

EVALUATING SEX PHEROMONE MONITORING
AS A TOOL IN THE INTEGRATED MANAGEMENT OF
VINE MEALYBUG, *PLANOCOCCUS FICUS* (SIGNORET)
(HOMOPTERA: PSEUDOCOCCIDAE).

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Dedicated to my father

Dirk Jacobus Kotze

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ABSTRACT

The vine mealybug, *Planococcus ficus* (Signoret) (Homoptera: Pseudococcidae) is a pest with significant economic impact on the grape growing industry in South Africa and other parts of the world. With the isolation and synthesizing of the vine mealybug sex pheromone in 2001, new control options for the integrated management of the vine mealybug have been created.

The status of sex pheromone monitoring as a tool in the integrated management of the vine mealybug has been evaluated from different perspectives. A significant quantitative difference in male vine mealybug trap catch numbers has been observed between wine and table grape vineyards and results indicated that there were differences in the susceptibility of grape cultivars to vine mealybug. Currently, the delta trap design is the accepted trap design for vine mealybug monitoring. No studies have yet been conducted to determine the optimum trap parameters like size or design. Population pressure may have an influence on the qualitative efficiency of various trap designs.

The basis for degree-day forecasting models has been established adequately. However, refinements need to be done and the incorporation of factors such as humidity and regionality also need to be considered. Daily maximum temperatures fluctuating around the upper developmental threshold temperature for prolonged periods of time seemed to suppress population numbers. Different vineyard management practices exist for wine and table grape production. While an action threshold of 65 vine mealybug males per trap per two-week period seems an acceptable threshold for table grape production, it may not be appropriate for wine grape (or raisin grape) production.

Using sex pheromone traps for population monitoring is a valid technique in the arsenal of management tactics against the vine mealybug. However, refinements and validation of research results must be done further to build credibility into the monitoring system.

Keywords: *Planococcus ficus*, vine mealybug, pheromone, monitoring, trap design, *Vitis vinifera*, degree-days, humidity

OPSOMMING

Titel: Die evaluering van seksferomoonmonitering as 'n instrument in die geïntegreerde bestuur van die wingerdwitluis, *Planococcus ficus* (Signoret) (Homoptera: Pseudococcidae).

Die wingerdwitluis, *Planococcus ficus* (Signoret) (Homoptera: Pseudococcidae), is 'n plaag wat 'n beduidend ekonomiese impak op die druivebedryf in Suid Afrika en ander dele van die wêreld het. Met die isolering en sintetisering van die seksferomoon van die wingerdwitluis in 2001, het nuwe beheeropsies vir die geïntegreerde bestuur van wingerdwitluis ontstaan.

Die stand van seksferomoonmonitering as 'n instrument in die geïntegreerde bestuur van die wingerdwitluis, is uit verskeie perspektiewe beoordeel. 'n Beduidende kwalitatiewe verskil in lokvalvangste van wingerdwitluismannetjies is opgemerk tussen wyn- en tafeldruive wat aandui dat daar verskille in die vatbaarheid vir wingerdwitluis in sommige druifkultivars kan wees. Die delta-lokvalontwerp word tans aanvaar as die standaard lokvalontwerp vir wingerdwitluismonitering. Optimale lokvalparameters, soos grootte en ontwerp, is egter nog nie nagevors nie. Bevolkingsdruk mag ook 'n invloed hê op die kwalitatiewe effektiwiteit van verskillende lokvalontwerpe.

Die basis vir graaddae-voorspellingsmodelle is voldoende gevestig. Die modelle moet egter verfyn word en daar moet oorweging geskenk word aan die opneming van faktore soos streeksgebondenheid en humiditeit in die model. Langdurige skommelings van daaglikse maksimum temperature om die boonste temperatuurontwikkelingsdrempel mag moontlik die witluisbevolking onderdruk. Wingerdbestuurspraktyke verskil vir wyn- en tafeldruifproduksie. 'n Aksiedrempelwaarde van 65 wingerdwitluismannetjies per lokval per twee weke-periode

blyk voldoende te wees vir tafeldruifverbouing, maar is moontlik nie optimaal vir wyndruif- of rosyndruifverbouing nie.

Die gebruik van seksferomoonlokvalle om bevolkingskattings mee te doen, is 'n geldige bestuurstechniek teen wingerdwitluis. Verfynings en bekragtiging van navorsingsresultate moet egter gedoen word om geloofwaardigheid in die moniteringstelsel in te bou.

Sleuteltermes: *Planococcus ficus*, wingerdwitluis, feromoon, monitering, lokvalontwerp, *Vitis vinifera*, graaddae, humiditeit

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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Scale insects (Homoptera) are amongst the most important pests of agricultural crops, debilitating the plant by loss of sap, contaminating the plant and its fruit with honeydew on which sooty mould frequently grows, transmitting plant viruses, and sometimes injecting toxins that stunt plant growth. Having life cycles of as short as one month in warm climates, mealybugs can rapidly attain very high numbers on their host plant. Fortunately, they are usually attacked by a wide range of natural enemies, in particular, encyrtid parasitic wasps and ladybird beetles (Cox, 1989).

1.2 Systematics

1.2.1 Nomenclatural history of the vine mealybug

Today, the vine mealybug is classified as the *Planococcus ficus* under the family Pseudococcidae within the suborder Coccoidea of the order Homoptera of the class Insecta. Although the positioning thereof in the family Pseudococcidae was never disputed, there was great confusion about the genus and species categories.

Throughout the years from 1869 to as recent as 1984 various combinations of four genus names and six species names were proposed. Ben-Dov (2001) and Cox (1989) list the history of the nomenclature. In 1869 it was described as *Coccus vitis* by Nedzilskii. This name reappeared during 1912 and 1942, but was then again rejected. In 1870 the name *Dactylopius vitis* was proposed by Lichtenstein and again in 1895 by Signoret. The genus

name *Dactylopius* was subsequently combined with species names *ficus* and *subterraneus*. From 1903 the genus name *Pseudococcus* appears with species names *ficus*, *vitis*, *citrioides*, *citri* and *praetermissus*. From 1950 the genus *Planococcus* appeared combined only with species names *citrioides*, *vitis* and *ficus* (Ben-Dov, 2001). The vine mealybug species was often confused with the citrus mealybug, *Planococcus citri*, and many authors listed *ficus* and *vitis* as synonyms for *citri* (De Lotto, 1975). Some evidence was later produced that *ficus* and *vitis* are forms specifically distinct from each other as well as from *citri* (De Lotto, 1975).

Eventually the species description as done by Signoret in 1875 was accepted and ascribed to Signoret (Walton, 2003a).

1.2.2 The family Pseudococcidae

The family Pseudococcidae comprises about 2000 species of which 109 mealybug species are found in South Africa, and 68 of those species, are only found in South Africa (Millar, 2002).

These insects are commonly known as mealybugs because they typically secrete a white, powdery or mealy wax that covers the body. All mealybugs are phytophagous, and they remove plant juice using their piercing-sucking mouthparts. Many species are important agricultural pests. Fruit infested with mealybugs becomes unmarketable. Their feeding may cause deformation or death of plant shoots, and some species can transmit plant virus diseases. Large populations contaminate foliage with their sticky honeydew excretions, which provide a substrate for sooty mould growth (Millar, 2002). About 20 species of Pseudococcidae are of economic importance on cultivated plants in South Africa (Annecke & Moran, 1982).

Fifty mealybug genera occur in South Africa, 13 of which have been recorded only from this country. Most of the endemic genera in South Africa are monotypic, and have been collected on one or only a few species of native plants. About half of the endemic genera are known only from the Western Cape Province of South Africa, where they are closely associated with plants of the Fynbos Biome (Millar, 2002).

1.2.3 The mealybug genus *Planococcus*

Planococcus is not satisfactorily distinguished from other genera. The evolution of mealybugs apparently involved the loss rather than gain of characters in the adult females, making phylogenetic analysis based on females intractable. Studies on males would probably lead to a better understanding of relationships, but associated males are not available from most species. One of the consequences of this arbitrary distinction of mealybug genera is that some species of *Planococcus* may be more closely related (by descent) to species currently placed in other genera than they are to other species of *Planococcus* (Cox, 1989).

Several species-groups, apparently monophyletic, can be distinguished amongst this assemblage of species (Cox, 1989).

1.2.3.1 The *citri*-group

This group contains those species like *P. citri* and *P. ficus*, that have marginal multilocular disc pores on the abdominal venter, tubular ducts on the venter of all abdominal segments and on the head and thorax, and flagellate dorsal setae. This group contains the type-species of the genus. All but one species occur in the Mediterranean Basin or the Afrotropical Region, although *P. citri*, *P. ficus* and *P. halli* have been transported to other parts of the world (Cox, 1989).

1.2.3.2 The *dendrobi*-group

All the species in this group are rotund, have stout legs and have multilocular disc pores and tubular ducts confined to the posterior abdominal segments. Species of this group occur in the Oriental Region and the Afrotropical Region (Cox, 1989).

1.2.3.3 The *dorsospinosus*-group

This group comprises those species that lack marginal multilocular disc pores and have conical dorsal setae with associated aggregations of trilocular pores. These species occur in the Oriental and Austro-oriental Regions and Japan (Cox, 1989).

1.2.3.4 The *mali*-group

These species are characterised by having a short, stout, almost conical, dorsal setae and a marginal group of tubular ducts adjacent to the anterior spiracles, while these ducts are absent, or in very low numbers, on the margins of the head and mesothorax. These species are reported to occur in Japan, Australia, New Zealand and the Oriental Region (Cox, 1989).

1.2.3.5 Species with no affinity with any of the above groups

Two remaining species do not have an affinity with any of the above groups. These are *Planococcus boafoensis* from the Afrotropical Region, and *Planococcus lilacinus* from the Oriental Region (Cox, 1989).

1.3 The vine mealybug, *Planococcus ficus* (Signoret)

1.3.1 The history of the vine mealybug in South Africa

The vine mealybug was first reported in South Africa in 1914 by De Charmoy who referred to it as *Pseudococcus vitis* (De Lotto, 1975). However, it is generally believed that the mealybug that was a pest on vines in the Cape up to the mid-1930s was *Pseudococcus obscurus*. Since the 1930s it has been reported that a different species, assumed to be *Planococcus citri*, the citrus mealybug, displaced *Pseudococcus obscurus* on vines (Annecke & Moran, 1982; Walton, 2003a). Only in 1975, after a survey done in the southwestern Cape, was it shown that the citrus mealybug was in fact rare on vines, and that the pest species on grapes is *Planococcus ficus*, a species that very likely occurs around the world wherever vines are grown (De Lotto, 1975; Annecke & Moran, 1982).

1.3.2 Identification of the vine mealybug, *Planococcus ficus* (Signoret)

Mealybug taxonomy is based mainly on the morphology of the adult female, since this stage is readily found on host plants. Adult males are seldom collected unless reared from immature stages or caught in sticky traps. As a result, relatively few have been described. The larvae of most mealybug species are also poorly known, for reasons such as the large number of descriptions required to cover all the immature stages (Millar, 2002). A few publications like those of Millar (2002) and Cox (1989) contain keys to the *Planococcus* and other genera of the Pseudococcidae, but to follow these, specimens have to be prepared and mounted on microscope slides according to specific procedures in order to observe the minute morphological features (Millar, 2002). The characters that are described below are used for field diagnostics only.

The adult female *Planococcus ficus* (Figure 1.1) is small with the body length about 4 mm,

the width slightly more than 2 mm and it is about 1.5 mm thick (Kriegler, 1954 in Walton, 2003a). The body is oval, segmented and slate-grey to pinkish, covered in a fine waxy layer, with a fringe of waxy, hair-like extensions around the body (Annecke & Moran, 1982; Picker *et al.*, 2002).



Figure 1.1. Vine mealybug male and female, with eggsac visible (indicated with the arrow) to the right of the female (UOCCE, 2003).

The wax becomes more abundant as the female ages, except on the longitudinal midline where it remains sparse, leaving the midline rather conspicuous and a little darker in colour than the rest of the upper surface of the body (Annecke & Moran, 1982). Females of most species are sessile (Hinkens *et al.*, 2001), but retain legs and antennae, and are capable of limited locomotion until egg development (Picker *et al.*, 2002).

The adult male (Figure 1.1) is about one mm in length (Annecke & Moran, 1982), a bit smaller than adult thrips and amber-brown in colour. It has a large, egg-shaped thorax with a

narrower abdomen, a single pair of wings with no noticeable veination and long antennae, prominent eyes which appear red to black (Haviland, 2003; Daane *et al.*, 2004b) and long filamentous anal setae and no mouthparts (Kriegler, 1954 in Walton, 2003a). It is also short-lived (Millar, 2002).

The vine mealybug closely resembles the citrus mealybug (*P. citri*) (Annecke & Moran, 1982; Daane *et al.*, 2004b) and living female individuals can be distinguished only by the number of short waxy filaments around the edge of the body: there are seventeen on each side of *P. ficus*, and eighteen in *P. citri* (Annecke & Moran, 1982). The vine mealybug is easily distinguished from other mealybugs during the nymphal and adult female stages. The males are indistinguishable from the widespread citrus mealybug (Plant Health Report, 2003).

1.3.3 General biology and life cycle

The vine mealybug exploits its host plant most successfully. In the Western Cape, during the winter when the vines are leafless and dormant, colonies of mealybug are abundant on the stems in sheltered spots beneath the bark (Berlinger, 1977; Annecke & Moran, 1982; Walton & Pringle, 2004a). All life stages can also be found to overwinter in the root system below the soil surface (Walton, 2003a; Hashim, 2003) (Figure 1.2). This is in contrast to the other mealybug species, which are only found on the above ground portions of the vines (Bettiga, 2002). Its underground habit provides it with an excellent refuge from parasitoids and contact insecticides (Berlinger, 1977; Bettiga, 2002; Daane *et al.*, 2002b; Hashim, 2003). As temperatures increase during spring (starting in September) the mealybugs move upward into the growing vine. This activity reaches a peak in November or December. This upward migration may continue throughout summer, whilst a residue of young-producing females remains on the stems (Annecke & Moran, 1982). In South Africa, the population numbers peak during summer (Walton, 2003a; Walton & Pringle, 2004a). A successional trend of

mealybug colonisation can be observed between different positions on vines. Vine mealybugs colonise new growth early in the season, followed by the leaves and eventually the bunches, towards the end of the season. High stem infestations early in the season normally result in high bunch infestation levels at harvest (Walton, 2003a; Walton & Pringle, 2004a). In the Mediterranean region and in California a smaller population increase is observed during autumn (Berlinger, 1977; Daane *et al.*, 2004b) while the population numbers are very low during winter (Berlinger, 1977; Walton, 2003a; Daane *et al.*, 2004b; Walton & Pringle, 2004a).



Figure 1.2. Vine mealybug females on root of vine (UOCCE, 2003).

Individual male flights are quite apparent during the early growth season. Numbers of males typically increase until harvest-time and then decline, as the *P. ficus* densities naturally decrease with vine senescence and late-season natural enemy activity (Walton *et al.*, 2004). The same tendency as in Western Cape vineyards was observed in studies in Californian vineyards (Daane *et al.*, 2002b).

Mealybug populations are usually constituted by individuals of different life stages and ages (Figure 1.3) (Annecke & Moran, 1982). The mature female is sessile and emits a sex pheromone to attract flying males (Hinkens *et al.*, 2001). The female lays eggs that hatch to first-instar nymphs (Figure 1.4).



Figure 1.3. Overlapping generations (crawlers, nymphs and adults) of Vine mealybug on a grapevine stem (Daane *et al.*, 2004a).

In the female, there are two moults. After the second one, the female is fertilised by the adult male. The female then passes through a protracted pre-oviposition period that is a feature of many mealybugs and scale insects. The female then begins to lay eggs in a sac of loosely woven wax threads (Annecke & Moran, 1982; Walton, 2003a). Crawlers hatch after 7-10 days at a mean temperature of 25°C (Walton, 2003b). Each female may produce up to 750 eggs (Annecke & Moran, 1982).

The first nymphal stage (crawler) (Figure 1.4), unlike the other nymphal stages, has well-developed legs and antennae. Crawlers are the primary means of mealybug dispersal since they move from one spot to another on the plant and they are easily spread by wind (as well as on farm equipment) (Ohmart, 2002; Bentley *et al.*, 2002).



Figure 1.4. Vine mealybug females deposit their eggs in ovisacs. The small orange crawlers, or first-instar nymphs, leave the ovisac and begin to feed (Godfrey *et al.*, 2005).

The life cycle of the male differs from that of the female in that there are four moults that separate the five nymphal instars. At the end of the second instar, the male insect begins to spin a small cocoon, about 1 mm in length, and the last three moults take place inside this cocoon. Feeding ceases during the second instar. The small winged adult male, hardly more than 1 mm in length, stays in the cocoon for the first 1-4 days of its life, depending on the season, before emerging to mate. A single male fertilises up to ten or more females although the ratio of males to females in the vine mealybug populations is about 1:1 (Annecke & Moran, 1982). The male mealybug causes no damage, as it has no feeding mouthparts (Walton, 2003b).

The developmental stages of the vine mealybug with some characteristics of each stage are presented in Table 1.1.

Table 1.1. Life stages of *Planococcus ficus* (after Kriegler, 1954 in Walton, 2003a; Annecke & Moran, 1982).

Females	Males	Characteristics/Colour
Egg	Egg	Light straw
First nymphal instar	First nymphal instar	Light to dark yellow, six antennal segments
Second nymphal instar	Second nymphal instar	Yellowish brown
Third nymphal instar	Third nymphal instar	Seven antennal segments
	Prepupa	One pair of lateral ocelli Visible wingbuds
	Pupa	Three pairs of lateral ocelli Wingbuds reaching to third abdominal segment
	Adult male	Wings fully developed
Immature female		
Adult female		Wingless Eight antennal segments

A single generation, from the hatching of the first eggs of one generation to that of the following, develops over a period of approximately one month in summer and up to four months during the period June to October (Annecke & Moran, 1982). Thus, it is possible to visualise six generations each year (Annecke & Moran, 1982). In South African vineyards, *P. ficus*, has between three and four generations per year (Annecke & Moran, 1982; Walton *et al.*, 2004).

1.3.4 Developmental biology

Initially, the only information available on the developmental biology of mealybug was from Kriegler (1954, in Walton, 2003a) who did developmental studies on *P. ficus* at different temperatures. Walton (2003a) extended these studies to get an understanding of the effect of temperature on the rate of development of the pest. In his study, the developmental times, fecundity and fertility of the vine mealybug were determined at various temperatures between 18°C and 30°C.

Walton (2003a) found that the time for development from egg to oviposition of adult female mealybugs (egg to adult plus pre-oviposition period) decreased with increasing temperatures. It was also observed that fecundity was directly influenced by temperature and reached a maximum number of eggs per female between 20 to 25°C. This is similar to observations made by Kriegler (1954, in Walton, 2003a).

Walton (2003a) observed that the net reproduction rate (R_0) of *P. ficus* reached a maximum at 21°C and that R_0 was greater than zero at all temperatures tested, indicating positive population growth. The maximum intrinsic rate of natural increase (r_m) for *P. ficus* occurred at 25°C. The ratio of females to males declined at the extremes of the temperatures tested. The higher numbers of males at high and low temperatures could possibly be ascribed to higher stress levels. This phenomenon was previously reported by Castagnoli & Simoni (1991, in Walton, 2003a) and may produce greater genetic variability, which could in turn increase the probability of survival.

By using quadratic regression of $1/t$ on temperature for *P. ficus*, Walton (2003a) estimated the minimum and maximum threshold temperature for development of *P. ficus* to be 16.59°C and 35.61°C respectively, while the optimum temperature for development was 27.84°C.

1.3.5 Crop hosts

The vine mealybug is polyphagous and feeds on a wide variety of fruit and ornamental plants. These include grape, ornamental and commercial fig, pomegranate, avocado, date palm, apple, quince and dahlia. It has also been reported that this pest feeds on mango, oleander, bamboo, walnut, *Dichrostachys glomerata*, mesquite, *Tephrosia purpurea*, sycamore, jujube, willow, cacao, and styrax (Cox, 1989; Ben-Dov, 2001; Plant Health Report, 2003).

Some authors list citrus as one of the crops that the vine mealybug occurs on (Hinkens *et al.*, 2001; Plant Health Report, 2003), however De Lotto (1975) states, “while the citrus mealybug may attack vines, the vine mealybug apparently does not thrive on citrus”. The vine mealybug is however a key pest in South African vineyards (*Vitis vinifera* L.) (Walton *et al.*, 2004).

1.3.6 Geographical distribution

Although the area of origin of the vine mealybug is uncertain (Anneck & Moran, 1982), it probably originated in the Mediterranean region (Blumberg *et al.*, 1995). The vine mealybug has a large distribution throughout the world (Figure 1.5). It has been recorded in Southern Europe, the Middle East, and parts of North Africa, South Africa, South America and North America (Ben-Dov, 2001; Millar *et al.*, 2002; Watson & Kubiriba, 2005).

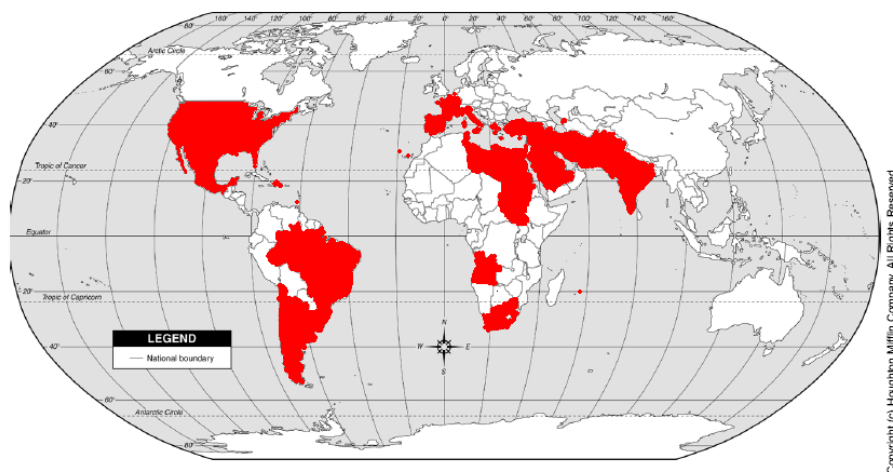


Figure 1.5. Global distribution of the vine mealybug. (Compiled from Ben-Dov, 2001; Millar *et al.*, 2002; Daane *et al.*, 2002a, 2004b; Kaydan *et al.*, 2004; Watson & Kubiriba, 2005.)

1.4 Economic importance of the vine mealybug

1.4.1 Damage potential

This pest has the potential to cause severe crop damage and loss due to the heavy production of honeydew, which also serves as a substrate for black sooty mould growth. High population densities can result in a loss of vine vigour. In grape producing areas where this insect has become established, total crop loss is possible if insecticide treatments are not applied (Bettiga, 2002, 2003; Plant Health Report, 2003). Damage has more specifically been associated with fruit and flower drop, wilting or general debilitation of the plant e.g. desiccation in the case of wine grapes; and, mainly, with plant and fruit appearance e.g. in the case of table grapes – because of the secretion of large amounts of honeydew on which sooty mould develops (Blumberg *et al.*, 1995; Walton & Pringle, 2004b). In southern California, severe vine mealybug infestations have also been reported to reduce vine growth and resulted in defoliation, bunch rots and even spur and cane death (Daane *et al.*, 2004b) (Figure 1.6).

In addition to the obvious damage, the vine mealybug as well as the other species of mealybugs is capable of transmitting grapevine leafroll viruses (Engelbrecht & Kasdorf, 1990), corky bark disease (Tanne *et al.*, 1989) and Shiraz disease (Walton, 2003a) between plants.



Figure 1.6. Damage to a grapevine due to vine mealybug infestation (Godfrey *et al.*, 2005).

Several factors make vine mealybug much more damaging and difficult to control than other mealybug species. Firstly, the vine mealybug reproduces at a higher rate than other species, enabling small numbers of mealybugs to reach damaging levels within one season. Females can each deposit up to 700 eggs (average is approximately 300). Vine mealybug has four to seven generations per year compared with two for the grape mealybug, *Pseudococcus maritimus* (Bettiga, 2002; Millar *et al.*, 2002; Daane *et al.*, 2004b). This greatly increases the population size, and it leads to overlapping generations (Figure 1.3). The overlap in generations complicates chemical control actions, since some insecticides are effective only against the nymphal stages.

Secondly, vine mealybug excretes much more honeydew than other species. This honeydew can cover leaves, canes, trunks and fruit, making entire clusters and vines a sticky mess (Figure 1.7).



Figure 1.7. The honeydew produced when vine mealybugs feed inside or above a cluster will cover the berries. Sooty mould grows easily on the sugary honeydew (Godfrey *et al.*, 2005).

The honeydew often becomes so thick it resembles soft candle wax. Fruit from heavily infested vines is not suitable for harvest. The stickiness of all the plant parts also facilitates spread of vine mealybug from vineyard to vineyard on equipment and workers' clothes (Millar *et al.*, 2002; Daane *et al.*, 2004b).

Thirdly, vine mealybug can feed on all parts of the vine throughout the year. It can be found on leaves (Figure 1.8), in clusters (Figure 1.9), under the bark, and even on the roots of grapevines (Figure 1.2). The occurrence of vine mealybug under bark or on the roots, provides it with protection from most foliar insecticides, from high summer temperatures, and from parasitoids and other natural enemies (Bettiga, 2002; Daane *et al.*, 2004b).



Figure 1.8 Vine mealybug adults and egg sacs on a leaf (UOCCE, 2003).



Figure 1.9 Vine mealybugs also infest clusters (Godfrey *et al.*, 2005).

Fourthly, the vine mealybug being exotic to California and South Africa has fewer natural enemies in the USA or South Africa than other mealybug species may have. Established populations will require repeated insecticide treatments to keep them at manageable levels (Daane *et al.*, 2004b).

Finally, vine mealybug has a wide host range. It may feed on subtropical (grapes, figs, apples) and tropical (dates, bananas, avocados, and mangos) crops as well as a number of common weeds, such as malva, burclover, black nightshade, sowthistle, and lambsquarter. However, in California and South Africa, grapevines appear to be its preferred host throughout the season (Daane *et al.*, 2004b; Millar *et al.*, 2002; Walton, 2003a).

1.4.2 Damage symptoms

There are a few signs that can be indicative of a vine mealybug infestation:

Ants travelling on drip irrigation wires and training wires, and/or streaming up and down the

trunk of the vine are frequently the first sign growers see of the infestation. The roots of certain weeds, like Common blackjack (*Bidens pilosa*), Khaki weed (*Tagetes minuta*) and Small mallow (*Malva parviflora*) may be inspected for the presence of mealybug. These observations may serve as early warning signs of potential infestations (Duncan, 2003; Napa, 2004; Walton, 2003b).

White cottony masses can be found on the trunk and cordons (Figure 1.10), especially in cracks in the bark, and can be showing up on fruit clusters and leaves (Figures 1.8 and 1.9) as the season progresses (Daane *et al.*, 2004b; Napa, 2004; Walton, 2003b).



Figure 1.10 Vine mealybugs can be found on the woody parts of the grapevine, including the trunk and cordon (Godfrey *et al.*, 2005)



Figure 1.11 A water-soaked look on the trunk and cordon (UOCCE, 2003).

Large drips and deposits of sticky honeydew can be found on the fruit clusters, accompanied by white residues of mealybugs and egg masses (Bentley *et al.*, 2002; Napa, 2004). A water-soaked look on the trunk (Figure 1.11) and black sooty-mould on the leaves can also be indicative of vine mealybug infestations (Daane *et al.*, 2004b; Napa, 2004).

1.5 Control measures

1.5.1 Chemical control

The vine mealybug can be found on all parts of the vine including the root system. This is different from the other mealybug species, which are only found on the above ground portions of the vines. A proportion of the population is always located on the root system making it more difficult to control and less susceptible to natural enemies. These root-living populations, in addition to those that live under the bark, make control with contact insecticide sprays difficult (Bettiga, 2002). Furthermore, mealybugs are covered with a protective wax secretion (Millar *et al.*, 2002). Sprays must be timed carefully to minimise disruption of mealybug natural enemies (Walton & Pringle, 1999) and indirectly, outbreaks of secondary pests such as mites. In addition, regulatory restrictions may limit continued use of the organophosphate insecticides that historically have been used for mealybug control (Millar *et al.*, 2002).

There is a formidable array of different agricultural chemicals registered for use against pests and diseases of vines in South Africa (Annecke & Moran, 1982). For the mealybugs on grapes alone, chemicals with eight different active ingredient components are being used. These active ingredient components have been taken up in a wide range of products (Nel *et al.*, 2002).

In South African vineyards a spraying regime in three stages, i.e. the dormant period before bud break, during the growing season and post-harvest is proposed. Dormancy spraying (after leaf drop and before budding) is recommended to protect natural enemies as much as possible (Walton, 2003b). Routine dormant spraying however should be avoided (Walton, 2003b). One or more applications of long-residual organophosphates e.g. chlorpyrifos is often applied (Walton *et al.*, 2004). After budding, there are problems with phytotoxicity on

young shoots and leaves (Walton, 2003b). During the growing season spraying with a short-residual organophosphate e.g. mevinphos is suggested (Walton *et al.*, 2004).

Post-harvest spraying is not recommended, since this is the period when the populations of natural enemies, which are a lot more susceptible to insecticides than mealybug, are at their highest. Spraying at this stage interferes with biological control for the next season. Post-harvest sprays are recommended only if monitoring records indicate that the infestation early in the season did not exceed 2% and that the outbreak really only occurred later. Spot applications are recommended, unless monitoring indicates that the infestation is so widespread throughout the block that spot sprays are not feasible (Walton, 2003b).

It seems that resistance to organophosphate and carbamate chemical sprays is already building up and research on this topic is being conducted in South Africa (De Wet & Moores, 2003).

1.5.2 Biological control

Many natural enemies associated with *Planococcus ficus* have been reported. These include the following parasitoids: one species in the Diptera (Chamameyidae), and at least eleven species in the Hymenoptera (Encyrtidae). Predators include at least three species in the Neuroptera (Chrysopidae) and at least nine species in the Coleoptera (Coccinellidae) (Annecke & Moran, 1982; Ben-Dov, 2001; Berlinger, 1977; Walton, 2003a;). Some of these species are hyperparasitoids. Many of these species occur in the Western Cape Province of South Africa (Walton, 2003a). Investigating available literature, Walton (2003a) found the dominant parasitoids recorded to be *Anagyrus* spp. (Figure 1.12), *Coccidoxenoides peregrinus* (Figure 1.13) and *Leptomastix dactylopii* (Figure 1.14), all three in the Hymenoptera (Encyrtidae). The dominant predators were *Nephus bineavatus*, *Nephus*

angustus and *Nephus quadrivittatus*, all three in the Coleoptera (Coccinellidae).



Figure 1.12 *Anagyrus pseudococci* female searching for vine mealybug (Godfrey *et al.*, 2005).



Figure 1.13 *Coccidoxenoides perminutus* (NHM, 2003). Formerly known as *C. peregrinus* (Davies *et al.*, 2004).

In a study done of the natural enemies of the vine mealybug in vineyards of the Western Cape of South Africa, Walton and Pringle (2004a) found that predatory beetles did not play a significant role in the biological control of the vine mealybug. The hymenopteran parasitoids, *Anagyrus* spp. (Figure 1.12) and *C. peregrinus* (Figure 1.13), however, played a major role in the biological control of the vine mealybug. Biological control was however not very effective as suppression of mealybug was only achieved towards the end of the season when damage to the crop had already been done (Walton & Pringle, 2004a).

Since recently *Cryptolaemus montrouzieri*, a Coccinellid beetle is also being used in Western Cape vineyards in biological control against *P. ficus* (Geldenhuys, 2004) (Figure 1.15).

Under optimal conditions, biological control can suppress mealybug populations to infestation levels below 1% of infested vines. Biological control can be enhanced by creating optimal

conditions for natural enemies (Walton, 2003b). The efficiency of the natural enemies in keeping the vine mealybug below economically injurious levels depends on two factors. One, patrolling ants, which collect the honeydew and effectively protect the mealybugs from natural enemies (Annecke & Moran, 1982; Daane *et al.*, 2002b); and two, the use of pesticides, some of which are particularly harmful to predators and parasitoids (Annecke & Moran, 1982). In addition, beneficial insects can also be encouraged by planting a cover crop that flowers early in the season (many natural enemies use pollen as an alternative food source) (Walton, 2003b).

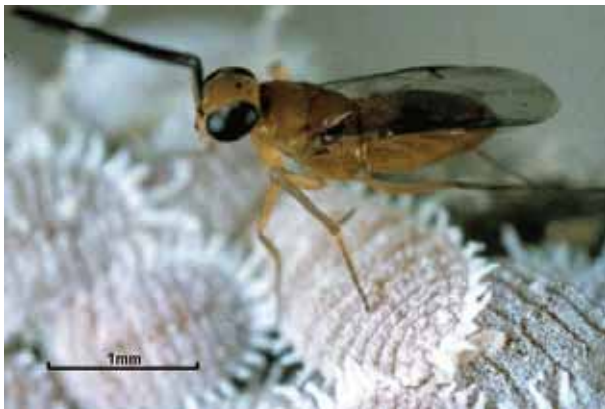


Figure 1.14 *Leptomastix dactylopii* female on mealybug host *Planococcus citri* (NHM, 2003).



Figure 1.15 *Cryptolaemus montrouzieri* larva (right) and adult (left) feeding on vine mealybugs (Godfrey *et al.*, 2005).

Biological control by means of augmentative releases of commercially available natural enemies can be applied if mealybug population numbers are low enough (infestation level <2%) (Walton, 2003b). While predators and parasitoids may help reduce the overall number of vine mealybugs, they alone will not provide sufficient control to keep populations below damaging levels (Daane *et al.*, 2004b).

1.5.3 Cultural control

Vine mealybug like the other mealybugs can be transported by vineyard equipment or people that are exposed to infested vines. The adult female mealybug and the immature stages are flightless and flight is therefore not a mechanism for spread. During harvest, mechanical harvesters, picking crews and a variety of harvesting equipment can transport mealybugs from infested to non-infested vineyards. Sanitation of all equipment leaving known mealybug infested vineyards will help prevent further spread (Bettiga, 2002). Sanitation practices include scheduling crews (e.g., irrigators, pruners, pickers, etc.) such that once they work in an infested vineyard they are finished for the day; destructing the prunings from an infested vineyard by shredding and mulching and then treated with a chemical like Lorsban; steam cleaning equipment that has contact with the infested vineyard; and hand harvesting grapes from infested vineyards instead of mechanical harvesters (Plant Health Report, 2003).

Vine mealybug can also be spread by infected nursery stock since adult females and nymphs may occur under the bark or on the roots of dormant plants or on the leaves of green growing plants. This is believed to be the method of spread in the north coast sites in California, USA. Insecticide treatments or hot water dips may be appropriate treatments for infested planting material from areas where vine mealybug is known to exist (Bettiga, 2002).

Mealybugs can survive on any live grapevine material on or under the soil surface. Where new vines are established on old vineyard soil, all live old vines and roots must be removed so that no mealybugs can survive. Old vines should be treated with herbicide directly after the last harvest to kill all roots. At least 6-8 weeks should be allowed after herbicide application before vines are removed and only certified planting material should be used when establishing new vineyards (Winetech, 2004).

1.5.4 Ant control

Biological control of the vine mealybug by predatory beetles and parasitic wasps is significantly reduced in the presence of ants (Addison & Samways, 2000). Honeydew excreted by the vine mealybug contains four sugars (sucrose, glucose, fructose and raffinose), sixteen free amino acids and three organic acids (citric, tartaric and oxalic acid) (Saleh & Salama, 1971). Ants feed on this sweet honeydew (Figure 1.16) excreted by mealybugs and thereby disturb the natural enemies as they attempt to feed on the mealybugs. In this way, the ants gain an easily accessible food source and, in turn, the mealybugs gain protection from predators and are able to reach very high numbers (Addison & Samways, 2000).



Figure 1.16. Ants tending vine mealybugs to obtain honeydew on which they feed (Godfrey *et al.*, 2005).

Some ants nest in the vines, while others nest on the ground. Direct chemical stem barriers are not effective against vine-nesting ants such as the cocktail ant *Crematogaster peringueyi* (Addison & Samways, 2000). All ants are beneficial if they remain on the ground, as they are

predacious and feed on other pests such as the pupae of fruit flies and false codling moth (Addison & Samways, 2000). Ants also physically move young mealybugs to desirable feeding areas of the vine in order to collect mealybug honeydew (Raisin Grapes, 1999).

A very effective method for controlling ground-nesting ants, snout-beetles and other crawling insects is stem banding (Figure 1.17), as they are still left to prey on other pests, but are not permitted access into the vine canopy (Addison & Samways, 2000).



Figure 1.17. Stem banding with a sticky substance prevents ground-nesting ants, snout-beetles and other crawling insects from accessing the vine canopy (Andrag, 2004; Photo: M.J. Kotze).

Currently, the only two chemicals available for controlling ants in vineyards are alpha-cypermethrin and chlorpyrifos (that can also be used against vine mealybugs). Alpha-cypermethrin is used on trellised vines only (Nel *et al.*, 2002). The spread of mealybugs can be reduced if ant populations are controlled (Raisin Grapes, 1999).

1.5.5 Weed control

A correlation has been observed between the occurrence of several weed species and mealybug infestation in vines. The following weeds found in South Africa may serve as hosts for mealybug: Common blackjack (*Bidens pilosa*), Khaki weed (*Tagetes minuta*), Small mallow (*Malva parviflora*), Flax-leaf fleabane (*Conyza bonariensis*), Black nightshade (*Solanum nigrum*), Thorn-apple (*Datura stramonium*), Sowthistle (*Sonchus oleraceus*) (Figure 1.18), Musk Herons Bill (*Erodium moschantum*) and White goosefoot (*Chenopodium album*) (Walton, 2003b).

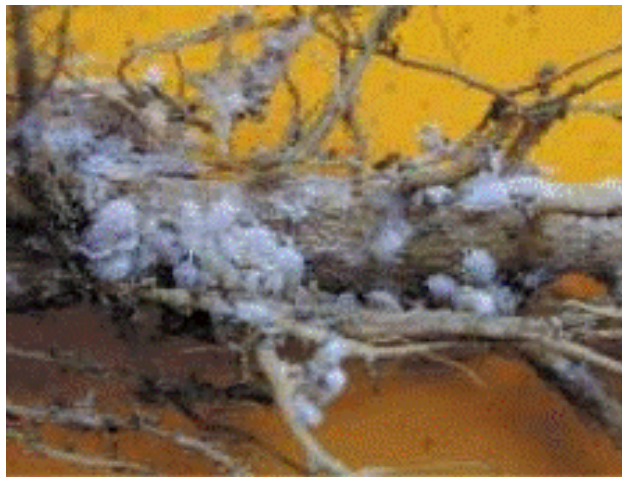


Figure 1.18. Mealybugs of an unidentified species on the roots of thistle (*Sonchus oleraceus*) (Walton, 2003b).

The most effective method of control is the planting of long flowering cover crops as recommended by Fourie *et al.* (in Walton, 2003b). Long flowering cover crops that do not host mealybug may reduce ant problems, may help to reduce the forming of dust, may serve as supplementary nutrition for natural enemies and may bind nitrogen. Weeds may also be controlled physically (shrub beaters) and chemically (herbicides) (Walton, 2003b). Weeds have to be controlled from early in the season, since they act as access routes to the vine for ants and do not contribute much to the quality of the soil. Ant control is impossible if weeds

grow into the vines (Walton, 2003b).

1.5.6 Legislative control

It is not clear what legislative regulation exists in countries including South Africa against the vine mealybug.

The California Department of Food and Agriculture has classified the vine mealybug as a category “B” pest which means that the pest is of quarantine significance and that it should be regulated at the discretion of the County Agricultural Commissioner (Bettiga, 2002). Subsequently the vine mealybug obtained full quarantine status in American states like the State of Washington (WASHINGTON, 2006).

1.6 Sampling and monitoring

Effective control of mealybug infestations in vineyards is complicated by several factors. Until recently, there were no simple and effective methods to monitor most mealybug species (Geiger & Daane, 2001). Monitoring methods consisted of time-consuming and often laborious examination of plant material for the presence of live mealybugs (Millar *et al.*, 2002; Walton *et al.*, 2004). In some vineyards, detection of honeydew and sooty mould or monitoring ant species that tend mealybugs may be a useful adjunct to direct sampling (Millar *et al.*, 2002). Because *P. ficus* has a clumped distribution and a cryptic lifestyle during much of the year, these visual monitoring methods are most effective during late summer when mealybugs are located in exposed locations and have higher densities. Unfortunately, this period and these conditions are often encountered after crop damage has already occurred and it is too late to apply control measures (Millar *et al.*, 2002; Walton *et al.*, 2004).

In general, pheromones and other semiochemicals however have provided tremendous return on the investment of identification and development, by giving researchers, consultants and growers access to cost-effective monitoring systems. The monitoring systems (and the knowledge that they have helped produce) have enabled more effective targeting of all major control tactics including pesticides, biopesticides, cultural control, and biotechnical control methods such as mating disruption or sterile male releases against many pest species (Suckling, 2000).

1.6.1 Visual sampling

Walton (2003a) developed a sampling system with known levels of error for estimating *P. ficus* population levels in commercial vineyards, enabling producers to decide on the necessity for and correct timing of interventions.

The technique is to select 20 evenly spaced plots each consisting of five vines per hectare; thus, a total number of 100 selected vines per hectare. Each of the vines, especially the new growth, is then inspected. The presence or absence of mealybug females (crawlers, nymphs and/or adult females) is noted. Even if there is only one female found on a vine, the vine is still noted as infested. The total number of infested vines out of the 100 indicates the estimated percentage mealybug infestation for that block or unit of a block. For example, if six vines out of the 100 are infested, the estimated percentage infestation for that hectare or unit of block is 6% (Walton & Pringle, 2004a; Winetech, 2004).

Walton (2003a) determined a 2% stem infestation level as the action threshold at which control intervention should be applied for *P. ficus* in South African vineyards. Stem infestation precedes bunch infestation, which facilitates planning of interventions such as parasitoid releases.

1.6.2 Semiochemical monitoring

The low tolerance for *P. ficus* in grapes and the importance of timely insecticide applications necessitate the use of a species-specific monitoring program that can quickly determine pest presence and density (Walton *et al.*, 2004). Even though an effective sampling method has been developed, visual sampling still remains a labour intensive process that consists of time-consuming examination of many individual vines (Geiger & Daane, 2001; Walton *et al.*, 2004).

A sex pheromone was first detected in Coccoidea in the red pine scale *Matsucoccus resinosae* (Bean & Godwin) (Homoptera: Matsucoccidae). Pheromones are probably present throughout the Coccoidea (Miller & Kosztarab, 1979). In 1975, Rotundo & Tremblay (in Miller & Kosztarab, 1979) reported on the existence of a sex pheromone of the *P. ficus*. Gravitz and Willson (in Miller & Kosztarab, 1979) reported on the sex pheromone of the *P. citri* in 1968. The sex pheromones appear to be specific and males can successfully discriminate between closely related species such as *Aonidiella auranti* and *A. citrina*, and *P. citri* and *P. ficus* (Miller & Kosztarab, 1979).

Bierl-Leonhardt *et al.* (1981) identified the female *P. citri* sex pheromone as (+)-(1*R*)-*cis*-2,2-dimethyl-3-isopropenylcyclobutanemethanol acetate. Dunkelblum *et al.* (2002) amongst others have successfully synthesized and tested the *P. citri* sex pheromone. Hinkens *et al.* (2001) reported on the chemical structure of the *P. ficus* sex pheromone and identified it as a single-component pheromone, the monoterpene ester, lavandulyl senecioate. The synthetic pheromone was developed and tested as a monitoring tool for *P. ficus* in Californian vineyards by Millar *et al.* (2002) and in South African vineyards by Walton *et al.* (2004).

The male vine mealybug responds well to the racemic lavandulyl senecioate (Hinkens *et al.*,

2001), and for practical purposes this is advantageous because the racemic compound can be produced economically in one step from commercially available intermediates (Hinkens *et al.*, 2001; Millar *et al.*, 2002). Thus, the availability or cost of the pheromone should not present a barrier to commercialisation of pheromone products for use in integrated pest management (IPM) programs (Millar *et al.*, 2002).

Because the pheromone is species-specific, no taxonomic expertise would be required to determine whether the trapped insects were vine mealybug, grape mealybug, or other unrelated species, which directly impacts on control decisions. This factor may be especially important in areas where vine mealybug is sympatric with a morphologically similar species, the citrus mealybug, *P. citri* (Millar *et al.*, 2002).

1.6.3 A second component in the vine mealybug sex pheromone

In studies done in Israel, Zada *et al.* (2003) detected a second pheromonal component in airborne collections from vine mealybug that was mass-reared on potato-sprouts. As reported above, the first component is (S)-lavandulyl senecioate (I); the second component is (S)-lavandulyl isovalerate (II). Compounds I and II displayed similar biological activities in laboratory assays, but with feral populations in the vineyard, only compound I attracted males. It was found that first generation laboratory raised females, only produce compound I and that first-generation laboratory raised males only respond to compound I. The amount of compound II increased gradually in the subsequent generations. It was suggested that rearing the vine mealybug in the artificial environment on potato sprouts had induced this change. Preliminary results in further studies by Zada *et al.* (2003) indicated that compound II was inhibitory to wild males. The addition of compound II to compound I significantly reduced trap catches of *P. ficus* males in vineyards.

1.6.4 Pheromone monitoring and population density

In an ideal monitoring trap, catch will always be directly proportional to the surrounding population, so that the catch provides a useful estimate of insect density. Lures need to be designed to attract insects in a predictable fashion, even if they occur at low population densities (Suckling, 2000).

Millar *et al.* (2002) in their development and optimisation of methods for monitoring the vine mealybug in Californian vineyards included a study to compare sex pheromone trap catches at different population densities in vineyards. They used the five-minute count sampling method (Geiger & Daane, 2001) in which all mealybug stages occurring anywhere on the vine (trunk, cordon, canes, leaves and fruit) were counted over a period of five minutes. They searched vine sections that showed indications of mealybug presence (e.g. ant activity, honeydew). Their results showed a significantly positive correlation between pheromone-baited trap catches and visual sampling methods for mealybug density. They did however not suggest or determine an economic injury level.

Walton *et al.* (2004) conducted a similar study in South African vineyards using a delta trap (Figure 1.19). The visual stem infestation monitoring method used was based on a methodology developed by Walton (2003a) and is explained elsewhere in this chapter. That study determined the economic action threshold to be at a 2% stem infestation level. They showed that stem infestation based on this sampling method was significantly correlated to trap counts. However, correlating trap catches to stem infestation levels by means of regression did not always provide results consistent with that of field studies.



Figure 1.19. Delta trap with pheromone lure baited with 0.01 mg lavandulyl senecioate, suspended from the roof of the trap (Photo: M.J. Kotze).

Possible reasons for this could be that the pheromone-baited traps were found to be more sensitive than visual monitoring procedures e.g. adult male *P. ficus* were trapped when there were few or no mealybugs found using the visual sampling method; and individual traps in the same block provided different ratios of male *P. ficus* caught, compared to stem infestation in that block. They have therefore started field studies to verify the suggested economic action threshold and to determine if variation in trap catches can be reduced by using a different trap design or placement. For the interim, because of the great variations found in individual trap counts, they suggested the use of more than one trap per vineyard block.

Subsequent research indicated an action threshold value of 65 vine mealybug males per trap over a period of two weeks. This number is accepted to represent a 2% female mealybug infestation on vines. The decision whether to control or not is based on the following decision tree (Winetech, 2004):

IF a trap count is below 65 males per trap over a period of two weeks, THEN

No control is required.

IF a trap count is more than 65 males per trap over a period of two weeks, THEN

Do physical monitoring (vine inspection)

If infestation exceeds 2%, THEN

Control should be applied in that part of the block.

If infestation in the block is less than 2% AND

There is a spot with heavily infested vines, THEN

A spot treatment can be applied to prevent infestation from spreading further.

IF a trap registers high counts (45-64 per trap) twice in a row, THEN

Do physical monitoring (vine inspection)

If infestation exceeds 2%, THEN

Control should be applied.

1.7 Principal objective

This dissertation describes the evaluation of the validity of using sex pheromone monitoring systems as tools in integrated management of the vine mealybug, *Planococcus ficus* (Signoret), which is a key pest of grapes (*Vitis vinefera*) across the world, and also particularly in South Africa (Walton, 2003a). The evaluation was done on the basis of three focuses: one, comparing an experimental pheromone lure formulation against a commercially available pheromone lure formulation; two, evaluating alternative trap designs against the commonly accepted norm of the delta trap design; and three, determining the relationship between male trap catches and selected climatic factors.

1.8 Objectives

The first objective of this study was to evaluate and compare different pheromone lure formulations for monitoring of vine mealybug. The effectiveness of an experimental pheromone lure formulation was compared with a commercially available standard pheromone lure formulation.

It was expected that the effectiveness of the experimental pheromone lure formulation would not differ from that of the commercially available pheromone lure formulation. The availability of another pheromone lure formulation will contribute to healthy economic competition in the cost management aspect of vine mealybug control options.

The second objective was to compare the effectiveness of the yellow delta trap to the yellow scale card sticky trap and the white scale card sticky trap; and also to investigate factors relating to trap design that could contribute to increased trap efficacy. Currently, it is widely accepted that semiochemical monitoring of the vine mealybug using the delta trap correlates best with population estimates as ascertained by visual stem infestation sampling methods in the vineyards (Millar *et al.*, 2002). In South Africa, Winetech (2004) (the Wine Industry Network of Expertise and Technology) recommends the use of delta traps with the pheromone lure.

It was expected that two trap types being evaluated against the delta trap would be as effective for monitoring vine mealybug male flight patterns as the yellow delta trap. With these hypotheses proven, cost (of the trap and of operating the trap) and ease of operation will be the driving factors in deciding which trap to use.

The third objective was to determine the relationship between male trap catches and the climatic variables of temperature, relative humidity and rainfall. Analysis of the temperature data was extended to determine the relationship between male trap catches and degree days.

1.9 Chapter arrangement

The materials and methods section is provided as the second chapter of the dissertation. Although it is customary to include materials and methods used in the particular chapter of the dissertation pertaining to a particular objective of the study, it was decided to extract and combine the information that is relevant to the first two objectives of the study in one chapter.

Chapter 3 deals with the first objective of the project, namely to compare the effectiveness of the experimental vine mealybug pheromone lure formulation with the commercially available formulation.

Chapter 4 deals with the second objective of the project, namely to compare two alternative trap designs (white scale card and yellow scale card), with the trap design commonly used in the industry.

The possible effect of climatic factors like temperature, relative humidity and rainfall (the third objective of the study) could play an important role in the effective monitoring of vine mealybug with pheromone lures. This topic is addressed in Chapter 5.

As the project data was being accumulated it became evident that the two alternative trap designs (yellow and white scale cards) potentially would not be as effective as the commonly

used trap design. The reasons for that were being speculated about and it was decided to suggest a new trap design and test that in the field in an experiment additional to the main project as described in this dissertation. The materials and methods used in this experiment and the results obtained are discussed in Chapter 6.

The concluding chapter, Chapter 7, presents the evaluation of the validity of sex pheromone monitoring of the vine mealybug as a tool in the integrated management of the pest. It incorporates the findings of the project, and also makes recommendations towards further studies.

CHAPTER 2: MATERIALS AND METHODS

2.1. Introduction

Some of the techniques employed in the study are relevant to several aspects of this study and topics are reported on in more than one of the chapters. In addition, the same field sites have been used for all aspects of the work. To avoid repetition, this chapter describes the methods and study sites common to those chapters.

2.2. Field study sites

Studies were conducted in two commercial vineyards in the Western Cape, South Africa. The first site, Irene, is a table grape vineyard near Paarl (Figure 2.1).



Figure 2.1. Table grape vineyards on Irene (Photo: M.J. Kotze).

The experiments were conducted in five blocks in the vineyard containing Sunred Seedless, Waltham Cross, Regal Seedless, Victoria and Majestic grape varieties. The block sizes ranged from 1.3 to 2.2 ha. All the blocks had a history of *Planococcus ficus* infestation, and two of the blocks also had a history of *Pseudococcus longispinus* infestation. Intra-row spacing in the vineyards was 3.25 m and the length of each plot was 7.5 m. (A plot is the area between two trellis poles and usually contains five vines). Canes were supported by a 7-wire 'Y- trellis' system, and all blocks were drip irrigated. The ground was clean cultivated (Figure 2.2). No sprays were applied during the growing season as all blocks were prepared during the winter dormant season. In some of the blocks all the vines had been debarked from ground level to the cordon. Just below the split of the two arms, a band of a sticky substance had been applied to deter ants, snout beetles, and other crawling insects from accessing the canopy (Figure 1.17).



Figure 2.2. The table grape vineyard blocks were clean cultivated and drip irrigated. The “Y”-structure of the trellis system can also be seen (Photo: M.J. Kotze).

Although the sticky bands are largely effective, it has been observed that ants travel in cracks in the support poles, and from there manoeuvre to where they find vine mealybug females to tend to.

The second study site, Hartenberg, is a wine grape vineyard near Stellenbosch (Figure 2.3). The experiments were conducted in three blocks in the vineyard containing Merlot (six years old), Cabernet Sauvignon (six years old) and Chardonnay (20 years old) grape varieties. The block sizes ranged from 3.75 to 4.7 ha. All the blocks had a history of *Planococcus ficus* infestation.



Figure 2.3. Wine grape vineyards at Hartenberg (Photo: M.J. Kotze).

Intra-row spacing was 2.5 m and the length of each plot was 7 m. Canes were supported by a 5-wire vertical trellis system, and all blocks were drip irrigated. In some of the blocks cover crop were grown (Figure 2.4). All blocks were prepared during the winter dormant season,

and spot treatments with Chlorpyrifos were applied during the growing season. This was done at low pressure on those vines where vine mealybug activity was detected.



Figure 2.4. All blocks were drip irrigated and cover crops were planted in some of the blocks. The vertical structure of the trellis system can also be seen (Photo: M.J. Kotze).

2.3. Experimental design

The aim of this study was to compare two different pheromone formulations as well as the efficacy of different trap designs using one of the pheromone formulations. The delta trap is the recommended trap design for vine mealybug monitoring with pheromones (Chempack, 2002; Daane *et al.*, 2004b; Winetech, 2004). The experimental design for both sites was a complete randomised block arrangement with five treatments and six replications (Figure 2.5). The treatments are described in Tables 2.1 and 2.2. Treatment T3 was used in both of the experiments.

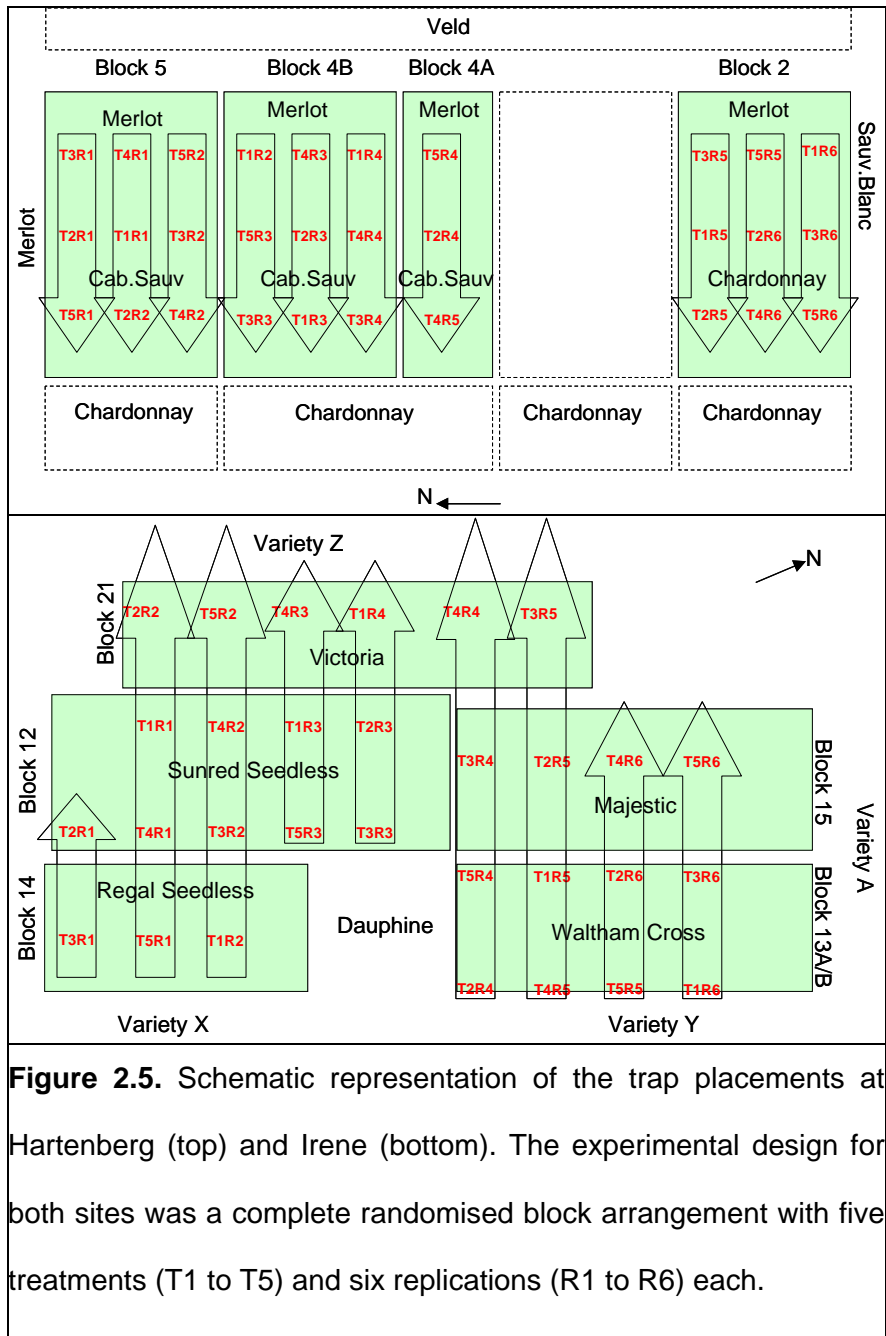


Figure 2.5. Schematic representation of the trap placements at Hartenberg (top) and Irene (bottom). The experimental design for both sites was a complete randomised block arrangement with five treatments (T1 to T5) and six replications (R1 to R6) each.

2.4. Material used

Material used consisted of yellow delta traps (Figure 2.7) with white sticky liners (20 cm x 18.4 cm; approximate sticky surface area, 320 cm²) in which two types of pheromone lures were used (Figure 2.6). The pheromone lure (T2, Table 2.1) from Chempack, Simondium, South Africa is commercially available and registered for use in monitoring vine mealybug (NDA, 2005). The lure itself is a grey rubber septum impregnated with 0.01 mg lavandulyl senecioate. The pheromone lure (T3, Table 2.1) from Insect Science

(ISSA), Tzaneen, South Africa is not yet commercially available. The lure consists of a white rubber septum impregnated with 0.01 mg lavandulyl senecioate. The formulation (physical structure of the lure unit) differs from the Chempack formulation. As a control treatment (T1, Table 2.1), a yellow delta trap without a pheromone lure was used.

Table 2.1. Description of treatments for the pheromone lure formulation comparison experiment (refer to Chapter 3).

Treatment number	Pheromone lure formulation	Trap type	Study objective
T1	No pheromone lure	Yellow delta trap	Control treatment for T2 and T3
T2	Commercial (Chempack) lure	Yellow delta trap	Pheromone lure formulation comparison (with T3)
T3	Experimental (ISSA) lure	Yellow delta trap	Pheromone lure formulation comparison (with T2)

Table 2.2. Description of treatments for the trap design comparison experiment (refer to Chapter 4).

Treatment number	Pheromone lure formulation	Trap type	Study objective
T3	Experimental (ISSA) lure	Yellow delta trap	Trap design comparison (with T4 and T5)
T4	Experimental (ISSA) lure	Yellow scale card	Trap design comparison (with T3 and T5)
T5	Experimental (ISSA) lure	White scale card	Trap design comparison (with T3 and T4)

To evaluate the efficacy of the different trap designs, three types of traps were used, each with the ISSA pheromone. These were yellow scale cards (T4, Table 2.2, Figure 2.8)

(12.4 cm x 7.5 cm; approximate sticky surface area, 180 cm²), white scale cards (T5, Table 2.2, Figure 2.9) (28 cm x 8.5 cm; approximate sticky surface area, 220 cm²) as well as the standard yellow delta trap (T3, Table 2.2, Figure 2.7) (20 cm x 18.4 cm; approximate sticky surface area, 320 cm²).

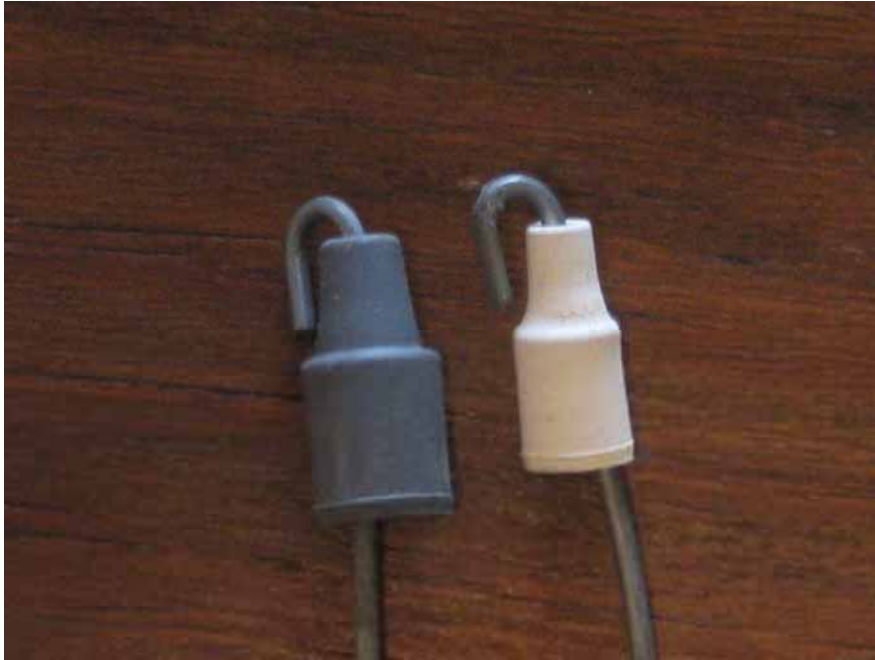


Figure 2.6. Chempack lure capsule (left) (T2, Table 2.1) and ISSA lure capsule (right) (T3, Table 2.1). Note also the application of the wire (Photo: M.J. Kotze).

The traps were secured in the cordon using the trellis wires for attachment according to the Chempack (2002) usage brochure and the vine mealybug trapping protocol as published by Winetech (2004). In the Irene vineyards, they were approximately 1.6 m above the ground and in the Hartenberg vineyards; they were about 0.6 m above the ground. The effective trapping range of the pheromone lure of 50 m suggests a difference in distance between traps of 100 m (Walton *et al.*, 2004). For South African vineyards it is recommended to place traps 100 m apart so that the pheromone activities of the traps do not interfere with each other (Winetech, 2004). For Californian vineyards the recommendation is to place traps 200-300 feet (60-90 m) apart (Daane *et al.*, 2004b). For this study, the traps were placed 60 m

apart, the lower end of the suggested range. Since the focus of the project was the comparison of lure and trap design and the treatments were placed in a randomised block design, it was felt that it was in order to have them this close apart.



Figure 2.7. Yellow delta trap with sticky liner. Lure capsule is suspended from the roof of the trap (Photo: M.J. Kotze).

The pheromone lures used throughout the study were from the same batch and were stored in a refrigerator at approximately 7°C until they were used. The night before the lures were to be replaced, they were taken out of the refrigerator and opened to reduce any possible “flash off” effect (Hodges *et al.*, 2004). The procedure for installation of the lure capsule in the delta trap was as follows: the end of a short piece of wire (approximately 8 cm) was inserted through the pheromone lure capsule and the other end was inserted through the roof of the delta trap to suspend the pheromone capsule just above the sticky trap liner on the base of the trap (Figure 2.7) (Winetech, 2004).

The delta trap has its own attachment wires for attaching it to the trellis wires. Suspending the lure capsule above the sticky liner is an alternative way to placing the lure in the centre of

the sticky liner that lies on the base of the trap (Chempack, 2002) as the capsule can become covered with glue and that can affect the release rate of the pheromone from the rubber capsule (Winetech, 2004).



Figure 2.8. Yellow scale card trap. Both sides of the trap are sticky. The lure capsule is suspended above the trap (Photo: M.J. Kotze).



Figure 2.9. White scale card trap. Only the outer sides of the trap is sticky. The lure capsule is suspended above the trap (Photo: M.J. Kotze).

In the case of the scale cards, a piece of wire (approximately 20 cm) was inserted through the pheromone lure and the capsule was positioned about 4-5 cm from the end of the wire. This end was placed through the hole at the top of the scale card (Figures 2.8 and 2.9). The other end of the wire was attached to the trellis wires to secure the trap in position. The pheromone lures were never handled with bare hands in order to prevent cross contamination between the two pheromone formulations.

The trap liners for the delta traps and the white scale cards were pre-glued by the manufacturer while the yellow scale cards were glued on both sides with Flytac just prior to use. Flytac is a solvent free, non-toxic, non-inflammable water based paste which becomes extremely tacky when dry (ISSA, 2002).

Traps were first hung out on 9 October 2004 just before bud break as recommended (Chempack, 2002; Winetech, 2004), and they remained in place for 16 weeks. They were then re-randomised and rotated to reduce any positional effects (Flechtmann *et al.*, 2000) and possible trap bias on trap catches (Herman *et al.*, 1994; Rojas *et al.*, 2004). The re-randomised traps remained in place for another 16 weeks. Trap liners and scale cards were exchanged every two weeks and pheromone lures were replaced every eight weeks (Chempack, 2002; Winetech, 2004). A total of 16 trap catch observations were made. Normally, it is recommended that monitoring continues until harvest (Chempack, 2002; Winetech, 2004). In this case, the study lasted until 21 May 2005, way past harvesting, to just before pruning started. This was to record the natural decline in numbers of vine mealybug males as they enter the overwintering period.

2.5. Data analysis

The data was analysed separately for each of the two vineyards, Irene and Hartenberg, as different grape types, table grapes and wine grapes respectively, were cultivated at the two vineyards. Data were $\log_{10}(x+1)$ transformed to stabilise the variance (Van Ark, 1981). Repeated measures analysis of variance (ANOVA) was used to compare season-long treatment differences for each sample date. Statistical analysis was performed with Statistica software (version 7.1).

On two separate dates, data from one trap type each was missing at Irene, and on one occasion data from one trap was missing at Hartenberg. As zero was a legitimate value in the trap catches, missing values were estimated using the following equation (Van Ark, 1981):

$$y = \frac{rB + tT - G}{(r - 1)(t - 1)}$$

Where: B, T and G are the treatment total, block total and grand total for the observations actually available; and r and t, the number of replications and number of treatments respectively.

2.6. Weather data

Daily weather data (minimum, maximum and mean temperature, relative humidity and rainfall data) for the study period were obtained. Each of the study sites had weather stations on site and weather data was downloaded from the service providers for the two sites.

This data was applied in the discussion of the climatic influences on monitoring. This data was also used for estimating the accumulated number of degree days in each area, enabling the estimation of the number of *P. ficus* generations per growing season in each area.

CHAPTER 3: COMPARISON OF PHEROMONE FORMULATIONS

3.1 Introduction

The chemical composition of the female vine mealybug sex pheromone was identified and synthesized in 2001 (Hinkins *et al.*, 2001). Thereafter the practical usage of the pheromone for monitoring purposes was investigated in California by Millar *et al.* (2002) and in South Africa by Walton *et al.* (2004).

Groundbreaking work in this field was done in the Californian study by Millar *et al.* (2002). Two insect-produced compounds, (S)-lavandulol and (S)-lavandulol seneciote (ratio about 2:5) were identified by Hinkins *et al.* (2001) from odours released by virgin female vine mealybugs. Laboratory bioassays indicated that the latter component was crucial for attraction of males, whereas the former was not. For their study, they prepared the synthetic pheromone to be used in a monitoring system. Grey rubber septa were used as dispensers. They tested for the optimal blend ratios and pheromone doses, field longevity of the lures and pheromone range. As the synthetic sex pheromone was prepared for usage in monitoring vine mealybug infestations in vineyards, comparisons of pheromone trap catches with mealybug population densities were conducted to verify the effectiveness and applicability of such a method (Millar *et al.*, 2002).

The South African study by Walton *et al.* (2004) followed after the Californian study and tested lure longevity and lure range under South African conditions. The correlation between vine mealybug male trap catches and vine mealybug female population density was also determined to verify the effectiveness of the sex pheromone baited traps as a monitoring technique for vine infestation levels. The lures were made from rubber septa that were

impregnated with a 100 µg dose of the racemic synthetic pheromone (Walton *et al.*, 2004). The outcome of research results in this field in South Africa was the development of a protocol for control of vine mealybug in vineyards together with a recommendation to grape growers to use the commercially available pheromone lure capsules distributed by Chempack in Simondium (near Paarl), South Africa (Walton *et al.*, 2003).

Insect Science (ISSA), a company based in Tzaneen, South Africa, and distributor of pest monitoring products, endeavours to register an alternative pheromone dispenser for the monitoring of vine mealybug in vineyards. This current project was initiated to conduct field tests with the new pheromone formulation (dispenser) as required by the regulatory body of agricultural remedies in South Africa.

The aim of the experiment was to compare the effectiveness of the new pheromone formulation with that of the commercially available and already registered formulation.

3.2 Materials and methods

The experiment consisted of three treatments each replicated six times. The yellow delta trap was used in each treatment. The first treatment was a control treatment without a pheromone lure (T1 in Table 2.1). The second treatment was the Chempack pheromone lure (T2 in Table 2.1, Figure 2.6) and the third treatment was the ISSA pheromone lure (T3 in Table 2.1, Figure 2.6). Traps were replaced every fortnight for 32 consecutive weeks resulting in 16 sets of data.

The procedures for preparation, installation and servicing of traps, the experimental design and the data analysis were described in Chapter 2.

3.3 Results

A total of 33 386.8¹ *Planococcus ficus* males was captured in the traps at Hartenberg and 21 971.9² at Irene. The total number of vine mealybug males trapped in the control traps were 130 (0.39% of total) and 19 (0.9% of total) for the Hartenberg and Irene sites respectively. Since these totals were negligible they were excluded from further analysis (Zada *et al.*, 2004). The mean number of trap catches (averaged over T2 and T3, Table 2.1) at the two different sites is presented in Figure 3.1.

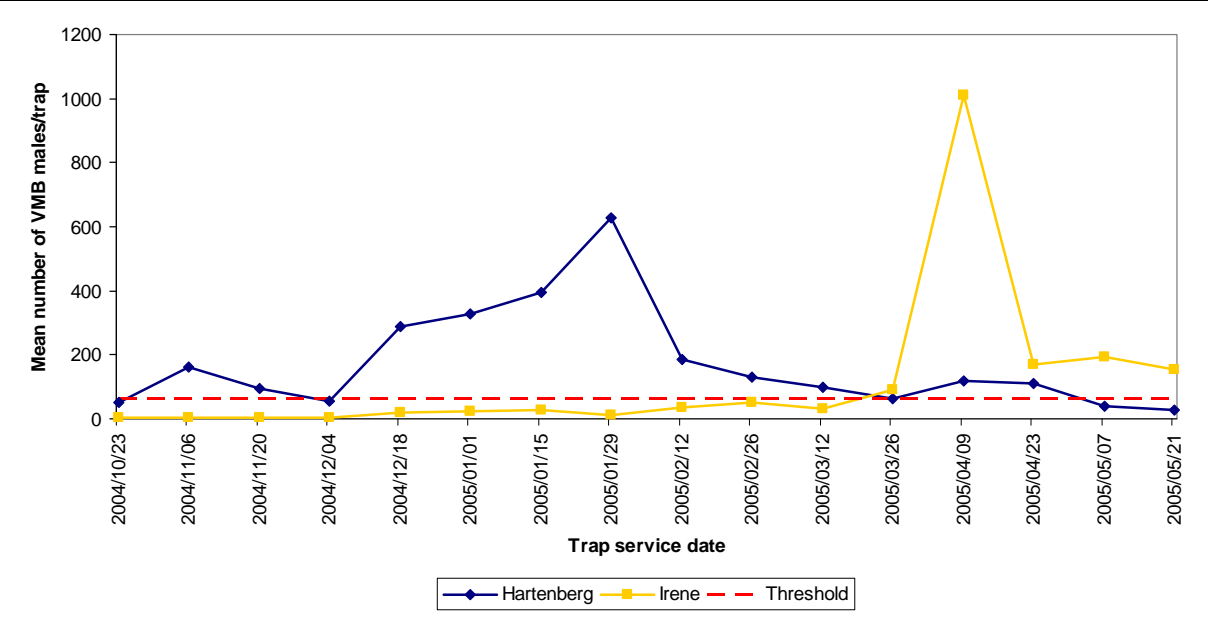


Figure 3.1. Mean number of *Planococcus ficus* males per trap during the 2004/2005 experimental period at Hartenberg and Irene. The dashed line shows the action threshold of 65 males per trap per two-week period.

¹ On 12/02/2005 data of one of the six ISSA (T3) traps was missing at Hartenberg. Missing data was calculated as described in Chapter 2 and resulted in non-integer numbers as is evident in this number.

² On 29/01/2005 data of one of the six Chempack (T2) traps was missing at Irene. Missing data was calculated as described in Chapter 2 and resulted in non-integer numbers as is evident in this number.

At Hartenberg, the wine grape vineyard, approximately 66% of the total number of catches was done during the period from mid-December 2004 to mid-February 2005 (Figure 3.1). This period coincides with the crop stages from pea sized berries to veraison. About 13% of the trap catches was recorded in the preceding period and 21% was recorded thereafter until the end of the study. During the period from 4 December 2004 to 12 February 2005 the maximum temperatures recorded for Hartenberg ranged between 19.2°C and 38.8°C with a median of 27.6°C.

At Irene, in the table grape vineyard, an increase in trap catches was observed from 26 March onwards to harvest, with a huge peak in trap catches during mid-April 2005 (Figure 3.1). The number of males trapped during the two fortnight periods from 23 March to 9 April amounts to approximately 55% of the total number of captured males. Higher than normal (compared to the period before the 9th of April) trap catches (approximately 28% as to 17%) were recorded thereafter until the end of the study. The 9th of April 2005 was already past the harvesting period. During the period from 12 March 2005 to 7 May 2005 (information not available until the end of the study) the maximum temperatures recorded for Irene ranged between 15.7°C and 40.7°C with a median of 29.1°C.

Data on vine mealybug trap catches for the previous (2003/2004) season (Figure 3.2) was obtained for Hartenberg (Snyman, unpublished data) and Irene (Venter, unpublished data). The trap catch peaks for both sites during the 2003/2004 season were similar to those in the 2004/2005 season. For both these seasons trap catches peaked just before harvest at Hartenberg, and just after harvest at Irene. Although there are quantitative differences in the trap catches between the two seasons, the similar trend in the trap catches across the growing seasons potentially indicates a consistent pattern provided by sex pheromone monitoring in the wine grapes and table grapes.

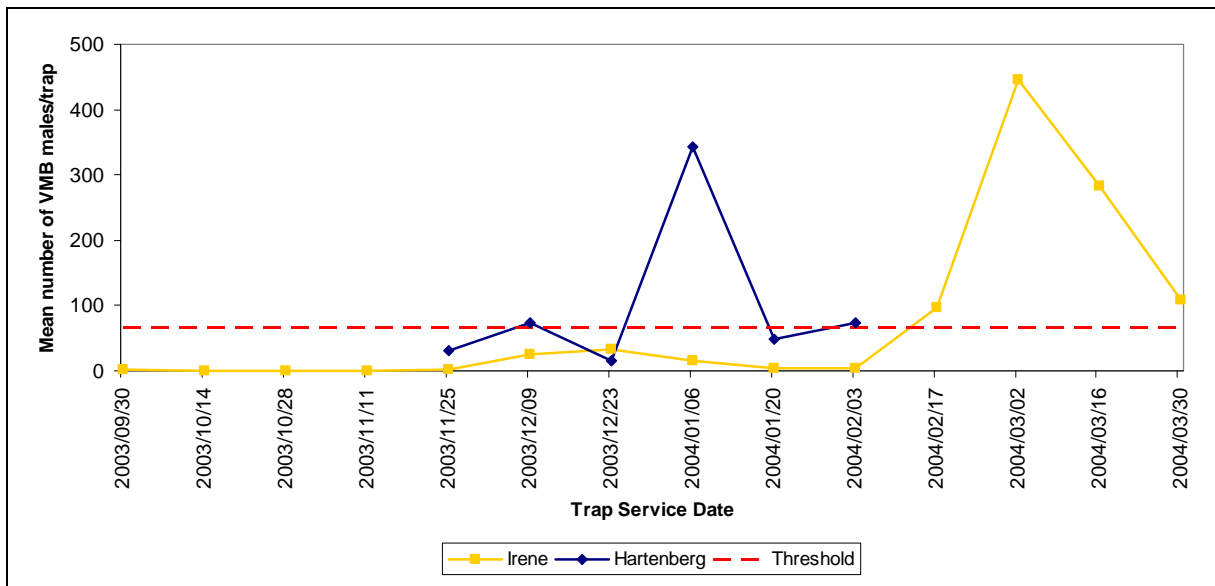


Figure 3.2. Mean number of *Planococcus ficus* males per trap for the 2003/2004 season at Hartenberg (Snyman, unpublished data) and Irene (Venter, unpublished data). The dashed line shows the action threshold of 65 males per trap per two-week period.

A significant difference in trap catches between grape types was observed when the data was combined for wine and table grapes ($F(1,22) = 48.143, p < 0.0001$) and hence further results are presented for each grape type separately.

3.3.1 Wine grapes

Figure 3.3 shows the comparative efficiency of the two pheromone formulations in wine grape vineyards at Hartenberg. The mean male trap catch was generally above the threshold of 65 males per trap per two-week period.

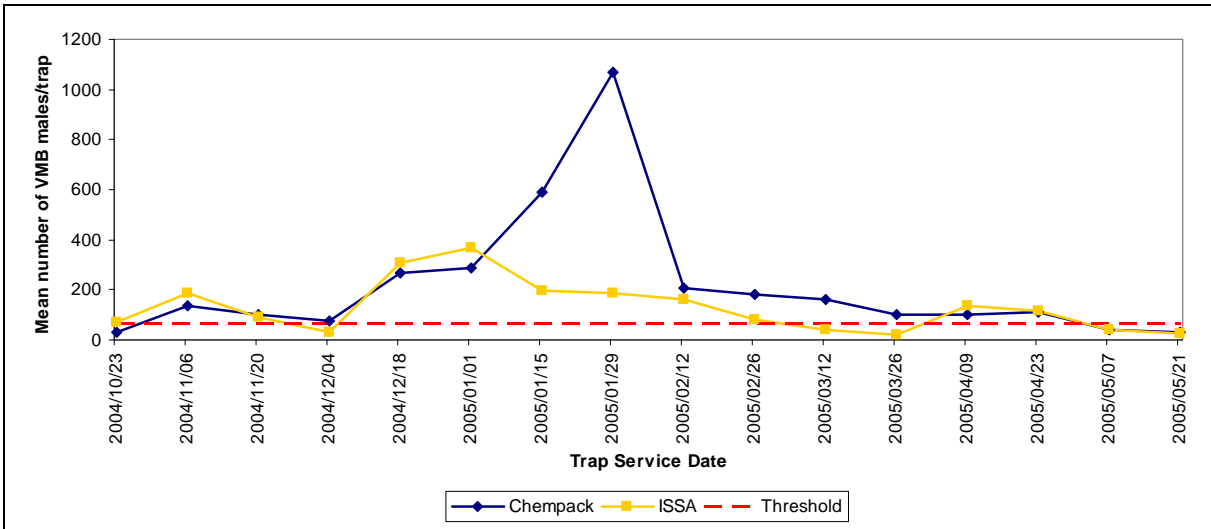


Figure 3.3. Mean number of *Planococcus ficus* males per trap per sampling period for two types of lure formulations in wine grape vineyards at Hartenberg. The dashed line shows the accepted action threshold of 65 males per trap per two-week period.

A higher number of vine mealybug males (approximately 1.7 times more) were captured at Hartenberg with the Chempack pheromone lure formulation (mean = 217.59 ± 33.34 , $n = 96$) than with the ISSA pheromone lure formulation (mean = 128.83 ± 15.73 , $n = 96$) (Figure 3.4).

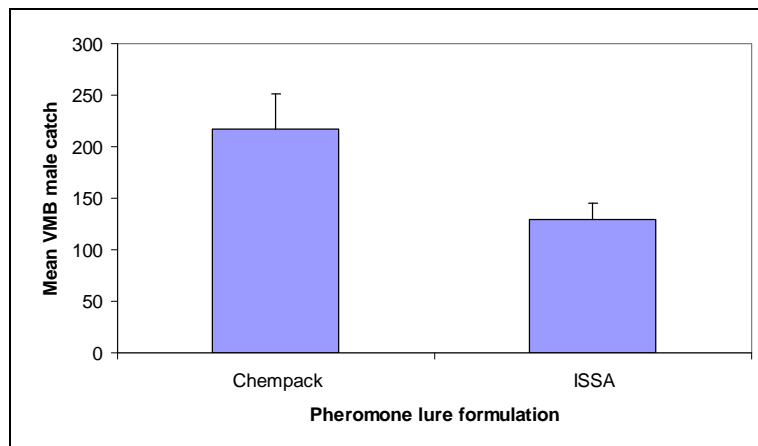


Figure 3.4. Mean (\pm SE) number of *Planococcus ficus* males trapped with two pheromone lure formulations in wine grape vineyards at Hartenberg.

When trap catch figures were analysed for the two pheromone lure formulations without taking any influencing factors into account, no significant difference in their effectiveness ($F(1,10) = 3.308$, $p = 0.098952$) was observed. However, various factors and interactions between factors indicated a significant difference in the effectiveness of the two formulations (Table 3.1).

A factor that produced a significant effect on the findings was time of sampling during the season ($F(3,30) = 33.497$, $p < 0.0001$). The experiment spanned the whole of the grape growing season from bud break to vine senescence, from spring through summer to autumn. Replacing of the pheromone dispenser also produced a significant effect on the findings ($F(1,10) = 26.827$, $p = 0.000413$). Each pheromone dispenser was retained in the field for eight weeks (four sets of data) after which it was replaced with a fresh pheromone dispenser. Despite the fact that the traps were re-randomised half way during the experiment, location as a stand-alone factor and even the interaction between location and the pheromone lure formulation, produced no significant effect. The main interactions between factors that produced significant effects were time x formulation, location x dispenser and location x time (Table 3.1). The interaction between time and formulation (Table 3.1) is graphically depicted in Figure 3.5. A downward trend in the effectiveness of the ISSA pheromone formulation is evident from this graph.

A series of Student t-tests (corrected for possible unequal variances) between the two pheromone lure formulations over the total period of time showed significant differences between trap catches for the two formulations on four occasions. It corresponds with the following dates: 15 January 2005 (t-value = 3.8114, $df = 9.3$, $p = 0.0034$), 29 January 2005 (t-value = 4.2833, $df = 9.9$, $p = 0.0016$), 12 March 2005 (t-value = 3.9916, $df = 7.9$, $p = 0.0026$) and 26 March 2005 (t-value = 4.3765, $df = 6.5$, $p = 0.0014$) (Figure 3.3).

Table 3.1. Repeated measures analysis of variance with pheromone formulation as categorised factor and with location, dispenser and time as within effects (giving 16 repeated measures) for wine grapes at Hartenberg.

Factor	Sum of Squares	Df	Mean Squares	F-value	p-value
Intercept	716.6979	1	716.6979	1481.231	< 0.0001
Formulation	1.6008	1	1.6008	3.308	0.0990
Error	4.8385	10	0.4839		
Location	1.8486	1	1.8486	2.726	0.1297
Location*Formulation	0.1509	1	0.1509	0.223	0.6472
Error	6.7812	10	0.6781		
Dispenser	5.2715	1	5.2715	26.827	0.0004
Dispenser*Formulation	0.1912	1	0.1912	0.973	0.3472
Error	1.9650	10	0.1965		
Time	2.8913	3	0.9638	33.497	< 0.0001
Time*Formulation	3.0194	3	1.0065	34.981	< 0.0001
Error	0.8631	30	0.0288		
Location*Dispenser	12.4156	1	12.4156	52.446	< 0.0001
Location*Dispenser*Formulation	1.3798	1	1.3798	5.829	0.0364
Error	2.3673	10	0.2367		
Location*Time	3.8360	3	1.2787	54.975	< 0.0001
Location*Time*Formulation	0.4651	3	0.1550	6.665	0.0014
Error	0.6978	30	0.0233		
Dispenser*Time	0.1562	3	0.0521	1.332	0.2823
Dispenser*Time*Formulation	0.0375	3	0.0125	0.320	0.8108
Error	1.1723	30	0.0391		
Location*Dispenser*Time	0.6561	3	0.2187	3.245	0.0356
Location*Dispenser*Time*Formulation	0.2675	3	0.0892	1.323	0.2852
Error	2.0218	30	0.0674		

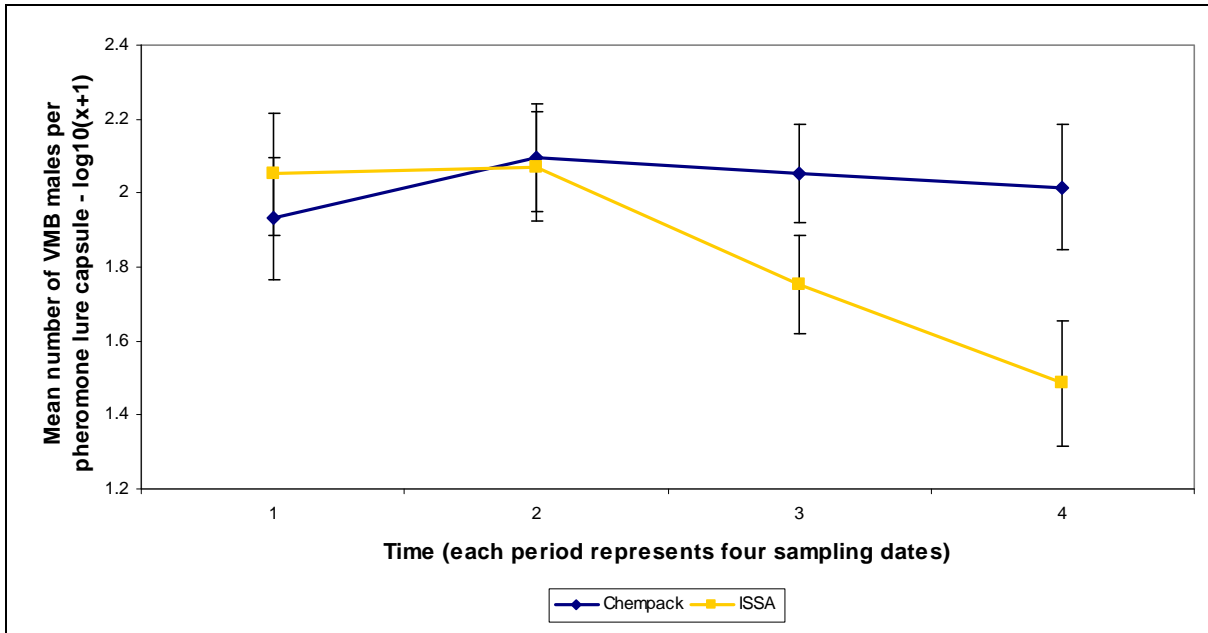


Figure 3.5. Mean (\pm SE) number of *Planococcus ficus* males trapped with two pheromone lure formulations in wine grape vineyards at Hartenberg expressing the interaction between time and formulation. Note the downward trend in effectiveness of the ISSA pheromone lure formulation over time.

A higher number of vine mealybug males were captured in Chardonnay vines (mean = 344.75 ± 69.18 , $n = 40$) than in Cabernet Sauvignon (mean = 128.59 ± 15.35 , $n = 120$) and Merlot (mean = 126.13 ± 21.35 , $n = 32$) vines (Figure 3.6).

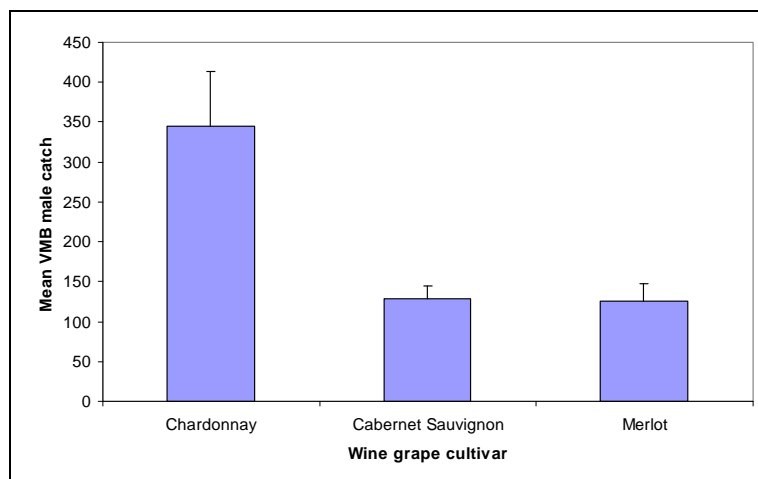


Figure 3.6. Mean (\pm SE) number of *Planococcus ficus* males trapped in three wine grape cultivars at Hartenberg.

3.3.2 Table grapes

The comparative efficiency of the two pheromone formulations in table grape vineyards at Irene is shown in Figure 3.7 (data is only shown for the first 12 sampling periods as the peak in the trap catch figures hides important trends during that period). The mean male trap catch was generally below the threshold of 65 males per trap per two-week period throughout the pre-harvest period.

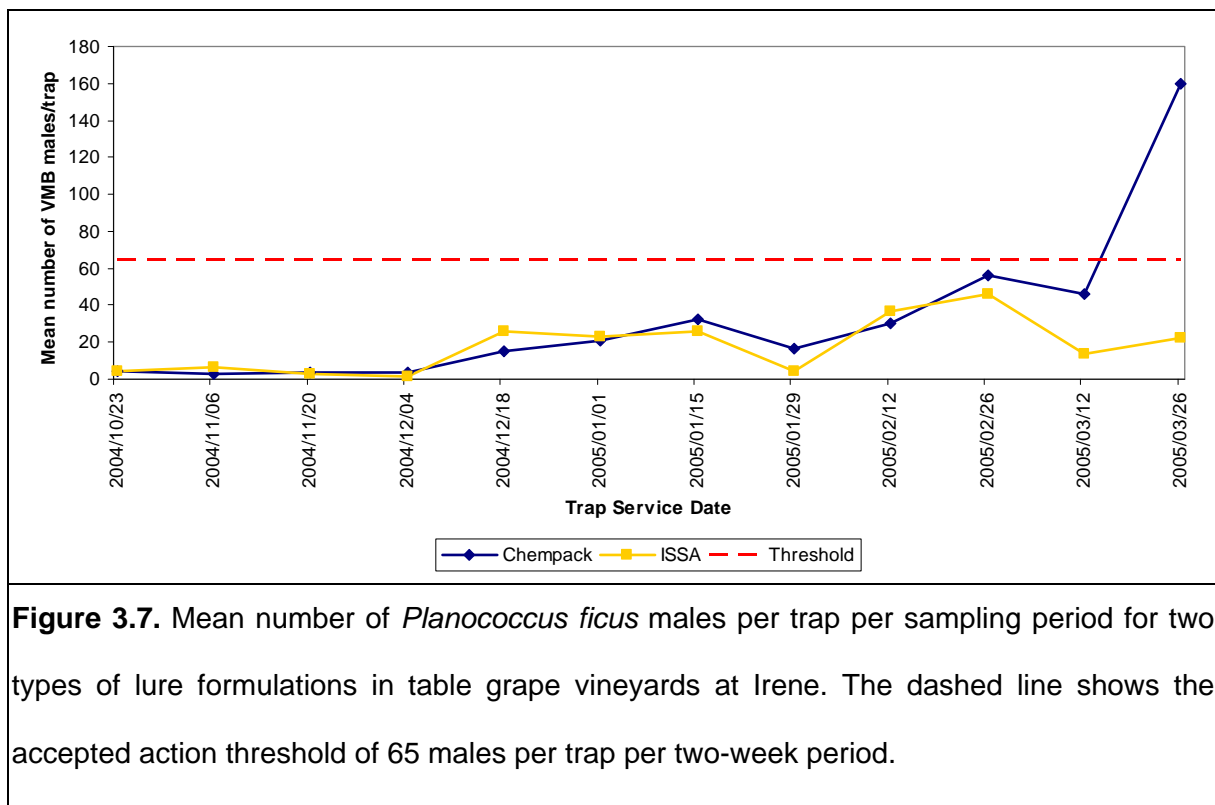


Figure 3.7. Mean number of *Planococcus ficus* males per trap per sampling period for two types of lure formulations in table grape vineyards at Irene. The dashed line shows the accepted action threshold of 65 males per trap per two-week period.

A slightly higher number of vine mealybug males (approximately 1.07 times more) were captured with the Chempack pheromone lure formulation (mean = 118.16 ± 27.33 , $n = 96$) than with the ISSA pheromone lure formulation (mean = 110.21 ± 28.49 , $n = 96$) (Figure 3.8).

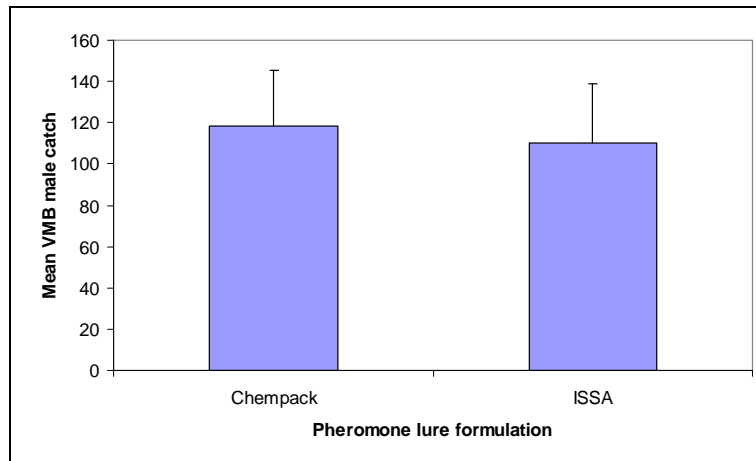


Figure 3.8. Mean (\pm SE) number of *Planococcus ficus* males trapped for two pheromone lure formulations in table grape vineyards at Irene.

Analysing the trap catch figures for the two pheromone lure formulations without taking any influencing factor into account, showed no significant difference in their effectiveness ($F(1,10) = 2.003$, $p = 0.187343$). However, various factors and interactions between factors influenced the findings and indicated a significant difference in the effectiveness of the two formulations (Table 3.2).

One of the factors that produced a significant effect on the findings was time of trapping during the monitoring period ($F(3,30) = 12.4773$, $p < 0.0001$). The experiment spanned the whole of the grape growing season from bud break to vine senescence, from spring through summer to autumn. Replacing of the pheromone dispenser also produced a significant effect on the findings ($F(1,10) = 136.1799$, $p < 0.0001$). Each pheromone dispenser was retained in the field for eight weeks (four sets of data) after which it was replaced with a fresh pheromone dispenser. A significant effect was produced by the change in location of the traps ($F(1,10) = 159.1901$, $p < 0.0001$) when the traps were re-randomised traps half way during the experiment.

The main interactions between factors that produced significant effects were time x formulation, location x time and dispenser x time (Table 3.2). The interaction between time and formulation (Table 3.2) is graphically depicted in Figure 3.9. A downward trend in the effectiveness of the ISSA pheromone formulation is evident from this graph.

A series of Student t-tests (corrected for possible unequal variances) between the two pheromone lure formulations over the total period of time showed significant differences between trap catches with the two formulations on only two occasions. It corresponds with the following dates: 29 January 2005 (t-value = 3.324, df = 8.7, p = 0.0077) and 26 March 2005 (t-value = 4.0911, df = 5.9, p = 0.0022) (Figure 3.7).

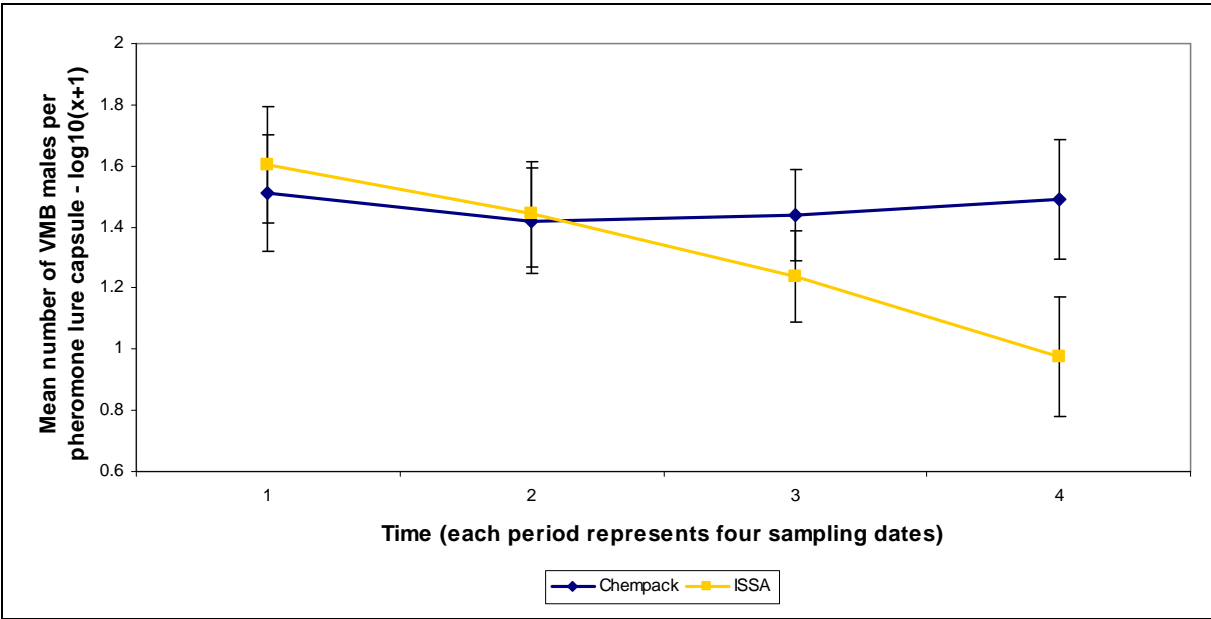
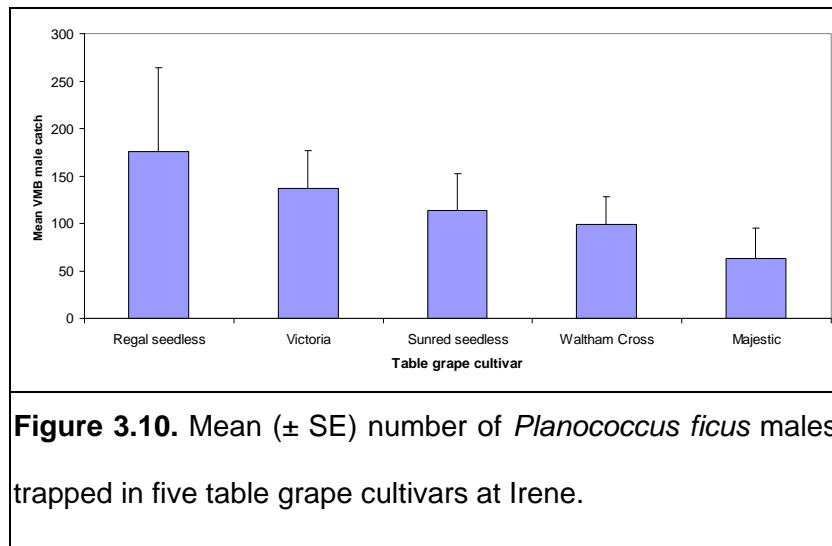


Figure 3.9. Mean (\pm SE) number of *Planococcus ficus* males trapped with two pheromone lure formulations in table grape vineyards at Irene expressing the interaction between time and formulation. Note the downward trend in effectiveness of the ISSA pheromone lure formulation over time).

Table 3.2. Repeated measures analysis of variance with pheromone formulation as categorised factor and with location, dispenser and time as within effects (giving 16 repeated measures) for table grapes at Irene.

Factor	Sum of Squares	Df	Mean Squares	F-value	p-value
Intercept	370.7528	1	370.7528	689.0990	<0.0001
Formulation	1.0778	1	1.0778	2.0032	0.1873
Error	5.3803	10	0.5380		
Location	46.4383	1	46.4383	159.1901	<0.0001
Location*Formulation	0.2932	1	0.2932	1.0050	0.3397
Error	2.9172	10	0.2917		
Dispenser	28.1492	1	28.1492	136.1799	<0.0001
Dispenser*Formulation	0.6907	1	0.6907	3.3414	0.0975
Error	2.0671	10	0.2067		
Time	2.7567	3	0.9189	12.4773	<0.0001
Time*Formulation	2.7102	3	0.9034	12.2671	<0.0001
Error	2.2093	30	0.0736		
Location*Dispenser	0.6314	1	0.6314	1.4094	0.2626
Location*Dispenser*Formulation	0.9100	1	0.9100	2.0313	0.1845
Error	4.4800	10	0.4480		
Location*Time	1.6706	3	0.5569	7.5607	0.0007
Location*Time*Formulation	0.0459	3	0.0153	0.2078	0.8902
Error	2.2096	30	0.0737		
Dispenser*Time	3.9217	3	1.3072	17.4194	<0.0001
Dispenser*Time*Formulation	0.2436	3	0.0812	1.0821	0.3716
Error	2.2513	30	0.0750		
Location*Dispenser*Time	2.5396	3	0.8465	14.6884	<0.0001
Location*Dispenser*Time*Formulation	0.8490	3	0.2830	4.9103	0.0068
Error	1.7290	30	0.0576		

Figure 3.10 depicts the mean number of the vine mealybug males captured per table grape cultivar: Regal Seedless (mean = 175.46 ± 88.96 , $n = 24$), Victoria (mean = 136.56 ± 40.51 , $n = 32$), Sunred Seedless (mean = 113.18 ± 39.28 , $n = 56$), Waltham Cross (mean = 98.70 ± 29.62 , $n = 56$) and Majestic (mean = 62.79 ± 32.27 , $n = 24$).



3.4 Discussion

A much higher number of vine mealybug males were trapped much earlier in the season at Hartenberg than at Irene (Figure 3.1). This number was above the action threshold value of 65 vine mealybug males per trap per two-week period (Winetech, 2004) for most of the season. It was despite the fact that the mean maximum temperature for the period of the study (October 2004 to May 2005) was 5.8°C higher at Irene than at Hartenberg. A variety of factors could have influenced these results of which vine and vineyard architecture potentially plays a large role.

The vine architecture for wine grapes (Hartenberg) and table grapes (Irene) differs considerably. The main stem, new growth areas and bunches are more exposed in table grape vines compared to wine grape vines (Walton, 2003a) (Figures 3.11 and 3.12). This is

mainly a function of the type of trellising used, the vine density per ha and the way the berries are packed in the bunch. The table grapes at Irene are trellised on a “Y”-structured trellis (Figures 2.2) and the wine grapes at Hartenberg are trellised on a 5-wire vertical trellis system (Figures 2.4). Vines in table grape blocks at Irene are more closely spaced at approximately 2000 vines per ha compared to approximately 2700 vines per ha in the wine grape blocks at Hartenberg. Wine grape berries in bunches usually are more tightly packed (Figure 3.11) than in table grape bunches (Figure 3.12) (Walton, 2003a) especially when considering the size of the bunch. This may lead to the creation of more refuge sites for mealybugs because of the presence of more leaves, stems and bunches per unit area (Walton, 2003a).

At Irene, a significant increase in vine mealybug males trap catch numbers were observed towards the end of the season, post harvest (Figure 3.1). Maximum temperatures after harvest were lower than early in the season and no special vineyard management activities were conducted as the vines were nearing senescence. Unfortunately, vine mealybug has never been monitored post harvest time, so there are no historical data to compare this occurrence to (Andrag, 2004). It is advisable to monitor for this type of increase in vine mealybug male trap catch numbers until very late in the season on an annual basis. It may be beneficial when deciding on the timing and nature of control mechanisms to be applied in preparation for the next growing season.

For both wine grapes (Hartenberg) and table grapes (Irene) the vine mealybug trap catch numbers for the 2004/2005 season followed the same trend as during the 2003/2004 season (Figure 3.2) with a peak in the December-February (wine grape) and February-March (table grape) trap catch numbers.



Figure 3.11. Merlot wine grapes. The berries are densely packed in the bunch and the bunches are protected under the foliage (Photo: M.J. Kotze).



Figure 3.12. Majestic table grapes. The berries are loosely packed in the bunches and the bunches are exposed within the “tunnel” formed by the trellis system (Photo: M.J. Kotze).

In both grape types, the effectiveness of the two pheromone lure formulations seems to be the same (Tables 3.1 and 3.2). The apparent differences as observed for wine grapes at Hartenberg, where 1.7 times more vine mealybug males were trapped with the Chempack pheromone lure formulation than with the ISSA pheromone lure formulation (Figure 3.4 as compared to Figure 3.8), were too small to be statistically significant. This can possibly be attributed to the small data set and the large variances observed in the data. The series of Student t-tests have shown individual differences for four of the 16 sampling dates at Hartenberg and for only two sampling dates at Irene. However, it is interesting to note that for both the grape types, the dates where individual differences were observed, always coincide with the last sampling dates the particular lure dispensers were used before they were replaced (Figures 3.5 and 3.9).

Despite the fact that the pheromone lure formulation treatments themselves did not show a statistically significant difference in effectiveness of the two types of lure formulations tested,

the factor relating to the replacement of fresh lure dispensers had a significant effect on the trap catch figures observed for both grape types. From Figures 3.5 and 3.9 it is evident that the ISSA pheromone lure formulation did not perform equally with the Chempack formulation. It can possibly be attributed to the differences in the physical characteristics of the dispensers (Figure 3.13).

The Chempack pheromone dispenser is made of a grey, relatively dense rubber material, whereas the ISSA pheromone dispenser is made of a whitish, more porous rubber material. A comparison of the size related attributes of the two formulations are provided in Table 3.3. The rubber of the ISSA pheromone dispensers seemed to be much more porous than that of the Chempack dispenser. It was observed that after only about three weeks in the vineyard, the ISSA pheromone dispenser showed signs of perishing (MJK, personal observation) (Figure 3.14).



Figure 3.13. The Chempack (grey) and ISSA (whitish) pheromone lure capsules. Note the physical differences between the two capsules (Photo: M.J. Kotze).



Figure 3.14. The extent to which some of the ISSA pheromone lure capsules perished during the eight week period (Photo: M. Carlsson).

It is possible that the release rate of the pheromone in the ISSA dispenser is higher than in the Chempack dispenser and thereby loses its effectiveness more rapidly (Figures 3.5 and 3.9). The surface area of the Chempack dispenser is about 1.4 times as much as that of the ISSA dispenser. It is possible that the combined effect of the potentially lower permeability of the Chempack rubber and the larger surface size regulates the release rate of the pheromone better than in the case of the ISSA pheromone rubber.

Table 3.3. Comparison of the physical attributes of the two types of pheromone lure formulations used.

Physical attributes of rubber septum	Chempack	ISSA
Approximate surface area	460 mm ²	320 mm ²
Approximate height	19 mm	16 mm
Approximate thickness of septum wall at base	1.5 mm	1.2 mm
Approximate thickness of septum wall at apex	1.5 mm	0.8 mm
Approximate outer diameter at base	9 mm	7 mm
Approximate outer diameter at apex	5 mm	4.2 mm

A significant effect on the effectiveness of the two pheromone lure formulations due to the duration of the monitoring period (time) as well as the interaction between time and formulation was also observed for both grape types. Until harvest time, for table grapes at Irene the mean male vine mealybug trap catch per trap per two week period was never above the action threshold of 65 for either of the two pheromone lure formulations, whereas for wine grapes at Hartenberg it was only thrice below the threshold value, twice for the ISSA pheromone formulation and once for the Chempack pheromone lure formulation.

Cultural practices for grape production vary depending on the intended use of the crop (e.g.

whether for table usage, producing wine or drying for producing raisins), the growing region, and the management preferences of the grower. Pest management priorities are also impacted by the intended use where, for example, control of pests that cause cosmetic damage to the fruit can be much more important in the production of table grapes than in the production of wine and raisin grapes (Raisin grapes, 1999). The low male vine mealybug trap catch figures observed until harvest time in the table grape vineyards at Irene can possibly be attributed to the choice of vineyard management practices that fit best with the intended use of the crop, which for the crop produced at Irene, is the export markets. At Irene, a strict vineyard management regime was followed by the vineyard manager to comply with Eurepgap regulations (Andrag, 2004). Actions were focused on preventative rather than corrective action. An array of control methods was applied and included the following: one, debarking of trunks of old established vines (Figure 1.17); two, applying a sticky stem barrier to all trunks, poles and wires planted in the ground (Figure 1.17); three, maintaining clean inter-row spaces without weeds (Figure 2.2); four, prophylactic spray applications of chlorpyrifos applied during the dormant season – never during the growing season; five, vine mealybug was strictly monitored with pheromone lures and manual stem infestation monitoring.

Millar *et al.* (2002) and Walton *et al.* (2004) observed a significant correlation between the number of vine mealybug males caught in pheromone traps and the female vine mealybug population found on the vines. It is currently accepted that the action threshold of 65 vine mealybug males per trap per two-week period corresponds with a 2% vineyard infestation by the female vine mealybug (Winetech, 2004). This threshold is suggested for all vineyards irrespective of the type of grapes produced. It does however seem that vine mealybug is less tolerated in table grapes than wine grapes. When superficially evaluating the appropriateness of action threshold, it seems that it may be in order for table grapes; however for wine grapes it may be too low. Walton *et al.* (2004) reported that studies are in

progress to verify the suggested action threshold albeit for different reasons.

The data analysis indicates that the re-randomisation of trap location did not have a significant effect on the trap catch numbers observed for wine grape vineyards at Hartenberg, as opposed to the trap catch numbers observed for table grapes at Irene where a significant effect due to location was recorded. In the case of the wine grape vineyards, it can be indicative of a few factors: one, a more uniform distribution of the vine mealybug throughout the blocks where monitoring took place; two, it was a function of the higher population pressure (vine mealybug population mainly far above the action threshold of 65 males per trap per two-week period); three, it can be because of higher interference of infestation levels of adjacent blocks where mealybug monitoring did not take place; four, a combination of any of the factors mentioned. In the case of the table grape vineyards, it can be because of an inverse of the factors mentioned for wine grapes, namely: one, a more clumped distribution of the vine mealybug infestation; two, a function of the lower population pressure observed until harvest; three, less interference of infestation levels of adjacent blocks; four, a combination of any of the factors mentioned.

It has been observed that certain wine grape cultivars are more susceptible to mealybug infestation than others (Le Roux, 1996 in Walton, 2003b). These observations indicated that the sensitive cultivars were Chardonnay, Cabernet Sauvignon, Cape Riesling, Pinotage, Shiraz and Merlot. The least sensitive cultivars were Colombar, Sauvignon blanc, Semillon, Hanepoot and Chenin blanc. Results of this study in which only three cultivars were used at Hartenberg, showed that Chardonnay had the highest vine mealybug trap catches, with the lowest trap catch numbers being recorded in Merlot vines (Figure 3.6). Unfortunately, this observation could not be correlated with the female vine mealybug infestation levels in the different cultivars.

It is uncertain whether similar studies were done on differences in infestation levels of table grape cultivars. In this experiment, the cultivars can be ranked from those where most male vine mealybugs were captured to those where least were captured: Regal Seedless, Victoria, Sunred Seedless, Waltham Cross and Majestic (Figure 3.10). Studies to identify table grape cultivars that are resistant to vine mealybug infestation, need to be conducted to confirm these observations.

3.5 Conclusion

The ISSA pheromone formulation was not as effective in the field as the Chempack pheromone formulation. Future research on vine mealybug should address the adaptation of the action threshold for wine, table and raisin grapes. Studies should also be conducted on the host suitability of different grape cultivars for vine mealybug.

CHAPTER 4: COMPARISON OF TRAP DESIGNS

4.1. Introduction

After the chemical composition of the female vine mealybug sex pheromone had been identified and synthesized in 2001 (Hinkens *et al.*, 2001), the practical usage of the pheromone for monitoring purposes was investigated in California by Millar *et al.* (2002) and in South Africa by Walton *et al.* (2004).

In the Californian study both a delta trap and a two-sided sticky trap were evaluated. It was concluded that the delta trap was superior to the two-sided sticky trap for catching vine mealybug males. However, the study done by Millar *et al.* (2002) focused on other aspects and did not pursue the reasons for the delta trap being superior to the two-sided scale trap. The South African study followed up on this, also using the delta trap for pheromone monitoring. The latter study included a technique to determine the infestation levels in the vineyards. Furthermore it was demonstrated that the vine mealybug male trap catch numbers positively correlated with the female population levels in vineyards (Walton *et al.*, 2004). Subsequently, an action threshold of 65 males per trap per two-week period was proposed (Winetech, 2004).

The University of California Cooperative Extension program proposes the use of the three dimensional delta traps (red in colour) as it provides increased adult male vine mealybug catches and lower “unwanted” insect catches as compared to flat traps (UOCCE, 2003). In South Africa, Winetech (2004) (Wine Industry Network of Expertise and Technology) also proposes the use of the delta trap, in this case yellow. The yellow scale card (the two-sided sticky trap used in the Millar *et al.* (2002) study) and a white scale card are recommended for

monitoring of the citrus mealybug, *Planococcus citri* and the grape mealybug, *Pseudococcus maritimus* (ISSA, 2002).

The aim of this current project was to investigate whether the yellow scale card and white scale card would be as effective as the yellow delta trap for monitoring the vine mealybug in vineyards. As usage of the delta trap is the norm in the industry for monitoring vine mealybug, and the male trap catch figures have been positively correlated with female vine mealybug infestation levels (Walton *et al.*, 2004), this present study did not verify trap design comparison results with infestation levels. Evaluating the effect of the colour of the traps was not an objective in the study.

4.2. Materials and methods

The two alternative trap designs used were the yellow two-sided non-disposable scale card and the white disposable scale card.

For this part of the study, three treatments with six replications each with the Insect Science (ISSA) pheromone were used. The first treatment, the delta trap with sticky liner (T3 in Table 2.2, Figure 2.7) was the standard. The second treatment was the two-sided yellow scale card (T4 in Table 2.2, Figure 2.8) with stickiness on both sides. The third treatment was the white scale card with two sticky outside surfaces (T5 in Table 2.2, Figure 2.9). The white scale card has a hollow inner area created once the card has been prepared for usage. The procedures for preparation, installation and servicing of traps and the experimental design and data analysis were described in Chapter 2.

4.3. Results

The trap design comparison experiment used the experimental ISSA pheromone lure formulation with each of the three trap designs (Table 2.2). Since the ISSA lure was shown to produce results inferior to that with the commercially available pheromone lure, all qualitative and quantitative analyses were compared to the results obtained with the delta trap with commercial pheromone lure formulation (T2 in Table 2.1) as standard.

For both grape types, the male vine mealybug trap catch for the first 12 datasets and the total number of data sets (16) were summed and the ratio between the sums calculated (Table 4.1). The distribution of numbers of male vine mealybug captured in the wine grape vineyards at Hartenberg was more evenly distributed than in the table grape vineyards at Irene. Towards the end of the monitoring season a huge increase in trap catch numbers was observed in the table grape vineyards at Irene (discussed in the previous chapter). Some further results presented for Irene will be based on data of the first 12 datasets only as important trends can only be displayed with the lower population numbers. It will however be evident from the graphs where the smaller dataset was used.

Table 4.1. The ratio of total number of *Planococcus ficus* males trapped between numbers obtained after 12 and 16 sampling periods shown for the different treatments in two grape types.

Treatment	Wine grapes				Table grapes			
	12 datasets	16 datasets	Ratio	n	12 datasets	16 datasets	Ratio	n
T2	19177.75	20888.75	1 : 1.09	96	2352	11343	1 : 4.82	96
T3	10493	12368	1 : 1.18	96	1280.85	10609.85	1 : 8.28	96
T4	1656	1710	1 : 1.03	96	193.2	1509.2	1 : 7.81	96
T5	3083	3408	1 : 1.11	96	686	4065	1 : 5.93	96

Note: T2 = Delta trap + Commercial pheromone lure formulation (PLF); T3 = Delta trap + Experimental PLF; T4 = Yellow scale card + Experimental PLF; T5 = White scale card + Experimental PLF

A significant difference was observed between the trap catch results for the three trap designs obtained with wine grapes at Hartenberg ($F(2,15) = 47.232, p < 0.0001$). A higher number of vine mealybug males were captured with the delta trap (mean = 128.33 ± 15.73 , $n = 96$) than with the yellow scale card (mean = 17.81 ± 2.76 , $n = 96$) or the white scale card (mean = 33.5 ± 4.73 , $n = 96$) (Figure 4.1).

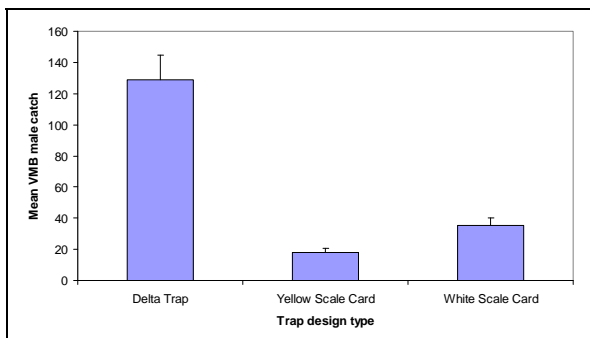


Figure 4.1. Mean (\pm SE) number of *Planococcus ficus* males trapped with three trap design types in wine grapes at Hartenberg.

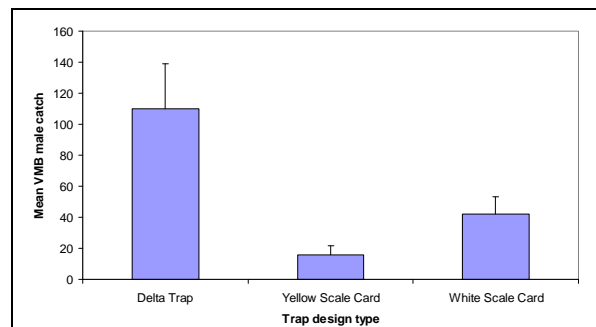


Figure 4.2. Mean (\pm SE) number of *Planococcus ficus* males trapped with three trap design types in table grapes at Irene.

At Irene, a significant difference was also observed between the trap catch results obtained with table grapes ($F(2,15) = 46.694, p < 0.0001$) for the three trap designs. A higher number of vine mealybug males were captured with the delta trap (mean = 110.21 ± 28.49 , $n = 96$) than with the yellow scale card (mean = 15.72 ± 5.75 , $n = 96$) or the white scale card (mean = 42.34 ± 10.93 , $n = 96$) (Figure 4.2).

Trap captures with the commercial pheromone lure in the delta trap can be regarded as the standard for comparison purposes. A quantitative comparison of the male trap catch obtained in wine and table grapes with the experimental pheromone lure formulation in a delta trap and yellow and white scale cards is provided in Figure 4.3.

To do a qualitative comparison of the trap catches obtained with the different trap designs, the trap catch data obtained with the experimental pheromone lure formulation and the three trap designs were again compared to the trap captures obtained with the commercial pheromone lure formulation in the delta trap. The treatment with the highest number of male vine mealybugs captured was selected as the standard and the actual data of the individual traps of the other treatments were standardised to the standard treatment. The mean male vine mealybug trap catch per trap design type per two-week period was determined and presented in Figure 4.4 for wine grapes at Hartenberg and Figure 4.5 for table grapes at Irene. Data for Irene is only shown for the first 11 sampling dates.

Correlation coefficients were determined for various combinations of treatments for data obtained in both wine and table grapes (Table 4.2). Mostly weak positive correlations existed between the combinations of treatments tested for wine grapes at Hartenberg. Strong positive correlations exist between all combinations of treatments tested for table grapes at Irene.

Table 4.2. Correlations (r-values) between vine mealybug trap catches for various combinations of treatments in wine and table grapes.					
		Wine grapes		Table grapes	
Treatment 1	Treatment 2	r	p-value	r	p-value
T2	T3	0.467	<0.0001	0.799	<0.0001
T2	T4	0.196	0.055	0.787	<0.001
T2	T5	0.389	<0.0001	0.877	<0.001
T3	T4	0.381	<0.0001	0.799	<0.001
T3	T5	0.712	<0.0001	0.790	<0.001
T4	T5	0.285	0.005	0.909	<0.001

Note: T2 = Delta trap + Commercial pheromone lure formulation (PLF); T3 = Delta trap + Experimental PLF; T4 = Yellow scale card + Experimental PLF; T5 = White scale card + Experimental PLF

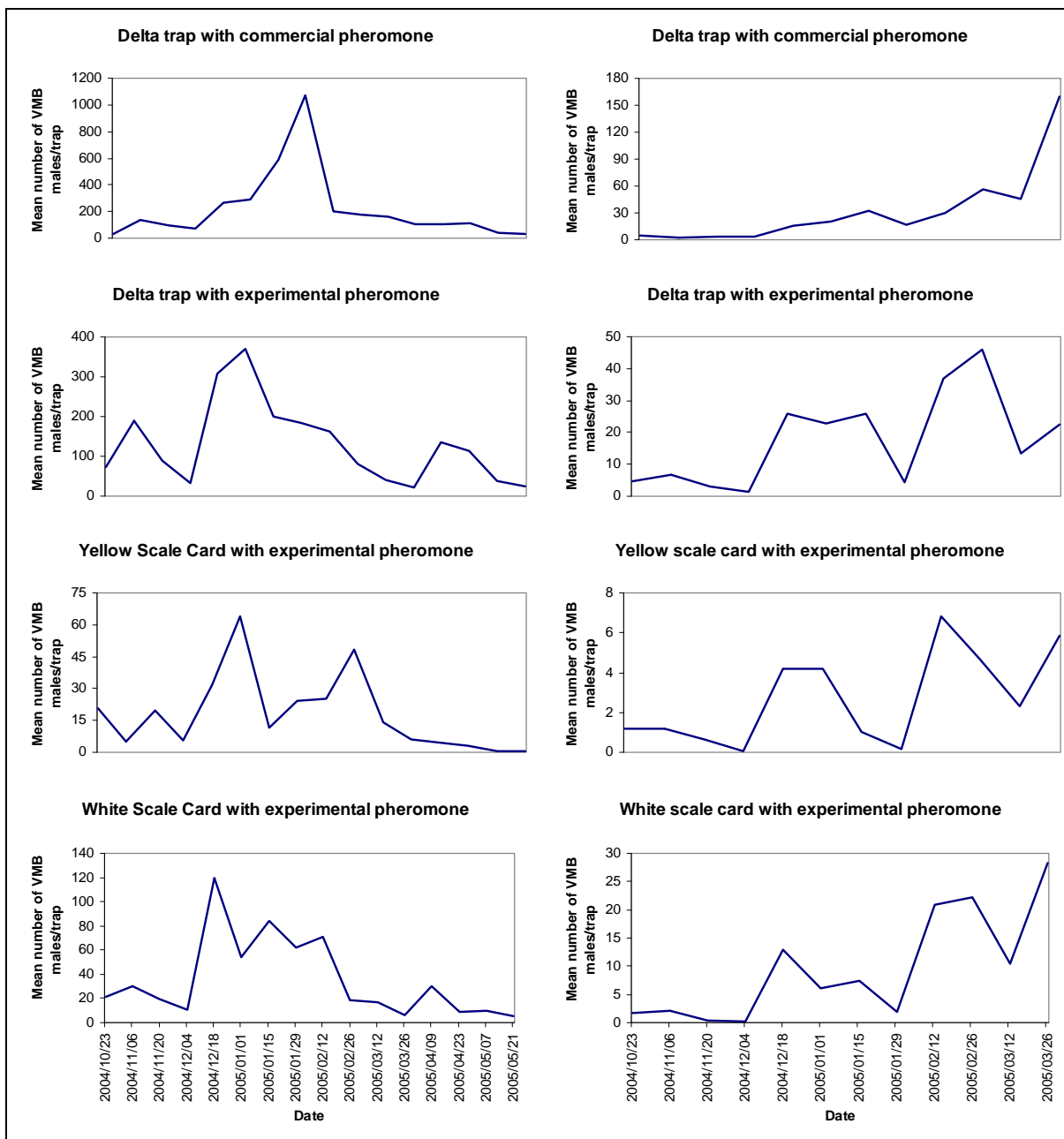


Figure 4.3. Mean number of *Planococcus ficus* males captured with the commercial pheromone lure formulation in a delta trap is contrasted with the mean number of males captured with the experimental pheromone lure formulation with a delta trap, and yellow and white scale cards. It is shown for both wine grapes (left column of graphs) and table grapes (right column of graphs).

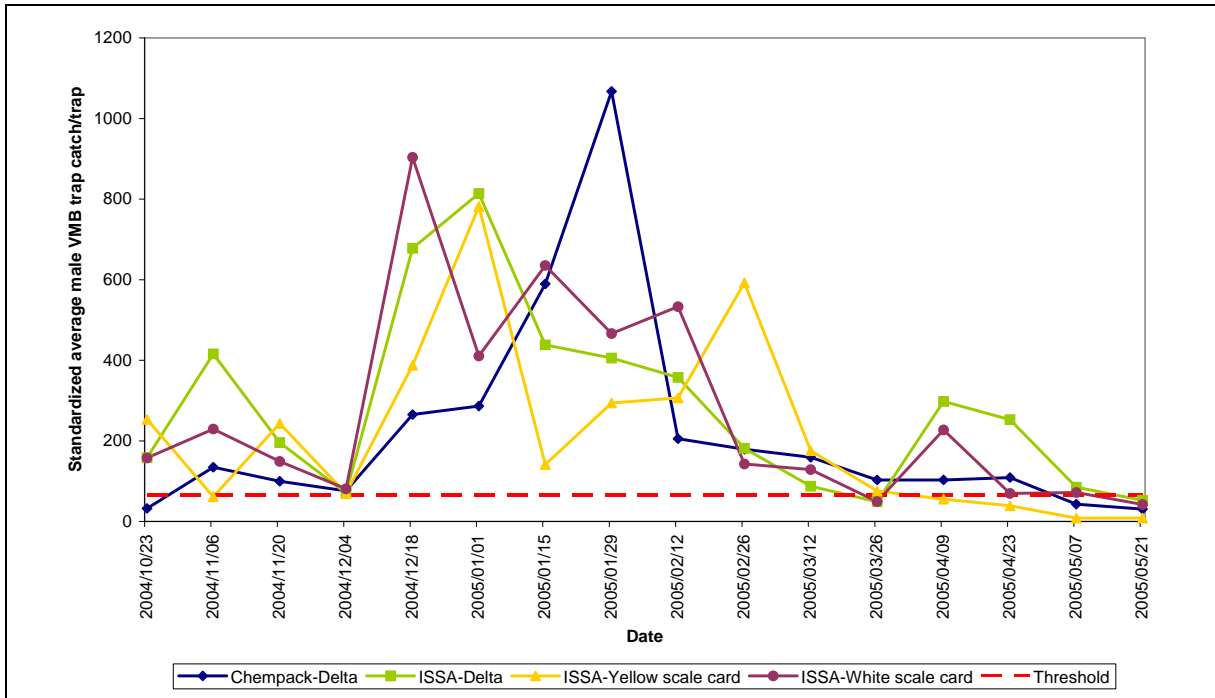


Figure 4.4. Standardised male *Planococcus ficus* trap catch numbers for combinations of different trap designs and pheromone lure formulations for wine grapes at Hartenberg. The dashed line shows the action threshold of 65 males per trap per two-week period.

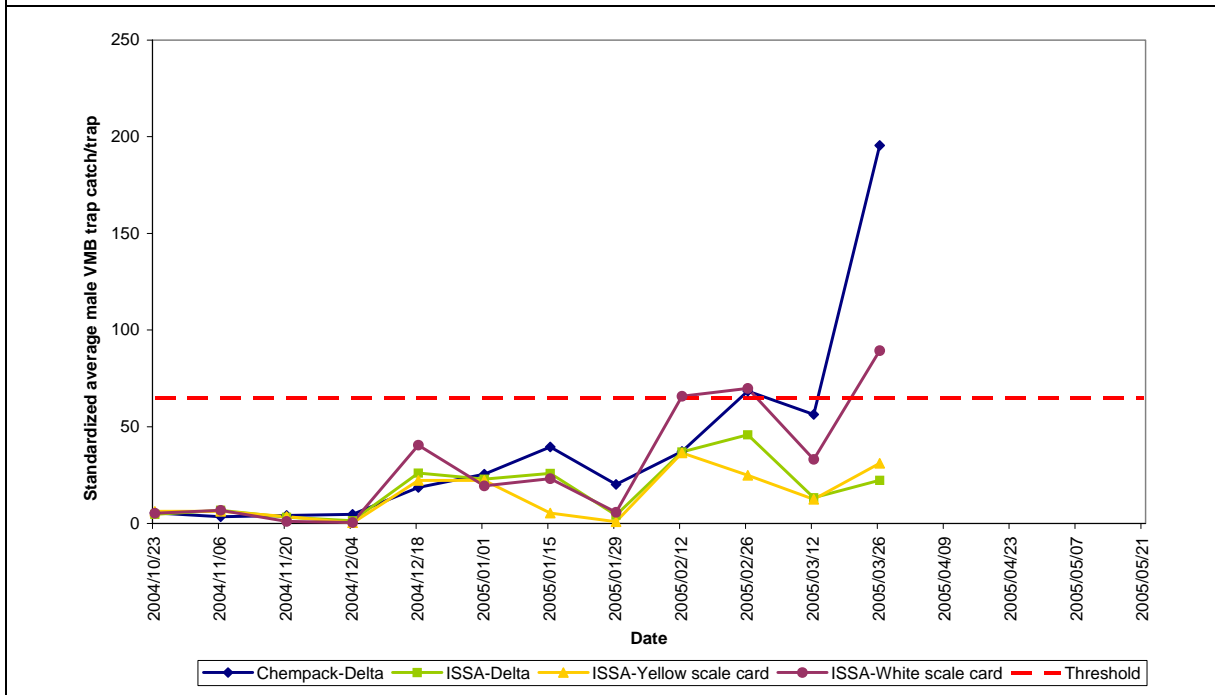


Figure 4.5. Standardised male *Planococcus ficus* trap catch numbers for combinations of different trap designs and pheromone lure formulations for table grapes at Irene. The dashed line shows the action threshold of 65 males per trap per two-week period.

4.4. Discussion

For most of the vine mealybug trapping season, the number of trap catches in the table grape vineyards at Irene was very low (Figure 3.1). It was only after harvest that the population numbers increased to such an extent that it resulted in total trap catch numbers similar to that observed in the wine grape vineyards at Hartenberg (Table 4.1).

In both wine and table grape vineyards, significantly more vine mealybug males were captured with the delta trap than with the yellow and white scale cards (Figures 4.1 and 4.2). When the trap catch figures are standardised to that corresponding with the sticky surface area for the delta trap, the quantitative efficiency of the scale card traps were still inferior to that of the delta trap.

The three trap designs can be evaluated from a quantitative and a qualitative perspective. When looking from the quantitative perspective, two types of differences can be observed, namely that attributed to the two grape types and that attributed to the trap design. The differences in trap catch abundance are evident in the differences in Y-axis scale (Figure 4.3). Previous studies have also shown a quantitative difference between table grapes and wine grapes (Walton *et al.*, 2004) as well as between the delta trap and a two-sided sticky trap (Millar *et al.*, 2002). The difference in trap catch abundance between the grape types can possibly be ascribed to the differences observed in vineyard management and vineyard architecture between the two grape types as described in Chapter 3.

A similar ratio of trap catches was observed for the different trap designs between wine and table grapes. Given the sum of the male vine mealybug trap catch per trap design type in wine grapes, the delta trap captured approximately 7.2 and 3.6 times more males compared with the yellow and white scale cards respectively (Table 4.1). In table grapes, the delta trap

captured approximately 7.0 and 2.6 times more males compared with the yellow and white scale cards respectively (Table 4.1). The sticky surface areas of the three trap design types differ (delta trap = 320 cm²; yellow scale card = 180 cm²; white scale card = 220 cm²). When standardising the trap catch figures to that obtained with the sticky surface area of the delta trap, the delta trap still captured more vine mealybug males. Approximately 4.1 and 2.5 times more vine mealybug were captured with the delta trap than with the yellow scale card and white scale card respectively for wine grapes; and approximately 4 and 1.8 times more than that of the yellow and white scale cards respectively for table grapes.

The difference in trap catch numbers between the three trap designs (Figures 4.1 and 4.2) can possibly be attributed to the positioning of the pheromone lure inside the trap. The pheromone lure capsule is installed within the cavity of the delta trap (Figure 2.7) where it is protected against direct ultraviolet light or rain (Branco *et al.*, 2004) compared to the scale card traps where the pheromone lure is installed in the open above the scale card (Figures 2.8 and 2.9). As the pheromone is released from the rubber capsule, there could be a pheromone concentration build-up in the hollow formed by the delta trap sides. This higher concentration build-up may secondarily impact on the pheromone release rate by altering the pheromone plume that is released through the openings of the delta trap sides. The size, direction and behaviour of the plume is influenced by the cubic size of the hollow within the delta trap, the size and form of the openings in the trap sides, the direction of the openings in relation to the direction of the prevailing winds. The pheromone concentration build-up in the hollow of the delta trap may simulate a possible build-up of pheromone emitted by the female vine mealybug living under the bark of the vine. The pheromone release from the lure capsules in the case of the yellow scale card and white scale card is potentially quicker and much more evenly dispersed with a different plume structure than with the delta trap. This may attribute to the lower trap catches observed with the scale cards.

It was originally anticipated that the yellow scale card and the white scale card would present similar results in male vine mealybug catches. Analysis of the data however showed that the white scale card had a higher quantitative efficiency than the yellow scale card. This can possibly also be attributed to the hollow which is formed between the upturned sides of the white scale card (Figure 2.9). There could also be a pheromone concentration build-up as in the case of the delta trap, albeit in a much smaller area.

In an ideal monitoring trap, catch will always be directly proportional to the surrounding population, so that the catch provides a useful estimate of insect density (Suckling, 2000). Walton *et al.* (2004) showed that delta trap catches can be regarded as a reliable estimate of vine mealybug population size. Quantitatively, the scale cards cannot be regarded as effective alternative trap types for the delta trap.

In order to evaluate the qualitative trap efficiency of the three trap designs, the trap catch numbers obtained with the different trap design types were standardised and superimposed on the same scale for both wine grapes (Figure 4.4) and table grapes (Figure 4.5). Qualitative trap efficiency can be demonstrated by how well the trap catches follow the trend in trap catches as obtained with the standard trap. In the case of the wine grape vineyards at Hartenberg (Figure 4.4), the four trap types collectively show synchronisation in the formation of three peaks in population numbers, namely one spanning the sampling period 23 October 2004 to 4 December 2004, the second large peak from 4 December 2004 to 26 March 2005 and the last peak from 26 March 2004 to 21 May 2005 at the conclusion of the experiment. Within the population peaks however, there is much variation in the trap catch patterns. In the case of the table grape vineyards at Irene (Figure 4.5), there is a discernable similarity in the trap catch pattern. The mostly dissimilar trap catch pattern for trap catches in the wine grape vineyards and the mostly similar pattern for trap catches in the table grape vineyards are confirmed by the series of correlation coefficients determined

between different combinations of treatments for both wine and table grapes (Table 4.2). The difference in trap catch patterns between Hartenberg and Irene can possibly be attributed to the difference in population pressure observed at the two sites. By implication, it seems that the pheromone monitoring of vine mealybug is more successful at lower population pressure than at higher population pressure. It was also suggested that mealybug density may influence the effectiveness of vine mealybug mating disruption programmes (Daane *et al.*, 2006).

The physical structure of the trap has a major effect on the trapping efficiency (Howse *et al.*, 1998 in Zada *et al.*, 2004). In studies with the citrus mealybug, *Planococcus citri* (Hemiptera: Pseudococcidae), Zada *et al.* (2004) observed that trap catches of males in various sticky traps were affected by both trap size and design. In general, they found that plate traps caught more males than delta traps, and large traps more than small ones. Branco *et al.* (2004) studied amongst other things the effect of trap design and trap size on male capture of two pine bast scale species, *Matsucoccus feytaudi* and *M. josephi* (Hemiptera: Matsucoccidae). Catches of *M. feytaudi* males were not affected by trap design, whereas *M. josephi* males were caught in greater numbers in delta traps. In both cases, large traps captured more males than smaller traps. This current study on pheromone monitoring of the vine mealybug did not evaluate trap size, but it did indicate that trap design played a role in trap efficiency.

Daane *et al.* (2005) investigated the effect of trap colour on male vine mealybug trap catches. Standard red pheromone traps were spray-painted (blue, green and yellow) and compared to red traps in a field experiment which were left in the field for a two week period. The least number of male vine mealybugs were captured in green traps while the most were captured in yellow traps, with red traps capturing slightly less than yellow traps. However, trap colour had no significant effect on the number of males captured. Flight activity of the

male vine mealybug seemed to be dependent on both temperature and time of day. The optimum time of flight was determined at 8h52 (during a two-week period in August, the Californian summer season) with the optimum flight temperature estimated at 14.82°C (Daane *et al.*, 2005). Although trap colour had no significant effect on the number of male vine mealybug captured, the sample size may have been too small and the duration of the experiment too short.

Suckling (2000) lists a few trap design and deployment variables which need to be considered when determining an effective monitoring mechanism. It includes lure (substrate and blend), physical shape, colour and durability of the trap, trapping surface, service frequency, position in the crop, and cost. It is advisable that the influence of the more obvious parameters that may influence the efficiency of the trap used for pheromone monitoring of vine mealybug be investigated. These include for example size of the trap with particular focus on size and position of the sticky area, cubic size of the hollow area or cavity, and size of the openings in the sides of the trap.

4.5. Conclusion

In the wine grape vineyards at Hartenberg, the qualitative and quantitative efficiency of the yellow and the white scale cards was lower than that of the delta trap which is currently accepted as the standard trap design for vine mealybug monitoring. In the table grape vineyards at Irene, the quantitative efficiency of the scale cards was lower than the delta trap, but the qualitative efficiency of the scale cards shows a strong positive correlation with the delta trap. Important though, the quantitative efficiency of the white scale card is significantly higher than that of the yellow scale card. This seems to indicate that the trap designs where some sort of a hollow or cavity is formed are more efficient than plate trap designs for monitoring of vine mealybug.

Future research should be aimed at evaluation of trap design and trap size in order to identify the optimal trap design for monitoring of vine mealybug. Further research should also confirm the effect of trap colour on trap catch numbers.

CHAPTER 5: CLIMATIC INFLUENCES ON MONITORING

5.1 Introduction

Climate is often the principal factor limiting the geographical distribution of a particular species population, and variations in climate and its short-term expression, “weather”, have profound effects upon pest abundance. Climatic factors, especially temperature, directly affect the survival, development, reproduction and movement of individual insects and thus the potential distribution and abundance of a particular species. Climate usually sets the limits within which everything else reacts (Cammell & Knight, 1992).

Two important climatic factors affecting the physiology of insects are temperature and moisture. These effects are often interactive (Cammell & Knight, 1992). Insects are poikilothermic, their temperatures approximate to and varying with ambient temperature (Cammell & Knight, 1992; Chapman, 1998). Typically there are upper and lower lethal temperature limits between which the organism survives and within the survival range there is a more restricted range for growth and development and an even further range for reproduction (Cammell & Knight, 1992; Chapman, 1998). Relative humidity tends to modify the effects of temperature (Cammell & Knight, 1992). The upper temperature at which death occurs depends on the species, the duration of exposure and its interaction with other factors, e.g. humidity (Cammell & Knight, 1992). Much work has been done on the effect of temperature on development of insects and it is well established that developmental time decreases with increase in temperature (Ashamo & Odeyemi, 2004).

Humidity may also affect the developmental rates of many species. Optimum conditions for many species lie in the range of 60%-80% relative humidity and insects will often respond to

slight differences in humidity by moving to the preferred humidity where possible. In many species there is a gradual lengthening of development time as relative humidity declines and this may be accompanied by a lower optimum temperature than occurs at higher humidities (Cammell & Knight, 1992).

The number of days required to complete development depends on the insect's temperature-dependent rate curve and the temperature regime it experiences. Development time for a particular species can be expressed more meaningfully in terms of physiological time in which a constant number of heat units between the developmental thresholds are required to complete development (Cammell & Knight, 1992). Physiological time is measured in degree-days ($^{\circ}\text{D}$). One degree-day is equal to one degree above the lower developmental threshold over 24 hours (Zalom *et al.*, 1983). Most methods (averaging, single triangulation, double triangulation, sine, and double sine) used to predict the developmental rates of individual life stages assume a linear relationship between development rates and temperature (Cammell & Knight, 1992; Zalom *et al.*, 1983). This type of model proved reasonably accurate for predicting developmental rates under field conditions, particularly when temperatures are within the most favourable range for development (Cammell & Knight, 1992). Bioclimatic data have been used extensively as the basis for pest forecasting systems since the early 1900's and a wide range of experimental and analytical techniques have been developed (Cammell & Knight, 1992).

The concept of degree-days has a history of more than 270 years. It is attributed to René A. F. Réaumur, the inventor of the Réaumur temperature scale where 0°R is the melting point of ice and 80°R is the boiling point of water (Bonhomme, 2000; Isaacs, 2000; Wang, 1960). Réaumur summed up the mean daily air temperatures for three consecutive months in his locality and found the sum to be a nearly constant value for the development of any plant from year to year. This summation of temperatures, published in 1735, was later

known as Réaumur's thermal constant of phenology (Wang, 1960). He apparently also said: 'The same grains are harvested in very different climates; it would be interesting to compare the sums of heat degrees over the months during which wheat does most of its growing and reaches complete maturity in hot countries, like Spain or Africa..., in temperate countries, like France..., and in the colder countries of the North (Réaumur, 1735 in Bonhomme, 2000; Durand, 1969 in Bonhomme, 2000). Throughout the years many adaptations of the original method was published and the areas of application were extended from botany to entomology, pathology, ornithology, zoology and other disciplines (Bonhomme, 2000; Wang, 1960).

Different methods of calculating degree-days are the result of refinements that have been sought for the calculation of degree-days. Most attempts at improvement have dealt with either sophistications of the calculation procedures (like Baskerville & Emin, 1969) or the inclusion of additional climatic parameters such as solar radiation (Idso *et al.*, 1978), photoperiod (Bonhomme, 2000) and relative humidity (Mashaya, 2001). In terms of growing degree-days relating to plants, edaphic parameters like soil moisture, could also be included to develop the "stress degree day" concept (Idso *et al.*, 1978). Interpretation of the estimated degree-days is often difficult and should be supplemented with precise descriptions of the method used (McMaster & Wilhelm, 1997) like that done by Spencer *et al.* (2000).

Planococcus ficus has the potential to rapidly spread to new areas due to its high reproductive potential. This characteristic is temperature-dependent and can differ according to the macroclimate of different grape-growing regions (Walton & Pringle, 2005). There is currently no known study of the effect of climatic factors on vine mealybug population abundance in grape vineyards. The aim of this study was to determine the relationships between *P. ficus* male trap catches and temperature, relative humidity and rainfall.

5.2 Materials and methods

The male vine mealybug trap catch data obtained with the commercial pheromone lure formulation and the delta trap was used in this aspect of the study (T2, Table 2.1, in Chapter 2). In all cases the data was $\log_{10}(x+1)$ transformed. Repeated measures analysis of variance (ANOVA) was used to compare treatment differences for each sample date for Hartenberg and Irene. Statistical analysis was performed with Statistica, version 7.1. Transformed trap catch data was correlated with the weather factors by means of Pearson correlations.

Daily weather data was obtained for both sites. For Hartenberg, the wine grape vineyard, it included minimum, maximum and mean temperature in °C; minimum, maximum and mean relative humidity (%); and rainfall in mm. Daily data for Hartenberg was available for the full duration of the experiment. For Irene, the table grape vineyard, it included minimum, maximum and mean temperature in °C; minimum relative humidity (%); and rainfall in mm. Daily data for Irene was only available until 7 May 2005. This date coincided with the fifteenth of sixteen sampling dates.

In degree day summations, the influence of the representational quality of the reference temperature used should be considered. The site at which temperatures are measured must be very near the site where observations are made (Bonhomme, 2000). For both experiments in the wine and table grape vineyards, the weather data stations were located within the vineyard blocks where the experiments were conducted. Accumulated degree-days were calculated using the single triangle method described by Zalom *et al.* (1983) (Table 5.1). A lower and upper temperature threshold for the development of *P. ficus* of 16.59°C and 35.61°C respectively, were used (Walton, 2003a; Walton & Pringle, 2005). The number of degree-days required to complete one generation is 235 degree-days (Walton,

2003a). When calculating accumulated degree-days, it is necessary to determine a starting date (known as biofix), i.e. the date to begin accumulating degree-days (Zalom *et al.*, 1983). A starting date of 9 October 2004 was used. To ensure an accurate degree-day calculation, it is preferable that the starting date coincides with the start of the male vine mealybug flight period. In the case of trap catches obtained at Irene, it presumably coincided with the start of the male flight period as only four male vine mealybugs per trap was captured during the first sampling period. At Hartenberg, however, the male flight period presumably started before that date as more than 32 males per trap was captured during the first sampling period.

Table 5.1. Equations for calculating degree-days by the single triangulation method (Zalom <i>et al.</i> , 1983).		
Condition	Equation number	Equation
If $T_{\max} > T_U$ AND $T_{\min} > T_U$	1	$^{\circ}D = T_U - T_L$
If $T_{\max} < T_L$ AND $T_{\min} < T_L$	2	$^{\circ}D = 0$
If $T_{\max} < T_U$ AND $T_{\min} > T_L$	3	$^{\circ}D = [6(T_{\max} + T_{\min} - 2T_L)] / 12$
If $T_{\max} < T_U$ AND $T_{\max} > T_L$ AND $T_{\min} < T_L$	4	$^{\circ}D = [6(T_{\max} - T_L)^2 / (T_{\max} - T_{\min})] / 12$
If $T_{\max} > T_U$ AND $T_{\min} < T_U$ AND $T_{\min} > T_L$	5	$^{\circ}D = \{[6(T_{\max} + T_{\min} - 2T_L)] / 12\} -$ $\{[6(T_{\max} - T_U)^2] / (T_{\max} - T_{\min}) / 12\}$
If $T_{\max} > T_U$ AND $T_{\min} < T_L$	6	$^{\circ}D = \{[6(T_{\max} - T_L)^2 / (T_{\max} - T_{\min})] -$ $[6(T_{\max} - T_U)^2 / (T_{\max} - T_{\min})]\} / 12$
T_{\max} = Maximum daily temperature; T_{\min} = Minimum daily temperature; T_U = Upper developmental threshold; T_L = Lower developmental threshold; $^{\circ}D$ = Degree-days.		

5.3 Results

Trap catch data for the two grape types, wine and table grapes, differed significantly ($F(1,10) = 30.136, p = 0.0003$) (Figure 5.1). Male vine mealybug trap catch numbers in wine grapes at Hartenberg were mostly above the action threshold of 65 males per trap per two-week period throughout the monitoring season. At Irene trap catches were below the action threshold in table grapes until after harvest time which lasted from February to the middle of March.

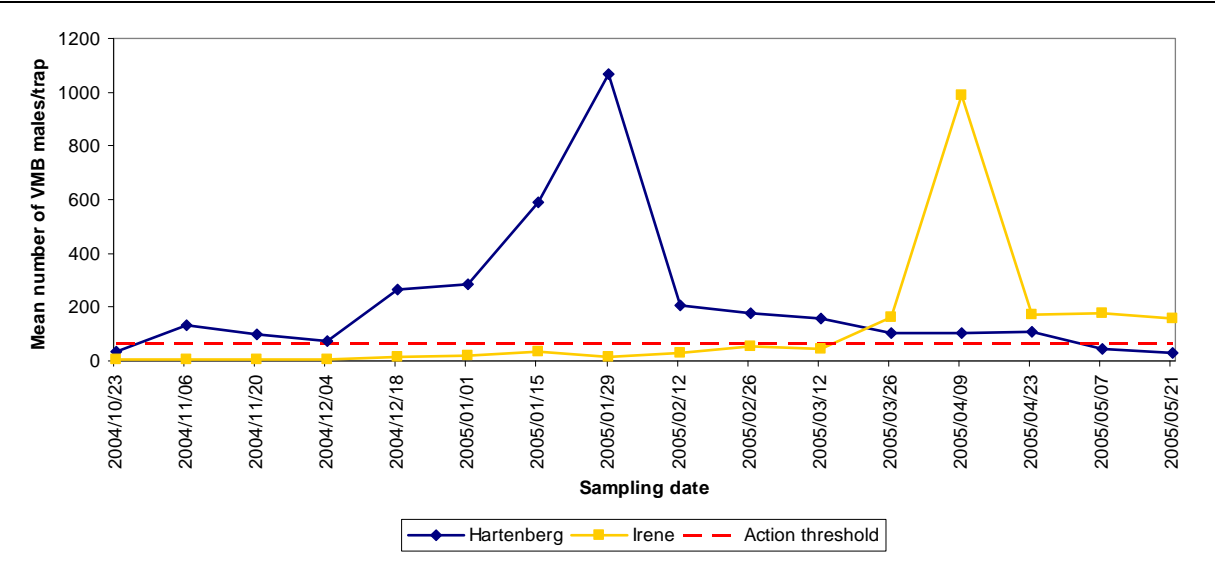


Figure 5.1. Mean male *Planococcus ficus* numbers per trap per sampling period in two locations. The dashed line shows the accepted action threshold of 65 males per trap per two-week period. (The data used were from the male trap catches with the delta trap and the commercially available pheromone, treatment T2).

5.3.1 Temperature

Strong, positive correlations were observed between mean numbers of vine mealybug males per trap and minimum, mean and maximum temperatures (°C) in wine grapes at Hartenberg (Figure 5.2, Table 5.2). Trap catch numbers in table grapes at Irene showed modest to strong positive correlations for the first 12 sampling dates, but changed to weak, negative correlations for the 15 sampling dates for which daily weather data was available (Figure 5.2,

Table 5.2). This change from positive to negative correlations could possibly be ascribed to the population explosion observed in Figure 5.1. Figure 5.3 shows a strong coefficient of determination (R^2) of 0.6476 for wine grapes at Hartenberg, but a negligible R^2 of 0.0546 for table grapes at Irene. Again the low coefficient of determination can possibly be attributed to the sudden population explosion as mentioned earlier.

A comparison of the lower and upper threshold and optimal temperature for development of the vine mealybug in relation to the minimum, mean and maximum temperature as observed at Hartenberg and Irene is provided in Figure 5.4.

5.3.2 Relative humidity

A modest to strong, negative correlation existed between mean numbers of males per trap and minimum, mean and maximum relative humidity in wine grapes at Hartenberg. However, a modest positive correlation existed for the same factors in table grapes at Irene (Figure 5.5; Table 5.2). The correlation coefficients between trap catch and temperature are almost the inverse of correlation coefficients between trap catch and relative humidity. This corresponds to the nature of the relationship between temperature and relative humidity.

5.3.3 Rainfall

The relationships between mean male vine mealybug trap catch and rainfall observed at Hartenberg and Irene are shown in Figure 5.6. The total precipitation over the whole period was 247 mm and 285 mm at Hartenberg and Irene respectively. Precipitation at Hartenberg was more evenly distributed than at Irene, where there were prolonged periods without precipitation. No meaningful correlation coefficients (Table 5.2) were observed between male trap catch numbers and rainfall for either of the two locations.

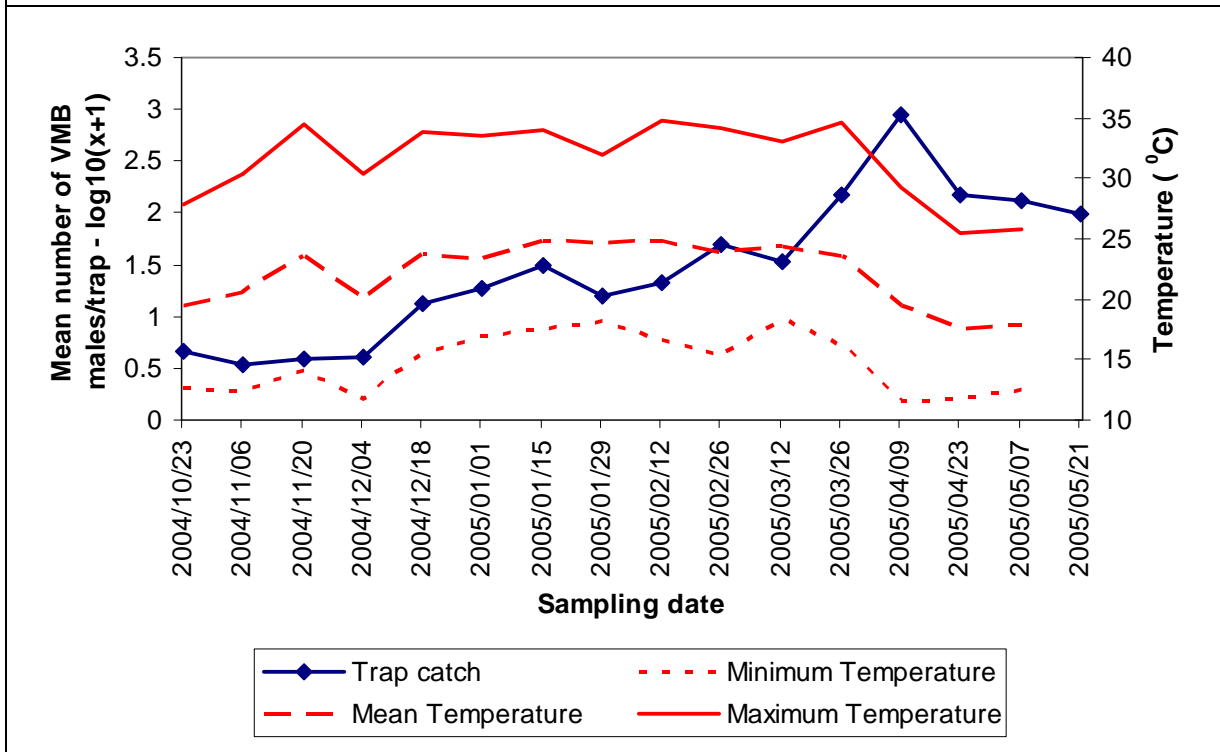
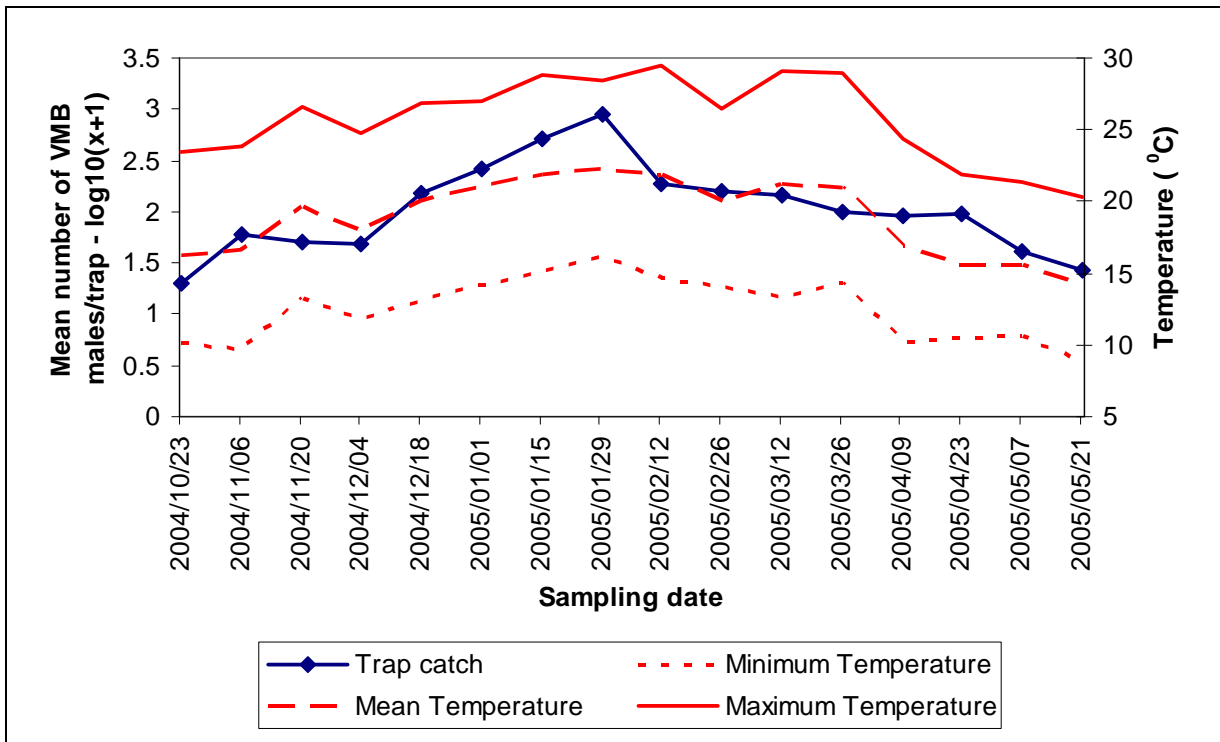


Figure 5.2. Mean male vine mealybug trap catch numbers and minimum, mean and maximum temperature (°C) per sampling date for wine grapes at Hartenberg (top) and table grapes at Irene (bottom).

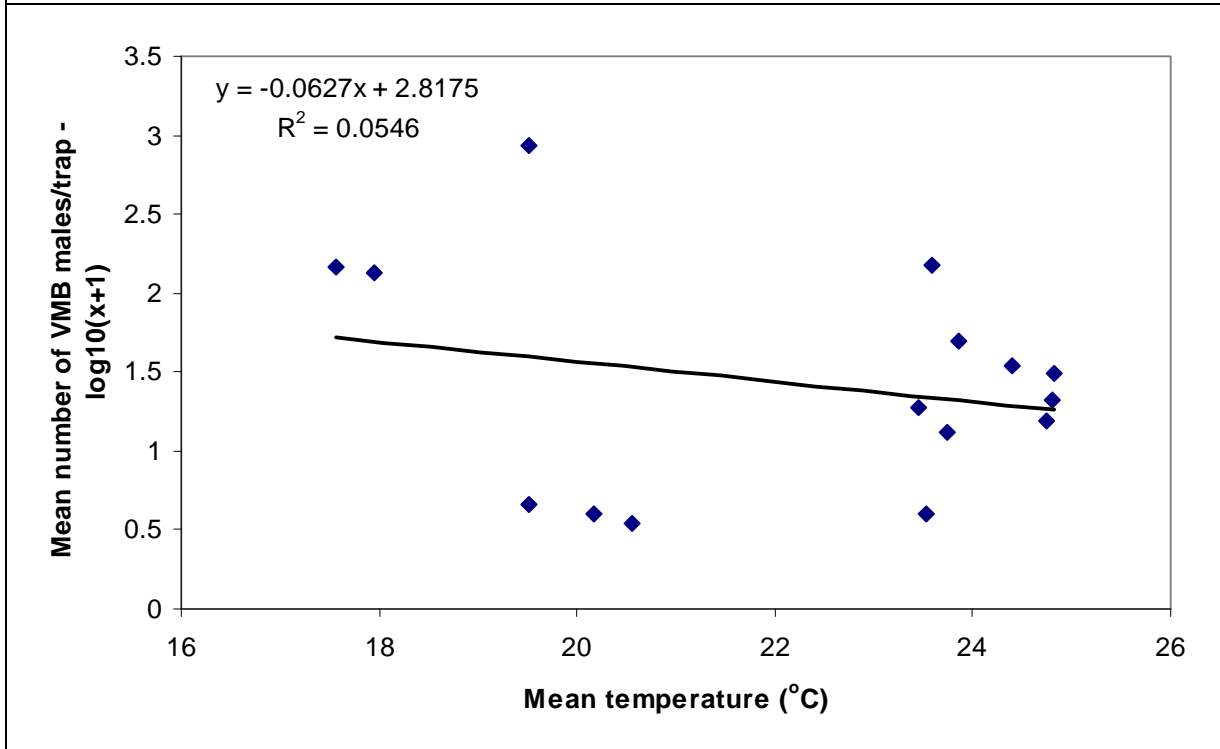
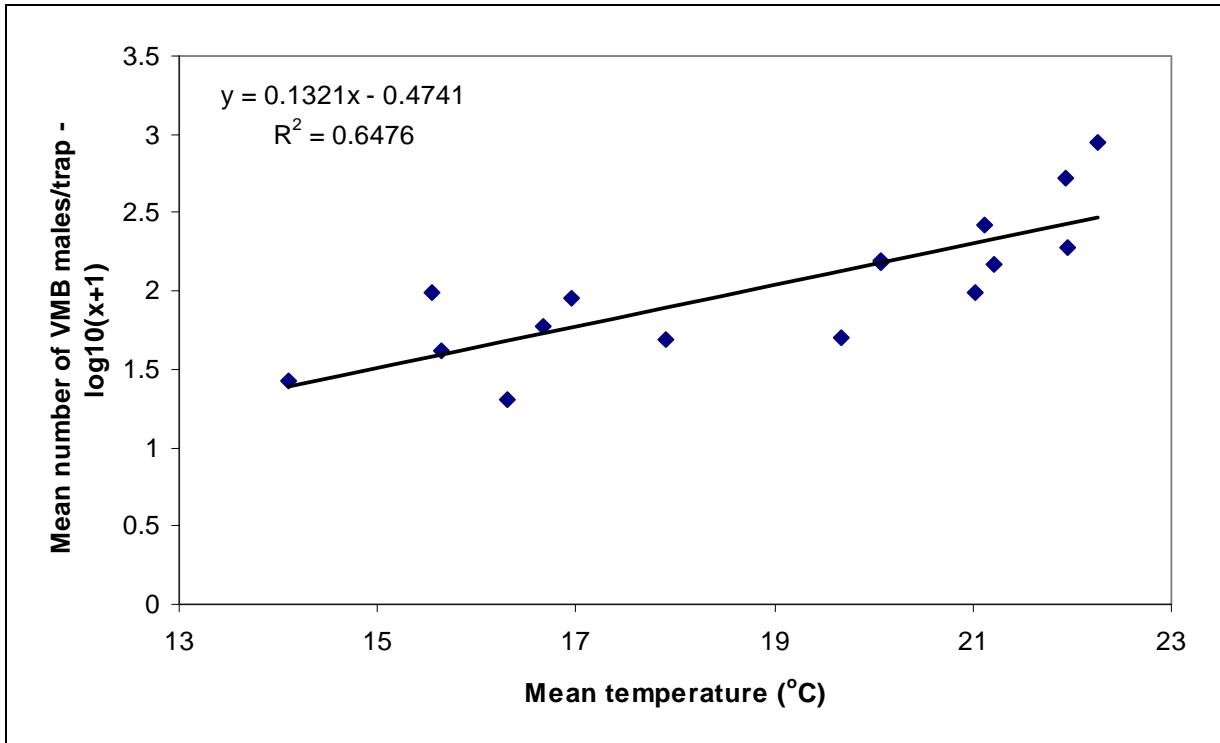


Figure 5.3. Relationships between mean male vine mealybug trap catch numbers (transformed) and mean temperature ($^{\circ}\text{C}$) for wine grapes at Hartenberg (top) and table grapes at Irene (bottom).

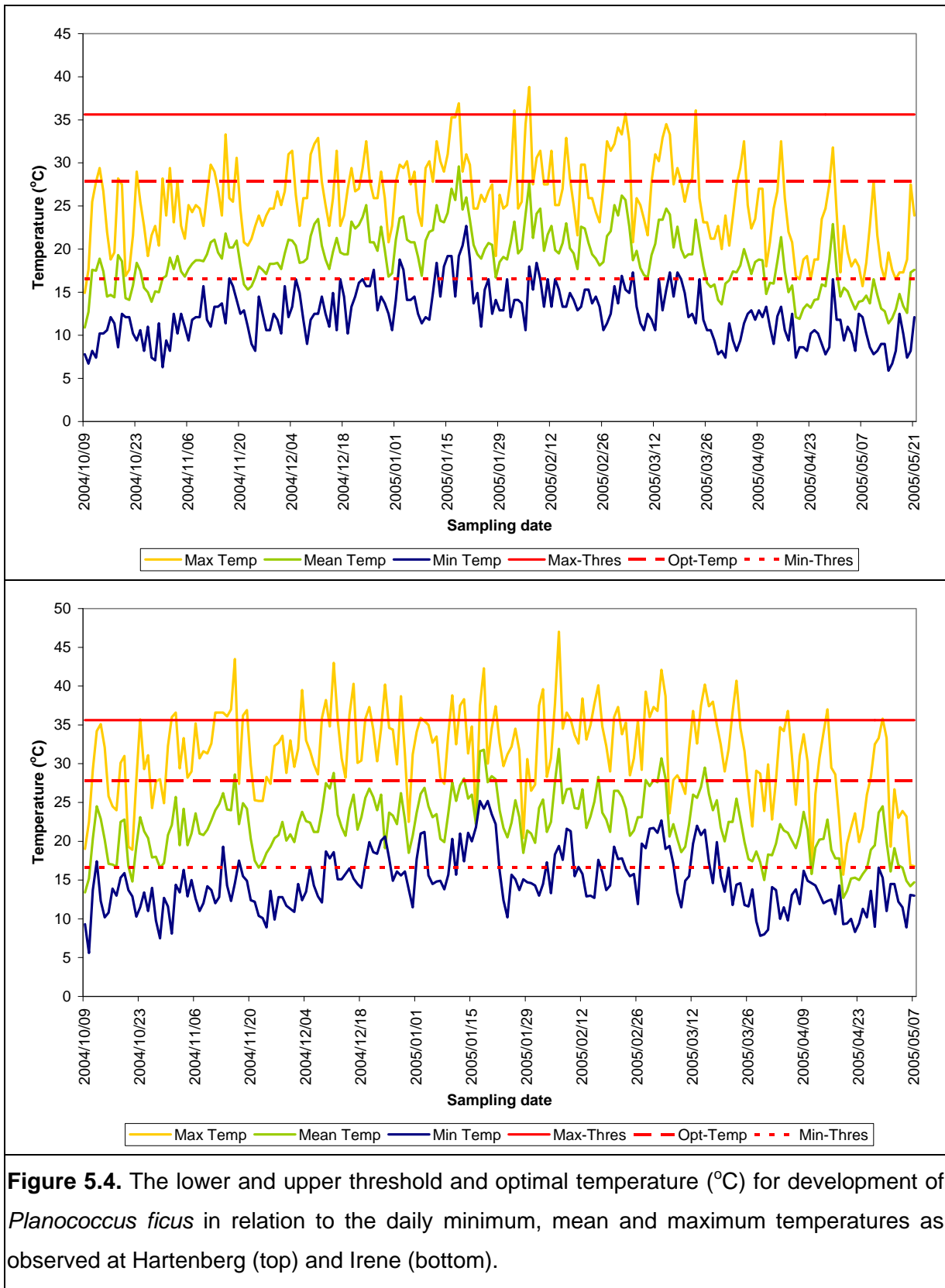


Figure 5.4. The lower and upper threshold and optimal temperature (°C) for development of *Planococcus ficus* in relation to the daily minimum, mean and maximum temperatures as observed at Hartenberg (top) and Irene (bottom).

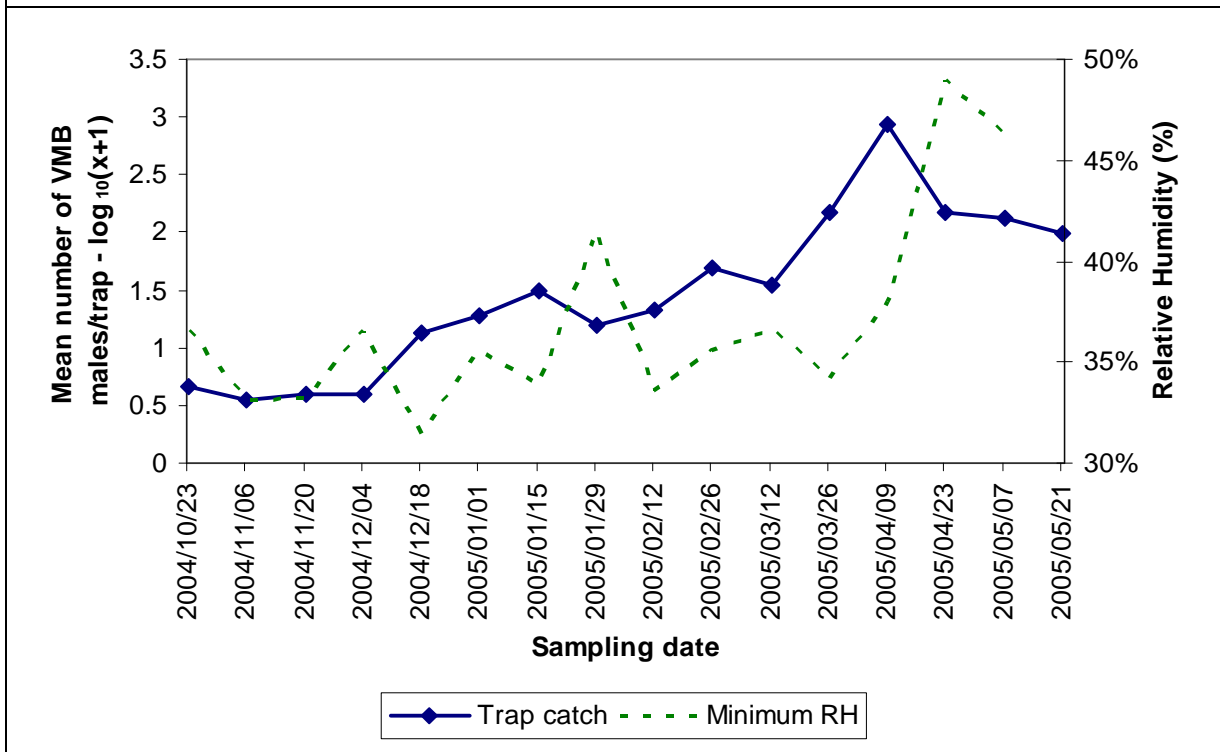
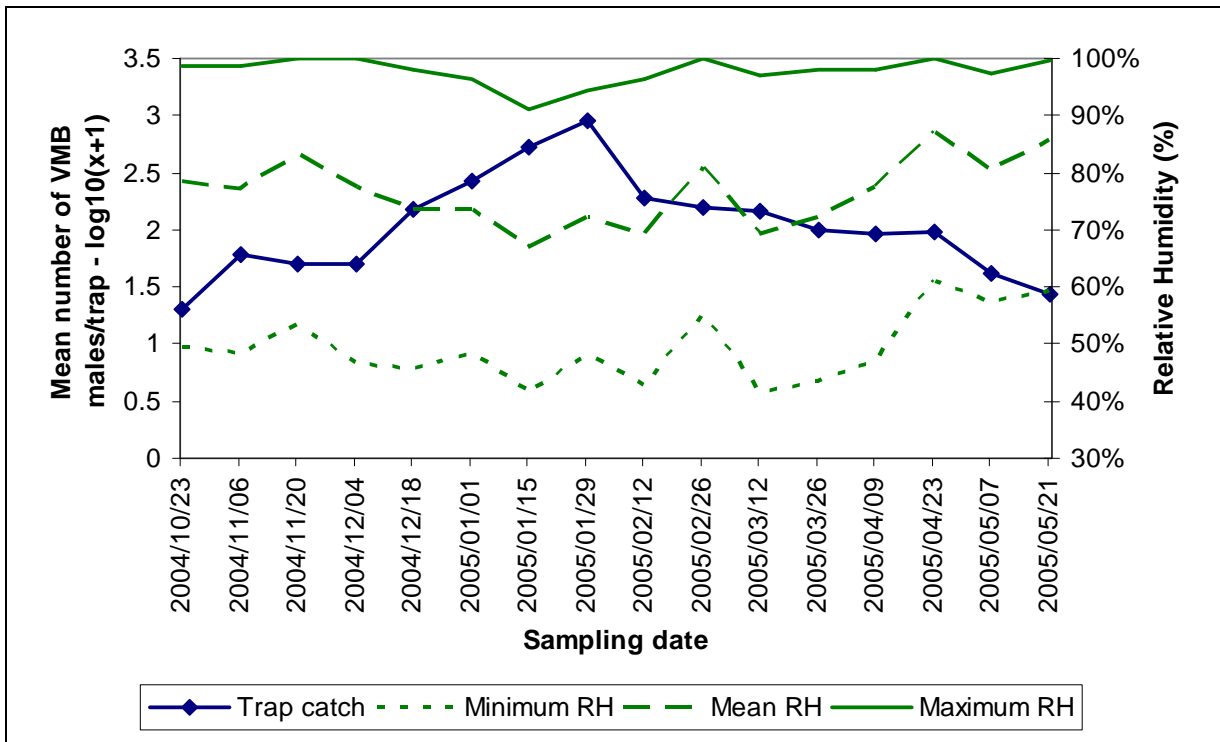


Figure 5.5. Mean male vine mealybug trap catch numbers and minimum, mean and maximum relative humidity (%) per sampling date for wine grapes at Hartenberg (top) and table grapes at Irene (bottom).

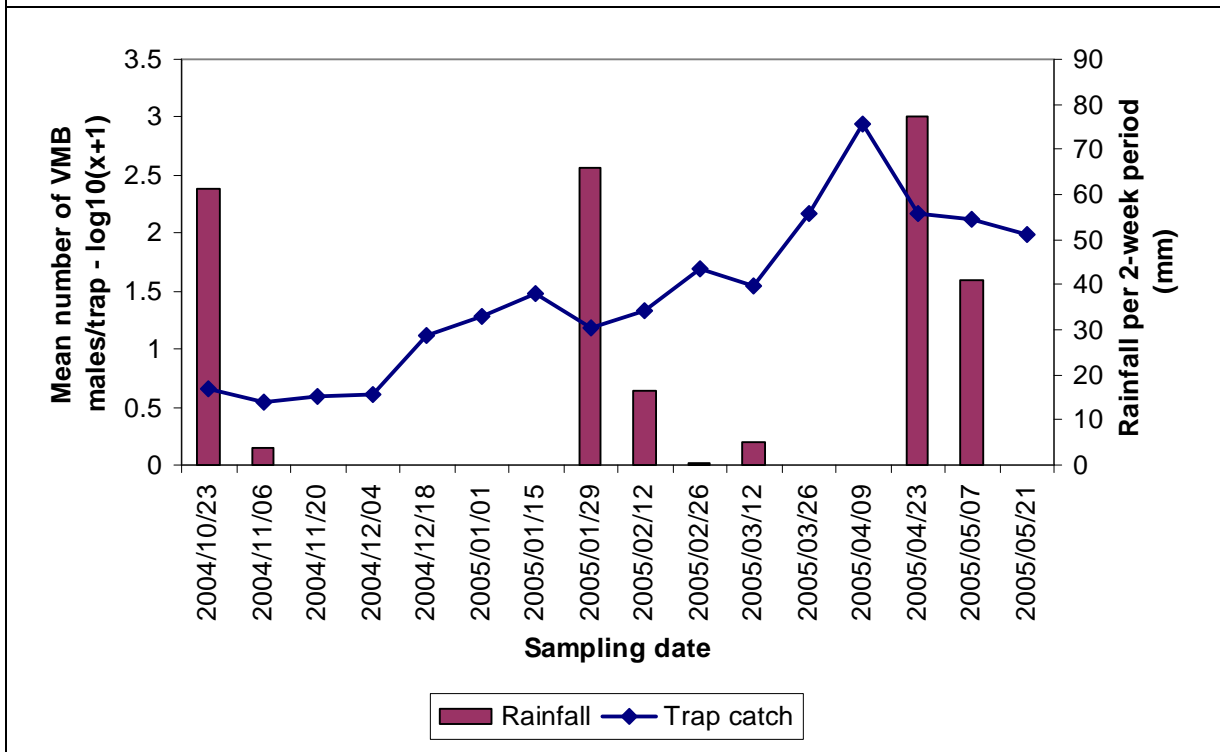
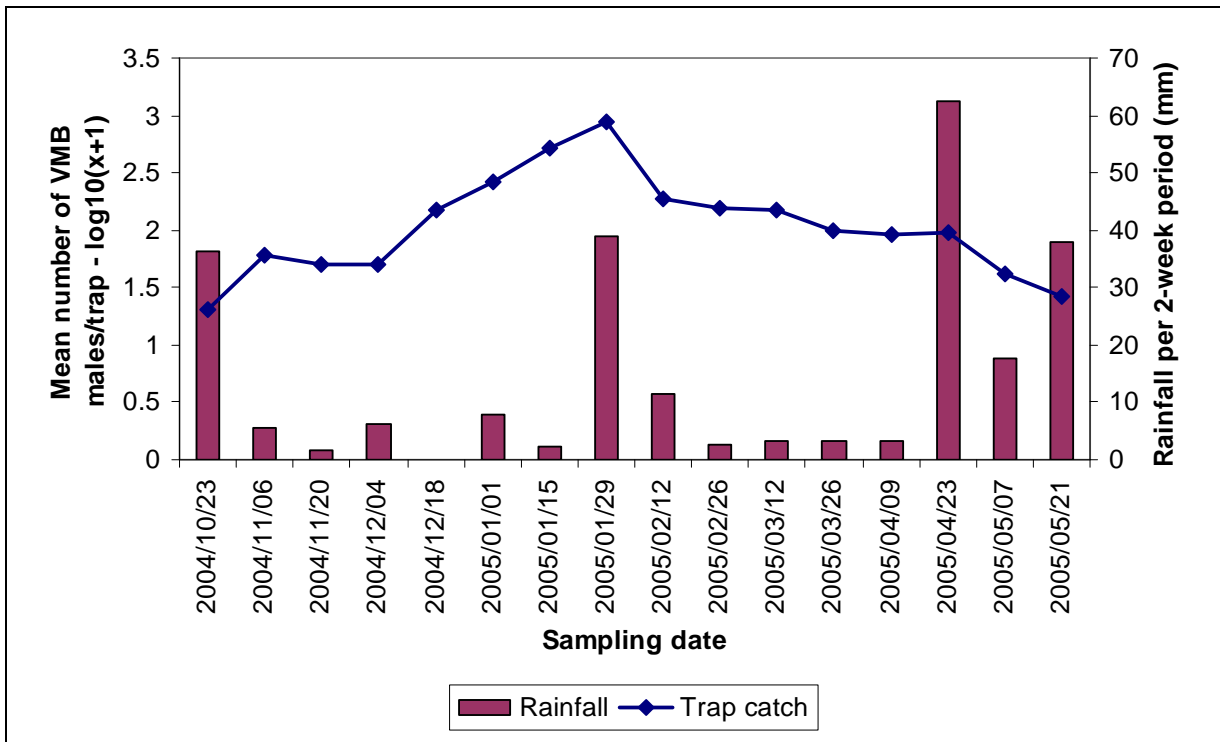


Figure 5.6. Mean number of vine mealybug males trapped and total rainfall (mm) per two-week period in wine grape vineyards at Hartenberg (top) and table grape vineyards at Irene (bottom).

Table 5.2. Correlations (r-values) between numbers of vine mealybug males trapped and temperature, relative humidity and rainfall in two vineyards.

	Hartenberg (wine grapes)	Irene (table grapes)	
	r (n=16)	r (n=12)	r (n=15)
Temperature			
Minimum	0.832	0.701	-0.065
Mean	0.805	0.663	-0.234
Maximum	0.719	0.638	-0.245
Relative humidity			
Minimum	-0.452	0.019	0.460
Mean	-0.647	-	-
Maximum	-0.741	-	-
Rainfall			
Rainfall	-0.139	-0.220	0.066

n = number of sampling dates

5.3.4 Degree-days

From the onset of the experiment the number of degree-days for the development of *P. ficus* accumulated gradually at Hartenberg, but rapidly at Irene (Figure 5.7). Contrary to that, the cumulative male vine mealybug trap catch for Hartenberg increased much more rapidly compared to the trap catch at Irene. The correlation coefficient between trap catch and mean degree-days per two-week period corresponds well with that observed between trap catch and temperature (Tables 5.2 and 5.3).

The correlation coefficients between trap catch and cumulative degree days were very weak for Hartenberg, and very strong for Irene. This probably has to do with the fact that the high trap catch peak at Hartenberg was present much earlier in the season than at Irene.

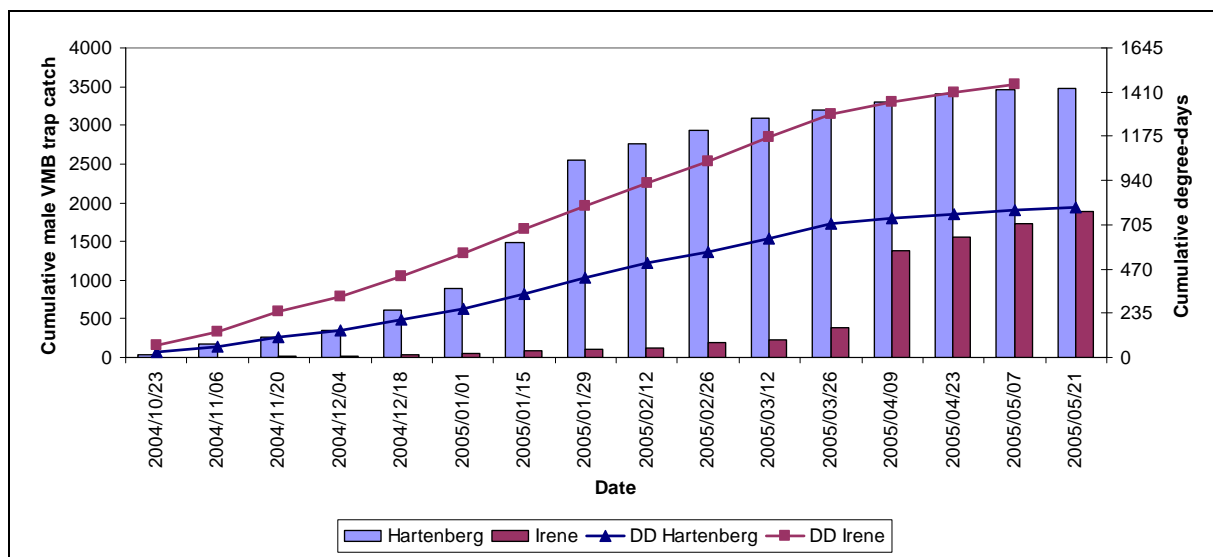


Figure 5.7. Cumulative *Planococcus ficus* male trap catches per sampling period and cumulative degree-days (DD) at Hartenberg and Irene for the duration of the study.

Table 5.3. Correlations (r-values) between numbers of vine mealybug males trapped and mean degree-days per two-week period and cumulative degree-days in two vineyards.

Degree-days	Hartenberg (wine grapes)		Irene (table grapes)	
	r (n=16)		r (n=12)	r (n=15)
Mean	0.800		0.757	-0.165
Cumulative	0.042		0.925	0.916

n = number of sampling dates

At Hartenberg, a strong negative correlation ($r = -0.808$) was determined between mean degree-days per two-week period and mean relative humidity.

The estimated numbers of vine mealybug generations at Hartenberg and Irene using the calculations method of Zalom *et al.* (1983) are shown in Figure 5.8. Hartenberg potentially had four generations, and Irene potentially had seven generations. Such a high number of generations for vine mealybug is not uncommon. Walton (2003a) observed up to six and five generations for the 1999-2000 and 2000-2001 seasons respectively.

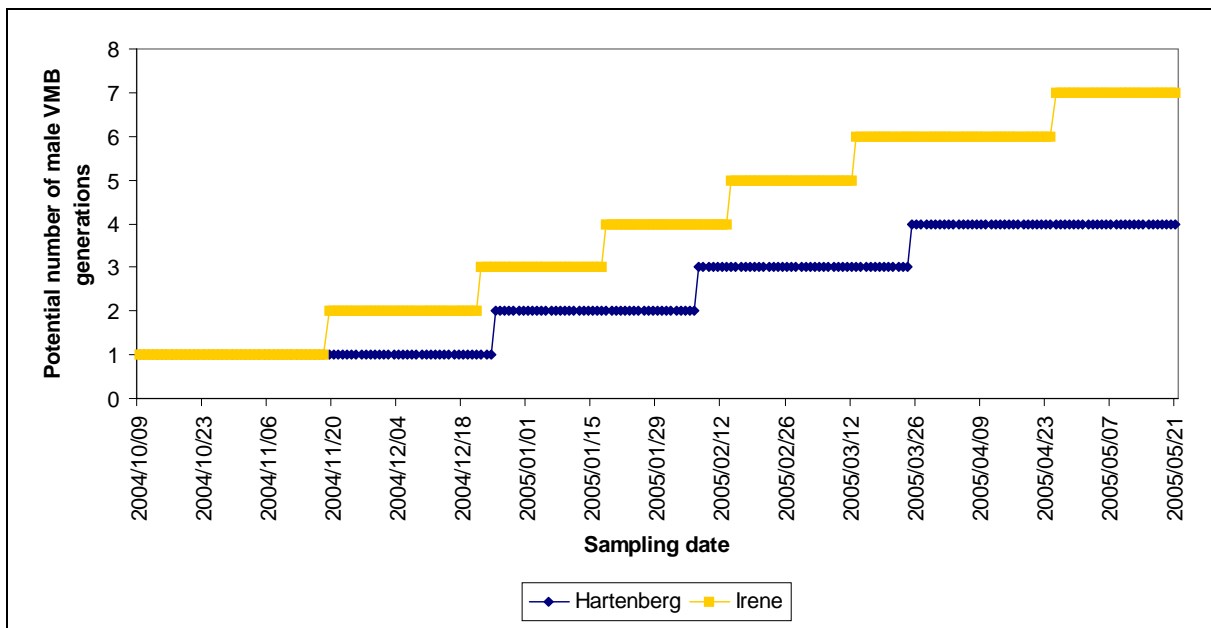


Figure 5.8. The number of potential vine mealybug generations over the period of the study for Hartenberg and Irene.

5.4 Discussion

The male vine mealybug trap catch peaks recorded in the wine grape vineyards at Hartenberg (Figure 5.1) are apparently typical of male vine mealybug trap catch trends (Venter, 2004). This trend was also evident during the 2003-2004 season (Figure 3.2). At Irene, until harvest time, the trap catch numbers were maintained below the action threshold for the 2004-2005 season as well as the previous season (Figures 5.1 and 3.2). However, a huge atypical peak in male numbers was observed at Irene at the end of the grape growing season (Figure 5.1). Five observations can be noted. One, for most of the season until post-harvest, the male vine mealybug trap catch numbers were below the action threshold of 65 males per trap per two-week period; this despite the fact that the minimum, mean and maximum temperatures for Irene were respectively 2.2 °C, 3.1 °C and 5.8 °C higher than at Hartenberg for the same period. Two, there was a positive, modest to strong correlation between the trap catch numbers and temperature for the period until the male trap catch peak occurred (Table 5.2). Three, the trap catch numbers increased dramatically when the temperature decreased (Figure 5.2). Four, the high population numbers were only recorded

over a short period of time, a maximum of four weeks (Figure 5.1). Five, the trap catch numbers seem to stabilise on a higher level after the huge trap catch peak than before the peak (Figure 5.1). It is very probable, as will be demonstrated below, that this peak in trap catch numbers at Irene can be attributed to the effects of temperature.

5.4.1 Temperature

The temperatures recorded at Hartenberg fluctuated over a narrower range than at Irene, namely between 5.9 and 38.8 °C as opposed to between 5.6 and 47 °C (Figure 5.4). At Hartenberg, until the end of October 2004 and from the beginning of April 2005, the mean daily temperature was mostly below the lower developmental threshold of 16.59 °C. During this time, little growth and development of vine mealybug would occur. However, for the period November 2004 to March 2005, the maximum daily temperature exceeded the upper development threshold of 35.61 only 5 times. The thermal ranges experienced per day were optimal for completing the development cycles of vine mealybug, as is evident in the trap catch peak in Figure 5.1 and also the strong, positive correlation between trap catch numbers and minimum and mean temperature (Table 5.2, Figure 5.3). The fact that only four potential generations of vine mealybug were observed at Hartenberg (Figure 5.8) could be due to the narrow thermal range observed. At Irene, until the middle of October 2004 and from approximately the middle of April 2005, the mean daily temperature was often below the lower developmental threshold of 16.59 °C. For 73% of the duration of the experiment, the daily minimum temperature was below the lower threshold. As at Hartenberg, it could be assumed that during this time little or no development would occur. Until the middle of March 2005, for 25% of the duration of the experiment, the daily maximum temperature was above the upper threshold. At temperatures above the upper threshold, a reduction in fecundity, egg hatch and survival may occur. Minimum temperatures too close or too far from an extreme maximum may result in that maximum causing greater mortality than it would if it were preceded by a minimum of intermediate distance (Cammell & Knight, 1992). This may

in part explain why trap catch numbers below the action threshold of 65 male vine mealybug per trap per two-week period were observed. Unfavourable hot weather conditions may restrict population growth and thus delay or prevent pest outbreaks (Cammell & Knight, 1992). From the end of March 2005, the mean daily temperature was lower (Figure 5.2) and often decreased below the lower threshold temperature (Figure 5.4). The lower temperatures cancelled out suppressed development and subsequently resulted in the trap catch “explosion” that was observed. Trap catch numbers after the post-harvest peak were more or less similar to the trap catch numbers observed after the mid-season peak at Hartenberg (Figure 5.1), which may indicate that stabilisation in the vine mealybug population due to more optimal temperatures for development took place. A similar effect of high temperatures was observed on the development of vedalia beetle, *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae), in the San Joaquin Valley, USA (Grafton-Cardwell *et al.*, 2005) and *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae), in a citrus orchard in Southern Brazil (Souza & Carvalho, 2002).

Analysis of daily temperature data in relation to the lower and upper development thresholds may potentially be used to determine when to apply insecticide spraying to control the post-harvest surge of vine mealybug in vineyards in areas where there are extreme temperature ranges like that which occurred at Irene in 2005. However, the same climatic factors that have an influence on the pest insect also have an impact on the natural enemy complex. Davies *et al.* (2004) observed that dry, hot conditions apparently impede successful biological control of *P. citri* by the hymenopteran parasitoid, *Coccidoxenoides perminutes* (Timberlake) (Hymenoptera: Encyrtidae), which is also a parasitoid to *P. ficus* (Figure 1.13). Careful investigation of the practicality of this suggestion thus needs to be done, as a spraying regime introduced soon after harvest could impact negatively on natural enemies. Walton & Pringle (2004a) observed that parasitoid populations peak about one month after the population peak of the host.

5.4.2 Relative humidity

As expected an inverse relationship between temperature (°C) and relative humidity (%) was observed for both vineyards; for Hartenberg, $y = -0.0166x + 0.9224$ ($R^2 = 0.638$) and for Irene, $y = -1.012x + 0.7607$ ($R^2 = 0.644$). It seemed that because of the long exposures to high temperature, humidity had the opposite effect on trap catch numbers (Figure 5.5, Table 5.2) because at low humidities insects are adversely affected by desiccation (Chapman, 1998). Humidity influences egg development in some insect species. Many eggs have to absorb water before they can complete their development. If there is sufficient moisture in the environment to prevent death through desiccation, but not enough for development to continue, the eggs may remain quiescent for some time. At any time during this period development will proceed if more water becomes available (Chapman, 1998). This could possibly have contributed to the suppression of insect numbers at Irene in the pre-harvest period. However, specific research would be required to verify this statement.

5.4.3 Rainfall

From the available data (Figure 5.6, Table 5.2), it seems that rainfall did not influence the male vine mealybug trap catch numbers. However, the 2004-2005 season was regarded as a very dry season, and the vines were water-stressed despite the fact that both vineyards were irrigated (Andrag, 2004; Snyman, 2004). This fact could possibly impact on the number of accumulated degree-days.

5.4.4 Degree-days

The effect of temperature on male trap catch numbers as discussed earlier can be seen in Figure 5.7. Despite the rapid accumulation of degree-days at Irene, the cumulative male trap catch numbers were much lower than at Hartenberg (Table 5.3).

Using only temperature factors in the calculation of degree-days, the number of potential vine mealybug generations amounts to four and seven for Hartenberg and Irene respectively (Figure 5.8). Walton (2003a) indicated that five to six generations could occur in vineyards. The difference in number of generations between areas in Walton's study was not as pronounced as in this present study which observed four and seven generations. As discussed earlier, the temperature ranges between minima and maxima and how often the mean temperature fluctuates around the optimal temperature for development, possibly plays an important role in determining the number of generations per location. Warmer conditions are likely to increase the abundance of some pest species where decreased development times will enable extra generations to occur (Cammell & Knight, 1992).

In insect pest management, degree-day accumulations often form the basis of forecasting models to predict pest outbreaks or the onset of a particular life stage, for example *Lobesia botrana* (Lepidoptera: Tortricidae) in Spain and Greece (Del Tio *et al.*, 2001; Milonas *et al.*, 2001), rice water weevil (Coleoptera: Curculionidae) in Southwestern Louisiana, USA (Zou *et al.*, 2004), and vine mealybug, *P. ficus* (Homoptera: Pseudococcidae) (Walton, 2003a). However, the strong negative correlation found between mean degree-days and mean relative humidity at Hartenberg potentially indicates that degree-day forecasting models need to incorporate relative humidity into the model (Allen, 1976).

Various methods ranging from simple to complex exist to calculate degree-days. Various authors list and compare a number of degree-day calculation methods (Baskerville & Emin, 1969; Higley *et al.*, 1986; McMaster & Wilhelm, 1997; Pruess, 1983; Wang, 1960; Zalom *et al.*, 1983). Methods that are often used are that of Baskerville & Emin (1969, in e.g. Walton, 2003a) and Allen (1976, in e.g. Mashaya, 2001). Comparing the single triangle method (Zalom *et al.*, 1983) as used in this study with two alternative methods (McMaster & Wilhelm, 1997) revealed percentage differences of 31% and -6% for temperature data at Hartenberg,

and -2% and 10% for temperature data at Irene. In the case of Hartenberg, it resulted in a difference from four to five generations of vine mealybug. These percentage differences may be a result of predictive limitations of the linear calculation models when temperatures are around the lower and upper thresholds (Cammell & Knight, 1992) as was often the case at both Hartenberg and Irene. The development curve is sigmoid rather than linear and therefore deviations from linearity are most pronounced towards the temperature extremes (Cammell & Knight, 1992).

Pruess (1983) warns that models that have been developed from limited data, often from a single location, may not hold in other locations. Allen (1976) compared degree-day calculations based on the modified sine wave method for four different geographical areas in the USA. He found that for one particular area where night temperatures tend to be constant the actual degree-days were overestimated. He therefore decided to build a deviation factor into the calculation method to account for such occurrences. This practice limits the general application of his method, but it demonstrates the point that regionality should possibly be considered. The notion of regionality is supported by Wolda (1988) when he stated that in tropical areas degree-day models are denied any role in describing and forecasting the appearance of seasonal insects in a given year because seasonal changes in temperature in such areas are minimal.

Degree-day forecasting serves as a good practical mechanism to estimate the emergence of the next generation of the pest insect and to ensure timely applications of a control measure, e.g. insecticide applications (Dent, 2000). The concept is easy to understand, and grape growers often have temperature (and even relative humidity) data readily available in order to calculate the cumulative degree-days for their vineyards. It may be worthwhile to test the best method applicable to a crop, a pest insect and a region and then to standardise on a method for that crop-pest-region complex.

5.5 Conclusion

Temperature was the climatic factor which influenced male vine mealybug trap catch numbers the most. Generally the increase in trap catch numbers correlated with an increase in temperature. However, given prolonged exposure to very high temperatures and a broad thermal range, an inverse relationship was observed where trap catch numbers increased with decreasing temperatures. Relative humidity had a significant effect on trap catch numbers and the effect thereof should be investigated further. Precipitation did not influence trap catch numbers. Thus, there are times in which weather conditions allow the survival of an insect, but they are unfavourable to its growth, development and reproduction (Cammell & Knight, 1992; Souza & Carvalho, 2002).

Degree-day estimations can be an important instrument in pest management e.g. predicting pest occurrence, scheduling pest management actions, and monitoring pest activity (Zalom *et al.*, 1983). It is however advisable that a particular method of calculation is standardised for the grape-vine mealybug-regional complex. If degree-day forecasting is to achieve its potential for practical applications, some compromise may be necessary between calculation precision and utility (Pruess, 1983).

CHAPTER 6: NEW TRAP DESIGN

6.1. Introduction

Sticky traps with pheromone lures are commonly used for monitoring of scale insect populations. These traps are usually in the form of plate traps (scale cards) or delta traps with sticky liners (Branco *et al.*, 2004; Millar *et al.*, 2002; Walton *et al.*, 2004; Zada *et al.*, 2004). Sticky traps are preferred to funnel or bucket traps to monitor scale insects because tiny males with a wingspan of only a few mm are easier to count on sticky surfaces. However, sticky traps often become saturated with captured insects (both the target insects and other insects) and dust, which decreases the effectiveness of the traps. Delta traps with sticky liners inside provide better protection of the lures against direct sunlight, rain and other weather elements. They may however, have a detrimental effect on the pheromone release by modifying the plume structure (Branco *et al.*, 2004).

Millar *et al.* (2002) reported a positive and significant correlation of male vine mealybug trap catches with female infestation levels on vine stems based on a 5-minute scouting technique developed by Geiger and Daane (2001). Walton (2003a) developed a different scouting technique and also reported a positive and significant correlation of male vine mealybug trap catches with female infestation levels (Walton *et al.*, 2004). The latter study formed the basis for the establishment of the action threshold of 65 vine mealybug males per trap per two-week period (Winetech, 2004).

Optimal trap parameters have not yet been determined for pheromone monitoring of *P. ficus* populations. This is evident in two respects: one, the established action threshold seems not to be conclusive (Walton *et al.*, 2004), and two, the physical parameters e.g. the size or

design of the trap have not been developed or compared experimentally to other designs.

Trap colour has recently been considered as a trap design parameter for vine mealybug and it has been suggested that trap colour has no effect on trap efficiency (Daane *et al.*, 2005). The delta trap suggested by Californian extension workers and researchers is red, while the one used in South Africa is yellow (UOCCE, 2003; Winetech, 2004). Colour could however potentially play a role as male vine mealybug apparently are day-fliers (Labuscagne, 2005; Daane *et al.*, 2005). Although yellow and white traps were evaluated in this study, colour was not a focus of the experiment and cannot be reported on.

Trap size as a factor that could optimise vine mealybug monitoring has also not yet been considered. Millar *et al.* (2002) used a delta trap and a white, two-sided sticky card trap normally used for California red scale, *Aonidiella auranti* (Maskell). They found the delta trap effectiveness to be superior to that of the two-sided sticky card, but they did not provide any suggestions as to the reasons thereof. Differences in size are inherent in the trap design, but that was not the focus of their study. Walton *et al.* (2004) did not use different trap designs and hence did not do any trap size comparisons. Zada *et al.* (2004) and Branco *et al.* (2004) found that large traps captured more citrus mealybug and pine bast scales respectively, than small traps.

Millar *et al.* (2002) used two sticky trap types on the extreme ends of a size continuum for monitoring of *P. ficus*, namely a delta trap with a “large” cavity and a flat plate-like trap without any cavity. The project reported on in this dissertation, evaluated a third trap type (Chapter 4) somewhere in the middle of the continuum which is smaller than the delta trap, closer in nature and usage to the plate-like trap, but also with a cavity or hollow formed (Figure 6.1). It can potentially be postulated that the presence of the cavity plays a role in the

superiority of the delta trap over the plate-like traps.

Since the influence of cavity shape and size, and the shape and size of the openings in the trap on the efficiency of the trap has not yet been studied, an experiment was done to determine if changing the physical design of the trap would affect trap catches.

The objective of the experiment was to evaluate effectiveness of the white scale card trap with the lure placed within the cavity formed between the sides of the trap (as opposed to the lure suspended above the trap) against the delta trap where the lure is also placed inside the trap. The new trap design was named, the “Bubble”-trap.

6.2. Materials and methods

The white scale cards are pre-glued on one side and once the trap is installed the glued side is on the outside. During the latter half of the experiment described in Chapter 4 a pilot study was done to ascertain what the level of trap catch was inside the hollow of the installed trap (Figure 6.1). This was done by putting Flytac on the inside of the hollow of the installed traps. The vine mealybug male trap catch was totaled separately for the inside and the outside of the white scale card and then summed. Data from Irene and Hartenberg was combined for this experiment. The result of this experiment was evaluated to decide whether to perform the following experiment described in this chapter.

The experiment was conducted at Hartenberg, the wine grape estate where the main project was executed. Six vineyard blocks (not the same as for the main project), two with Shiraz, two with Cabernet Sauvignon, one with Pinotage and one with Merlot, were used. The sizes of the blocks ranged from 0.85 to 4.28 ha. All blocks had a history of vine mealybug

infestation. Intra-row spacing was 2.5 m for all the blocks, except one where intra-row spacing was 2 m. The length of each plot was 7 m. Canes were supported by a 5-wire vertical trellis system, and all blocks were drip irrigated. In some of the blocks cover crop were grown. All blocks were prepared during the winter dormant season, and spot sprays of chlorpyrifos was applied during the growing season and at low pressure on those vines where vine mealybug activity was detected.

The experimental design was a complete randomised block arrangement with two treatments and three replications. The treatments were a delta trap with the ISSA pheromone lure formulation and a white scale card also with the ISSA pheromone lure formulation. One trap was placed per block and installed in the middle of the block. The traps were secured in the cordon using the trellis wires for attachment (Chempack, 2002; Winetech, 2004). The material used consisted of three yellow delta traps with white sticky liners in which the experimental pheromone lure from ISSA was used. All details pertaining to the delta trap are as described in Chapter 2. The white scale card was pre-glued on one side. Just before placement of the traps, the un-glued side of the trap was glued with Flytac, a solvent free, non-toxic, non-inflammable water based paste which becomes extremely tacky when dry (ISSA, 2002).

The pheromone lures were stored in a refrigerator at approximately 7°C until they were used. The lures were taken out of the sachets just before use, thereby not allowing for any possible “flash off” effect (Hodges *et al.*, 2004). The same lures were used during the whole experiment which lasted 12 weeks. Twelve weeks is the maximum duration of the lure’s effectiveness (Chempack, 2002; Millar *et al.*, 2002; Walton *et al.*, 2004).

The important aspects during installing the pheromone lures within the cavity were to employ

a mechanism that was fairly easy to use and stable in the wind. Initially, an intricate set of wiring was employed to secure the lure within the cavity. This method was discarded because it was very time consuming to set up and to replace the cards; and also because the capsule was not stable enough in the bubble – it got stuck against the sticky sides of the bubble with the slightest of wind. Thereafter, the mechanism that was deployed involved a little wooden bar with the wire to which the pheromone capsule was secured, going through it. The card was attached to the wooden bar with thumb nails. Although it was still a bit cumbersome to replace the cards, it did keep the sticky sides away from the capsule (Figure 6.2 as contrasted to Figure 6.1).



Figure 6.1. The white scale card with the pheromone lure capsule installed above the card (Photo: M.J. Kotze).



Figure 6.2. The “Bubble”-trap with the pheromone lure capsule installed within the bubble (Photo: M.J. Kotze).

The traps were hung out on 19 February 2005 and were re-randomised every two weeks when the trap was serviced to avoid positional effects (Branco *et al.*, 2004; Flechtmann *et al.*, 2000). The duration of the experiment was 12 weeks and delivered six sets of data.

The data obtained was $\log_{10}(x+1)$ transformed to stabilise the variance (Van Ark, 1981). Repeated measures analysis of variance (ANOVA) was used to compare treatment differences for each sample date. Student t-tests were performed to determine differences at different sampling dates. Statistical analysis was performed with Statistica, version 7.1.

6.3. Results

The trap catch on the inside of the trap with the pheromone lure dispenser above the scale card (Figure 6.1) was 19.8% of the total trap catch for the whole period (Figure 6.3). The vine mealybug trap catch on the inside of the “Bubble” (Figure 6.2) trap amounted to 44% of the total trap catch (Figure 6.3).

Analysing the trap catch figures for the two trap types without taking any influencing factor into account, showed no significant difference in their effectiveness ($F(1,4) = 2.786$, $p = 0.170$). However, there was a significant difference in the effectiveness of the two trap types at different times of sampling (Table 6.1). The experiment was conducted very late in the season and spanned the period from harvest time to vine senescence.

A series of Student t-tests (corrected for possible unequal variances) between the two trap types over the total period of time showed significant differences between trap catches for the two trap types on only one occasion, namely the last sampling date: 30 April 2005 (t-value = -5.2832, $df = 3.2$, $p = 0.0062$) (Figure 6.4).

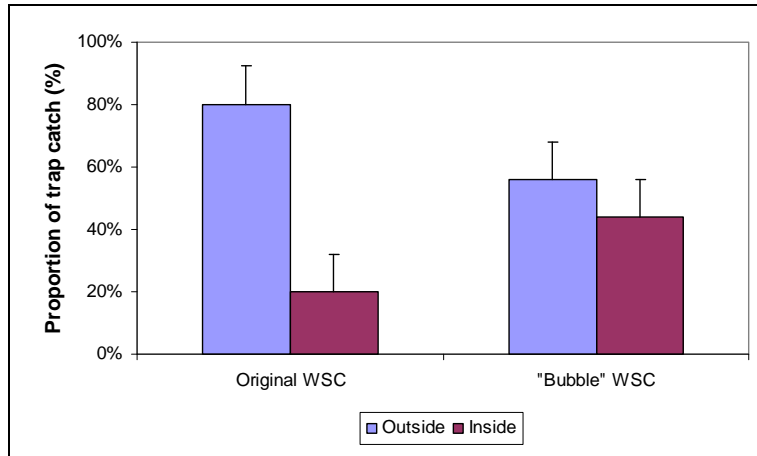


Figure 6.3. The proportion (\pm SE) of vine mealybug trap catch on the inside and the outside of the original white scale card (left) and the "Bubble"-trap (right).

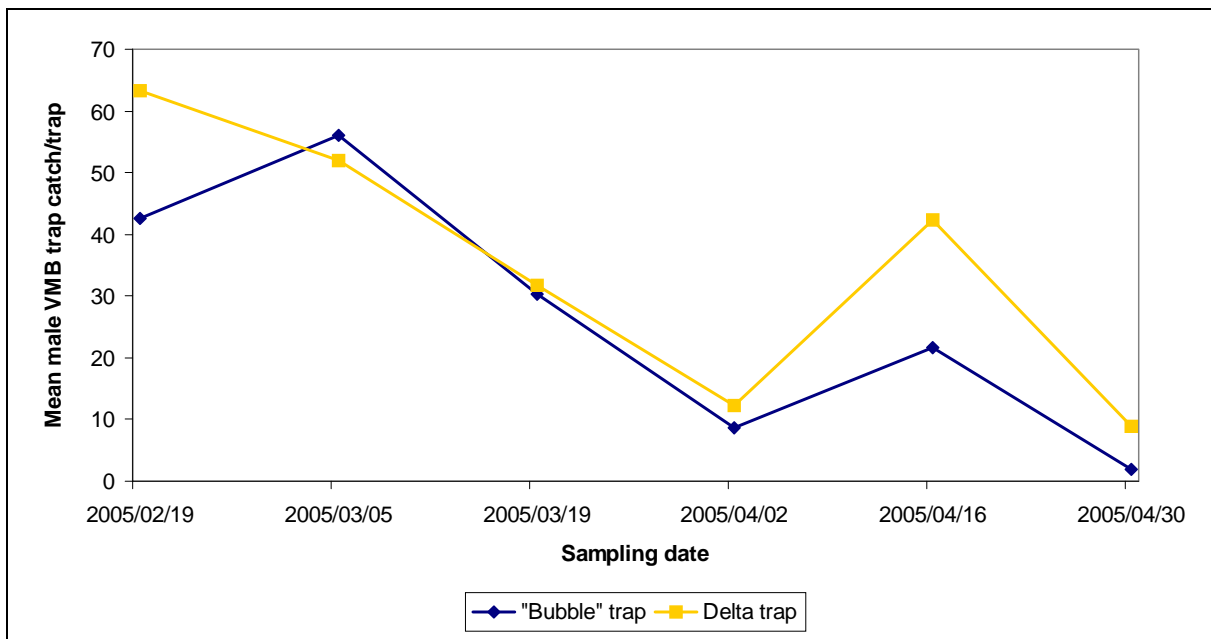


Figure 6.4. A comparison of the mean number of *Planococcus ficus* males per trap per two-week period for the two types of traps.

Table 6.1. Repeated measures analysis of variance with trap type as the categorized factor and with sampling time as within effect (giving six repeated measures).

Factor	Sum of Squares	Df	Mean Squares	F-value	p-value
Intercept	56.18685	1	56.18685	222.4754	0.000118
Trap type	0.70370	1	0.70370	2.7863	0.170395
Error	1.01021	4	0.25255		
Time	4.56803	5	0.91361	4.7023	0.005313
Time*Trap type	0.32325	5	0.06465	0.3328	0.887134
Error	3.88577	20	0.19429		

6.4. Discussion

It was initially anticipated that there would not be a significant difference between the quantitative trap efficiency of the yellow scale card and the white scale card (Chapter 4). Soon after the assessments of the trap catch data started, it became evident that there would potentially be a significant difference in trap efficiency. The situation was carefully observed as more data sets became available. The physical characteristics of the two traps when installed were compared (Figures 2.8 and 2.9). The two most obvious differences related to the colour of the trap and the presence of the hollow or cavity formed between the sides of the installed white scale card. The higher percentage of the male trap catch on the inside of the trap to that on the outside of the trap (44% compared to 19.8%; Figure 6.3), could indicate the importance of the presence of a hollow or cavity inside the trap with the pheromone lure placed inside this cavity.

Although the statistical analysis of the data indicated a similarity in the efficiency of the “Bubble”-trap and the delta trap, it cannot be accepted as a conclusive result. The data was collected over a short period (12 weeks, with only six sets of data), it was late in the season and mainly post-harvest, and the infestation levels were low. The mean number of male

catches per trap service date ranged between 2.0 and 56.0 and between 9.0 and 63.3 for the “Bubble”-trap and the delta trap respectively. The sample size and number of male trap catches, seemed to impact on the quantitative efficiencies of the trap (Cameron *et al.*, 2001). Correlation between the male trap catch figures and the female population levels in the vineyard blocks had not been determined. Despite the aforementioned, the similarity in the quantitative efficiency of the “Bubble”-trap and the delta trap may be indicative of the importance of the size of the cavity or hollow in the trap wherein the pheromone lure is placed. It may also indicate that the colour of the trap is not very important, and thus confirm the findings of Daane *et al.* (2005).

The design of the “Bubble” trap is experimental only. It was not practical to use, but it served the purpose of the experiment. The main purpose of the design was to modify the usage parameters of the existing white scale card trap sufficiently to demonstrate the potential importance of certain trap features like presence of a cavity and size of the cavity. These features should be focused on in a new trap design rather than commercialising the design of the “Bubble” trap.

6.5. Conclusion

The results of the experiment provide a possible answer to why the white scale card is quantitatively a more efficient trap than the yellow scale card. It highlights the importance of the presence and size of a cavity in the trap in which the pheromone lure can be placed. It indicates considerations towards a new trap design that can possibly be a cheaper alternative than the existing delta trap. The best colour trap to use, optimum trap size with optimum size and shape of the cavity in the trap as well as the optimum size, shape and position of the openings in the trap should be studied further. Male trap catches should then also be correlated to female infestation levels in the vineyard.

CHAPTER 7: SUMMARY OF RESEARCH RESULTS

7.1. Summary

In 1979, Miller & Kosztarab wrote: “Monitoring populations of both parasites and scale pests using kairomone and sex pheromone traps in integrated pest management programs is feasible. It is reasonable to imagine effective models that will predict population fluxes of the various scale insect components of a crop system, and to have an integrated management system that will utilize scalcicides, oils, pheromones, pheromone inhibitors, insect growth regulators, kairomones, natural enemies, regulation of the nutritional quality of the host, and other as yet unexplored agents of scale insect control.”

In recent years, pheromones and other semiochemicals have provided tremendous return on the investment of identification and development, by giving researchers, consultants and growers access to cost-effective monitoring systems. The monitoring systems (and the knowledge that they have helped produce) have enabled more effective targeting of all major control tactics including pesticides, biological and cultural control, and biotechnical control strategies such as mating disruption or sterile male releases (Suckling, 2000).

All aspects of integrated management, including the value and validity of sex pheromones as monitoring tools for the vine mealybug, *P. ficus* (Signoret), can be incorporated into a model to define requirements and functions, and to design the implementation of control tactics for this pest. The “Integration Definition for Function Modelling”-technique (IDEF, 1993) could be used for such a model (Figure 7.1). IDEF0 is a modelling language (semantics and syntax) with associated rules and techniques for developing structured graphical representations of a system or enterprise (IDEF, 1993).

During the 1970s, the U.S. Air Force Program for Integrated Computer Aided Manufacturing (ICAM) sought to increase manufacturing productivity through systematic application of computer technology. The need for better analysis and communication techniques for people involved in improving manufacturing productivity was identified. As a result, a series of techniques known as the IDEF techniques which included the IDEF0 modelling technique which is used to produce a "function model" was developed. A function model is a structured representation of the functions, activities or processes within the modelled system or subject area. IDEF0 (Integration DEFinition language 0) is based on SADT™ (Structured Analysis and Design Technique™), developed by Douglas T. Ross and SofTech, Inc. In its original form, IDEF0 includes both a definition of a graphical modelling language (syntax and semantics) and a description of a comprehensive methodology for developing models (IDEF, 1993).

IDEF0 may be used to model a wide variety of automated and non-automated systems. For new systems, IDEF0 may be used first to define the requirements and specify the functions, and then to design an implementation that meets the requirements and performs the functions. For existing systems, IDEF0 can be used to analyse the functions the system performs and to record the mechanisms (means) by which these are done. Currently, the IDEF0 techniques are widely used in the government, industrial and commercial sectors, supporting modelling efforts for a wide range of enterprises and application domains (IDEF, 1993).

The definitions of the five basic elements of IDEF0 are the following: *function*, an activity, process, transformation that must be accomplished; *input*, the data or objects that are transformed by the function into the output; *output*, the data or objects produced by the

function; *controls*, conditions required to produce correct output, the data or objects modelled as controls may be transformed by the function, creating output; *mechanisms*, the means used to perform a function (IDEF, 1993).

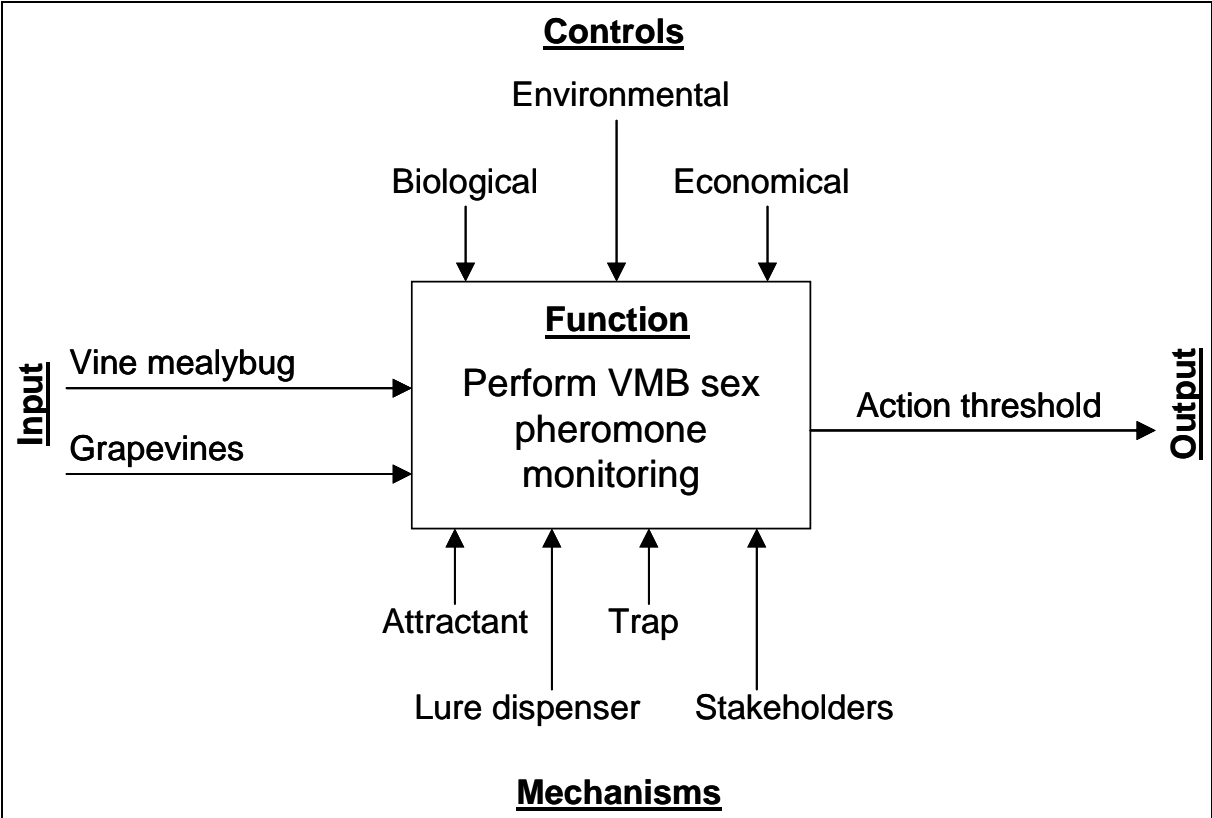
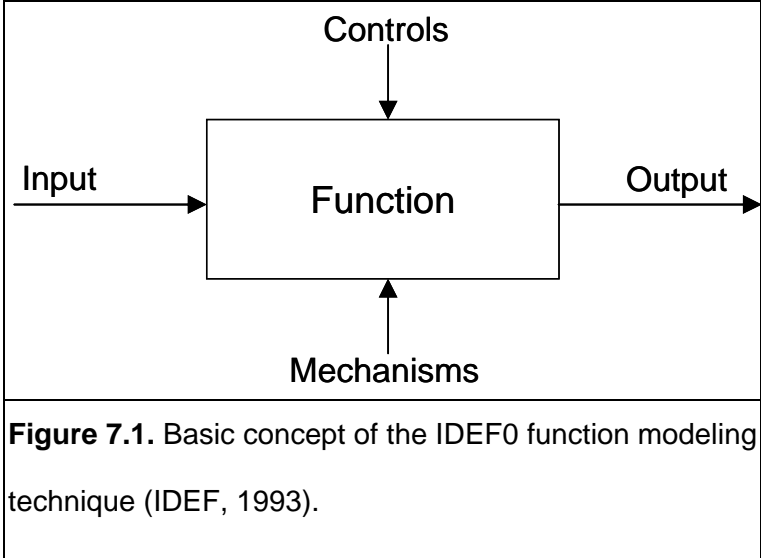


Figure 7.2. Schema for evaluating the validity of using a sex pheromone monitoring system as tool in the integrated management of the vine mealybug, *Planococcus ficus*.

Figure 7.2 shows aspects of the vine mealybug sex pheromone monitoring system that have been mapped onto the generic schema of IDEF0. The discussions that follow will describe the different aspects of this diagramme, i.e. function, input, mechanisms, controls and output.

7.1.1. Function: Performing vine mealybug sex pheromone monitoring

After the isolation and synthesizing of the vine mealybug sex pheromone in 2001, monitoring methods were developed and tested in California and South Africa (Millar *et al.*, 2002; Walton *et al.*, 2004). In South Africa, a protocol was published by Winetech (Wine Industry Network of Expertise and Technology) for the control of mealybugs in vineyards (Winetech, 2004). It can be seen as the “How to”-guide for mealybug control. It covers aspects about cultural control, biological control, ant control, and chemical control. In terms of sex pheromone monitoring, it covers aspects such as when to start monitoring, trap type, trap service frequency, and trap placement, area coverage and optimum distances between traps. It also provides guidance about interpreting the trap catch numbers, and integrating sex pheromone monitoring into a chemical control programme. The monitoring protocol is simple, easy to understand and implementable by grape growers and researchers alike. The experiments reported on in this dissertation were based on this protocol.

Much has been done regarding training and educating grape growers and their workers, and extension workers, in grape growing regions of South Africa and the USA. This is evident in the number of workshops and seminars conducted with growers and publications in agricultural magazines and periodicals. Training and education should not be seen as once-off interventions, and ongoing training and awareness campaigns should be launched and maintained for a number of years to support growers in changing their control practices away from only chemical controls to integrated pest management control strategies. Reaching growers with effective information remains a challenge, whether it is related to pheromone-

based pest management or other tactics (Suckling, 2000).

7.1.2. Input

7.1.2.1. Vine mealybug, *Planococcus ficus*

The vine mealybug is a pest with significant economic impact on the grape growing industry in the world. Once established, it cannot be eradicated, but needs effective management actions to control its impact and spread. Traditionally, chemical control mechanisms are applied, but a vast array of integrated control mechanisms exists that can effectively control vine mealybug infestations. Sex pheromone monitoring became an important part of the vine mealybug management programme.

7.1.2.2. Grapevine, *Vitis vinifera*

The experiments reported on in this dissertation showed a significant quantitative difference in the male vine mealybug trap catch numbers between wine and table grapevines. The same trend was observed in the studies done by Walton (2003a). Up till now, most research focused on establishing a vine mealybug sex pheromone monitoring protocol. The next level of research should include a focus on the grapevine type e.g. wine, table and raisin grapes, and the changes that may be required to the protocol due to the vine and vineyard management practices pertaining to each grapevine type.

7.1.3. Mechanisms

7.1.3.1. Attractant

A sex pheromone was first detected in a coccoid in the red pine scale *Matsucoccus resinosae* in 1966, in *Planococcus citri* in 1968 and in *Planococcus ficus* in 1975 (Miller &

Kosztarab, 1979). In 2001, it was isolated and synthesized for monitoring purposes (Hinkens *et al.*, 2001). Sex pheromones appear to be specific; males can successfully discriminate between females of closely related species such as *Planococcus citri* and *Planococcus ficus* (Miller & Kosztarab, 1979). Because the pheromone is species-specific, no taxonomic expertise would be required to determine whether the trapped insects were vine mealybug, grape mealybug, or other unrelated species, which directly impacts control decisions (Millar *et al.*, 2002). This is a great advantage for enabling independent use of the monitoring system by farmers and extension workers.

Attractants that are highly selective and inexpensive, make them useful tools for pest detection, assessment of pest density to establish pest thresholds, assessment of pest phenology to determine the appropriate timing for pesticide applications, and assessment of insecticide resistance (Knight & Weissling, 1999).

The synthetic vine mealybug sex pheromone's effectiveness and ease of manufacturing also led to studies on mating disruption of the vine mealybug. The interim research results were promising. It seems that a mating disruption programme will be compatible with biological control. Results suggested that mating disruption may not be the most effective tool to quickly lower high density mealybug populations. The longevity of the product delivery method (either in microcapsules or dispensers) still needs to be solved (Daane *et al.*, 2006).

7.1.3.2. Lure dispenser

Lures for use in traps have commonly been formulated on rubber septa or other passive carriers as a practical and cost-effective reservoir for the semiochemical. However, the release rate from many substrates cannot be readily controlled, and changes significantly over time and with temperature (Suckling, 2000). In the pheromone lure formulation

experiment described earlier, the effectiveness of an experimental pheromone lure formulation was field-tested against a commercially available pheromone lure formulation and it was shown to be inferior to the commercially available formulation. The reasons pertain mostly to the physical structure of the dispenser and the material it was made of. These findings will prohibit the registration of the experimental pheromone formulation and more work needs to be done on the type of material the dispenser is made of and the release rate of the pheromone compound from the dispenser.

Synthetic lures can sometimes be developed to out-compete calling females, by offering higher release rates or longer pheromone release periods than used by calling females. In monitoring of various moth species, it has been noted that earlier emergence of males can lead to a changing rate of competition between traps and virgin females. In such a case, the proportion of the male moth population caught in traps is reduced over time, after females emerge (Suckling, 2000). Research needs to be conducted on an ongoing basis to determine whether a changing rate of competition between synthetic lures and virgin female attraction would possibly influence the efficiency of sex pheromone lures for monitoring of vine mealybug.

7.1.3.3. Trap

The delta trap design is currently the accepted trap design for use in vine mealybug sex pheromone monitoring (Winetech, 2004). The effectiveness of the yellow scale card (plate trap) and the white scale card was evaluated under field conditions and was compared with the delta trap in order to investigate a cheaper and easier to use alternative to the delta trap. Quantitatively, both the yellow and white scale cards were shown to be less effective than the delta trap. However, it seemed that qualitatively, when male trap catch numbers are low, there is comparable trap efficiencies between the three trap design types evaluated. The

white scale card was significantly more effective than the yellow scale card. The reason for that seems to lie in the presence of a cavity within the trap where there is potentially a build-up of a higher pheromone concentration than in a trap without this cavity. The comparison results of the “Bubble”-trap with the delta trap, indicated that there was some ground for investigating the role of the cavity in the trap. Further research opportunities lie in the study of the role of the cavity (presence, size and shape) of the trap in the qualitative and quantitative efficiency of the trap design and confirming the effect of trap colour on the trap efficiency.

7.1.3.4. Stakeholders

Various stakeholders are interested in an effective vine mealybug sex pheromone monitoring technique. First and foremost is the grape grower; monitoring will be most effective if the grape grower can conduct all activities of the technique on the farm, and can react quickly to the trap catch results. Counting of the male vine mealybug trapped on the sticky trap liner requires a stereo-microscope and some expertise in identification of the insect. Although the pheromone is species-specific and may not trap other mealybug species, the untrained eye may struggle to distinguish e.g. between vine mealybugs and thrips. The vineyard manager at the table grape vineyard, Irene, was trained in identification of the vine mealybug, and conducted interpretation of trap catch results himself. The vineyard manager at the wine grape vineyard, Hartenberg, has not been trained in identification of the vine mealybug and relies on external expertise. The reliance on external parties for determining trap catch results and the concomitant delay in reacting on recommendations based on these results may impact negatively on the adoption of monitoring systems before applying chemical control.

The next group of stakeholders in the monitoring practices is the extension workers and

organisations like the Agricultural Research Council in South Africa. From a consulting point of view, they can provide advice, monitoring services and expertise in identification of vine mealybug for counting purposes. Their services however, would not come free of charge, and although it may provide a revenue stream to them, grape growers may not see it as a necessity to engage with them in this regard.

Another group of stakeholders is the researchers. Sex pheromone monitoring has established itself as an important practice in integrated pest management. Researchers will always want to refine the techniques to produce an optimum protocol in terms of appropriateness, cost effectiveness and practicality. Ultimately they should endeavor to establish the use of the monitoring technique by the grape growers on the farm. Another research objective is the developing of sound and practical pest outbreak forecasting models. This can only be done from a regional and long term perspective. This is on a much larger scale than the scale on which grape growers work. Vine mealybug sex pheromone monitoring can be an important element in developing vine mealybug outbreak forecasting models.

Detailed stakeholder analysis may be performed in order to rank future research priorities.

7.1.4. Controls

7.1.4.1. Biological

7.1.4.1.1. Host plant resistance

Host plants often show varying degrees of susceptibility to a particular scale insect (Miller & Kosztarab, 1979). Flanders (1970, in Miller & Kosztarab, 1979) suggested that some plants are genetically immune and never susceptible, some fluctuate from immune to susceptible, and some are always susceptible. The plants fluctuating from immune to susceptible have

“pheno-immunity”. This is an environmentally induced, physiological resistance to a particular coccid. He hypothesized that meteorological or edaphic factors can modify the physiology of the plant and alter its immunity to scale attack. Scale populations can also be suppressed by natural enemies and adverse environmental factors. It has previously been observed that certain wine grape cultivars are more susceptible to mealybug infestation than others (Le Roux, 1996 in Walton, 2003b). Of the three wine grape cultivars used in these experiments Chardonnay vines were observed to be the most sensitive. Dedicated studies are necessary to confirm the levels of resistance observed in this study.

7.1.4.1.2. Physiological time (degree-days)

Walton (2003a) calculated the developmental time of one generation of vine mealybug at 235 degree-days. Different methods of degree-day calculations have been applied using a duration of 235 degree-days, and resulted in differences of up to 31% between the various methods of calculations. This can warp the identification of the onset of the next generation and wrong decisions can be made. Conceptually, degree-day estimations can be used as a pest-outbreak forecasting technique. However, a standard calculation method needs to be determined for the pest-plant complex. Potentially the effect of region should also be incorporated into the model, to evaluate the pest-plant-region complex. Such a model should be field tested and correlated with actual male flight periods.

7.1.4.1.3. Male trap catch as indicator of female population levels

In an ideal monitoring trap, catch will always be directly proportional to the surrounding population, so that the catch provides a useful estimate of insect density (Suckling, 2000). Both Millar *et al.* (2002) and Walton *et al.* (2004) observed a good correlation between male vine mealybug trap catch numbers and female population numbers albeit with different visual sampling techniques. Trap efficiency can also vary with population density, generally

decreasing as density and competition from natural pheromone sources increases (Miller and McDougall, 1973 in Chittamuru, 2000). Therefore, it will be advisable to investigate the effect of population pressure on these correlations.

7.1.4.2. Environmental conditions

Catches in pheromone traps are often significantly affected by environmental conditions such as wind speed, temperature, rainfall and humidity and even moonlight (Miller and McDougall, 1973 in Chittamuru, 2000).

7.1.4.2.1. Temperature

Temperature was the climatic factor which influenced male vine mealybug trap catch numbers the most. Generally the increase in trap catch numbers correlated with an increase in temperature. However, given prolonged exposure to very high temperatures and a broad thermal range, an inverse relationship can be seen where trap catch numbers increase with decreasing temperatures. The temperature range for development of the vine mealybug has been established to be between 16.59 °C and 35.61 °C (Walton, 2003a) and it seemed that temperature fluctuation around the upper developmental threshold level for an extended period of time suppressed populations. The effect of this observation on pest outbreak forecasting models based on degree-day estimations, needs to be investigated.

7.1.4.2.2. Relative humidity and rainfall

The effect of relative humidity on vine mealybug populations has not yet been investigated. Although this study did not focus on the impact of climatic factors on male vine mealybug trap catches, it was observed that relative humidity had a significant effect on trap catch numbers and this effect should be investigated further. Rainfall during the study period was

not an important climatic factor influencing the trap catch numbers. It must however be noted that in the Western Cape, South Africa, the summer season of 2004/2005 was regarded as an extremely dry season. Although grapevines were irrigated at both study sites, the grapevines did experience water stress.

7.1.4.3. Economic factors

The cost of monitoring can be evaluated from a number of angles, namely direct costs and potential costs. Direct costs would include the cost of the monitoring material, the trap servicing cost, and consultancy cost if the grape grower needs to buy in expertise in any aspect of monitoring. The potential costs include the potential cost of delayed response to monitoring findings. Any potential cost is much more difficult to determine and justify.

The following estimate of monitoring costs has been based on experience gained during this present study. The estimate assumes that the grape grower has the expertise available to successfully identify the male vine mealybug on the sticky liner and process the data once counted. The cost of the delta trap-sticky liner-pheromone lure trapping unit was approximately R54.00. Given the trap service frequency (changing trap liners) every two weeks, and the pheromone lure replacement every eight weeks, a trapping season of 32 weeks and the recommendation that the trap density should be one trap per hectare; the total cost per hectare would be approximately R189.00. On a farm of 100 ha, the costs for the material would amount to R18 900 for the season. If the white scale card could be used as an effective trap the cost would be about R28.50 per trap unit, and R17 400 for the season. The cost of servicing the traps can be estimated at a rate per hour of the person performing the task. Servicing includes the preparation for trap liner/pheromone lure replacement, and the actual replacement of trap liners/lures, the counting of the trap catch and processing of the data. Servicing of 60 traps per trap service date during this study

required about 13 hours of work every two weeks. Servicing of traps on a farm of 100 ha, for a trapping season of 32 weeks, would then cost about R10 400 given an hourly rate of R30.00. The total direct cost of monitoring vine mealybug for a season of 32 weeks on a 100 ha farm comes to R29 300, or R293 per ha.

If a farm worker is used to conduct this task, then the cost per hour would possibly be less than if someone is contracted in to perform the task. Consultancy time would have to be bought at a much higher rate than that of a farm worker or a trap service contractor, if the grape grower does not have the expertise on the farm to identify and count trap catches.

The cost of pheromone monitoring must be compared to the cost of visual monitoring. Visual monitoring is a much more labour intensive process. Some grape growers may still see the cost of monitoring as too high and not delivering enough value. A possible field of future study is the role of vine mealybug monitoring in an integrated management model in which dynamic economic injury levels and action threshold levels are used.

7.1.5. Output: Action threshold

During the field tests done for the comparison of the pheromone lure formulations, a number of observations were made which warrant further study. The viticultural methods applied in table grape growing and wine grape growing differ notably. Female vine mealybug infestation levels were much lower in table grape vineyards than in wine grape vineyards (Walton, 2003a; Walton *et al.*, 2004). Male vine mealybug trap catch levels were also much lower in table grape vineyards than in wine grape vineyards (this study). An action threshold of 65 vine mealybug males per trap per two-week period seemed to fit the numbers of male trap catch generally observed. In the wine grape vineyards, it seems to be too low compared to the trap catch numbers generally observed. The owner of the table grape vineyard was very

concerned when the trap catch figures reached 45-50 (Kirsten, 2004), whereas the vineyard manager of the wine grape vineyard was not concerned about trap catch figures reaching 400 (Snyman, 2004). His reliance was rather on a visual stem monitoring system where a person walked the vineyard blocks and rows on a daily basis observing damage symptoms. Spot sprays of chlorpyrifos were then applied according to a particular spraying regime.

The currently accepted action threshold should be refined to account for the type of grape (wine, table and raisin), and vineyard management practices.

7.2. Conclusion

Using sex pheromone traps for population estimates is a valid technique in the arsenal of control tactics against the vine mealybug. Since 2001, important research has been conducted to determine the basic elements of monitoring and it has been consolidated effectively into a monitoring protocol. Refinements and validation of research results must be done to build further credibility into the monitoring system.

When tracing the history of the vine mealybug, the damage it does and the management strategies formulated for its control, it seems that the single most important event relates to the isolation and synthesization of the female sex pheromone. New management options became available thereafter. It seems sensible to consolidate all known and new management guidelines in an easy-to-use expert system. With the grape grower in mind, it will be much more valuable to integrate vine mealybug management guidelines with other pests' management guidelines into a holistic expert system that focuses on the crop and not a single pest.

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