ASSESSMENT OF TOXIC BAITS FOR THE CONTROL OF ANTS (HYMENOPTERA: FORMICIDAE) IN SOUTH AFRICAN VINEYARDS

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DECLARATION

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

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ABSTRACT

Ant infestations comprising the Argentine ant *Linepithema humile* (Mayr), common pugnacious ant *Anoplolepis custodiens* (F. Smith) and cocktail ant *Crematogaster peringueyi* Emery are a widespread pest problem in South African vineyards. Integrated Pest Management (IPM) programmes aimed at suppressing the problematic honeydew excreting vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) on grapes must include ant control to optimize the effectiveness and efficacy of mealybug natural enemies. If ants are eliminated, natural enemies are able to contain mealybugs below the Economic Threshold Level (ETL). Current strategies for ant control are limited and generally include the application of long term residual insecticides that are detrimental to the environment, labour intensive to apply and can disrupt natural biological control if applied incorrectly. A more practical method of ant control using low toxicity baits was therefore investigated. Field bait preference and bait acceptance assessments aimed at determining bait repellency and palatability, respectively, were carried out during spring, summer and autumn in three vineyards of the Cape winelands region during 2007/08. Five toxicants comprising gourmet ant bait (0.5%), boric acid (0.5%), fipronil (0.0001%), fenoxycarb (0.5%) and spinosad (0.01%) dissolved in 25% sugar solution were tested against a 25% sucrose solution control. Gourmet ant bait was significantly more preferred and accepted by all ant species than the other baits. Laboratory bait efficacy assessments using four insecticides (gourmet, boric acid & spinosad) at concentrations of 0.25; 0.5; 1; 2 and 4 times the field dose and fipronil at 0.015625; 0.03125; 0.0625; 0.125; 0.25 times the field dose were carried out. Results revealed that boric acid (2%), gourmet ant bait (2%) and fipronil (1.0 X 10^{-5}%) exhibited delayed toxicity for *L. humile* and *C. peringueyi* while spinosad (0.01%) showed delayed action on *L. humile*. Field foraging activity and food preference tests were also carried out for the three ant species during 2007/08. Foraging activity trials revealed that vineyard foraging activity of *L. humile* is higher relative to *A. custodiens* and *C. peringueyi*. This means fewer bait stations are required for effective *L. humile* control making low toxicity baits a more affordable and practical method of controlling *L. humile* than the other two ant species. Food preference trials showed that *L. humile* and *C. peringueyi* have a high preference for sugar while *A. custodiens* significantly preferred tuna over other baits. However, all ant species had a preference for wet baits (25% sugar water, 25% honey, tuna &

agar) as opposed to dry ones (fish meal, sorghum grit, peanut butter & dog food). This research concludes that low toxicity baits show potential in ant pest management and can offer producers with a more practical, economical and environmentally friendly method of ant control which is compatible with vineyard IPM programmes.

OPSOMMING

Mierbesmetting wat uit die Argentynse mier *Linepithema humile* (Mayr), die gewone malmier *Anoplolepis custodiens* (F. Smith) en die wipstertmier *Crematogaster peringueyi* Emery bestaan, is ’n plaagprobleem wat wydverspreid in Suid-Afrikaanse wingerde voorkom. Programme vir geïntegreerde plaagbeheer (GPB) wat daarop gemik is om die wingerdwitluis *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) – wat ’n probleem is weens die heuningdou wat dit afskei – op druiwe te beheer, moet mierbeheer insluit om sodoende die uitwerking en doeltreffendheid van die witluis se natuurlike vyande die beste te benut. As miere uitgeskakel kan word, sal dit vir die natuurlike vyande moontlik wees om die witluis sodanig te beheer dat dit onder die ekonomiese drempelvlakke (EDV) bly. Huidige strategieë om miere te beheer, is beperk en sluit gewoonlik die toediening van insekdoders in wat lank neem om in die grond af te breek, wat skadelik vir die omgewing is, waarvan die toediening arbeidsintensief is en wat die natuurlike biologiese beheer kan versteur indien dit verkeerd toegepas word. Daarom is ’n meer praktiese metode ondersoek waar miere deur die gebruik van lae toksisiteit lokase beheer word. Ondersoeke na lokaasvoorkeure en lokaasaanvaarbaarheid in die praktyk, wat daarop gemik is om te bepaal of die lokaas onderskeidelik afstootlik en smaaklik bevind word, is oor lente, somer en herfs in drie verskillende wingerde in die Kaapse wynlandstreek gedurende die 2007/08-seisoen uitgevoer. Vyf gifstowwe, bestaande uit gourmet ant bait (0.5%), boorsuur (0.5%), fiproniel (0.0001%), fenoksiekarb (0.5%) en spinosad (0.01%) wat in ’n 25%-suikeroplossing opgelos is, is getoets teenoor ’n kontrole wat uit ’n 25%-sukrose-oplossing bestaan. Al die mierspesies het gourmet ant bait bo die ander lokase verkies en aanvaar. In die laboratorium is ondersoeke gedoen om die doeltreffendheid van die lokase te bepaal deur vier insekdoders (gourmet ant bait, boorsuur en spinosad) te gebruik in konsentrasies van 0.25; 0.5; 1; 2 en 4 keer die dosis in die praktyk en fiproniel teen 0.015625; 0.03125; 0.0625; 0.125; 0.25 keer die dosis in die praktyk. Resultate het getoon dat boorsuur (2%), gourmet ant bait (2%) en fiproniel (1.0 X 10^{-5}%) vertraagde toksisiteit getoon het vir *L. humile* en *C. peringueyi,* terwyl spinosad (0.01%) ’n vertraagde uitwerking getoon het op *L. humile*. Toetse om kossoekaktiwiteite in die praktyk en die voedselvoorkeure van die drie mierspesies te ondersoek, is ook gedurende die 2007/08-seisoen gedoen. Proewe oor kossoekaktiwiteite het getoon dat hierdie aktiwiteite in die wingerd by *L. humile* hoër

is in verhouding met *A. custodiens* en *C. peringueyi*. Dit beteken dat minder lokaasstasies nodig is om *L. humile* doeltreffend te beheer en lei daartoe dat lae toksisteit lokaas ’n beter manier is om *L. humile* te beheer as die ander twee mierspesies. Proewe oor voedselvoorkeure het aangedui dat *L. humile* en *C. peringueyi* ’n groot voorkeur toon vir suiker, terwyl *A. custodiens* ’n duidelike voorkeur vir tuna het. Alle mierspesies het egter ’n voorkeur vir nat lokaas (25% suikerwater, 25% heuning, tuna en agar), eerder as droë lokaas (vismeel, sorghumgruis, grondboontjiebotter en hondekos) getoon. Uit hierdie navorsing word afgelei dat lae toksisteit lokaas potensiaal toon in mierbeheer en dat dit produsente ’n meer praktiese, ekonomiese en omgewingsvriendelike metode van mierbeheer kan bied wat met GPB-programme in die wingerd versoenbaar is.

DEDICATION

I dedicate this thesis to my dad Lawrence Nyamukondiwa. Without his support, hard work, role modeling and most of all sacrifices he made for me, this work would have been incomplete.

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CHAPTER 1

GENERAL INTRODUCTION

Ecology and significance of ants

Ants (Hymenoptera: Formicidae) are eusocial insects, characterized by cooperative brood care, overlapping generations of workers within a colony, and highly developed caste systems (Wilson 1971). They are omnipresent, diverse and found in very large numbers, constituting about 80% of animal biomass in tropical ecosystems (Hölldobler & Wilson 1990). Ants are widely used as bioindicators (Kaspari et al. 2003), and they play a key role in ecosystem health because of their dominant contribution to biodiversity and their effects on key ecological processes (Leston 1973; Gotelli & Ellison 2002). Ants can be useful as pollination agents, biological control agents, seed dispersal agents in addition to improving soil structure by enhancing aeration, incorporating soil organic matter thereby increasing decomposition and nutrient availability (Folgarait 1998).

Ants are classified as both agricultural and household pests. They are disease vectors of economic significance in addition to being a key landscape pest (Jahn & Beardsley 1996). In poultry runs, ants disturb hens sitting on eggs driving them off nests and killing their newly hatched chicks (Steyn 1954). *Anoplolepis custodiens* (F. Smith) has been reported to disturb or even kill chickens in certain areas of the Free State Province, South Africa, therefore justifying control measures around chicken runs (Steyn 1954). *Crematogaster peringueyi* Emery infests trees, timber and poles, which eventually become weakened and break (Prins et al. 1990). Ants also threaten apiculture as they irritate bees until they desert their hives (Skaife 1961). In the kitchen and pantry, *Linepithema humile* (Mayr) is an unmitigated nuisance, swarming over foodstuffs and making them unfit for human consumption (Skaife 1961).

Ants in vineyards

Ant infestations are a widespread problem in vineyards of the Western Cape Province. According to a survey of ants in Western Cape vineyards (Addison & Samways 2000), forty two species of ants were recorded. The most significant ant pests comprised the Argentine ant *Linepithema humile*, cocktail ant *Crematogaster peringueyi*; two species of pugnacious ants; the common pugnacious ant *Anoplolepis* *custodiens* and the black pugnacious ant *A. steingroeveri* Forel and the little ubiquitous white-footed ant *Technomyrmex albipes* Smith (Addison & Samways 2000).

Linepithema humile is one of the world's worst ant species (Vega & Rust 2001) often with damaging economic and ecological impacts (Holway et al. 2002). It has spread from South America to many regions of the world and has become established throughout the southern states of California (Mallis 1982) and has reached pest status in multiple environments (Human & Gordon 1997; Vega & Rust 2001; Holway et al. 2002). It was first recorded in Cape Town, South Africa in 1908 (Joubert 1943) and is native to Argentina (Vega & Rust 2001; McGynn 1999). It prefers habitats with permanent sources of water and their populations steadily decline with increasing distance into adjacent drier vegetation (Holway 2005), indicating that this ant is prone to desiccation (Witt & Giliomee 1999). Certain aspects of the biology of this species have contributed greatly to its cosmopolitan distribution and development of its pest status in Mediterranean climates throughout the world (Markin 1968). Workers are approximately 3mm long with a uniform honey brown colour. They appear dark brown when seen foraging and have a single segment (node) in the pedicel and are monomorphic (all workers are approximately the same size). This species primarily nests in the soil; single colonies are polygynous (containing many queens) and can have many thousands of workers. Often, many individual nests are interconnected (polydomous) to form enormous "supercolonies". The ant's unicolonial nest structure, high population density, and efficient use of resources provide an advantage in interspecific competition (Human & Gordon 1996; Holway 1998; Chen & Nonacs 2000). As a result, *L. humile* displace native ants and other invertebrate and vertebrate species (Sanders et al. 2001; Suarez et al. 2002). This ant is inactive in winter because of its high susceptibility to cold. It becomes more active in warmer temperatures in spring through summer and reaches a peak in March (Skaife 1961) in South Africa. Colonies reproduce by sociotomy (budding) usually in spring (Markin 1968).

Anoplolepis custodiens is approximately 3-10mm long, medium to dark brown in colour and has 3 polymorphic worker castes (Steyn 1954). It is a ground nesting ant whose nest entrances are characterized by a mound of excavated earth. It is a behaviorally dominant ant that is very successful in interspecific competition and exhibits no intraspecific competition (Hölldobler & Wilson 1990).

Crematogaster peringueyi is arboreal (Kriegler & Whitehead 1962) often constructing its nests within grape vines and is approximately 5mm in length. It is black in colour, monomorphic and is characterized by the ability to cork its heart shaped abdomen over its thorax when disturbed (Kriegler & Whitehead 1962). It is an economic pest of grapes in South Africa and is found in most Western Cape vineyards (Kriegler & Whitehead 1962; Addison & Samways 2000) where it forms mutualistic relationships with mealybugs.

In agroecosystems, ants are indirect pests in that they stimulate pest outbreaks (Veeresh 1990; Thompson 1990 & Delabie 1990). By consuming honeydew from mealybugs and other scale insects, foliage inhabiting ants increase the survival of honeydew producing pests and consequently increase their damaging effects on crops (Way 1963; Buckley 1987; Jordano et al. 1992; Jahn & Beardsky 1996; Styrsky et al. 2007).

The vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) is regarded as one of the most devastating pests of the grape industry in South Africa (Joubert 1943; Kriegler & Whitehead 1954; Walton 2001; 2004; Walton & Pringle 2004). Biological control of *P. ficus* by predatory beetles and parasitic wasps is significantly reduced in the presence of ants (Kriegler & Whitehead 1962; Myburgh et al. 1973; Urban et al. 1980). Ants tend honeydew excreting Hemiptera thereby preventing small predators and parasitoids from attacking scale insects and mealybugs (Bartlett 1961; Way 1963). This results in an increase in both pest populations.

It is very difficult to separate ant infestations from mealybug problems in vineyards. Consequently, most growers often apply control measures for the hemipterans and ignore the ants (Daane et al. 2004). *Planococcus ficus* feeds on grapes, trunk, canes or leaves in addition to the vine's roots, where it finds some protection from unfavorable temperatures and natural enemies (Walton 2001; 2003). This mealybug excretes vast amounts of honeydew- a sugary substance, on which sooty mould grows (Flaherty et al. 1991) consequently decreasing grape bunch quality (Geiger & Daane 2001). Excessive mealybug infestations may result in desiccation of bunches leading to premature senescence (Annecke & Moran 1982). Furthermore, *P. ficus* is a vector of grapevine leafroll virus (Englebrecht & Kasdorf 1990), a devastating vine disease in the Western Cape Province which has resulted in the large scale removal of virus-infested vines throughout the Province. Because there is no treatment for viral diseases, the only control option for vine leafroll virus is controlling *P. ficus* (the vector) and attendant ants.

Integrated Pest Management programmes aimed at suppressing honeydew excreting mealybug pests on grapes must include ant control in order to optimize the effectiveness and efficacy of natural enemies. Natural enemies are capable of keeping hemipteran pests below Economic Threshold Levels (ETL) (Moreno et al. 1987; Walton & Pringle 2003) unless their efficacy is reduced by ants or broad spectrum pesticides (Flaherty et al. 1991). As a result, all key vine foraging ant species must be controlled in order to conserve mealybug natural enemies.

Review of ant control methods

Most ant control chemicals are registered for use during the growing season (Anonymous 2007) and these may therefore have negative effects on natural enemies, which also reach their peak at this time of the year (Walton 2003). These chemicals are often incorrectly applied as ground sprays or are applied as trunk bands that kill foragers by contact (Addison 2002). With chemical stem barriers, only limited control can be achieved because the queen or queens and the vast majority of workers in the nest are not affected (Baker et al. 1985; Knight & Rust 1990; Nelson & Daane 2007; Styrsky & Eubanks 2007). Direct ground chemical sprays pose a risk to beneficial organisms through leaching into groundwater and volatilization. Chemical stem barriers have been found to be effective against various ant pests, among them *L. humile* and *A. custodiens* in vineyards (Addison 2002). They are considered an ecologically sound method of ant control as ants are not killed but rather left to forage on the ground, where they are beneficial predators of other pests (Samways & Tate 1984; Moreno et al. 1987; Stevens et al. 1995; James et al. 1998). Although acceptable for IPM, growers find this application labour intensive and research has indicated *Anoplolepis* species are not effectively controlled by this method if infestations are severe (Ueckermann 1998). Furthermore, this method is also not very practical for the control of arboreal ants like *C. peringueyi*. When using trunk barriers, vines need to be skirt pruned in order to prevent the ants from using alternative routes into canopy. This becomes labor intensive and is not practical for bush vines (those that are not grown using the trellise system). It is also not practical in nurseries which need to be virus vector free, and therefore, has not been adopted by many growers. Habitat modifications have also been tested for controlling ant pest problems. Exclusion of moisture sources has been indicated as one of the ways to control *L. humile* populations (Soeprono & Rust 2004). Volunteer ground cover showed no significant effect on *L. humile* infestations in citrus orchards (Stevens et al. 2007) while cover crops showed no significant effect on *A. custodiens* infestations in vineyards (Addison & Samways 2006). Baker et al. (1985) revealed that ants can be controlled using water filled ditches around citrus groves while Stevens & Pereira (2003) reported on successful biological control of *S. invicta* using the entomopathogen *Thelohania solenopsae*. With the introduction of the Scheme for Integrated Production of Wine (IPW), ant control needs to be cost effective, environmentally friendly, practicable and highly compatible in an IPM program (Anonymous 2000). Therefore IPM is strongly being emphasized.

Development of low toxicity baits

Low toxicity baits are those compounds that provide <15% mortality after 24 hour exposure, and >89% mortality at 20 days (Stringer et al. 1964). Low toxicity baits may offer a more effective method of controlling ants in vineyards and orchards. The recruitment and food-sharing behavior of ants can be exploited to their disadvantage by spreading a toxicant through the colony (Hooper-Bui & Rust 2000). Ant baits generally contain these components:

- Attractant, usually food (sugar, protein) or pheromone which makes the bait acceptable and readily picked up.
- Palatable carrier, usually water or agar, which gives the physical structure or matrix to the bait.
- Toxicant, which should be non repellent and delayed in action, effective over at least a ten fold dosage range.
- Other materials, such as emulsifiers, preservatives, waterproofing or antimicrobial agents added for the purpose of formulation.

Each of these components play a critical role in the bait's effectiveness (Hooper-Bui & Rust 2000) thus ideal baits should strike a balance for these critical factors. Ideally, baits should be highly attractive, palatable and should be effective at multiple low doses therefore allowing bait distribution via trophallaxis (Stringer et al. 1964). This makes bait development highly challenging. Relatively very few toxicants are suitable for use as ant baits (Stringer et al. 1964). Out of over 7 000 toxicants tested for imported fire ant (*Solenopsis* species), only six were commercialized in the USA (Banks et al. 1992). By 1998, only four were still being used commercially (Collins & Callcott 1998).

Baits are fast becoming an indispensable tool in urban and agricultural pest control. Boron containing compounds such as borax (sodium tetraborate decahydrate) and boric acid have been used since the 1900s against ants (Rust 1986). Low concentrations of boric acid (<1%) dissolved in sucrose water have been shown to be slow acting and non repellent, thereby enhancing long term ingestion by *L. humile* (Klotz & Moss 1996; Klotz et al. 1997). Recruitment tests for *L. humile* have shown preference for 50>25>10% sucrose solutions (Klotz et al. 1998). However, higher sucrose baits (>25%) result in crystallization of the sugar thus interfering with bait delivery. Hence 25% sucrose solution is the ideal matrix for formulating toxic baits for sugar feeding ants. The delayed action of boric acid promotes a thorough distribution of the active ingredient within the nest, leading to death of the entire colony (Klotz et al. 1998). Soil mixes of granular fipronil have also been used to prevent *L. humile* from colonizing potted plants (Costa & Rust 1999) and when mixed with sugar water, fipronil provided effective control of *L. humile* at low toxicity doses (Hooper-Bui & Rust 2000). Before using toxic baits, the biology of the ants and their foraging behavior must be well understood. This will give an insight into the type of bait, volume of bait that a station should contain and the number of bait stations needed per unit area. Furthermore, a clear indication of the food requirements for the ants is critical in bait formulation. The optimal percentage of carbohydrate, protein and fat as the bait's feeding stimulant should therefore be specific to the species of ant and the nutritional requirements of the colony. The use of toxic baits for the control of ants in vineyards has not yet been investigated in South Africa.

Bait delivered in stations minimises environmental exposure to the toxicant and therefore reduces the risk to non target organisms. Toxic baits have great value for agricultural pest management since they are easy to apply, do not need specially trained manpower to apply, and the bait concentrations used are very low, allowing recruitment and trophallaxis. This, together with the fact that they are containerized and easy to place into the field (no additional tools or machinery required) makes it a practical, economical and environmentally friendly method of ant control which can be integrated into vineyard IPM and IPW programmes. However baits of low toxicity have a few setbacks mainly highlighted in agroecosystems as opposed to urban settings. Sugar baits are quickly affected by microbial growth consequently affecting bait palatability (Silverman & Brightwell 2008). Secondly, when baits are placed in agroecosystems, precipitation, irrigation and evaporation can easily affect bait palatability and effectiveness by concentrating or diluting the bait. Furthermore, water leakage through irrigation pipes can negatively affect product performance (Silverman & Brightwell 2008). Ant behavior depends upon a number of environmental conditions, one of which is the availability of alternative food sources. Effectiveness of baits in vineyards is limited by abundant food resources like honeydew, prey items and nectar which presumably are more attractive to foragers than low toxicity baits (Kiss 1981; Volkl et al. 1999; Daane et al. 2006). Furthermore plants may augment food (for omnivores like *L. humile*) and shelter (for arboreal ants like *C. peringueyi*) (Lach 2003). This contributes to colonies' growth and may complicate their control using low toxicity baits.

Objective of the study

This project was aimed at addressing problems associated with ant control in vineyards and nurseries. The objectives of the project were as follows:

1. Determining whether various baits were more or less preferred by three ant species relative to a control containing no toxin (chapter 2). Non-preference of a bait could indicate repellency. Preference/non-preference is the first behavior an ant exhibits when encountering a bait and was measured in terms of number of ants at bait stations.
2. Determining bait acceptance of various baits by three ant species relative to a control containing no toxin (chapter 3). Acceptance of a bait indicates palatability when quantified in terms of grams bait removed and takes place once the bait is a preferred food source.
3. Determining bait efficacy in small-scale field experiments and laboratory bioassays (chapter 4). This measures delayed toxicity of the accepted baits against the ant species.
4. Investigating bait field application in terms of foraging activity and food preferences (protein versus sugar & solid versus wet diet) for three ant species (Chapter 5). This will in turn indicate optimum bait density and distribution patterns and optimize bait attractiveness by tailoring bait attractants to suit different ant dietary requirements.

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CHAPTER 2

BAIT PREFERENCE BY ANTS (HYMENOPTERA: FORMICIDAE) FORAGING IN SOUTH AFRICAN VINEYARDS

INTRODUCTION

The honeydew producing vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) is a serious pest problem in South African vineyards (Whitehead 1957; Urban & Bradley 1982; Walton 2001; 2003). This phloem feeding insect excretes sugar rich honeydew on which sooty mould grows and which attracts ants. Interactions between honeydew excreting hemipterans and ants have been widely documented in both natural and agroecosystems (Bartlett 1961; Way 1963; Buckley 1987). Current ant control methods include insecticidal sprays and chemical stem banding (Addison 2002), which can affect natural enemy numbers and effectiveness (Kriegler & Whitehead 1962; Myburgh et al. 1973; Urban & Bradley 1982; Flaherty et al. 1992). Furthermore, chemical stem barriers are labour intensive to apply, are not practical for use in vine nurseries and are not effective for controlling arboreal ants like *Crematogaster peringueyi*. Thus an alternative approach to ant control had to be investigated. Slow acting toxic baits can provide good ant control because they allow for toxicant distribution to nestmates via trophallaxis. Bait delivery systems are target specific and therefore minimise disruption of biological control and prevent contamination of the crop with pesticides.

For bait toxicants to be successful in controlling target ant pests, it is imperative that the bait must not be repellent to ant foragers (Stevens et al. 2002) and it must be preferred over competing natural food sources (Nelson & Daane 2007). Furthermore, the bait should have an optimized bait attractant and toxicant that does not deter feeding, mass recruitment and trophallaxis (Goss et al. 1990). Thus determining bait preference is essential for the effectiveness of low toxicity ant baits. Markin (1970) showed that 99% of food entering the Argentine ants' nest was honeydew and nectar making sugar an ideal bait matrix for this ant species. Ant preference for sugar baits containing borates and other toxic compounds have been studied extensively both in agricultural (Klotz et al. 2003; 2004; Rust et al. 2004; Daane et al. 2006) and in urban settings (Klotz et al. 1998; 2002).

This study compared the preference of *Linepithema humile*, *C. peringueyi*, and *Anoplolepis custodiens* to low toxicity ant baits dissolved in 25% sugar solution. The goal of this study was therefore to determine whether or not specific toxic baits were more/less/equally attractive to three ant species than a 25% sugar solution control under field conditions. Results obtained may be used as a guideline for formulating non-repellent low toxicity ant baits for the three ant species.

MATERIALS AND METHODS

Trial sites

To determine ants' preference for toxic baits dissolved in 25% sugar solution, bait preference tests were carried out in three vineyards in the Stellenbosch winelands region. Joostenberg farm (33.80S; 18.81E) was used for *L humile* bait preference trials, Plaisir de Merle farm (33.87S; 18.94E) for *A. custodiens* trials while La Motte farm (33.88S; 19.08E) was used for *C. peringueyi* bait preference trials (Figure 1).

Bait preference tests

Five bait toxicants serially diluted in 25% sugar solution plus a 25% sugar solution control was assessed for their attractiveness to *L. humile*, *C. peringueyi* and *A. custodiens*. These baits included: (1) gourmet ant bait (0.5%); (2) boric acid (0.5%); (3) fipronil (0.0001%); (4) fenoxycarb (0.5%); (5) spinosad (0.01%); and (6) sugar solution (25%) (Table 1). These toxicants were chosen in co-ordination with technical personnel from chemical companies serving on research advisory panels of the wine industry and from available literature. Toxicant concentrations were determined by a comprehensive review of literature. Since vineyards usually contain more than one pest ant species (Addison & Samways 2000), the concentration most often sited was used for all ant species as using different concentrations in commercial baits would be impractical. The toxicant mixtures were added to cotton plugs and were held in place in small petri dishes (70mm diameter by 7mm height). The petri dishes were then randomly assigned to positions in the choice test arena.

Choice test arenas were made of plastic containers (270mm diameter by 65mm height) with 6 (125mm long by 8mm diameter) plastic tubes that extended through six openings at 60° in the inside of the choice test arena (Figure 2). The tubes directed all ants to the centre of the choice test arena before they could forage on a bait of their choice. Ants were free to move in and out of the choice test arenas during the test period.

In the vineyard, active ant nests were chosen and five choice test arenas were placed close to each of the five active ant nests (five replicates). Ants were allowed to forage on the sugar baits for four hours. The same procedure was repeated over three days and over three different seasons: autumn (April 2007), spring (October/November 2007) and summer (January/February 2008) for each of the three ant species. A different section of the vineyard was used during the three different trial days for each of the three ant species.

Data collection and analysis

The trial was arranged in a Completely Randomized Block Design (CRBD) replicated five times, repeated over three days per season for each of the three different ant species. The number of ants feeding at each of the five ant baits plus the 25% sugar solution control was recorded at hourly intervals up to four hours. Since the data were repeated measures (over days) non linear count data, using standard ANOVA in this case was inappropriate because the data would not assume normality. The number of ants feeding at each bait station over the three days was analyzed for each ant species and for every season separately using Generalized Estimating Equations (GEE) (Liang & Zeger 1986) assuming a Poisson distribution with an identity link function in SAS Enterprise 3.0 (2004). This was followed by Tukey-Kramer post hoc tests to separate differences between means.

RESULTS

Argentine ants

Despite placing the toxicants dissolved in 25% sugar solution, some ants were not attracted to the baits and were observed foraging on natural mealybug honeydew that was in close proximity to the bait arenas. During the test period, no acute toxicity was observed for the five different toxicants on the three ant species. Nevertheless, bait treatment preference was highly significant in autumn and spring but not significant in summer (Table 2) (Figure 3). Gourmet ant bait was the most attractive and significantly differed from the rest of the treatments in autumn. Day was significant for *L. humile* bait preference in autumn and summer but was not significant in spring (Table 2) (Figure 4). Bait preference in autumn was highest on day 2 which was not significantly different from day 1 but differed significantly from day 3. In summer, bait preference was highest on day 2 and significantly differed from the rest of the days. Number of *L. humile* at bait stations significantly decreased over time (hours) in autumn but significantly increased over time in spring and summer (Table 2) (Figure 5).

Common pugnacious ants

Some ants were observed foraging on honeydew and prey items regardless of toxic baits being placed within their vicinity. Despite this, bait treatments were highly significant for *A. custodiens* bait preference in autumn, spring and summer (Table 3) (Figure 6). All baits were not repellent (zero ant counts) but gourmet ant bait, fipronil and spinosad were significantly the most preferred baits. There were no significant day differences in bait preference in autumn, spring and summer (Table 3) (Figure 7). Hour was highly significant for *A. custodiens* across all the seasons; autumn spring and summer (Table 3) (Figure 8). The number of ants at bait stations significantly increased with time across all the seasons.

Cocktail ants

These ants were generally found in low numbers in the trial sites and were also observed foraging on natural honeydew in the vines, despite being exposed to the 25% sugar water bait toxicants. Gourmet ant bait, fipronil and spinosad were the most preferred baits across all seasons. Bait treatments were highly significant in autumn, spring and summer (Table 4) (Figure 9). Day was also significant in autumn but not during spring and summer (Table 4) (Figure 10). Bait preference in autumn was highest on day 2 and differed significantly from day 1 and 2. The number of *C. peringueyi* at bait stations increased significantly with time in autumn, spring and summer (Table 4) (Figure 11).

DISCUSSION

One of the most vital features of low toxicity baits is that they should be preferable over a vast array of competing natural food sources like honeydew and nectar, which are highly abundant in agroecosystems. Furthermore, these baits should be non-repellent to foraging ants (Stringer et al. 1964). Despite setting up choice test arenas, some ants were observed foraging on mealybug honeydew on the vines indicating that honeydew possibly is more attractive than the toxicants dissolved in 25% sugar solution. Results of this trial suggested that bait toxicants (1) gourmet ant bait, (2) boric acid, (3) fipronil, (4) fenoxycarb and (5) spinosad when dissolved in 25% sugar solution show potential in future options for ant pest control. However, in spring, boric acid and fenoxycarb were significantly less attractive for *L. humile* than the control. Furthermore, boric acid and spinosad were also significantly less attractive than the control for *C. peringueyi* in spring. These two cases indicate a significant degree of non-preference for the toxicants mentioned and will not be practical for incorporation into ant baits.

Gourmet ant bait was significantly the most preferred toxic bait across all the seasons and ant species possibly because of its bait attractant which mimics honeydew. Previous research has indicated that *L. humile* forages predominantly on sugar (Markin 1970; Baker et al. 1985). Similarly, since *C. peringueyi* and *A. custodiens* are mealybug tending ant species, honeydew forms a significant part of their diet, thus gourmet ant bait would form the best alternative. There was a day difference for *L. humile* bait preference in autumn and summer and for *C. peringueyi* in autumn. The reason for this unexpected trend is unknown. Since weather over the three days was checked and assumed uniformity, the difference might be microclimatic habitat conditions having some impact on ant foraging. A more likely explanation is that since a different area of the vineyard was used for each day, the differences could be attributed to different ant nest densities in the area, which would exert different pressures on the ants for finding food. The number of ants at bait stations over time was also highly significant and number of foragers generally increased with time. This increase might have been attributed to pheromone calling and consequently increase in recruitment. Goss et al. (1990) concluded that selection of the best food source by ants is not because foragers assess the food quality but rather is an indirect consequence of individual foragers laying more pheromones to a better food source. Only the inability of foragers to maintain foraging trails reduces foraging at bait stations. Greenberg & Klotz (2000) also supported this by concluding that addition of synthetic trail pheromones to bait stations increased *L. humile* recruitment and consumption of sucrose water baits.

Results of the present study demonstrated the potential for insecticides dissolved in sugar solution in ant control and conform to the findings by Collins & Callcott (1998); Rust et al. (2000) and Klotz et al. (2000, 2003), who indicated that low amounts of relatively non-toxic insecticides dissolved in sucrose solution are not repellent to foraging ants. Research still needs to be done on use of pheromone incorporated baits and use of more attractive bait/matrix combinations. Unfortunately we were unable to obtain these pheromones from International suppliers for this study due to unavailability of stock. Furthermore, research on timing of bait delivery needs to be done to complement these results, before baits can be used for ant control in vineyards. Nelson & Daane (2007) hypothesized that spring is the optimum time to apply toxic baits mainly because at this time, colonies are busy developing their brood and this gives the ants a chance to transfer toxicants to developing ant larvae and therefore disrupt colony growth. Furthermore, alternative sugars in the form of mealybug honeydew and grape juice that are present during other seasons are not available in spring, leaving ants with no option but to forage on baits. During this trial, there was generally higher bait preference for *A. custodiens* during spring, possibly because of the same reasons put forward by Nelson & Daane (2007). However, bait preference for *L. humile* and *C. peringueyi* peaked in summer, as this is also when colonies are at their peak and would be more difficult to control at this time.

Table 1: Insecticides used in low toxic baits, which were tested in small-scale vineyard experiments against three ant species *Linepithema humile*, *Anoplolepis custodiens* and *Crematogaster peringueyi*.

Trade Names	Active Ingredient (A.I)	Grams pure active ingredient	Manufacturing Company
Gourmet ant bait	boric acid	20g/L	Innovative Pest Control Products, Boca Raton, USA
Borax	boric acid	200g/kg	Pakco Private Limited, Durban, South Africa
Regent	fipronil	200g/L	BASF, Stellenbosch, South Africa
Insegar	fenoxycarb	250g/kg	Syngenta, South Arica
Tracer	spinosad	120g/L	Dow Agrosciences, South Africa

Table 2: Summary of the effects of treatment day and time on bait preference of *Linepithema humile* during three seasons in small field trials

Season	Effect	χ^2	d.f	p
Autumn 2007	Bait treatments	274.94	5	<0.0001
	Time (hours)	404.66	3	<0.0001
	Day	11.77	2	<0.01
Spring 2007	Bait treatments	1059.4	5	<0.0001
	Time (hours)	133.96	3	<0.0001
	Day	0.64	2	0.7277
Summer 2008	Bait treatments	6.50	5	0.2603
	Time (hours)	298.2	3	<0.0001
	Day	30.23	2	<0.0001

Table 3: Summary of the effects of treatment day and time on bait preference of *Anoplolepis custodiens* during three seasons in small field trials

Season	Effect	χ^2	d.f	p
Autumn 2007	Bait treatments	75.49	5	<0.0001
	Time (hours)	751.14	3	<0.0001
	Day	3.67	2	0.1599
Spring 2007	Bait treatments	715.70	5	<0.0001
	Time (hours)	146.35	3	<0.0001
	Day	10.87	2	0.0734
Summer 2008	Bait treatments	141.36	5	<0.0001
	Time (hours)	49.28	3	<0.0001
	Day	8.92	2	<0.1115

Table 4: Summary of the effects of treatment day and time on bait preference of *Crematogaster peringueyi* during three seasons in small field trials

Season	Effect	χ^2	d.f	p
Autumn 2007	Bait treatments	80.95	5	<0.0001
	Time (hours)	35.77	3	<0.0001
	Day	19.04	2	<0.0001
Spring 2007	Bait treatments	137.84	5	<0.0001
	Time (hours)	384.26	3	<0.0001
	Day	12.75	2	0.2382
Summer 2008	Bait treatments	151.33	5	<0.0001
	Time (hours)	539.86	3	<0.0001
	Day	3.11	2	0.2112

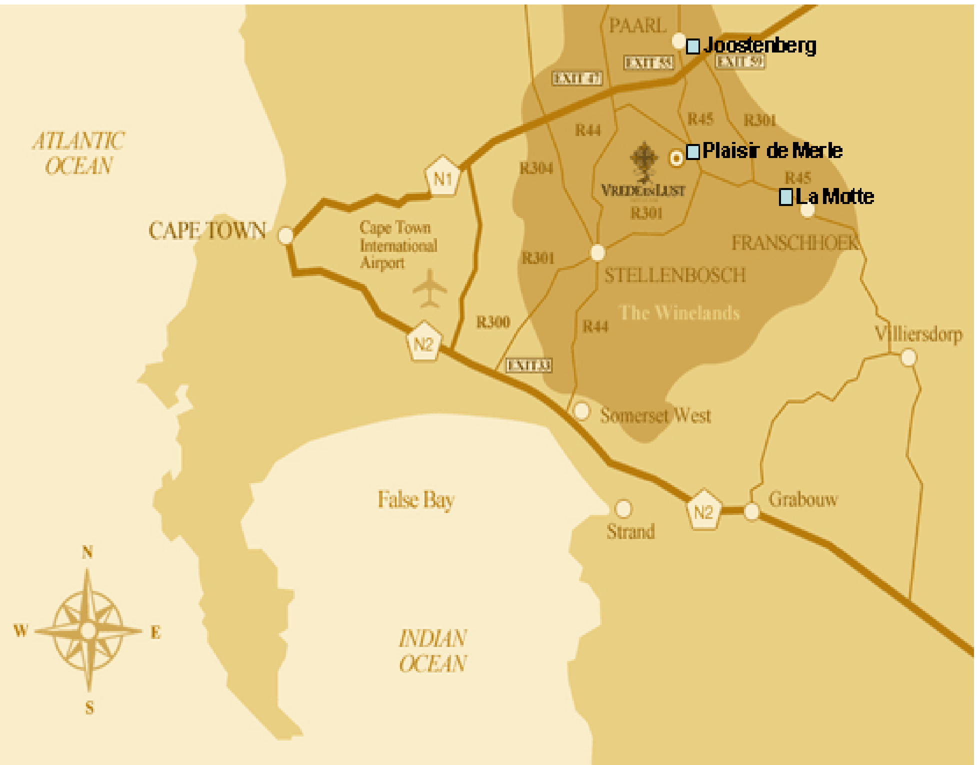

Figure 1: Map showing areas used as study sites for bait acceptance tests. Argentine ant (Joostenberg farm); cocktail ant (La Motte farm) & common pugnacious ant (Plaisir de Merle farm) (Vrede en Lust 2008).

Figure 2: Choice test arena (with lid open) showing common pugnacious ants foraging on the toxic baits during the bait acceptance tests

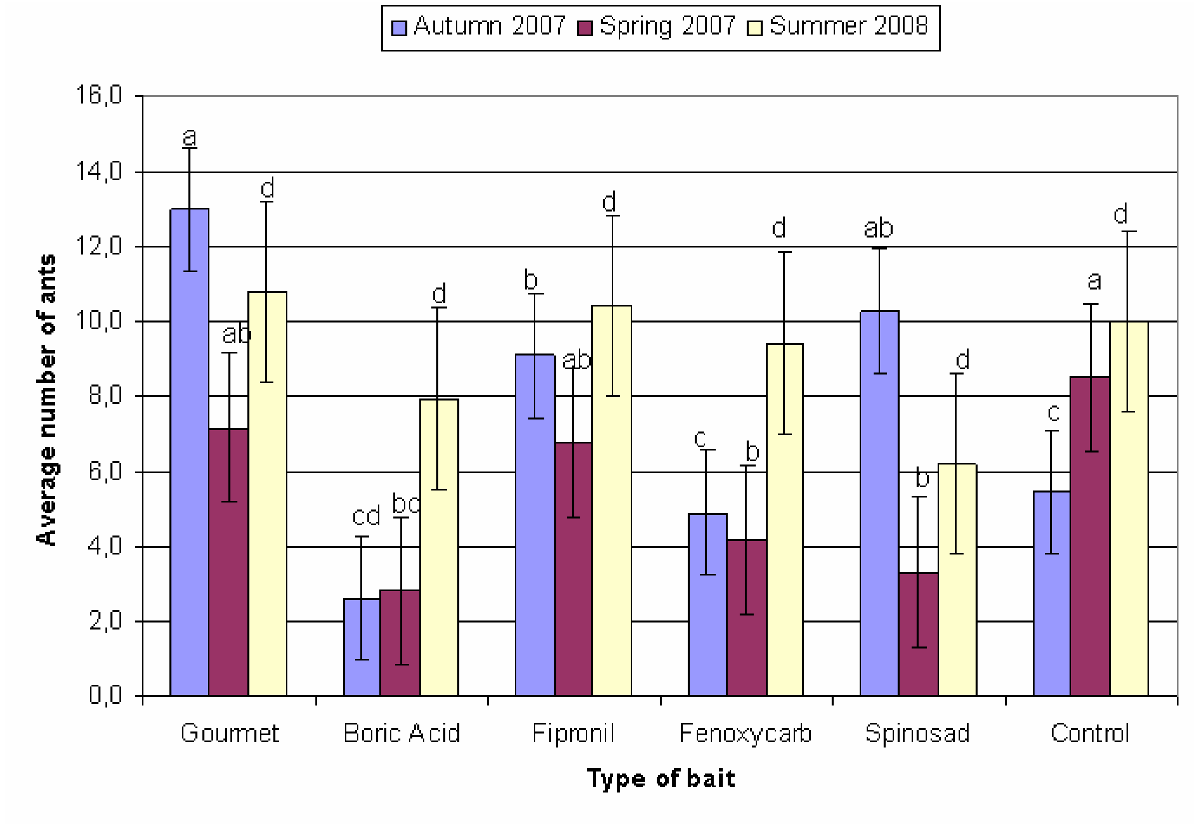

Figure 3: *Linepithema humile* bait preference in autumn, spring and summer. Seasons were analyzed separately and means with the same letter are not significantly different (bars represent ±95% CI).

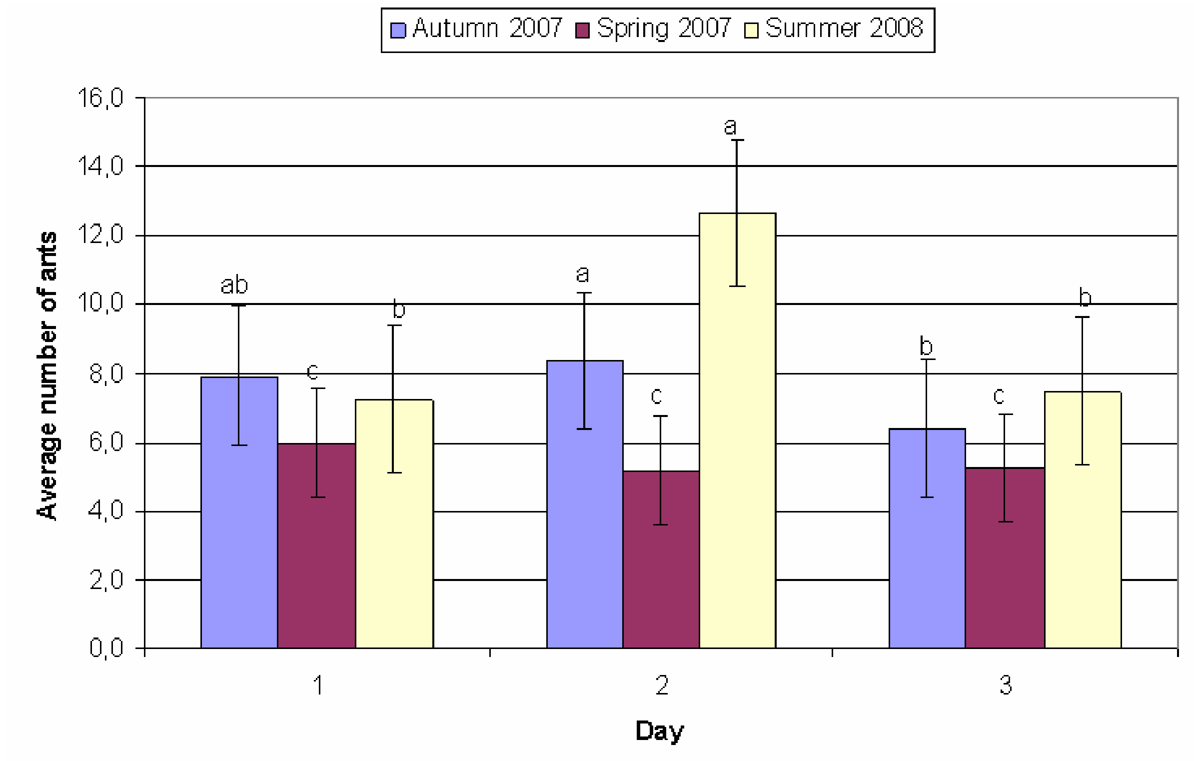

Figure 4: Average number of *Linepithema humile* on bait during three different days in autumn, spring and summer. Seasons were analyzed separately and means with the same letter are not significantly different (bars represent ±95% CI).

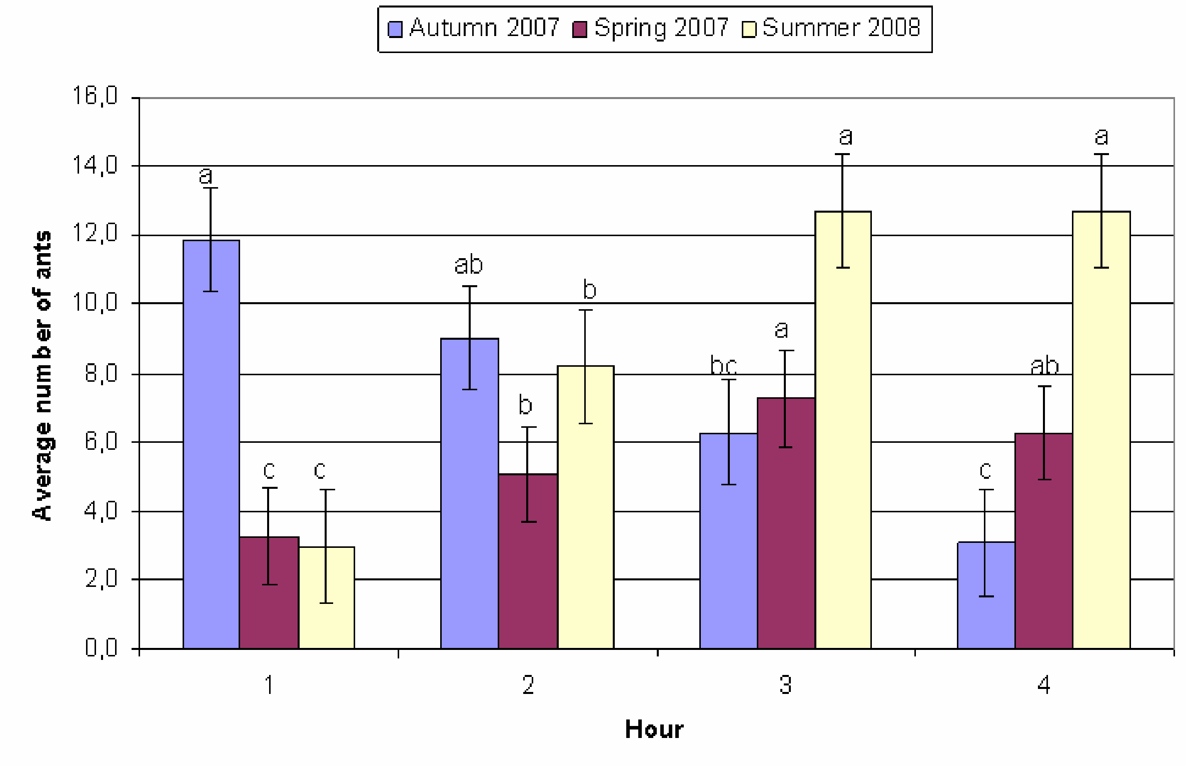

Figure 5: Average number *Linepithema humile* on bait over time in autumn, spring and summer. Seasons were analyzed separately and means with the same letter are not significantly different (bars represent ±95% CI).

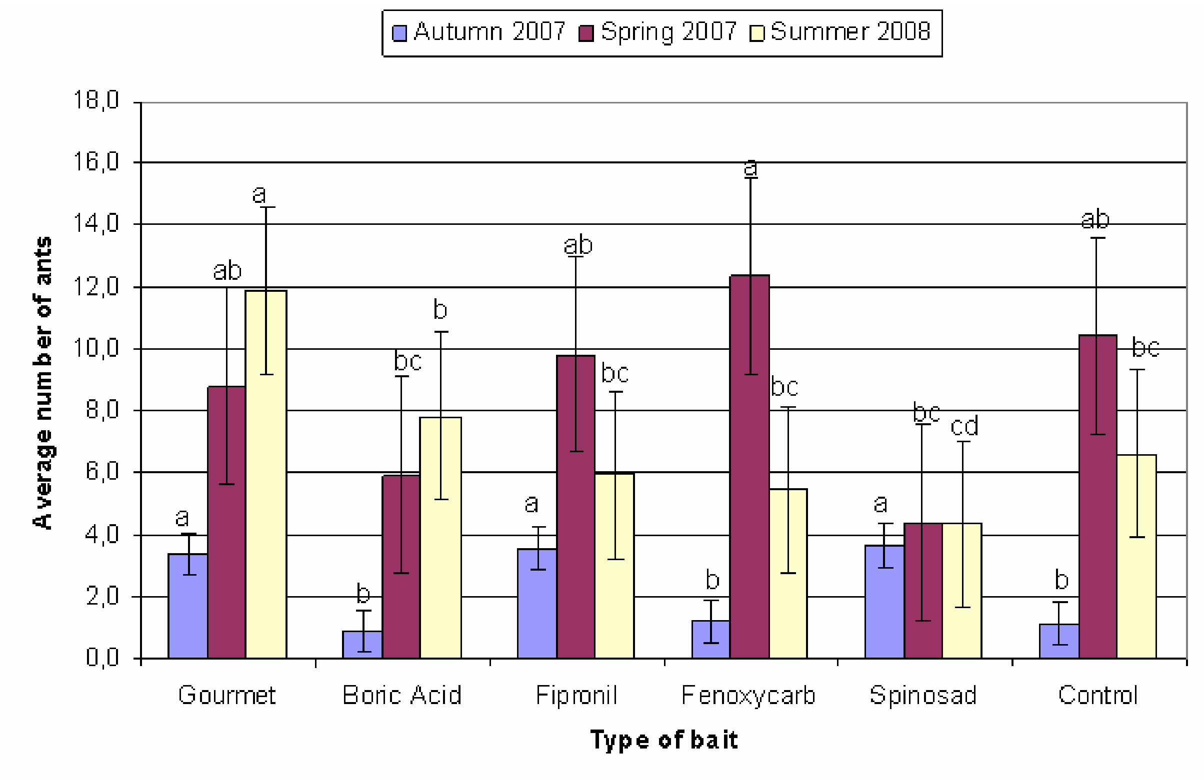

Figure 6: Average number of *Anoplolepis custodiens* on bait in autumn, spring and summer. Seasons were analyzed separately and means with the same letter are not significantly different (bars represent ±95% CI).

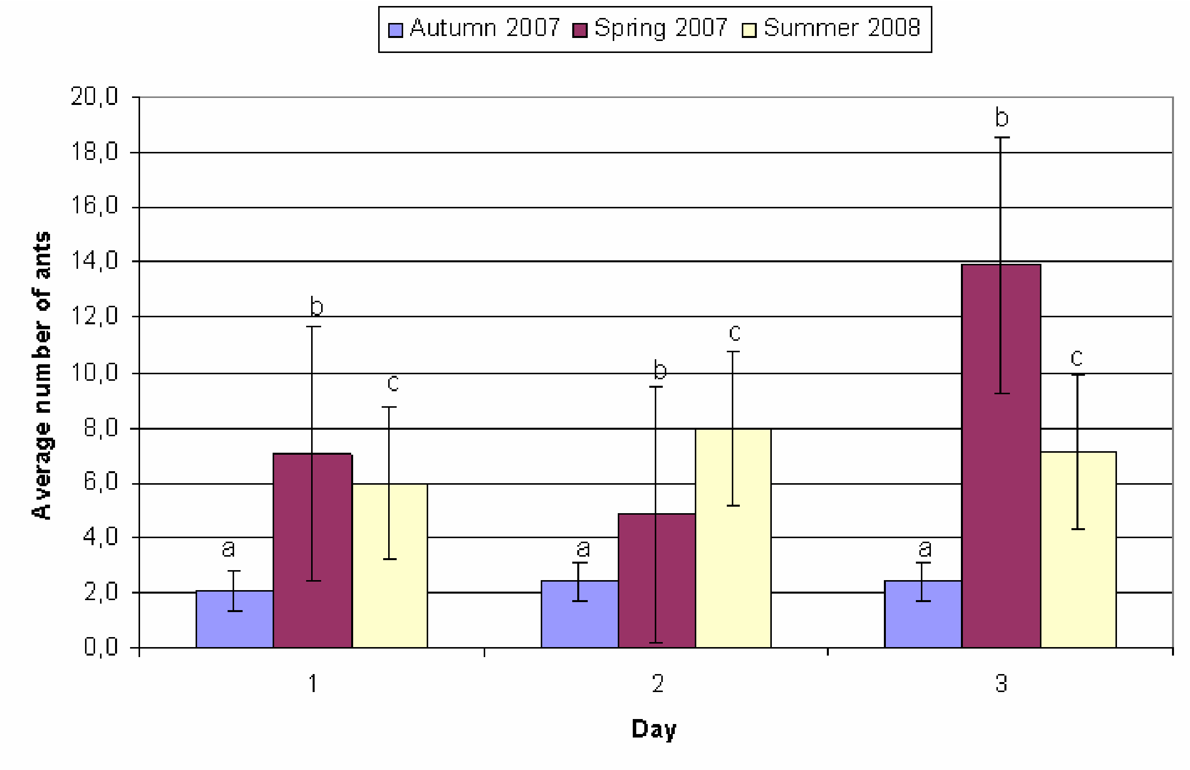

Figure 7: Average number of *Anoplolepis custodiens* on bait during three different days in autumn, spring and summer. Seasons were analyzed separately and means with the same letter are not significantly different (bars represent ±95% CI).

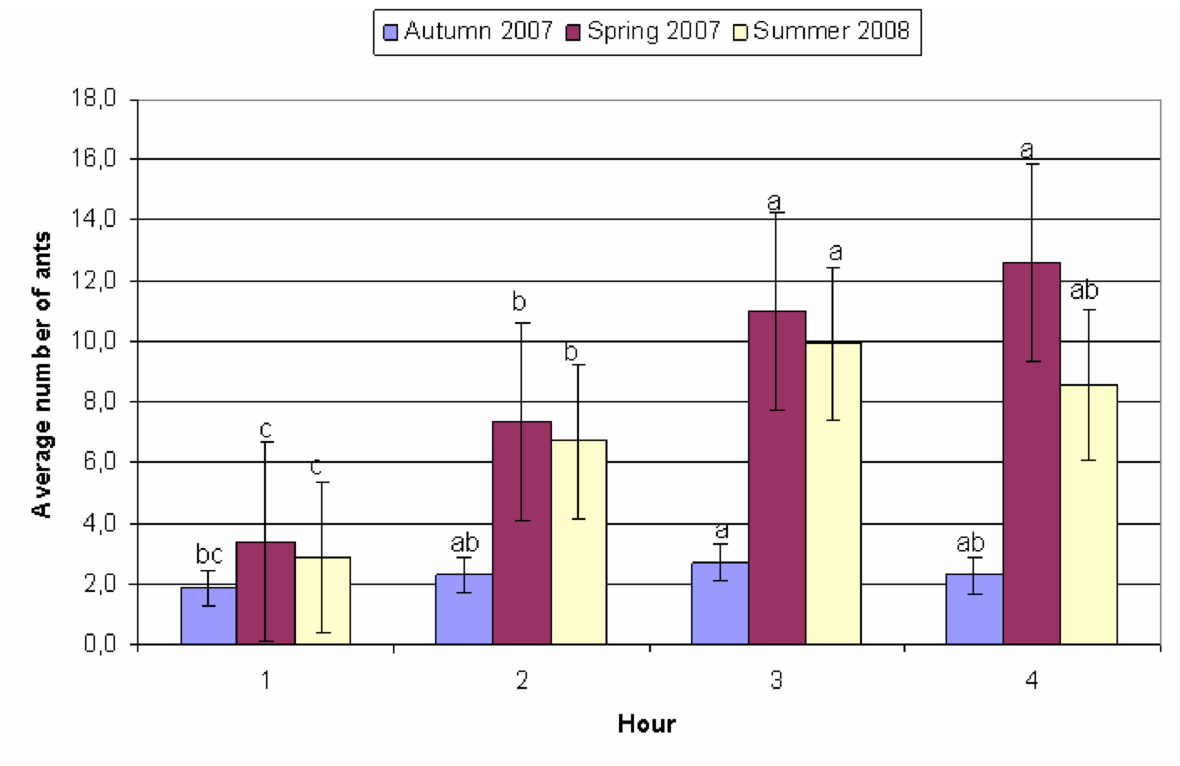

Figure 8: Average number of *Anoplolepis custodiens* on bait over time in autumn, spring and summer. Seasons were analyzed separately and means with the same letter are not significantly different (bars represent ±95% CI).

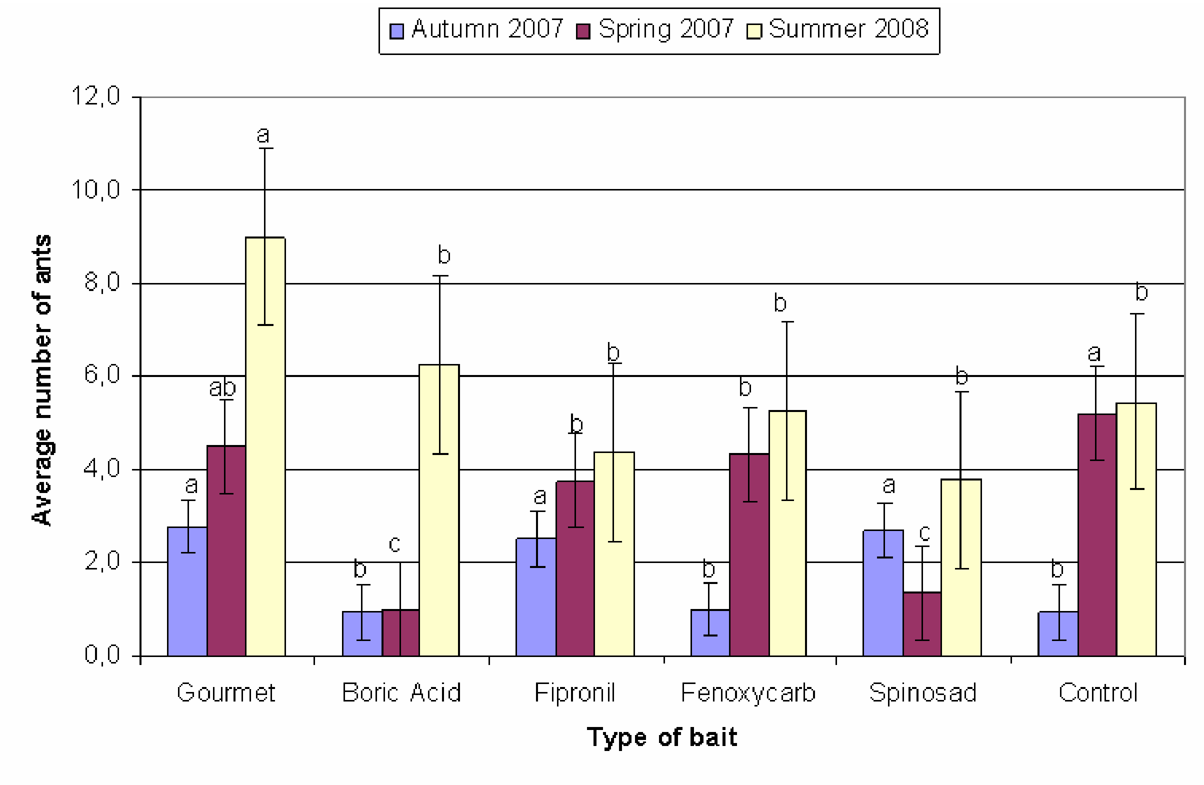

Figure 9: Average number of *Crematogaster peringueyi* on bait in autumn, spring and summer. Seasons were analyzed separately and means with the same letter are not significantly different (bars represent ±95% CI).

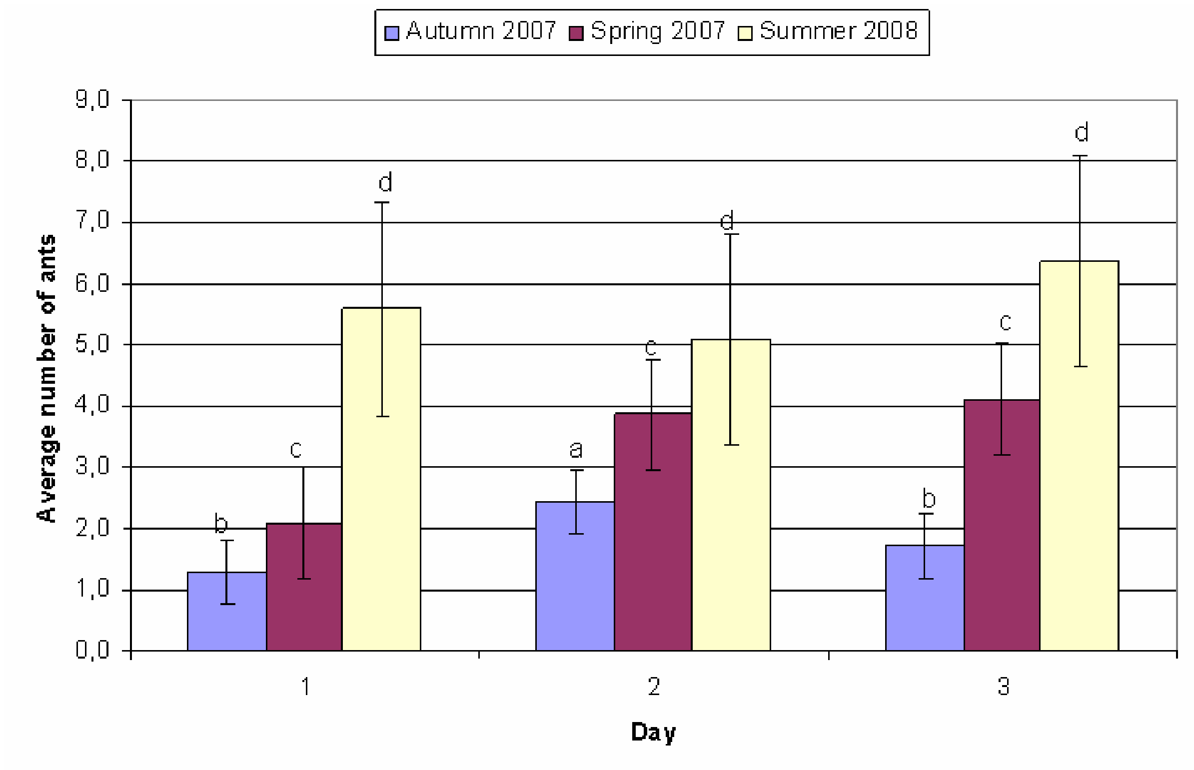

Figure 10: Average number of *Crematogaster peringueyi* on bait during three different days in autumn, spring and summer. Seasons were analyzed separately and means with the same letter are not significantly different (bars represent ±95% CI).

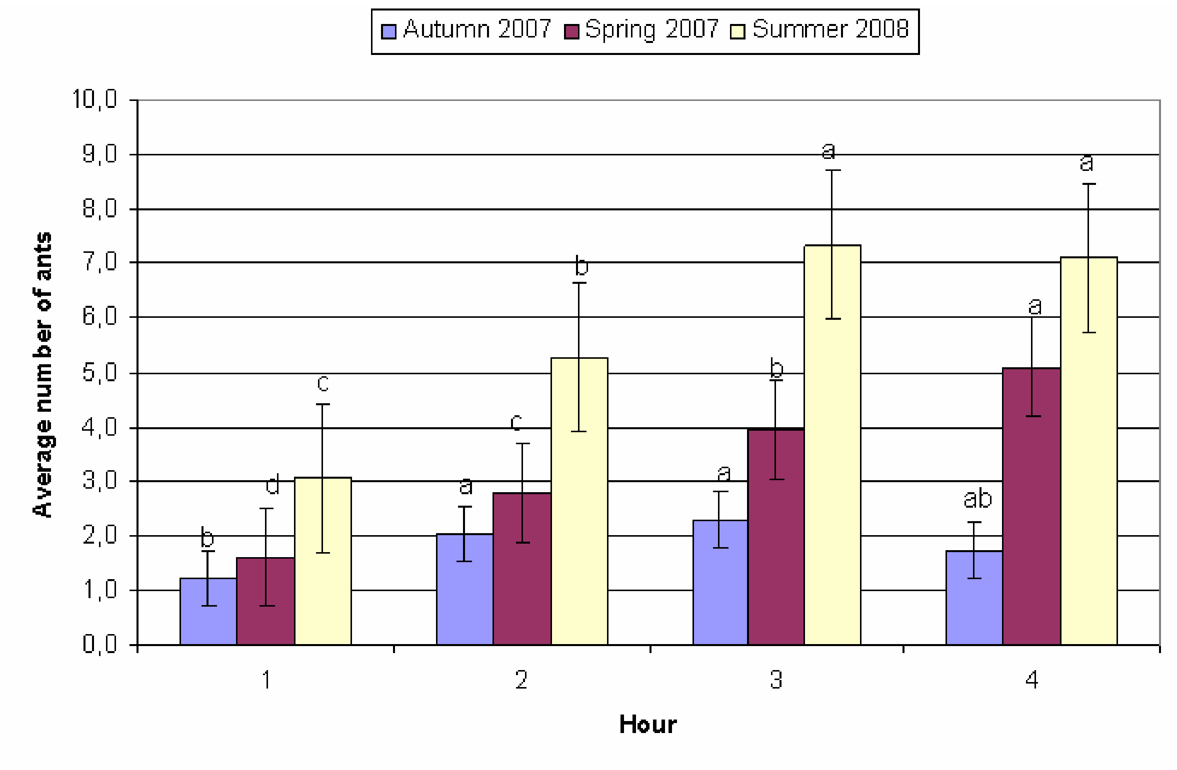

Figure 11: Average number of *Crematogaster peringueyi* on bait over time in autumn, spring and summer. Seasons were analyzed separately and means with the same letter are not significantly different (bars represent ±95% CI).

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CHAPTER 3

BAIT ACCEPTANCE ASSESSMENTS FOR ANTS (HYMENOPTERA: FORMICIDAE) FORAGING IN WESTERN CAPE VINEYARDS

INTRODUCTION

Integrated Pest management (IPM), aimed at suppressing problematic honeydew excreting mealybug pests on grapes must include ant control in order to optimize the efficacy of mealybug natural enemies. If ants are controlled, coccinellid predators and parasitic Hymenoptera are able to keep mealybug levels below Economic Threshold Levels (ETL) (Moreno et al. 1987; Walton & Pringle 2003). Ants, including the Argentine ant *Linepithema humile* (Mayr), common pugnacious ant *Anoplolepis custodiens* (F. Smith) and the cocktail ant *Crematogaster peringueyi* Emery are economic pests in South African vineyards (Whitehead 1957; Urban & Bradley 1982; Addison & Samways 2000). These ants form mutualistic relationships with the vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), a key pest of vines in the Western Cape Province (Whitehead 1957; Annecke & Moran 1982; Walton 2001; 2003; Walton & Pringle 2004). Current strategies for ant control are limited and generally include the application of long term residual insecticides on the main trunk of vines. These are labour intensive to apply and growers have therefore been slow to adopt this method. Growers are interested in alternative pest control methods because the effectiveness of insecticide sprays is limited by protected locations of ants and mealybugs; ant colonies are found underground. Furthermore, insecticidal sprays against ants only affect actively foraging workers but not the vast majority of eggs, larvae and queens that reside in the nest giving the ant populations a chance to recover quickly after insecticidal sprays (Nelson & Daane 2007; Styrsky & Eubanks 2007). Ant bait acceptance assessments were therefore investigated.

Bait acceptance is critical for the success of low toxicity ant baits. Optimized ant baits should be highly palatable to allow ants to sufficiently consume the bait. Low toxicity baits have been used in the control of ants (Blachly & Foster 1996; Rust et al. 2000; Klotz et al. 2004; Greenberg et al. 2006) and different active ingredients have already been tested and accepted by ants in the vineyard agroecosystem (Klotz et al. 2003; Tollerup et al. 2004).

The aim of this study was to assess the palatability of various bait toxicants dissolved in 25% sugar solution to *L. humile*, *C. peringueyi* and *A. custodiens*. The goal was therefore to determine whether or not specific toxic baits were more/less/equally taken by the three ant species than a 25% sugar solution control under field conditions. These results will follow on from bait preference tests conducted in Chapter 2 by assessing whether preferred baits are also those which are most often consumed.

MATERIALS AND METHODS

Study sites

To determine bait acceptance of different toxicants formulated in a 25% sugar solution, bait acceptance tests were carried out in three vineyards in the Cape Winelands region. Bait acceptance tests were carried out at Joostenberg farm (33.80S; 18.81E) for the Argentine ant, Plaisir de Merle farm (33.87S; 18.94E) for the common pugnacious ant and La Motte farm (33.88S; 19.08E) for the cocktail ant (Figure 1, Chapter 2).

Bait acceptance tests

Under field conditions, five toxicants were tested: Gourmet ant bait (0.5%), boric acid (0.5%), fipronil (0.0001%), fenoxycarb (0.5%), spinosad (0.01%) and a 25% sugar solution control (Table 1, Chapter 2). Reasons for choice of toxicants plus toxicant concentrations are laid out in Chapter 2.

Serially diluted bait toxicants were added to cotton plugs in small petri dishes and these dishes were randomly assigned to positions in choice test arenas. Choice test arenas were made of plastic containers (270mm diameter by 65mm height) with six (125mm long by 8mm diameter) plastic tubes that extended through six openings at 60° in the inside of the choice test arena (Figure 2, Chapter 2). These tubes provided ants with access to the centre of the arena before they could forage on the respective toxic baits.

Each of five choice test arenas (five replicates) were placed 10 metres away from each other and each near an active ant nest in an ant infested vineyard. The arenas were monitored for ant foraging for 2 hours and then left in the vineyard for 24 hours. The arenas were covered with a plastic lid and a brick was used to secure the arena on top to avoid disturbance by wind or wild animals. To account for evaporation, a 25% sucrose solution control was placed in a protected location where ants were unable to forage and feed on the bait. The experiment was repeated during three days and over three different seasons; autumn (April 2007), spring

(October/November 2007) & summer (February 2008) for each of the three ant species in their three respective vineyards (Table 1). A different section of the vineyard was used during the three different trial days for each of the three ant species.

Data collection and analysis

The experiment was arranged in a Completely Randomized Block Design (CRBD), replicated five times and repeated over three days for each season and for each ant species. Since the results (amount of bait consumed) were measured under different conditions (days), using standard Analysis of Variance (ANOVA) would be inappropriate because it fails to model the correlation between the repeated measures. Initial bait mass, final bait mass and evaporative loss was measured and actual bait consumption was calculated as follows: Initial bait mass (g) - Final bait mass (g) - Evaporative loss (g). The amount of bait consumed for each serially diluted bait toxicant was analyzed for each ant species and for each season using repeated measures ANOVA in Statistica 7 (2004) and Tukey-Kramer's post hoc tests were used to separate differences between means.

RESULTS

Argentine ant bait acceptance

When the arenas were left in the vineyard and checked after 24 hours, no other Arthropod species were found foraging on the bait toxicants, indicating that only ant foraging accounted for any decrease in bait mass. Average daily temperatures and rainfall were checked and these weather parameters (not shown here due to large file size) showed no apparent differences in pattern during the trial days for all the respective ant trial sites.

Toxic bait treatment acceptance was highly significant for *L. humile* in autumn ($F_{(5, 20)}$= 7.3362, p<0.0001). Gourmet ant bait and spinosad were significantly more accepted by *L. humile* than the other treatments while boric acid, fipronil and fenoxycarb were not significantly different from the 25% sugar solution control in autumn (Figure 1). Furthermore, amount of toxic bait taken by *L. humile* on the three different days was also significant in autumn ($F_{(2,8)}$= 52.623, p<0.0001). *Linepithema humile* consumed the most amount of bait on day 1 and the least on day 2 (Figure 2).

In spring, there was no significant difference between the means of all the bait treatments and that of the 25% sugar solution control (F($_{(5,\ 20)}$= 1.6666, p=0.18868) (Figure 1). However, bait acceptance on the three different days was significant ($F_{(2,8)}$= 9.6571, p<0.01) (Figure 2).

In summer, bait treatment effects were not significant ($F_{(5,\ 20)}$= 1.9951, p=0.12328) but day was significant ($F_{(2,8)}$= 9.0108, p<0.01). Ant bait intake on day 1 was significantly different from days 2 and 3 which were not significantly different from each other (Figure 2).

Cocktail ant bait acceptance

There was no significant day effect on *C. peringueyi* bait acceptance in autumn ($F_{(2,8)}$= 0.06954, p=0.93338). Treatment (baits) were significant ($F_{(5,20)}$= 5.3928, p<0.01) for this ant species in autumn. Means for boric acid, fenoxycarb and spinosad did not differ significantly from that of the 25% sugar solution control while gourmet ant bait and fipronil were significantly more accepted than the rest of the treatments in autumn (Figure 3).

Treatment (bait) effects were not significant for *C. peringueyi* bait acceptance in spring ($F_{(5,20)}$= 0.46258, p=0.79936). Day differences in *C. peringueyi* bait acceptance were highly significant in spring ($F_{(2,8)}$= 98.436, p<0.0001). Bait acceptance on all three days were significantly different from each other in spring (Figure 4).

There was neither a treatment (bait) effect ($F_{(5,20)}$= 3.4482, p=0.2078) nor a day effect ($F_{(2,8)}$= 0.50417, p=0.62199) on *C. peringueyi* bait acceptance in summer.

Common pugnacious ant bait acceptance

There was a significant difference between treatments in ant bait acceptance in autumn ($F_{(5,20)}$= 7.2274, p<0.001). Gourmet ant bait, fipronil and spinosad were significantly different from boric acid, fenoxycarb and the 25% sugar solution control (Figure 5). Day effect on bait acceptance was not significant in autumn ($F_{(2,8)}$= 1.9027, p=0.83037).

In spring, there was no treatment (bait) effect on *A. custodiens* bait acceptance ($F_{(5,\ 20)}$= 0.4964, p=0.77519) (Figure 5). Rather, there was a day difference in bait acceptance ($F_{(2,8)}$= 21.484, p<0.001). *Anoplolepis custodiens* bait acceptance was the least on day 1 and was almost constant for day 2 and 3 (Figure 6).

Neither treatment (baits) ($F_{(5, 20)}$= 2.0239, p=0.1188) nor day effects ($F_{(2,8)}$= 0.18038, p=0.83826) were significant for *A. custodiens* bait acceptance in summer.

DISCUSSION

One of the most important attributes of low toxicity baits for controlling ants is that toxicants must be highly palatable, should not hinder feeding and be non-repellent (Stringer et al. 1964). Field trials with low toxicity ant baits (insecticide plus 25% sugar solution) indicated potential for certain insecticides in vineyard ant pest control. Three insecticides, gourmet ant bait, fipronil and spinosad showed great potential for ant control in autumn. Gourmet ant bait was the most palatable ant bait across all the ant species in autumn for the reasons already discussed in chapter 2. Research has shown that boric acid, fenoxycarb and fipronil, when dissolved in sugar solution are palatable to foraging ants (Klotz et al. 1997; Collins & Callcott 1998; Klotz et al. 1998; Stevens et al. 2002; Greenberg & Klotz 2000). In the same manner, these results have confirmed previous research, and these low toxicity baits have shown high palatability to the ants. In autumn, spring and summer there was a significant difference in the amount of bait consumed by *L. humile* on three different days. This was also true for *A. custodiens* and *C. peringueyi* in spring. These day differences were also found in the bait preference trials and are discussed in chapter 2. Ant bait consumption was generally higher in spring than it was in autumn or summer. This might have been caused by plenty of alternative food sources in the form of honeydew for ants in the vineyard in summer and autumn making baits less attractive to ants during these months. I hypothesize that honeydew produced by *P. ficus*, some grape juice and nectar that are highly abundant in summer were far more attractive to ants than the sucrose bait solutions, and hence the lower bait consumption in summer.

Neither the bait treatments nor the days were significant for *A. custodiens* and *C. peringueyi* in summer. In addition to vineyard food abundance, this might also have been attributed to colonies' seasonal changes in dietary requirements. Ant colony food requirements are directly linked to the level of brood production. Stein et al. (1990) revealed that *S. invicta* prefers carbohydrates during cooler seasons to provide energy for workers and shifts to protein based foods in warmer seasons when colony growth is at its peak. Rust et al. (2000) also noted that foraging *L. humile* preferred protein over sugars in summer and that protein preference dropped during cooler seasons. I hypothesize that *C. peringueyi* and *A. custodiens* follow the same trend.

Table 1: Sampling dates on which bait acceptance trials were conducted in three vineyards in the Stellenbosch/Simondium area during 2007/08 for three ant species.

Ant species	Autumn	Spring	Summer
Argentine ant	07/04/07	26/09/07	04/02/08
	08/04/07	27/09/07	14/02/08
	10/04/07	01/02/07	18/02/08
Common pugnacious ant	15/04/07	03/11/07	21/02/08
	16/04/07	04/11/07	28/02/08
	17/04/07	06/11/07	04/03/08
Cocktail ant	15/04/07	03/11/07	21/02/08
	16/04/07	04/11/07	28/02/08
	17/04/07	06/11/07	04/03/08

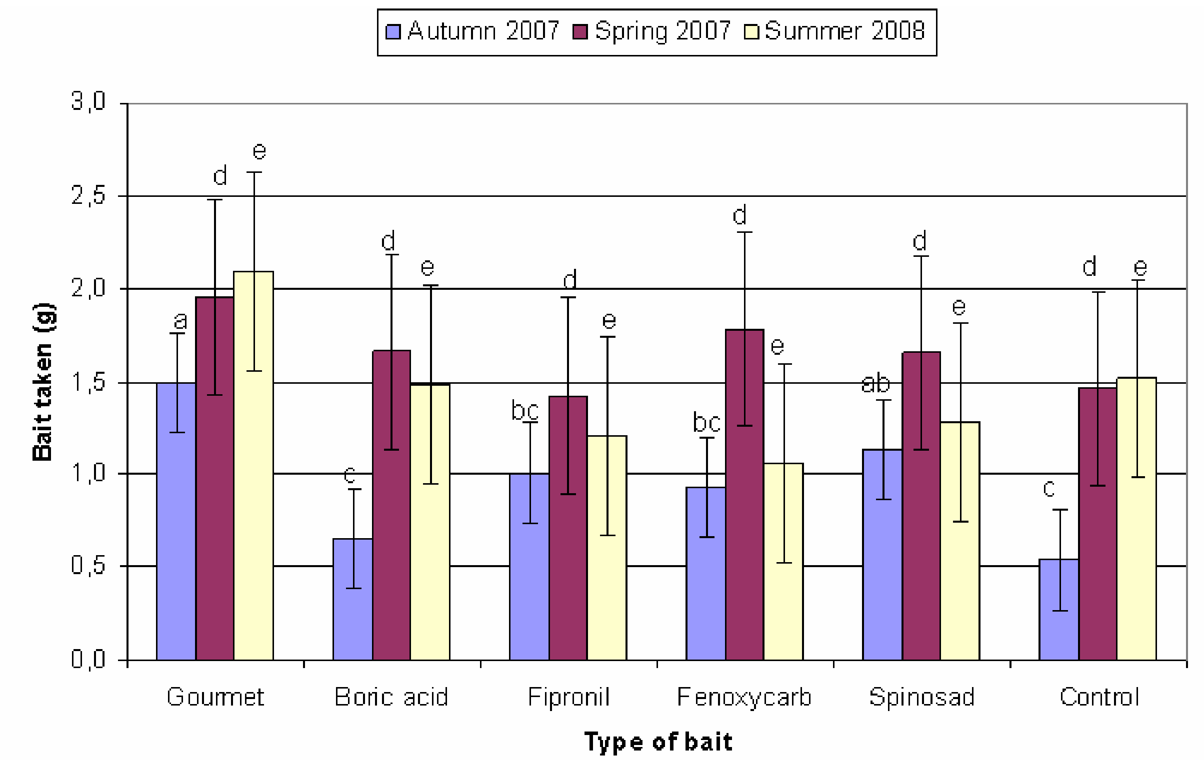

Figure 1: Average bait consumption by *Linepithema humile*, in autumn, spring and summer. Seasons were analyzed separately and means with the same letter are not significantly different (bars represent ±95% CI).

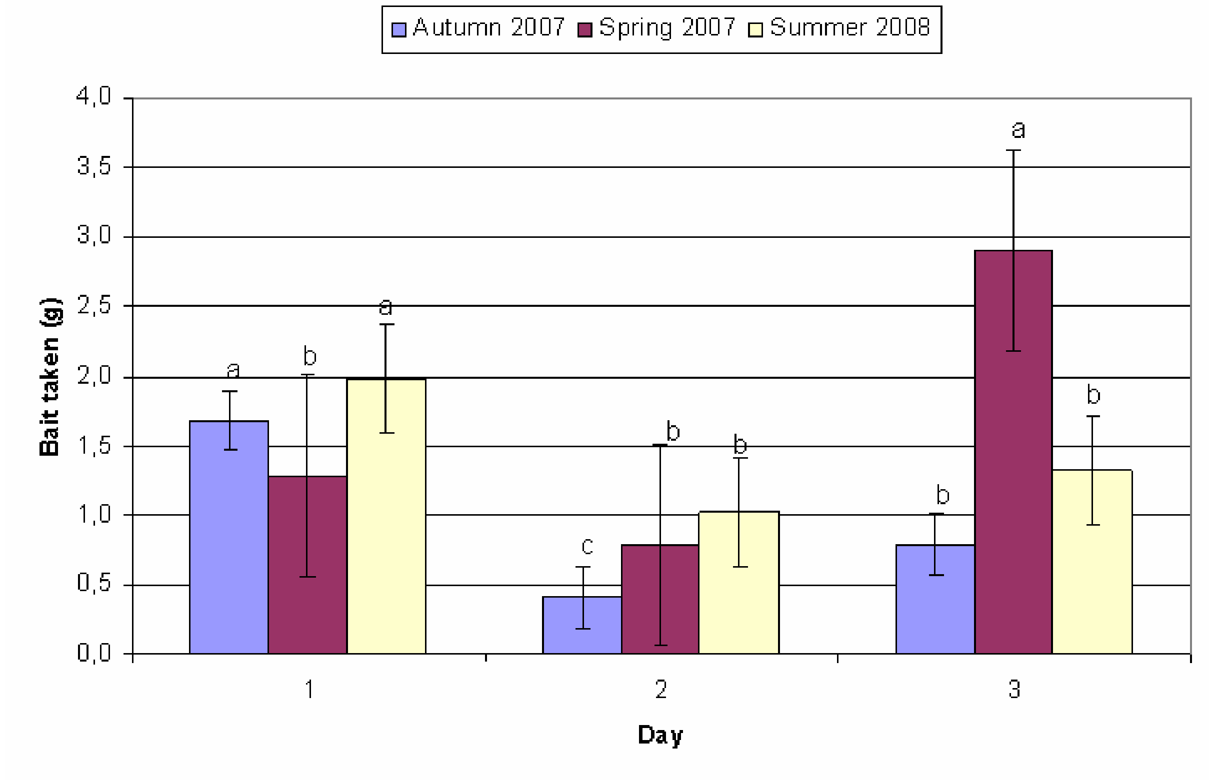

Figure 2: Average bait consumption by *Linepithema humile* over 3 different days in autumn, spring and summer. Seasons were analyzed separately and means with the same letter are not significantly different (bars represent ±95% CI).

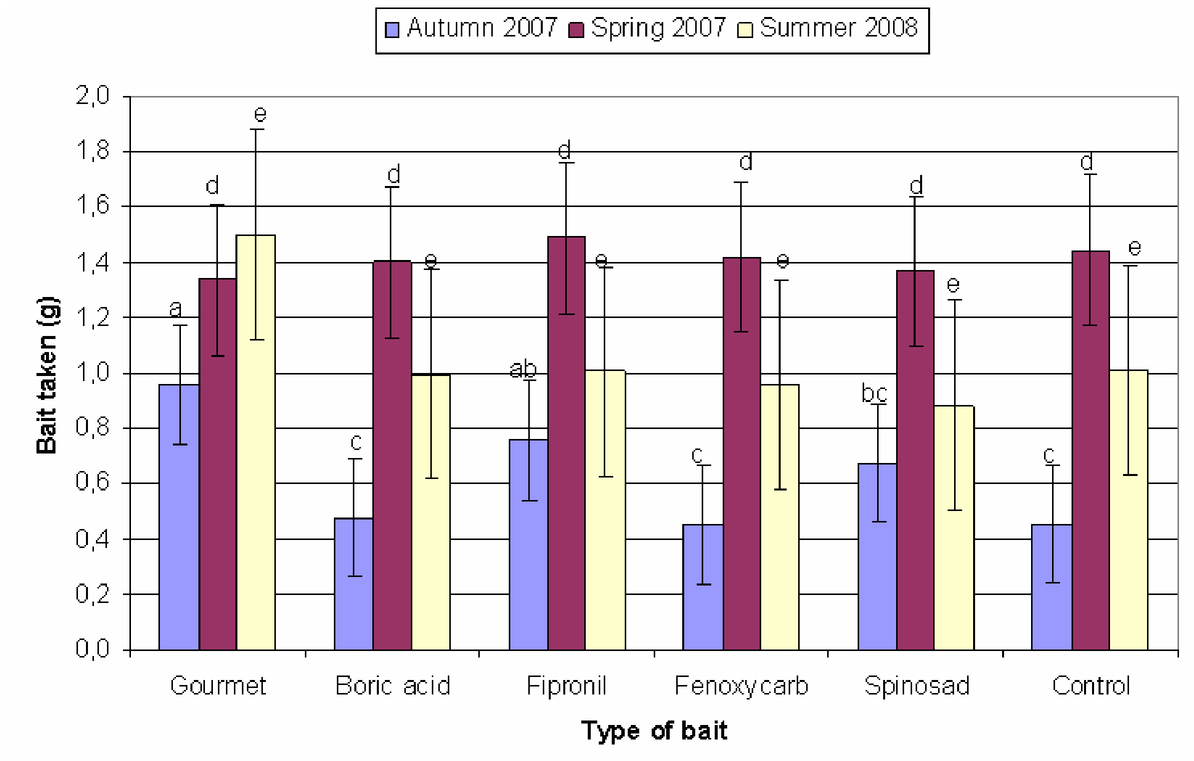

Figure 3: Average bait consumption by *Crematogaster peringueyi*, in autumn, spring and summer. Seasons were analyzed separately and means with the same letter are not significantly different (bars represent ±95% CI).

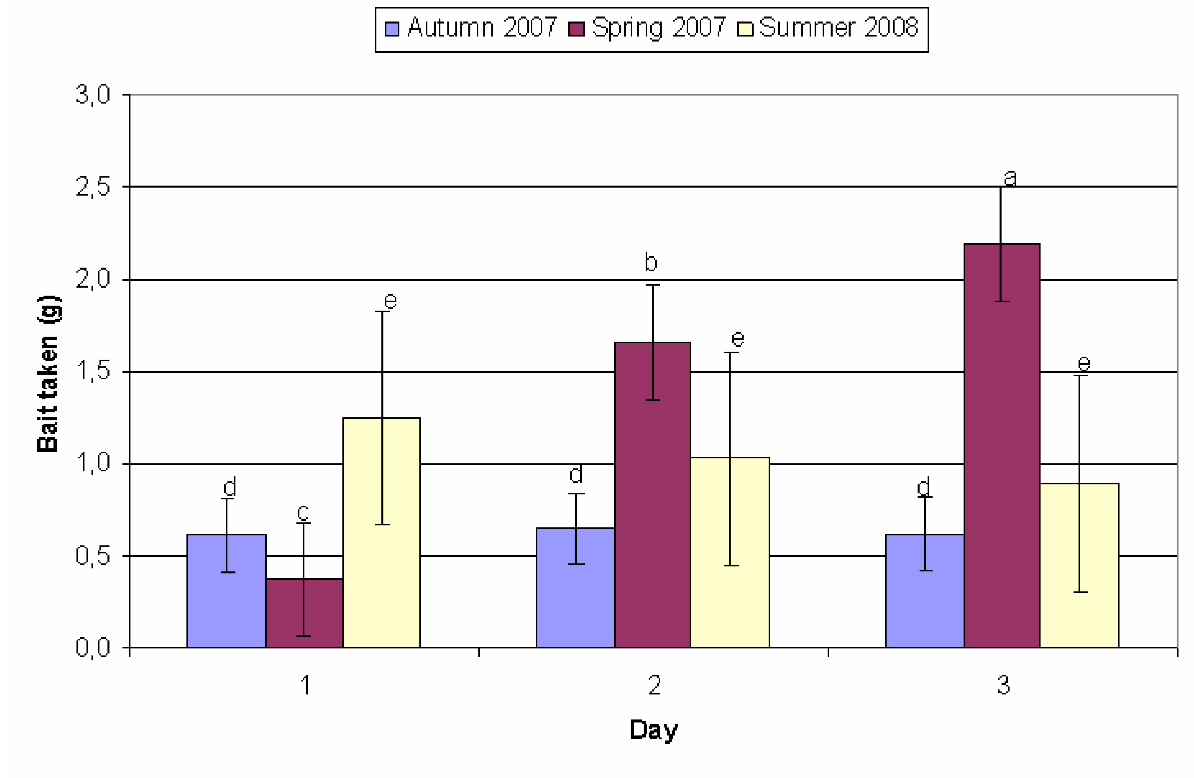

Figure 4: Average bait consumption by *Crematogaster peringueyi* over 3 different days in autumn, spring and summer. Seasons were analyzed separately and means with the same letter are not significantly different (bars represent ±95% CI).

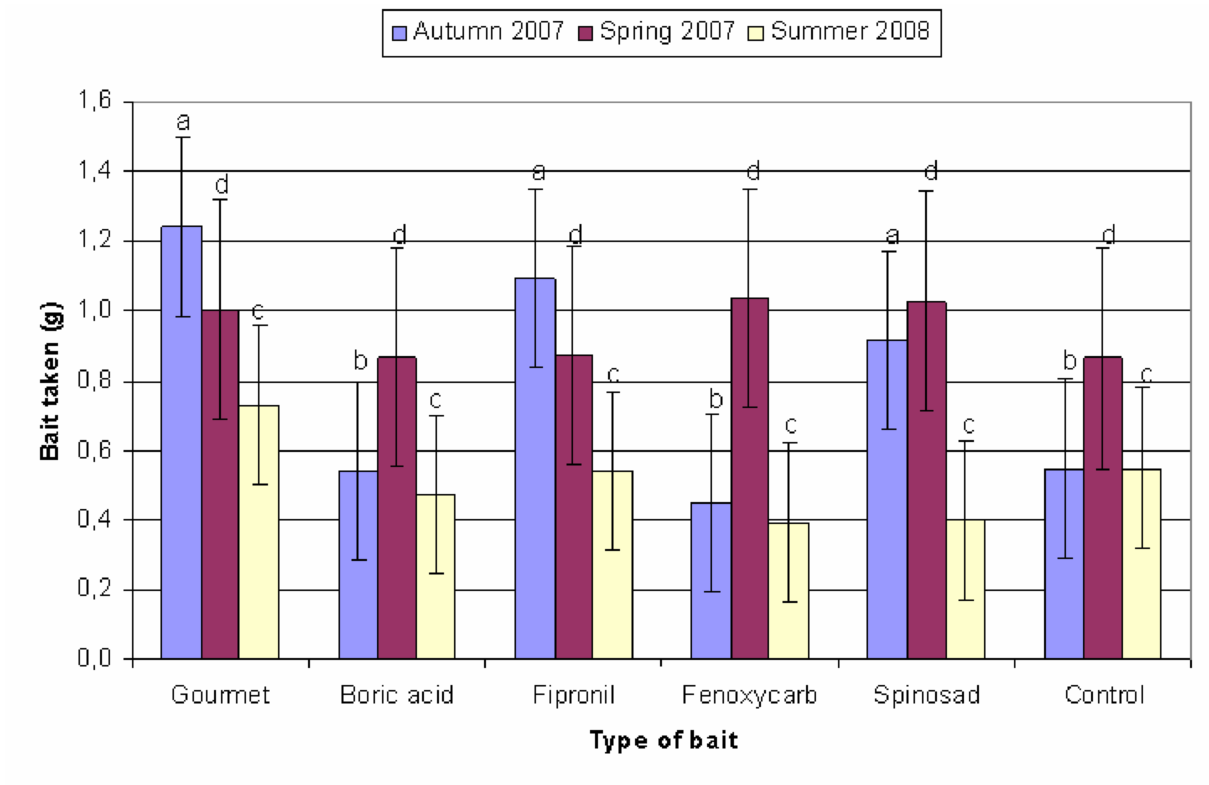

Figure 5: Average bait consumption by *Anoplolepis custodiens*, in autumn, spring and summer. Seasons were analyzed separately and means with the same letter are not significantly different (bars represent ±95% CI).

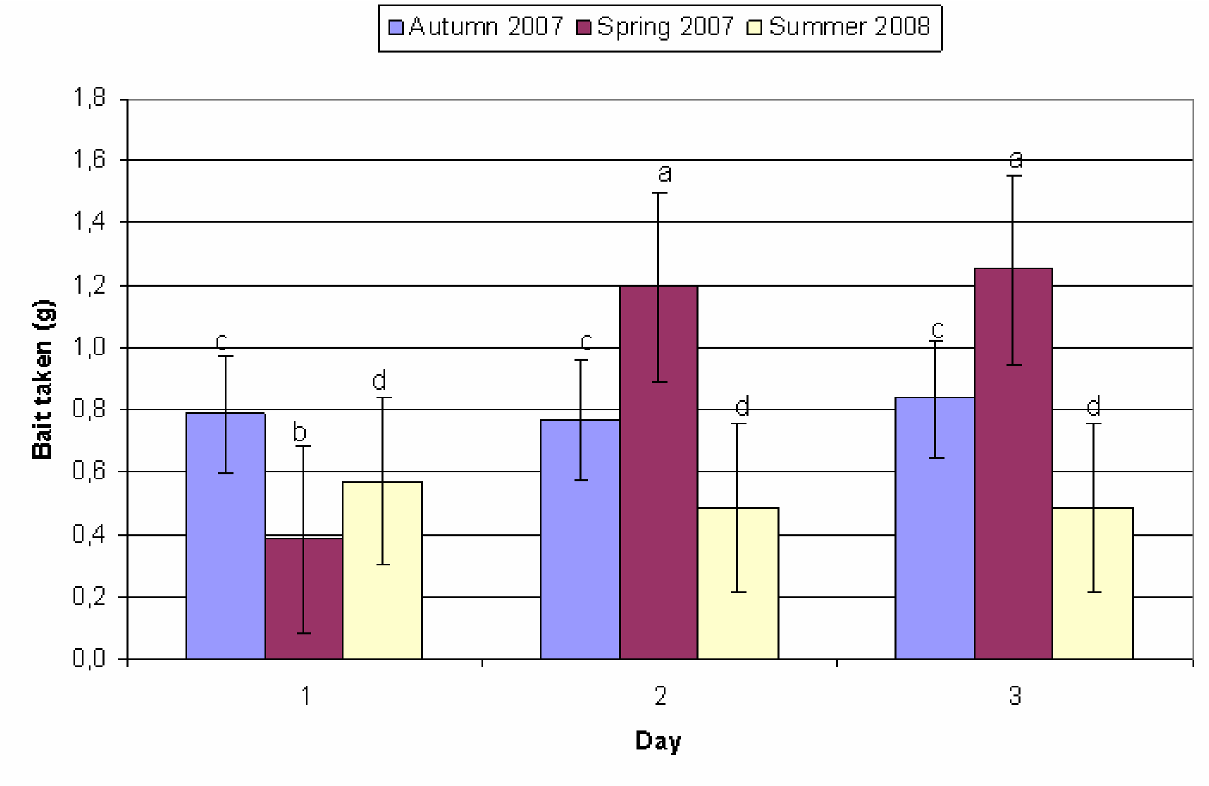

Figure 6: Average bait consumption by *Anoplolepis custodiens* over 3 different days in autumn, spring and summer. Seasons were analyzed separately and means with the same letter are not significantly different (bars represent ±95% CI).

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CHAPTER 4

EFFICACY OF LOW TOXIC INSECTICIDE BAITS ON VINEYARD FORAGING ANTS (HYMENOPTERA: FORMICIDAE)

INTRODUCTION

Argentine ants *Linepithema humile* (Mayr), common pugnacious ants *Anoplolepis custodiens* (F. Smith) and cocktail ants *Crematogaster peringueyi* Emery are agricultural pests of economic importance in Western Cape vineyards (Addison & Samways 2000). By feeding on hemipteran honeydew, these ants reduce the efficacy of predators and parasitic Hymenoptera in controlling the vine mealybug *Plannococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), a devastating pest in Western Cape vineyards (Kriegler & Whitehead 1962; Myburgh et al. 1973; Urban & Mynhardt 1983; Walton 2001; 2003). Current strategies to control pest ants are limited and include the application of residual insecticides such as chlorpyrifos and alpha-cypermethrin (Addison 2002) which are detrimental to the environment and upset natural enemy numbers if applied incorrectly. Furthermore, these chemicals only target foraging workers and consequently the entire colony is not controlled (Baker et al. 1985; Davis & Schagen 1993). Baits containing insecticides of relatively low toxicity may offer a more practical method of ant control which is compatible with vineyard Integrated Pest Management (IPM) practices. Ants redistribute food via trophallaxis which results in baits of low toxicity affecting a much larger proportion of the ant colony (Baker et al. 1985; Klotz et al. 2002; Daane et al. 2006) and consequently better pest control. However, one of the greatest challenges is formulating toxicants into sufficiently low toxicity baits (Baker et al. 1985; Silverman & Roulston 2001). The majority of known insecticides cannot be used as bait toxicants for control of ants because of their high toxicity and rigid efficacy requirements (Stringer et al. 1964). According to Stringer et al. (1964), baits of low toxicity should: (1) exhibit delayed toxicity and be effective over a ten-fold, preferably hundred-fold dosage range, (2) not be repellent to foraging ants, (3) be rapidly shared between ants through trophallaxis before killing the recipient. A limited number of insecticides display all three critical prerequisite characteristics, therefore very few candidate compounds are available for use as toxic ant baits. Of a total of 7100 compounds tested as fire ant (*Solenopsis invicta*) bait toxicants, only six became commercialized (Banks et al. 1992) and two of these have since been deregistered. Another desirable feature of prospective baits is their ability to be formulated in water when baiting ants that predominantly forage on honeydew and nectar. *Linepithema humile* prefers liquid sucrose baits (Baker et al. 1985; Rust et al. 2000) making sugar solution an ideal matrix for toxic baits.

This study was aimed at evaluating the efficacy of five different insecticide baits on two vineyard dwelling ants, *L. humile* and *C. peringueyi*. The main objective was to determine whether or not specific toxic baits were sufficiently toxic to the two ant species under laboratory conditions. Emphasis was placed on speed of toxic action in order to determine the best compounds that meet the low toxic bait criterion by Stringer et al. (1964), who defined delayed toxicity for fire ants (*Solenopsis invicta*) as ≤15% kill at 24 hours and ≥89% kill at 20 days (Stringer et al. 1964). However, in recent studies, this definition has been used with no modifications but with varying experimental duration from anything between 10 – 18 days (Williams et al. 1980; Williams & Lofgren 1981; Williams 1983; Vander Meer et al. 1985; Tamashiro & Haverty 1987; Knight & Rust 1991; Klotz et al. 2004). Consequently, I used 16 days. *Anoplolepis custodiens* was omitted during these bioassays also due to time constraints. The Insect Growth Regulator (IGR) fenoxycarb was also tested against *L. humile* and *C. peringueyi* but due to its mode of action, the results for this chemical are not reported in this chapter.

MATERIALS AND METHODS

Insect colonies

Linepithema humile workers, queens and brood were collected by scooping sections of an ants' nest and the surrounding soil while *C. peringueyi* were collected by cutting sections of branches infested with ants from vines. *Linepithema humile* were collected from Joostenberg farm (33.805S, 18.81E) and *C. peringueyi* were collected from La Motte farm (33.885S, 19.08E) (Figure 1, Chapter 2). These ants were then transferred to artificially constructed ant cages with a wooden base and sides, 500mm length, 300mm width and 25mm height with the top covered with glass. The cages were connected to a separate cylindrical container (120mm height and 70mm diameter) using a 10mm diameter plastic tube, which served as the foraging arena for ants. The ants would move freely from their nests via the plastic tube to and from the foraging arena. Ants were supplied with 25% sucrose solution on cotton wool every 48 hours and with noctuid moth larva or tuna once a week for protein. For the *L. humile* colony, the nest was sprinkled with water every 48 hours to maintain high humidity which is required for the species to prosper. Colonies were maintained for at least one month before the bioassays were carried out.

Insecticides

Four insecticides comprising gourmet ant bait, boric acid, fipronil (Regent) and spinosad (Tracer) (Table 1, Chapter 2) were tested for efficacy using a 25% sucrose solution as a control. The five insecticides were chosen in coordination with technical personnel from chemical companies serving on research advisory panels of the wine industry and from available literature. Table 1 lists the physical and chemical properties of the insecticides used in the experiment.

Toxicity tests

To determine the toxicity of each insecticide, worker *L. humile* and *C. peringueyi* were confined and fed on serially diluted toxicants (Table 1, Chapter 2) mixed with 25% sugar solution in small plastic containers (110mm X 110mm X 40mm). Three insecticides (gourmet, boric acid & spinosad) were used at concentrations 0.25; 0.5; 1; 2 and 4 times the field dose while fipronil was used at 0.015625; 0.03125; 0.0625; 0.125; 0.25 times the field dose. Field dose here refers to commonly used doses from literature, as these chemicals are not yet registered in South Africa. Test ants were starved for 24 hours prior to being subjected to toxic baits. Ants were subjected to the different toxic baits on cotton wool for 24 hours, after which the bait was removed. The ants were then fed on 25% sugar solution for the remainder of the test period. This was done in order to ensure that any ant mortality recorded was due to delayed toxicity and not because of toxin accumulation. Temperature was maintained at ±25°C and humidity at ±70%. Each treatment plus control was replicated 4 times for each ant species with 20 experimental units (ants) per replication.

Data collection and analysis

Worker ant mortality was recorded at 24, 48, 96, 192 and 384 hours after toxin exposure and the experiment was terminated after 16 days. To account for control mortality, data were corrected using Abbott's formula (Abbott 1925). Mortality for each ant species (*L. humile* and *C. peringueyi*) and for each treatment was pooled separately and dose-mortality regressions, median lethal doses (LD_{50}s) and their fiducial limits (FL) (Finney 1971) were computed using POLO Probit analysis (Raymond 1985).

RESULTS

Argentine ants

For each of the treatments and doses tested, ant mortality generally increased with time (Figure 1). Mortality with boric acid was low for the three lowest doses tested and did not exceed 15% after 24 hour exposure to this toxicant (Figure 1A). However, only the field dose and those above it resulted in ≥89% mortality after 384 hours. *Linepithema humile* mortality stabilized from 48 hours onwards with the highest mortality recorded after 364 hours.

For gourmet ant bait, at 24 hours, only the lowest dose used resulted in <15% mortality after 24 hours but mortality at this dose did not satisfy the condition of ≥89% mortality after the 384 hour test period (Figure 1B). The highest dose of gourmet ant bait (2%) resulted in ≥89% mortality after the 384 hour test period. Mortality was low initially but generally stabilized after 96 hours (Table 2).

Compared to the other bait toxicants, *L. humile* was relatively more susceptible to fipronil. At 24 hours, only the lowest fipronil concentration used recorded a mortality of <15% and a mortality of ≥89% after 384 hours (Figure 1C). All the other doses did not meet the conditions for low toxic baits as set by Stringer et al. (1964).

Spinosad exhibited some positive results as a toxicant against *L. humile* (Table 2). The lowest dose used in the bioassays gave a mortality of ≤15% at 24 hours but mortality did not exceed 89% after 384 hours. Only the field dose and those concentrations above this value gave a mortality that came close to ≥89% after 384 hours (Figure 1D) (refer to table 2 for summary of results).

Cocktail ants

Mortality of *C. peringueyi* after 24 hours exposure to boric acid did not exceed 15% for the two lowest doses used (Figure 2A). However, mortality at these doses did not reach ≥89% after 384 hours. Only 1% and 2% boric acid resulted in ≥89% kill after 384 hours. Mortality was generally low across all the doses after 24 hours but significantly increased and stabilized after 48 hours and was highest after 384 hours (Table 3).

Crematogaster peringueyi and *L. humile* exhibited a similar mortality trend response to gourmet ant bait. The lowest two doses used satisfied the condition of <15%

mortality after 24 hours but failed to reach ≥89% mortality after 384 hours. Only 2% gourmet ant bait resulted in ≥89% mortality after 384 hours (Figure 2B). Mortality was low across all doses after 96 hours but generally increased after 192 hours reaching its maximum after 384 hours (Figure 2B).

As with *L. humile*, only the lowest dose of fipronil exhibited sufficient delayed toxicity on *C. peringueyi*. The lowest fipronil dose tested resulted in <15% mortality of *C. peringueyi* after 24 hours and ≥89% mortality after 16 days (Figure 2C). All the other doses resulted in rapid mortality of *C. peringueyi* and thus fell short of the conditions set by Stringer et al. (1964) (refer to table 3 for summary of results).

Spinosad was also part of the toxic baits tested for *C. peringueyi* but due to poor performance, it was difficult to graph the results and therefore, the results are not reported here.

DISCUSSION

Important prerequisites for toxicants of ant baits are that the toxicant must exhibit delayed action over a wide range of doses, be readily transferred between ants via trophallaxis and be able to kill the recipient while not being repellent to foraging ants.

From the bioassay results, boric acid exhibited delayed toxicity on both ant species. Boric acid is a stomach poison that has been used to control ants for more than a century (Rust 1986). Ant baits that contain boric acid use a liquid bait matrix, mostly sugar water and exploit the natural feeding habits of ants that forage on honeydew or nectar (Klotz & Williams 1996). Boric acid disrupts water regulation causing ants to ingest more of the bait leading to dehydration (Klotz et al. 1996). Liquid baits containing 0.5% boric acid in 25% sucrose solution showed delayed action and provided approximately 80% reduction in the number of *L. humile* workers foraging at monitoring stations in an urban setup (Klotz et al. 1998). This toxicant has shown potential as toxicity ant bait in previous experiments (Rust 1986; Klotz et al. 1996; Rust et al. 2004; Nelson & Daane 2007). *Linepithema humile* was more susceptible to boric acid than *C. peringueyi*. After 48 hours of toxic bait consumption, the LD_{50} for *L. humile* and *C. peringueyi* varied substantially. This may be attributed to the differences in size of the two ant species. *Crematogaster peringueyi* is larger than *L. humile* and may thus require higher doses to cause the same mortality effect as in *L. humile*. Secondly, greater susceptibility of *L. humile* may be due to more active feeding, leading to more toxin ingestion or a better toxin spread within the test insects. A specialized adaptation of their crop allows *L. humile* workers to consume nearly an equivalent of their weight of sugary liquids at a single feeding (Markin 1970; Reierson et al. 1998). Bait matrix requirements, sugar versus protein, could also account for the differences in mortality of the two species. Research has shown that *L. humile*'s diet is predominantly sugar (Baker et al. 1985; Rust et al. 2000). However, food preference assessments (Chapter 5) revealed that *C. peringueyi* also forage predominantly on sugar. Hence we attribute more susceptibility of *L. humile* to its specialized crop allowing toxin accumulation.

Gourmet ant bait contains boric acid as the active ingredient. It essentially comprises the same toxicant as boric acid but the only difference is its attractant. Gourmet ant bait uses a special insect attractant that mimics honeydew and enhances bait acceptance by a variety of sugar feeding ants. However, gourmet ant bait generally gave a lower mortality than boric acid. Since these two products were formulated differently, I hypothesize they were manufactured on different dates and this may have affected their efficacy. Delayed action exhibited by gourmet ant bait conforms to those obtained by Greenberg et al. (2006) on *L. humile*. As with boric acid, *L. humile* was more susceptible to this bait than *C. peringueyi* possibly because of the same reasons; size differences and different rates of bait uptake.

The use of fipronil as low toxicity ant bait has been well documented (Collins & Callcott 1998; Costa & Rust 1998). Fipronil is a neurological inhibitor that disrupts the insects' central nervous system by blocking neuron receptors (Collins & Callcott 1998). This compound has been formulated effectively as a low toxic bait to control ant species such as *Solenopsis invicta*, *L. humile* and *Anoplolepis gracilipes* (Barr & Best 2002; Collins & Callcott 1998). Solutions of 1 X 10^{-4}% fipronil in 25% sugar solution significantly reduced the number of *L. humile* foraging around urban structures (Klotz et al. 2002; Vega & Rust 2003). In this experiment, both ant species were highly susceptible to the fipronil treatment. Ant mortality was very high after 24 hours of exposure to toxic bait. Only the lowest dose used exhibited some degree of delayed action on both ant species. The reason for this might be that fipronil is highly toxic (Collins & Callcott 1998; Barr & Best 2002). Ripa et al. (1999) discovered that fipronil incorporated sugar baits reduced the amount of sucrose consumption plus bait transfer within *L. humile* populations. This led to the conclusion that fipronil affected workers within 24 hours leading to a reduction in trail deposition and recruitment, restriction in trophallaxis and a limitation in bait consumption.

Spinosad recorded very low toxicity on *L. humile* in the first four days (96 hours) of the experiment. Mortality, however, significantly increased after 192 hours of toxic bait consumption indicating that this toxicant is ideal for incorporation into *L. humile* toxic baits. This toxicant is derived from naturally occurring entomopathogenic bacteria which are highly effective at low doses, hence its potential as a low toxicity ant bait. Spinosad causes ant mortality through activation of the acetylcholine nervous system. Continuous activation of motor neurons will eventually cause insect mortality through exhaustion (Thompson et al. 2000; Saldago 1997). However, most previous research on spinosad had only focused on red imported fire ants *S. invicta* (Barr 2001).

Results of this experiment revealed that boric acid (2%), gourmet (2%) and fipronil (1.0 X 10^{-5}%) show potential in *L. humile* and *C. peringueyi* control while spinosad (0.01%) show promising results as *L. humile* low toxicity baits. Research has revealed that these doses are non-repellent and readily palatable to ants (Saldago 1997; Collins & Callcott 1998; Rust et al. 2004; Greenberg et al. 2006). However, these results are not conclusive. Field efficacy trials should follow these laboratory bioassays before baits can be fully implemented in ant pest management. More research should also consider testing the acceptability of different sugars as phagostimulants for *L. humile* and *C. peringueyi*.

Table1: Physical and chemical properties of selected bait toxicants (Kidd & James 1991).

Insecticide (Trade name)	Active Ingredient (AI)	Field dose (%)	Formulation	LD_{50} (rat oral mg/kg)	Solubility in water @ 20°C
Gourmet ant bait	boric acid	0.5	SC	42.5	Completely miscible with water
Borax	boric acid	0.5	WP	3500-4100	50g/L
Regent	fipronil	0.0001	SC	91-103	1.9mg/L
Insegar	fenoxycarb	0.5	WP	>5000	6mg/L
Tracer	spinosad	0.01	SC	3500-5000	290mg/L

SC= Soluble Concentrate, WP= Wettable Powder

Table 2: LD_{50} values for *Linepithema humile* workers fed on four toxicants dissolved in 25% sugar solution.

Treatment	Time (hours)	LD_{50}	95% Fiducial Limits (FL)
Boric acid	48	0.548	0.225-0.904
	96	0.379	0.110-0.654
	192	0.293	0.087-0.495
	384	0.246	0.030-0.491
Gourmet ant bait	96	0.713	0.328-1.191
	192	0.366	0.092-0.648
	384	0.225	0.010-0.487
Fipronil	24	0.032	0.025-0.040
Spinosad	96	1.227	0.717-2.007
	192	0.736	0.397-1.148

Table 3: LD_{50} values for *C. peringueyi* workers fed on three toxicants dissolved in 25% sugar solution.

Treatment	Time (hours)	LD_{50}	95% Fiducial Limits (FL)
Boric acid	48	1.835	1.090-3.706
	96	1.307	0.829-2.027
	192	1.024	0.362-1.958
Gourmet ant bait	384	0.424	0.069-0.836
Fipronil	24	0.046	0.031-0.063
	48	0.022	0.016-0.028

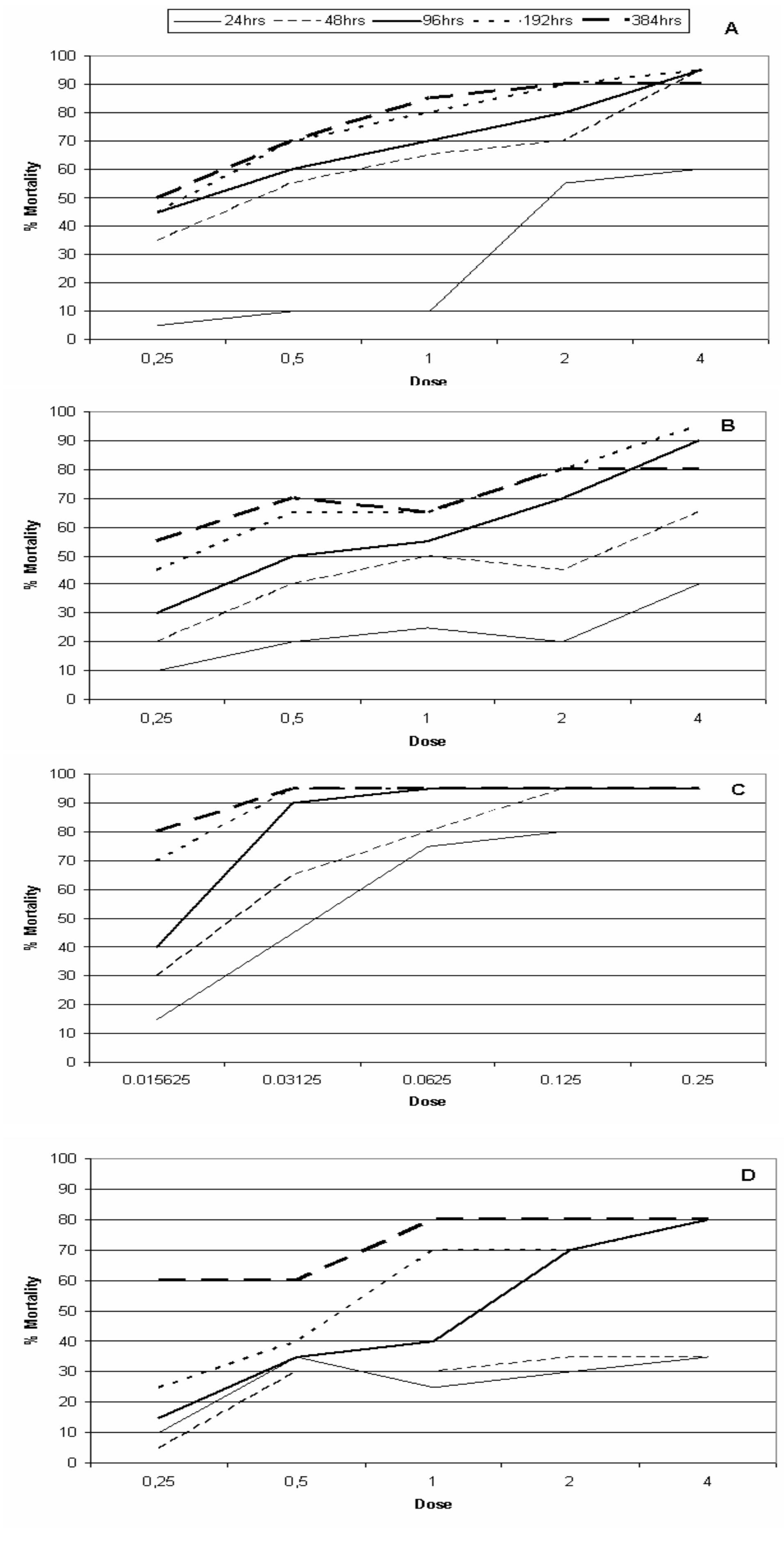

Figure 1: Efficacy of different doses of insecticide baits and time on *Linepithema humile* mortality: A, Boric acid; B, Gourmet; C, Fipronil; D, Spinosad

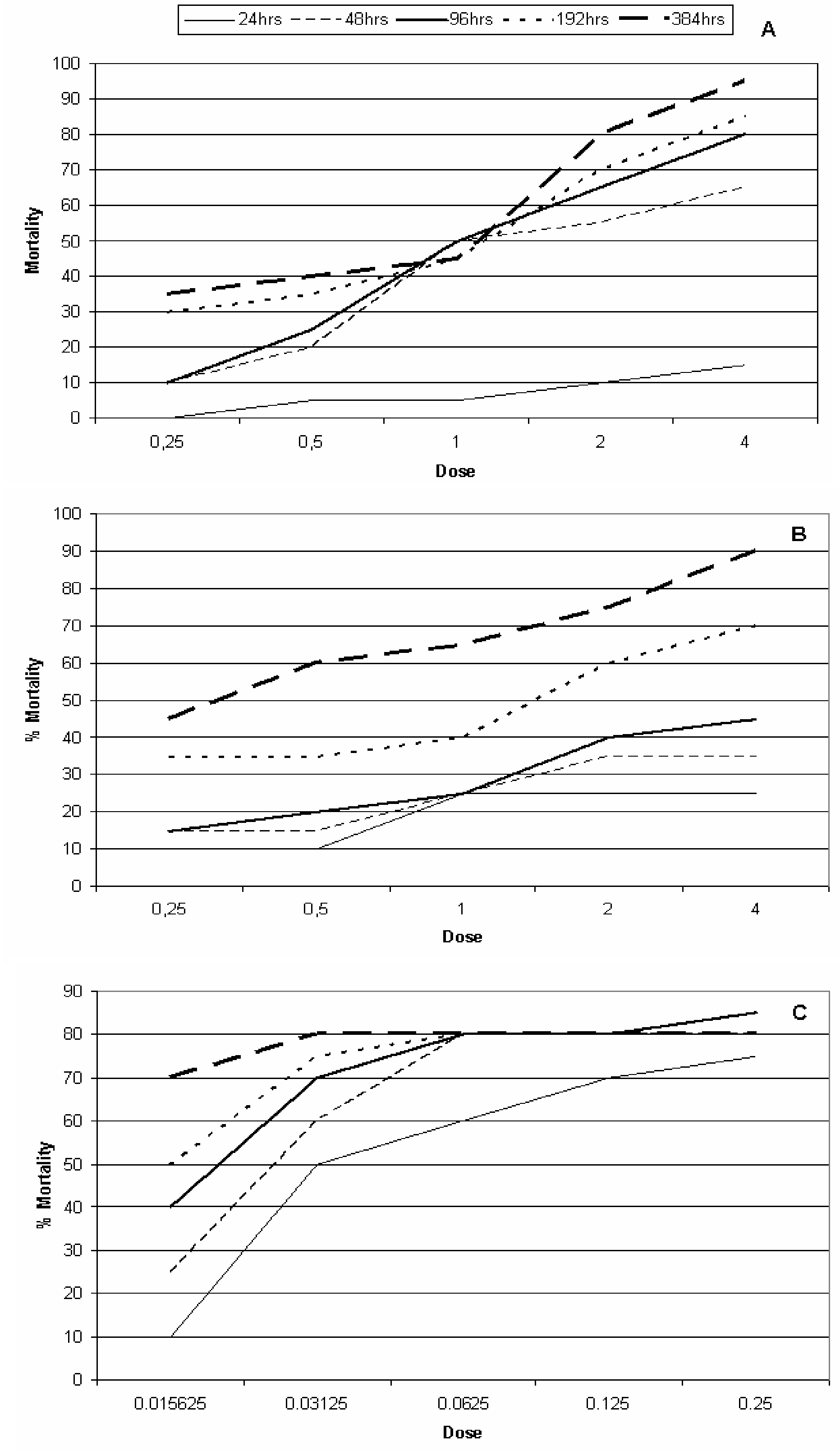

Figure 2: Efficacy of different doses of insecticide baits and time on *C. peringueyi* mortality: A, Boric acid; B, Gourmet; C, Fipronil

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CHAPTER 5

FOOD PREFERENCE AND FORAGING ACTIVITY OF ANTS (HYMENOPTERA: FORMICIDAE) IN VINEYARDS: INITIAL RECOMMENDATIONS FOR FIELD APPLICATIONS

INTRODUCTION

In vineyards, the mutualism between ants and the vine mealybug *Plannococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) results in population explosions of both insects, therefore causing pest injury and economic loss (Kriegler & Whitehead 1962; Way 1963; Myburgh et al. 1973; Buckley 1987; Styrsky & Eubanks 2007). The Argentine ant *Linepithema humile* (Mayr), cocktail ant *Crematogaster peringueyi* Emery and the common pugnacious ant *Anoplolepis custodiens* (F. Smith) are some of the problematic ants associated with the vine mealybug in South African vineyards (Whitehead 1957; Urban & Bradley 1982; Addison & Samways 2000). Effective control of these ants using baits of low toxicity is limited by inadequate knowledge on ant foraging activity and bait station density. Density of bait stations per unit area may however vary depending on the ant species and the level of ant infestation. In previous studies, Daane et al. (2006) dispensed liquid baits at 85-620 baits/ha. While at high densities, baits resulted in fewer *L. humile*, fewer mealybugs and less damage to grape clusters. Similarly, to determine optimal density of bait stations in vineyards, ant foraging activity needs to be evaluated. Ant foraging ranges and behavior is affected by a number of factors, amongst them: temperature, circadian rhythm, competition, food availability, food particle size and photoperiod (Oster & Wilson 1978; Hölldobler & Wilson 1990). Daane et al. (2006) revealed that *L. humile* mainly follow irrigation pipes and trellis wires when foraging for honeydew and do not move across rows in vineyards. Heavy utilization of trellis wires as foraging trails has also been observed in South African vineyards (Pia Addison, personal communication).

Ant foraging patterns and foraging activity are not well understood and have not been thoroughly examined in South African vineyards. The challenge is selecting an appropriate insect marker, which should ideally be cheap, non-toxic (to the environment and insect), be easily applied and be observed clearly. Furthermore, insect markers should not hinder, irritate or interfere with the insect's normal behavior, lifespan, growth and reproduction (Hagler & Jackson 2001). However, similar research has been done, both in urban and agricultural settings outside South Africa. Oil soluble dyes like calco red have been added to peanut butter to study foraging behavior of the imported fire ant *Solenopsis richteri* (Wilson et al. 1971) and in a separate experiment, similar dyes have been used to mark *Solenopsis invicta* (Juan et al. 2002). To determine Argentine ant foraging distance, Markin (1968) placed feeding stations labeled with P^{32} near one nest and sampled ants various distances away from the station. This research showed that Argentine ants can forage up to 45m. Similarly, Ripa et al. (1999) found up to 21% of marked *L. humile* 54m away from the feeding station; Vega & Rust (2001; 2003) found marked ants up to 61m from feeding stations. However, ant foraging activity in urban settings may differ from a vineyard setup because of abundance of food resources in vineyards. Furthermore, level of infestation by honeydew-producing mealybugs would, presumably, also impact on foraging activity. Although *L. humile* can forage for long distances, these ants generally nest in close proximity to food sources and relocate nests when nearby food sources become exhausted (Suarez et al. 2001; Tsutsui et al. 2003; Heller & Gordon 2006). At Letaba citrus orchards, *A. custodiens* has been observed to forage for 111m during peak honeydew production periods and up to 50m in April, when the mealybugs are abundant (Steyn 1954). No research has been carried out to ascertain *C. peringueyi* foraging activity.

One other factor limiting the effectiveness of toxic baits in the control of vineyard ants is inadequate research on ant bait carriers and attractants. Different ant species have different dietary requirements and consequently this must be taken into account in formulating baits for control of these ants. Proportions of fat, carbohydrates and proteins in the bait differ from one species to the other depending on the colonies' nutritional needs (Rust et al. 2000). Physical state of the bait, liquid versus protein and particle size of the carrier also affects the rate of collection of the bait (Hooper-bui & Rust 2000). This requirement makes the development of effective baits a highly challenging task. Most of the research on ant food preference studies has been done on *L. humile* (Baker et al. 1985). In choice tests, Hooper & Rust (1997) indicated that the southern fire ant *Solenopsis xyloni* preferred anchovy over tuna, sardine and mealworms. To date, no food preference studies have been done in South African vineyards to determine ideal food requirements for *L. humile*, *A. custodiens* and *C. peringueyi*.

This research was aimed at determining the foraging activity of vineyard ants (*L. humile*, *C. peringueyi* & *A. custodiens*) using liquid baits labeled with a marker. This would indicate whether or not intraspecific competition plays a role in the foraging activity of the three ant species. Furthermore, this research was also aimed at determining whether or not specific bait matrices were more/less/equally attractive to three ant species under field conditions. Results of this trial will help us to better determine bait station density and bait distribution patterns and will help us determine the best attractant to use when formulating toxicants for the control of these three ant species. This information can therefore be used to assist with practical field applications of these baits.

MATERIALS AND METHODS

Study sites

To determine food preferences and how far each ant travels from a food source up to a predetermined distance, ant food preference trials were carried out during spring (October 2007) as this is the optimum time for application of baits since ant populations will still be low (Nelson & Daane 2007). Foraging distance evaluations were carried out during summer (December 2007, January & February 2008) in the Stellenbosch winelands region. Ideally this should also have been conducted in spring but selection of a suitable marker for the ants delayed this experiment. *Linepithema humile* trials were carried out at Joostenberg farm (33.80S; 18.81E), *C. peringueyi* at La Motte farm (33.88S; 19.08E) while *A. custodiens* trials were carried out at Plaisir de Merle farm (33.87S; 18.94E) (Figure 1, Chapter 2). Irrigation in all the vineyards was by drip irrigation. Plaisir de Merle comprised young trellised white Chenin blanc wine grapes; La Motte vineyard comprised very old trellised white Chenin blanc wine grape variety while Joostenberg farm had young trellised red Pinotage wine grapes.

Experiment 1: Determination of ant foraging activity

Trial layout and sampling methods

Five bait stations were placed along five transects in a vineyard block approximately (100m X 100m) and ants were allowed to feed on 25% sugar solution that had been labeled with 0.25% calco red (N-1700®, Passaic Color and Chemical Company, Paterson, New Jersey). The calco red labeled sugar water was soaked in cotton wool and held in place on petri dishes which were placed 10m away from each other along the edge of the vineyard floor. In the study of ant foraging behavior, previous research has placed baits at five rows between treatment rows (Daane et al. 2006), which was regarded as being sufficient enough to prevent cross-infestations. Ants were allowed to forage on the calco red labeled sugar water for one week before ant sampling was done. The labeled sugar was replenished once during the week because the bait easily dried out due to crystallization of the sugar component.

After seven days, thirty pitfall traps arranged 1, 2, 4, 8, 16 & 32m away from the bait station and running along the vine rows were set along each of the five transects. Pitfall traps consisted of plastic containers (35mm diameter X 60mm height). The pitfall traps were drilled into the soil and the soil surface was leveled so that the trap rim was flush with the soil surface (Figure 1). Approximately 4ml of three parts concentrated glycerol and seven parts 70% ethyl alcohol was placed in each of the pitfall traps to preserve the captured ants as used by Majer (1978). This liquid is relatively non volatile. Pitfall traps were left in the vineyard for 48 hours after which the traps and their catch were collected for laboratory analysis.

During the same time when pitfall traps were being collected, tuna baits were used to sample vine dwelling ants. Roughly one teaspoon of shredded tuna chunks were placed in small plastic containers (70mm diameter X 7mm height) at the crutch of vines (Figure 2) above each of the pitfall traps along each of the five transects. Ants were left to forage on the tuna for 30 minutes after which all ants feeding on the tuna bait were collected separately by sweeping them in different containers containing 70% ethyl alcohol as a preservative.

Ant samples collected from both pitfall and tuna traps were taken to the laboratory for analysis. Calco red positive ants were detected by crushing the ants' abdomen on white paper towels and observing its coloration through a microscope. A pink coloration of the abdomen denoted the presence of calco red.

Experiment 2: Food preference assessments

Eight bait matrices were assessed for their attractiveness to *L. humile*, *C. peringueyi* and *A. custodiens* during spring 2007. These included: (1) 25% sugar solution; (2) agar (in 25% sugar solution) (Warren Chemical specialists, Cape Town, South Africa); (3) tuna (Pick'n pay, Kensington South Africa); (4) honey (Fleures® honey products, Pretoria, South Africa); (5) dog food (Boss® chicken beef and beef platter, Promeal Private Limited, South Africa); (6) dry fish meal; (7) dry sorghum grit; (8) 25% peanut butter (in distilled water) (Nola Yum Yum®, Nola, Randfontein, South Africa). Tests were conducted in vineyards using choice test arenas like those described in chapter 2 (Figure 2). Five choice test arenas were used (5 replicates) and eight bait matrices were placed in small petri dishes which were randomly assigned to positions in the choice test arenas. Active ant nests were selected in the vineyards and each arena was placed close by, with approximately 10m distance between arenas. Ants were allowed to forage on the baits and the experiment was replicated at five nests for each of the three ant species.

Data collection and analysis

Proportion of ants testing positive for calco red for each of the respective distances of the two trapping methods and for each of the three ant species was calculated. Since the results (proportion of calco red positive ants) were measured under different conditions (months), using standard ANOVA in this case was inappropriate because it fails to model the correlation between the repeated measures. Proportion of ants that carried the dye labeled sugar water at each of the various distances from the bait source for the two trapping methods was therefore calculated for each ant species using repeated measures ANOVA in Statistica 7 (2004). Tukey-Kramer's post hoc tests were used to separate differences between treatment means. During the food preference tests, number of ants foraging at each bait station was recorded at hourly intervals up to four hours. Assumption of homogeneity of variances was tested and results were subjected to Analysis of Variance (ANOVA) in Statistica 7 (2004). Tukey-Kramer's post hoc test was again used to separate means.

RESULTS

Experiment 1: Determination of ant foraging activity

Argentine ants

There were no significant month differences in *L. humile* foraging distance during the three different trial dates both on the ground and on the vine (Table 1). However, distance foraged by this species was highly significant both on the ground and in the vine (Table 1). The transport of the calco red labeled ant bait decreased with distance from the bait source for both ground and vine sampled ants (Figure 3). There was a significant drop in foraging activity after 2m in the vines and after 8m on the ground (Figure 3).

Common pugnacious ants

This ant species was more predominant on the ground than it was on the vines (Figure 4). Nevertheless, a considerable number of ants were baited on the vines, where they were observed tending mealybugs. There were no significant month differences in foraging activity during the three trial days (during December, January & February) both on the ground and on the vine (Table 2). However, distance foraged by *A. custodiens* during the three different months was significant both on the ground and on the vine (Table 2). Transport of the labeled ant bait also decreased with increasing distance from the bait source on the ground while on the vine there was no difference in number of marked ants until after 8m (Figure 4). A significant drop in *A. custodiens* foraging activity occurred on the ground after 2m (Figure 4).

Cocktail ants

Detection of the calco red labeled sugar water again decreased with distance from the bait source (Figure 5). Distance foraged by *C. peringueyi* was highly significant for both ground sampled and vine sampled ants (Table 3). I observed *C. peringueyi* were more predominant on the vines as opposed to the ground. There were no month differences in *C. peringueyi* foraging activity during the three trial days (during December, January & February) both on the ground and on the vine (Table 3). Transport of the calco red labeled sugar water significantly dropped after 4m (in the vines) and after only 1m on the ground (Figure 5).

Experiment 2: Food preference assessments

Argentine ants

Field trials revealed that treatment baits and hour were highly significant for this ant species (Table 4). Generally, *L. humile* was more attracted to sugar based baits as opposed to protein based baits. A 25% sugar solution was the most attractive bait and differed significantly from the rest of the treatments (Figure 6). *Linepithema humile* significantly preferred liquid baits (25% sugar water, honey & agar) over solid baits (Figure 6). Hour was significant for *L. humile* and foragers significantly increased with time (Figure 7).

Cocktail ants

There were no significant differences in this ant's preference for 25% sugar solution as compared to 25% honey. These two baits were the most attractive to *C. peringueyi* and significantly differed from the rest of the treatments (Figure 6). Both bait treatments and hour were significant for this ant species (Table 4). Hour was highly significant and the number of *C. peringueyi* at food baits significantly increased with time (Figure 7).

Common pugnacious ants

Bait treatments were also significant for this ant species during the field trials (Table 4). *Anoplolepis custodiens* was significantly attracted to tuna and this bait differed significantly from the rest of the treatments (Figure 6). Hour was also significant for *A. custodiens* food preference (Figure 7), with increase in number of ants with time.

Discussion

From the foraging activity trials, it was observed that *L. humile* had the greatest proportion of individuals positive for the calco red at each of the respective distances from the bait source, *A. custodiens* was intermediate and *C. peringueyi* the least. *Linepithema humile* are successful and aggressive competitors that generally dominate rapidly over other ant species (Hölldobler & Wilson 1990). They are unicolonial, meaning that ants from one colony are not aggressive to non nestmates (Passera & Keller 1994). Markin (1968) revealed that after five days, the exchange of worker ants between neighboring nests exceeds 50%. In this manner, *L. humile* can easily form supercolonies that can saturate an entire habitat. Working as a supercolony compounded by their dominance over other ant species found in the vineyards, this can account for its high foraging activity. This result corroborates the research by Nelson & Daane (2007) which indicated that *L. humile* is extremely vagile. In citrus orchards, Markin (1967, 1968) and Ripa et al. (1999) indicated that *L. humile* foraging seldom exceeds 61m. However, this research was limited to 32m as placing baits at more than 32m apart would most certainly not result in effective control (Daane et al. 2006). Results of *L. humile* foraging on the ground were almost consistent with those of ants foraging on the vine. The proportion of individuals testing positive for calco red were almost the same (for the two sampling methods) at each of the respective distances from the bait source. The reason why so many ants were observed on the vine could be that *L. humile* is a sugar feeding ant species (Markin 1970a; Baker et al. 1985). This trial was done in summer when honeydew excretion by mealybugs was at its peak, thus allowing *L. humile* to concentrate their foraging activity on the vines.

The proportion of *A. custodiens* testing positive for calco red at each of the respective distances from the bait source was intermediate between *L. humile* and *C.*

peringueyi. *Anoplolepis custodiens* is a heat-loving behaviorally dominant ant species that competes successfully with other ant species (Hölldobler & Wilson 1990). This ant can easily outcompete many local ant species for honeydew (Samways 1999). In research carried out in guava and citrus orchards, *A. custodiens* displayed high dominance over other ant species (Samways 1990) and in a survey of Western Cape vineyards (Addison & Samways 2000) this ant species also exhibited dominance over other vineyard ants. Previous research has revealed that *A. custodiens* does not show intra-specific competition amongst colonies (Steyn 1954) and this ant species forms supercolonies that reduce competition within the species (Hölldobler & Wilson 1990). This high dominance and lack of intraspecific competition might account for widespread foraging activity of this ant species. The fact that this species likes nesting on the periphery of vineyards where there are hard undisturbed soils, no vegetative cover with lots of insolation (Steyn 1954) would explain why a smaller number of ants were sampled from the vine indicating that this ant forages predominantly on the ground and seldom on the vine, where they tend honeydew excreting Hemiptera.

The number and proportion of *C. peringueyi* sampled on the ground were very low and inconsistent. This might have been because this ant species nests and forages on the vine and rarely forages on the ground, making it an arboreal species (Kriegler & Whitehead 1962). Results showed that the proportions of calco red positive ants foraging at each of the respective distances from the bait source were low and that the foraging territory for this ant species was relatively small. No research has been done to assess the dominance of *C. peringueyi* and its reactions in interspecific and intraspecific competition.

Markin (1970b) estimated that 99% of *L. humile*'s diet consists of honeydew and nectar. Sucrose is the main ingredient of honeydew (Tennant & Porter 1991) and forms a significant part of nectar (Baker & Baker 1985). Baker et al. (1985) carried out food preference trials on *L. humile* and concluded that this ant preferred 25% sugar solution or honey over other solid protein based foods like tuna. Similarly, Silverman & Roulston (2001) compared the consumption of gel and liquid sucrose formulations by *L. humile* and results indicated that this species preferred foraging on the gel formulations. Results of the present study support the above findings except the gel (agar) result. *Crematogaster peringueyi* is a vineyard ant that forages on mealybug honeydew (Kriegler & Whitehead 1962; Addison & Samways 2000). This fact may explain why this ant species was most attracted to honey and 25% sugar solution during the food preference tests. It therefore also appears that *C. peringueyi* prefers sugar over protein. *Anoplolepis custodiens* was most attracted to tuna over the rest of the treatments. *Anoplolepis custodiens* is a highly predatory ant species that is a major predator of insects in South Africa (Löhr 1992; Steyn 1954). Its predatory nature might account for this ant being most attracted to tuna. From the food preference trials, it generally appeared that vineyard foraging ants have a preference of wet foods (25% sugar water, agar, tuna & honey) over dry matrices (fish meal, sorghum grit, dog food & peanut butter). This is typical of vineyard ants that forage on mealybug honeydew. Furthermore, the number of foragers at bait stations was significant and food preference tests showed that ant foragers increased with time. Increase in foraging numbers would have been caused by pheromone calling and consequently increase in ant recruitment with time.

High attractiveness of 25% sugar solution to *L. humile* and *C. peringueyi* indicates that liquid sugar baits are the most appropriate attractants/carriers for baiting these two species in future pest control. This bait matrix has the advantage that it is cheap, readily available but is prone to drying out (due to sugar crystallization) and fungal attack. Furthermore, when formulating toxicants for baiting *A. custodiens*, wet protein attractants like tuna is the most ideal bait matrix for maximum effectiveness and should be taken into account. The large foraging ranges for *L. humile* compared to *C. peringueyi* and *A. custodiens* have implications for bait distribution density. To be economically viable, Daane et al. (2006) recommended that baits should be dispensed at 85 baits/ha or less. Results reveal that at 81 baits/ha (one bait every 9m), low toxic baits will reach ±80% of the *L. humile* target pest population. Consequently, high foraging activity plus unicoloniality makes *L. humile* more susceptible to toxic baits at low bait densities that are relatively economical. At the same rate (81 baits/ha), only ±30% *A. custodiens* and ±20% *C. peringueyi* will be reached. Lower foraging activities for *A. custodiens* and *C. peringueyi* imply higher bait densities for effective ant control. At high densities, baits of low toxicity will less likely be adopted by growers because of the costs of materials and maintenance involved. However, bait distribution density also depends upon the size of the ant infestation. Thus all these factors must be considered including monitoring for pest intensity before determining optimum bait density.

Results of this trial are not conclusive. More research needs to be done before we can fully understand bait distribution patterns and bait station density. Foraging behavior of ants depends upon a number of environmental factors, one of which is the availability of food sources. Foraging activity field trials were done in summer when honeydew excreted by *P. ficus* and grape juice from the ripening grape clusters was at its peak. Therefore this is the time when ant baits would be at their least effective. I hypothesize that ants forage for shorter distances during this time of the year, since food resources will be highly abundant. Nelson & Daane (2007) hypothesized that ant foraging on 25% sugar water peaks in spring. Thus further research should focus on repeating the study in spring and autumn to deduce whether increased labeled bait acceptance will translate to increased bait movement in the vineyard. Furthermore, more research should focus on assessing the dominance of *C. peringueyi* which is one of the factors that will determine this ant's foraging behavior. Food preference may also differ depending on season and consequently more research still needs to be done on species seasonal food requirements (protein, lipids & carbohydrates).

Table 1: Summary of the effects of distance and month on *Linepithema humile* foraging distance in small field trials.

Sample from	Effect	F-value	d.f	p
Ground	distance	44.63898	5	<0.0001
	month	0.00330	2	0.996704
Vine	distance	44.53758	5	<0.0001
	month	0.42629	2	0.666932

Table 2: Summary of the effects of distance and month on *Anoplolepis custodiens* foraging distance in small field trials.

Sample from	Effect	F-value	d.f	p
Ground	distance	15.502	5	<0.0001
	month	1.73194	2	0.237155
Vine	distance	1.580643	5	<0.01
	month	8.611527	2	0.10120

Table 3: Summary of the effects of distance and month on *Crematogaster peringueyi* foraging distance in small field trials.

Sample from	Effect	F-value	d.f	p
Ground	distance	7.728028	5	<0.001
	month	4.403968	2	0.051322
Vine	distance	18.45683	5	<0.0001
	month	4.36035	2	0.052401

Table 4: Summary of the effects of bait and hour on food preference for *Linepithema humile*, *Crematogaster peringueyi* and *Anoplolepis custodiens*.

Ant species	Effect	F-value	d.f	p
L. humile	bait	10.74995	7	<0.0001
	hour	5.27113	3	<0.01
C. peringueyi	bait	20.54866	7	<0.0001
	hour	5.66825	3	<0.01
A. custodiens	bait	19.00829	7	<0.0001
	hour	6.7310	3	<0.01

Figure 1: Pitfall trap in flush with the ground, used in trapping ground foraging ants.

Figure 2: *Crematogaster peringueyi* foraging on tuna baits used to sample vine dwelling ants.

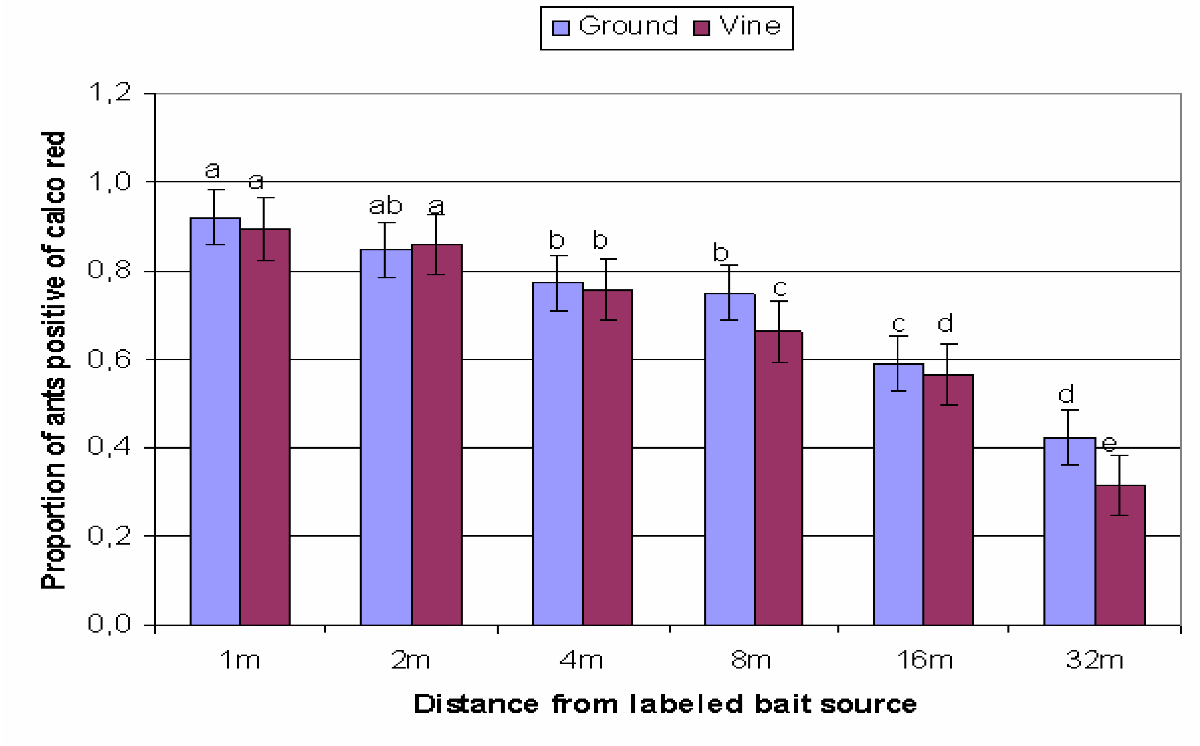

Figure 3: Proportion of *Linepithema humile* positive of a calco red labeled bait source at different distances from the bait source in a vineyard at Joostenberg farm. Ground and vine data were analyzed separately and means with the same letter are not significantly different (Bars represent ±95% CI).

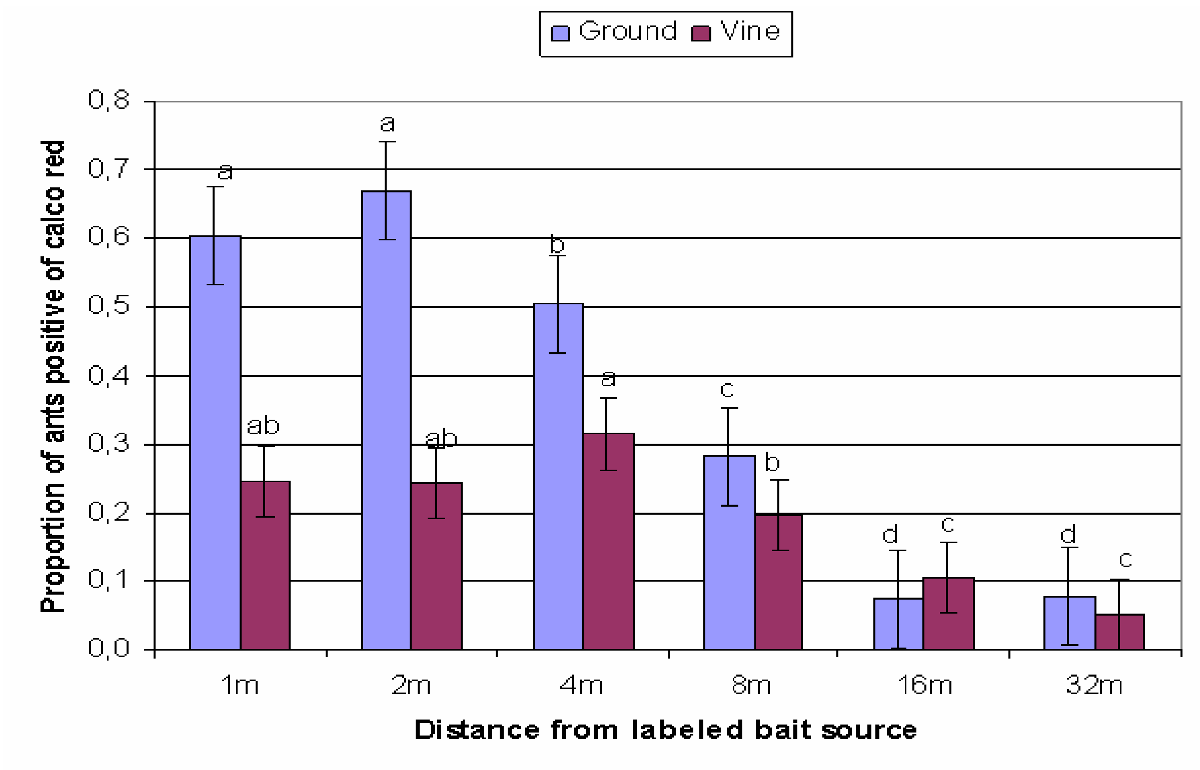

Figure 4: Proportion of *Anoplolepis custodiens* positive of a calco red labeled bait source at different distances from the bait source in a vineyard at Plaisir de Merle farm. Ground and vine data were analyzed separately and means with the same letter are not significantly different (Bars represent ±95% CI).

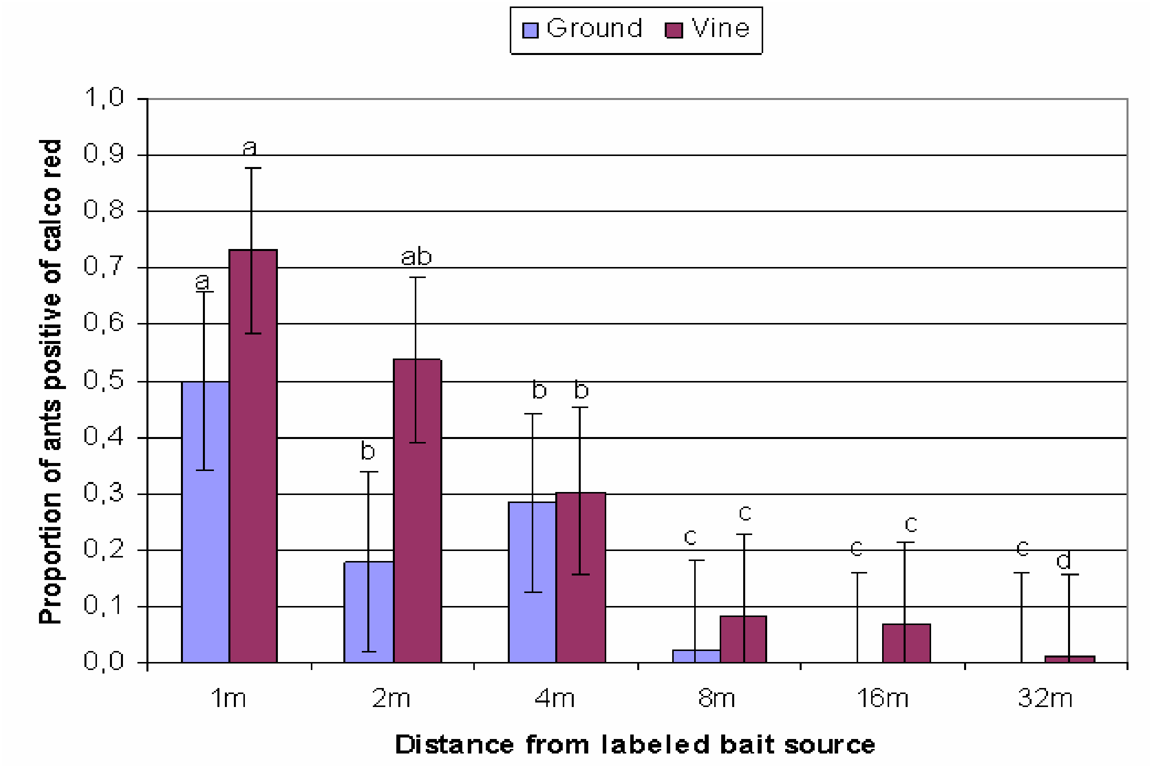

Figure 5: Proportion of *Crematogaster peringueyi* positive of a calco red labeled bait source at different distances from the bait source in a vineyard at La Motte farm. Ground and vine data were analyzed separately and means with the same letter are not significantly different (Bars represent ±95% CI).

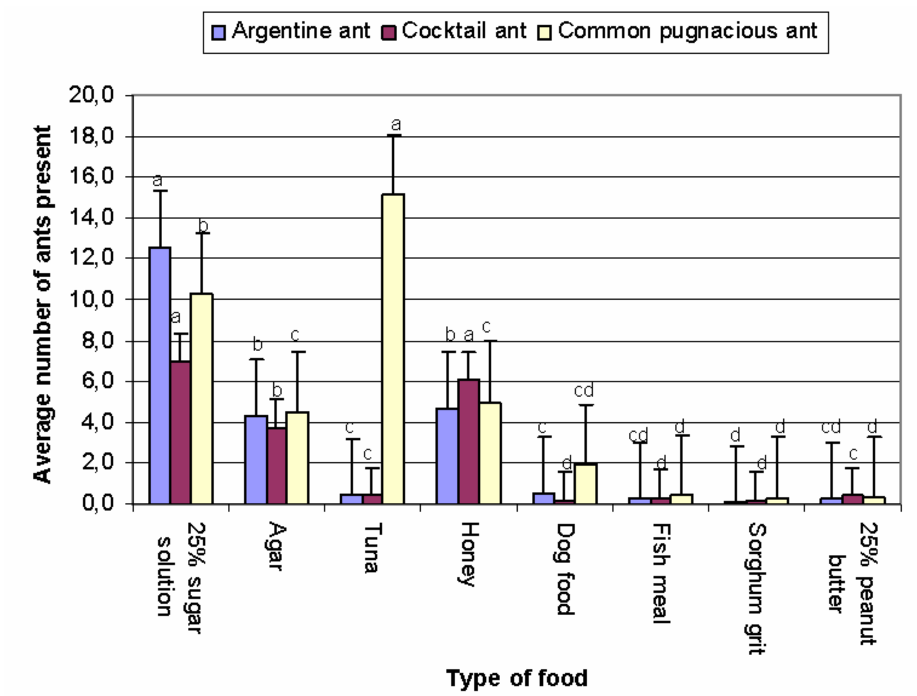

Figure 6: Average number of *Linepithema humile, Crematogaster peringueyi* and *Anoplolepis custodiens* on different food baits during October 2007. Ant species were analyzed separately and means with the same letter are not significantly different (bars represent ±95% CI).

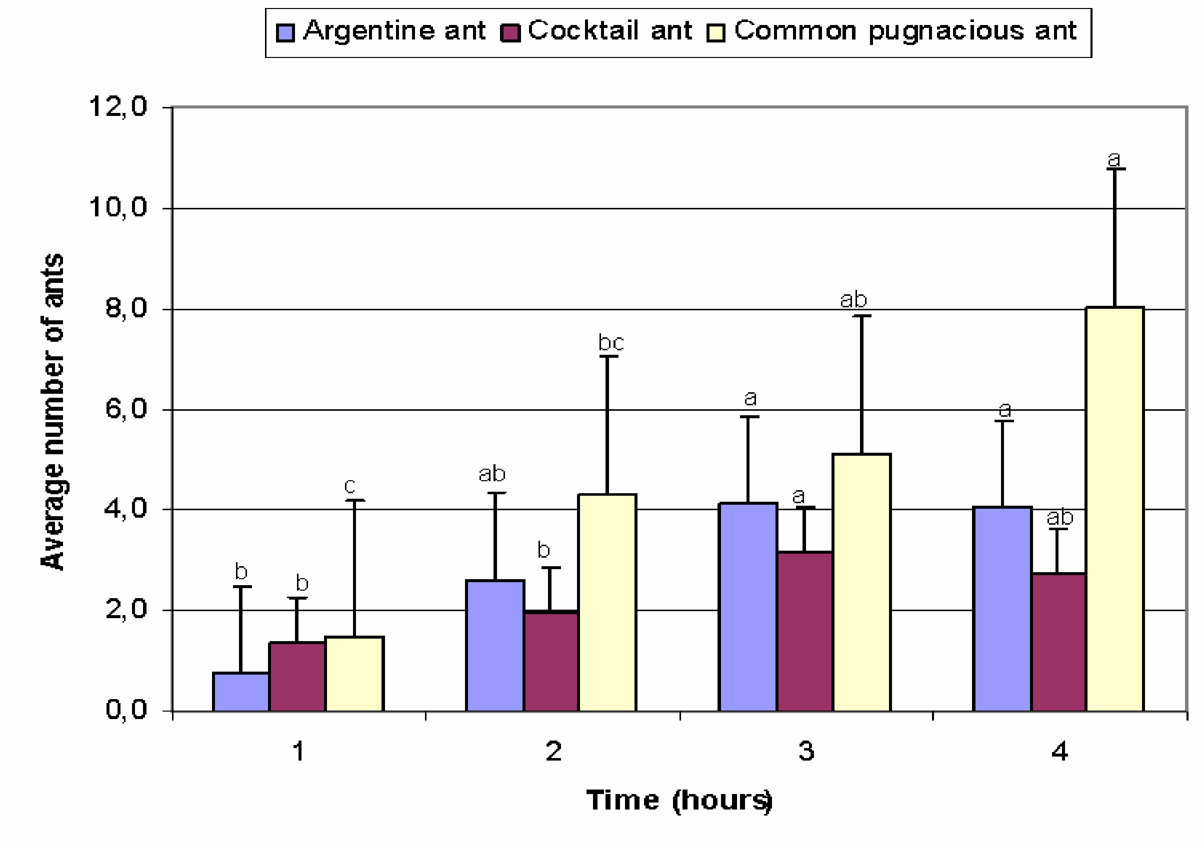

Figure 7: Average number *Linepithema humile, Crematogaster peringueyi* and *Anoplolepis custodiens* on food baits over time in spring. Ant species were analyzed separately and means with the same letter are not significantly different (bars represent ±95% CI).

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CHAPTER 6

GENERAL DISCUSSION

Baits of low toxicity were tested as an alternative approach to ant control in vineyards. They are an environmentally friendly method of ant control which is target specific and can be integrated into vineyard IPM and IPW programmes. Under field conditions, bait preference (Chapter 2) and bait acceptance (Chapter 3) tests were carried out for the three ant species in three different vineyards. This was meant to determine bait repellency and palatability, respectively. Five toxic baits (gourmet ant bait, boric acid, fipronil, fenoxycarb & spinosad) were tested. Chapter 4 was aimed at assessing the efficacy of the different toxicants to *L. humile* and *C. peringueyi* in the laboratory with reference to conditions set by Stringer et al. (1964). Chapter 5 describes foraging activity and food preferences for the three ant species, with the aim of providing initial guidelines for field applications.

Chapter 2: Bait preference experiments

To be effective in ant control, ant baits should be non repellent and not deter feeding of ant foragers. In agroecosystems, ant baits are sometimes challenged by huge constraints; high quality natural food in the form of floral nectar, honeydew and prey items (Daane et al. 2006; Rust et al. 2003). Therefore, if toxic baits are to be effective, they need to be non-repellent and attractive enough to compete with these naturally available food sources. This led to bait preference tests being carried out for the three ant species using five different toxicants dissolved in 25% sugar solution. I conclude that small amounts of selected insecticides formulated in sugar water have the potential to control ant populations as shown by previous research (Rust et al. 2000; Klotz et al. 2000, 2003, 2004). Gourmet ant bait was significantly more attractive than the other baits across all ant species and during spring, summer and autumn, possibly because of its attractive bait matrix that mimics honeydew. Honeydew comprises more complex and varied carbohydrate sources than the sugar water and consequently is more attractive to ant foragers (Kiss 1981; Volkl et al. 1999). However, boric acid, fenoxycarb and spinosad were significantly less attractive to *L. humile* as compared to 25% sugar solution control during spring. Furthermore, boric acid and spinosad were also less attractive to *C. peringueyi* than the sugar water control during spring. These formulations are therefore not ideal for low toxicity ant baits. Effectiveness of baits of low toxicity may be affected by the season upon which they are deployed. Nelson & Daane (2007) suggested spring as the best time to apply sugar baits mainly because at this time, colonies are busy developing their brood and this gives the ants a chance to transfer toxicants to developing larva and disrupt colony growth. Furthermore, alternative sugars in the form of honeydew and grape juice are not available during spring, leaving ants to concentrate their foraging on baits. Bait preference was essentially high for *A. custodiens* in spring. I also assume spring is the best time to deploy baits of low toxicity in South African vineyards, as colonies are still small (Addison & Samways 2006; Nelson & Daane 2007) and could therefore be more easily controlled, requiring less baits. Research has furthermore indicated that pheromones can increase ant recruitment at baits (Goss et al. 1990; Greenberg & Klotz 2000). Therefore I recommend further research be done in investigating incorporation of pheromones into toxic baits to increase attractiveness further.

Chapter 3: Bait acceptance experiments

Bait acceptance is highly significant for the success of low toxicity ant baits. Chapter 2 revealed that selected low toxicity baits were equally or more attractive to ant foragers as compared to a 25% sugar solution control. But to be efficacious, ants must sufficiently consume the bait. Results for this study showed that bait toxicants dissolved in 25% sugar solution were equally or more acceptable to *L. humile*, *C. peringueyi* and *A. custodiens* when compared to a 25% sugar water control. Gourmet ant bait was the most accepted bait for all ant species and seasons for reasons already mentioned. Present results confirm studies by Klotz et al. (1997); Collins & Callcott (1998); Klotz et al. (1998); Greenberg et al. (2006). Bait acceptance results directly reflected on bait preference results. Toxic baits that were highly preferred translated into the most acceptable baits across the seasons for the three ant species.

Chapter 4: Bait efficacy experiments

One of the key attributes of candidate toxicants to be incorporated into toxic baits is that they should exhibit delayed action and be readily transferred between ants via trophallaxis before killing the recipient (Stringer et al. 1964). To test for this key characteristic, bait efficacy bioassays were carried out in the laboratory using five candidate toxicants on two ant species *L. humile* and *C. peringueyi*. In this study, we defined delayed toxicity as ≤15% kill at 24 hours and ≥89% kill at 20 days (Stringer et al. 1964). Delayed action is critical because it gives foragers ample time for mass recruitment, trail following and to spread a toxicant throughout the entire colony. This is also important, particularly for *L. humile* workers which lay trail pheromones from the nest to the food source (Van Vorhis Key & Baker 1986). Gourmet ant bait (2%), boric acid (2%), fipronil (1.0 X 10^{-4}) and spinosad (0.01%) exhibited delayed toxicity on *L. humile* while gourmet ant bait (2%), boric acid (2%) and fipronil (1.0 X 10^{-4}) showed delayed action on *C. peringueyi*. Furthermore, these toxicants were effective over a wide dosage range implying that the toxicants will still remain lethal to recipients even after dilution via trophallaxis. Another desirable feature of the bait toxicants was their solubility in water. This is especially critical when baiting ants that forage predominantly on honeydew (Baker et al. 1985). All toxicants were soluble when formulated in 25% sugar water.

Fenoxycarb and spinosad were also part of the candidate toxicants tested but the latter did not perform well against *C. peringueyi* hence the results were omitted. Furthermore, due to the mode of action of fenoxycarb, efficacy results for this toxicant were also omitted in these results. Though some of the toxicants exhibited delayed action, assumptions should not be made that these laboratory results will truly reflect what happens when toxic bait is placed in the field. More efficacy trials should be carried out in a field situation to complement these laboratory results before baits of low toxicity can be made available for ant management in commercial agriculture.

Chapter 5: Food preference and foraging activity

Control of ants using baits of low toxicity cannot be effective without knowledge of bait distribution patterns and bait station densities which are determined by ants' foraging activities. Furthermore, success of toxic baits also depends upon attractiveness of bait carriers. This chapter assessed foraging activity and food preferences for the three ant species under field conditions. Results showed that *L. humile*'s foraging activity is high relative to the other two ant species. This finding is consistent with research by Markin (1967, 1968) and Ripa et al. (1999) on *L. humile*. Field trials also revealed that movement of ant bait by *C. peringueyi* and *A. custodiens* in the vineyard is low. Daane et al. (2006) recommended that baits should be dispensed at ≤85 baits/ha to be economically viable. Foraging activity indicated, for example, that at 81 baits/ha (one bait every 9m), baits of low toxicity will reach ±80% of the target *L. humile* pest population. Consequently more bait stations need to be dispensed for more effective pest control. Furthermore at this rate only ±30% *A. custodiens* and ±20% *C. peringueyi* foragers will be exposed making baiting at this density not as economically viable for the control of these two species. I hypothesize that bait density also depends upon the size of the ant infestation and the size of mealybug infestation. I recommend ant monitoring be considered as a factor to determine bait distribution patterns. .

Food preference trials revealed that vineyard foraging ants significantly preferred wet bait attractants over dry ones making liquids the most ideal carriers for baiting these ants. *Linepithema humile* was significantly attracted to 25% sugar water while *C. peringueyi* was significantly attracted to 25% sugar water and honey. *Anoplolepis custodiens* was significantly attracted to tuna but was also attracted to 25% sugar water. Thus future bait formulations should be tailor made to suit these specific food requirements if baits are to be successful for ant management. I propose that further research be done on ant seasonal diet requirements for South African vineyard ants.

In conclusion, ant control using low toxicity baits can reduce non-target exposure and increase contact of non foraging pest ants with the toxicant, thus the entire colony can be controlled. In situations where there is high ant pressure, this method could be used to supplement chemical stem barriers to make the latter more effective. Toxic baits also give the growers an alternative to labour intensive chemical stem barriers. The cost-efficacy of baits will depend on the final formulation of the bait, and whether bait stations will be used to ensure that no non-target organisms gain access. A suitable bait station design should also therefore be investigated.

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