

# **ANT MANAGEMENT IN WESTERN CAPE VINEYARDS**

By

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## DECLARATION

This study represents original work by the author and has not been submitted in any form to another University. Where use was made of the work of others it has been duly acknowledged in the text.

A handwritten signature in black ink, appearing to read 'P. Addison', with a large, sweeping flourish above the name.

P. ADDISON

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## GENERAL INTRODUCTION

Ants fulfil various and sometimes important ecological functions, such as myrmecochory, pollination, nutrient recycling, soil improvement and predation of pest insects (Way & Khoo 1992). Many ants are, however, also pests in agriculture, urban and natural environments. *Linepithema humile* (Mayr), *Oecophylla smaragdina* Fabricius, *Wasmannia auropunctata* (Roger) and *Solenopsis invicta* Buren are some widely-distributed ants that displace indigenous species and thereby reduce biodiversity of both plants and arthropods (Donnelly & Gilliom 1985, Andersen 1992, de Kock *et al.* 1992 and Human & Gordon 1996). *L. humile*, *Technomyrmex albipes* (F. Smith), *Pheidole megacephala* (Fabricius) and *Monomorium pharaonis* (Linnaeus) are cosmopolitan pests that have invaded homes, offices, factories, food establishments and, particularly the latter species, hospitals (de Kock & Gilliom 1989, Prins *et al.* 1990, Knight & Rust 1991, Williams & Vail 1994 and Klotz *et al.* 1996). *Anoplolepis custodiens* (F. Smith) disturbs and even kills chickens in certain areas of the Free State Province, necessitating control measures around chicken runs. *Melissotarsus*, *Crematogaster* and *Camponotus* species often infest trees, timbers and poles, which eventually become weakened and break (Prins *et al.* 1990).

### Ants as pests in agriculture

In agriculture, the most economically-significant damage caused by ants is indirect, although direct feeding damage does occur (Veeresh 1990, Thompson 1990 and Delabie 1990). Ants tend honeydew-excreting homopterans and thereby prevent small predators and also parasitoids from attacking aphids, scale and mealybugs (Way 1963). Crops primarily affected by this mutualistic association are grapes (Kriegler & Whitehead 1962, Myburgh *et al.* 1973 and Phillips & Sherk, 1991), guava, mango, citrus (Samways 1981, 1982, Moreno *et al.* 1987, Veeresh 1990 and James *et al.* 1996), cocoa, coffee (Room 1971, Leston 1973 and Delabie 1990), pineapple and sugarcane (Reimer *et al.* 1990). Often, ant-tended Homoptera are also known vectors of certain pathogens. Although ants are not known to transmit these pathogens, if they are not controlled effectively, vector control becomes increasingly difficult. For example, scale insects protected by *Acropyga* spp. are vectors of several root diseases on coffee and cocoa in South America (Fowler *et al.* 1990), while *Crematogaster* spp. were found to aid the

distribution of cocoa swollen-shoot virus transmitted by the mealybug *Planococcoides njalensis* Laing (Hemiptera: Pseudococcidae) in Ghana (Hanna *et al.* 1956).

### **Ants as pests in South African vineyards**

The Argentine ant, *Linepithema humile*, the two pugnacious ant species *Anoplolepis custodiens* and *A. steingroeveri* (Forel), the cocktail ant *Crematogaster peringueyi* Emery and the little ubiquitous white-footed ant *T. albipes* were found to be associated with mealybugs on vines in South Africa (Whitehead 1957 and Urban & Bradley 1982). The most widely distributed mealybug in vineyards in the Western Cape Province is *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) (Walton 2003). Grape growers spent around R19 million on the chemical control of *P. ficus* during 2001 (Vaughn Walton, personal communication). Several mealybug species, including *P. ficus*, have been implicated as being vectors of grape vine leafroll virus in South Africa (Engelbrecht & Kasdorf 1990) and thereby cause additional, indirect damage which cannot be quantified at this stage. As there is currently no treatment for vine viruses, one of the primary control options is to limit the distribution of the vector and attendant ants. To date, several chemicals have been registered for mealybug control (Nel *et al.* 1999). However, the majority of these are registered for use during the growing season, and many of these chemicals could therefore have detrimental effects on the natural enemies of mealybug, which reach their highest numbers at this time of year (Walton 2003). Chlorpyrifos, a chemical widely used for mealybug control, has been proven to be highly toxic to the parasitoid *Coccidoxenoides peregrinus* (Timberlake) (Hymenoptera: Encyrtidae) and the previously undescribed predatory beetle *Nephus 'boschianus'* (Coccinellidae: Scymnini), two natural enemies currently reared for augmentative releases against *P. ficus* (Walton & Pringle 1999, 2001). Furthermore, *P. ficus* colonies have been found to infest the roots of several weed species growing in vineyards (Walton 2001), where they would be completely unaffected by non-systemic chemical sprays on vines. The above factors could explain why mealybugs appear to be increasingly more difficult to control, but also highlight the importance of effective ant management as a primary control strategy to aid the biological control of mealybug.

Epigaeic ants, such as *L. humile*, *A. custodiens* and *A. steingroeveri*, are the prime mutualists associated with mealybugs, and are the most difficult to control (Urban & Bradley 1982). Arboreal ants, such as *C. peringueyi*, agitate farm workers harvesting

grapes and infest irrigation pipes, causing blockages. The indigenous ants *A. custodiens* and *A. steingroeveri*, although occurring in large numbers in vines, are also effective predators of pest insects, such as the pupae of the Mediterranean fruit fly *Ceratitidis capitata* (Wiedemann) (Diptera: Tephritidae) in the soil (Samways 1982). Although their beneficial effect as predators has never been measured in vines, this should be borne in mind when planning ant control strategies. Both these species are also efficient seed dispersal agents in indigenous Cape vegetation (Bond & Slingsby 1983). Ideal management practices effectively control ants where needed, whilst still allowing them to perform important ecological and economic functions.

Dominant ants have the potential to becoming serious economic pests. Steyn (1954) and Leston (1973) described dominant ants as being broad-spectrum predators, regularly tending Homoptera and having colonies made up of several nests (polydomous), often with each nest containing several queens (polygynous). This makes them ideally suited to reproduce prolifically and renders them almost unmanageable under favourable conditions. Most ant control experiments on vines in South Africa have been directed against *L. humile* (Dürr 1953, Joubert & Walters 1955, Whitehead 1958 and 1961, Schwartz 1988). Early trials showed that organochlorine insecticides such as DDT, dieldrin and chlordane gave good control as soil and stem treatments, and were a good alternative to baiting, which was the standard practice 50 or so years ago (Dürr 1953, Joubert & Walters 1955, Whitehead 1957 and 1961). After the withdrawal of DDT and dieldrin, Urban & Mynhardt (1983) and Schwartz (1988) tested various sticky stem barriers such as Plantex, Formex and Rever Ant and physical barriers such as Sper. While Schwartz (1988) achieved good control with polybutene-based sticky barriers, Urban and Bradley (1983) warned that certain problems with phytotoxicity could result from polybutenoids and a geofabric backing (Bidim) on vines. During the two-year assessments of Formex on Bidim against *L. humile* on vines, Schwartz (1988) observed no signs of phytotoxicity, but stated that this treatment became expensive as a result of having to use the Bidim backing.

With the introduction of the Scheme for Integrated Production of Wine (Anonymous 2000), Integrated Pest Management (IPM) is strongly emphasized and grape growers are obliged to manage pests with more regard for the possible negative environmental impact that inappropriate control could have. According to Samways (1981), Moreno *et*

*al.* (1987) and James *et al.* (1996), direct stem barriers are an effective, environmentally-friendly method for controlling epigeic ants. This method is also believed to be more beneficial to the natural enemies of Homoptera than blanket insecticidal spray treatments, yet it still allows ants to continue feeding on possible insect pests on the ground. Although acceptable for IPM, growers find the application of stem treatments labour-intensive and often *Anoplolepis* spp. are not effectively controlled when there is high ant pressure (Ueckermann 1998). However, this method is believed to be the most suitable control option for the Integrated Pest Management of wingless vine pests.

The application of baits around nests and at the base of vine stems has also been investigated in preliminary field trials, but was found to be ineffective (Ueckermann 1998). Possible reasons for this were described in a review by Cherrett (1990) and include the following:

- the difficulty in locating all nests, which are often concealed;
- the necessity for controlling nests in a wide area, as ants can forage over vast distances from outside of the area where they cause the damage;
- baits must be formulated to be attractive to a particular species, therefore requiring much research; and
- although workers are killed, queens often survive, enabling the colony to recover.

Although effective baits have been described for controlling *L. humile* (Samways 1985, Baker *et al.* 1985, Blachly & Forschler 1996, Klotz *et al.* 1996 and James *et al.* 1996), no extensive bait trials have reported success against *Anoplolepis* spp. Indeed, most bait trials have been conducted in and around buildings against *L. humile* and not in an agricultural context (Baker *et al.* 1985, Knight & Rust 1991, Blachly & Forschler 1996, Klotz *et al.* 1996 and 1998). Despite the possible environmental problems that could be associated with toxic baits, such as leaching of chemicals into the soil and killing beneficial insects, an ant-specific (containerised) bait with low mammalian toxicity could be of great value in vineyards and should be investigated.

## **Aims**

The aim of this study is to address ant control in vineyards in terms of IPM. No detailed studies of the distribution of ants in vineyards in the Western Cape have been carried out. It is therefore not known which ants forage in vineyards, nor how many species are associated with the vine mealybug, nor their current geographical distribution. It was



necessary, therefore, to fill these gaps in our knowledge before ant control strategies could be devised (Chapter 1).

Since no registered treatments were available to control epigaeic ants in vineyards at the start of this study, it was necessary to establish a cost-effective, practical and environmentally-friendly method for ant control that is also acceptable for an integrated pest management programme. Several direct chemical stem treatments were tested for their efficacy against *L. humile*, *A. custodiens* and *A. steingroeveri* (Chapter 2).

Due to the increasing interest in organic production in agriculture, and as a result of more persistent chemicals being replaced with less persistent and more expensive chemicals, it is becoming increasingly important to investigate non-chemical management options. The studies of Way (1953), Steyn (1954), Myers (1957), Prins *et al.* (1990) and Way & Khoo (1992) suggest that there is merit in investigating the effects of a vegetative ground cover for deterring *A. custodiens* from nesting. Brian & Brian (1951) have already established that *Myrmica rubra* Linn. produces an inferior and reduced brood in shaded nests as opposed to colonies nesting in insolated soil. *L. humile* was found to be negatively correlated with vegetation density in Portugal, although this was ascribed to its strong association with human-disturbed vegetation and no mention was made of soil temperature as a possible factor (Way *et al.* 1997). An investigation of the effect that cover crops can have on *A. custodiens* will indicate if this practice can be used for suppressing ants in vineyards. Many growers sow cover crops to promote vine health and growth. Also, cover crops can have the potential to increase biodiversity in many agricultural crops, including vineyards (Teddars 1983, Altieri & Schmidt 1985, Bugg & Waddington 1994 and Costello & Daane 1998). Although non-chemical methods may not be the sole way to effectively control particularly high ant infestations, they may be supplementary management tools which help prevent high infestations from developing. It is hoped that once these aspects have been investigated, a more sustainable answer to ant control in South African vineyards can be found (Chapter 3).

*A. custodiens* foraging behaviour and morphology in the different ground cover treatments are measured to establish if cover crops have any more subtle effects on *A. custodiens* populations in shaded soils versus unshaded soils (Chapter 4). It is already

known that *A. custodiens* can exhibit extreme dominance over other ant species in agricultural crops infested with homopterans (Way 1953 and Samways 1981). However, it may be possible to manipulate ant abundance in vineyards by providing additional vegetative diversity in the form of cover crops. The combination of additional niches for non-target ants and habitat modification to deter target ants (in the form of reduced soil temperatures to limit nesting) could result in a more even ant species distribution. This theory states that reduced vegetative structure may lead to lower ant species diversity and increased dominance (Greenslade & Greenslade 1977 and Majer & de Kock 1992). Not much is known about *A. custodiens* foraging behaviour relative to more widely studied species such as *S. invicta*. It is known, for example, that certain species of *Anoplolepis* make use of group transport when foraging for food items (Hölldobler & Wilson 1990). Steyn (1954) established foraging distances in and around citrus orchards at Letaba for *A. custodiens* and found that there was a size difference between foragers outside of orchards compared to foragers within orchards. To add to the current information available, ant species diversity was measured in four ground cover treatments. Head capsule measurements were made of workers foraging within the vineyard in the four ground cover treatments as well as in natural vegetation close to the vineyard. Furthermore, ant activity was monitored in the vineyard to establish optimum time for applying chemical stem treatments.

The General Discussion aims to connect the four chapters as an integrated thrust towards finding a method, or combination of methods, suitable for ant control within the context of the Scheme for Integrated Production of Wine.

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## CHAPTER 1

### A SURVEY OF ANTS (HYMENOPTERA: FORMICIDAE) THAT FORAGE IN VINEYARDS IN THE WESTERN CAPE PROVINCE, SOUTH AFRICA\*

#### ABSTRACT

*This study aimed to establish which species of ants are associated with mealybug *Planococcus ficus* and which species are dominant in the major vine-growing areas of the Western Cape Province. During 1998/99, twenty two vineyards were surveyed in the Stellenbosch/Paarl, Klein Karoo, Worcester, Swartland, Olifants River and Hex River Valley regions using pitfall traps to sample epigeic ants and tuna bait traps to sample arboreal ants. Each vineyard was sampled intensively for two consecutive weeks shortly before harvest. Forty two species of ants were recorded during the survey. The most widely distributed ant species, which are potentially dominant and associated with mealybug outbreaks in vineyards in the Western Cape, are *Anoplolepis custodiens*, *A. steingroeveri* and *Linepithema humile*. *Crematogaster peringueyi*, *Crematogaster* sp. 2 and *C. melanogaster* are three arboreal species potentially dominant in vines only. Dominance indices for *Pheidole* sp. 1 and *Pheidole* sp. 2 were low compared to the more aggressive *Anoplolepis* spp. and *L. humile*, indicating that the former two species are not of economic significance. Edge effects occurred in five of the surveyed vineyards for three ant species. These edge effects indicate specific preferences of the ants for certain abiotic and microclimatic factors in vineyards, but could also be the result of interspecific competition.*

#### INTRODUCTION

Pest management in South African vineyards is no longer based only on chemical control. With the introduction of the Scheme for Integrated Production of Wine (Anonymous 2000), emphasis is being placed on Integrated Pest Management (IPM). The biological control of mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), which is one of the principal pests on vines, is given high priority. It has already been shown that the efficacy of biological control of mealybug on vines by

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coccinellid predators and parasitic Hymenoptera is significantly reduced by the presence of ants, which tend mealybug (Kriegler & Whitehead 1962, Myburgh *et al.* 1973, Urban & Mynhardt 1983 and Philips & Sherk 1991). The Argentine ant, *Linepithema humile* (Mayr), the two pugnacious ant species *Anoplolepis custodiens* (F. Smith) and *A. steingroeveri* (Forel), the cocktail ant *Crematogaster peringueyi* Emery and, to a lesser degree, the little ubiquitous white-footed ant *Technomyrmex albipes* (F. Smith) are the most common ants associated with mealybug on vines in South Africa (Whitehead 1957 and Urban & Bradley 1982). This study aimed to establish which other ant species were associated with mealybug and which species dominate in the major vine-growing areas of the Western Cape Province. This has implications for ant control since ants are either predominantly arboreal or epigaeic, which necessitates different methods of control. Epigaeic ants have been found to be the most troublesome in vineyards (Urban & Bradley 1982). In IPM programmes, chemical stem-barriers, that prevent ants from accessing vines, are an acceptable method of control as ants are not eradicated, but left to prey on other pests such as the pupae of the Mediterranean fruit fly, *Ceratitidis capitata* (Wiedemann) (Diptera: Tephritidae) and false codling moth, *Cryptophlebia leucotreta* Meyrick (Lepidoptera: Olethreutidae) in the soil (Samways 1982). Stem banding is not effective for arboreal species such as the cocktail ant.

## **MATERIAL AND METHODS**

### *Sites*

During 1998/99, 22 vineyards were surveyed in the Stellenbosch/Paarl, Klein Karoo, Worcester, Swartland, Olifants River and Hex River Valley regions. The location and cultivar of each vineyard is indicated in Table 1. The vineyards were of varying age and size and were all infested with mealybug. Irrigation was mainly by drip or micro-irrigation, with some vineyards being dry-land (Koelenhof and Malmesbury) or flood irrigated (Kys). All vineyards were trellised and all were wine grape vineyards, except those in the Hex River Valley, Paarl, and one vineyard at Riebeek-Kasteel, which were table grape vineyards. Ground cover included weeds of varying density at the time of the survey. Some vineyards had cover crops planted between the rows during winter. These cover crops were treated with herbicide at the beginning of the growing season where weeds predominate during summer.

### *Sampling methods and trial layout*

Each vineyard was sampled intensively for two consecutive weeks shortly before harvest. The harvest period varied from February until March, according to location and cultivar. This is the time of year when ants appear to be most active in the vines (personal observation).

Each vineyard was sampled using 42 pitfall traps similar to those described by Majer (1978). Each trap consisted of a polystyrene test tube (18 x 150mm) containing approximately 4 ml of seven parts 70 % ethyl alcohol and three parts pure glycerol. The test tubes were sunk into holes in the ground prepared with a metal rod. No outer case was used. The soil was levelled around the test tubes so that the edge was even with the soil surface.

Traps were arranged in transects along six, non-adjacent vine rows as shown in Fig. 1 to establish whether any edge effects occurred that might influence the distribution of ant species within each vineyard. Traps were removed after one week and replaced with fresh traps which were left for another week. Pitfall trapping was found to be suitable for survey purposes because it sampled both nocturnal and diurnal ant species.

In addition, tuna baits were used to sample dominant and arboreal ants more selectively in a method similar to the one described by Andersen (1992). One teaspoon of shredded, tinned tuna was placed in the crutch of 12-30 vines nearest the pitfall traps in each vineyard once during the second week of sampling. Placement of baits took approximately 10 - 15 min. The baits were left for 30 min., after which all ants feeding on the tuna were collected by sweeping them into separate containers filled with 70% ethyl alcohol. Collection of ants and baits took approximately 15 - 20 min.

Sorting and counting of pitfall and bait trap contents was carried out in the laboratory, and specimens were mounted for identification. The Berger-Parker dominance index was used to express the proportion of the total catch that is ascribed to the dominant species (Southwood 1978). Standard error of means for pitfall trap catches were calculated and ANOVA, LSD was performed on the data to determine edge effects. Data were transformed [ $\log(x+1)$ ] to normalize variance.

The level of mealybug infestation was estimated in the six rows where sampling for ants took place after the first week of sampling and classified into three divisions for each of the 22 vineyards according to the following criteria:

- Low: A few infested vines were distributed in patches, but mostly honeydew and attendant ants were not visible, grape bunches were all marketable.
- Medium: A few infested vines were distributed in patches, honeydew and sooty mould was always visible which attracted some ants and left grape bunches unmarketable.
- High: Infested vines were evenly distributed and plentiful, honeydew and sooty mould was visible which attracted large numbers of ants and left grape bunches unmarketable.

## RESULTS AND DISCUSSION

Forty two species of ants were recorded during the survey (Table 2). Six species were dominant in one or more vineyards, and an additional three arboreal species were dominant on vines (Table 3). The distribution of the three most widely distributed, dominant ants, *A. steingroeveri*, *A. custodiens* and *L. humile* is shown in Fig. 2. The average temperatures in the various areas for the two week study period ranged from 13.2 – 19.5°C (minimum temperature) and from 27.2 – 35.1°C (maximum temperature). Average temperatures for each area are shown in Tables 2A and B (Chapter 4). Although the total seasonal rainfall varied considerably between each area (Tables 2A and B, Chapter 4), the average rainfall during the two week study period was 0mm in all areas except Bonnievale (0.18mm), the Hex River Valley (0.06mm) and Lutzville (0.95mm).

### ***Anoplolepis custodiens***

This was the most widely distributed dominant ant, particularly in the Klein Karoo, Hex River Valley and Worcester areas (Table 2, Table 3). It was dominant wherever it occurred, and was observed to tend mealybugs, often in highly infested vineyards. In terms of abundance, this species yielded the highest numbers in traps, with over 16 000 individuals being trapped during the two week sampling period on one farm, N1-Worcester. It dominated pitfall and bait traps where it occurred (Table 3).

### ***Anoplolepis steingroeveri***

Although widely distributed, this ant was replaced as the dominant species by one *Pheidole* species in two vineyards in the Worcester and Swartland areas, and another *Pheidole* species in a vineyard in the Olifants River area (Table 2). Its distribution appears to be concentrated closer to the West Coast and does not extend far into the Breede River valley. *A. steingroeveri* was also observed to tend mealybug in vineyards moderately to highly infested with mealybug (Table 4). These observations confirmed the findings of Urban & Bradley (1982) who also associated *Anoplolepis* spp. with severe mealybug infestations. It dominated in pitfall and bait traps (Table 3), but was often found only in pitfall traps in the Worcester, Swartland and Olifants River areas, particularly in vineyards that were dominated by other ant species (Table 2). *A. steingroeveri* was often seen nesting and foraging on dirt roads and open bare ground amongst sparse natural vegetation, particularly small Karoo bushes, immediately adjacent to the vineyards. Edge effects were noted in vineyards in Riebeek-Kasteel (R2 – Swartland), Kys and Rawsonville (R2 – Worcester). In these three vineyards, *A. steingroeveri* was recorded in significantly greater numbers two metres outside of the vineyards in the roads than in the remaining traps placed inside the vineyards (Fig. 3A, B and C). Possible reasons for this could be that this ant has specific preferences for certain abiotic and microclimatic factors that occur outside of the vineyards. Such factors could include soil type, moisture and ground cover. An observation made by Steyn (1954) suggested that *A. custodiens* could be deterred from nesting in citrus orchards by planting cover crops, because these ants find soil that does not receive much sun and soil that is very sandy unsuitable for rearing their brood. Other reasons for the observed edge effects could include interspecific competition between other ants or the use of insecticides within the boundary of the vineyard.

### ***Linepithema humile***

This species was recorded in at least one vineyard in all areas except in the Olifants River region. According to observations made by Pasfield & Braithwaite (1950), Markin (1970) and De Kock *et al.* (1992), *L. humile* prefers moist environments, indicating that this ant is prone to desiccation (Witt & Giliomee 1999), which could result in its patchy distribution within the Western Cape (Fig. 2). It is therefore possible that *L. humile* has not been able to establish itself in the outlying, drier northern regions of the South Western Cape and is limited to microclimates that are favourable, ie. vineyards with

irrigation or vineyard situated against mountains the rainfall is higher or the environment is moisture. *Tetramorium quadrispinosum* Emery was found as a subdominant ant wherever *L. humile* occurred, as it seems to be well adapted to co-exist with *L. humile* due to its habit of closing its nest entrances, which protects it against invasion (Witt & Gilliomee 1999). *L. humile* dominated in pitfall and bait traps, except on two occasions where it was found only in pitfall traps in the Klein Karoo and Hex River Valley (Table 2). Although *L. humile* was reported as being very aggressive towards other ants and easily displaced indigenous ant species such as *A. custodiens* (Donnelly & Gilliomee 1985 and De Kock 1990), it was dominated by *Technomyrmex albipes* (F. Smith) in one vineyard in the Hex River Valley. A possible reason for this could be that the noticeably low mealybug infestation in this vineyard might not have been able to sustain large *L. humile* colonies, thereby giving other ant species a chance to dominate (Table 4). Total ant catch in this vineyard was the lowest of all vineyards sampled (630 ants). As only ten *T. albipes* individuals were recorded in bait traps in vines during the entire survey, it was not possible to establish whether this ant tended mealybugs or not. Urban *et al.* (1980) suggested that *T. albipes* could be important in contributing to mealybug infestations, as this ant was found in the presence of mealybug, but was not widely distributed. *T. albipes* was found to be an occasional pest on citrus, where it was associated with red scale outbreaks (Samways *et al.* 1982). In the present study, *T. albipes* did not have a high dominance index in pitfall traps, and was not widely distributed as a dominant ant (Table 3). Although a dominance index of 100% was found in bait traps in one vineyard, only two ants were recorded here. This ant is therefore not a potential pest as it does not occur in high numbers and is not widely distributed. In another vineyard in the Hex River Valley where *L. humile* dominated and the mealybug infestation was low, more ants were recorded 2m outside on the road than within the vineyard (Fig. 3D). Total ant catch in this vineyard was relatively low (1158 ants). These results suggest that vineyards with a low honeydew source are not the ideal environment for *L. humile* to forage and reach high numbers in, and in such situations it may well be dominated by other ant species. The edge effect could also, however, be the result of insecticide sprays.

### ***Pheidole* spp.**

Three species of *Pheidole* were found foraging in vineyards, of which *Pheidole* sp. 1 (possibly *capensis*) and *Pheidole* sp. 2 dominated. *Pheidole* sp. 1 was observed to tend

mealybugs, and dominated pitfall and bait traps in two vineyards in the Worcester and Swartland areas. However, dominance indices were not high in comparison to the *Anoplolepis* spp. or *L. humile* (Table 3). *Pheidole* sp. 1 was not found in the Hex River Valley or in the Olifants River region (Table 2). *Pheidole* sp. 2 and *Pheidole* sp. 3 were not widely distributed, being found only in the Swartland and Olifants River region. While *Pheidole* sp. 2 was collected in both pitfall and bait traps, *Pheidole* sp. 3 was not recorded from bait traps. It was not possible to establish whether either species tended mealybugs due to low numbers in the field. In Lutzville, *Pheidole* sp. 2 (the dominant ant) was found in greater numbers in traps within the vineyard than two metres outside on the road (Fig. 3E), suggesting a possible association with mealybugs. This could, however, also be the result of a habitat feature within the vineyard. Of the three *Pheidole* species, only *Pheidole* sp. 1 and *Pheidole* sp. 2 are potentially dominant, although their occurrence was limited at the time of this survey and dominance indices are low. These species are therefore not of economic significance as they were mostly out-competed by more aggressive species.

#### ***Crematogaster* spp.**

None of the four *Crematogaster* spp. dominated on the ground in the vineyards sampled. A possible reason for this could be their arboreal habits, which make pitfall traps an ineffective trapping method for these species in vineyards. Dominance indices for *C. peringueyi*, *C. melanogaster* and *Crematogaster* sp. 2 in tuna bait traps are relatively high in comparison to the *Anoplolepis* spp. and *L. humile* (Table 3). These *Crematogaster* species were not widely distributed and were dominated by *Anoplolepis* spp., *Pheidole* spp. or *T. albipes* when looking at overall dominance (both pitfall and bait traps). They were not trapped in vineyards where *L. humile* occurred. *C. peringueyi*, the species most often trapped during this survey, was trapped in all regions except in Stellenbosch, the Hex River Valley and the Olifants River Valley. Observations confirmed that *C. peringueyi* was associated with mealybugs. The absence of *Crematogaster* spp. in the Hex River Valley confirmed the findings of Kriegler & Whitehead (1962) and Urban & Bradley (1982), who suggested that intensive spraying of insecticides for mealybug control in table grape vines in this area was a possible reason for the absence of these arboreal ants. *Crematogaster* spp. were observed on vines on three farms not included in the survey in the Stellenbosch and Paarl areas.



## IMPLICATIONS FOR ANT CONTROL

According to this survey, the most widely distributed, ground-dwelling ant species that are potentially dominant and associated with mealybugs in vineyards in the Western Cape Province, are *A. custodiens*, *A. steingroeveri* and *L. humile*. These epigaeic species can be controlled effectively in vineyards by chemical stem-barriers for a period of up to 110 days, although the *Anoplolepis* spp. are generally more difficult to control, possibly owing to their larger size (Ueckermann 1998). *C. peringueyi*, *Crematogaster* sp. 2 and *C. melanogaster* are arboreal species potentially dominant in vines and are possible pests in areas such as the Klein Karoo, Worcester, Olifants River and Swartland regions. In these areas they have been observed to infest holes in vines and irrigation pipes and are often a nuisance to workers harvesting grapes. The current recommendation for the control of *Crematogaster* spp. includes full cover spray applications (chlorpyrifos EC at a concentration of 400ml/100l water) of vines during winter (Nel *et al.* 1999).

The edge effects occurring in five of the vineyards indicate specific preferences of the ants for abiotic and microclimatic factors that prevail in or adjacent to specific vineyards, but could also be the result of interspecific competition or insecticide use within the vineyards. More research is being undertaken to establish these preferences, specifically regarding percentage ground cover, soil type and irrigation type (see Chapter 4). This will provide a better understanding of ant distribution and could have possible implications for ant management. As the planting of cover crops is a recommended practice for the Integrated Production of wine in vineyards, ground cover manipulation for ant management would be an added benefit if this proves to be an ant deterrent (see Chapter 3).

Table 1. Location and cultivar of vineyards sampled for ants in various wine growing regions of the Western Cape Province, South Africa.

Area	Farm	Co-ordinates	Altitude (m.a.s.l)	Grapevine cultivar
Stellenbosch	ARC Experimental farm	33.54S 18.52E	164	S.A. Riesling
Koelenhof	Muratie Wine Estate	33.53S 18.53E	342	Pinotage
Paarl	De Hoop	33.45S 18.56E	± 340	Dauphine
Montagu	Goedemoed	33.41S 19.50E	927	Unknown white
Montagu	Goedemoed	33.41S 19.50E	927	Unknown white
Klaasvoogds	Middelplaas	33.49S 19.59E	220	Chenin blanc
Robertson	Goree	33.49S 19.47E	180	Chardonnay
Bonnievale	Nordale Wine Estate	33.56S 20.06E	115	Sauvignon blanc
Rawsonville	Servele	33.42S 19.19E	880	S.A. Riesling
Rawsonville	Merwida	33.42S 19.19E	880	Sauvignon blanc
Nuy Valley	Glen Oaks	33.39S 19.37E	328	Chenin blanc
Nuy Valley	Kloppersbosch	33.39S 19.37E	328	Pinotage
Malmesbury	Carinus Bros.	33.24S 18.40E	117	Sauvignon blanc
Riebeek-Kasteel	Botmansdrif	33.29S 18.55E	250	Chenin blanc
Riebeek-Kasteel	Grensplaas	33.29S 18.55E	250	Sultanina
Tulbagh	Lemberg Wine Estate	33.15S 19.09E	190	Harslevelü
Lutzville	ARC Experimental farm	31.36S 18.26E	32	Pinot gris
Vredendal	Tlakaan	31.39S 18.27E	± 30	Pinotage
Kys	Houmoed	31.42S 18.34E	± 30	Sultanina
Hex River Valley	Naudésig	33.30S 19.37E	490	Barlinka
Hex River Valley	Welgemoed	33.30S 19.37E	490	Dauphine
Hex River Valley	Skottelploeg	33.30S 19.37E	490	Barlinka

Table 2. Ants foraging in vineyards in six vine growing regions of the Western Cape Province, South Africa.

Species	Klein Karoo					Stellenbosch			Worcester				Swartland				Hex River Valley			Olifants River			
	M1	M2	K	R	B	S	Ko	P	N1	N2	R1	R2	M	T	K1	K2	H1	H2	H3	L	V	Ky	
<i>Aenictus rotundatus</i> Mayr									○■														
<i>Anoplolepis custodiens</i> (F. Smith)	●▲	●▲		●▲	●■				●▲	●▲		○■				●▲							
<i>Anoplolepis steingroeveri</i> (Forel)							●▲					○■				●▲					○■	●▲	●■
<i>Camponotus fulvopilosus</i> (De Geer)																					○▲	○□	
<i>Cardiocondyia emeryi</i> Forel															○■						○■		○■
<i>Cardiocondyia shuckardi</i> Forel		○■			○■				○■	○■	○■				○■			○■	○■				○■
<i>Crematogaster liengmei</i> Forel													○▲										○▲
<i>Crematogaster melanogaster</i> Emery															○■								○▲
<i>Crematogaster peringueyi</i> Emery	○▲	○■										○▲			○▲								○▲
<i>Crematogaster</i> sp. 2	○■														○▲								○▲
<i>Dorylus helvolus</i> (Linnaeus)	○■			○■							○■	○■			○■	○■							○■
<i>Hypoponera</i> sp. 1				○■				○■	○■							○■							
<i>Linepithema humile</i> (Mayr)				●■				●▲		●▲					●▲						○■	●▲	
<i>Lepisiota capensis</i> (Mayr)														○■									○■
<i>Lepisiota laevis</i> (Santschi)												○■											○■
<i>Leptogenys castanea</i> (Mayr)			○■		○■																		
<i>Messor capensis</i> (Mayr)	○■												○■	○■		○■							○■
<i>Monomorium havilandi</i> Forel		○■																					○■
<i>Monomorium macrops</i> Arnold								○▲										○■	○■				
<i>Monomorium rhopalocerum</i> Emery				○■																			○■
<i>Monomorium schultzei</i> Forel		○■			○■	○■																	○■
<i>Monomorium</i> sp. 1 (subopacum-complex)				○■	○■	○■			○■	○■	○■	○■											○■
<i>Monomorium</i> sp. 4 (subopacum-complex)				○■									○■	○■									○■
<i>Monomorium</i> sp. 6 (monomorium group)													○■	○■									○■
<i>Monomorium</i> sp. 7															○▲								○■
<i>Ocymyrmex barbiger</i> Emery		○■							○■	○■						○■							○■
<i>Oligomyrmex</i> sp. 1				○■																			○■
<i>Pheidole</i> sp. 1					○■							●▲		●▲	○■	○■							○■
<i>Pheidole</i> sp. 2														○▲		●▲							●▲
<i>Pheidole</i> sp. 3														○■									○■
<i>Solenopsis punctaticeps</i> Mayr			○■	○■	○■	○■																	
<i>Technomyrmex albipes</i> (F. Smith)	○■	○■						○■	○■							○▲	○▲						○■
<i>Tetramorium bevisi</i> Arnold	○■	○■		○■								○■			○■	○■							○■
<i>Tetramorium erectum</i> Emery										○■													○■
<i>Tetramorium frigidum</i> Arnold						○■	○■	○■	○■		○■	○■											○■
<i>Tetramorium pusillum</i> Emery				○■	○■	○■			○■	○■	○■		○■	○■	○■	○■							○■
<i>Tetramorium quadrispinosum</i> Emery	○■			○■		○■			○■	○■	○■		○■	○■	○■	○■							○■
<i>Tetramorium regulare</i> Bolton				○■					○■														○■
<i>Tetramorium solidum</i> Emery							○■		○■														○■
<i>Tetramorium</i> sp. 2 (poweri-complex)							○■																○■
<i>Tetramorium</i> sp. 3 (oculatum-complex)		○■																					○■
<i>Tetramorium</i> sp. 4 (squaminode-group)					○■																		○■

Total number of species: 42

M1 – Montagu (vineyard 1), M2 – Montagu (vineyard 2), K – Klaasvoogds, R – Robertson, B – Bonnievale, S1 – Stellenbosch, Ko – Koelenhof, P – Paarl, N1 – Nuy Valley (Kloppersbosch), N2 – Nuy Valley (Glen Oaks), R1 – Rawsonville (Merwida), R2 – Rawsonville (Servede), M – Malmesbury, T – Tulbagh, K1 – Riebeeck-Kasteel (Botmansdrif), K2 – Riebeeck-Kasteel (Gransplaas), H1 – H3 (Skottelploeg, Welgemoed and Naudésig), L – Lutzville, V – Vredendal, Ky – Kys.

- - present and dominant (overall dominance ie. pitfall and tuna bait traps)
- - present, not dominant
- - caught in pitfall traps only (ground foragers)
- - caught in tuna bait traps only (arboreal)
- ▲ - caught in both pitfall and tuna bait traps

Table 3. Dominance indices of common ants foraging in vineyards sampled in the Western Cape Province, South Africa.

Species	Pitfall traps		Arboreal bait traps	
	Ave. % dominance * (pitfall traps)	No. of vineyards where species was dominant	Ave. % dominance * (tuna bait traps)	No. of vineyards where species was dominant
<i>Anoplolepis custodiens</i>	93.8	7	100	5
<i>Anoplolepis steingroeveri</i>	84.4	4	86.7	3
<i>Crematogaster melanogaster</i>	-	0	78.9	1
<i>Crematogaster peringueyi</i>	-	0	95.4	2
<i>Crematogaster</i> sp. 2	-	0	82.4	2
<i>Linepithema humile</i>	80.1	6	99.8	5
<i>Pheidole</i> sp. 1	35.4	2	43.5	1
<i>Pheidole</i> sp. 2	50.1	2	-	0
<i>Technomyrmex albipes</i>	28.5	1	(100)**	1

\* % Dominance calculated according to the Berger-Parker dominance index (Southwood, 1978). The index is based only on the number of vineyards where the ant species was dominant.

\*\* Only two individuals caught.

Table 4. Estimated level of mealybug infestation, dominant ant with actual numbers caught and cultivar of 22 vineyards sampled in various wine growing regions of the Western Cape Province, South Africa.

Area	Dominant ant	Actual nr. individuals sampled	Estimated level of mealybug infestation	Grapevine cultivar
Montagu	<i>Anoplolepis custodiens</i>	10 880	High	Unknown white
Montagu	<i>Anoplolepis custodiens</i>	1450	High	Unknown white
Robertson	<i>Anoplolepis custodiens</i>	1954	High	Chardonnay
Bonnievale	<i>Anoplolepis custodiens</i>	3656	High	Sauvignon blanc
Nuy Valley	<i>Anoplolepis custodiens</i>	16 248	High	Chenin blanc
Nuy Valley	<i>Anoplolepis custodiens</i>	5499	High	Pinotage
Hex River Valley	<i>Anoplolepis custodiens</i>	5323	High	Barlinka
Riebeek-Kasteel	<i>Anoplolepis steingroeveri</i>	2038	Medium	Sultanina
Vredendal	<i>Anoplolepis steingroeveri</i>	3875	High	Pinotage
Kys	<i>Anoplolepis steingroeveri</i>	478	Low	Sultanina
Koelenhof	<i>Anoplolepis steingroeveri</i>	1747	High	Pinotage
Paarl	<i>Linepithema humile</i>	630	Low	Dauphine
Stellenbosch	<i>Linepithema humile</i>	1923	Medium	S.A. Riesling
Klaasvoogds	<i>Linepithema humile</i>	4045	Medium	Chenin blanc
Tulbagh	<i>Linepithema humile</i>	2089	Low	Harslevelü
Rawsonville	<i>Linepithema humile</i>	2606	Low	Sauvignon blanc
Hex River Valley	<i>Linepithema humile</i>	811	Low	Barlinka
Malmesbury	<i>Pheidole</i> sp. 1	503	Low	Sauvignon blanc
Rawsonville	<i>Pheidole</i> sp. 1	307	Medium	S.A. Riesling
Riebeek-Kasteel	<i>Pheidole</i> sp. 2	237	Low	Chenin blanc
Lutzville	<i>Pheidole</i> sp. 2	873	Low	Pinot gris
Hex River Valley	<i>Technomyrmex albipes</i>	181	Low	Dauphine

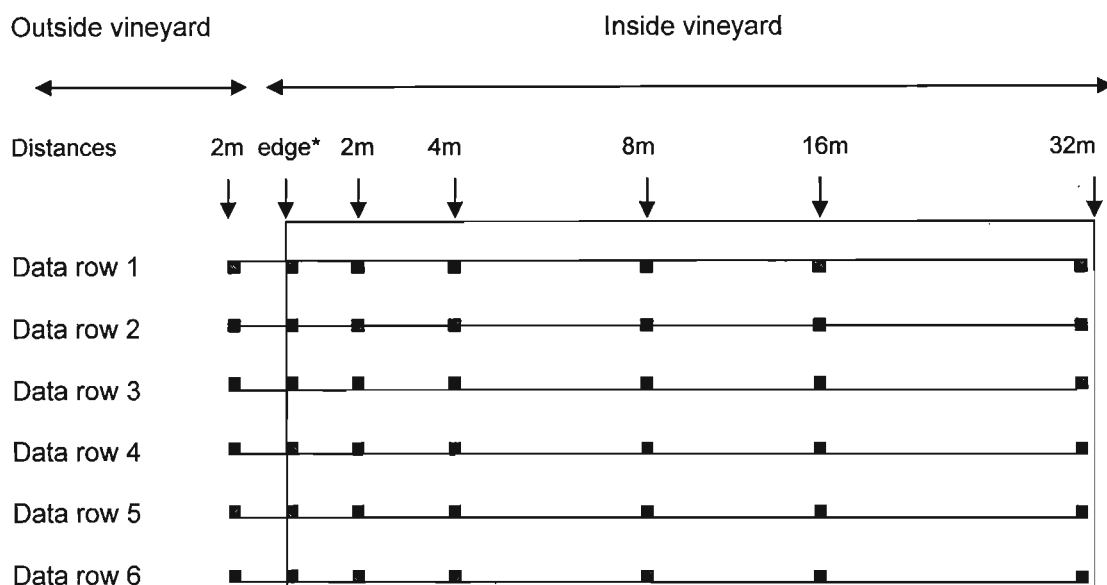


Fig. 1. A section of a vineyard showing the distances at which traps were placed along 6 vine rows sampled during the survey. Traps were placed close to the base of the vine stem at each point where there is a square on the diagram. Approximately two rows (eight meters) separated each of the six data rows.

\* Edge refers to the first trellising posts at the start of each of the vine rows.

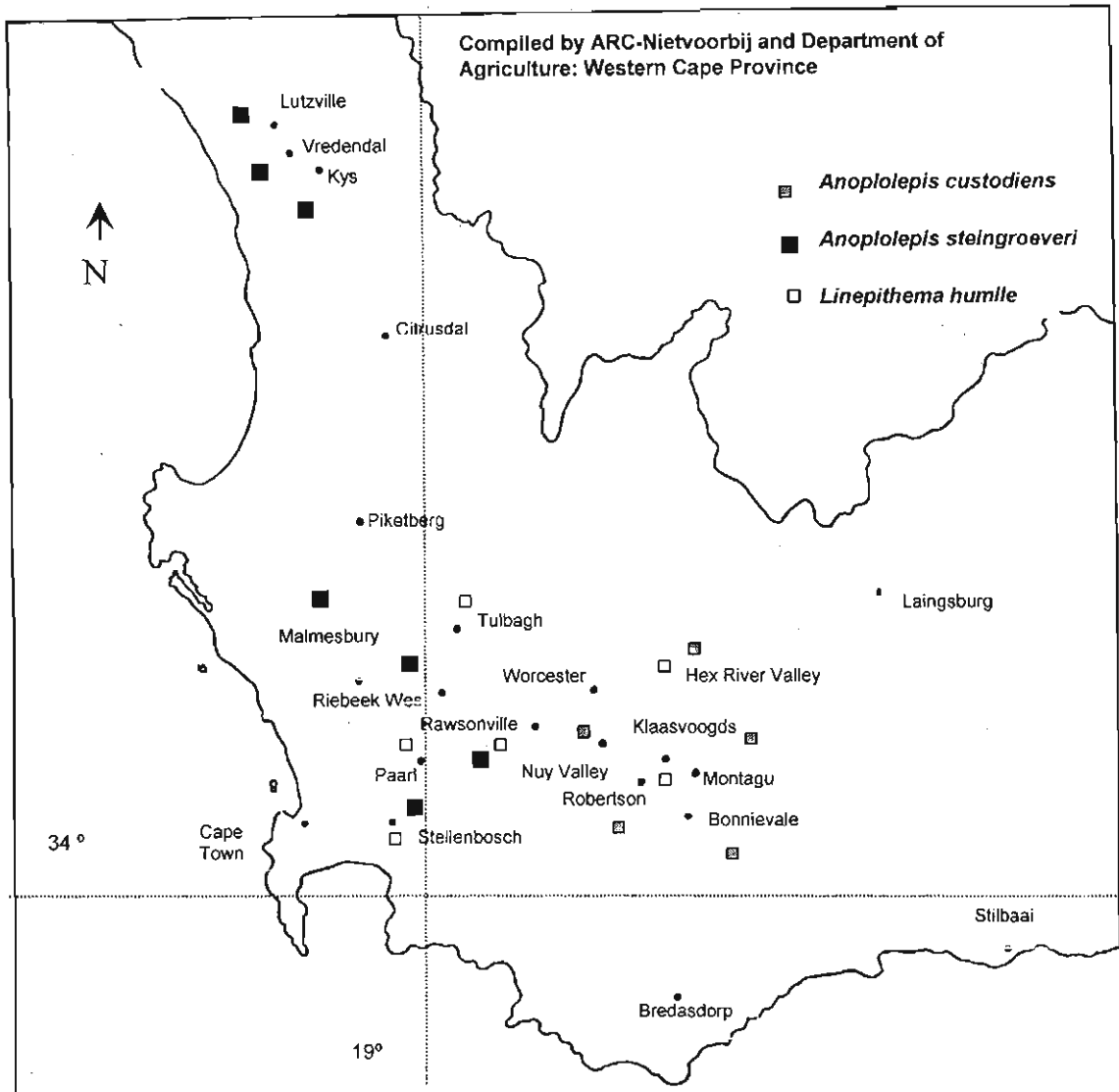


Fig. 2. Distribution of *Anoplolepis steingroeveri*, *A. custodiens* and *Linepithema humile* in Western Cape vineyards.

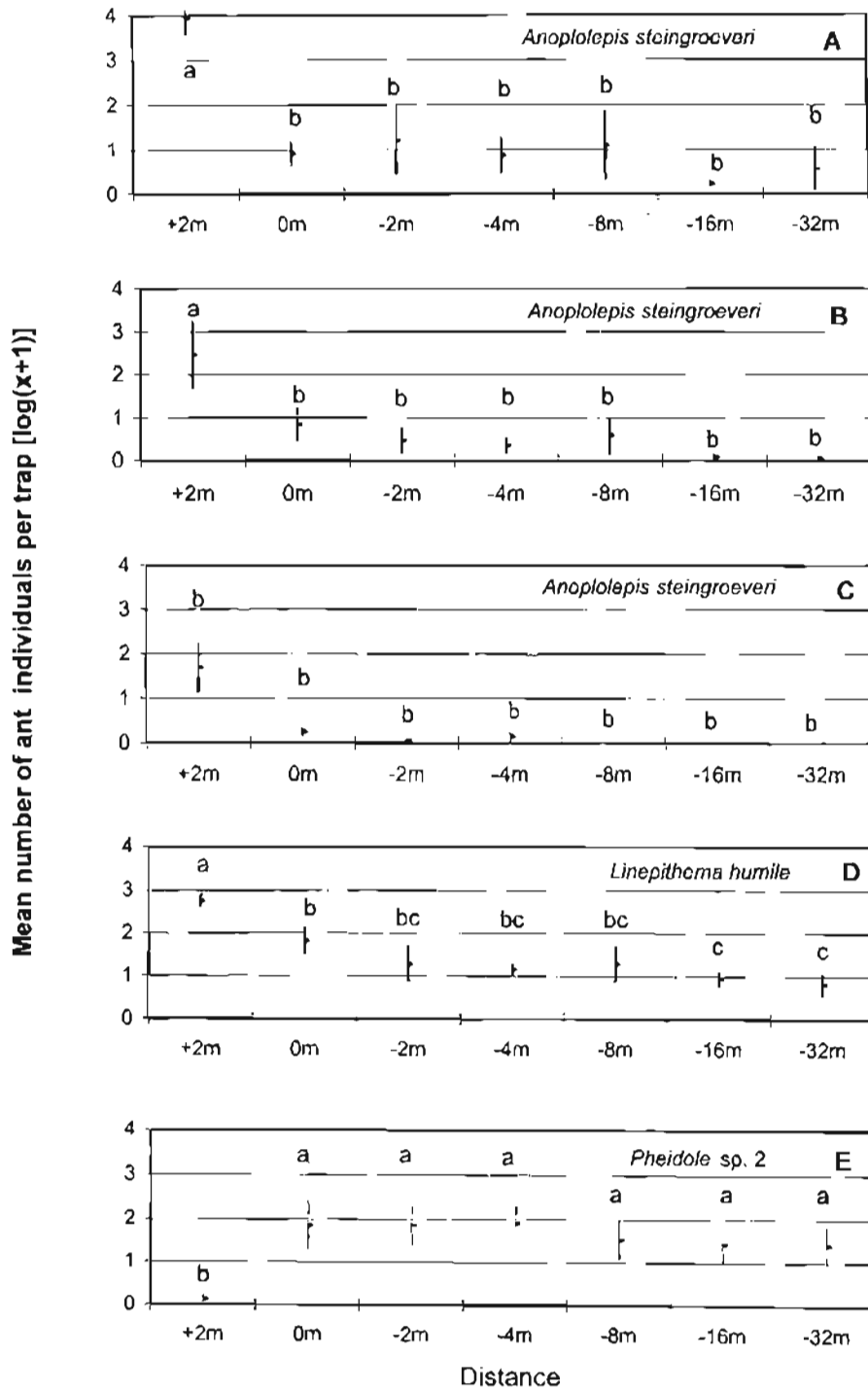


Fig. 3. Mean number of ant individuals trapped during two weeks at various distance inside (-) and outside(+) of vineyards, showing significant edge effects in the following areas: A - Riebeek-Kasteel; B - Kys; C - Rawsonville; D - Hex River Valley; E - Lutzville. Letters above each line which are the same in each graph do not differ significantly (ANOVA, LSD where  $P \leq 0.05$ ).



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## CHAPTER 2

### CHEMICAL STEM BARRIERS FOR THE CONTROL OF ANTS (HYMENOPTERA: FORMICIDAE) IN VINEYARDS \*

#### ABSTRACT

*Honeydew-feeding ants significantly reduce the efficacy of biological control of the mealybug *Planococcus ficus* in vines. Two trials were conducted to find a cost-effective method for ant control that is environmentally friendly, practicable and acceptable in an integrated pest management programme. Thirteen chemical stem barriers were assessed for two ant species, *Linepithema humile* and *Anoplolepis custodiens*, in four field trials during two years. Four of the treatments that showed high efficacy in the field trials were also evaluated in two simulated field trials for *L. humile* and *Anoplolepis steingroeveri* due to high variability in pre-treatment counts that occurred in the field trials. Treatments showing the highest efficacy against *L. humile* and *A. custodiens* in field trials were the chlorpyrifos-impregnated band and the terbufos slow-release band. Alphacypermethrin SC at 10mℓℓ was effective against *L. humile* and has subsequently been registered as a chemical stem barrier on vines. The treatment showing the highest efficacy against *A. steingroeveri* in the simulated field trial was alphacypermethrin SC at 20mℓℓ. In the simulated field trial, a decline in ant infestation was observed in all treatments, including the control, five to six weeks after application of treatments. The most likely explanation is that chemical stem barriers result in ant mortality, although other reasons for this decline are discussed. It is recommended that suitable bioassay techniques, which expose ants to the treated substrate for a limited period, thereby simulating field conditions, be developed in order to determine if chemical stem barriers result in ant mortality.*

#### INTRODUCTION

Honeydew-feeding ants significantly reduce the efficacy of biological control of the mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) in vines by coccinellid predators and parasitic Hymenoptera (Kriegler & Whitehead 1962, Myburgh

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*et al.* 1973 and Urban & Mynhardt 1983). These ants, while feeding on honeydew excreted by the mealybug, deter natural enemies from controlling mealybug populations. The ants that are most abundant in vineyards and are known to feed on honeydew in South Africa are the common pugnacious ant *Anoplolepis custodiens* (F. Smith), the black pugnacious ant *A. steingroeveri* (Forel), the Argentine ant *Linepithema humile* (Mayr) (all epigaeic species) and the cocktail ant *Crematogaster peringueyi* Emery (an arboreal species) (Whitehead 1957, Urban & Bradley 1982 and Addison & Samways 2000). With the introduction of the Scheme for Integrated Production of Wine (Anonymous 2000), it was necessary to find a cost-effective method for ant control that is environmentally friendly, practical and acceptable in an integrated pest management programme. This study was conducted to assess various chemical stem treatments against the three epigaeic ant species, *L. humile*, *A. steingroeveri* and *A. custodiens*. Chemical stem barriers have been found to be effective against various ant pests, including *L. humile* and *A. custodiens*, in citrus orchards and are considered to be a suitable method of ant control in terms of IPM as ants are left to forage on the orchard floor where they fulfil important ecological functions such as feeding on other pest insects (Samways & Tate 1984, Moreno *et al.*, 1987, Stevens *et al.* 1995 and James *et al.* 1998).

## MATERIALS AND METHODS

### *Field trials*

**Treatments:** Concentrations and application methods of chemicals tested are listed in Table 1. Two synthetic pyrethroids (alphacypermethrin and betacyfluthrin) and one organophosphate (chlorpyrifos) were tested as direct stem sprays. These were applied directly above the irrigation pipes (approximately 40cm above the ground) as a 10cm wide band using a ring spray attached to a knapsack spray pump (Fig. 1). An approximate dosage of 50mℓ/vine of the spray mixture was applied to each stem. Slow-release chlorpyrifos-impregnated bands, which measured 4cm in width, were fastened around stems by stapling them onto vines directly above the irrigation pipes. Another slow-release band consisted of a cotton material stocking filled with granulated terbufos, also an organophosphate. This band, measuring approximately 2cm in diameter, was fastened around the base of vine stems as the granule required moisture to release the chemical. The fruit on vines exposed to the latter treatment were analysed for organophosphorous residues by the Department of Health (Forensic chemistry

laboratory, Cape Town) shortly before harvest, due to the volatility [vapour pressure: 34.6mPa (25 °C)] of this chemical (Tomlin 1997).

One insect and three plant extracts, intended for use by resource-limited producers, were also tested. Using methods described by Elwell & Maas (1995), the leaves and fruit of the syringa tree (*Melia azedarach* Linn.), the leaves and stems of the tomato plant (*Lycopersicon esculentum* Linn.), five moderate-sized garlic cloves (*Allium sativum* Linn.) and ants, the same species as the pest ant species, were crushed and soaked in 1ℓ water with 5mℓ liquid soap (Sunlight liquid) for 12 hours. Quantities of these ingredients are given in Table 1. The ants were collected during December during both years and consisted mainly of workers. The solutions were strained through filter paper (milk filters, 191mm in diameter), stored in a closed glass container until the following morning and used as direct stem sprays. The terbufos slow-release band, crushed garlic, syringa and tomato extracts were each tested during one year only as the extracts were found to be ineffective. The terbufos slow-release band was only tested for one year as the company involved did not want to pursue registration at the time due to safety aspects (regarding the application of the bands) which needed to be addressed first.

**Sites:** Four field trials, two to assess the control of *A. custodiens* in Robertson (33°50'S 19°56'E) and two to assess the control of *L. humile* in Simondium (33°10'S 18°55'E), were carried out during the 1997/98 and 1998/99 seasons. These sites were different to those used in Chapter 1 for the survey. *A. custodiens* and *L. humile* were the most significant ones in the Robertson and Simondium vineyards, respectively. The same vineyards were not re-used for trials during the following year due to chemical residues possibly still being present from the last year. All trials were carried out in established, trellised, wine grape vineyards. The vineyards were micro-irrigated and weeds were trimmed where necessary to prevent ants from gaining alternate access to vines. All vineyards were naturally infested with mealybug.

#### **Trial layout:**

- Robertson – *Anoplolepis custodiens*: Five replicates of eleven treatments and one untreated control (Table 2) were randomised in a complete block design. Each replicate consisted of the five to six vines occurring between two adjacent trellis poles. Pre-treatment ant counts were made on 4 December 1997 and on

18 November 1998. Treatments were applied on 9 December 1997 and on 3 December 1998, respectively. Trials were evaluated until after harvest when ant activity decreased, 115 and 139 days after application of treatments in 1998 and 1999, respectively.

- Simondium – *Linepithema humile*: Five (1997) and four (1998) replicates of eleven treatments and one untreated control (Table 2) were randomised in a complete block design. Pre-treatment counts were made on 27 November 1997 and on 25 November 1998. Treatments were applied on 11 December 1997 and on 17 December 1998, respectively. Trials were evaluated until after harvest, 135 and 117 days after application of treatments in 1998 and 1999, respectively.

**Method of evaluation:** Pre-treatment and post-treatment ant counts were made by classifying vines as infested if one or more of the target ants were seen moving up or down the entire length of vine stems (approximately 60cm) during a 5 to 10 sec stem observation. All the treated vines per replicate were used to collect data. Stem observations were done weekly during 1997/98 and every second week during 1998/99 until ant activity decreased. Observations were done in the morning in Simondium, and around mid-morning in Robertson. The number of days after which 25% or more of the vines per plot were infested with the target ants was used to measure the efficacy of treatments. This is the guideline that is currently used by producers as an action threshold to determine if ant control is necessary. ANOVA, LSD was performed on the data. Data were analysed separately for each year and for each trial. All treatment plots in both areas were assessed for mealybug infestation by scanning the stems, leaves and bark for adults and crawlers, and classifying the vine as infested or not infested at the end of each growing season during May 1998 and 1999. Mealybug infestations were then averaged to obtain a percentage for each of the four vineyards.

### *Simulated field trials*

Due to the uneven distribution of ants in vineyards resulting in some plots having low or zero ant counts in pre-treatment evaluations, a simulated field trial was conducted in order to achieve even and high pre-treatment ant counts for testing chemical stem barriers. Five treatments were evaluated, alphacypermethrin SC at 20mℓℓ water, betacyfluthrin EC at 30mℓℓ water, chlorpyrifos EC at 41mℓℓ water, the chlorpyrifos-impregnated band and an untreated control. Two vineyards in the Stellenbosch area,

one infested with *L. humile* and one infested with *A. steingroeveri* were used. Twenty-five cm lengths of old vine stems were attached to dowel sticks at one end and plastic feeding trays (6cm in diameter) at the other end (Fig. 2). The dowel sticks were used to vertically secure stems in the ground. Stems were distributed in ten groups of five each (ten replicates, five treatments), throughout each vineyard directly in front of planted vines where ant activity was noticed. The five stems in each group were placed 30-40cm apart. The feeding trays were filled with syrup daily (approximately 10mm) until all trays were infested with ants, resulting in a 100% pre-treatment infestation, and subsequently on a daily basis until the end of the trial. The ten stems of each of the three treatments were then dipped (to ensure that all stems obtained equal distribution of chemical) into the respective chemicals, while the bands were stapled around the middle of the remaining ten stems (the ten stems of the control were left untouched). All stems were replaced in the same locations. Feeding by the relevant ant species continued throughout the trial. Stems were treated in February 1999 against *L. humile* and in March 1999 against *A. steingroeveri*. Feeding trays were inspected at weekly or every two week intervals and classified as infested or uninfested with one or more ants during 5 sec observations. A 9 x 1 Chi-squared test was used to test for treatment differences in the trial assessed for *L. humile* and a 6 x 1 Chi-squared test was used for the same purpose in the trial assessed for *A. steingroeveri* (Snedecor & Cochran 1967). This test was decided upon due to the binary nature of the data. The average percentage infestation during ten and seven sampling dates, of *L. humile* and *A. steingroeveri* respectively, were thus compared to the control and to each other, resulting in a Chi-square value that is either significant or not significant at  $p \leq 0.05$ .

## RESULTS

### *Field trials*

Ant activity as measured in the pre-treatment counts was variable between treatment plots and much lower during the first year than during the second year for both ant species (Table 2). Treatments were regarded as effective if they succeeded in keeping 75% or more vines free of ants for 90 days, the approximate time between the start of ant activity in vines and harvest. The average mealybug infestation during May 1998 and 1999 is shown in Table 2.



**Robertson:** *Anoplolepis custodiens* was not present in high numbers in vines treated with chlorpyrifos EC, alphacypermethrin EC and SC at 20mℓℓ water, both concentrations of betacyfluthrin EC and the chlorpyrifos-impregnated band during the first year (Table 2). These treatments did not differ significantly from the control as a result of low ant infestations and, therefore, their effectiveness cannot be evaluated using these results (Fig. 3A). During the second year, only the chlorpyrifos-impregnated band and the terbufos slow-release band resulted in acceptable control. No organophosphate residues were found on grapes from the terbufos-banded vines. These bands could have been effective for a longer period, but as evaluations ceased 139 days after application of treatments, this could not be determined.

**Simondium:** *Linepithema humile* was excluded from vines by chlorpyrifos EC, alphacypermethrin EC and SC at both concentrations, betacyfluthrin at 20mℓℓ water, the chlorpyrifos-impregnated band and the crushed ant extract during the first year (Fig. 3B). Alphacypermethrin EC and SC at 20mℓℓ water were still effective at the last sampling date during the first year. Alphacypermethrin SC at 10mℓℓ water, betacyfluthrin EC at 20mℓℓ water, the chlorpyrifos-impregnated band and the terbufos slow-release band were effective during the second year, indicating that these treatments are effective against high ant infestations. Although the higher concentrations of alphacypermethrin EC and SC were less effective than the lower concentrations during the second year, these differences were not significant. However, the higher concentrations of betacyfluthrin EC were significantly less effective than the lower concentrations during both years. The chlorpyrifos-impregnated band was still effective at the last sampling date during 1998/99.

More treatments were, therefore, effective in excluding *L. humile* than *A. custodiens*. In vineyards with high ant activity the chlorpyrifos-impregnated band, the terbufos slow-release band and alphacypermethrin SC at 10mℓℓ water were consistently effective in excluding target ants for three months or longer. The latter was only effective against *L. humile*.

#### *Simulated field trials*

***Linepithema humile:*** All treatments differed significantly from the control and from each other, as shown by the relevant Chi-square values (Table 3A). The lowest average percentage infestation over 10 sampling dates was found in the chlorpyrifos band

treatment which was therefore most successful in excluding *L. humile* (Table 3A). Figure 4A shows the percentage infestation of *L. humile* for each observation. The percentage infestations varied for each treatment and for each sampling date, where initial reductions only took place in the two synthetic pyrethroid treatments, betacyfluthrin and alphacypermethrin, on 12 March 1999. After this date numbers increased once again in these two treatments. The % infestation in the chlorpyrifos band treatment remained zero on all observations except on two occasions, on 1 April and on 7 May 1999. Infestations in this treatment never exceeded 40%. A general reduction in all treatments, including the control, was observed on 9 April, after which a general increase took place until 7 May in all treatments.

***Anoplolepis steingroeveri*:** All treatments differed significantly from the control at the last sampling date and all treatments, except chlorpyrifos EC and the chlorpyrifos-impregnated band, differed significantly from each other (Table 3B). The most effective treatment in excluding *A. steingroeveri*, as indicated by the average percentage infestation, was alphacypermethrin SC (Table 3B). Figure 4B shows the percentage infestation of *A. steingroeveri* for each observation. A general decrease in percentage infestation took place on 14 April 1999, two weeks after the application of treatments, for the betacyfluthrin, alphacypermethrin and chlorpyrifos treatments. One week later, all treatments showed a reduction in infestation, including the control, after which numbers increased again, remaining high until the last sampling date.

## DISCUSSION

From the field trials and simulated field trials it can be seen that *L. humile* is easier to control than the *Anoplolepis* spp. One possible reason for this could be the larger size and longer legs of the *Anoplolepis* spp. compared to the smaller *L. humile* (Arnold 1915), resulting in less contact with the treated area when walking up and down the vine stem.

### *Field trials*

Some unusual trends were observed during the two years of testing chemical stem barriers against *L. humile*, where betacyfluthrin EC at 30mℓℓ water was consistently less effective than the lower concentration of 20mℓℓ water. Results also indicated that alphacypermethrin SC at 20mℓℓ water was less effective than the lower concentration of 10 mℓℓ water during the second year. Although this difference was not significant, the

higher concentration does not meet with the efficacy requirement of a minimum of 90 days ant exclusion. No explanation can be given for this unusual trend.

### *Simulated field trials*

None of the treatments met with the requirements of a 25% infestation or less for *A. steingroeveri*. The percentage infestation for all treatments often reached over 80% for *A. steingroeveri*, but always remained at 60% or below for *L. humile* for all treatments (Fig. 4). Alphacypermethrin SC at 20m $\mu$ l/l water gave the best control out of the four treatments for this ant and was significantly better than the chlorpyrifos-impregnated band, which significantly out-performed all treatments in the second year (high ant infestation) of field trials. This difference in results can possibly be explained by observations where an established trail of ants was never seen moving over bands in the field trial, only an occasional single ant. In the simulated field trial, such established trails were more common, possibly due to the easily accessible food source. The same trend was not observed for *L. humile*, where the chlorpyrifos-impregnated band always gave acceptable control in field trials and simulated field trials, even with high ant infestations. A decrease in ant infestation occurred five to six weeks after application of treatments for all five treatments, including the control, in the simulated field trials for both ant species. A possible reason for this could be that treatments were killing ants and that the effect was only being noticed at this time. In Australian field trials, testing controlled-release chlorpyrifos stem bands against *Iridomyrmex rufoniger* gp. spp. in citrus trees, a decline in ant activity was also observed and ascribed to the bands killing ants (James *et al.* 1998). From laboratory bioassays conducted in the same study, it was shown that these bands can result in ant mortality after exposure for 16 h, and it is therefore possible that other stem treatments also cause ant mortality. Since the reduction in numbers also occurred in the untreated control, it is assumed that ants from one colony may have fed on vines with different treatments. The subsequent increase in numbers can be explained by a new invasion of ants from other nests or a recovery of the ant populations. From visual observations it was noticed that the nest density of *A. steingroeveri* was much higher than that of *L. humile*, which could explain why the population reduction was shorter-lived for *A. steingroeveri* than for *L. humile*. Another possible reason for the decline in numbers could be that feeding trays were lacking syrup for an extended period between sampling dates. This occasionally occurred as a result of bees feeding on the syrup. Bees were particularly abundant in the vineyard where *A. steingroeveri* was being monitored. However, this would not explain the

gradual decrease and subsequent gradual increase in numbers over three to five sampling dates. The first reduction in numbers was probably not due to unfavourable weather conditions as temperatures were more or less constant on each sampling date and average rainfall was low for that month (5mm).

The simulated field trial has several advantages over a conventional field trial. A pre-treatment infestation of 100% can be obtained for all treatments. Assessments are less time consuming and more accurate. High ant activity can be maintained throughout the evaluation period by the continuous availability of an energy rich, easily obtainable food source, which allows for very thorough testing of treatments. A simulated field trial can give quick results under field conditions and does not require the establishment and maintenance of laboratory colonies. Finally, standardisation is easy and it is possible to test treatments that have been variably weathered, thereby allowing their efficacy over time to be assessed simultaneously. Weathering of treatments will take place at the same rate as in the field as the substrate (vine stems) used is the same. However, it is recommended that the same treatments be grouped together instead of replicates in order to prevent cross-infestation of ants between treatments from taking place. This is important if treatments result in ant mortality. The feeding trays should be screened to prevent bees from feeding, but still allow the ant's access to the food source.

## CONCLUSION

More treatments effectively control *L. humile* than *A. custodiens* or *A. steingroeveri* for the required period of approximately three months. Subsequent to the start of these trials, alphacypermethrin SC at 10mℓℓ water was registered as a direct stem treatment in vineyards against *L. humile*, while alphacypermethrin SC at 20mℓℓ water has been registered against the *Anoplolepis* spp. The two treatments that provided the best control against all three ant species, the chlorpyrifos-impregnated band and the terbufos slow-release band, may be impractical and expensive for use in commercial vineyards due to high planting density ( $\pm 2000$  vines ha<sup>-1</sup>). However, if they are effective for more than one year, their use on farms may be reconsidered, and their continued efficacy for *L. humile* and the *Anoplolepis* spp. in vineyards needs to be determined.

## RECOMMENDATIONS FOR FUTURE WORK

More practical control methods should be investigated for use in vineyards, such as toxic baits with low mammalian toxicity, for example, boric acid in a sugar solution (Klotz *et al.* 1998). Although toxic baits can control *L. humile* effectively, it is more difficult to find a suitable bait that controls the *Anoplolepis* spp. Future research is also needed to establish suitable and practical bioassay techniques, which expose ants to the treated substrate for a limited period thereby simulating field conditions, and more chemical stem treatments need to be assessed for ant mortality after exposure.

Table 1. Treatments and application method used in Robertson against *Anoplolepis custodiens* and in Simondium against *Linepithema humile* during 1997 and 1998.

Active ingredient, formulation*** and grams pure active ingredient	Concentration in mL/L (unless otherwise stated)	Application method
<sup>1</sup> Alphacypermethrin SC (100g/L)	10	Stem spray
Alphacypermethrin SC (100g/L)	20	Stem spray
Alphacypermethrin EC (100g/L)	10	Stem spray
Alphacypermethrin EC (100g/L)	20	Stem spray
<sup>2</sup> Betacyfluthrin EC (50g/L)	20	Stem spray
Betacyfluthrin EC (50g/L)	30	Stem spray
<sup>3</sup> Chlorpyrifos EC (480g/L)	41	Stem spray
Crushed ant extract	5g/L	Stem spray
Crushed garlic extract	15g/L	Stem spray
Syringa plant extracts	225g/L	Stem spray
Tomato plant extracts	140g/L	Stem spray
*Chlorpyrifos-impregnated band	-	Stem band
**Terbufos slow-release band	-	Stem band

<sup>1</sup> Fastac (Cyanamid), <sup>2</sup> Bulldock (Bayer), <sup>3</sup> Dursban (Efekto), \* Suskon Blue Ribbon (UAP Crop Care), \*\* Donor (Quest Developments).

\*\*\* Formulations: SC=suspension concentrate, EC=emulsifiable concentrate.

Table 2. Pre-treatment ant counts of *Anoplolepis custodiens* in Robertson and of *Linepithema humile* in Simondium during 1997/98 and 1998/99 and percentage mealybug infestation as calculated at the end of each season in 1998 and 1999.

Active ingredient and Concentration	Pre-treatment counts (% infested vines) *			
	<i>Anoplolepis custodiens</i>		<i>Linepithema humile</i>	
	1997/98	1998/99	1997/98	1998/99
Control	8 ab	70 c	14 a	75 ab
Alphacypermethrin SC (10mℓℓ)	15 ab	88 abc	8 a	84 a
Alphacypermethrin SC (20mℓℓ)	10 ab	100 a	16 a	67 ab
Alphacypermethrin EC (10mℓℓ)	28 a	81 abc	12 a	73 ab
Alphacypermethrin EC (20mℓℓ)	15 ab	71 c	12 a	60 b
Betacyfluthrin EC (20mℓℓ)	8 ab	88 abc	12 a	62 ab
Betacyfluthrin EC (30mℓℓ)	10 ab	80 bc	13 a	72 ab
Chlorpyrifos EC (41mℓℓ)	17 ab	74 bc	17 a	71 ab
Crushed ant extract	7 b	75 bc	12 a	57 b
Crushed garlic extract	Not tested	92 ab	Not tested	71 ab
Syringa plant extracts	20 ab	Not tested	12 a	Not tested
Tomato plant extracts	22 ab	Not tested	8 a	Not tested
Chlorpyrifos-impregnated band	5 b	87 abc	10 a	77 ab
Terbufos slow-release band	Not tested	88 abc	Not tested	66 ab
<b>% Mealybug infestation</b>	<b>33.0 <sup>1</sup></b>	<b>68.4 <sup>2</sup></b>	<b>19.4 <sup>1</sup></b>	<b>40.8 <sup>2</sup></b>

\* Numbers followed by the same letter in a column do not differ significantly ( $p \leq 0.05$ ) (excluding % mealybug infestation).

<sup>1</sup> As calculated in May 1998.

<sup>2</sup> As calculated in May 1999.

Table 3A. Comparison between average percentage infestation (using Chi-square values, df = 9) of five treatments tested against *Linepithema humile* during ten sampling dates in a simulated field trial in Stellenbosch during 1999.

Treatments	% Infestation	Control	Chlorpyrifos EC	Alphacypermethrin SC	Betacyfluthrin EC
Control	81	-	-	-	-
Chlorpyrifos EC	20	94.6 *	-	-	-
Alphacypermethrin SC	30	57.6 *	54.2 *	-	-
Betacyfluthrin EC	40	65.8 *	124.7 *	54.6 *	-
Chlorpyrifos band	6	92.0 *	34.9 *	60.0 *	56.4 *

\* indicates significant differences between treatments ( $p \leq 0.05$ ).

Table 3B. Comparison of average percentage infestation (using Chi-square values, df = 6) of five treatments tested against *Anoplolepis steingroeveri* during seven sampling dates in a simulated field trial in Stellenbosch during 1999.

Treatments	% Infestation	Control	Chlorpyrifos EC	Alphacypermethrin SC	Betacyfluthrin EC
Control	86	-	-	-	-
Chlorpyrifos EC	77	42.6 *	-	-	-
Alphacypermethrin SC	42	115.3 *	58.9 *	-	-
Betacyfluthrin EC	50	134.2 *	69.1 *	21.23 *	-
Chlorpyrifos band	79	52.6 *	11.7	80.4 *	96.0 *

\* indicates significant differences between treatments ( $p \leq 0.05$ ).



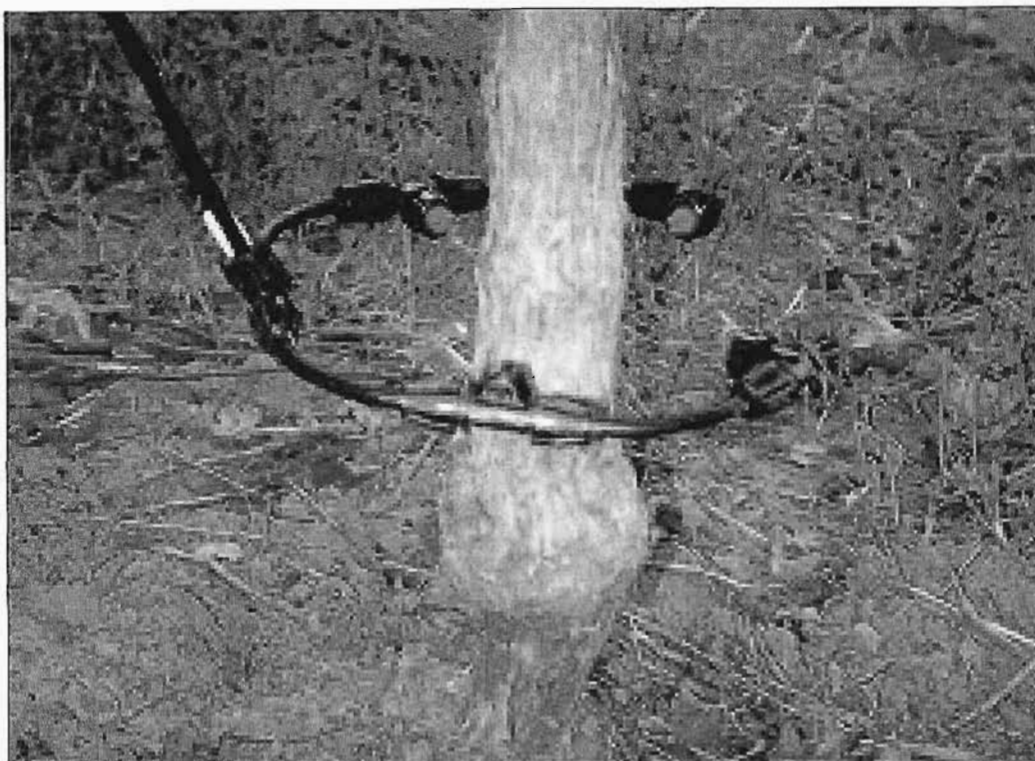


Fig. 1. Ring spray attachment with four nozzles fastened onto a knapsack spray pump (not shown here), which was used to apply chemical stem treatments around vine stems.

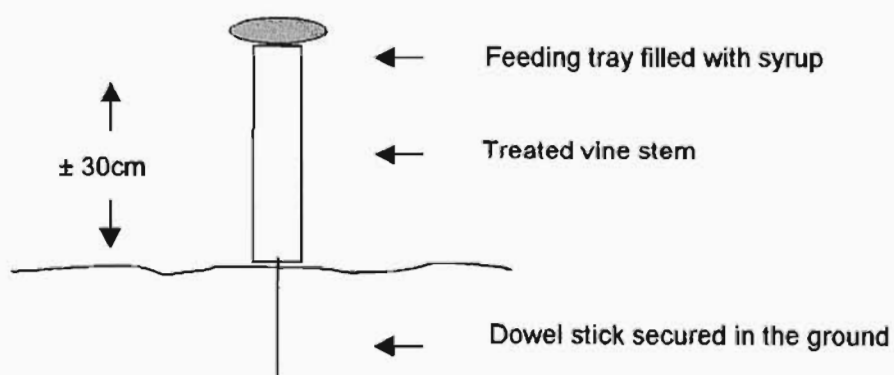


Fig. 2. Schematic representation of an old vine stem used in the simulated field trial to test chemical stem barriers against ants.

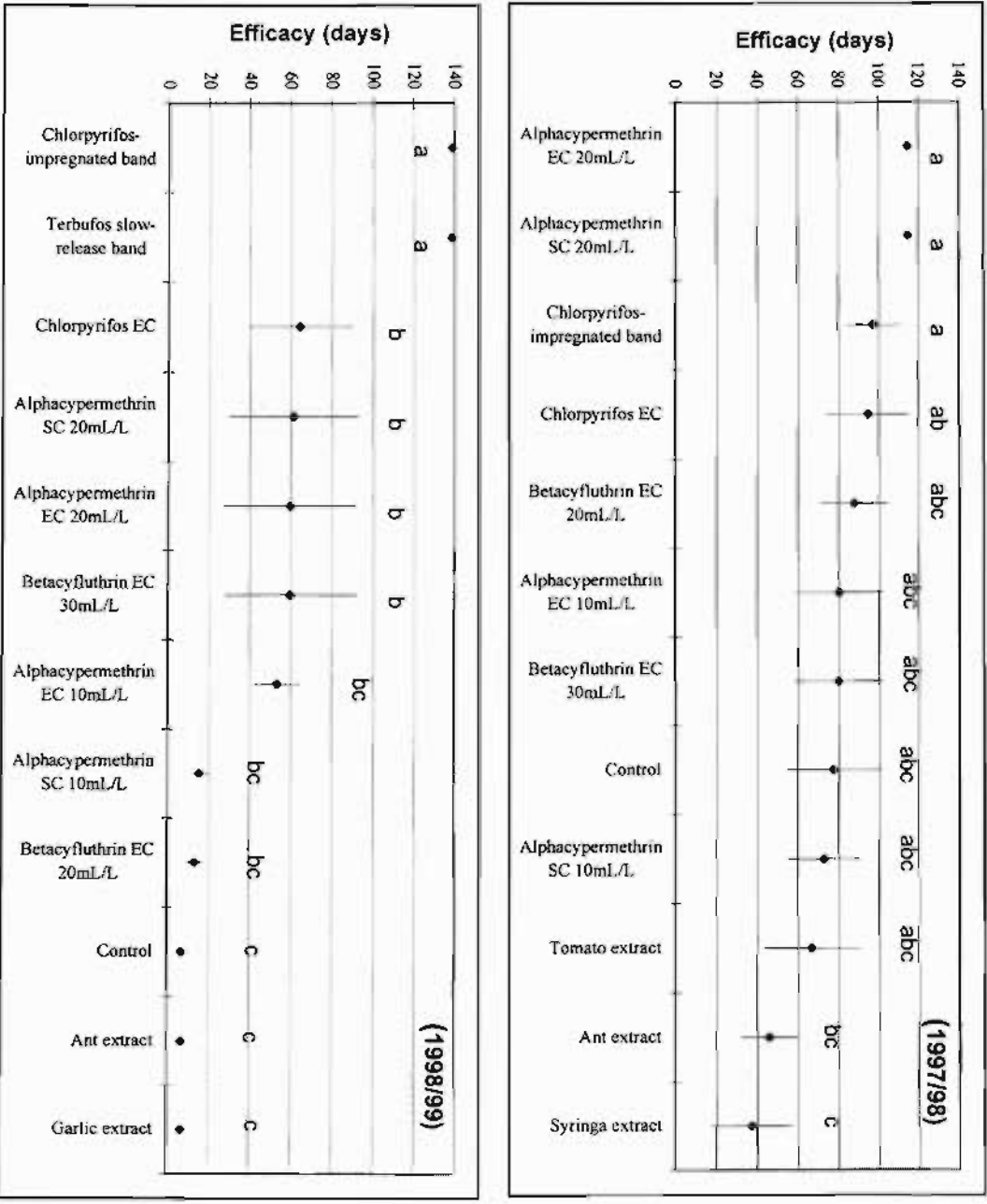


Fig. 3A. Efficacy (number of days after application of treatments until ants infest 25% or more vines) assessed during two years for *Anoplolepis custodiens* in Robertson. Error lines represent plus/minus standard errors. Treatments not designated by the same letter are significantly different ( $p \leq 0.05$ ).

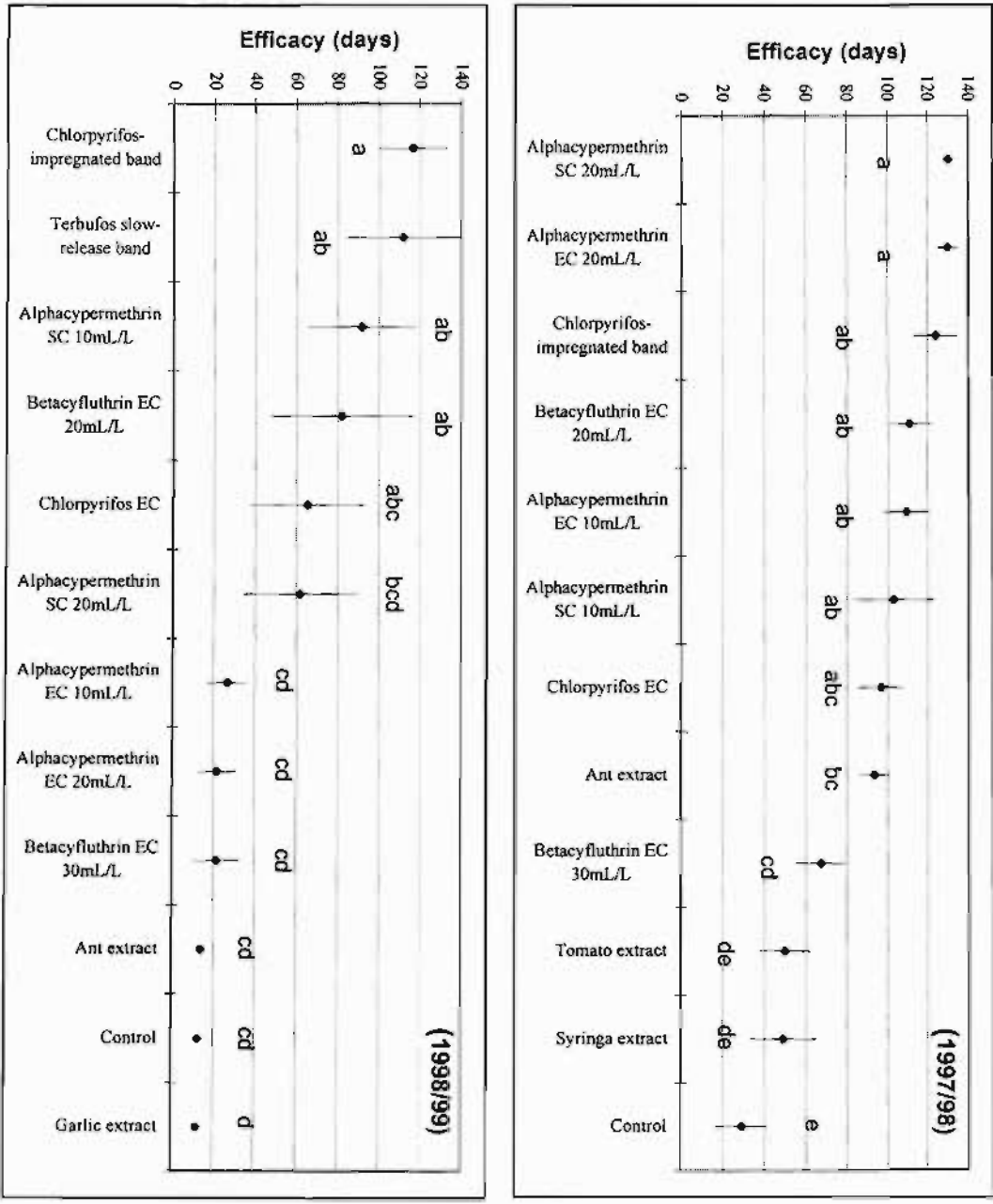


Fig. 3B. Efficacy (number of days after application of treatments until ants infest 25% or more vines) assessed during two years for *Linepithema humile* in Simondium. Error lines represent plus/minus standard errors. Treatments not designated by the same letter are significantly different ( $p < 0.05$ ).

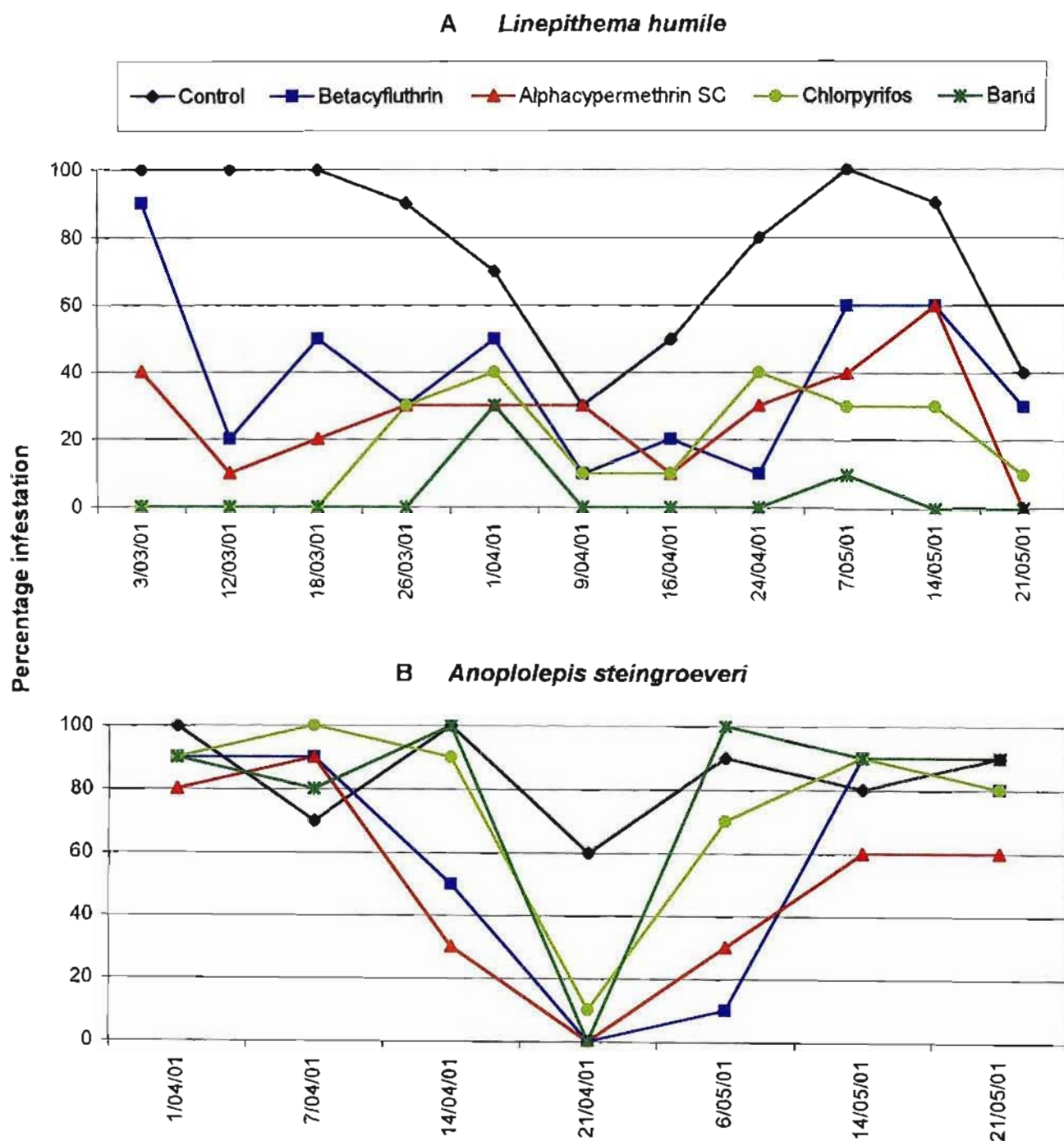


Fig. 4. Percentage infestation by *Linepithema humile* and *Anoplolepis steingroeveri* during eleven and seven weeks, respectively, in a simulated field trial where five treatments were tested in Stellenbosch during 1999.

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## CHAPTER 3

### INTEGRATED PEST MANAGEMENT USING COVER CROPS FOR MANAGING THE ANT-MEALYBUG (FORMICIDAE: PSEUDOCOCCIDAE) MUTUALISM IN WESTERN CAPE VINEYARDS

#### ABSTRACT

*Vegetative ground cover has been observed to deter the common pugnacious ant Anoplolepis custodiens from nesting in shaded soils in a number of studies. In view of this, the aim of this study was to determine whether cover crops: 1) can reduce local A. custodiens infestations in vineyards in the Western Cape Province as a result of a lowering of soil temperatures; 2) provide winter refugia for natural enemies of the mealybug Planococcus ficus. If both these effects occur, there would therefore be potential for using cover crops in the integrated management of vine mealybug from two points of view. Four ground cover treatments were established in a wine grape vineyard: Creeping vetch Vicia dasycarpa, triticale Triticale v. Usgen 18, and one permanent cover crop, consisting of a seed mixture during the first season and a pure stand of dwarf fescue Festuca sp. during the second season. These were compared to a control plot kept free of ground cover. A. custodiens activity, number of nest entrances, ant and mealybug vine infestations, mealybug natural enemy numbers and soil temperature and moisture were monitored. Results showed that cover crops appear to have no significant effect on either ant nor mealybug infestations, nor on the number of ant nest entrances, nor on natural enemy numbers, despite the fact that cover crops did reduce soil temperature and increase soil moisture significantly. Ant activity in the triticale plots even had significantly higher average ant abundances, indicating that this cover crop should therefore not be used in vineyards with ant and mealybug infestations. The primary regulator of ant infestations in this vineyard was the food source in the form of mealybug honeydew and possibly seeds from triticale. The results indicate that current ant control methods will have to be employed until such a time that there are other alternatives.*

## INTRODUCTION

Biological control of the mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), a major pest of vines in South Africa, is disrupted by ants, which tend it for honeydew (Kriegler & Whitehead 1962, Myburgh *et al.* 1973, Urban & Mynhardt 1983). In the Western Cape Province, the Argentine ant *Linepithema humile* (Mayr), the two pugnacious ants *Anoplolepis custodiens* (F. Smith) and *A. steingroeveri* (Forel), the cocktail ant *Crematogaster peringueyi* Emery and the little ubiquitous white-footed ant *Technomyrmex albipes* (F. Smith) are associated with this mealybug (Addison & Samways 2000). A *Pheidole* species (possibly *capensis*) also occasionally tends the vine mealybug (Addison & Samways 2000).

As ants are sensitive to anthropogenic disturbance, their local geographical distribution appears to be dynamic and influenced by various factors, including interspecific competition, regional climatic differences and habitat changes (De Kock *et al.* 1992). Vegetative ground cover was observed to deter *A. custodiens* from nesting in shaded soils in a number of studies (Way 1953, Steyn 1954, Myers 1957, Prins *et al.* 1990 and Way & Khoo 1992). A possible reason for this is that this ant species is unable to breed successfully if a certain nest temperature has not been reached (Way 1953, Steyn 1954 and Myers 1957). In an extensive study in Scotland, where the effects of soil temperature on *Myrmica rubra* Linn. were established, Brian & Brian (1951) determined that the brood in shaded nests were much retarded and there was less brood and colonies were failing to reproduce. This suggests that a leafy, dense cover crop, which cools the soil, may prevent *A. custodiens* and possibly other ant species from nesting there. This type of habitat modification may be a viable alternative or supplement to chemical control.

Certain ant species, such as the *Anoplolepis* spp., are difficult to control in vines solely with chemical stem treatments (Addison 2002), the method of ant control that is currently accepted for the Scheme for Integrated Production of Wine Grapes in South Africa (Anonymous 2000). In these guidelines, planting cover crops is strongly recommended for soil amelioration. Cover crops can reduce the need for herbicides, as they compete directly with weeds. Weeds, which ants use to enter the vine canopy, are one of the factors reducing the efficacy of chemical stem barriers. Certain low-growing cover crops could reduce this infestation pathway by out-competing tall weeds.



Mealybugs have also been found to infest the roots of weeds in vineyards, in particular broad-leaf species (Walton 2001). This is an additional food source for ants, with the mealybug being difficult to control as its populations are subterranean. One option is to apply full cover chemical weed control regularly. Another, more environmentally-friendly option, is to plant cover crops which out-compete weeds successfully (Fourie *et al.* 1997).

Other benefits derived from planting cover crops are that they are believed to serve as an alternative refuge for natural enemies (van Emden 1990). An increase in natural enemies has been found on various cover crops in agricultural systems (Teddars 1983, and Bugg & Waddington 1994) including vineyards in the USA and Germany (Altieri & Schmidt 1985 and Hofmann 2000). It is also thought that cover crops, by reducing dust levels in vineyards, protect small predators and parasitoids from abrasion to their exoskeleton (Pettigrew 1998). The combination of reduced ant nesting and increased natural enemy populations in vineyards could help in the control of the mealybug in an integrated and environmentally-friendly way.

No formal studies have been undertaken to establish the effect of cover crops on pest ants in South African vineyards. Also, little is known of natural enemies of mealybug on cover crops in the country. One observational study on vines in the USA monitored the effect of cover crops in attracting predatory ants to control insect pests of vines (Altieri & Schmidt 1985) but there was no mention of possible control of pest ants using cover crops. Conlong (1995) likewise investigated intercropping maize and sorghum with sugarcane to attract epigaeic predators to control the sugarcane stalk borer *Eidana saccharina* Walker (Lepidoptera: Pyralidae) in South Africa. Way & Khoo (1992) suggested that vegetative ground cover plays an important role in both attracting beneficial ants and suppressing pest ants such as *A. custodiens*. Moreover, they caution that the dynamics involved must be fully understood before such tools can be used successfully in pest management.

This study was conducted to determine whether cover crops: 1) can reduce local *A. custodiens* infestations in vineyards in the Western Cape Province as a result of a lowering of soil temperatures, and 2) provide winter refugia for mealybug natural enemy populations in vineyards. If these effects occur, there would therefore be some potential

for using cover crops in the integrated management of vine mealybug from two points of view.

## MATERIALS AND METHODS

### *Site and trial layout*

The trial site was in the Bonnievale area on the farm Morgensonndt (33.26S 20.01E) in a nine year old, trellised Chenin blanc vineyard infested with vine mealybug *P. ficus* and the common pugnacious ant *A. custodiens*. Irrigation was by micro-jets. Plant spacing was 1.2m apart, while rows were 2.4m apart. A middle section of the vineyard was selected for the trial, approximately 1.5 ha<sup>-1</sup> in size. The vineyard was surrounded by orchards on two opposite borders separated from the vineyard by dirt roads, approximately 4m wide. The farm was situated between the Breede River and a country road, on the other side of which was a narrow strip of vineyards and a montane region with natural vegetation. The grower had not used cover crops previously in this vineyard and controlled weeds by mechanical and chemical methods. A 4 x 4 latin square design was used, with columns and rows randomised (Fig. 1). This design was chosen to minimize any possible edge effects, such as additional infestations from the dirt roads. Edge effects have previously been recorded from ants in vineyards (Addison & Samways 2000). Each plot was 950m<sup>2</sup> in size (11 rows x 6 subplots, consisting of the 5 vines between the trellis poles). The data area was 144m<sup>2</sup> (5 rows x 2 subplots) and was situated in the centre of each plot.

### *Cover crops used*

Two winter cover crops (those treated with herbicide in spring), creeping vetch *Vicia dasycarpa* (Fabaceae) and triticale *Triticale* v. Usgen 18 (Graminae), and one permanent cover crop, consisting of a seed mixture during the first season (Table 1) and a pure stand of dwarf fescue *Festuca* sp. (Poaceae) during the second season, were compared to control plots kept free of ground cover using herbicides and a tractor-drawn rotary mower. Triticale is currently the most widely used cover crop by grape growers in the Western Cape and is sown in the inter-row only leaving the vine row free of ground cover. Vetch provides a very dense vegetative cover over both row and inter-row due to its creeping nature, whereas fescue is a permanent low-growing cover that remains green throughout the year and is sown only in the inter-row. Seed planting densities are given in Table 1. Seeds were obtained from Agricol (Pty) Ltd., Brackenfell. Due to the

poor growth of the permanent mixture during the 2001/2002 season, it was decided to sow a pure stand of dwarf fescue, the strongest grower of the mixture, during the 2002/2003 season.

Cover crops were sown on 25 April 2001 and again on 10 April 2002. Soil preparation occurred approximately one month prior to sowing. The soil was disked to a depth of about 15cm. Immediately after sowing the seeds, they were raked into the soil using a tractor-drawn furrow plough. Treatments (vetch, triticale and control) were sprayed with herbicide (glyphosate 360 g/l at 6l/ha) on 6 September 2001 and on 17 September 2002. The vine rows of the permanent cover crop mixture were also sprayed with herbicide to control weeds growing on the berm (vine row). The inter-row is the 1.4m wide strip where the tractor can move, while the vine row is the 1m wide strip on which the vines are planted. One month after spraying the treatments, the control plots were mechanically mowed with a rotary mower, while the permanent cover crop plots were mowed to a height of approximately 30cm above the soil surface to slash high-growing weeds. During the first week in April 2002 the control plots were again slashed with a rotary mower.

#### *Soil temperature and moisture measurements*

Four 2-channel soil temperature and moisture loggers (MCS 486-TSM, Mike Cotton Systems, Cape Town) were used. This device uses gypsum blocks to measure soil moisture as described by Toome (2002), which he believes to be a suitable method for use in orchards and vineyards. Before being used, the loggers were calibrated for soil moisture with soil taken from the trial site as per manufacturer's instruction. This was done to ensure greater accuracy of the readings. One logger was placed into each of four treatments, approximately one meter from a trellis pole into the inter-row. The sensors were moved alternately to 10cm and 30cm below the soil surface on a monthly basis. Every second month, the sensors were moved to another section of the plot in the first replicate of each treatment. Monitoring started on 27 June 2001 and continued until March 2002. Readings were taken at hourly intervals. On 7 January 2002, the loggers were removed to download the data, and replaced one week later. Loggers were again removed on 11 March 2002 when the soil was disked in preparation for sowing. Loggers were replaced on 9 April 2002 after cover crops were sown, but data for the second year were lost on two loggers due to corrosion and sensor failure. Due to missing data for two treatments, all data for the second year were omitted. A *t* test was

used to analyse the data separately for each depth. Accumulated degree day units (Baskerville & Emin 1969) were calculated in each treatment using temperatures above 9.7°C, which is the lower threshold of development for *A. custodiens* medium worker pupae as calculated by Steyn (1954) for laboratory colonies. No upper threshold of development for *A. custodiens* was calculated in Steyn's study and for the purposes of comparing temperatures between treatments only, the following formula was therefore used:

$$\text{Accumulated } ^\circ\text{D} = \sum [(\text{hourly temperature reading} - 9.7)/24],$$

where:  $^\circ\text{D}$  = degree days.

### *Ant activity sampling*

Foraging activity of epigaeic ants was monitored using pitfall traps similar in design to the one described by Majer (1978). Traps consisted of polystyrene test tubes (18 x 150mm) containing approximately 10ml of a mixture of seven parts 70% ethyl alcohol and three parts pure glycerol. An outer case consisting of irrigation pipe, approximately 160mm in length was permanently sunk into the ground and used as a trap sleeve to facilitate changing of traps. The test tubes were sunk into the casing in the vine row and the ground levelled so that the edge was even with the soil surface. A total of 64 traps were used, four traps in each of 16 plots. The traps were changed every two weeks, except when unfavourable weather conditions prevailed and caused the changing of traps to be postponed. All ant species caught were then sorted for identification and counted in the laboratory. Sampling started on 19 June 2001, and was continuous for the duration of the trial, which ended in March 2003.

### *Ant nest entrance counts and foraging distance*

All active nest entrances were counted in the vine row and inter-row in each of the four subplots in the data area as indicated in Fig. 1. In each of these subplots, an area of 1 x 1m was designated for the counts (directly opposite pitfall traps). This was done during March 2001 (pre-treatment counts) and then subsequently during November 2001 and 2002 and March 2002 and 2003. Nest entrances were considered active when ants emerged after the nest entrance was probed with a twig, or when ants were seen moving in and out of the entrance. Nest entrances were often located at the base of vine stems, forming a suspended nest entrance made of plastered soil particles that went some way up the stem. On two sampling dates, November 2001 (spring) and

March 2003 (autumn), the number of suspended nest entrances in each of the treatments was therefore counted. To establish the average foraging distance (end point) of *A. custodiens* from nest entrances in this vineyard, a detailed observation was conducted during April 2003. The average distance that 120 ants (30 per treatment) travelled from 120 randomly-selected nest entrances to the point where they ended after a 2 min observation period was recorded in the four treatments. Since no definite foraging columns could be detected for *A. custodiens* workers leaving the nests (Steyn 1954 and personal observation), the observations were limited to 2 min each. This was regarded as sufficient time to establish an average foraging distance as the ants either turned back in the direction of the nest or stopped to collect honeydew in the vines.

#### *Ant and mealybug infestations in the vine canopy*

*A. custodiens* and *P. ficus* infestations were monitored in the vines during April 2001 (pre-treatment counts), February 2002, November 2002 and March 2003. The vines on either side of the inter-rows in the data area of each of the 16 plots were monitored (Fig. 1). As the number of vines in each subplot was not always the same, a total of 624 vines were monitored. Monitoring was done by inspecting the leaves (10 per vine), stems and fruit of vines. Vines were classified as infested or not infested. Pearson's correlation coefficient was used to calculate the correlation between ant and mealybug vine infestations in each treatment.

#### *Mealybug root infestations*

Two assessments to monitor mealybug root infestations on weeds and cover crops were carried out during September 2001 and 2002. Five plant samples were collected from each of four sub-plots in each of the data areas of each treatment. A total of 80 samples were therefore collected per treatment. The roots of the plants were examined in the field for the presence of mealybug species, which were then placed into 70% ethanol and sent away for identification.

#### *Mealybug natural enemy counts*

Monitoring was done using yellow, sticky Bug Traps™ (Agribiol, Vlaeberg) measuring 200mm x 100mm and natural enemies identified in the laboratory. One trap was placed into the data area of each replicate, resulting in a total of 16 traps. Traps were therefore situated roughly 36m apart in each row, and 26m apart between each replicate (Fig. 1). Identifications were made to species level where possible. Monitoring started during

June 2001, after which traps were changed monthly. This was done continuously for the duration of the trial.

### *Additional food source*

Aphids may be an additional food source for the ants and, as large numbers of aphids were noticed on weeds in this vineyard, an assessment of their presence on cover crops and weeds was undertaken. Monitoring took place on 12 September 2002. Five samples were taken in the inter-rows of each of four subplots in the data areas, resulting in a total of 80 samples for each treatment. The samples were placed into marked plastic bags and taken back to the laboratory where all aphids were collected and placed into 70% ethanol in marked plastic containers for later counting. Samples were sent away for identification.

Data from pitfall trap catches, nest entrance counts, ant and vine mealybug infestations and yellow bug traps for natural enemies were analysed using analysis of variance (ANOVA) and least significant differences (LSD) calculated to compare treatments (SAS Institute, 1999).

## **RESULTS**

### ***Cover crops***

Triticale grew to a height of approximately 1m (Fig. 2a). Initially, plants were leafy and dense, while as the season progressed and their maximum height was reached, they started to dry out. Once triticale was treated with herbicide in spring, the plants eventually broke at the base and formed a thick layer on the soil surface during summer. The vetch grew to a maximum height of about 20cm, and tended to spread over inter-row and vine row to form a dense, leafy layer leaving little of the soil surface exposed (Fig. 2b). Once treated with herbicide, the vetch formed a dense layer over the entire soil surface during summer. Both triticale and vetch were effective in competing with weeds. The permanent mixture and fescue (Fig. 2c) did not perform optimally during the two years. During the first year, only some of the fescue seeds from the seed mixture germinated. As these plots were not treated with herbicide in the inter-row, a green layer of weeds was left to grow in the inter-row during winter and much of summer, interspersed with a few fescue plants. Control plots were largely free of weeds during summer, although an estimated 40% of the soil surface became covered with weeds as

winter progressed (Fig. 2d). Control plots were characterised by weeds such as yellow sorrel (*Oxalis pes-caprae* L.), wild radish (*Raphanus raphanistrum* L.), wild mustard (*Rapistrum rugosum* L.), sowthistle (*Sonchus olearceus* (L.)), small mallow (*Malva parviflora* L.), red pigweed (*Amaranthus thunbergii* Moq.) and white goosefoot (*Chenopodium album* L.). Weeds were identified from Fourie (1996).

### **Soil temperature and moisture measurements**

Mean maximum and minimum soil temperatures (Tables 6 and 7, respectively) over the entire monitoring period, as measured in the inter-rows, were significantly higher in the control at both 10cm and 30cm than in the other treatments. Average soil temperatures throughout the season are graphed in Fig. 7. Accumulated heat units, calculated at 10 and 30cm respectively, were highest in the control (1078°D and 1370°D), intermediate in triticale (880°D and 933°D) and vetch (856°D and 859°D) and lowest in the fescue (801°D and 787°D). Mean percentage soil moisture over the entire monitoring period was significantly lower in control plots than in fescue or vetch plots, but not in triticale plots (Table 8). Average monthly soil moisture percentage remained lower in control plots than in fescue and vetch plots and was more or less the same in control and triticale plots, except during September/October (Fig. 8), which most likely resulted in triticale plots being significantly drier at 30cm than the control plot when assessed over the full trial period (Table 8).

### **Ant activity**

Ant activity started to increase in November 2001 and October 2002 and reached a peak in February during both years (Fig. 3). Few ants were caught between June and September. There was no significant interaction between ant activity in the various treatments between years ( $F = 1.28$ ,  $df = 6$ ,  $P = 0.26$ ) and therefore accumulated, average ant numbers over the two year monitoring period were used to compare ant activity between treatments. Ant activity was significantly higher ( $P \leq 0.05$ ) in the triticale plots than in the other treatments, which did not differ significantly from each other (Fig. 3).

### **Ant nest entrance counts and foraging distance**

The distribution of the nest entrances is given in Fig. 5. No significant differences in the number of nest entrances were found between ground cover treatments on any of the sampling dates (Table 4). There were significantly more nest entrances on the vine row

than in the inter-row on each sampling date (Table 4). This was also seen for the pre-treatment counts taken on March 2001. In November, the percentage of suspended nest entrances making up the total nest entrance count on the vine row was as follows: Fescue (67%), vetch (67%), control (38%) and *Triticale* (32%), while in March they were as follows: Control (34%), vetch (32%), fescue (21%) and *Triticale* (21%). From measuring the average distance that 120 ants travelled from nest entrances the following was observed: In the triticale plots, ants travelled an average of 34cm, in fescue plots the average distance was 32cm, in vetch plots 25cm and in control plots 26cm. The shortest foraging distance during a two-minute observation period was 10cm and the longest was 79cm.

### ***Ant and mealybug infestations in the vine canopy***

Ant infestation in the vines in the pre-treatment count was variable between treatments with the fescue plots having a significantly higher infestation than the control plots (Fig. 4a). However, percentage increase in the fescue plots was significantly lower than in the vetch and control plots (Table 2). During November 2002, the ant infestation in the vines in the vetch plots was significantly lower than in the other treatments (Fig. 4a). By March of the following year, however, no significant differences were found between any of the treatments.

The percentage mealybug infestation in the fescue and triticale was significantly higher than in the vetch and control treatments in pre-treatment counts (Fig. 4b). This variation in the pre-treatment counts was the result of a highly infested patch occurring in the top left hand corner of the vineyard, which affected only one fescue and triticale plot each. The mealybug infestation decreased after the first year, unlike the ant infestation, since regular chlorpyrifos treatments were applied during winter for mealybug control. The increased population in March 2003 showed no significant difference between treatments (Fig. 4b).

Correlations between ant and mealybug infestations in the vines showed that when ant activity was highest (February), the best correlations were obtained and that these correlations became poorer as the season progressed and ant activity began to decrease (Table 3). The poorest correlations were generally obtained in the control plots, which also had the least ant activity.



### ***Mealybug root infestations***

From the assessments conducted on the roots of cover crop and weed samples, only two mealybugs were found on small mallow *M. parviflora* in the control plot during the first year, while one mealybug was found on small mallow and three on sowthistle *S. olearceus* during the second year. The mealybugs could not be identified to species level as they were nymphs, but belonged to the Pseudococcidae.

### ***Mealybug natural enemy counts***

Three species of natural enemies were monitored, namely, the three endo-parasitic wasps *Coccidoxenoides peregrinus* (Timberlake) (Hymenoptera: Encyrtidae), *Anagyrus* sp. (Hymenoptera: Encyrtidae) and *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae). Monthly counts of natural enemies are given in Fig. 6. Activity was variable between treatments from month to month and therefore not one treatment showed a significant and consistent trend. The highest mean number of parasitoids was found in the control plots, although this difference was not always significant when compared with other treatments. (Table 5).

### ***Additional food source***

The majority of honeydew-excreting homopterans sampled during September 2002 were aphids, and only these will therefore be considered here. Samples were identified as follows: *Tetraneura nigriabdominalis* (Sasaki), *Aphis craccivora* Koch and *Uroleucon sonchi* (Linnaeus). The total number of aphids sampled on each cover crop and on the weeds of the control plots was as follows: Control (592), triticale (103), vetch (7) and fescue (5).

## **DISCUSSION**

### ***Ant activity in cover crops***

The poor germination of the fescue seeds can be attributed to the implements available to the farmer, which were not optimal and caused the fine seeds to be buried too deeply in the soil.

The reason for the increased ant activity in the triticale plots was most likely due to the ants utilizing the seeds from the cover crop as an additional food source, since A.

*custodiens* is known to disperse seeds in fynbos (Bond & Slingsby 1983). This could have resulted in larger nests, which would explain why ant activity was higher in this treatment. The high ant activity in this treatment is, however, not reflected in the ant infestation in the vines (Fig. 4a), percentage increase in ant infestation after two years (Table 2) or in the number of nest entrances (Table 4), as all these measurements in the triticale plots showed no significant differences to the other treatments. From March of the first year and during March of the second year, ant activity in the triticale plots declined relative to the other treatments, possibly as a result of the seeds being buried during soil preparations (disking). Weeds from the control plot also provided an alternate food source for ants in the form of honeydew from aphids, although average ant activity in control plots was the lowest. It therefore appears that weeds, by being hosts to aphids, did not significantly attract ants into these plots.

#### *Soil temperatures and ant activity*

Significantly higher soil temperatures and a greater number of accumulated heat units in the control did not increase ant nesting or ant activity significantly. A maximum reduction in soil temperature of 3°C was brought about by the permanent mixture (weeds) ground cover at 30cm depth, but this does not appear to have been enough to reduce ant nesting or ant activity. It is also apparent that soil moisture had no effect on nesting preference, as significant differences in soil moisture did occur between control and triticale at 30cm, but no significant differences were found in the number of ant nest entrances between any of the treatments. A maximum difference of 5.21% soil moisture was therefore also not enough to affect ant distribution in this vineyard. Possible reasons for a higher number of ant nest entrances being recorded on the vine row than in the inter-row are that the vine rows are not affected by continuous tractor movement, which could result in disturbance of nest entrances. Nesting on vine rows had the added benefit of being closer to the mealybug honeydew.

#### *Ant foraging distances*

In spring (November), at the end of the rainy season, a higher percentage of suspended nest entrances were recorded than at the end of the dry, growing season (March), probably as a result of ants trying to prevent flooding of the nests. This could also indicate that foraging trails at the end of the growing season, when mealybug populations are high, are slightly longer, extending from the rest of the vine row or inter-row with more ants from nests in the inter-row utilizing mealybug honeydew.

Observations in Letaba citrus orchards showed that the longest *A. custodiens* foraging trails (111m) were recorded during peak manna production periods as ants from extra-orchard nests also engaged in honeydew collecting at this time (Steyn 1954). From observations on foraging distances in April in this study, it seems that there is not much need for ants to travel long distances to find food in vineyards and that their primary food source (honeydew from mealybugs) is located in the vine canopy near the nests. Steyn (1954) noted that at Letaba, *A. custodiens* workers foraged mainly between their nests and a particular citrus tree, which was sometimes as far as 50m away. This was because there was some limitation in availability of nesting sites in the orchards due to routine orchard operations and regular flooding. Since destructive operations such as disking only occurred once a year in the vineyard of this study, there was no reason for ants to nest outside of the vineyards, although a few nests were in the dirt roads on either side of the vineyard. Ants from these nests foraged on the edges of the vineyard, resulting in higher ant infestations in the plots next to the roads (personal observation). The short foraging trails observed in this study also indicates that there is not much cross-infestation of ants between treatment plots, as the data areas were located 24m apart in each row, and 19m apart in each column of the latin square.

During November 2002, the ant infestation in the vines in the vetch plots was significantly lower than in the other treatments (Fig. 4a). This could have been due to the vetch plants forming a dense, dry layer by November and restricting movements of the ants between their nests and the vines. The percentage increase in the mealybug infestation after two years showed the same trend as the percentage increase in ant infestation, with the higher increases occurring in the vetch and control plots, and the lower increases occurring in the triticale and fescue plots (Table 2). However, these differences were rarely significant and showed no particular trend for any one ground cover treatment. Since no significant differences in either ant or mealybug infestations in the vines were found after two years, cover crops appeared to have no effect on the ant or mealybug infestation in the vine canopy in this study. The level of mealybug infestations found on the roots was not high enough to test the theory that mealybug root infestations can be reduced by cover crops controlling weeds. Furthermore, it was not possible to establish whether the mealybugs found on the weed roots were *P. ficus*.

### *Parasitoid activity*

It was possible to classify only *C. peregrinus* and *L. dactylopii* to species level. According to Prinsloo (1984), the genus *Anagyrus* is poorly defined taxonomically and many of its species cannot be determined with certainty. The highest mean numbers of endo-parasitic wasps were always recorded in control plots (Table 5), although this difference was not always significant. This was possibly due to the larger variety of weeds found there during winter which could have provided the natural enemies with a greater variety of habitats to utilize as refuges. Weeds were also found to increase natural enemy diversity by van Emden (1990). The occurrence of the wasps was apparently not related to the presence of ants or mealybugs in the vine canopy, probably because the level of ant and mealybug infestations in the canopy was not consistent for any one treatment over the course of the study. The level of ant infestations in the vine canopy at the end of the study period was severe, which would have hampered natural enemy activity in the vine canopy. Since only the level of natural enemy activity in the vine canopy was of interest in this study (as this was where they would have affected mealybug populations), mealybug natural enemies were not sampled in cover crops and weeds. Although greater differences in natural enemy numbers between ground cover treatments could have been established, this would have been of no use for the integrated control of vine mealybug unless there was a significant movement of natural enemies into the vine canopy.

## **SIGNIFICANCE OF RESULTS FOR INTEGRATED PEST MANAGEMENT IN VINEYARDS**

From these results the hypotheses stated at the onset of the trial can therefore be addressed as follows: 1) Triticale consistently caused a significant *increase* in ant activity during both years, although this did not lead to a significantly higher ant or mealybug infestation in the vines in this treatment. Furthermore, none of the cover crops had any effect on ant nesting, when compared to the control, despite the fact that cover crops did lower the soil temperatures significantly. It can be concluded that the reduction in soil temperature and the increase in soil moisture that cover crops caused, was not enough to affect ant infestations and that the main driver regulating ant abundance in this trial was the food source. 2) None of the cover crops caused a significant increase in the number of natural enemies. Similar results were obtained from a study conducted in California where cover crops had no significant effect on

spider species densities on vines with and without ground covers (Costello & Daane 1998). In the current study, the highest number (although mostly not statistically significantly higher) of endo-parasitic wasps was found in the control plots. This may indicate that a more diverse planting could be of more value to natural enemies in vineyards in that they provide a greater variety of refugia to choose from. Weeds may therefore not be as detrimental as earlier thought, provided that they do not harbour underground mealybug populations. 3) Since the cover crops used in this trial did not reduce ant infestations and did not increase mealybug natural enemy abundance in the vine canopy, these ground covers did not have any effect on the mealybug infestations after two years. It appears, therefore, that there is no potential for the use of either, a green, permanent ground cover, vetch or triticale in the integrated management of vine mealybug. This lack of ant response to vegetation cover was also emphasized in Australia, where ants were found to be poor indicators for monitoring grassland condition (New 2000). Likewise, Samways (1983) found that habitat modification for dominant *Pheidole* spp. was not a suitable primary method for managing these species, and that stem barriers are an ecologically more appropriate method of management. It is recommended that triticale (where it is used as a horticultural ground cover) be treated with caution, as the potential for increased ant infestations is a possibility in vineyards already infested with *A. custodiens* and planting this cover crop could be to the detriment of an integrated mealybug control program. Current ant control methods will therefore still have to be employed until a suitable alternative to planting cover crops is found.

Table 1. Seed planting densities used for two years in a vineyard in Bonnievale where four ground cover treatments were compared.

<b>Cover crop (2001/2002)</b>	<b>Concentration</b>
Triticale	100kg ha <sup>-1</sup>
Permanent mixture:	
-Permanent dwarf fescue	16kg ha <sup>-1</sup>
-Creeping red Harold	8kg ha <sup>-1</sup>
-SR-4-200	8kg ha <sup>-1</sup>
-Santiago medic	8kg ha <sup>-1</sup>
Creeping vetch	50kg ha <sup>-1</sup>
<b>Cover crop (2002/2003)</b>	<b>Concentration</b>
Triticale	100kg ha <sup>-1</sup>
Permanent dwarf fescue	30kg ha <sup>-1</sup>
Creeping vetch	50kg ha <sup>-1</sup>

Table 2. Percentage increase, relative to pre-treatment counts, in ant and mealybug vine infestations after two years in a vineyard in Bonnievale where four ground cover treatments were compared.

Treatments	Ant infestation (% increase), mean $\pm$ SE *	Mealybug infestation (% increase), mean $\pm$ SE *
Control	47.8 $\pm$ 7.0 a	17.4 $\pm$ 6.2 ab
Triticale	35.5 $\pm$ 6.3 ab	0.8 $\pm$ 7.0 b
Fescue	25.7 $\pm$ 7.4 b	6.9 $\pm$ 5.7 ab
Vetch	42.0 $\pm$ 7.2 a	19.1 $\pm$ 6.1 a

\* Numbers in a column followed by the same letter are not significantly different ( $p \leq 0.05$ ), ANOVA, LSD.

Table 3. Correlations between ant and mealybug infestations in a vineyard in Bonnievale on four sampling dates where four ground cover treatments were compared.

Treatments	Pearson Correlation Coefficient (P = 0.05)			
	April 2001	February 2002	November 2002	March 2003
Control	0.07	0.81	0.19	0.67
Triticale	0.39	0.95	0.35	0.71
Fescue	0.53	0.93	0.61	0.63
Vetch	0.51	0.84	0.74	0.85

Table 4. Mean number of nest entrances ( $\pm$  standard error) on five sampling dates (the first being pre-treatment counts) in a vineyard in Bonnievale where four ground cover treatments were compared.

Treatment	March 2001	November 2001	March 2002	November 2002	March 2003
Control	4.00 $\pm$ 0.60 a	0.50 $\pm$ 0.23 a	9.81 $\pm$ 1.10 a	0.87 $\pm$ 0.22 a	2.78 $\pm$ 0.54 a
Triticale	3.25 $\pm$ 0.54 a	0.65 $\pm$ 0.19 a	11.00 $\pm$ 1.46 a	0.84 $\pm$ 0.25 a	2.21 $\pm$ 0.65 a
Fescue	4.18 $\pm$ 0.70 a	0.81 $\pm$ 0.22 a	8.75 $\pm$ 1.01 a	0.75 $\pm$ 0.19 a	3.68 $\pm$ 0.87 a
Vetch	2.68 $\pm$ 0.40 a	0.18 $\pm$ 0.08 a	8.37 $\pm$ 0.85 a	0.53 $\pm$ 0.17 a	2.87 $\pm$ 0.67 a
Row	4.46 $\pm$ 0.41 A	0.92 $\pm$ 0.17 A	10.95 $\pm$ 0.83 A	1.25 $\pm$ 0.17 A	4.76 $\pm$ 0.58 A
Inter-row	2.59 $\pm$ 0.36 B	0.15 $\pm$ 0.05 B	8.01 $\pm$ 0.72 B	0.25 $\pm$ 0.07 B	1.01 $\pm$ 0.19 B

Numbers in a column followed by the same letter do not differ significantly (ANOVA, LSD where  $P \leq 0.05$ ). Row and inter-row counts were analyzed over all treatments.

Table 5. Transformed means ( $\log\{x+1\} \pm$  standard error) of mealybug natural enemies caught in sticky yellow Bugtraps® from July 2001 to March 2003 in a vineyard in Bonnievale where four ground cover treatments were compared.

Treatments	<i>Anagyrus</i> sp.	<i>Coccidoxenoides peregrinus</i>	<i>Leptomastix dactylopii</i>
Control	0.60 $\pm$ 0.10 a	1.00 $\pm$ 0.09 a	0.26 $\pm$ 0.07 a
Triticale	0.53 $\pm$ 0.09 a	0.91 $\pm$ 0.09 ab	0.14 $\pm$ 0.04 b
Fescue	0.48 $\pm$ 0.05 a	0.96 $\pm$ 0.10 a	0.18 $\pm$ 0.05 ab
Vetch	0.50 $\pm$ 0.09 a	0.75 $\pm$ 0.09 b	0.11 $\pm$ 0.05 b

Numbers in a column followed by the same letters do not differ significantly (ANOVA, LSD where  $P \leq 0.05$ ).



Table 6. Mean difference between maximum soil temperature readings (°C) between ground cover treatments in a Chenin blanc vineyard in Bonnievale from June 2001 until February 2002.

Treatments	Triticale		Vetch		Permanent mix	
	10 cm	30 cm	10 cm	30 cm	10 cm	30 cm
Control	2.20 (P=0.01)	1.87 (P=0.01)	2.02 (P=0.01)	2.49 (P=0.01)	2.25 (P=0.01)	3.0 (P=0.01)
Triticale			-0.19 (P=0.58)	0.61 (P=0.01)	0.05 (P=0.88)	1.12 (P=0.01)
Vetch					0.24 (P=0.02)	0.51 (P=0.01)

\*If the probability (P) is less than 0.05, the difference of the mean is significant. Minus numbers next to means indicate that the readings of treatments in the left column are smaller than those in the top row.

Table 7. Mean difference between minimum soil temperature readings (°C) between ground cover treatments in a Chenin blanc vineyard in Bonnievale from June 2001 until February 2002.

Treatments	Triticale		Vetch		Permanent mix	
	10 cm	30 cm	10 cm	30 cm	10 cm	30 cm
Control	1.49 (P=0.01)	1.13 (P=0.01)	1.80 (P=0.01)	1.98 (P=0.01)	2.59 (P=0.01)	2.60 (P=0.01)
Triticale			0.32 (P=0.05)	0.85 (P=0.01)	1.11 (P=0.01)	1.46 (P=0.01)
Vetch					0.79 (P=0.01)	0.62 (P=0.01)

\* If the probability (P) is less than 0.05, the difference of the mean is significant. Positive numbers next to means indicate that the readings of treatments in the left column are larger than those in the top row.

Table 8. Mean difference between soil moisture readings (%) between ground cover treatments in a Chenin blanc vineyard in Bonnievale from June 2001 until February 2002.

Treatments	Triticale		Vetch		Permanent mix	
	10 cm	30 cm	10 cm	30 cm	10 cm	30 cm
Control	-0.57 (P=0.07)	3.81 (P=0.01)	-3.26 (P=0.01)	-5.15 (P=0.01)	-5.21 (P=0.01)	-4.63 (P=0.01)
Triticale			-2.68 (P=0.05)	-8.97 (P=0.01)	-4.63 (P=0.01)	-8.44 (P=0.01)
Vetch					-1.95 (P=0.02)	-0.52 (P=0.54)

\*If the probability (P) is less than 0.05, the difference of the mean is significant. Minus numbers next to means indicate that the readings of treatments in the left column are smaller than those in the top row.

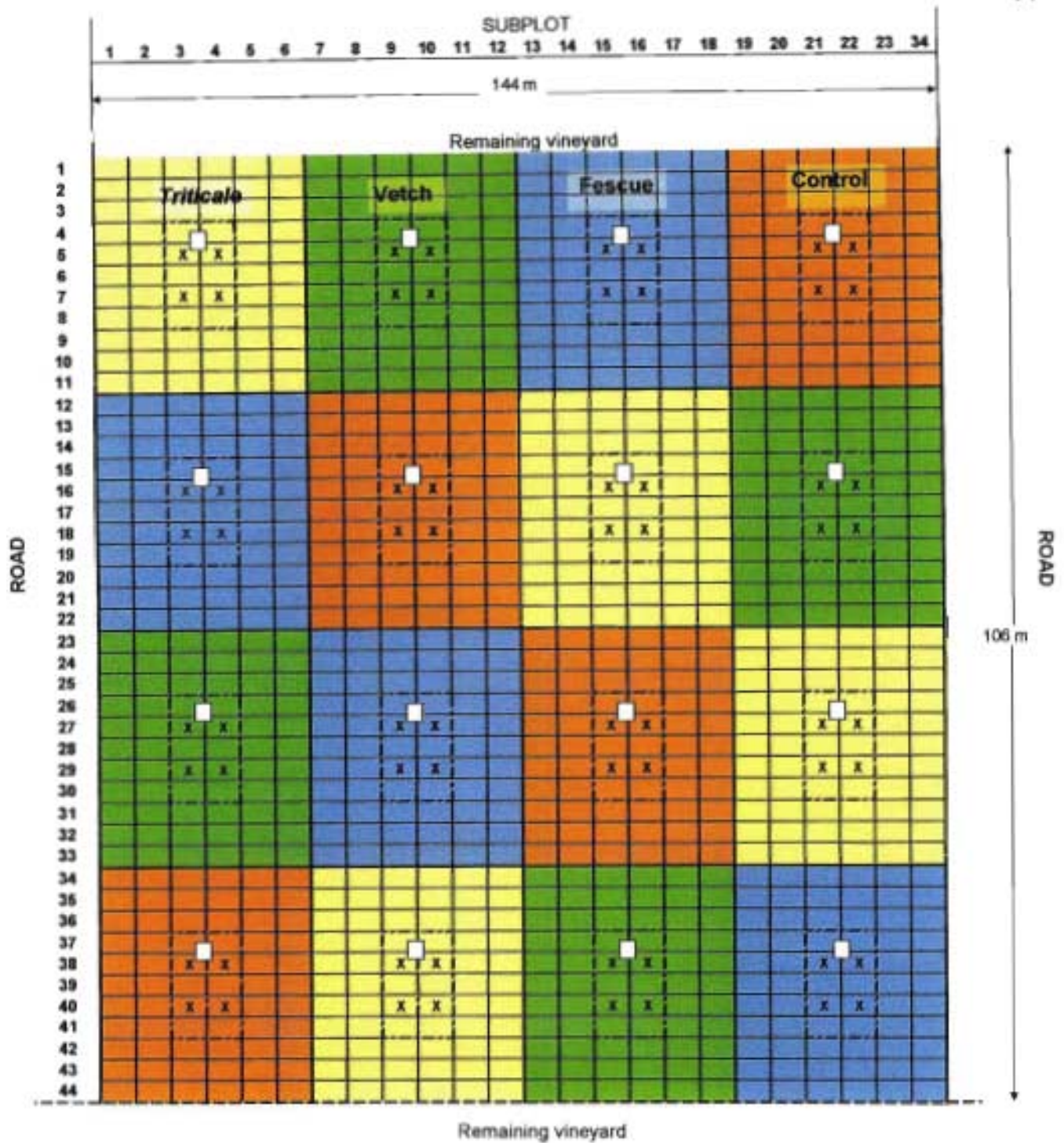


Fig. 1. Trial layout for four ground cover treatments in a vineyard in Bonnievale. White squares mark where yellow bug traps were placed to monitor natural enemies. X marks where pitfall traps were placed on the berm of the vine row. X also indicates where plant samples for mealybug and homopteran inspections were collected in the inter-row. The broken line combined in a square in the centre of each plot indicates the data area (144 m<sup>2</sup>).



Fig. 2a. Triticale is a tall-growing genus hybrid between wheat and rye, and is left as a dry mulch during summer.

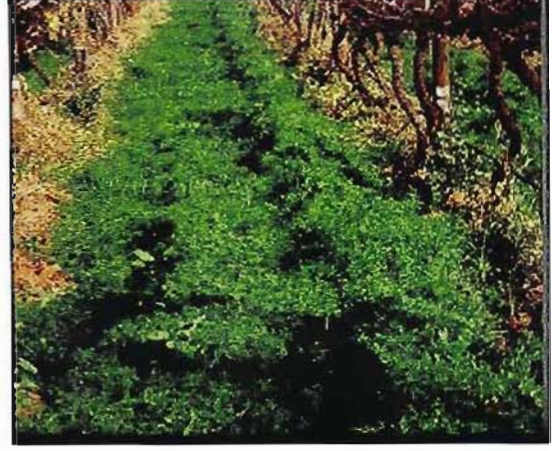


Fig. 2b. Vetch is a low-growing, leguminous plant with a creeping habit. It is left as a dry mulch during summer.



Fig. 2c. Fescue is a permanent, low-growing plant which remains green throughout the summer.



Fig. 2d. The control plot is kept free of weeds for most of the year.

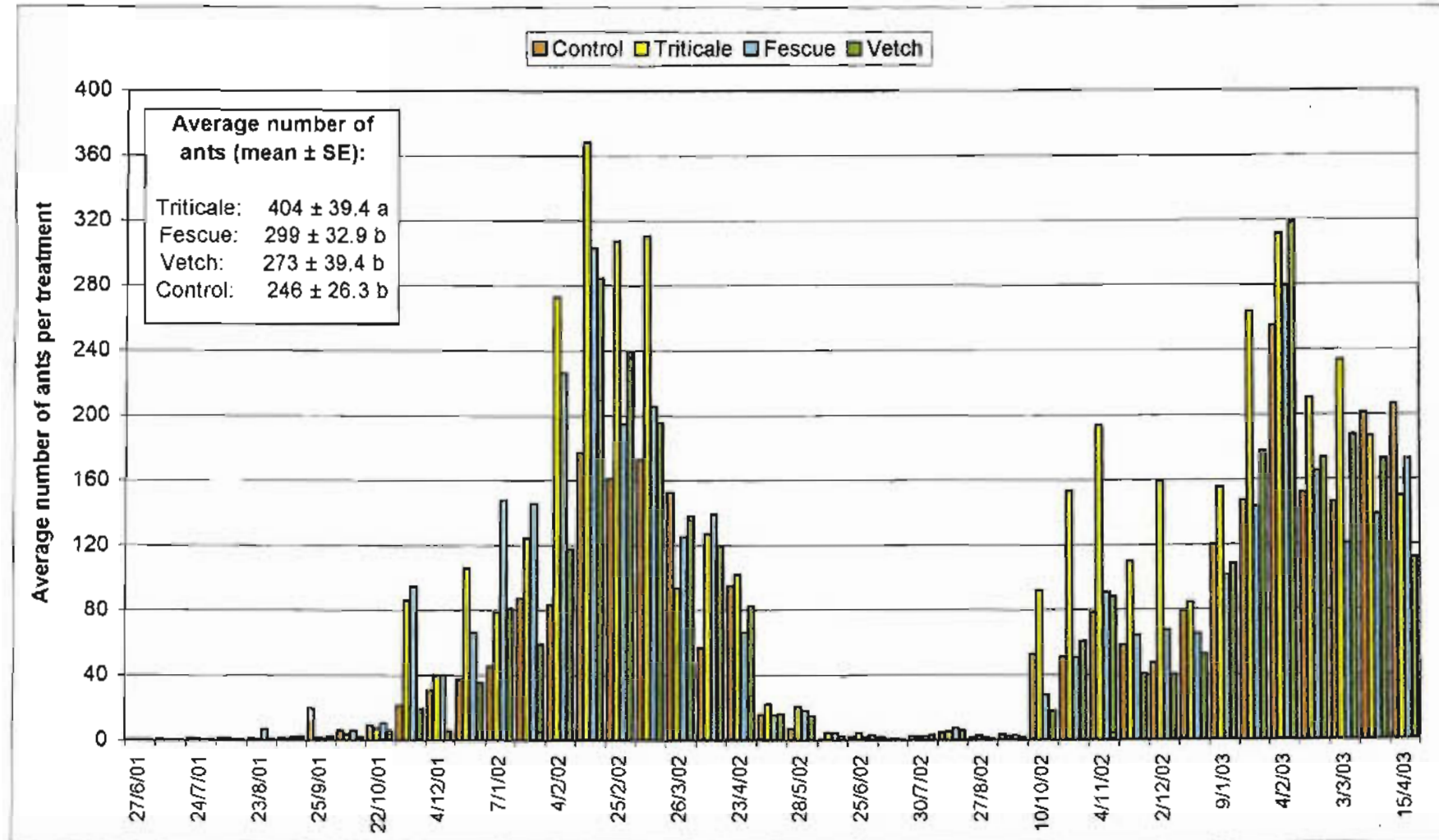


Fig. 3. Average number of *A. custodiens*, as measured using pitfall traps, for four ground cover treatments established in a vineyard in Bonnievale during two years. Mean number of ants ( $\pm$  standard error) are indicated. Numbers with the same letter do not differ significantly ( $P \leq 0.05$ ), ANOVA, LSD.

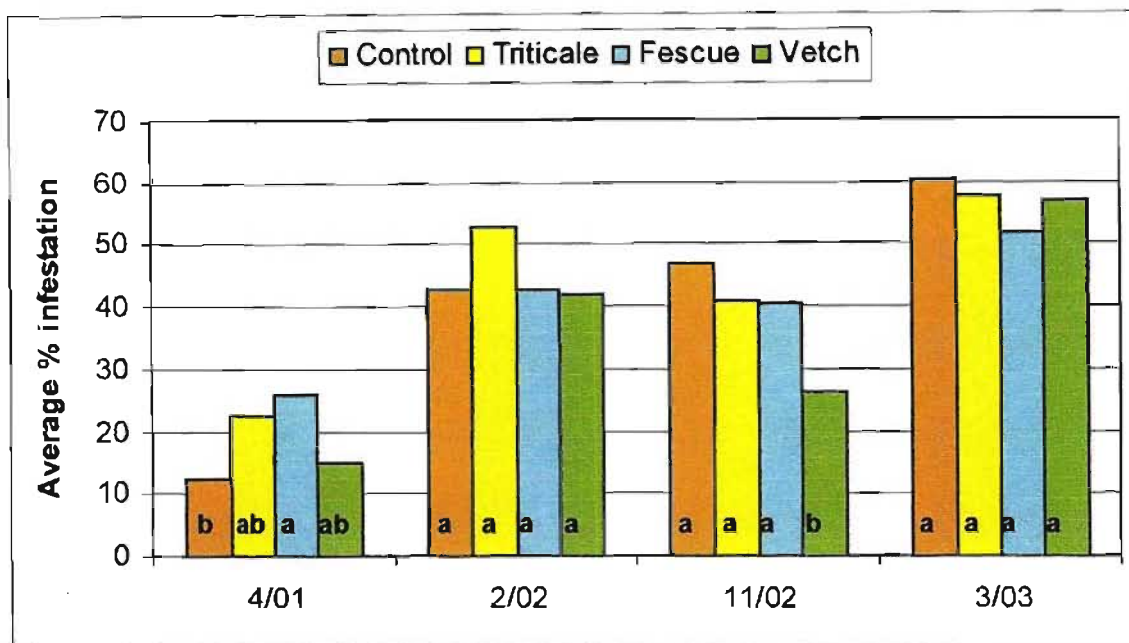


Fig. 4a. Average percentage *A. custodiens* infestation in vines, as monitored on four sampling dates, in a vineyard in Bonnievale where four ground cover treatments were compared. April 2001 represents the pre-treatment sampling date. Letters that differ on each column indicate a significant difference ( $p \leq 0.05$ ), analysed for each date separately (ANOVA, LSD).

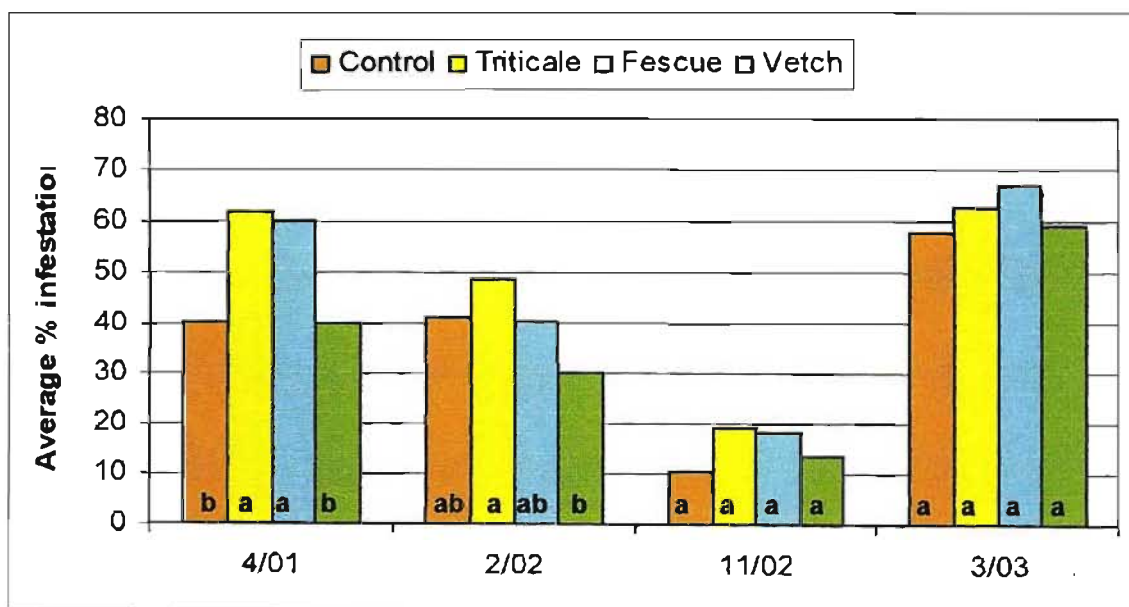


Fig. 4b. Average percentage *P. ficus* infestation in vines as monitored on four sampling dates, in a vineyard in Bonnievale where four ground cover treatments were compared. April 2001 represents the pre-treatment sampling date. Letters that differ on each column indicate a significant difference ( $p \leq 0.05$ ) between treatments, analysed for each date separately (ANOVA, LSD).

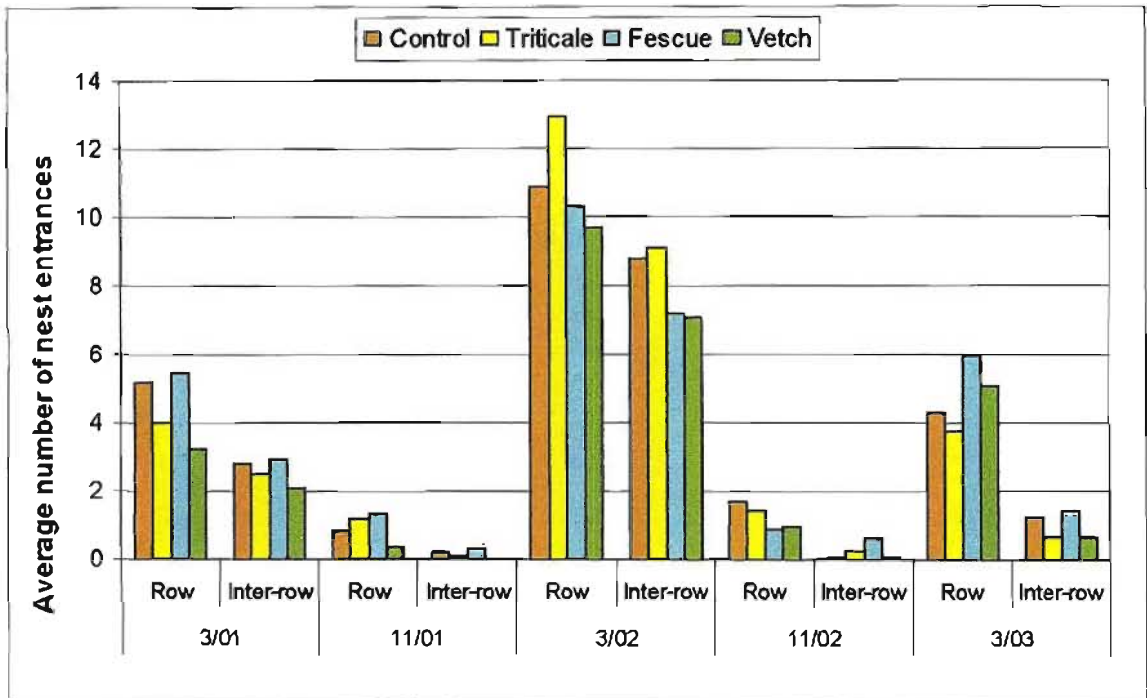


Fig. 5. Average number of *A. custodiens* nest entrances as monitored on five sampling dates in four ground cover treatments (in the row and in the inter-row) in a vineyard in Bonnievale. March 2001 refers to the pre-treatment counts.

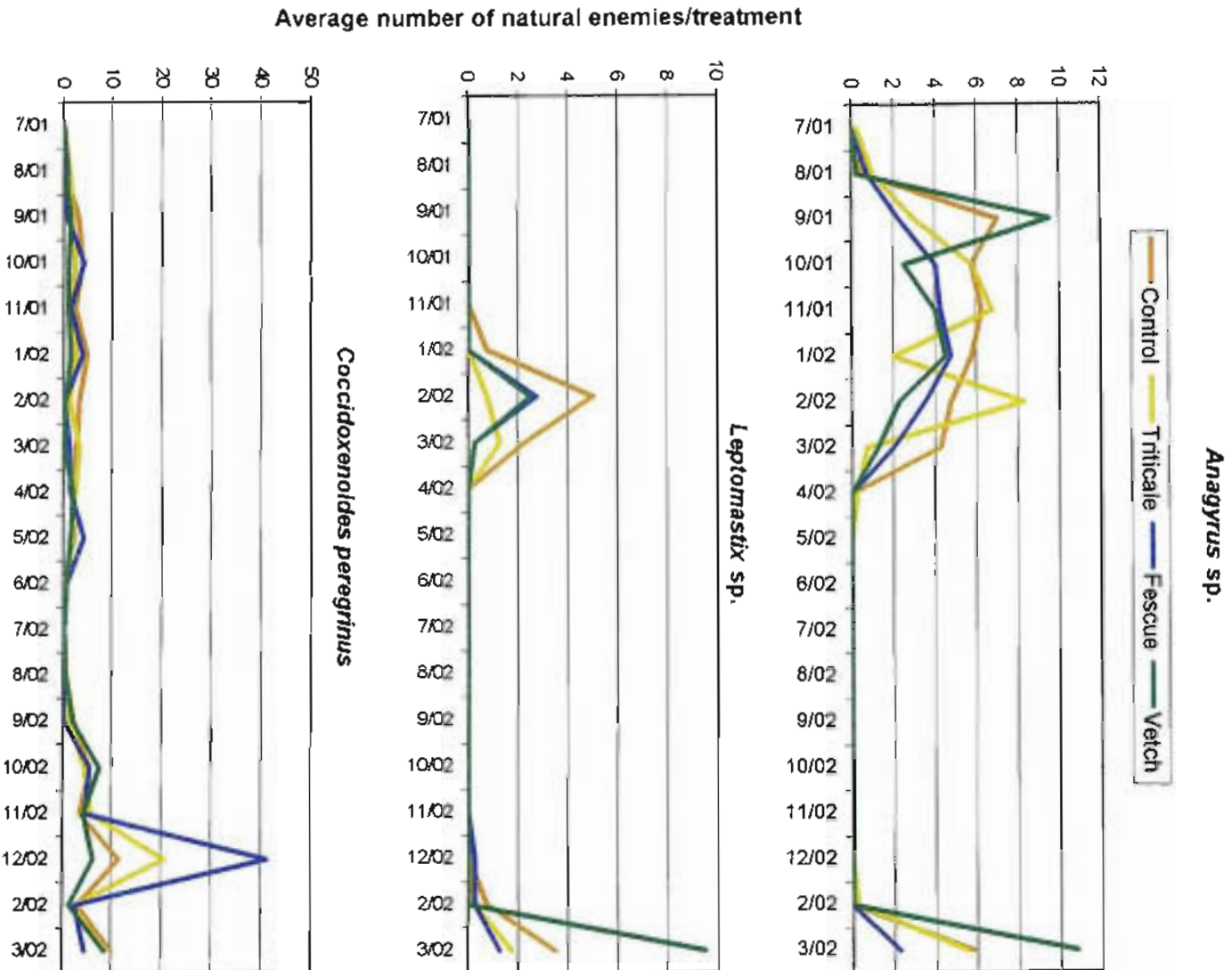


Fig. 6. Natural enemy counts from July 2001 to March 2003 in a vineyard in Bonnievale where four ground cover treatments are compared.

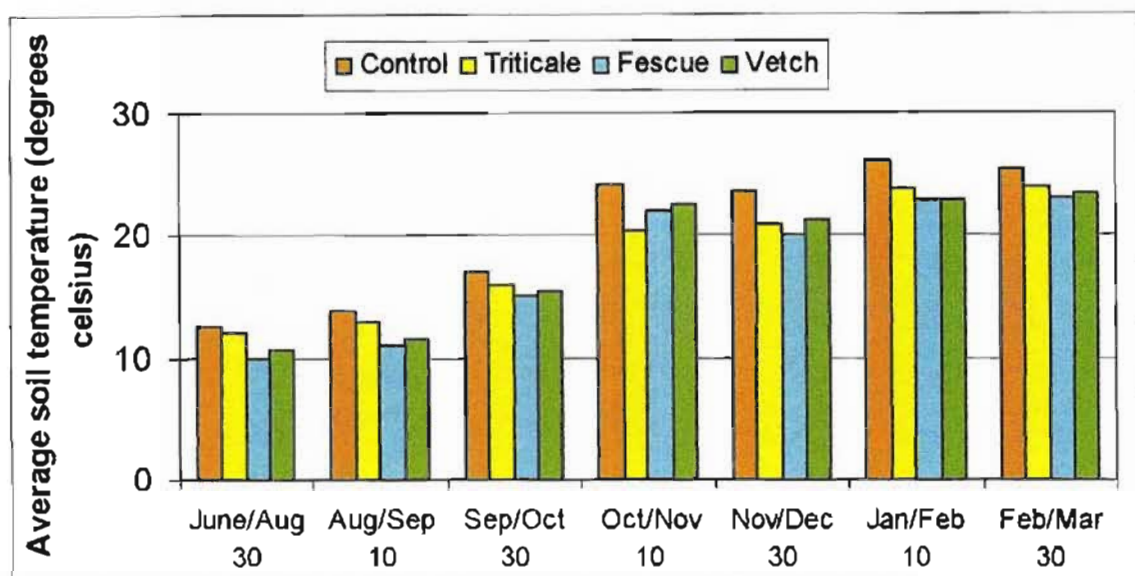


Fig. 7. Average soil temperature in a vineyard in Bonnievale, where four ground cover treatments were compared. Readings were taken from June 2001 – March 2002 (10 and 30 refer to soil depth in cm).

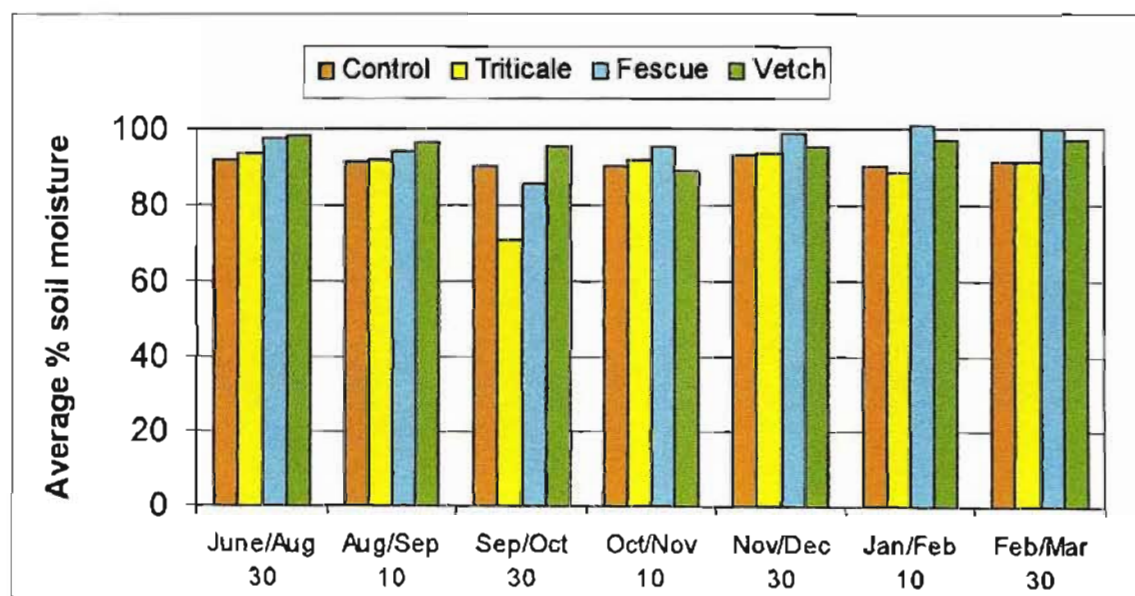


Fig. 8. Average percentage soil moisture in a vineyard in Bonnievale, where four ground cover treatments were compared. Readings were taken from June 2001 – March 2002 (10 and 30 refer to soil depth in cm).



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## CHAPTER 4

### VARIATION IN ANT (HYMENOPTERA: FORMICIDAE) DIVERSITY, FORAGING BEHAVIOUR AND MORPHOLOGY IN DIFFERENT STRUCTURAL HABITATS ASSOCIATED WITH VINEYARDS

#### ABSTRACT

*The aim of this study was to establish whether planting cover crops in vineyards 1) increases ant species diversity in vineyards by providing additional niches, 2) affects foraging behaviour in dominant *Anoplolepis custodiens* workers, and 3) causes a variation in head capsule size of *A. custodiens* workers from different structural habitats as a result of behavioural or microclimatic changes. Ants are detrimental to biological control of grape vine mealybug *Planococcus ficus*. Creeping vetch *Vicia dasycarpa*, triticale *Triticale v. Usgen 18*, and one permanent cover crop, consisting of a seed mixture during the first season and a pure stand of dwarf fescue *Festuca sp.* during the second season were established in a vineyard in Bonnievale. These were compared to a control plot kept free of ground cover. Epigeic ants were sampled in the vineyard for two years, from June 2001 to March 2003, and species diversity compared between ground cover treatments. Head capsule measurements were taken and compared between the four ground cover treatments in the vineyard and, as an additional control, in natural vegetation close to the vineyard. During March and April 2003, *A. custodiens* foragers were collected within the four ground cover treatments and natural vegetation. The head capsules of these ants were measured and the size-frequency distribution of workers compared. This study found that cover crops supported more non-target ant species when compared to the control plot and that this difference was most likely the result of increased vegetation complexity. Furthermore, cover crops supported a smaller number of large workers than the control plot or the natural vegetation, which could be the result of the diet utilized by the ants in the different microhabitats, but could also be the result of lowered soil temperatures in the cover crop plots. Seasonal ant activity patterns monitored over two years suggest that October is the optimum time for applying chemical stem barriers against *A. custodiens*.*

## INTRODUCTION

The mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) is widely distributed in Western Cape vineyards and a major pest in vines (Kriegler & Whitehead 1962, Myburgh *et al.* 1973, Urban & Mynhardt 1983 and Walton 2003). Ants tend it for honeydew and so disrupt biological control of mealybugs by parasitoids. In particular, *Anoplolepis custodiens* appears to be the most aggressive mealybug-tending ant in vineyards, dominating other ant species wherever it occurs (Addison & Samways 2000). Although habitat modification has little to recommend it as a form of management for such dominant ants, it may be used as a way of encouraging competitive species (Samways 1983). *A. custodiens* is widely distributed in the Western Cape, but is most commonly found in the dry Klein Karoo and Breede River Valley regions, where it is associated with severe mealybug outbreaks on vines (Addison & Samways 2000). Chemical stem barriers are effective in excluding this species for approximately two to three months, depending on ant pressure, although more success was achieved with chemical stem barriers on other pestiferous ants (Addison 2002).

Foraging behaviour by ants can be affected by various factors, such as temperature, competition, photoperiod, circadian rhythm, food availability and food particle size (Oster & Wilson 1978, Bernstein 1979, and Hölldobler & Wilson 1990). The food source appears to be one of the main drivers which regulate *A. custodiens* infestations in vineyards, the most abundant of which is honeydew (Chapter 3). The ambient and soil surface temperature at which *A. custodiens* is active in the southern Karoo (33.07S 22.16E to 32.54S 23.10E) has been determined and is influenced by competitive interactions with *A. steingroeveri* (Dean 1992). In Dean's (1992) study, both ant species were recorded in natural vegetation (dwarf shrublands), where *Anoplolepis* spp. made use of a wide variety of foods, such as live and dead animal matter, as well as honeydew and nectar.

The planting of cover crops in vineyards is recommended for soil amelioration (Anonymous 2000). The aim of this study was to establish whether cover crops 1) increase ant species diversity in vineyards by providing additional niches; 2) affect foraging behaviour in dominant *A. custodiens* workers; and 3) cause variation in head

capsule size of *A. custodiens* workers from different structural habitats as a result of behavioural or microclimatic changes.

## MATERIALS AND METHODS

### *Site and sampling layout*

The trial site was in the Bonnievale area on the farm Morgensonndt (33.26S 20.01E) in a nine year old, trellised Chenin blanc vineyard infested with vine mealybug *P. ficus* and the common pugnacious ant *A. custodiens*. Irrigation was by micro-jets. Plant spacing was 1.2m apart, while rows were 2.4m apart. A middle section of the vineyard was selected for the trial, approximately 1.5ha<sup>-1</sup> in size. The vineyard was surrounded by orchards on two opposite borders separated from the vineyard by dirt roads, approximately 4m wide. The farm was situated between the Breede River and a country road, on the other side of which was a narrow strip of vineyards and a montane region with natural vegetation. The natural vegetation was characterized by sparsely-growing shrubs typical of Karoo vegetation. The grower had not used cover crops previously in this vineyard and controlled weeds by mechanical and chemical methods. A 4 x 4 latin square design was used, with columns and rows randomised (Fig. 1 of Chapter 3). This design was chosen to minimize any possible edge effects, such as additional infestations from the dirt roads. Edge effects have previously been recorded from ants in vineyards (Addison & Samways 2000). Each plot was 950m<sup>2</sup> in size (11 rows x 6 subplots, consisting of the 5 vines between the trellis poles). The data area was 144m<sup>2</sup> (5 rows x 2 subplots) and was situated in the centre of each plot.

### *Cover crops used*

Two winter cover crops (those treated with herbicide in spring), creeping vetch *Vicia dasycarpa* (Fabaceae) and triticale *Triticale* v. Usgen 18 (Graminae), and one permanent cover crop, consisting of a seed mixture during the first season (Table 1) and a pure stand of dwarf fescue *Festuca* sp. (Poaceae) during the second season, were compared to a control plot kept free of ground cover using herbicides and a tractor-drawn rotary mower. Triticale is currently the most widely used cover crop by grape growers in the Western Cape and is sown in the inter-row only leaving the vine row free of ground cover. Vetch provides a very dense vegetative cover over both row and inter-row due to its creeping nature, whereas fescue is a permanent low-growing cover that remains green throughout the year and is sown only in the inter-row. Seed planting

densities are given in Table 1. Seeds were obtained from Agricol (Pty) Ltd., Brackenfell. Due to the poor growth of the permanent mixture during the 2001/2002 season, it was decided to sow a pure stand of dwarf fescue, the strongest grower of the mixture, during the 2002/2003 season.

Cover crops were sown on 25 April 2001 and again on 10 April 2002. Soil preparation occurred approximately one month prior to sowing. The soil was disked to a depth of about 15cm. Immediately after sowing the seeds, they were raked into the soil using a tractor-drawn furrow plough. Treatments (vetch, triticale and control) were sprayed with herbicide (glyphosate 360 g/l at 6l/ha) on 6 September 2001 and on 17 September 2002. The vine rows of the permanent cover crop mixture were also sprayed with herbicide to control weeds growing on the berm (vine row). The inter-row is the 1.4m wide strip where the tractor can move, while the vine row is the 1m wide strip on which the vines are planted. One month after spraying the treatments, the control plots were mechanically mowed with a rotary mower, while the permanent cover crop plots were mowed to a height of approximately 30cm above the soil surface to slash high-growing weeds. During the first week in April 2002 the control plots were again slashed with a rotary mower.

#### *Species diversity and ant activity sampling*

Epigaeic ants were sampled using pitfall traps similar in design to the one described by Majer (1978). Traps consisted of polystyrene test tubes (18 x 150mm) containing approximately 10ml of a mixture of seven parts 70% ethyl alcohol and three parts pure glycerol. An outer case consisting of irrigation pipe, approximately 160mm in length was permanently sunk into the ground and used as a trap sleeve to facilitate changing of traps. The test tubes were sunk into the casing in the vine row and the ground levelled so that the edge was even with the soil surface. A total of 64 traps were used, four traps in each of 16 plots. The traps were changed every two weeks, except when unfavourable weather conditions prevailed and caused the changing of traps to be postponed. All ant species caught were then sorted for identification using a reference collection and counted in the laboratory. Sampling started on 19 June 2001, and was continuous for the duration of the trial, which ended in March 2003. To determine the dominant species, log abundance/rank plots were drawn using total ant counts from four replicates, accumulated over the study period.



### *Head capsule measurements*

Sampling took place in the vineyard and, as an additional control, also in a section of natural vegetation, approximately 900m away from the vineyard in the montane region opposite the farm. Traps were changed every two weeks during March and April 2003. At this time, the cover crops had been in the soil for two years. In the natural vegetation, sixteen test tubes were sunk into the soil in close proximity to nests. The soil was levelled so that the edge was even with the soil surface. In the vineyard, the same traps were used as for ant diversity sampling. Two hundred, randomly selected *A. custodiens* workers per ground cover treatment and in natural vegetation were used. A further 50 of the smallest workers from each of the ground cover treatments only were also collected. The length and width of the head capsules (Fig. 1) were measured using a digital camera with measuring function and software (PhotoLib 3.03) mounted onto a stereo microscope. Ants were decapitated and mounted onto slides covered with sticky tape prior to being measured to facilitate exact positioning of head capsules. Once the head capsules were measured, the following formula (Southwood 1978) was used to determine whether the samples of 200 randomly selected workers and 50 of the smallest workers were sufficient:

$$n = (\text{Standard Deviation}/0.05 \times \text{Mean})^2,$$

where 0.05 is a predetermined standard error of the mean.

Head capsule size was calculated by multiplying head width by head length and data were subjected to an unpaired t-test to compare head capsule size between workers from different ground cover treatments and natural vegetation. The 200 randomly selected workers were analysed separately from the 50 smallest workers.

## **RESULTS AND DISCUSSION**

### *Species diversity*

A total of nine ant species were recorded from this vineyard. *A. custodiens* showed extreme dominance in all ground cover treatments (Fig. 2). Extreme dominance and aggression is characteristic of this ant and was found in previous studies on coconut palms (Way 1953) and in citrus orchards (Samways 1981). The least number of ant species were found in the control plot, while the most were found in the fescue plot (Fig. 2). Although fescue seeds did not germinate well (see Chapter 3), the green cover

(weeds) that was established by limiting herbicide treatments could have played a role in attracting non-target ant species. Conversely, the naturally barren control plot limited ant diversity due to a lack of growing plants and therefore less opportunity for finding a suitable food source. It is also possible that the micro-environment that was formed by planting cover crops is more favourable for non-target ants. The soil in the cover crop plots was significantly cooler and wetter than in the control plots (Chapter 3).

#### *Ant activity*

The seasonal activity of *A. custodiens* and *Tetramorium pusillum*, the most abundant non-target ant species, is shown for the four ground cover treatments (Fig. 3). *A. custodiens* showed minimal activity between June and September, but activity increased greatly in November 2001 and again in October 2002. This sudden increase could have been the result of a combination of two factors, namely, honeydew availability and ambient temperature. According to a seasonal population study on mealybugs in the Robertson area, visibility of mealybugs on the stems, bunches and leaves increased greatly from October, to reach a peak during January (Walton 2003). An average daily maximum temperature of 26°C was reached during November 2001 and October 2002 in this study (Fig. 3). However, the average daily maximum temperature never went under 11.3°C, which was established as being the temperature under which *A. custodiens* ceases to be active in the southern Karoo (Dean 1992). At Letaba, it was determined that ant activity on citrus stems increased with increasing temperatures from 9.4°C to 27.8°C (Steyn 1954). Foraging in this vineyard could have, therefore, taken place during daytime in winter months as well, although few ants were caught. From the data available here, it therefore appears that honeydew availability was the main factor for limiting *A. custodiens* activity during winter, over temperature.

Although *T. pusillum* was also less active between June and September, the difference in activity was not as marked as with *A. custodiens*, probably as it makes use of a broader range of food sources and therefore is not dependent on one food source only. Species in the genus *Tetramorium* have been classified by Andersen (1995) as being opportunists and occurring in environments that are stressed or disturbed and where competition from other ant species is therefore limited.

### *Head capsule measurements*

It was determined that the sample size of 200 all-sized and 50 smallest workers was, indeed, sufficient. Workers (all-sized and smallest) had significantly larger head capsules in both natural vegetation, and in control plots, than in triticale and vetch plots (Tables 2 and 3). No significant difference was found in head capsule size between workers from natural vegetation, control plots or fescue plots. The size-frequency distribution (Fig. 4) indicates that *A. custodiens* exhibits continuous polymorphism, described by Oster & Wilson (1978) as minors, medias and majors occurring together simultaneously. Furthermore, these researchers suggest that species expand their physical polymorphism when they occupy habitats with few competitors. This would hold true for *A. custodiens* as it is an aggressive species and displayed extreme dominance over the other ant species. The foraging strategy employed by *A. custodiens* would best be described as foraging workers leaving the colony to retrieve prey singly but utilizing a broader food source range, such as larger food items, through recruitment i.e. group transport (Oster & Wilson 1987 and Hölldobler & Wilson 1990). In this study, the size-frequency distribution of workers in the natural vegetation contains more of the larger individuals than those in the ground cover treatments of the vineyard, while the control plots contain more of the larger individuals than the cover crop treatments (indicated by the median). This can be explained using two hypotheses, as suggested by Oster & Wilson (1978) and Brian & Brian (1951): 1) *A. custodiens* size-frequency distribution approximates the size-frequency distribution of its prey. Ants in natural vegetation therefore make use of a broader range of food sources, such as honeydew from scale insects and termites (personal observation), than they would in a vineyard with a monoculture of vines with mealybugs. The control plots in vineyards resemble natural vegetation in that there is a greater diversity of ecological niches in the form of weeds, as opposed to a pure stand of cover crop. Similar results were found in Letaba citrus orchards, where the larger *A. custodiens* workers were more prolific outside of orchards where there was a greater diversity of animal and plant life, than within orchards (Steyn 1954). 2) However, workers in the fescue plots were smaller than those in the control plots, although not significantly so. The fescue plots were characterised by a permanent growth of weeds, as opposed to the control plots where weeds were controlled chemically. It appears therefore, that prey type is not the only determining factor of worker size, but that temperature could also play a role. In this study, soil temperatures in the cover crop plots were significantly lower than in the control plots (Chapter 3). It is to be expected, although this was not measured in this

study, that the soil temperature in natural vegetation would be higher than in control plots, as the vegetation is low-growing and sparsely distributed and the shading effect of the vines does not come into play here. Both shortage of food and a reduction in soil temperature have been ascribed to causing smaller workers in a study conducted in Scotland on *Mymrica rubra* Linnaeus (Brian & Brian 1951).

### **IMPLICATIONS FOR ANT MANAGEMENT**

This study found that although cover crops may not have affected ant infestations significantly as a method of habitat modification for ant management in vineyards (Chapter 3), they did have an effect on worker size and the size-frequency distribution of foragers. A cover crop such as vetch, therefore, supported more non-target ant species and resulted in significantly smaller workers than in the control plots. Since it is not practically possible to lower soil temperature any further in vetch plots, which could have resulted in a significant effect on ant activity and brood development, the only option is to supplement vetch as a cover crop by denying the ants access to honeydew from mealybugs (chemical stem barriers). The combination of lowered soil temperature and limited food supply could extend the efficacy of chemical stem barriers for *A. custodiens*.

From these results, *A. custodiens* start foraging from October, which corresponds with the appearance of mealybug in vines. This is therefore the time of year when monitoring should start and chemical stem barriers should be applied. Efficacy was found to be between 60 and 100 days after application of treatments for *A. custodiens*, depending on ant pressure (Addison 2002). If ants therefore start foraging in October, and ant pressure is high, a second stem application may be required to exclude ants until harvest.

Table 1. Seed planting densities used for two years in a vineyard in Bonnievale where four ground cover treatments were compared.

<b>Cover crop (2001/2002)</b>	<b>Concentration</b>
Triticale	100kg ha <sup>-1</sup>
Permanent mixture:	
-Permanent dwarf fescue	16kg ha <sup>-1</sup>
-Creeping red Harold	8kg ha <sup>-1</sup>
-SR-4-200	8kg ha <sup>-1</sup>
-Santiago medic	8kg ha <sup>-1</sup>
Creeping vetch	50kg ha <sup>-1</sup>
<b>Cover crop (2002/2003)</b>	<b>Concentration</b>
Triticale	100kg ha <sup>-1</sup>
Permanent dwarf fescue	30kg ha <sup>-1</sup>
Creeping vetch	50kg ha <sup>-1</sup>

Table 2. Comparison between all-sized worker head capsule size (width x length) from various habitat types (df = 398).

Habitat type (means in mm)	Natural vegetation (1.52)			Control (1.56)		
	t-value	P value	SE of difference	t-value	P value	SE of difference
Control (1.56)	0.48	0.62	0.076	-	-	-
Triticale (1.28)	3.85	0.0001*	0.062	3.46	0.0006*	0.08
Fescue (1.53)	0.13	0.89	0.076	0.31	0.76	0.09
Vetch (1.37)	2.00	0.05*	0.073	2.08	0.04*	0.09

\* indicates means that are significantly different.

Table 3. Comparison between smallest worker head capsule size (width x length) from various habitat types associated with vineyards (df = 98).

Habitat type (means in mm)	Control (0.632)		
	t-value	P value	SE of difference
Triticale (0.716)	3.72	0.0003*	0.022
Fescue (0.631)	0.07	0.941	0.015
Vetch (0.683)	3.06	0.0029*	0.016

\* indicates means that are significantly different.

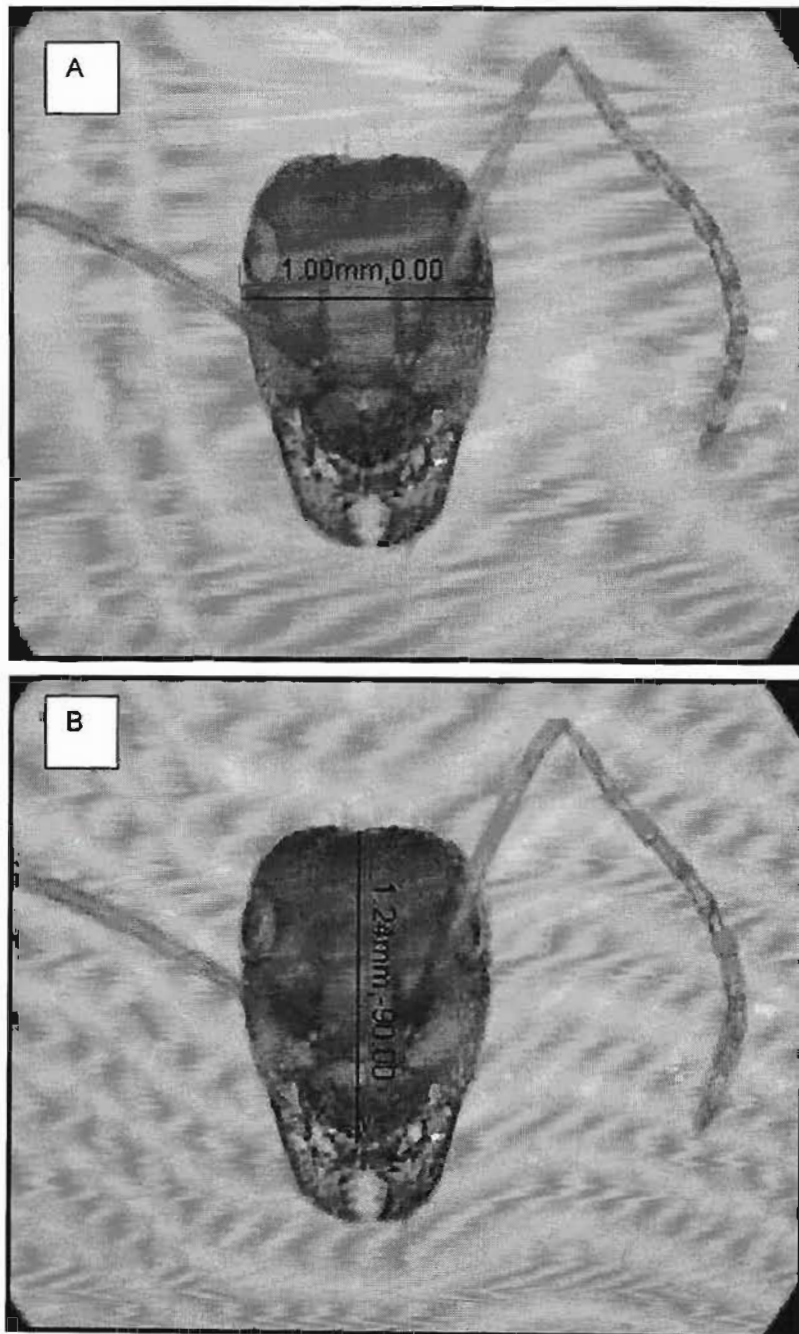


Fig. 1. Positioning of head capsule measurements is shown for width (A) and length (B).

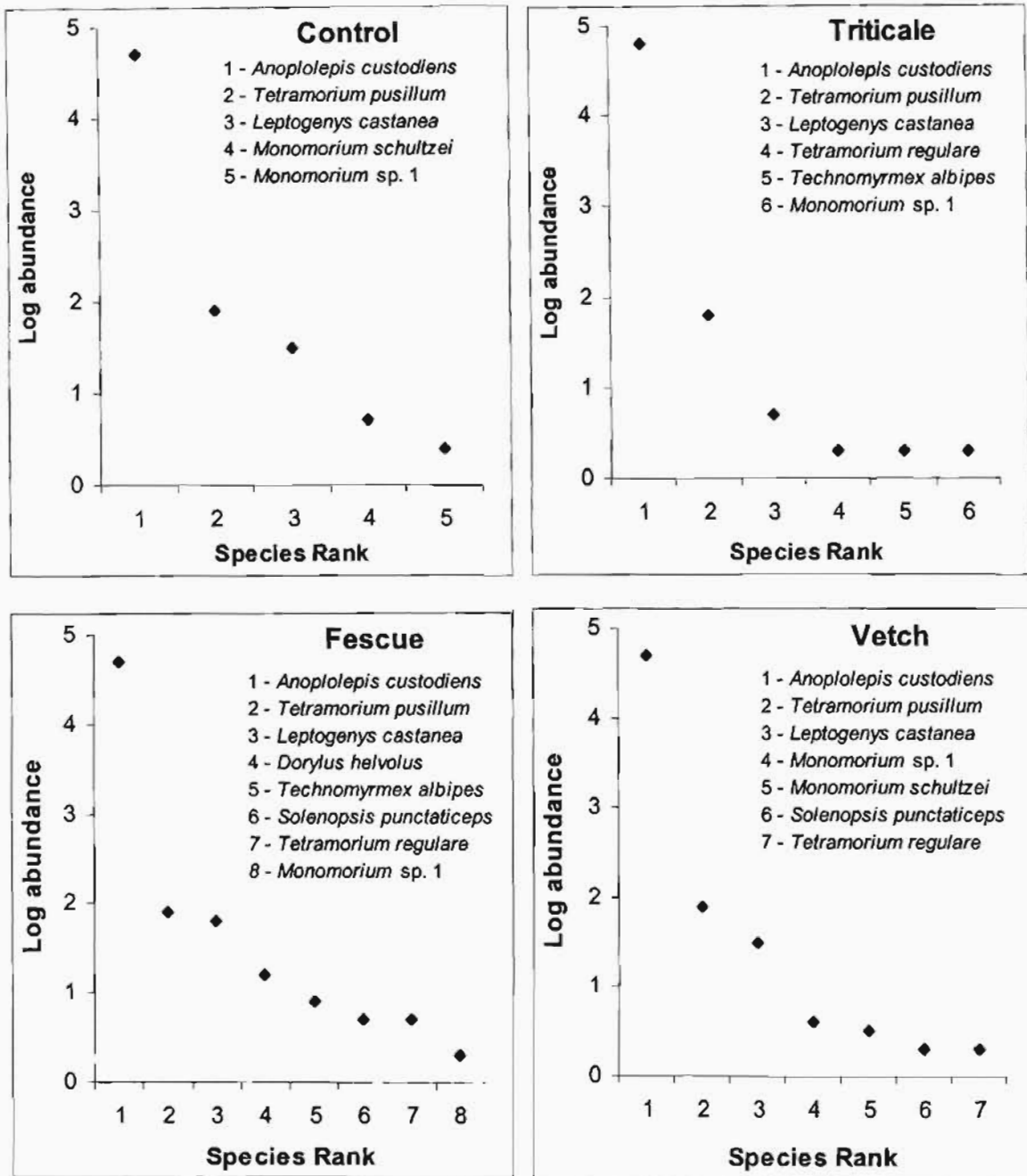


Fig. 2. Abundance/rank plots of the ant assemblages found in four different ground cover treatments, which were established in a vineyard in Bonnievale.



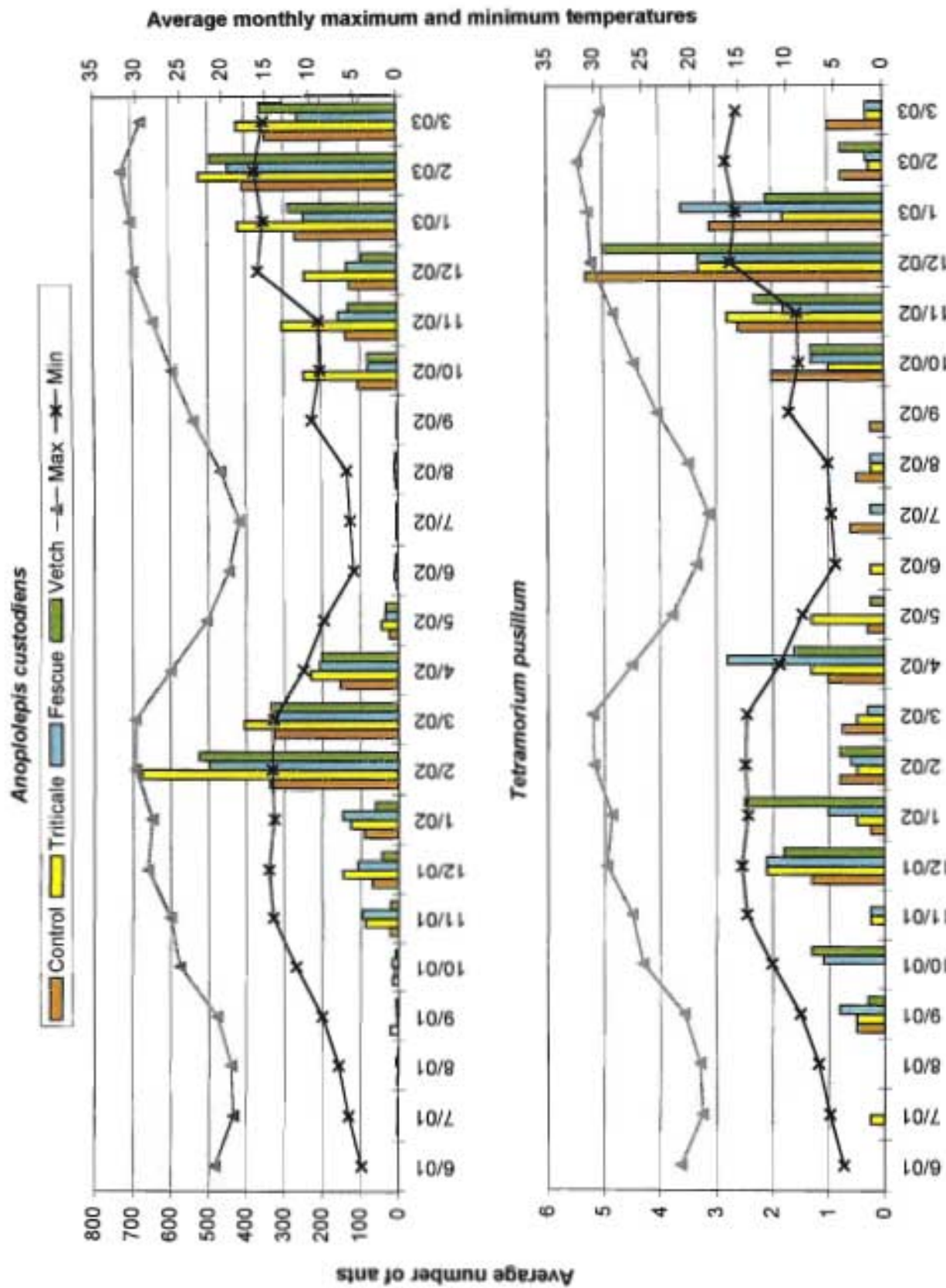


Fig. 3. Average number of ants (histograms), measured using pitfall traps, for four ground cover treatments established in a vineyard in Bonnievale during two years. Average monthly minimum and maximum temperatures are indicated by the lines.

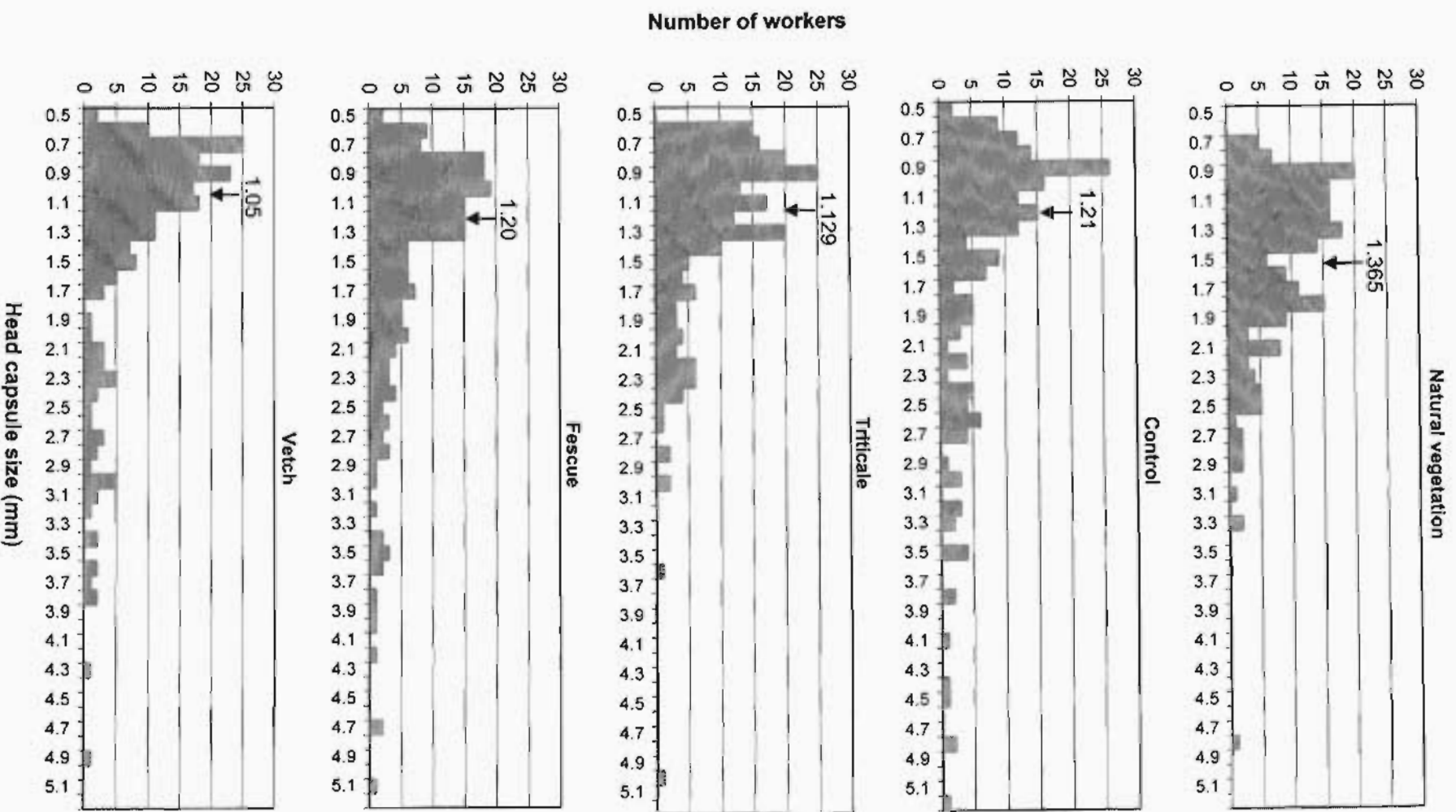


Fig. 4. The size-frequency distribution of 200 all-sized *A. custodiens* workers from different structural habitats associated with vineyards. Arrows indicate the median

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## GENERAL DISCUSSION

*Planococcus ficus* is a key pest in vineyards in South Africa, with grape producers having spent approximately R19 million on chemical mealybug control during 2001 (Walton, personal communication). This study was therefore initiated as certain dominant ant species, notably *L. humile* and *Anoplolepis* spp., were the main factor in preventing the effective biological control of vine mealybug *P. ficus* (Urban & Bradley 1982). These ants belong to a group known as coccidicolous ants (honeydew-feeders) (Wheeler 1910). In vineyards, coccidicolous ants feed primarily on the honeydew excreted by mealybugs (Whitehead 1957), and due to the sedentary nature of these pests the ants can obtain large amounts of food and are therefore able to reach high numbers, necessitating control measures. According to Wheeler (1910), ants derive their food from several sources: Primitive ants, such as *Dorylus helvolus*, are carnivorous, while more advanced species have adapted to utilizing various food sources, apart from honeydew. These include other insects (such as their own offspring and pest insects), excretions from plants (e.g. extrafloral nectaries), fungal hyphae and the seeds of plants. Ants can also be classified according to the structure of their nests: Ants nesting in the soil (epigaeic ants), in the cavities of plants (arboreal ants), in suspended nests (limited to tropical forests), in unusual sites (e.g. human dwellings) and in accessory structures (Wheeler 1910). This study concentrated primarily on epigaeic honeydew-feeders, as they have the potential to indirectly cause the most economic damage in agricultural crops (Way 1963 and Urban & Bradley 1982).

At the commencement of this study, it was not known which ants foraged in vineyards, how many were associated with mealybug or what their distribution was. A suitable ant control method could not be investigated before the target ants, and therefore their nesting habits, were known. No chemical treatments for epigaeic ants in vineyards were registered at the start of the study, and a suitable method that was cost-effective, environmentally-friendly, practical and acceptable for an Integrated Pest Management program needed to be found. In addition, a more sustainable method for ant control as an alternative to chemical control was required, due to insecticides becoming less persistent and more expensive.

This study provided the first published information on which ant species forage in Western Cape vineyards, which of these were associated with mealybugs as honeydew feeders, thereby protecting the mealybugs from their local natural enemies and increasing their impact as pests. Sixty nine percent of all ant species sampled during the survey were caught only in pitfall traps, indicating that the majority of ants found foraging in vineyards are beneficial and do not forage in the vine canopy where they could become potential pests (Chapter 1). This finding could, however, have been influenced by the efficacy of the trapping methods used. The study further determined that epigaeic ant species regularly tended mealybug populations the most, and were therefore of economic importance (Chapter 1). This was also found by Urban & Bradley (1982). With this knowledge, it was possible to develop specific pest management strategies to minimize the impact of these species on mealybug populations. Chapter 2 describes the development of chemical stem banding experiments, with the eventual registration of two treatments for the control of three of these problem ant species. Due to emphasis being placed on more environmentally-friendly management practices, research on the use of cover crops to reduce ant nesting and increase mealybug natural enemies, thereby enhancing integrated mealybug control in vineyards, was undertaken (Chapter 3). This led to the testing of the hypothesis that vegetative ground cover could be detrimental to epigaeic ant populations, while enhancing the diversity of non-target ants (Chapter 4). The following sections summarise the outcomes of this study in more detail, with more emphasis being placed on implications for control of target pest ant species.

### **Chapter 1: A survey of ants that forage in vineyards in the Western Cape Province**

The results of this study recorded a total of forty two ant species in six grape-growing regions in the Western Cape Province. Due to the relatively large variety of ants found in vineyards, the necessity for identifying pest ant species becomes important, as the majority of the ant species caught during the survey were seed harvesters or predators. Six species were observed to tend vine mealybug *P. ficus*. These were the epigaeic species *Linepithema humile*, *Anoplolepis custodiens*, *A. steingroeveri*, *Technomyrmex albipes* and *Pheidole* sp. 1, and the arboreal species *Crematogaster peringueyi*. These ants, with the exception of *Pheidole* sp. 1, were observed to tend mealybug in studies conducted by Whitehead (1957) and Urban & Bradley (1982), but their distributions in the Western Cape were not recorded in detail by these authors. It was therefore not

possible to compare ant distribution patterns recorded during the current study to previous work in vineyards. In the current study, *L. humile*, *A. custodiens* and *A. steingroeveri* were regarded as economically-significant due to their wide distribution and abundance.

Edge effects, where significantly more ants were caught outside of the vineyard than within the vineyard, were found to occur in four of the vineyards sampled,. Three of these vineyards were infested with *A. steingroeveri* and one with *L. humile*. The edge effects indicated that certain conditions within these vineyards made foraging unsuitable. Factors that were identified as most likely causing the edge effects were very low mealybug infestations within vineyards and therefore an insufficient food source to support many colonies. Soil type, moisture, irrigation type and vegetative ground cover were also thought to possibly play a role, which implies that the manipulation of such factors, if it is practically possible, could result in the control of certain ant species. For example, flood irrigation is common along the Olifants River region, but few ants were found foraging within such vineyards. Chapters 3 and 4 further investigated the use of cover crops as a means of habitat modification for ant management.

## **Chapter 2: Chemical stem barriers for the control of ants in vineyards**

Full cover applications of insecticides (chlorpyrifos) during winter were the only registered treatment for ant control at the start of this study, but this was found to be effective only against arboreal nesting ants, such as *C. peringueyi*. The survey (Chapter 1) confirmed that epigaeic ant species are the most problematic in vineyards, and that control should therefore be aimed at *L. humile* and *Anoplolepis* spp. The two field trials that were conducted against *L. humile* and *A. custodiens* in this study showed that direct chemical stem treatments, particularly alphacypermethrin, and two controlled-release chemical bands (terbufos and chlorpyrifos), were an effective method of ant control, but that *A. custodiens* was generally more difficult to control than *L. humile*. Samways & Buitendag (1986) describe a chemical band, alphacypermethrin sprayed onto a backing material ("Sper"), which is effective against ants on citrus in South Africa. Chemical stem treatments have also been evaluated on citrus using chlorpyrifos against *L. humile* in California, USA, (Moreno *et al.* 1987) and using alphacypermethrin (Stevens *et al.* 1995) and a chlorpyrifos-impregnated band (James *et al.* 1998) against *Iridomyrmex* spp. in Australia, with good results. However, none of the chemical stem treatments evaluated



during the current study had been tested on vines. Unnecessary chemical treatments could result in a reduction in ant biodiversity within vineyards with unknown consequences. It would be useful to establish the effect ants have on other vine pests, such as snout beetles (Curculionidae) and mediterranean fruit fly (Tephritidae), and if they are significant biocontrol agents.

Due to a low and variable ant infestation during the pre-treatment counts of the first year of field testing, possibly as a result of unfavourable climatic conditions, the results of the first year can be regarded as inconclusive. Such patchy distributions are often encountered when working with ants and Homoptera (Myers 1957 and Walton 2001). This unpredictable variability in the relevant ant species populations led to the development of an alternative method for evaluating chemical stem treatments against ants. Two simulated field trials, which made use of feeding trays placed on top of 30 cm sections of vine stumps, were conducted against *L. humile* and *A. steingroeveri*. This method provided a high pre-treatment count for all treatments and made evaluation of ant infestations on stems easier. The results of these trials indicated that direct chemical stem treatments may result in ant mortality. In a study conducted by James *et al.* (1998), ant mortality was established for two *Iridomyrmex* spp. after exposure to weathered chlorpyrifos controlled-release bands for 16 hours. However, more research is needed to establish suitable bio-assay techniques which simulate field conditions (short exposure) to determine whether or not direct stem treatments result in mortality of *L. humile* and *Anoplolepis* spp.

Both the simulated field trials and field trials high-lighted the difficulty in controlling *Anoplolepis* spp. The results of field trials show that alphacypermethrin SC at 20mℓℓ effectively excludes *A. custodiens* for approximately 60 days, while it is effective against *L. humile* at a concentration of 10mℓℓ for over 90 days under high ant pressure. Subsequent to these trials being conducted, alphacypermethrin SC (Fastac) and the controlled-release chlorpyrifos band (Suskon Blue Ribbon) have been registered for the control of the epigaiec ant *L. humile*, while only alphacypermethrin SC was registered to control *Anoplolepis* spp. in vineyards as stem treatments. The moderate efficacy of alphacypermethrin against *A. custodiens*, and also the difficulty in controlling *Anoplolepis* spp. in general, has a significant cost implication, as treatments may need to be applied twice per growing season when infestations are high.

### **Chapter 3: Integrated Pest Management using cover crops for managing the ant-mealybug mutualism in Western Cape vineyards.**

No formal studies have previously been undertaken to determine the effect of a cover crop on pest ants in South African vineyards. Furthermore, no information has been published as to whether the cover crops that are recommended for soil amelioration in South African vineyards provide any benefit as an alternate refuge for mealybug natural enemies during winter. If both these hypotheses proved to be correct, then cover crops could be recommended for integrated mealybug control in vineyards. However, the results of this study showed that ant activity in triticale was the only treatment which showed significantly higher ant activity, relative to the other ground cover treatments, possibly due to the seeds being utilized as an additional food source by the ants. Furthermore, none of the ground cover treatments showed ant activity which was lower than the control plots. There was no significant difference in either ant and mealybug infestations in the vine canopy, nor number of ant nest entrances. Few significant differences were found between treatments of mealybug natural enemy numbers over two years between the ground cover treatments. This was despite a statistically significant reduction in soil temperature of 3°C in cover crop plots relative to the control plots. Habitat modification was also found to be ineffective against dominant *Pheidole* spp. (Samways 1982), while New (2000) found that ants may not be sufficiently sensitive to floristic change to employ them in monitoring grassland condition in Australia.

Despite a considerable number of studies having shown that there is potential for a vegetative ground cover, such as cover crops, to benefit Integrated Pest Management (Way 1953, Steyn 1954, Tedders 1983, Altieri & Schmidt 1985, van Emden 1990, Bugg & Waddington 1994 and Hofmann 2000), this study proves otherwise for the ant-mealybug mutualism in South African vineyards. However, biological control of vine mealybug is rarely effective in situations of high pest pressure (Walton 2003). It is therefore possible that cover crops could be more effective if the ant and mealybug infestations are less severe, as this would give natural enemies a better chance of parasitizing mealybugs. Furthermore, from this study, cover crops did not have an apparent influence on pest ants, but no detailed examinations of *A. custodiens* colony fitness or ant diversity were carried out. These aspects were discussed further in Chapter 4. It can be concluded, however, that cover crops are not recommended as a

curative ant management method, but could be useful in young vineyards as a preventative management method. Nonetheless, cover crops will not be effective without chemical stem barriers, since the importance of breaking the ant-mealybug mutualism is apparent.

#### **Chapter 4: Variation in ant diversity, foraging behaviour and morphology in different structural habitats associated with vineyards.**

A variety of cover crops are used in Western Cape vineyards, and comprise a variety of different structural habitats. However, almost nothing is known about the effect these cover crops have on arthropod communities. From Chapter 3, it was found that no significant trends could be detected in the impact of cover crops on ant, mealybug or mealybug natural enemy populations in vineyards. Worldwide, research has been carried out on the effect of increased plant diversity on ant communities within agroecosystems (Room 1971, Lobry de Bruyn 1993, Roth *et al.* 1994 and Bestlemeyer & Wiens 1996). From these various studies, it was found that as vegetation complexity increases, so does ant species diversity and that farmlands supported fewer species than more complex vegetation types. In their study, Roth *et al.* (1994) further supported the hypothesis that reduced vegetation complexity results not only in reduced ant species diversity but also in a few ant species becoming more dominant. However, Bestlemeyer and Wiens (1996) concluded that highly degraded sites may have conservation value for rare, arid-adapted species in the Argentine Chaco, and that historical and biogeographic influences could play a role in determining species diversity of certain sites. It appears, therefore, that factors other than vegetative complexity could influence ant species diversity in agroecosystems, such as soil type and condition of neighbouring vegetation, although in the above mentioned studies, vegetation complexity was cited as the primary factor.

In comparing ant species diversity in four ground cover treatments (vetch, fescue, triticale and an unplanted control plot), it was found from this trial that three out of eight species were shared between all four habitats. *A. custodiens*, *Tetramorium pusillum* and *Leptogenys castanea* were always ranked first, second and third, respectively, in each of the four habitats. *A. custodiens* as being a dominant, aggressive ant was further illustrated in this chapter, which showed that *A. custodiens* was extremely dominant over other ant species in vineyards with high mealybug infestations (see also Chapter 1).

The control plot supported the lowest number of ant species (five), while the fescue plot supported the most (eight). The higher number of ant species in the fescue plot could be explained by the greater variety of weeds in this plot all year round, while the control plot was kept free of weeds for most of the year. Although the number of ant species did differ between habitats, many of the lower ranked species occurred in small numbers, where total accumulated numbers during two years came to between two and 18. The beneficial impact of these non-target species could therefore not have been significant.

The size-frequency distribution of foragers outside of vineyards was greater than within various ground cover treatments in the vineyard. This can be supported by data from Oster and Wilson (1978), which correlated food particle size with head capsule size of foraging fire ant *Solenopsis invicta* workers. Since honeydew from mealybugs is an easily accessible and readily available food source for ants in vineyards, there is no need to expend energy on producing large workers. However, in natural vegetation a larger variety of food particles were most likely utilized, which was seen by the presence of a greater number of larger foragers. It was concluded that these size differences were probably not only the result of the type of food utilized by the ants, but were most likely also the result of the reduced temperature in the cover crop plots.

Foraging was significantly higher in triticale plots than in the other ground cover treatments, but between June and September foraging was minimal in all treatments. It appears, therefore, that foraging behaviour of *A. custodiens* was affected mainly by food availability. That honeydew was the primary food source of *A. custodiens* in this vineyard was also indicated by the short foraging distances that ants travelled from their nest (Chapter 3). This further suggests that chemical stem barriers are indeed the most effective way to manage ants. However, since the foraging distances measured in this study were only done once during the two seasons that the trial was running and for a relatively short period of time (2 min observations), it is possible that the measurements were not that representative.

The latest research on ant control is focussing on containerized toxic baits (Stevens *et al.* 2002). The results of this study showed that these baits would have to be more attractive than honeydew and just as easily accessible. The implication for ant control is that since the average foraging distance of ants is possibly relatively short, many bait

stations would be required and could be as much as one per vine. Baiting could therefore be a more labour intensive control method than chemical stem treatments, as bait stations would have to be maintained at regular intervals. This short foraging distance further indicates that there could be interspecific competition between *A. custodiens* colonies. However, no fighting was detected between workers of different colonies, while Steyn (1954) also found no evidence of colony division or antagonism between *A. custodiens* workers from different colonies foraging on the same tree in Letaba citrus orchards.

Foraging almost ceased between June and September, but then quickly increased from October to February, when a peak was reached. This was the time when mealybug visibility increased in the vine canopy in a study conducted in the same areas the previous season (Walton 2003). The same period of low ant activity was found from a study conducted on a dominant Australian ant pest, *Iridomyrmex rufoniger*-group of species, and was found to be strongly affected by seasonal factors (Stevens *et al.* 1998). Chemical stem barriers should first be applied during October, when foraging activity starts to increase and colonies are still small. It is then necessary to maintain the efficacy of chemicals stem treatments throughout the foraging season to ensure that the mutualism between ant and homopteran remains severed. This can be done by controlling high-growing weeds, which the ants can use to enter the vine canopy. Monitoring ants throughout the foraging season is also important to establish when chemical stem treatments start to fail.

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## CONCLUSIONS

- Forty two species of ants were recorded from twenty two vineyards in six grape-growing regions of the Western Cape Province. These ants were mostly seed-harvesters or predators, while six of these species were observed to tend mealybug.
- The epigaeic ants *Anoplolepis custodiens*, *A. steingroeveri* and *Linepithema humile* were found to be economically significant pests due to their mutualistic association with mealybug, abundance and wide geographic distribution.
- Chemical stem treatments were found to be an effective method to control epigaeic ants, which is also an acceptable method for integrated pest management. A chlorpyrifos-impregnated band, a terbufos slow-release band or alphacypermethrin SC as a direct stem spray were found to be the most effective treatments.
- A simulated field trial provided an evenly high pre-treatment count of target ant species, and was found to be a useful tool to evaluate chemical stem treatments against them. Results of this trial indicated that chemical stem treatments may cause ant mortality, which could further the efficacy of such treatments.
- *Anoplolepis* spp. were found to be more difficult to control than *L. humile*, and alternative, non-chemical management options are therefore needed as a more sustainable method for managing these ants.
- Chemical stem barriers would not be effective against arboreal *Crematogaster* spp., and it is recommended that other options of control be investigated for these species, e.g. toxic baits.
- Although lower soil temperatures were measured in cover crop plots in a ground cover trial within a vineyard, no significant differences were found in the number of *A. custodiens* nest entrances between the four ground cover treatments (vetch, fescue, triticale and unplanted control) over two years.
- *A. custodiens* and *P. ficus* infestations in the vine canopy showed no significant trends, while few significant trends were found between mealybug natural enemy numbers in the four ground cover treatments.
- The only measure which showed a significant difference was higher ant activity, as measured by pitfall traps, in triticale plots. This was attributed to the ant's

utilizing the seeds from this cover crop as an additional food source. Triticale is therefore not recommended in vineyards with ant problems.

- Habitat modification by planting cover crops in vineyards cannot be recommended as a curative management practice for controlling *A. custodiens*. Vetch or fescue can be used in combination with chemical stem barriers as a supplement. However, high infestations could be inhibited from developing in younger vineyards by using cover crops with or without chemical stem treatments.
- Foraging distances, which were measured in the four ground cover treatments, were found to be relatively short (an average of 34cm). This indicates that the main food source for the ants was honeydew from mealybugs on the vine immediately above the nest.
- Species diversity was measured using rank/abundance plots. These showed that *A. custodiens*, *Tetramorium pusillum* and *Leptogenys castanea* were always ranked first, second and third, respectively, in each of the four ground cover treatments.
- It is concluded that the differences in ant species diversity between four ground cover treatments were not marked, due to the extreme dominance by *A. custodiens*.
- Cover crops did not affect the foraging behaviour of *A. custodiens*, while the main driver for regulating population density was most likely a combination of food availability and soil temperature.
- Foraging activity of *A. custodiens* almost ceased between June and September, but started to increase in October. Chemical stem treatments should therefore be first applied no later than October, and their efficacy maintained throughout the summer season by controlling high-growing weeds.