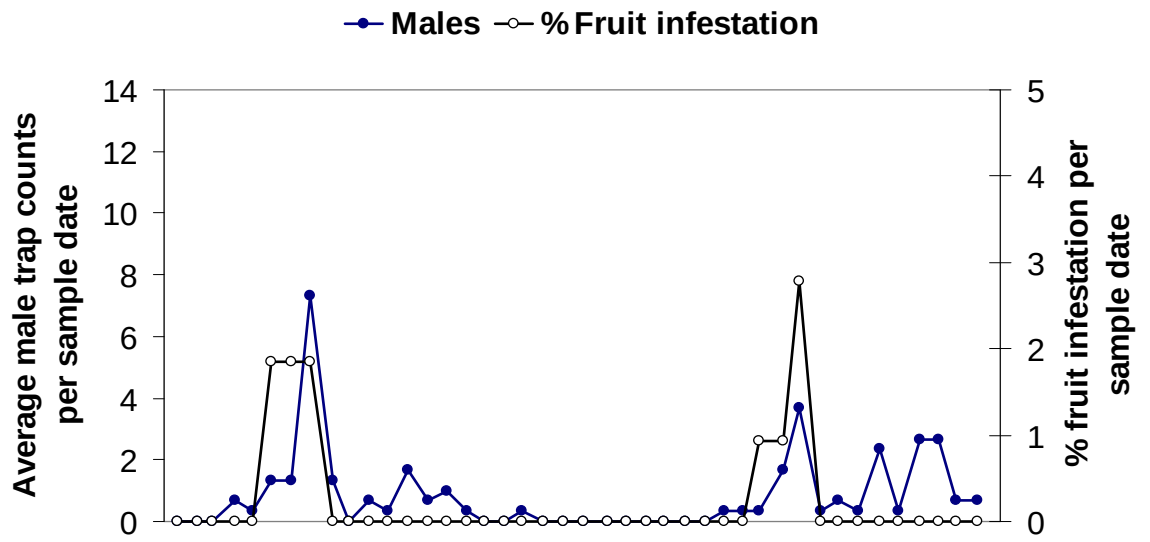


Fig. 3.4. Elgin region adult male *Pseudococcus viburni* trap counts (left – hand scale) and percent fruit infestations (right – hand scale) for each of two apple orchard blocks sampled (A: Elgin 1 & B: Elgin 2)

A.



B.

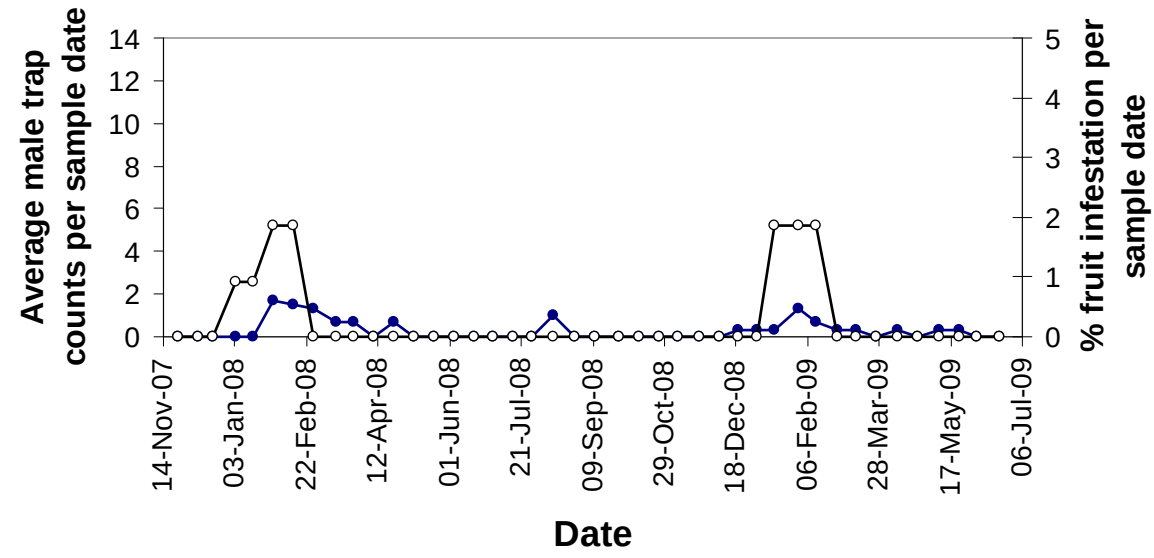


Fig. 3.5. Ceres region adult male *Pseudococcus viburni* trap counts (left – hand scale) and percent fruit infestations (right – hand scale) for each of two orchard blocks sampled (A: Ceres apples & B: Ceres pears)

Data collected from *P. viburni* pheromone trap counts showed peaks and troughs of male flight activity which may have represented changes in mealybug seasonal population age structure rather than density. This was similar to observations by Walton *et al.* (2004) who investigated the male flight activity of a related mealybug species, *P. ficus*. The summer peak flight periods coincided with the fruit season and

were the most important sampling dates, hence this analysis (Fig. 3.3) provided a better fit to the data set (r^2 = 0.454).

3.4. CONCLUSION

The results from this study showed that pheromone-baited traps could be a handy tool for use by farmers to aid in decision making with regards to fruit destined for various markets and targeting of control actions against *P. viburni* to prevent future infestations in ensuing seasons. The action threshold for intervention against *P. viburni,* as estimated by the regression model, may seem like an underestimation when compared to that of *P. ficus* in grape vineyards but it is very realistic. This can be explained by the fact that *P. viburni* and *P. ficus* are two different hemipteran pests occurring on different types of crops where the former does not occur in high population densities but is destructive and more cryptic in nature than the latter. The sensitivity of the traps in capturing male *P. viburni* when and where visual monitoring procedures report absence of colonies on the host plant will provide an effective tool for early warning of damaging *P. viburni* population levels. Placement of several traps per block should provide a more accurate count of the average male trap catches in agreement with findings by Walton *et al.* (2004). However the exact number of traps has yet to be determined.

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CHAPTER 4

LABORATORY STUDIES ON THE DEVELOPMENTAL BIOLOGY OF *PSEUDOCOCCUS VIBURNI* (SIGNORET) (HEMIPTERA: PSEUDOCOCCIDAE).

4.1 INTRODUCTION

Pseudococcus viburni (obscure mealybug) is a common and serious pest of apples and pears in South Africa (Myburgh *et al.,* 1975; Swart, 1977; Wakgari & Giliomee, 2003). The obscure mealybug is a polyphagous, cosmopolitan pest with a worldwide distribution (Ben-Dov, 1994). It is important to know under what conditions economic pest population levels may become destructive (Watson, 1964). Longevity, fecundity and capacity of insects to increase in numbers are influenced by temperature (Andrewartha & Birch, 1954; Romoser, 1981). Rate of development, which is regulated by temperature, is the most important factor influencing the intrinsic rate of increase of colonizing species (Romoser & Stoffolano, 1998). Insects may have a wide geographic distribution but they generally become pests in those areas where optimal environmental conditions occur (Romoser & Stoffolano, 1998).

There is currently no information available on the development biology of *P. viburni* in South Africa. Knowledge of these factors is therefore necessary not only to maintain laboratory colonies during mass-rearing and release programmes, but also to understand the population ecology and impact of these factors on the target pest in the field (Sandanayaka *et al.*, 2009). The aim of this study was to determine the development biology of *P. viburni* at a range of temperatures in the laboratory. This information is aimed at improving our understanding of the effect of temperature on the rate of development of the pest. This information can also be used to make a

comparison with the development biology of important natural enemies, such as *Pseudaphycus maculipennis,* which has already been studied (Sandanayaka *et al.,* 2009).

4.2. MATERIALS AND METHODS

4.2.1. Mass rearing of P. viburni

Several authors have described the mass-rearing of mealybugs on butternut pumpkins (*Cucurbita moschata*) (Duchesne ex Poir). Compared to other media or artificial diets butternuts are preferred due to their long shelf-life, low price, all-year round availability, convenience and viability (Jayanthi & Verghese, 2002). Krishnamoorthy & Singh (1987) and Walton & Pringle (2005) described mass-rearing of the citrus mealybug, *Planococcus citri* (Riso) and vine mealybug, *P. ficus* (Signoret) respectively, on butternut pumpkins. However *P. viburni* has also been reared and maintained on sprouting potatoes (*Solanum tuberosum* L.) (Charles *et al.,* 2004; Sandanayaka *et al.,* 2009).

In the present study, *P. viburni* were reared and maintained on butternut pumpkins. The parental stock (F_0) of *P. viburni* was originally sourced from several apple and pear growing sites in the Western Cape Province, South Africa. The mealybug colony was maintained at the Department of Conservation Ecology and Entomology Insectary from 2007 in wooden rearing bins (650 X 350 X 590 mm) (Fig. 4.1D).

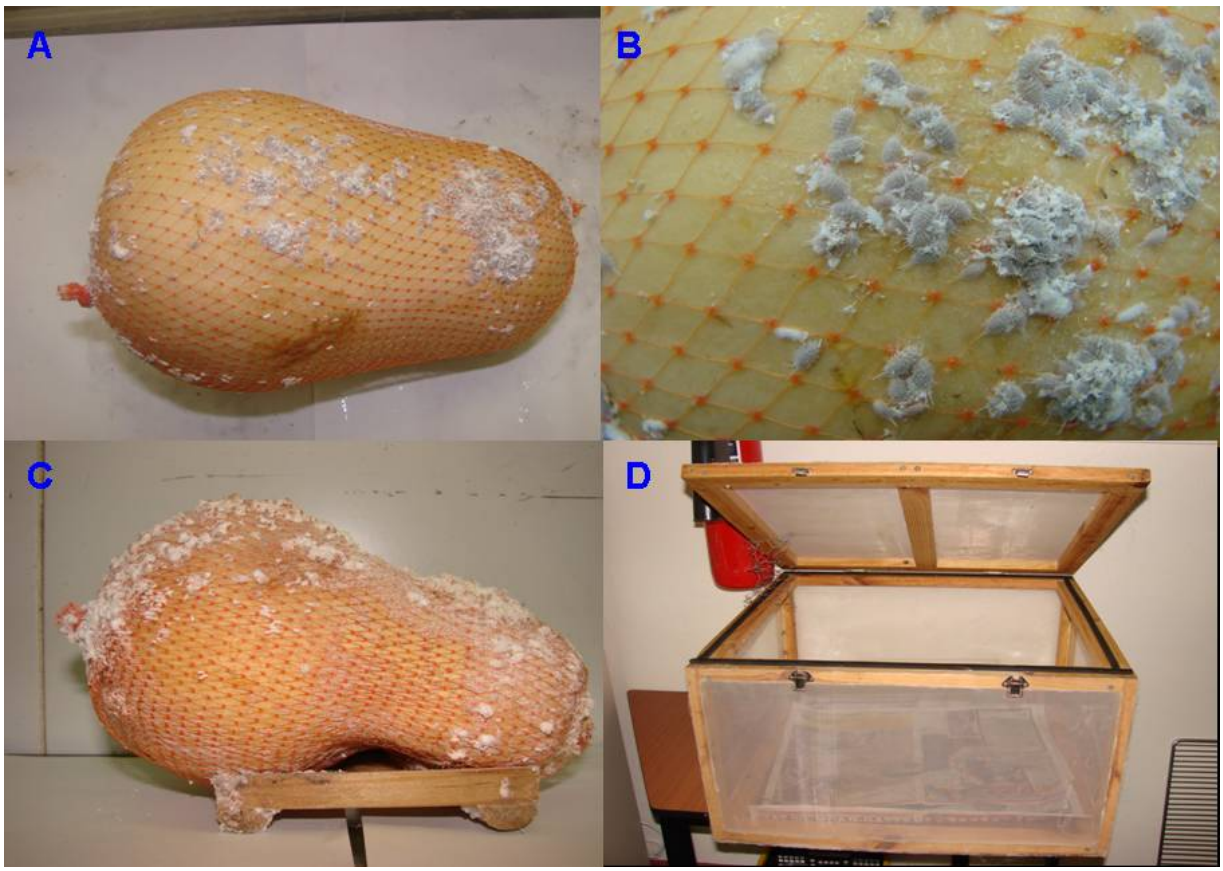

Fig 4.1.A, Orange nylon sleeves wrapped around the butternut; B, Adult female *Pseudococcus viburni* on surface of butternut; C, butternut firmly resting on mini wooden stake; D, The wooden rearing bin with lid.

The butternuts were commercially sourced, disinfected in 6 – 14% sodium hypochlorite solution and dried before being transferred to the colony. Orange nylon sleeves were wrapped around the butternuts as a way of manipulating the butternut surface area for mealybugs to clutch onto and enhance colony establishment (Fig. 4.1A). *P. viburni* is known for its cryptic nature and preference for secluded spaces on the host plant. The butternuts were placed and firmly confined onto mini wooden stakes in such a way that they were not resting directly on the surface/floor of the wooden rearing bin (Fig. 4.1C). This also ensured that the butternuts would not roll around and squash the delicate mealybugs during routine maintenance or colony access. The mini wooden stakes also allowed air circulation around each butternut and therefore prolonged quality of the butternut pumpkins. The wooden rearing bin had an access lid tightly secured by petroleum jelly to prevent entry of ants and

contamination by parasitoids as well as other insects (Fig. 4.1D). The rearing bin was covered with a nylon gauze mesh for ventilation and to minimize crawler and adult male escape, held in place by glue. The floor of the rearing bin was lined with newspapers on which honeydew and dead mealybugs accumulated. The colony was stored and reared at room temperature of 25 ± 1°C, with an average 16L: 8D photoperiod throughout the year. The rearing rooms were cleaned on a weekly basis. Butternuts were replaced depending on their palatability, physical condition and quality with regards to decay and drying out. The colony increased as mealybugs dispersed naturally to fresh butternuts.

4.2.2. Developmental biology

The methods described by Walton & Pringle (2005) were used. Modifications were done where necessary for this study. The culture of mass-reared *P. viburni* from the insectary colony was used. The investigations on the developmental biology of *P. viburni* were conducted in special temperature and light controlled growth chambers and growth rooms (Fig. 4.2.C&D). The developmental times, fecundity and fertility were determined at 18, 20, 25, 27 & 30°C. Relative humidity ranged from 60 – 90% and a photoperiod of L16:D8 was used for all experiments. A group of ovipositing adult female *P. viburni* of comparable age were obtained from the stock colony and introduced onto the leaves (Fig. 4.2A) and crutches (fork in the trunk) (Fig. 4.2B) of potted apple trees (Kripps Red) approximately one meter in height and left for 24 hours before removal . Potted apple trees were used instead of butternut pumpkins so as to more accurately reflect natural conditions under which *P. viburni* occurs.

Fig. 4.2. A group of adult ovipositing female mealybugs on leaf (A) and crutch (B); temperature, light & humidity controlled growth chamber (C) and growth room (D).

At least 25 eggs deposited by these adult females were initially retained on each of four plants per treatment. Records were then kept for five individual mealybugs specifically isolated to a separate section on each host plant at each temperature treatment. This therefore provided data for 20 mealybugs from each temperature treatment. To ensure that the same individuals were observed for the duration of the study, barriers of petroleum jelly were used to restrict mealybug movement and escape. The development of each individual mealybug from egg incubation, nymphal stages through to adult death was monitored on a daily basis. Records were kept on pre-oviposition period duration, daily fecundity and longevity of the surviving females mealybugs at each temperature treatment from the time of adult emergence until death. Mealybugs lost due to escape or injury were omitted from the analysis. The analysis of the development of male *P .viburni* was omitted because male mealybug

records were insufficient and unreliable, as a result of loss of males after emergence and the nature of host trees making it difficult to monitor their development.

4.2.3. Temperature-development curve fitting

The minimum threshold temperature for development of *P. viburni* was determined by regressing 1/*Time* (the reciprocal of time to development completion (in days) from egg to adult or rate of development) on temperature and then solving the regression equation for 1/*Time* = 0 (Campbell *et al.,* 1974). The following function by Brière *et al.* (1999) was used for regression of development rate on temperature in instances where development rate declined at temperatures higher than the optimum temperature.

$$y = (Ax)(x - T_0)(T_L - x)^{0.5},$$

where, *A* is a positive empirical constant, *(T_0)* is the lower temperature threshold and *(T_L)* is the lethal (or upper) temperature threshold. This is the model of Brière *et al.* (1999) that is given by Roy *et al.* (2002). This was solved in Statistica (StatSoft, 2008). The Levenberg-Marquardt least squares fitting routine was used. Estimates for T_0 and T_L were obtained from a preliminary quadratic fit. The optimum temperature was estimated by solving for *x* in $\frac{dy}{dx} = 0$. (Brière *et al.,* 1999)

4.3. RESULTS AND DISCUSSION

4.3.1. Mass rearing of P. viburni

The technique described above successfully produced a large, healthy and continuous *P. viburni* colony for 3 years. Mass rearing of *P. viburni* using this method was straight-forward, non-labour intensive, cost-effective and necessary to ensure sufficient insect quantities for the development biology study.

4.3.2. Developmental times

The developmental time of *P. viburni* from egg to oviposition including the pre-oviposition period of adult female mealybugs decreased from 132.33 days at 18°C to 47.80 days at 25°C. At 27°C it increased to 68.73 days (Table 4.1). Our results differed from findings by Islam *et al.,* (1995) who reported that the developmental periods of *P. viburni* decreased from 93.35 to 36.80 days as the rearing temperature increased from 18 to 30°C. Our results also slightly differed from Heidari (1989) who reported a developmental period of 30 days at 26 °C. The maximum number of eggs oviposited per female were recorded at 25°C while the least were recorded at 18°C. Results in table 5.1 showed that fecundity was directly influenced by temperature. This is similar to the conclusion by Walton & Pringle (2005), who conducted laboratory studies on the development of *P. ficus* at a range of constant temperatures.

Table 4.1. Developmental times in days (± S.E.) for eight developmental stages and fecundity of *Pseudococcus viburni* on Kripps Red potted apple plants at five temperatures (± 0.5 °C).

Developmental Stage	Temperature				
	18°C	20°C	25°C	27°C	30°C
Egg	19.22 (1.8)	16.39 (1.8)	8.40 (1.7)	11.75(1.7)	7.41 (1.9)
1st Nymphal	26.19 (1.9)	22.17 (1.9)	11.21 (1.8)	17.00 (2.0)	16.00(2.1)
2nd Nymphal	28.00 (2.1)	25.12 (1.9)	15.10 (1.9)	19.90 (2.4)	17.29 (2.9)
3rd Nymphal	28.92 (2.1)	24.57 (2.1)	10.42 (2.2)	15.75 (2.7)	
Adult Female	61.25 (2.7)	59.25 (2.7)	42.00 (2.6)	30.17 (3.1)	
Egg to adult female	102.33 (2.1)	88.25 (2.1)	45.13 (2.2)	64.40 (2.7)	
Pre-oviposition period	30.00 (2.7)	20.50 (2.7)	2.67 (2.6)	4.33 (3.1)	
Eggs per female	87.50 (2.7)	118.63 (2.7)	240.00 (2.6)	147.50 (3.1)	

Further development of *P. viburni* beyond the second nymphal stage at 30°C was arrested contrary to reports by Islam *et al.* (1995) that *P. viburni* completed development at 33°C. However, Islam *et al.* (1995) further stated that *P. viburni* thrives in and is more adapted to lower temperatures than other mealybug species. Results from this study also showed that the rate and time of development for the

three nymphal stages of female *P. viburni* decreased from 102.33 days to 45.13 days at 18°C and 25°C, respectively (Table 4.1). These results suggest that the time and rate of development of *P. viburni* is longer and slower than that of other mealybug species under similar temperature conditions. Walton and Pringle (2005) reported developmental times of 44.46 days at 18°C to 24.61 days at 27°C for the three female nymphal stages of *P. ficus.*

4.3.3. Temperature thresholds for development

The regression (Fig. 4.3) of 1/*Time* on temperature for *P. viburni* was $y = 0.00005x(x - 16.00009)(27.96934 - x)^{0.5}$. A good fit was obtained (r = 0.9530, d.f. = 27, 31, $P < 0.001$). The minimum and maximum threshold temperatures for development of *P. viburni* were estimated to be 16.00009°C (95% Confidence Interval: 15.422 – 16.578) and 27.96934°C (95% Confidence Interval: 27.636 – 28.303), respectively. Our lower level threshold is higher than that of Karamaouna & Copland (2009) quoting Islam (1993) who stated that the lower developmental threshold temperature for *P. viburni* was 11.4°C. By solving, $\frac{dy}{dx}(0.00005x)(x-16.00009)(27.96934-x)^{0.5} = 0$ (Brière *et al*., 1999, Roy *et al.,* 2002), the optimum temperature for development of *P. viburni* was estimated to be 24.715 °C.

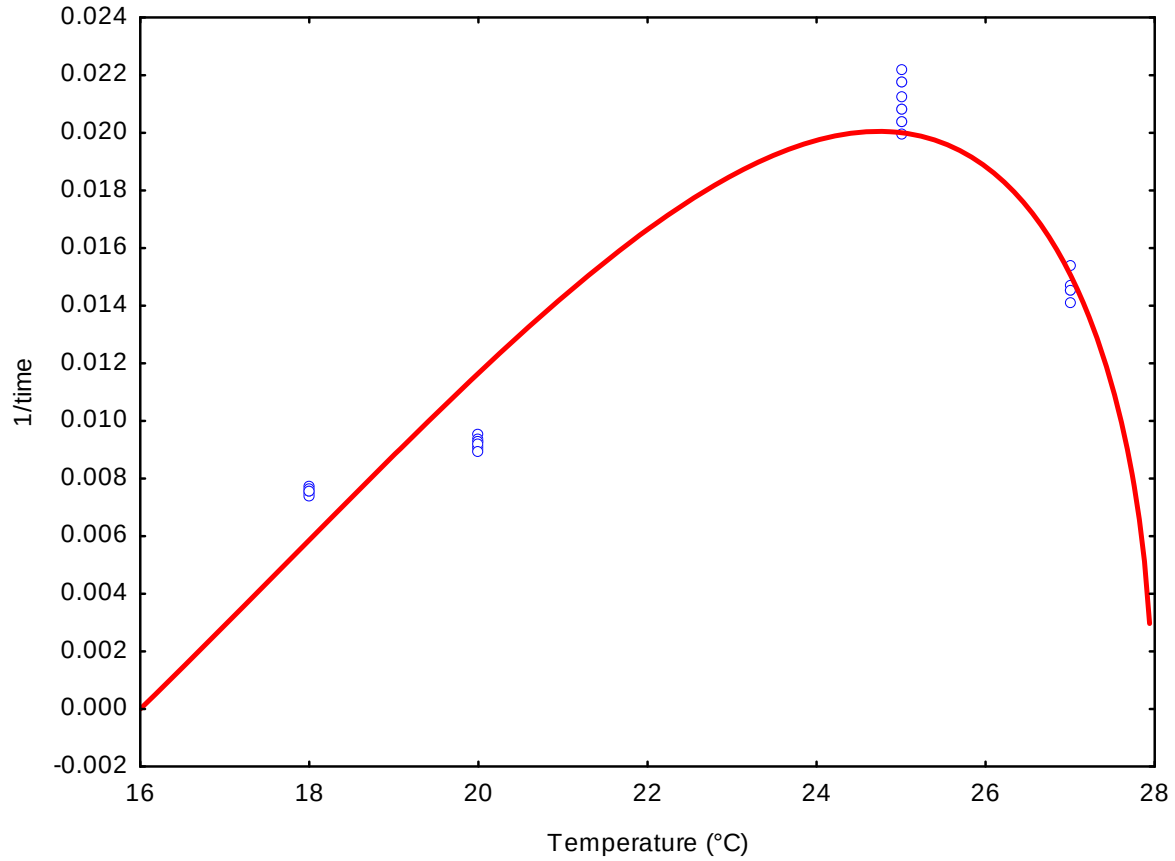

Fig. 4.3. Developmental rate (1/*Time*) of *Pseudococcus viburni* at a range of temperatures.

4.4. CONCLUSION

Based on this model we can conclude that 30°C is a lethal temperature for *P. viburni* confirming the observation that there was no further development beyond the second instar at this temperature. Results from the current study support findings by Panis (1986) who stated that high temperatures not only delay egg hatch but larval development in *P. viburni* as well. Results from this study also confirm reports by Daane & Bentley (2002), Varela et al., (2006) and Daane *et al.,* (2008) who stated that *P. viburni* has a narrower tolerance to temperatures compared to other closely related species such as the grape mealybug (*P. maritimus*) and as a result, is limited to the cooler grape-growing regions of the Central Coast of California. Walton and Pringle (2005) demonstrated that *P. ficus* has a wider temperature tolerance and is able to complete development between the temperature ranges of 16.59 and

35.61°C. *P. ficus* is predominant in the Breede River Valley – a hot region planted to wine grapes in the Western Cape Province, South Africa (Walton & Pringle, 2005). This data can be used for conducting life-table studies of *P. viburni*. However we propose that further research be done on appropriate methods of handling and prevention of loss or escape of male mealybugs during monitoring.

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CHAPTER 5

GENERAL DISCUSSION

Temporal patterns of occurrence similar to those reported by Swart (1977), Panis (1986) and Varela *et al.* (2006) were observed for the seasonal phenology of *P. viburni* in pome fruit orchards in the present study. Although *P. viburni* was always present and active all year round on different parts of the host plant, the highest populations occurred during the warm summer months while the lowest activity was during the cold winter months when mealybugs were overwintering in secluded locations of the woody parts of host trees. The management implications of these results are such that any effective control method should be implemented early in the season and targeted against the surviving overwintering population which, according to Swart (1977), is responsible for fruit infestation later in the season. Further research should therefore focus on development of alternative control techniques that are capable of searching, accessing and penetrating the secluded overwintering locations where mealybugs are protected and out of reach of insecticides or parasitoids, for example entomopathogenic nematodes (Dent, 2000).

Two natural enemies of mealybugs, namely, *Pseudaphycus maculipennis* and *Coccidoxenoides perminutus,* were found to be active in apple and pear orchards in the Western Cape. The former is a primary parasitoid of *P. viburni* (Sandanayaka *et al.,* 2009), is commercially available in the Netherlands (Walton, 2006) and is used for biological control of *P. viburni* in New Zealand pip fruit orchards (Charles *et al.,* 2004). However, the status of *C. perminutus* as a parasite of *P. viburni* still needs to be confirmed despite reports by Walton (2006) that local *C. perminutus* strains in California attack *P. viburni*. From the present study, indications are that this could also be the case, but alternate monitoring methods should be used to prevent

possible loss of *C. perminutus*-infested mealybugs, should they drop to the orchard floor before collection. The seasonal abundance trends of the two natural enemies revealed that their lifecycle is synchronized with that of the host, suggesting that temperature is important as a limiting factor in the population dynamics and seasonal occurrence pattern of these organisms. This information will therefore be vital in guiding the implementation of future mass rearing and release of natural enemies for inundative biological control and pest monitoring. Our failure to find mealybug predators during the current study could be attributed to the sampling method used. For future surveys we propose the use of *P. viburni*-infested butternuts placed in emergence cages similar to those used by Walton *et al.* (2004) and left in orchards for longer periods of up to one to two months after which natural enemies are identified and counted. Producer resistance to this method was the reason why this method was not employed in the present study. Radiation of mealybugs for sterility should be investigated to allay fears by growers of introduction of mealybug infestations into their orchards when conducting research of this nature.

The rate of *P. viburni* parasitism at harvest was approximately 46.52% with *P. maculipennis* and *C. perminutus* constituting 98.966% and 1.034%, respectively, of the parasitoids recovered from mealybug mummies. *P. maculipennis* is therefore a potential candidate for future augmentative biological control of *P. viburni* in pome fruit orchards in South Africa. We also propose further investigations regarding the status of *P. maculipennis* in areas such as Ceres, given the fact that there was no evidence of its occurrence in this region. Weather data showed that mean daily temperatures in Ceres were lower than in Elgin and Stellenbosch almost all year round, dropping to as low as 5°C, conditions which may be unsuitable for development and establishment of *P. maculipennis* because it has a lower

temperature threshold of 20°C (Anonymous, 2009), while that of a closely related species, *P. flavidulus,* is approximately 9.3 °C (Karamaouna & Copland, 2009).

The application of insecticides also played an important role in the population dynamics of both mealybugs and their natural enemies. The implications of this are such that the incidence of natural enemies as well as percentage parasitism would have been higher if insecticide applications were not as frequent. We therefore suggest pesticide applications based on effective monitoring. This would reduce spraying and allow establishment of natural enemies as well as promote sustainable, environmentally-friendly and cost-effective pest management. We also propose that bioassays be done to determine the susceptibility of natural enemies, in particular *P. maculipennis*, to current pesticides applied in South African pome fruit orchards. Alternative methods to insecticides are currently being investigated against the major pome fruit pests. These include codling moth, *Cydia pomonella* (L.) using the mating disruption, the sterile insect technique (Addison, 2005), and biological control (de Waal, 2008; Wahner 2008), banded fruit weevil, *Phlyctinus callosus* (Schoenherr) using entomopathogenic nematodes (Ferrieria & Malan, 2009); phytophagous orchard mites using biological control (Pringle 2001); and *P. viburni* using entomopathogenic nematodes (Stokwe & Malan, 2009).

An early monitoring tool for *P. viburni* in pome fruit orchards based on pheromone-baited traps was successfully developed. There was a positive and significant relationship between fruit infestation and number of adult male *P. viburni* caught in pheromone-baited traps. An action threshold of 2.5 male *P. viburni* per trap per two weeks corresponds to an economic threshold of 2% fruit infestation, as suggested by Swart (1977). This method will thus provide accurate information earlier in the

season, is quicker, more convenient and less labour intensive compared to current visual sampling and monitoring techniques. This would therefore imply better, sustainable, efficient and cost-effective management of *P. viburni* where insecticide spray applications are scheduled timeously, appropriately and implemented only when necessary. This is important particularly for limiting insecticide residues on fruit destined for various export markets, each of which have their own minimum residue levels. *P. viburni* is no longer a phytosanitary concern for existing export markets, based on the availability of identification keys (Wakgari & Giliomee, 2004). In the event that new export markets become available to producers in future, an accurate monitoring method will however be critical.

Laboratory studies on the development of *P. viburni* at a range of temperatures showed that 30°C is lethal for *P. viburni* development contrary to reports by Islam *et al.* (1995). The time and rate of development of *P. viburni* was also observed to be longer and slower than that of other mealybug species under similar temperature conditions. Based on the information gathered from this study the environmental conditions under which *P. viburni* population levels may become destructive as well as threshold developmental temperatures are now known. It is now also possible to accurately determine the regions were the pest is better adapted and where infestation increases can be expected given optimal temperature conditions. This information is also essential for guiding future maintenance of laboratory colonies during mass rearing and release programmes, conducting life-table studies and degree day modeling. Furthermore, linking these findings to studies on the seasonal phenology of *P. viburni*, indications are that temperature greatly influenced the seasonal abundance mealybugs. Mealybug abundance in orchards was higher in

summer when mean temperatures where near optimum while in winter the opposite was observed due to temperatures falling below the lower threshold levels.

The information gathered in the current study can therefore be used as a baseline for future research into biological control and improving integrated management of *P. viburni* in pome fruit orchards.

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APPENDIX A

Table A.1. A list of insecticides and fungicides sprayed and frequency of application in the Ceres pear orchard during 2007/08 and 2008/09 growing seasons.

	2007				
July	**August**	**September**	**October**	**November**	**December**
Prothiophos X 2 Mineral oil X 2	Copper-Oxychloride X 1 *Nonyl phenol ethoxylate X 1	Mineral oil X 1 Chlorpyriphos X1 Copper-Oxychloride X1 Mancozeb X 2	Beta Cyfluthrin X 1 Mancozeb X 2	Methyl parathion X 1	*Nonyl phenol ethoxylate X 1

	2008					
January	**June**	**July**	**September**	**October**	**November**	**December**
Methoxyfenoxide X 2 *Nonyl phenol ethoxylate X 4 Abamectin X 1	Prothiophos X 1 Mineral oil X 1	Prothiophos X 1 Mineral oil X 1	Chlorpyriphos X 1 Mineral oil X 1 Copper-Oxychloride X 1 Mancozeb X 2 Dodine X 2	Beta Cyfluthrin X 1 Mancozeb X 2	Thiacloprid X 1 Mancozeb X 1 Methyl parathion X 1 Myclobutanil(triazole) X 1	Azinphos methyl X 1 Thiacloprid X 2 *Nonyl phenol ethoxylate X 1

	2009
January	
Methoxyfenoxide X 2 *Nonyl phenol ethoxylate X 1 Abamectin X 1	

* Denotes wetting agent.
NB: records only available from July 2007

Table A.2. A list of insecticides and fungicides and frequency of application in the Ceres apple orchard during 2007/08 and 2008/09 growing seasons

	2007				
July	**August**	**September**	**October**	**November**	**December**
Prothiophos X 2	Copper-Oxychloride X 1	Mineral oil X 1	Beta Cyfluthrin X 1	Methyl parathion X 1	*Nonyl phenol ethoxylate X 4
Mineral oil X 2	*Nonyl phenol ethoxylate X 1	Chlorpyriphos X 1	Mancozeb X 5	Myclobutanil triazole X 1	Mancozeb X 1
		Copper-Oxychloride X 1	Trifloxystrobin X 1	Bifenthrin X 1	Trifloxystrobin X 1
		Mancozeb X 2	Myclobutanil(triazole) X 3	Mancozeb X 3	
			Carbaryl X 2		
			Naphthylacetic acid X 1		

	2008							
January	**March**	**April**	**June**	**July**	**September**	**October**	**November**	**December**
*Nonyl phenol ethoxylate X 1	Mercaptothion X 1	Mercaptothion X 1	Prothiophos X 1	Prothiophos X 1	Mineral oil X 1	Mancozeb X 4	Mancozeb X 2	Azinphos methyl X 1
Abamectin X 1	Nonyl phenol ethoxylate X 1		Mineral oil X 1	Mineral oil X 1	Chlorpyriphos X 1	Myclobutanil(triazole) X 1	Flusilazole X 1	*Nonyl phenol ethoxylate X 3
Mineral oil X 1					Mancozeb X 4	Carbaryl X 1	Beta Cyfluthrin X	Indoxacarb X 1
Thiacloprid X 1					Copper-Oxychloride X 1	Beta Cyfluthrin X 1	*Nonyl phenol ethoxylate X 1	Polysulphide sulfur X 1
					Dodine X 3	Trifloxystrobin X 3		Mancozeb X 1
						Naphthylacetic acid X 1		
						Dodine X 1		

	2009
January	
Thiacloprid X 1	
*Nonyl phenol ethoxylate X 2	
Abamectin X 1	
Mineral oil X 1	

* Denotes wetting agent.
NB: records only available from July 2007.

Table A.3. A list of insecticides and fungicides and frequency of application in the two apple blocks in Elgin during 2007/08 and 2008/09 growing seasons.

	2007						
January	**February**	**May**	**August**	**September**	**October**	**November**	**December**
*Phenyl-ethylene X 3	*Phenyl-ethylene X 1	*Phenyl-ethylene X 1	Mineral oil X 1	Prothiophos X 1	Bupirimate X1	Carbaryl X 1	Indoxacarb X 1
Azinphos methyl X 2	Fenarimol X 1	Difenoconazole X 2	Cyanimide X 2	Mancozeb X 6	Mancozeb X 8	Indoxacarb X 1	Thiacloprid X 1
	Azinphos methyl X 1	Copper – Oxychloride X 1	Prothiophos X 1		Flusilazole X 1	Chlorpyriphos X 1	Mancozeb X 3
					Cyprodinil X 1	Thiacloprid X 1	Mineral Oil X 2
					Trifloxystrobin X 2	Mancozeb X 8	
					Novaluron X 1		
					Endosulfan X 1		
					Fenarimol X 2		
					Lambda-cyhalothrin X 1		
	2008						
January	**February**	**March**	**May**	**September**	**October**	**November**	**December**
Mancozeb X 1	Mancozeb X 1	Mineral oil X 1	*Phenyl-ethylene X 2	Cyanimide X 1	Flusilazole X 1	Lambda-cyhalothrin X 1	*Phenyl-ethylene X 3
Methoxyfenoxide X 2	Methoxyfenoxide X 1	*Phenyl-ethylene X 1		Prothiophos X 1	Mancozeb X 4	Mancozeb X 5	Chlorpyriphos X 1
Mineral oil X 2				Mancozeb X 3	Bupirimate X 1	Novaluron X 1	Mancozeb X 3
				Bupirimate X 1	Pyraclostrobin + diathianon X 1	Indoxacarb X 1	Thiacloprid X 1
					Novaluron X 1	Fenarimol X 1	Methyl parathion X 1
						Thiacloprid X 1	Fenarimol X 2
	2009						
January	**February**	**March**	**May**				
Methyl parathion X 1	Thiacloprid X 1	Methoxyfenoxide X 1	*Phenyl-ethylene X 2				
Thiacloprid X 1	Methoxyfenoxide X 1						
Mancozeb X 1							

* Denotes wetting agent.

Table A.4. A list of insecticides and fungicides and frequency of application in the two pear blocks in Stellenbosch during 2007/08 and 2008/09 growing seasons.

	2007						
January	**March**	**May**	**June**	**September**	**October**	**November**	**December**
Azinphos methyl X 3	Copper-Oxychloride X 1	Copper-Oxychloride X 2	Mercaptothion X 1	Cyanimide X 1 Chlorpyriphos X 1	Novaluron X 1 Chlorpyriphos X 1 Mancozeb X 3 Flusilazole X 3	Mancozeb X 2 Methoxyfenoxide X 2	Azinphos methyl X 1 Methoxyfenoxide X 1 Mancozeb X 1
	2008						
January	**July**	**September**	**October**	**November**	**December**		
Azinphos methyl X 3	Chlorpyriphos X 1	Cyanimide X 1 Chlorpyriphos X 1	Prothiophos X 1 Mancozeb X 3 Flufenoxuron X 1 Flusilazole X 1	Benomyl X 1 Mancozeb X 3 Flusilazole X 2 Flufenoxuron X 2	Azinphos methyl X 1 Mancozeb X 1		
	2009						
January							
Methoxyfenoxide X 1							