EFFECTS OF ANT PREDATION ON THE EFFICACY OF BIOLOGICAL CONTROL AGENTS: *HYPENA LACERATALIS* WALKER (LEPIDOPTERA: NOCTUIDAE); *FALCONIA INTERMEDIA* DISTANT (HEMIPTERA: MIRIDAE) AND TELEONEMIA SCRUPULOSA STÅL (HEMIPTERA: TINGIDAE) ON *LANTANA CAMARA* (VERBENACEAE) IN SOUTH AFRICA

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Abstract

Lantana camara L. (Verbenaceae) remains a highly invasive and ecologically damaging weed in South Africa, despite some 50 years of biological control efforts. Lack of success has been ascribed to varietal differences, climate and predation of agents but these have not been tested. In this study, the effects of ant predation were tested on populations of three biological control agents for *L. camara*.

Colonies of two species, *Crematogaster* sp. 1 and 2 were investigated. *Crematogaster* sp. 1 colonies were offered no choice between immature stages of the agents *Hypena laceratalis* Walker (Lepidoptera: Noctuidae), *Falconia intermedia* Distant (Hemiptera: Miridae) or *Teleonemia scrupulosa* Stål (Hemiptera: Tingidae) on lantana shoots. Density-dependent predation on *F. intermedia* and *T. scrupulosa* nymphs on lantana shoots was tested using *Crematogaster* sp. 2 colonies. In choice experiments *Crematogaster* sp. 2 colonies were offered *F. intermedia* or *T. scrupulosa* nymphs on potted lantana plants. Preliminary food trials confirmed that colonies foraged for protein, thereby validating results of no-choice experiments.

Crematogaster sp.1 foragers removed 50% of *F. intermedia* nymphs, followed by 45% of *H. laceratalis* larvae and only 9% of *T. scrupulosa* nymphs. Foragers recruited most actively to *H. laceratalis* larvae and significantly more *H. laceratalis* biomass was removed than either *F. intermedia* or *T. scrupulosa*. A trade-off existed in prey size selection because larger larvae provided considerably more biomass but required forager cooperation and a longer time to subdue than did smaller prey. This increases both forager energy expense and mortality risk by other predators. This study showed that all Crematogaster sp. 1 colonies removed small (≤10mm) *H. laceratalis* larvae more frequently than larvae larger than 10mm. Thus, of these biological control agents, predators probably prefer small *H. laceratalis* larvae.

Significantly more *F. intermedia* than *T. scrupulosa* nymphs were removed by *Crematogaster* sp. 1, while *Crematogaster* sp. 2 colonies removed comparable numbers of both agent species. *Falconia intermedia* nymphs' fast movement triggered a predatory response by these ant species. In contrast, the relatively

immobile behaviour of *T. scrupulosa* nymphs was identified as a highly effective predator avoidance strategy. Since *T. scrupulosa* nymphs are unable to escape predators by moving, they appear to depend on the presence of alternative prey attracting predator attention. At high agent and/or forager density, *T. scrupulosa* nymphs attempted escape, but foragers identified them as prey once they moved and caught them. Predation on *F. intermedia* was also density dependent in that at high nymph and/or forager densities, escape routes were congested and nymphs were more easily caught. Survival of *F. intermedia* and *T. scrupulosa* nymphs in particular was low on ant-accessed shrubs in choice experiments and high on ant-excluded shrubs.

It is likely that ants significantly depress *F. intermedia* populations in the field since besides predation, ant foragers probably interrupt *F. intermedia* feeding and ovipositioning. The combination of parasitism and predation on early instar larvae may explain why *H. laceratalis* occurs across lantana's range in South Africa but populations remain low. It is unlikely that *T. scrupulosa* nymphs are habitually preyed on by ant species unless they attract attention by being mobile.

Although biological control of *L. camara* is influenced by climate and physiological defence mechanisms, this study has shown that predation by two ant species severely impacts leaf-feeding agents for *L. camara*. Thus, it is recommended that future selection of additional agents to control lantana should exclude leaf-feeding agents.

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Chapter I

1 General Introduction

The noxious woody shrub, *Lantana camara* Linnaeus (Verbenaceae) of tropical and sub-tropical Central and South American origin, has invaded warm temperate to tropical regions from South Africa to African countries north of the Equator, Hawaii, Australia, New Zealand and Northern China (Henderson 2001, Day *et al*. 2003a). The distribution of lantana in South Africa (Fig. 1.1) extends from the coastal regions of the Southern, Eastern and Western Cape through to regions of the Gauteng, Mpumalanga, Limpopo and KwaZulu-Natal provinces (Oosthuizen 1964; Stirton 1977; Baars & Neser 1999).

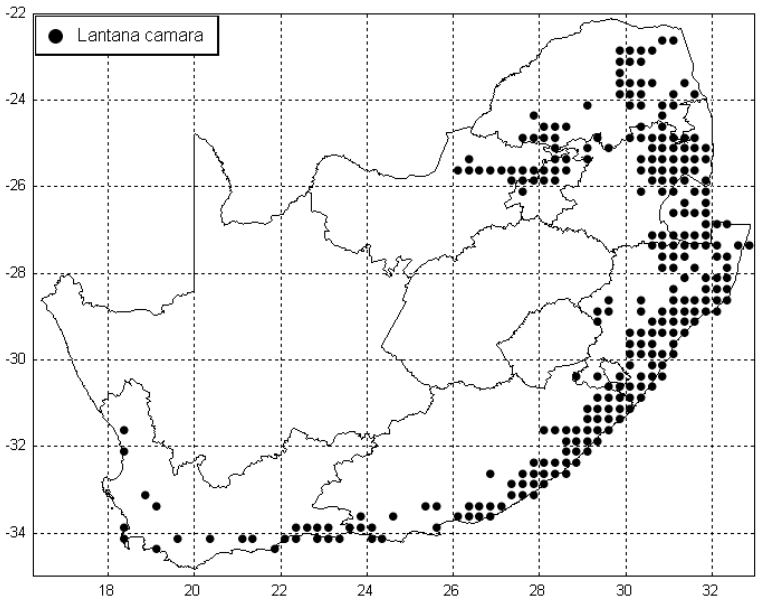

Fig. 1.1: Recorded localities of *Lantana camara* in South Africa (Henderson 2008).

This weed develops into a climbing vine and often forms dense thickets impenetrable to people, animals and vehicles. Besides its propensity to smother and kill native vegetation, *L. camara* can increase soil fertility in areas that normally have low fertility, which encourages further exotic weed invasions, and the woody stems create hotter bush fires (Humphries & Stanton 1992 *In* Day *et al*. 2003a). *Lantana camara* poses a threat to livestock farming in that the leaves of many strains are highly toxic, and this shrub out-competes desirable pasture grass species. This highly invasive weed can be found growing on mountain slopes, in pastures, riverbeds, commercial

and indigenous forests and it is quick to colonise any ecologically disturbed areas (Spies & Du Plessis 1987, Day *et al*. 2003a).

1.1 Biology of Lantana camara

Introduced to South Africa as an ornamental plant, the cultivation of numerous varieties of *L. camara* (for ornamental purposes) has allowed this plant to evolve into a polyploid species complex through both deliberate and natural hybridisation (Stirton 1977, Spies & Stirton 1982). *Lantana camara* is considered an unstable species with numerous varieties, differing in morphology, physiology and genetic composition (Spies 1984) to the extent that some say the varieties should be referred to as distinct weed species (Cilliers & Neser 1991).

The stems, leaves and fruit of *L. camara* contain pentacyclic triterpenes and other toxic compounds, although varieties of *L. camara* differ in degree of toxicity (Neser & Cilliers 1989). If consumed, the toxins cause photosensitivity and more fatally, an obstruction in the liver which interrupts the excretion of bile. This in turn causes ruminal stasis further poisoning the animal and leading to intestinal haemorrhage (Pass 1986). Livestock can die in 1-4 days (Kellerman *et al*. 1996). Cattle lose their appetite; become light sensitive; their skin becomes inflamed (particularly the muzzle, ears and udders) and they may experience paralysis of the legs (Oosthuizen 1964).

Lantana flowers prolifically and the inflorescence colours vary from white, yellow, pink, red to violet and combinations thereof. Original forms of *L. camara* have round, glossy, fleshy fruits that are purple-black when ripe. Feeding, predominantly by birds, on the fruit promotes the rapid spread of *L. camara* (Day *et al*. 2003a, Stock 2005; Heystek 2006).

1.2 Control measures

Lantana camara has been declared a major weed in many regions of the Palaeotropics, and landowners in South Africa are obliged under the Conservation of Agricultural Resources Act (Act no. 43 of 1983), to control lantana.

Common methods of controlling *L. camara* include mechanical (e.g. felling), regular burning, herbicide (foliar, cut stump and coppice growth applications) and biological control. Regular burning, using low- to moderate intensity fires, reduces the number of plants and returns minerals to soil for pasture establishment and assists as a pre-treatment for herbicides (Baars & Neser 1999; Day *et al*. 2003a). However fire is impractical for infestations in or near indigenous forests, plantation areas and mountainous areas. Similarly, not all sites are suitable for mechanical control (e.g. steep hillsides) and reinfestation by regrowth from stumps or seedlings is rapid, rendering the exercise a failure unless followed up by another treatment or complementary control measure (Baars & Neser 1999; Day *et al*. 2003a; Heystek 2006).

Marais *et al*. (2004) estimated the cost of mechanically clearing a high density (75-100%) lantana infested site to be R1000 per hectare and the additional costs in keeping the area cleared were estimated to be R600 per hectare per annum. The cost of clearing the same tract of land using herbicides was estimated as R572 per hectare, with follow-up sprays also required. Thus, burning, mechanical and herbicidal methods often pose an ecological threat to the environment in which they are practiced, and are labour intensive and expensive (Cilliers & Neser 1991; Baars & Neser 1999; Heystek 2006). Biological control is an environmentally compatible and cost-effective alternative, though the pest status of lantana requires an integrated control approach. Municipal clearing of lantana-infested land should allow refuge areas for biocontrol agents and should be complimentary with the population dynamics of the established agents. Recommendations also include physical removal of lantana from initially small areas, accompanied by the planting of fast-growing indigenous species that can increase the shade at a disturbed site to prevent the re-establishment of the weed (Stock 2005).

1.2.1 Biological control

The enemy release hypothesis (ERH) suggests that plant species, on introduction to an exotic location, experience a decrease in regulation by their natural enemies (herbivores, fungi), resulting in a rapid increase in abundance and distribution (Keane & Crawley 2002). The ERH is also referred to as the herbivore escape, predator escape or ecological release hypothesis (Williamson 1996). Successes of classical biological control are often attributed to the ERH in that natural enemies of alien plants are expected to experience similar competitive relief from *their* natural enemies (parasites, pathogens and predators) when released in exotic locations. The ERH predicts that specialist enemies of the study species are absent from the exotic region; host-switching by specialist enemies of native congeners will be rare and generalists have greater impact on the native competitors (Keane & Crawley 2002). Unfortunately, while the ERH may apply to *L. camara* in South Africa, it does not seem so for the natural enemies of this potent weed. Alien plant species that become invasive outside their native region often have other intrinsic properties (e.g. allelopathic advantage and/or faster uptake of limiting resources) that promote their success over competitors (Spies & Du Plessis 1987, Keane & Crawley 2002). Alternatively, human interference (e.g. eutrophication, overgrazing and climate change) can reduce the adaptation of indigenous species to the current environment, thereby decreasing their competitive advantage over exotic species that are better-adapted to the disturbed environment (Keane & Crawley 2002). However, some natural enemies of the weed may not possess competitive traits of typical pest species (e.g. rapid reproduction rate and broad climatic tolerance), without which the ERH allows them to survive but not necessarily thrive.

The first attempt to control *L. camara* by biological methods was in 1902, when Albert Koebele, an entomologist under the Commissioner of Agricultural of the Provisional Hawaiian Government, shipped 23 natural insect enemies of *L. camara* from Mexico to Hawaii. Mr Koebele did not conduct host specificity tests but sent only the insects that he considered safe for release. Many of the insects sent were found to be heavily parasitized upon arrival but eight species became established in the Honolulu area and later spread to neighbouring islands (Perkins & Swezey 1924 *In* Davis *et al*. 1966). *Teleonemia scrupulosa* (nee *T. lantanae* Distant) Stål (Hemiptera: Tingidae) was among the first established insect agents and although

Octotoma scabripennis Guérin-Méneville (Coleoptera: Chrysomelidae) was also introduced in 1902, it did not become established in Hawaii (Swezey 1925). Swezey (1924 *In* Davis *et al*. 1966) noted that the combined attack by different insect guilds greatly reduced seed production and thereby the potential spread of lantana. Yet despite extensive efforts since 1961 in South Africa, *L. camara* is still not under the desired level of control. It is still spreading, invading pristine areas, and the overall impact of its natural enemies is considered negligible (Baars 2002). Fungal pathogens are thought to have great potential as agents against invasive weeds, yet they remain underexploited (Thomas & Ellison 2000). To date only one fungus, *Mycovellosiella lantanae* (Chupp) Deighton (Mycosphaerellaceae), has been released in South Africa for the control of lantana (Den Breeÿen 2004). Over the last century, 24 agents have been introduced to South Africa to control *L. camara*, and although the 14 species described in Table 1 became established, few have significantly suppressed the spread of *L. camara* (Day & Neser 2000, Baars 2002; Zalucki *et al*. 2007). *Teleonemia scrupulosa*; *O. scabripennis* and *Uroplata girardi* Pic (Coleoptera: Chrysomelidae) are the only consistently effective agents in South Africa (Cilliers & Neser 1991; Baars 2003). New potential agents such as *Longitarsus columbicus columbicus* Harold (Chrysomelidae: Alticinae) and *Coelocephalapion camarae* Kissinger (Coleoptera: Brentidae) are continuously being sought to target different parts of the plant and to offset the population crashes incurred every winter (Baars 2001; Baars 2002 Baars & Heystek 2003; Heystek & Olckers 2003; Baars *et al*. 2006).

Table 1: Status of agents successfully established on *Lantana camara* in South Africa (Baars and Neser 1999; Zalucki *et al*. 2007).

Order/Family	Natural enemy	Origin	Released	Mode of attack	Status	Damage inflicted
Coleoptera						
Chrysomelidae	*Octotoma scabripennis* Guèrin-Mèneville	Mexico via Hawaii via Australia	1971	Leaf miner	Established in warm moist eastern areas, locally abundant inland	Extensive defoliation localised
	Uroplata girardi Pic	Paraguay via Hawaii via Australia	1974; 1983	Leaf miner	Abundant in warm moist inland KZN regions in low numbers	Extensive coastal defoliation
Diptera						
Agromyzidae	*Calycomyza lantanae* Frick	Trinidad via Australia; Florida, USA	1982; 1989	Leaf miner	Widely established in low numbers. Heavily parasitized	Unknown
	Ophiomya lantanae (Froggatt)	Mexico via Hawaii, RSA	1961	Leaf miner	Widely established, abundant	Low impact, high parasitism
	O. camarae Spencer	Florida	1997	Leaf miner	Established	Effect unknown
Hemiptera						
Tingidae	*Teleonemia scrupulosa* Stål	Mexico via Hawaii via Australia via Mauritius; Florida, USA	1961; 1971; 1984; 1989	Flower and leaf sucker	Widely established in large numbers across lantana range, severe sporadic damage	Complete defoliation & complete abortion of flowers
Miridae	*Falconia intermedia* Distant	Jamaica	1999	Leaf sucker	Established on eastern coastal belt and warm areas	Severe when high infestations
Lepidoptera						
Gracillariidae	*Aristaea onychote* (Meyrick)	Africa	N/A	Leaf-blister miner	Widely established but present in low numbers	Possible high parasitism levels
	Cremastobombycia lantanella Busck	Mexico		Leaf miner	Established	Minor damage
Noctuidae	*Hypena laceratalis* (Walker)	Africa; Asia; Australia	N/A	Leaf feeder	Widely est., damage to seedlings and new growth	Larvae active from late summer, high parasitism
Pterophoridae	*Lantanophaga Pusillidactyla* (Walker)	Mexico via Caribbean	1984	Flower and seed feeder	Established range unknown; present in low numbers	Low abundance and possible high parasitism
Pyralidae	*Salbia haemorrhoidalis* Guenèe	Cuba via Hawaii	1962	Flower and fruit feeder	Widely established in low numbers	Unknown
Tortricidae	*Epinotia lantana* (Busck)	Hawaii	1984	Flower peduncle & shoot tip borer	Established range unknown	Insignificant contribution to control
Mycosphaerellaceae						
	Mycovellosiella lantanae (Chupp)	Brazil	2001	Leaf pathogen	Established	Effect unknown

1.2.2 Establishing biocontrol agent populations

Poor establishment of, and control by, biological control agents of *L. camara* in South Africa is attributed to: agents having high host specificity that maladapted them to lantana's extreme genetic variability in the country of introduction; the broad climatic range that lantana invades; high levels of parasitism and predation on the biological control agents established on lantana (Cilliers & Neser 1991, Baars & Neser 1999; Day & Neser 2000; Baars 2003).

It is widely accepted that natural enemies can co-evolve with their hosts to the degree that certain strains of natural enemies thrive only on certain strains of the host species. Since lantana strains were introduced from several different countries of origin, it is challenging to find appropriate biological control agents for all the different varieties in South Africa. Hybridisation of varieties has exacerbated the problem. Differing levels of toxicity in the varieties of lantana can conceivably also render certain natural enemies impotent (Neser & Cilliers 1989; Day & Neser 2000). Genetic variability of *L. camara* presents natural enemies with both morphological and physiological barriers to exploitation. Genetic diversity has allowed this tropical weed to extend its distribution into temperate habitats where the plant loses its leaves in winter, contrary to the non-deciduous condition in tropical habitats (Cilliers & Neser 1991; Baars & Neser 1999).

Lantana has become naturalised across a broad climate in South Africa, from the temperate winter rainfall regions to the tropical, summer rainfall provinces and from the coast to areas that are over 1500m above mean sea level (Henderson 2001; Heystek 2006). Whereas, many of the established control agents have restricted distributions, favouring warm, wet regions at low altitudes (Baars 2002, Heystek 2006). At the end of each winter, biological control agents' population numbers are typically low since insects either over-winter in diapause (as eggs and/or adults), or continue developing and reproducing at a slow metabolic rate (Julien 1995; Byrne *et al*. 2002). Winter can further affect the mortality of leaf-feeding agents (50% of established agents for *L. camara*) since some *L. camara* varieties in cold, dry regions of South Africa lose their leaves, depriving foliage feeders of food and shelter (Day & Neser 2000; Baars & Heystek 2003; Heystek & Olckers 2003). Variability in the

availability of a host's resources can alter an insect's survival, fecundity and development rate, and thus the intrinsic rate of increase of the insect population (Gassmann 1996; Baars 2002). Furthermore, while lantana recovers from its deciduous, dormant winter state fairly quickly, there is a lag in agent population recovery (Broughton 2000; Day *et al*. 2003b). This discrepancy is due to lantana compensating for leaf loss by energy reserves available in its root mass (Broughton 2000; Stock 2005), whereas the rate of insect reproduction (number of generations per annum) is positively related to ambient temperature. As a result populations of many agents in the field reach 'effective damaging levels' only by mid-summer (Baars 2002).

Other major factors thought to hinder the establishment of biological control agents for *L*. *camara* are parasitism and predation on the agents by native natural enemies (Cilliers & Neser 1991, Baars & Neser 1999; Baars 2002; Heystek 2006). Worldwide, there have been numerous reports of parasitism of lantana insects, particularly dipteran and lepidopteran agents (Davis *et al*. 1966; Day *et al*. 2003a; Baars & Neser 1999; Broughton 2000). In South Africa parasitism, of lepidopteran biocontrol agents in particular, has significantly impeded the programme's success (Neser & Cilliers 1989; Cilliers & Neser 1991; Baars & Neser 1999; Baars 2003; Heystek 2006). Indigenous parasitoid species were reared from all but one moth species, *Lantanaphaga pusillidactyla* (Walker). Their damage to lantana was rated 'minimal' unless populations were abundant, which was seldom the case (Baars & Neser 1999; Baars 2003).

It is suggested that parasitism is more likely to impact species whose intrinsic reproductive potential is the limiting factor affecting population expansion because parasitism commonly occurs at the earliest life stages, when the agents are most vulnerable (Day *et al*. 2003a). However, susceptibility of agents to parasitism and the degree to which parasitism limits agent populations have rarely been studied and accounts in the literature are conflicting (Day *et al*. 2003a). This has led some to view parasitism as an unsupported reason for failure of agents, since screening in quarantine should prevent diseases and parasites from being released with the agents (Buckingham 1992; Day *et al*. 2003a). Technically this should be true; however it is more likely that introduced agents are attacked by native parasitoid species that have

wide host ranges or that parasitise indigenous species closely related to the agent (Cilliers & Neser 1991; Day *et a*l. 2003a). In the latter case, the effects of parasitism could be mitigated by ensuring that agents selected have no comparable species native to the countries of introduction (Harris 1980 *In* Day *et al*. 2003a). However, lantana-feeding insect species of American origin are seldom closely related to Old world species. Thus, any parasitism in the target country is likely to be by generalist species – making it very difficult to predict which biocontrol agents will escape either parasitism or predation (Day *et al*. 2003a).

1.3 Predatory behaviour of ants

Ants are highly efficient predators and due to the co-operative behaviour of the individuals of many species when foraging, they can prey on organisms more than 10 times the mass of an individual ant (Traniello 1989) and could decimate a small insect population once discovered. Thus, species of biocontrol agent that lay their eggs in clumps or the larvae/nymphs of which are gregarious, may be particularly susceptible to predation by recruiting species of ants. Hawkins (1988) examined parasitoid species richness patterns in Great Britain. He found that host-plant architecture and the herbivores' feeding niches were the major determinants of parasitoid species diversity. Hawkins (1988) states that the following three factors interact simultaneously to determine parasitoid species richness: degree of concealment, the extent of physical protection by host-plant tissue and the herbivores' mobility. Well concealed herbivore species (e.g. stem-, seed-borers and flower-head inhabitants) benefit from low visibility and high physical protection. Though some parasitoid species locate concealed hosts by chemical cues and host sound vibrations, they are often unable to penetrate the plant tissue. Some herbivores are able to escape parasitoids by moving within their host-plant region (e.g. wood-borers). Root-feeding herbivores have the lowest parasitoid diversity because they are both visually concealed and acoustically protected by soil. Relatively immobile herbivores, such as leaf miners, are generally restricted to one leaf and thus unable to escape parasitoids that cue in to the chemicals associated with their mines. Their mines are also highly visible and leaves offer little physical protection from parasitoids. Therefore leaf miners are usually the most susceptible herbivores to parasitoids and hence support

the richest parasitoid diversity. In contrast, and only due to physical protection by host-plant tissue, gall formers support far fewer parasitoid species. Though the galls are highly visible, parasitoids are deterred or slowed by the texture and thickness of the gall tissue and parasitoid efforts can be wasted since galls do not reveal whether or not they are inhabited by the herbivore host. Finally, Hawkins (1988) found that parasitoid richness increased with decreasing mobility of herbivore hosts. Many parasitoids locate their hosts by chemical cues produced by the host-plant when herbivores feed or by kairomones in the frass of herbivores. Thus, herbivores that can move away from such cues should be more difficult to locate by parasitoids. Although the above reference refers to attack by parasitoids, the principles are applicable to ant predation, as confirmed by similar results using ant species (Fowler & MacGarvin 1985; Ito & Higashi 1991).

It has long been thought that predation is more intense in tropical than in temperate ecosystems. The most commonly cited evidence is that important predatory taxa are more diverse in the tropics (Dyer 2002). Although ant predation varies seasonally in temperate forests, evidence suggests that no such variation exists in tropical forests (Horstmann 1975, 1977 *In* Floren *et al*. 2002; Harada 2005) or in South Africa (Robertson 1985). This equates to higher predation pressure by ants in the tropics. Floren *et al*. (2002) investigated arboreal ant species as key predators in a Malaysian tropical lowland rainforest, in trees that are neither myrmecophilous nor myrmecophytic. They found that predatory ant species always represented more than two thirds of the ant individuals in a tree. *Crematogaster* species comprised 21 of the 143 ant species collected. Of these, 16 species were predaceous (76%). Floren *et al*. (2002) found ants to be the most abundant and important predators in tropical lowland forest canopies, in the lower vegetation and at ground level. Two size classes of caterpillar (1cm and 2-2.5cm) were offered to ants experimentally. Ants attacked large caterpillars (2-2.5cm) in 51% of the trials, whereas in 40% of the trials ants ignored large caterpillars, attacking small caterpillars (1-2cm) instead. In only 9% of the trials were caterpillars ignored entirely. Ants preyed on other species of caterpillar, whether they were hairy or not and they preyed intensively on other arthropods (termites and leaf mining insects).

1.3.1 Ant predation on biological control agents

Several species of biological control agents have not attained population sizes that result in damaging levels of herbivory, or simply have not established due to mortality and/or disturbance by native general predators, particularly ant species (Hoffmann 1982; Robertson 1985; Robertson & Hoffmann 1989; Taylor 1989 *In* Broughton 2000; Cilliers & Neser 1991; Kluge 1994; Kluge and Caldwell 1996; Hoffmann *et al*. 1998; Martinez-Ferrer *et al*. 2002; van Klinken *et al*. 2003; Boughton & Pemberton 2008). However, few studies have been conducted that quantify the extent to which ant predation could affect agent populations. A study by van Klinken *et al*. (2003) assessed the establishment of two biocontrol agents on *Prosopis* species (Mimosoideae: Leguminosae) in Australian rangelands. While the leaf-tying moth, *Evippe* sp. (Lepidoptera: Gelechiidae) established across Australia where releases were made, the hemipteran agent, *Prosopidopsylla flava* Burckhardt (Hemiptera: Psyllidae) failed to establish at most sites. Strong circumstantial evidence suggested ants as an important factor in preventing establishment. The nymphs of *P. flava* are free living and produce no excretions that may reduce predation by ants which were abundant at all release sites. A poor match in relative humidity was the other possible explanation, though microclimate was expected to have a moderating effect. Nymphs live between appressed pinnules of growing tips distorted by nymphal feeding, which provide a humid microclimate. However, this cryptic behaviour was not sufficient to protect them from foraging ants.

An arboreal ant species, *Pseudomyrmex gracilis* (Fabricius), was implicated in the failure of establishment of *Austromusotima camptozonale* (Hampson) (Lepidoptera: Crambidae), a foliage-feeding moth agent for the control of *Lygodium microphyllum* (Schizaeaceae) in Florida (Boughton & Pemberton 2008). On several occasions the ant was seen preying on late instar *A. camptozonale*. Within 10-15 minutes of opening boxes to release the agent, *P. gracilis* had infested *L. microphyllum* clumps and either consumed *A. camptozonale* larvae immediately or (more commonly) carried them away, presumably to their nests. Other predatory ant species encountered at the release sites were *Pseudomyrmex ejectus* (Smith), *Crematogaster pilosa* (Emery) and *Camponotus planatus* Roger (Boughton & Pemberton 2008).

Studies have shown that predation on biocontrol agents, especially on their eggs, can be an important factor suppressing population growth of biocontrol agents in South Africa (Pettey 1948; Hoffmann 1982; Robertson 1985; Cilliers & Neser 1991; Kluge 1994; Kluge and Caldwell 1996; Hoffmann *et al*. 1998; Heystek 2006). Failure by the arctiid moth, *Pareuchaetes pseudoinsulata* Rego Barros to establish on triffid weed, *Chromolaena odorata* (L.) King and Robinson, in South Africa was ascribed to predation by the rich native ant fauna (Kluge 1994; Kluge and Caldwell 1996). Kluge (1994) looked at the fate of *P. pseudoinsulata* eggs at three sites near Durban, South Africa. He discovered that 65%; 65% and 52% of the eggs were eaten by ant species. Ant genera implicated as preying on the eggs were four species of *Lepisiota* Santchi (nee *Acantholepis* Mayr); *Anoplolepis* (one species); *Camponotus*; *Crematogaster* (two species); *Plagiolepis* (one species); *Myrmicaria* (one species); *Polyrachis* (one species) and *Tetroponera* (two species). Eggs of *P. pseudoinsulata* are laid in batches which are typically vulnerable to ant predation since once they are located, the ants will remove every edible egg unless the species has evolved a resistance mechanism to deter egg predation (e.g. unpalatable egg cases). To circumvent this problem, a closely related moth species, *Pareuchaetes aurata aurata* (Butler), was chosen as the next agent for *C. odorata* because it scatters its eggs singly on the ground. This means that ants can only collect one egg at a time if they find them. Unfortunately, *P. aurata aurata* also failed as a result of both ant predation and a microsporidian disease (Kluge & Caldwell 1996).

Similarly, egg predation by ants was an important factor in the failure of the moth, *Tucumania tapiacola* Dyar (Pyralidae) to establish in South Africa on jointed cactus, *Opuntia aurantiaca* Lindley (Hoffmann 1982). The next agent imported to South Africa for the control of *O. aurantiaca* and *Opuntia ficus-indica* (L.) Miller was the pyralid moth, *Cactoblastis cactorum* (Bergroth). Although it damages *Opuntia* species in South Africa, *C. cactorum* provides far less control than predicted and one of the main reasons is egg predation by ant species (Hoffmann *et al*. 1998). Robertson (1985) investigated the role of ant predation, regarding the effectiveness of *C. cactorum* as a biological control agent in South Africa. Robertson's (1985) work was based on an earlier study by Pettey (1948) in which he showed that *C. cactorum* was attacked by ant species at all life stages except the adult. Nearly 40 years later, Robertson (1985) demonstrated that the main cause of *C. cactorum* egg mortality was

predation by the ant species *Crematogaster liengmei* Forel, *Camponotus niveosetosus* Mayr, *Monomorium albopilosum* Emery, *Monomorium* sp., *Pheidole* sp. and *Tetramorium erector*. Predation by these species was found to account for 74% and 72% of *C. cactorum* egg mortality on *O. aurantiaca* in summer and winter respectively. Ant predation by the same assemblage accounted for 57% and 54% of *C. cactorum* egg mortality on *O. ficus-indica* in summer and winter respectively. *Crematogaster liengmei* was observed taking first instar larvae of *C. cactorum* soon after hatching and those that had not yet burrowed into the relative safety of *Opuntia* cladodes (Robertson 1985). Members of the speciose ant genus *Pheidole* and of the ant *Anoplolepis steingroeveri* (Bolton) were major predators of *C. cactorum* larvae post the cladode penetration stage. Other factors responsible for larval mortality at the post-penetration stage were: dispersal, disease, shortage of food and temperature extremes. The most important mortality factor for *C. cactorum* pupae, which pupate in the soil surrounding *Opuntia* stands, was predation by the driver ant, *Dorylus helvolus* (Linnaeus). Robertson & Hoffmann (1989) reported a greater diversity and density of ants visible on *O. aurantiaca*. This may explain why significantly more *C. cactorum* eggs survived on *O. ficus-indica* than on *O. aurantiaca*, since Bownes (2002) reported greater ant predation on herbivores where ant diversity is high.

1.3.2 *Crematogaster* – an influential ant

Ants are one of the most diverse and abundant insect families in the world. They drive most of the terrestrial world as soil turners and energy channellers, and are among the leading predators of small invertebrates (Hölldobler & Wilson 1990). Careful consideration was thus required in choosing an ant species that would be highly indicative of the effect of 'ant predation' on the populations of biological control agents of *Lantana camara* in South Africa. In summary, species of the genus *Crematogaster* were chosen to represent the effect of agent predation because the genus exhibits the following traits: Vast geographic range; large temperature tolerance; among the most abundant arboreal ants in the world; often dominant species in communities; aggressive predators and defenders of their territory (nests and food resources)

The genus *Crematogaster* was first described by Lund in 1831 but accrued numerous names by other authors throughout its vast geographic range from the artic circle to the southern most reaches of Tierra del Fuego, Tasmania and South Africa The lower and upper limits of temperature at which *Crematogaster* foragers will feed are 11-40 °C (France) and 14-62°C (Desert, western United States). Members of this genus are often multi-nested and have the potential for rapid population growth. Workers of several species are reported to be capable of producing females (workers) thelytokously in the physical absence of the queen. This allows and co-ordinates the development of secondary nests (usually in the same tree) to the main nest, in which the queen resides. *Crematogaster* species rank among the most abundant arboreal ants in the world. Species of this genus are often dominant in, and responsible for organising, their local communities. They influence the abundance and composition of other ant species, as well as that of plants and other arthropods. Dominant ant species compete with and displace other dominant ant species, thereby promoting the success of their (different) favoured ant and other arthropod associates (Hölldobler & Wilson 1990).

In tropical Africa, many tree crop pests have patchy distributions through their positive associations with some dominant ant species and their negative association with other dominant ant species. Insects can be arranged in an 'ant impact' hierarchy in which "flitting" insects are more affected than "flying" or endophytic species. Manipulation of the dominant species population levels results in changes in overall population levels of many pests. The competing dominant ant species on Ghanian cocoa farms belong to the genera *Crematogaster* and *Oecophylla* (Leston 1973). A similar mosaic pattern exists in tropical Australia where *Crematogaster* sp. and *Oecophylla smaragdina* (Fabricius) play key roles in determining their communities' composition. In the Florida Keys the arboreal ant species, *Crematogaster ashmeadi* Mayr and *Xenomyrmex floridanus* Emery are dominant in the small mangrove islands, where each competitively excludes the other (Cole 1983).

One way in which *Crematogaster* species influence their communities is by the trunk trails they lay. These are recruitment pheromone trails laid by foraging workers to rich persistent food sources (e.g. aphid herds and seedfalls) and they often link multi-nests (separate from the main nest). However their trails are not exclusive, so other

ant species use these odour trails whether intentionally or accidentally, since the dominant species in a community could override other species trails. In southern Europe, workers of *Camponotus lateralis* (Olivier) sometimes follow *Crematogaster scutellaris* (Olivier) in large numbers to their feeding sites and share the resource (Hölldobler & Wilson 1990). *Crematogaster* species also often engage in parabiosis with other ant species, in which the same nest and odour trails are utilized by colonies of different species that nevertheless keep their brood separate. Colonies of the rainforest species, *Crematogaster limata parabiotica* Forel, are reported to have parabiotic associations with two different ant species: *Camponotus femoratus* Fabricius and *Monacis debilis* (Emery) (Hölldobler & Wilson 1990).

Yet another important way in which *Crematogaster* as a dominant species can affect its communities at the level of other insect orders is by their tendency to harvest aphids and other honey-producing homopterans. They will disturb or kill predators and parasitoids, or anything seemingly disturbing their sugar resource (e.g. honey producing coreid species, aphids). In this way they often reduce the efficacy of natural enemies of scale insects (DeBach *et al*. 1951 *In* Martinez-Ferrer *et al*. 2002; Bownes 2002; Helms & Vinson 2002).

Crematogaster is commonly known as the 'cocktail ant' because they have the unique ability to bring their abdomens over their heads and spray venom into the area where they are biting. Victims as large as humans are quickly deterred from climbing trees in which these ants nest due to the ferocity of their attack *en masse* and small invertebrates are crippled or paralysed by the venom. Members of the genus *Crematogaster* are predominantly arboreal, constructing carton nests of leaf-matter, living under bark or inhabiting hollow branches of live trees (Wheeler 1910; Arnold 1915; Hölldobler & Wilson 1990). They forage in a patrol-like fashion on all foliage around and in contact with the tree in which they nest. Several species of *Crematogaster* have been seen foraging on lantana in Zimbabwe midlands, Mpumalanga, KwaZulu-Natal, and the Eastern Cape (*pers*. *obs*.). Along with 9 other ant genera, *Crematogaster* are frequently found on plants of the family Verbenaceae (Hölldobler & Wilson 1990).

1.4 Aim of this study

The focus of this project is to quantify the extent to which predation by two native *Crematogaster* (ant) species on three insect biological control agents for *L. camara* may impede agent efficacy in South Africa.

Care has been taken in choosing the biocontrol agents investigated, in order to maximise the opportunities for examining effects of predation by ants. The results of this project may then be used when selecting new biocontrol agents, to infer which are more likely to be susceptible to detrimental predation by ants. When choosing which *L. camara* control agents to focus on, a combination of species was selected that represent different life cycles (e.g. holo- and hemimetabolous agent species) as well as species that differ behaviourally in response to ant predation. Other reasons for choosing particular agents are indicated for each species. One leaf-feeding moth *Hypena laceratalis* Walker (Lepidoptera: Noctuidae) and two hemipteran agents, *Falconia intermedia* Distant (leaf sucking mirid) and *Teleonemia scrupulosa* (sap-sucking tingid) were chosen as study species.

Both hemipteran agents are effective at controlling *L. camara* in South Africa wherever large field populations occur. The control agent, *Falconia intermedia*, was released fairly recently, so the affect of predation on this species has not yet been formally investigated. However, Heystek (2003, unpublished data) conducted preliminary experiments in which he exposed some lantana plants, on which *F. intermedia* was cultured, to ants and excluded ants from other plants. He observed nymphs of *F. intermedia* being carried away by *Pheidole* sp. and *Camponotus* sp., and populations of the agent were lower on plants exposed to ants than those from which ants were excluded. These results have not been analyzed so it is unknown whether ant predation can significantly reduce the agent's populations. However, he observed the same genera taking *F. intermedia* nymphs at a study site in Tzaneen (Mpumalanga Province, South Africa). Adults of both *F. intermedia* and *T. scrupulosa* have been seen in webs of a variety of spiders in the Eastern Cape Province (*pers. obs.*). *Teleonemia scrupulosa* is severely damaging to *L. camara* where field populations are high, but it is unknown why populations remain low in other areas (Baars & Heystek 2003). Though the eggs of *T. scrupulosa* are laid in clusters, they are protected by an

egg case and are thus fairly well concealed. However, the nymphs are often gregarious and slow moving, which could make them vulnerable to predation by ants (Johnson & Lyon 1991).

To date there have been few follow-up studies on lepidopteran biological control agents for lantana (Baars 2002; Baars & Heystek 2003), though it is suspected that they are heavily preyed on by ants, as in the case of *C. cactorum* on *O. aurantiaca* (Robertson 1985). *Hypena laceratalis* was chosen for this study, largely based on availability, since other lantana lepidopteran agents were rare in the field, whereas *H. laceratalis* occurs throughout the distribution area of lantana in South Africa. Ants have been shown to preferentially prey on caterpillars up to a certain size (Floren *et al*. 2002) and since this is such a large species (up to 35mm body length) it is possible to test ant predation at a wide range of body lengths.

1.5 Life histories of the targeted agents

1.5.1 *Falconia intermedia* Distant (Hemiptera: Miridae)

This agent was first released in South Africa in April 1999 and thus far appears to be effective only at sites within its preferred climatic zone (Baars *et al*. 2003; Heshula 2005). The eggs of *F. intermedia* are usually laid singly, although occasionally in clusters of up to five eggs. The female inserts eggs into a leaf vein or laminar epidermal tissue on the undersides of leaves and then fastens and covers the eggs with resin-like excrement (Baars *et al*. 2003). The five nymphal instars are highly mobile and cluster gregariously on the undersides of the leaves where they feed. Feeding by the nymphs results in chlorotic spots on the lamina that cause stippling on the upper leaf surface. Extensive feeding damage results in the leaves turning silvery-white, desiccating and abscising prematurely. Adult *F. intermedia* are highly mobile and disperse widely by flying (Baars 2002; Baars *et al*. 2003; Day *et al*. 2003). In addition to dispersing well, *F. intermedia* is a good agent because it has a high rate of population increase (multiple generations per year); adult females produce eggs as long as they live (ca. 3 weeks); high damage levels are achieved by individuals and this agent has a narrow host range (Baars 2002). At high population density, 20 eggs

per leaf were found on average, and leaf damage on a scale of 0-12 was rated as 9. However, the minimum damage at the same sites was sometimes scored at 0.5 (Heystek 2006). In climatically suitable areas, feeding damage can cause a reduction of 40-100 % in fruit production and seedlings are severely damaged (Heystek & Olckers 2003). The main short-coming is that all life stages of *F. intermedia* are dependent on leaves for feeding and moisture (particularly the eggs), such that they experience population crashes in areas where lantana is deciduous in winter or where rainfall is highly variable. However, *F. intermedia* is established in the warm, moist areas of South Africa and the current lack of insect pressure there means this agent should make a significant impact on lantana (Baars 2002). In a study by Heystek & Olckers (2003), parasitism was undetected but predation by spiders and ants was noted.

1.5.2 *Teleonemia scrupulosa* Stål (Hemiptera: Tingidae)

This tingid was one of the first natural enemies deliberately introduced as a biological control agent for lantana in Hawaii (1902), Australia (1936) and to South Africa in 1961 (Heystek 2006). It remains one of the most effective agents, and by the early 1980s populations were established throughout South Africa (Cilliers 1987a&b; Heystek 2006). This species appears to favour sunny exposed sites to very humid conditions (Harley 1973; Heystek 2006). Despite laboratory results indicating preferences for certain varieties (Harley 1973; Cilliers 1987a), *T. scrupulosa* was found on 14 lantana varieties in the field, albeit at differing abundances (Baars & Heystek 2003).

Teleonemia scrupulosa inserts its egg cases into the midrib or main veins of the undersides of leaves, petioles, flower stalks and young stems, with the operculum protruding from the plant tissue. Clusters of 10-30 eggs are laid though not all are necessarily laid at the same time, so hatching may extend over several days (Cilliers 1987a). During summer (24-29°C) the incubation period is 6-8 days but as temperatures drop (16-21°C) incubation increases to 11-22 days and below16°C eggs may over-winter (Cilliers 1987a).

The five nymphal instars are gregarious and can be found congregated on dorsal and ventral sides of lantana leaves. The adults are adept fliers and are active in the summer months (Cilliers 1987a&b; Baars 2002). Damage caused by the feeding of *T. scrupulosa* on lantana leaves is considered severe and indirectly reduces plant growth and flowering intensity (Cilliers 1987b; Baars & Neser 1999; Heystek 2006). Nymphs and adults of *T. scrupulosa* are capable of surviving winter by feeding on small buds on lantana stems (Cilliers 1987a; Baars 2002).

1.5.3 *Hypena laceratalis* Walker (Lepidoptera: Noctuidae)

The green, spherical eggs of this moth are laid singly on the undersides of leaves and on green stems (*pers. obs*). The larvae of *H. laceratalis* are solitary external feeders that move between leaves, and their feeding damage causes epidermal windows to form on the leaves but the upper epidermis is left intact. The larvae are bright green, and small larvae (2-8mm) can be found higher up in lantana stands. As they grow (up to 35mm) they gradually move down, feeding on lower leaves before dropping off the plant to pupate in the leaf litter (Baars 2003; Day *et al*. 2003a). *Hypena laceratalis* is one of two biocontrol agents that are indigenous to Africa and believed to have extended their host range from native Verbenaceae to *L. camara* (Oosthuizen 1964; Cilliers & Neser 1991). This moth occurs throughout the range of lantana in South Africa. In a recent study Baars (2003) found *H. laceratalis* present at all but 5.7% of the sites surveyed. Though most populations were rated 'occasional' and its frequency rated between 'rare' and 'abundant', this species was usually the most abundant lepidopteran at the sampled sites. Their feeding impact was rated as partial- to minor damage where high populations occurred, but the impact was generally rated as minimal over its range. Larvae collected from the field were occasionally infected with a bacterial disease and were frequently parasitized by nine indigenous parasitoid species (Baars 2003).

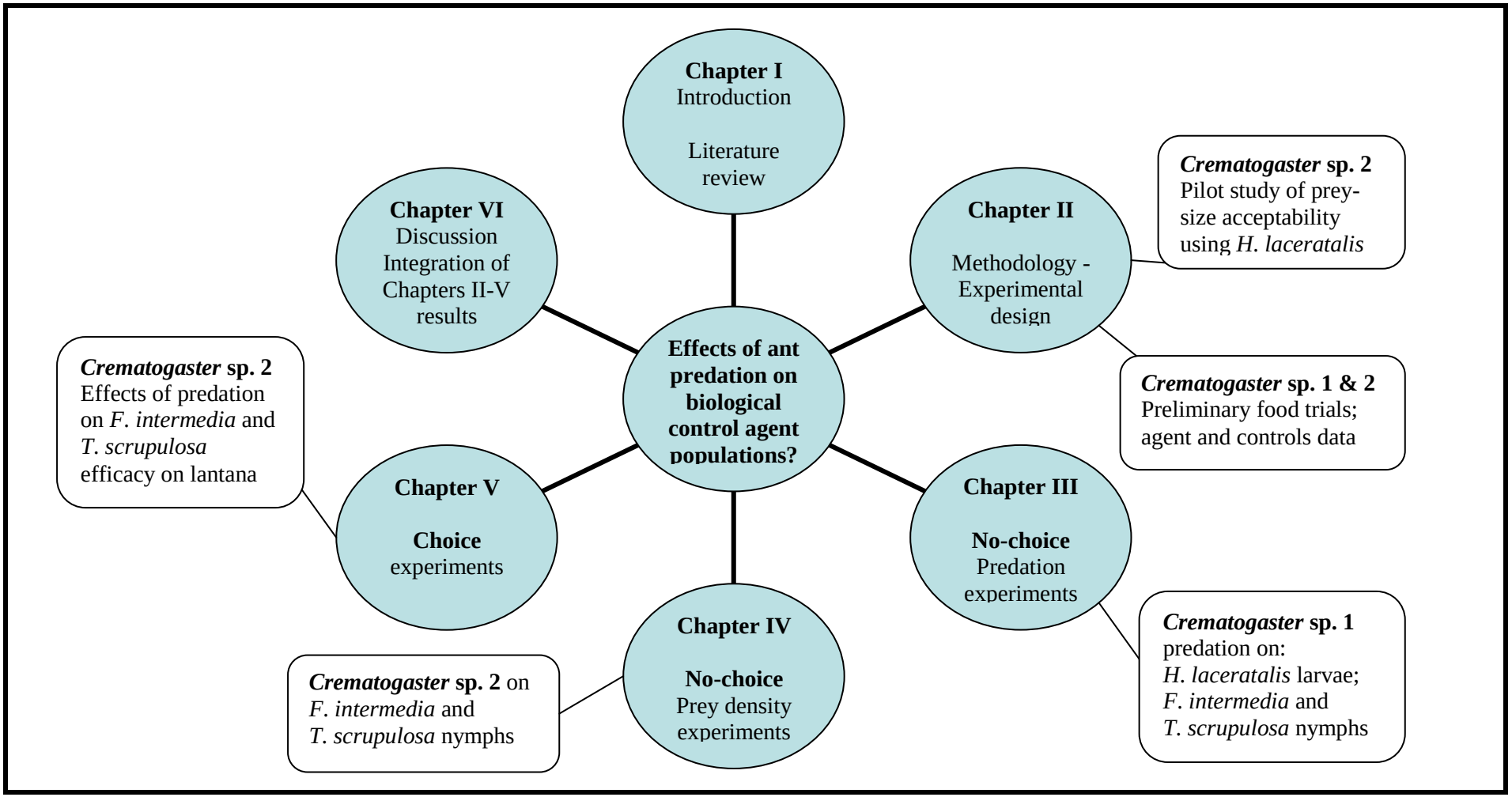

Fig. 1.2: Thesis plan

Chapter II

2 Methodology

Preface

Development of the experimental methods for the remainder of this thesis is discussed in this chapter, as well as recruitment rates by *Crematogaster* sp. 1 and 2 during preliminary food trials. In addition, recruitment rates by both *Crematogaster* sp. 1 and 2 to each biological control agent tested are compared with recruitment to the controls for each experiment. However, predation rates and recruitment rates are compared between agents for *Crematogaster* sp. 1 in chapter III and for *Crematogaster* sp. 2 in chapter IV.

2.1 Ant colonies

A colony of *Crematogaster* sp. 1 was collected from Amanzimtoti, KwaZulu-Natal (30°03’51.12”S; 30°52’44.76”E) and brought to Grahamstown (33°18’42.12”S; 26°31’54.48”E) where it was tested in the laboratory. Once it was evident that the species adapted well to laboratory conditions and could be used in the experimental design, more colonies were sought closer to Grahamstown. The warm-adapted, *Crematogaster* sp. 1 (Fig. 2.1) could be found only as far south as East London (33°58’58.51S; 27°55’08.90”E), from where a further four colonies were collected. As an indicator of diet and behavioural variation within the genus, seven *Crematogaster* sp. 2 (Fig. 2.1) colonies were collected from Bathurst (33° 29′ 0″ S; 26° 50′ 0″ E) and used in both choice and no-choice experiments to compare with the results of the experiments using the tropical *Crematogaster* sp. 1. Predatory arthropods are often more active at higher temperatures (Crawley 1992) and as *Crematogaster* sp. 1 and 2 are of very similar size, it allowed a comparison of potential prey preferences. Workers of both *Crematogaster* species 1 and 2 (Fig. 2.1) measured 4mm in length, though the tropical species, *Crematogaster* sp. 1 appears more robust than *Crematogaster* sp. 2. The taxonomy of the speciose *Crematogaster* genus in Southern Africa was most recently addressed by Arnold (1915) and is in

need of revision. For that reason, the species will be referred to as *Crematogaster* species 1 and 2. *Crematogaster* sp. 2 colonies were labelled A, B, C, D, E, F, G and H (Table 2.1) to distinguish them from *Crematogaster* sp. 1 colonies 1 to 5.

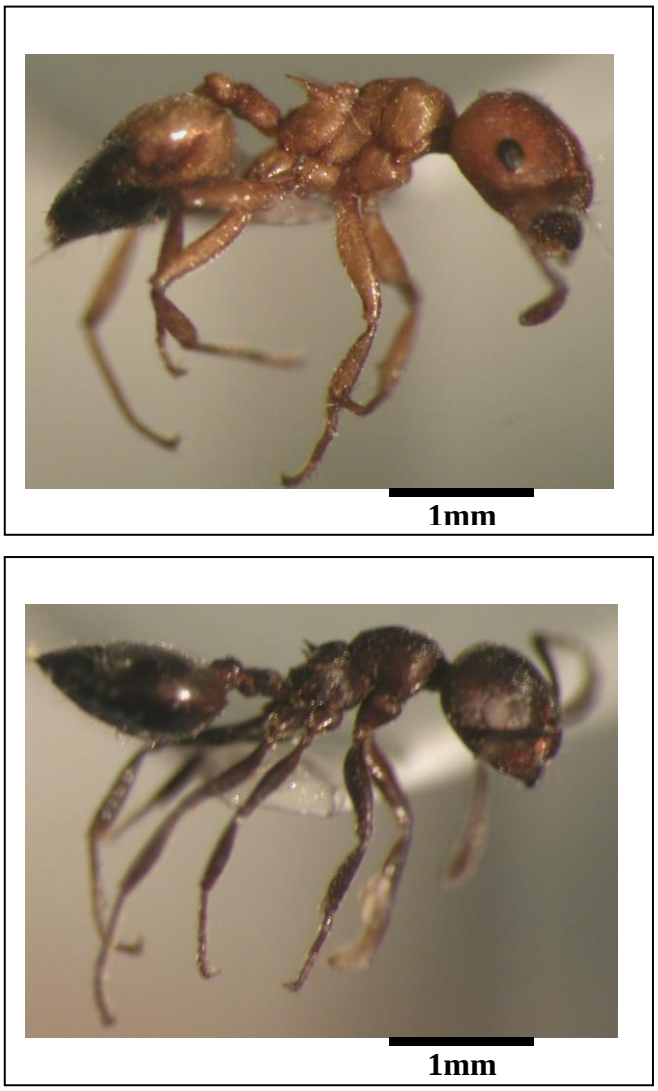

Fig. 2.1: Rust-coloured, Crematogaster sp. 1 (top) and molasses-coloured Crematogaster sp. 2 (bottom) workers.

2.1.1 Quantification of nest sizes

Nest volumes of the colonies used in this study were measured by submerging the carton nests in plastic bags and immersing them in a container (33 X 44cm area) of water. Prior to measurement, a waterline was marked on the container, such that the difference between the new waterline and the original indicated the amount of water (measured in cm) that the nest displaced. The amount of water displaced (cm) was then multiplied by the area of the container to establish the nest volume (measured in

litres). The *Crematogaster* sp. 1 nests used in this study ranged from 1.3 to 10L (Table 2.1) and were likely to contain variable numbers of workers available for foraging, assuming worker density is a simple correlate of nest size, as is the case for other ant species (Mikheyev & Tschinkel 2004, Tschinkel 2005). Nests of *Crematogaster* sp. 2 colonies ranged from 0.9 to 2.36L in volume. The data presented in this chapter were scaled to a common nest volume of 1L to standardise the comparison of agent predation results for *Crematogaster* sp. 1 and 2. With the exception of section 4.3.6 'Comparison of agent predation between *Crematogaster* species' (chapter IV), the data were not scaled in chapters III and IV. These chapters present results for experiments using *Crematogaster* sp. 1 and 2 respectively.

Scaling the data was achieved by dividing the forager recruitment numbers of each colony by their nest volume to determine how many foragers would have responded had it been a 1L volume nest. In taxonomic ant studies (Mikheyev & Tschinkel 2004, Tschinkel 2005), ant nests are destructively censored. However, since *Crematogaster* species carton nests can be large and contain all castes, or are small and dispersed, containing sometimes all castes (as in founder colonies) or only foragers (aerial nests protecting workers foraging far from the main nest), an accurate census of numbers of workers per nest size would require the destruction of many (20>) nests and thus is beyond the scope of this thesis (Wheeler 1910; Arnold 1915).

Table 2.1: *Crematogaster* species 1 and 2 colony sizes, measured by submerging the nests, to determine the nest volume by the amount of water displaced by it.
(Container area X cm water displaced = nest vol. in L).

Colony species and number	**Colony size (L)**
Crematogaster sp.**1** N1	7.26
Crematogaster sp.**1** N2	8.64
Crematogaster sp.**1** N3	10.09
Crematogaster sp.**1** N4	1.31
Crematogaster sp.**1** N5	1.31
Crematogaster sp.**2** NA	1.91
Crematogaster sp.**2** NB	2.36
Crematogaster sp.**2** NC	1.09
Crematogaster sp.**2** ND	1.45
Crematogaster sp.**2** NE	0.91
Crematogaster sp.**2** NF	1.00
Crematogaster sp.**2** NG	2.00
Crematogaster sp.**2** NH	1.77

2.2 Biological control agent cultures

While performing laboratory experiments using *Crematogaster* sp. 1, populations of *Hypena laceratalis*, *Falconia intermedia* and *Teloenemia scrupulosa* were cultured on caged *Lantana camara* plants in a room in the Zoology Department, Rhodes University. Temperatures in this room varied between 33°C during the day and 18°C at night. Grow lux lighting was used with a day length of 14 hours. Agent cultures of *H. laceratalis*, *F. intermedia* and *T. scrupulosa* were later cultured on caged *L. camara* plants in an enclosed, 10X30m plastic-covered tunnel while the prey density experiments using *Crematogaster* sp. 2 were performed. Irrespective of the location, throughout this study, plants were watered daily and replaced before most of the leaves showed signs of significant feeding damage. Adult *H. laceratalis* were provided with sucrose-water on a string hung inside the cage and they were sprayed with a fine mist of water daily to prevent them from dehydrating since adult moths no longer feed on the plant. Adult *T. scrupulosa* were supplied with bouquets of *L. camara* flowers as the pollen promotes fecundity in this species (Radunz 1971 *In* Cilliers 1987a). Great care was taken to ensure that scale insects, especially *Phenacoccus parvus* Morrison (Hemiptera: Pseudococcidae), did not infest the agent cultures since scale insects reduce food quality by feeding on the plants and exude honeydew which covers the surfaces of the leaves, rendering them less accessible for feeding on by the biocontrol agents. *Falconia intermedia* appear to be more affected by the presence of scale insects than do either of the other agents studied here, possibly due to their egg-laying strategy, in which eggs are inserted into a leaf vein or laminar epidermal tissue on the undersides of leaves and then cemented and covered by resin-like excrement (Baars *et al*. 2003). Whereas *T. scrupulosa* eggs too are inserted into the midrib or main veins of the undersides of leaves, the egg operculum protrudes from the plant tissue (as noted in chapter 1), such that the emerging nymph is less likely to be smothered by honey-dew surrounding the egg. In addition, *T. scrupulosa* eggs can also be laid in flower stalks, petioles and young stems, where honey-dew is infrequently excreted (Cilliers 1987a). The eggs of *H. laceratalis* can be laid on the undersides of leaves and on green stems, and their spherical shape makes them unlikely to be completely covered by honeydew.

2.3 No-choice experiment conditions

No-choice, biocontrol agent predation experiments using *Crematogaster* sp. 1 were performed in a laboratory in which the ambient temperature varied between 20 and 26°C. No-choice, prey density experiments using *Crematogaster* sp. 2 were performed in an enclosed 10X30m tunnel in which the temperature range for the duration of these experiments was between 20°C and 28°C. Other than the location in which experiments were performed, the set-up for all no-choice experiments and preliminary experiments was identical. All nests were suspended by string from retort stands. Bunsen-burner stands were used as podiums on which different food choices were made available to the ant nests, via lines of string (walkways) connecting the nests to the podiums (Fig. 2.2). To prevent the ants from escaping, all the stands were surrounded by moats of soapy water. Ants were offered sucrose water; seeds (parrot seed mix) and protein (tuna or termites) daily for colony nutrition and their diet was supplemented weekly (with apple, peach, banana, bread, egg, molasses and freshly killed insects) to accommodate the broad diet of many ant species (Bhatkar & Whitcomb 1970). Colonies always had access to fresh water, located at the base of the retort stand and once a week or on particularly hot days the nests were sprayed with a mist of water to ensure the carton nests did not desiccate. Nests were spaced at a distance apart such that no signs of antagonism from neighbouring colonies were observed. Signs of aggression are easily visible in that members of this genus raise their abdomens vertically, in preparation to spray formic acid from the acidophore (terminal end of the abdomen), in response to negative stimulation. Colonies of *Crematogaster* sp. 1 and 2 were collected as needed and released once tested.

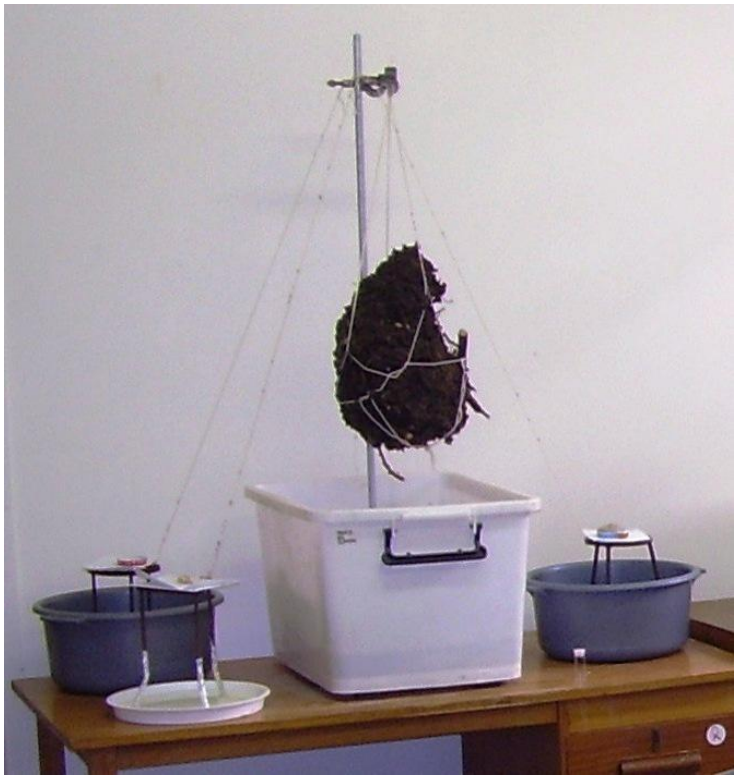

Fig. 2.2: Experimental arrangement of *Crematogaster* species nest in the laboratory, showing string walkways and the podiums on which food sources were offered.

2.4 No-choice experimental design

Biological control agent predation experiments are multi-faceted in that they involve testing the effects of live insect species on other insect species under laboratory conditions. Therefore predation experiments were carefully planned and trials were performed to practise and improve on the methods.

2.4.1 Preliminary experiments

Aims of the preliminary food choice experiments performed prior to all agent trials were to: 1) avoid obtaining false negative results in agent trials by ensuring that ant colonies were indeed foraging for protein at the time, and 2) test the experimental design for flaws prior to testing agents which were at times in scarce supply. Although agent cultures were maintained, prey density experiments required 60 nymphs each and if ant colonies were inactive (which they were occasionally), time would have been wasted setting up the experiments. Preliminary food experiments entailed switching around the food groups available to the ants, namely: sucrose

(sugar); starch and protein and recording the numbers of foragers recruited to the different food sources. Preliminary trials were performed up to two days prior to predation experiments as it is not known how rapidly colony nutritional needs may change.

2.4.2 Units of measurement

The recruitment rate is defined here as the number of ant foragers passing a point on the walkway (10cm from the top of the retort stand) in the direction of a particular treatment during one minute (No. ants per min.). The recruitment rate was recorded ten times per preliminary food trial treatment, making each experiment approximately 30 minutes long. Whereas for all agent predation trials and prey density experiments, recruitment rates of each colony were recorded fifteen times for each of the three treatments. Thus, each experiment took approximately 45 minutes. Recruitment results for *Crematogaster* sp. 1 to food and agent trials are shown in Figures 2.3 to 2.8 and recruitment results for *Crematogaster* sp. 2 in Figures 2.9 to 2.16. Observation of the insects during experiments aimed to determine what agent behaviours (if any) elicited a predatory response in the ants as well as how easily foragers could catch the immature agents once detected. At the end of each experiment the remaining agents were counted to determine how many had been removed during the experiment. The number of agents removed was then divided by the experimental time to obtain the predation rate (No. agents removed per minute).

2.4.3 No-choice experiment procedure

In testing the recruitment to, and predation on, biological control agents by *Crematogaster* sp. 1 and sp. 2 ants, foragers were offered 1) agents on a lantana cutting in a petri-dish; 2) an empty petri-dish (Control 1) or 3) a lantana cutting in a petri-dish (Control 2). The petri-dish kept the cuttings vertical, providing access for agents and ants to dorsal and ventral leaf surfaces. Two terminal leaf pairs without an inflorescence or buds were used as lantana cuttings. The ends of cuttings were wrapped in small pieces of slightly moist paper towel to prevent rapid leaf desiccation, which could disturb agent feeding since two of the agent test species are sap suckers.

Due to varying availability of agents over time, *Crematogaster* sp. 1 colonies were offered 20-30 immature agents. There were two exceptions to this: a shortage of *H. laceratalis* nymphs once meant that only 15 larvae were offered to colony 4, and on another occasion 60 *F. intermedia* nymphs being offered to colony 2. The motivation for the second irregularity was to investigate whether ant recruitment and agent removal increased with increased prey availability (Chapter IV). Similarly, in experiments using *Crematogaster* sp. 1 (Chapter III) the experimental time varied between about 60 and 100 minutes, depending on how slowly the ants were moving. To adjust for these irregularities in time and agent numbers, predation rates (No. agents removed per hour) and percentages of agents removed (Chapter III, Tables 3.1 - 3.3) were calculated and used for comparisons between agents and ant species. It is acknowledged that irregularities are undesirable when comparing data statistically. Thus every prey density experiment (Chapter IV) was performed using exactly the same number of nymphs per density treatment and the same experimental time. The different nymph densities tested using *Crematogaster* sp. 2 were 60; 30 and 10 per two terminal lantana leaf-pairs. In one predation experiment, *Crematogaster* sp. 1, colony 2 was offered 60 *F. intermedia* nymphs and although this colony was tested for double the time of the other colonies (which were offered ca. 30 *F. intermedia* nymphs) foragers did not remove all the nymphs. Thus *Crematogaster* sp. 2 colonies were offered 60 and 30 nymphs to quantify the effect of increased agent density on foraging by ants. Colonies were offered the 10 nymph option to determine whether foragers would recruit less to smaller prey densities.

2.4.4 Agent life-stages offered

Only immature stages of each agent were tested in this study because eggs are difficult to manipulate without the risk of disturbing them (breakage/desiccation) while setting up the experiment. Furthermore, except for those of *H. laceratalis*, eggs of the hemipteran agents are well protected from predation by an egg case and by being covered with frass in the cases of *T. scrupulosa* and *F. intermedia*. Adults were not tested because they disperse too readily (and are therefore not very vulnerable to ant predation) and adult *H. laceratalis* do not feed on lantana. Finally, since the immature stages are potentially vulnerable for the longest duration, it seemed most pertinent to focus on immature agents.

2.4.5 Immature agent size classes

Nymphs of both *F. intermedia* and *T. scrupulosa* were offered in equal proportions from three size classes: small (1st and 2nd instars), medium (3rd instar) and large (4th and 5th instars). Nymphs were weighed according to their size class and their masses (mg) plotted for each species (Figures 2.17 and 2.18). Similarly, a range of *H. laceratalis* larvae (2 – 25mm) to represent those offered to the ant colonies, were weighed and their masses (mg) plotted on a scatterplot (Fig. 2.19). *Hypena laceratalis* larvae and the hemipteran nymphs were weighed using a Satorius Electronic Microbalance (MC1 – 98648-003-48).

2.4.6 Pilot prey acceptability experiments

Hypena laceratalis larvae exhibit several defensive behaviours, namely: wriggling; biting; thrashing of the posterior segments to throw ants away/off the larva and escape - which usually involves finding a leaf edge from which to dangle off from a silken thread while the predator departs. The aims of the experiments were to determine a) an acceptable prey size (larva length) limit that *Crematogaster* sp. 2 colonies (n = 3) could successfully subdue and b) how long it took the ants to subdue the larva. These experiments were performed in the tunnel, using three *Crematogaster* sp. 2 colonies, and as they were performed over the same time period as the prey density experiments, the temperature ranges were the same. The experimental setup entailed a *L. camara* cutting (4 leaf-pairs) fixed vertically on a podium, attached to the nest via a string walkway (as in the no-choice experiments). A larva of either 4mm, 8mm, 12mm or 16mm was placed on a leaf and observed until one of the following conclusions: 1) foragers subdued/removed the larva; 2) the larva escaped or 3) foragers rejected the larva. In accordance with the prey subduing protocol by Dyer (2002), if the larva was touched and rejected by 10 foragers then it was considered undesirable or unmanageable by *Crematogaster* sp. 2 foragers. Once the larva was pinned down by foragers and no longer able to move, it was considered 'subdued'. This was followed by lymph imbibing and dismantling of the larva by foragers to transport it back to the nest.

2.5 Design of choice experiments

These choice experiments were performed using five *Crematogaster* sp. 2 colonies in a 10X30m plastic-covered tunnel where the minimum and maximum temperatures experienced were 6°C and 39°C respectively, although data were captured only between 20 and 28°C. Day length, temperature and humidity were allowed to vary with ambient conditions. Seven potted *L. camara* shrubs having approximately 300 leaves, of the variety from Whitney farm (33°40'43"S, 26°35'49"E) were arranged around a *Crematogaster* sp. 2 colony and all except control 3 were covered by mosquito net material to prevent infestation by flying insects or spiders. Three plants were attached via string to the ant colony. These plants contained 1st and 2nd instars of 80 *F. intermedia* nymphs (FA) or 80 *T. scrupulosa* nymphs (TA) or no insects (control 1). The remaining four plants were inaccessible to *Crematogaster* foragers. Three of these plants also contained 1st and 2nd instars of 80 *F. intermedia* nymphs (F) or 80 *T. scrupulosa* nymphs (T) or no insects (control 2). The third control plant was covered and inaccessible to ant colonies. This control was designed to test whether covered plants grew less due to decreased sunlight. Thus insect survival and feeding activity could be compared between ant excluded plants (F and T) and ant inoculated plants (FA and TA). Similarly, the damage by each insect species to *L. camara* shrubs could be compared with and between control plant 1 (covered, ants excluded), control plant 2 (covered, ants inoculated) and control 3 (uncovered, ants excluded). Plants were given 500ml of water daily and *Crematogaster* sp. 2 colonies were always provided with water and sucrose-water on paper towelling.

2.5.1 Units of measurement

Numbers of agents that survived the week-long experiment were recorded, as well as the percentage feeding activity, estimated by the proportion of leaves displaying feeding damage. The plant growth parameters for each labelled branch (three per shrub) were numbers of leaf-pairs and length (cm), measured one day prior to commencement of the experiments. Numbers of leaf-pairs and branch lengths were recorded on the last day of the experiment and the differences calculated were then analysed statistically. Recruitment rates of foragers to the three treatments (FA; TA and C2) were recorded for 10 repetitions both morning and afternoon for the first five

days of the experiment. On the second afternoon of the experiment, leaves of all shrubs were scouted for five minutes to ensure an acceptable number of nymphs had survived the inoculation procedure. Details of the choice experiment procedures are in chapter V of this study.

2.6 Statistical analyses

Scatterplots were created (using Statistica) to test for significant correlations between the following: nest volume, predation rate and recruitment rates. Predation on each agent was tested using the same five *Crematogaster* sp. 1 colonies. Thus there were five nest volumes in total (n = 5) and each agent predation experiment resulted in one removal rate per colony (n = 5) but 15 recruitment rates (replicates) per experiment. To meet the statistical requirement of comparing equal replicates per variable, correlations were calculated using one 'average recruitment' rate per experiment (n = 5), obtained by dividing the sum of recruitment numbers by 15.

Recruitment and predation rates were not normally distributed, so a parametric test could not be used to analyse these data. The recruitment data is a measure of the frequency of ant foragers passing a point in time, so it is continuous and measured on an ordinal scale, while predation rates of agents are not continuous numbers. The test chosen was a non-parametric ANOVA, namely the Kruskal-Wallis by Ranks and Median Test. Kruskal-Wallis assesses the hypothesis that the different samples in the comparison were drawn from distributions (populations) with the same median or from the same distribution. Thus, the interpretation of the Kruskal-Wallis test is nearly identical to that of the parametric one-way ANOVA, except that it is based on ranks rather than means. Therefore no standard deviation or error figures are reported for the data. Instead, the H-value indicates variability of the data. When viewing the graphs, it should be understood that overlap of data does not exclude the possibility of significant differences between the datasets overall. The Kruskal-Wallis test does not calculate means of the data, so the equivalent of standard deviations are represented on the graphs as boxes (median quartile) encompassing 25% of the values smaller, and 25% greater, than the median value. A small median quartile indicates little variation in the data and vice verse. The median rank represents the value most

frequently recorded for that dataset. The remaining 50% of values (ranks) are distributed as 25% of the values between the minimum value in the data set and median quartile and 25% between the median quartile and the maximum value. The strength of the Kruskal-Wallis test lies in the fact that this test ranks the data before analysing it. Replacing the actual data with ranks reduces variability in the data but does not reduce the number of data points (as is the case when means are used in tests). All data points are compared and the results tend to be more conservative than parametric tests (Rosner 2000).

2.7 Results

When nest size (volume) was compared with average recruitment by each colony to each agent, no significant correlations resulted. Similarly, when nest volume was compared with predation rates by each colony on each agent, no significant correlations were found. Finally, no significant correlations resulted when average recruitment by all colonies was compared with their corresponding predation rates on each agent. Thus it was concluded that nest size (volume) and recruitment did not significantly influence predation rates of the colonies. This legitimised the use of different sized colonies for comparing predation rates by the same species of ant on the agents tested. However, recruitment numbers for each colony have been scaled to those of a 1L nest size by the method described above (2.1.1) for comparing results between *Crematogaster* sp. 1 and 2.

2.7.1 Preliminary food trials using *Crematogaster* sp. 1

A food trial was performed on each ant colony prior to each agent predation trial. The results below are the combined recruitment results of each *Crematogaster* sp. 1 colony (n = 5) to three food sources. Therefore the number of recruitment rates analysed is 50 (5 X 10 recordings) for each of the three food sources (n total = 150 per experiment).

Using a multiple comparisons Kruskal-Wallis test, it was determined that *Crematogaster* sp. 1 colonies recruited significantly more foragers to the sucrose source than to the starch source ($p = 0.0025$), while recruitment by foragers to the protein source was not significantly different to the sucrose, nor starch sources during these preliminary food trials (Fig. 2.3).

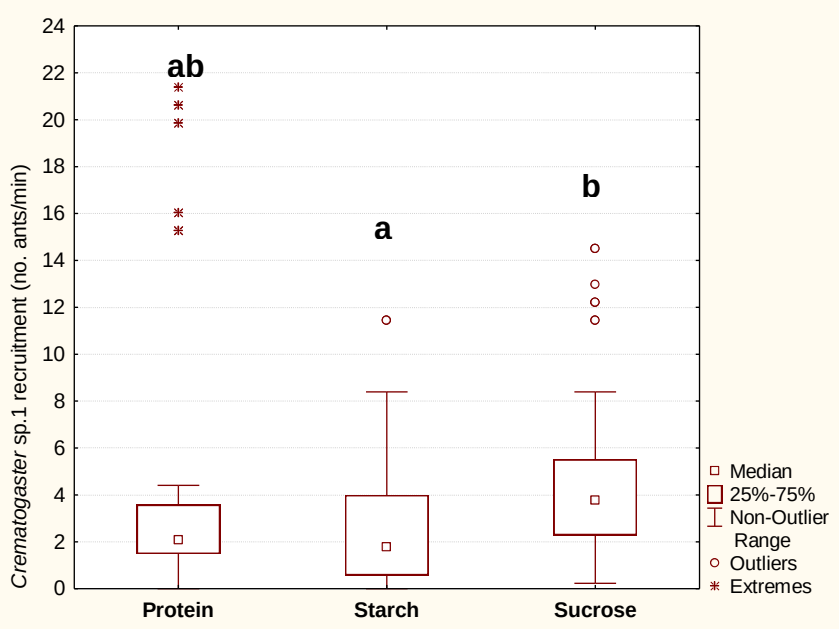

Fig. 2.3: Recruitment rates (scaled to a 1L nest size) of five *Crematogaster* sp. 1 colonies to food sources prior to *Falconia intermedia* predation trials ($H = 11.6562$, $n = 50$, $p = 0.0029$). Letters on graphs indicate significant relationships.

Preliminary food trials showed that prior to *T. scrupulosa* predation trials colonies recruited significantly more foragers to the sucrose source than to both starch ($p < 0.0001$) and protein ($p = 0.0032$) sources. Recruitment rates to starch and protein sources were not significantly different (Fig. 2.4).

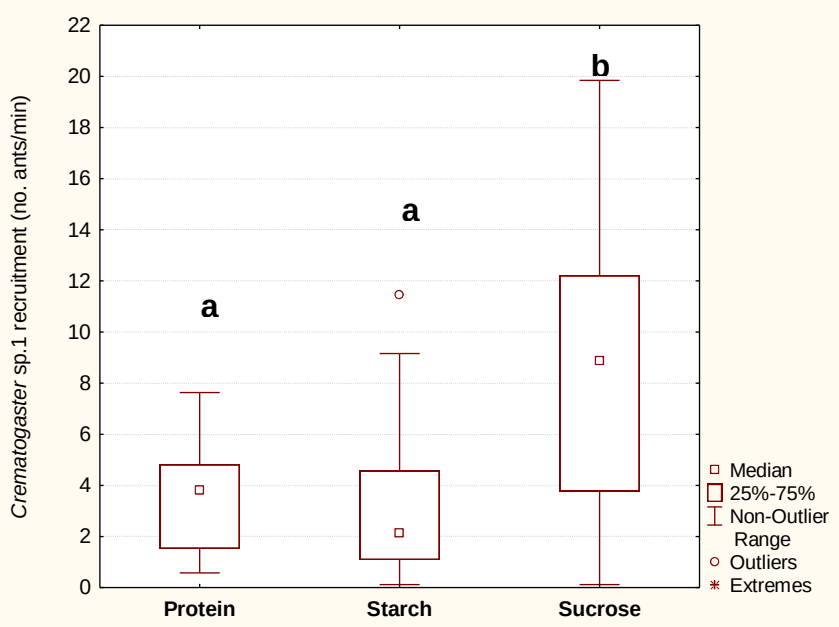

Fig. 2.4: Recruitment rates (scaled to a 1L nest size) of five *Crematogaster* sp. 1 colonies to food sources prior to *Teleonemia scrupulosa* predation trials ($H = 21.6753$, $n = 50$, $p < 0.0001$). Letters on graphs indicate significant relationships.

Similarly, during preliminary food trials for the *H. laceratalis* predation experiments, colonies recruited significantly more foragers to the sucrose source than to both starch ($p < 0.0001$) and protein ($p = 0.0307$) sources (Fig. 2.5). Recruitment to protein was significantly higher than to starch ($p = 0.0006$).

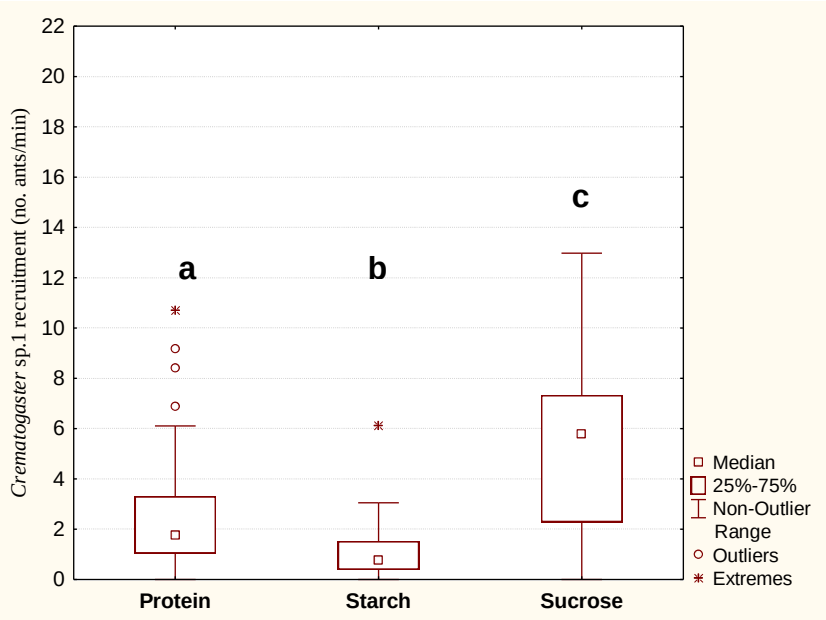

Fig. 2.5: Recruitment rates (scaled to a 1L nest size) of five *Crematogaster* sp. 1 colonies to food sources prior to *Hypena laceratalis* predation trials ($H = 40.2529$, $n = 50$, $p < 0.0001$). Letters on graphs indicate significant relationships.

2.7.2 Agent predation trials using *Crematogaster* sp. 1

The recruitment results for each treatment of the no-choice, agent predation experiments, using *Crematogaster* sp. 1 colonies (n = 5) were scaled for each colony to that of a model 1L colony nest size (*2.1.1*) for comparative purposes and combined for each agent tested. Therefore the number of recruitment rates analysed is 75 (5 X 15 recordings) for each of the three food sources (n total = 225 per experiment).

Recruitment of foragers by *Crematogaster* sp. 1 colonies to *F. intermedia* nymphs (F) and to control 2 (only lantana cuttings offered) exhibited much intra- and inter-colony variation as compared with recruitment to control 1 (empty petri-dish). However, overall recruitment rates of foragers to the treatments did not differ significantly for these agent predation experiments (Fig. 2.6).

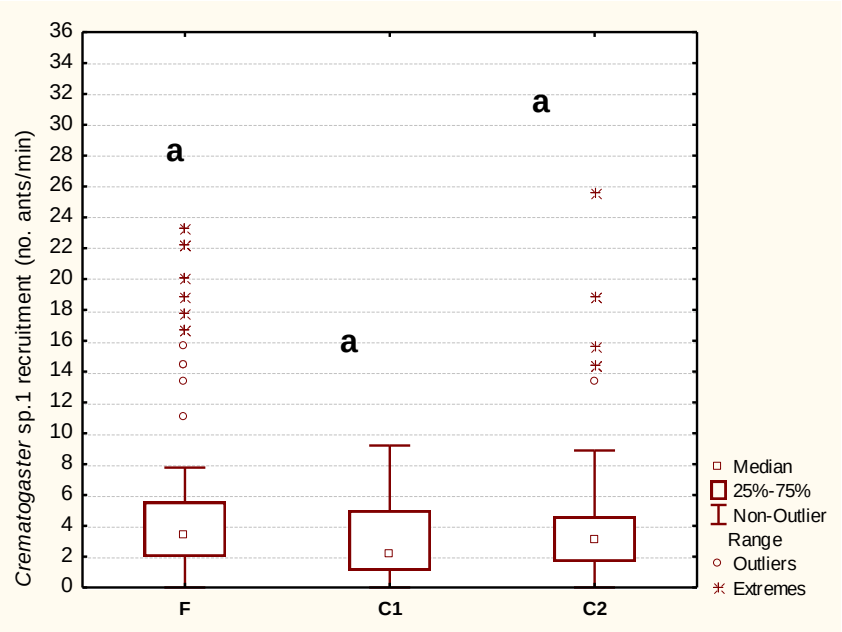

Fig. 2.6: Recruitment rates (scaled to a 1L nest size) of five *Crematogaster* sp. 1 colonies to *Falconia intermedia* nymphs (F) and to Controls 1 (empty) and 2 (leaves only) during no-choice predation experiments (H = 5.02597, n = 75, p = 0.0741). Letters on graphs denote significant relationships.

Forager recruitment varied both between and within colonies for the *T. scrupulosa* treatment in these agent predation experiments. However, when all colonies were analysed together (Fig. 2.7), significantly more foragers were recruited to the *T. scrupulosa* treatment than to control 2 ($p = 0.0231$). Recruitment to control 1 did not differ significantly from recruitment to control 2 or to the *T. scrupulosa* treatment.

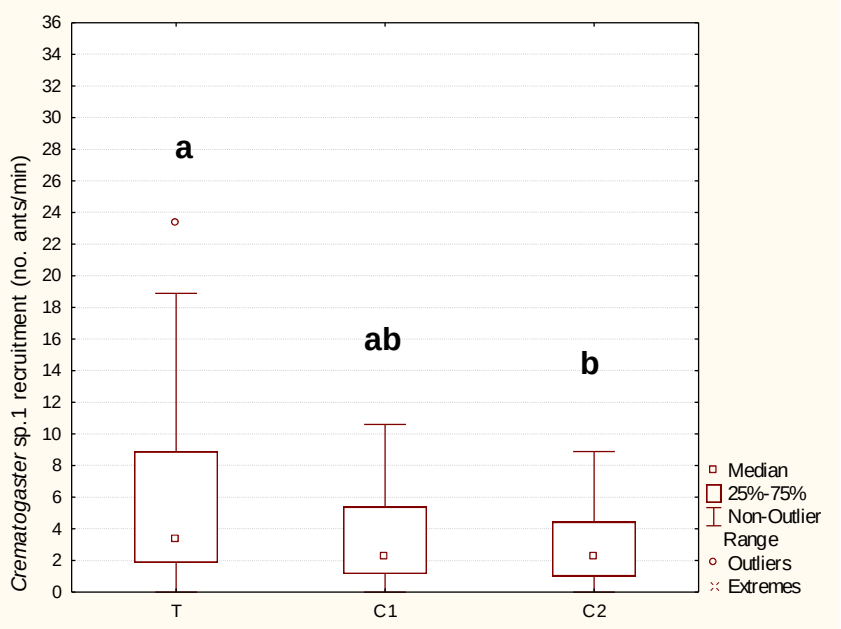

Fig. 2.7: Recruitment rates (scaled to a 1L nest size) of five *Crematogaster* sp. 1 colonies to *Teleonemia scrupulosa* nymphs (T) and Controls 1 (empty) and 2 (leaves only) during no-choice predation experiments ($H = 8.14486$, $n = 75$, $p = 0.017$). Letters on graphs denote significant relationships.

Significantly more foragers were recruited by *Crematogaster* sp. 1 colonies to *H. laceratalis* larvae (Fig. 2.8), than to control 1 ($p < 0.0001$) and control 2 ($p = 0.0007$). Similarly, significantly more foragers were recruited to control 2 than to control 1 ($p = 0.0216$).

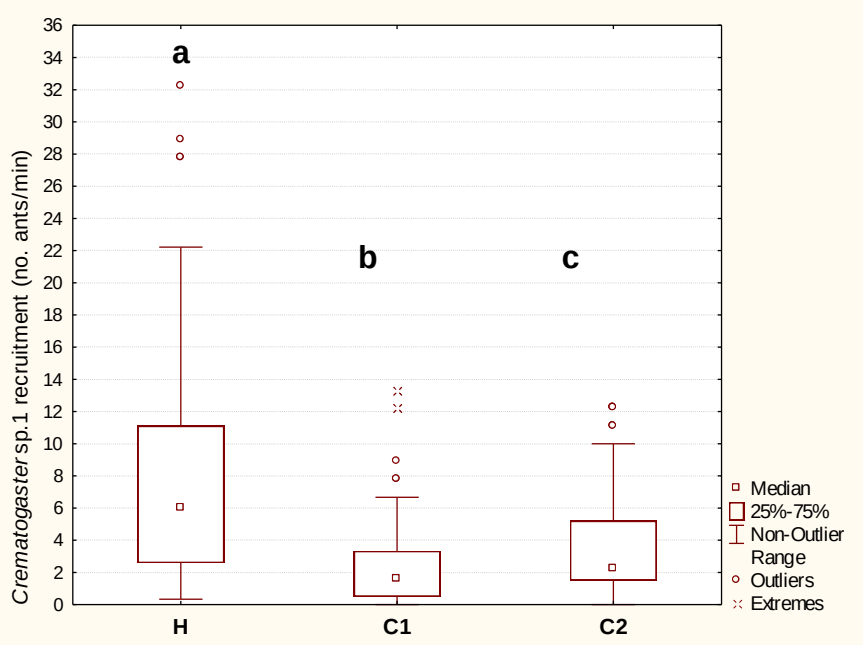

Fig. 2.8: Recruitment rates (scaled to a 1L nest size) of five *Crematogaster* sp. 1 colonies to *Hypena laceratalis* larvae (H) and to Controls 1 (empty) and 2 (leaves only) during no-choice predation experiments ($H = 41.00483$, $n = 75$, $p < 0.0001$). Letters on graphs denote significant relationships.

2.7.3 Preliminary food trials using *Crematogaster* sp. 2

Recruitment rates of *Crematogaster* sp. 2 colonies to protein and sucrose sources were not significantly different (Fig. 2.9). However, significantly more foragers were recruited by colonies to both the protein and sucrose sources than to the starch source ($p < 0.0001$).

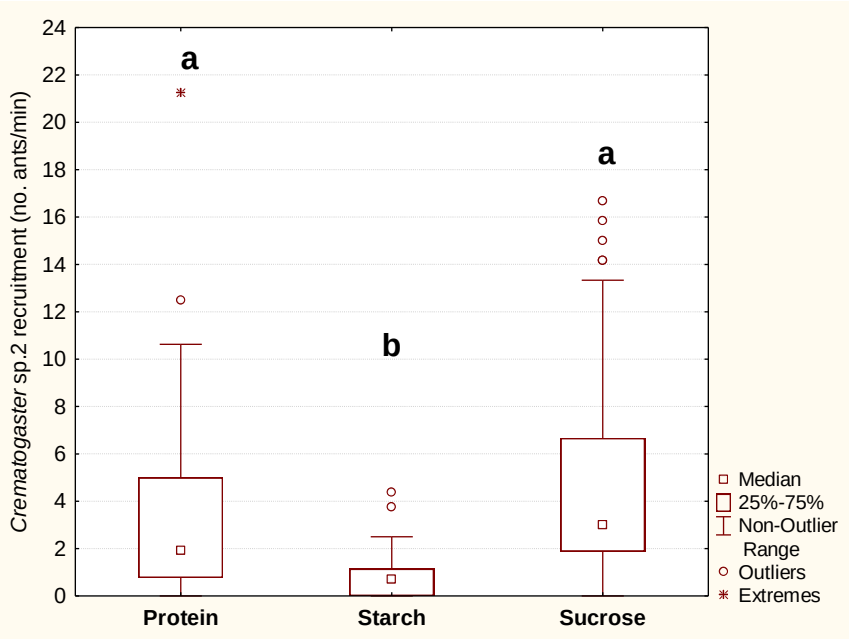

Fig. 2.9: Recruitment rates (scaled to a 1L nest size) of five *Crematogaster* sp. 2 colonies to food sources prior to *Falconia intermedia* prey density experiments ($H = 45.3164$, $n = 50$, $p < 0.0001$). Letters on graphs indicate significant relationships.

In the food trials prior to testing predation on *T. scrupulosa* nymphs (Fig. 2.10), significantly more foragers were recruited to the sucrose source than to either the protein or starch sources ($p < 0.0001$). In addition, significantly more foragers were recruited to the protein than to the starch source ($p < 0.0001$).

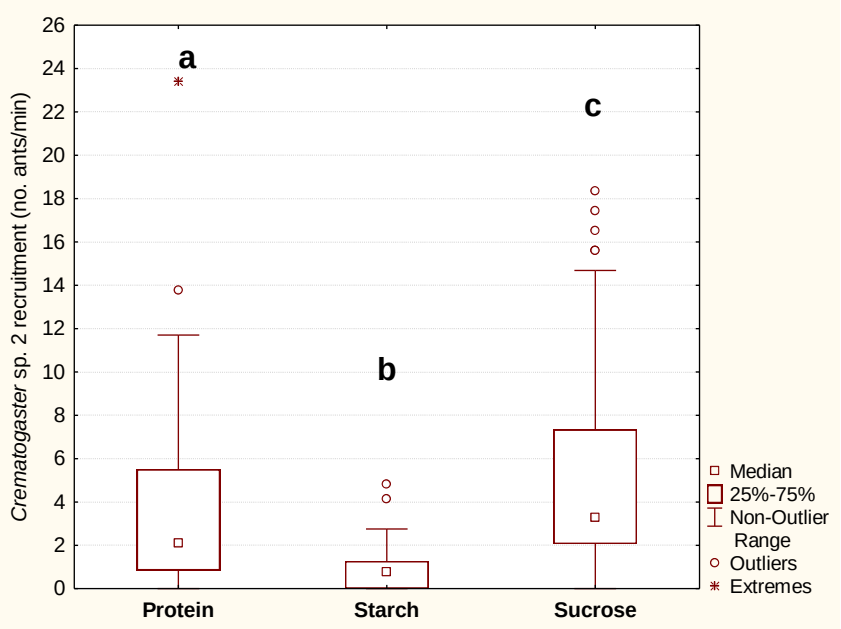

Fig. 2.10: Recruitment rates (scaled to a 1L nest size) of five *Crematogaster* sp. 2 colonies to food sources prior to *Teleonemia scrupulosa* prey density experiments ($H = 31.4767$, $N = 50$, $n = 50$, $p < 0.0001$). Letters on graphs indicate significant relationships.

2.7.4 *Falconia intermedia* density trials using *Crematogaster* sp. 2

Significantly more foragers were recruited by *Crematogaster* sp. 2 colonies to the 10 *F. intermedia* nymphs offered than to either control 1 ($p < 0.0001$) or to control 2 ($p < 0.0001$), while recruitment to the controls did not differ significantly (Fig. 2.11).

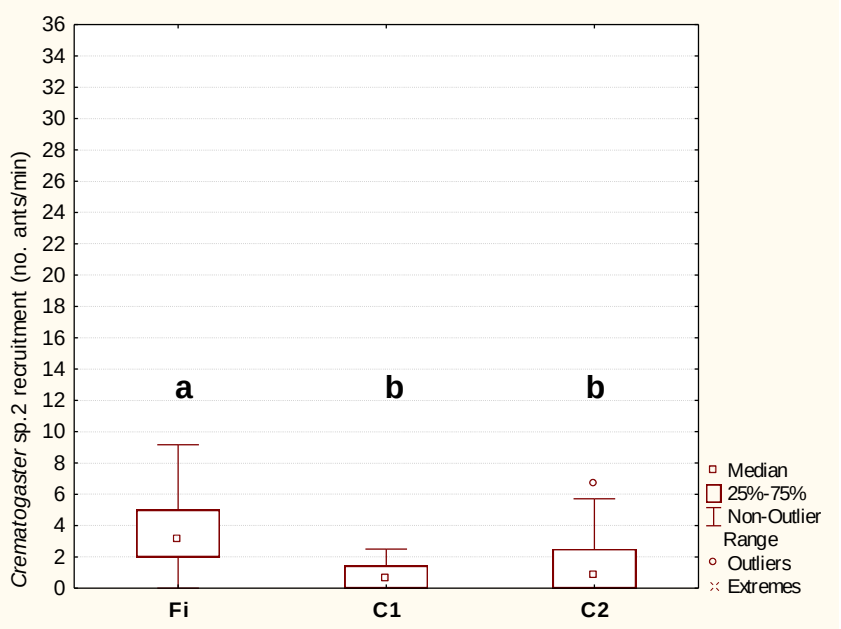

Fig. 2.11: Recruitment rates (scaled to a 1L nest size) of five *Crematogaster* sp. 2 colonies to 10 *Falconia intermedia* nymphs (Fi) and Controls 1 (empty) and 2 (leaves only) during no-choice prey density experiments ($H = 83.3609$, $n = 75$, $p < 0.0001$). Letters on graphs denote significant relationships.

Similarly, significantly more foragers were recruited to the 30 *F. intermedia* nymphs offered than to either control 1 or control 2 ($p < 0.0001$), while recruitment to the controls did not differ significantly (Fig. 2.12).

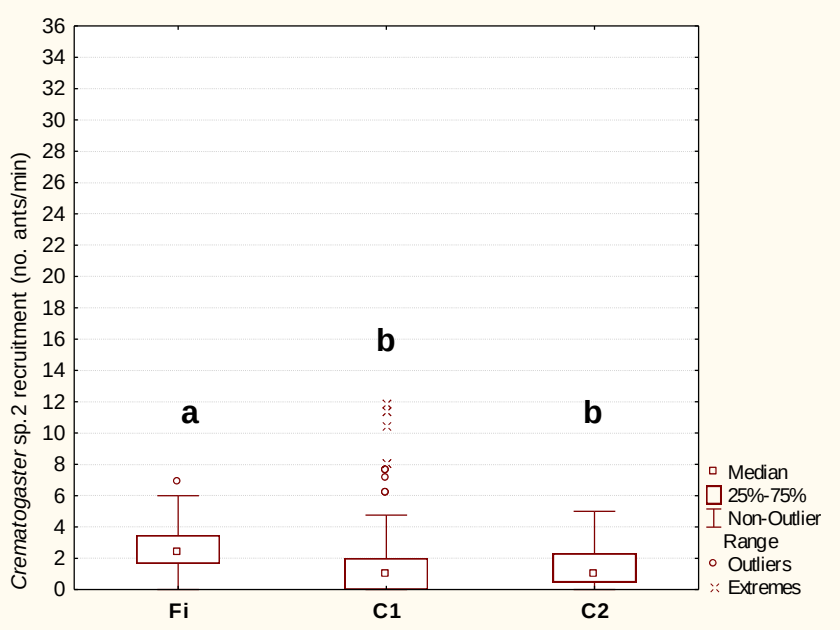

Fig. 2.12: Recruitment rates (scaled to a 1L nest size) of five *Crematogaster* sp. 2 colonies to 30 *Falconia intermedia* nymphs (Fi) and Controls 1 (empty) and 2 (leaves only) during no-choice prey density experiments ($H = 26.9088$, $n = 75$, $p < 0.0001$). Letters on graphs denote significant relationships.

Once again, significantly more foragers were recruited to the 60 *F. intermedia* nymphs offered than to either control 1 or to control 2 ($p < 0.0001$), while recruitment to the controls was not significantly different (Fig. 2.13).

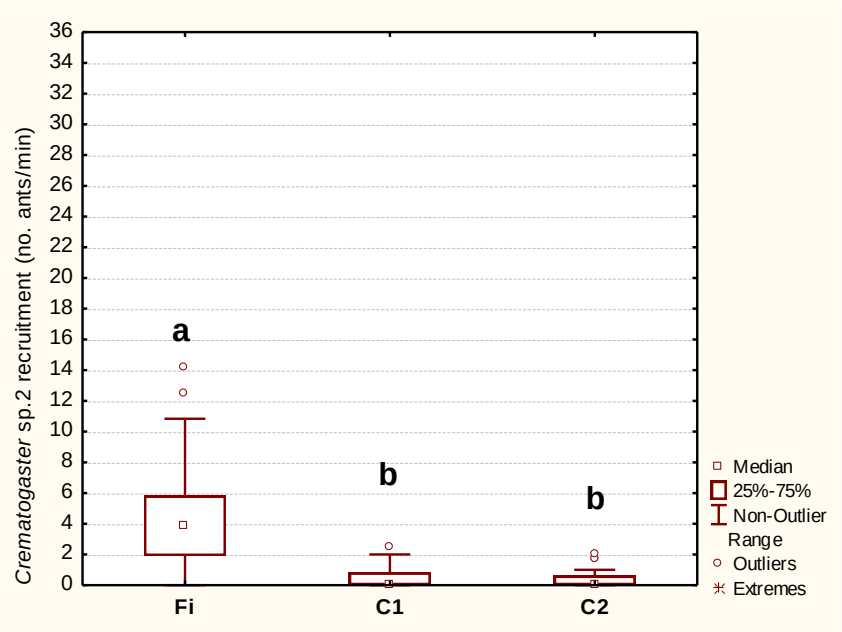

Fig. 2.13: Recruitment rates (scaled to a 1L nest size) of five *Crematogaster* sp. 2 colonies to 60 *Falconia intermedia* nymphs (Fi) and Controls 1 (empty) and 2 (leaves only) during no-choice prey density experiments ($H = 119.0094$, $n = 75$, $p < 0.0001$). Letters on graphs denote significant relationships.

2.7.5 *Teleonemia scrupulosa* density trials using *Crematogaster* sp. 2

Significantly more foragers were recruited by *Crematogaster* sp. 2 colonies to the 10 *T. scrupulosa* nymphs offered than to either control 1 ($p < 0.0001$) or to control 2 ($p = 0.0024$), while significantly more foragers were recruited to control 2 ($p = 0.0113$) than to control 1 (Fig. 2.14).

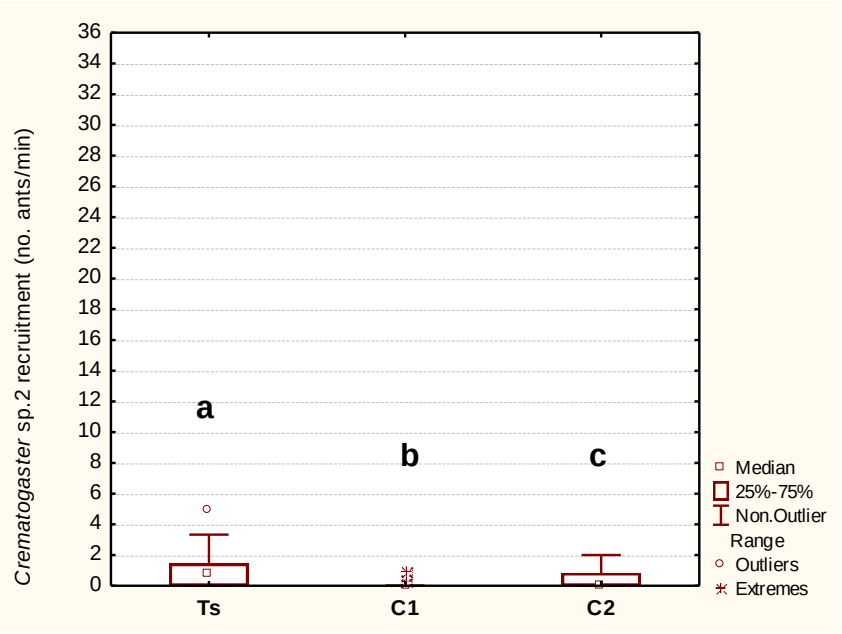

Fig. 2.14: Recruitment rates (scaled to a 1L nest size) of five *Crematogaster* sp. 2 colonies to 10 *Teleonemia scrupulosa* nymphs (Ts) and Controls 1 (empty) and 2 (leaves only) during no-choice prey density experiments ($H = 47.5223$, $n = 75$, $p < 0.0001$). Letters on graphs denote significant relationships.

Significantly more foragers were recruited to 30 *T. scrupulosa* nymphs than to controls 1 ($p = 0.004$) or 2 ($p < 0.0001$), which did not differ significantly (Fig. 2.15).

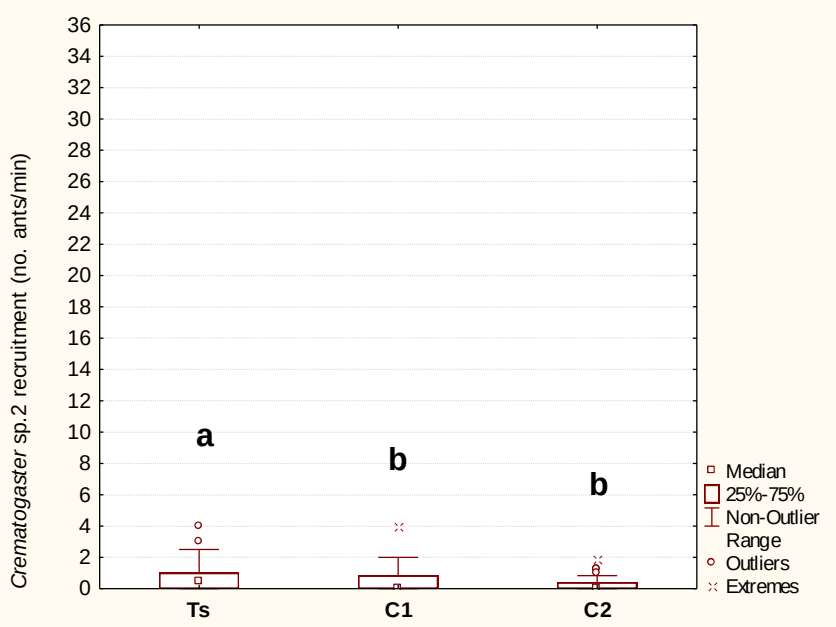

Fig. 2.15: Recruitment rates (scaled to a 1L nest size) of five *Crematogaster* sp. 2 colonies to 30 *Teleonemia scrupulosa* nymphs (Ts) and Controls 1 (empty) and 2 (leaves) during no-choice prey density experiments ($H = 26.513$, $n = 75$, $p < 0.0001$). Letters on graphs denote significant relationships.

Significantly more foragers were recruited to 60 *T. scrupulosa* nymphs offered than to either control 1 or to control 2 ($p < 0.0001$), while significantly more foragers were recruited to control 2 ($p = 0.0087$) than to control 1 (Fig. 2.16).

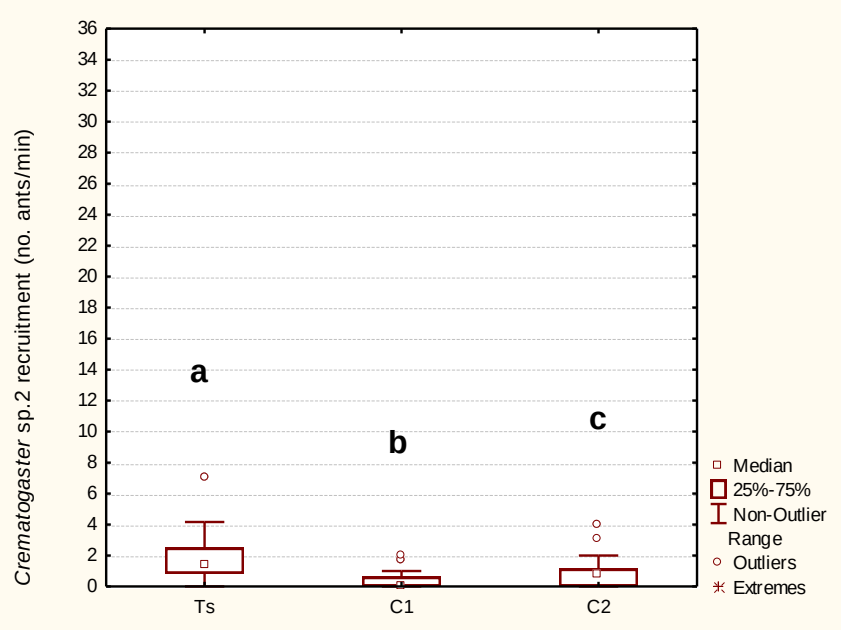

Fig. 2.16: Recruitment rates (scaled to a 1L nest size) of five *Crematogaster* sp. 2 colonies to 60 *Teleonemia scrupulosa* nymphs (Ts) and Controls 1 (empty) and 2 (leaves) during no-choice prey density experiments ($H = 72.7611$, $n = 75$, $p < 0.0001$). Letters on graphs denote significant relationships.

2.7.6 Immature agent size classes

Exact numbers of the different size class nymphs removed were not obtained for the predation experiments using *Crematogaster* sp. 1. However, exact numbers of the different size class nymphs removed were obtained during each of the three prey density experiments, using *Crematogaster* sp. 2 colonies (A-E). This scatterplot (Fig. 2.17) was then used to determine the mass of nymphs of each size class, removed by each *Crematogaster* sp. 2 colony (Chapter IV; Table 4.2). The average masses of *F. intermedia* nymphs in the three size classes were determined by dividing the sum of masses in that size class by the number of individuals measured for that size class. Average masses are as follows: small (0.03mg), medium (0.14mg) and large (0.3mg).

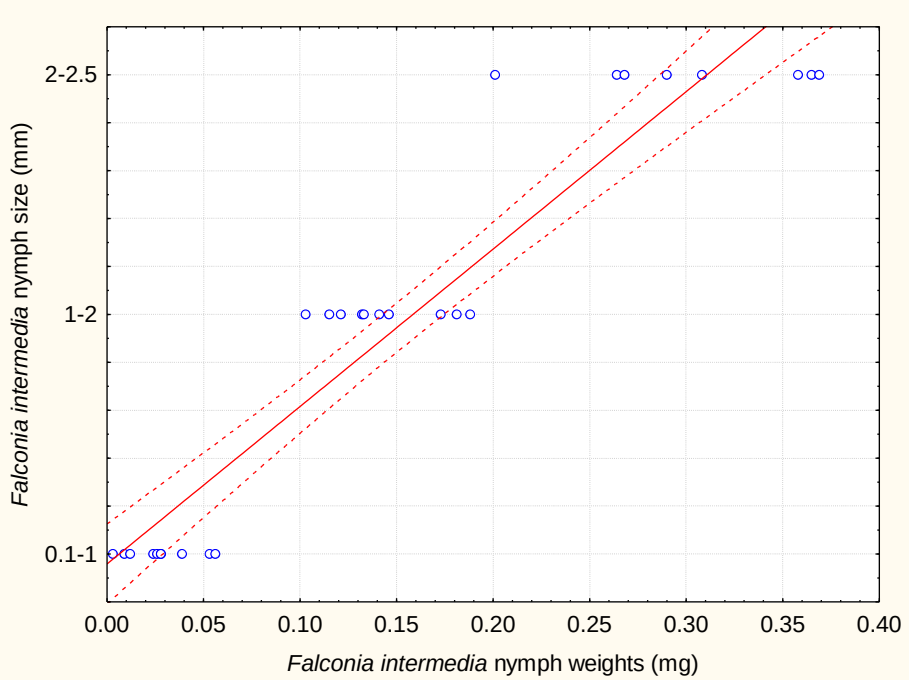

Fig. 2.17: Scatterplot showing the significant relationship between increased *Falconia intermedia* nymph mass with increased size ($p < 0.0001$, $r^2 = 0.8979$, $y = 101.9585 + 6.5702*x$, 0.95 Confidence interval).

Similarly, exact numbers of the different size class nymphs removed were only obtained during each of the three prey density experiments, using *Crematogaster* sp. 2 colonies (1-5) but not during experiments using *Crematogaster* sp. 1. This scatterplot (Fig. 2.18) was then used to determine the masses of nymphs in each size class, removed by each *Crematogaster* sp. 2 colony (Chapter IV; Table 4.3). The average masses of *T. scrupulosa* nymphs in the three size classes were determined by dividing the sum of masses in that size class by the number of individuals measured for that size class. Average masses are as follows: small (0.19mg), medium (0.47mg) and large (1.18mg). Note that medium sized *T. scrupulosa* nymphs weigh more than large *F. intermedia* nymphs (Fig. 2.17).

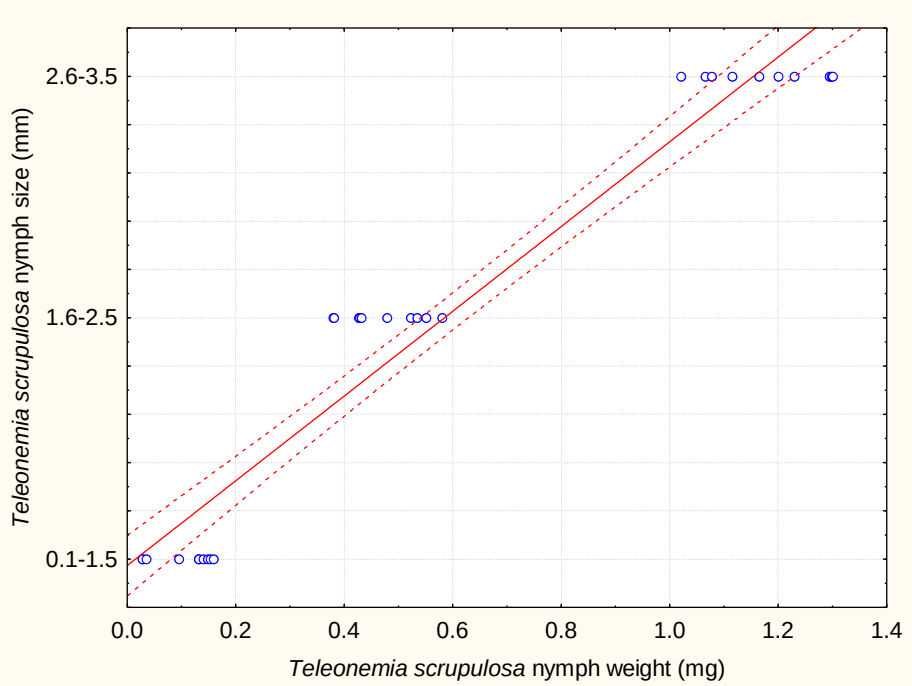

Fig. 2.18: Scatterplot showing the significant relationship between increased *Teleonemia scrupulosa* nymph mass (mg) with increased size ($p < 0.0001$, $r^2 = 0.9406$, $y = 100.9733 + 1.7555*x$, 0.95 Confidence interval).

Crematogaster sp. 1 colonies were offered larvae ranging in length from 2mm to 25mm. The lengths of larvae removed were determined by measuring the lengths of the surviving and drowned larvae and inferring from the recorded lengths of larvae offered in each predation experiment which length larvae were removed. A mass to length curve (Fig. 2.19) was produced by weighing 40 larvae ranging in length from 4mm to 26mm. This graph was then used to determine the total biomass of larvae removed by each *Crematogaster* sp. 1 colony during the agent predation experiments (Table 2.2). Note that early instar *H. laceratalis* larvae weigh ten times the mass of the largest *F. intermedia* and *T. scrupulosa* nymphs by the time larvae are 9mm and 11mm in body length respectively.

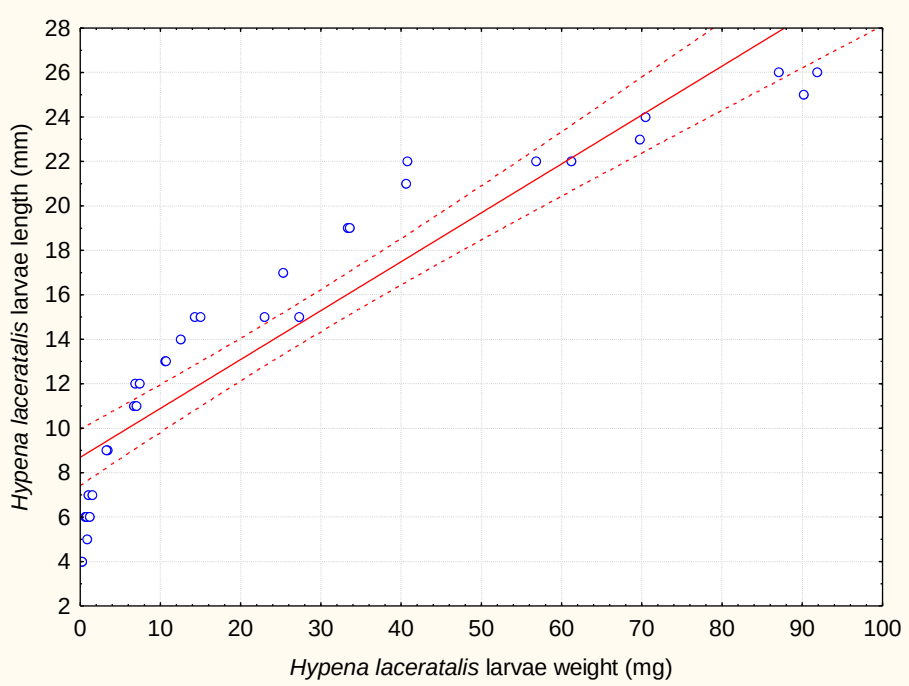

Fig. 2.19: Scatterplot showing the significant relationship between increased *Hypena laceratalis* larvae mass with increased length ($p < 0.0001$, $r^2 = 0.8591$, $y = 8.6886 + 0.22*x$, 0.95 Confidence interval).

Table 2.2: Numbers of the different length *Hypena laceratalis* larvae removed by *Crematogaster* sp. 1 and the total biomass (mg) removed by each colony.
* All masses are hereafter reported in milligrams (mg) for consistency.

Length (mm)	Nest 1	Nest 2	Nest 3	Nest 4	Nest 5
2	1	9	3	1	
3	1		5		1
4	4	1	1		
5	1		3		1
6	3				
7				1	
8	3				
9	1			1	
13				3	
15			2		
Biomass removed (mg)*	15 290	2 510	44 760	36 800	1 150
Biomass removal rate (mg/min.)	**220**	**30**	**500**	**370**	**20**

2.7.7 Pilot prey acceptability results

In the tunnel experiments using *Crematogaster* sp. 2 foraging ants, it was determined that larvae 16mm in length and larger were unacceptable prey for this ant species (n = 3). Although foragers encountered (touched) larvae of this size class and became active when the larva moved, larvae of this size shook and bit off up to seven simultaneously attacking ants. Larvae combined retaliation defence mechanisms with escape such that they would often end up dangling off a silken thread until foragers had departed. It is possible that if 10 or more foragers attacked a larva this size on the ground (or a surface from which it could not dangle), the foragers would overcome the larva. However, this size class is considered unmanageable under normal foraging conditions. Up to14 foragers were required to subdue a 12mm long *H. laceratalis* larva in an average of 13 minutes. This size class is considered acceptable prey whose only defence is escape. *Hypena laceratalis* larvae of 8mm in length required 5 to 7 foragers to subdue them in an average time of 5 minutes. A single forager could remove a 4mm long larva immediately once it had the larva in its mandibles. Larvae of this length were unable to out-manoeuvre foragers due to their small size, unless they were close enough to a leaf edge from which to dangle off. All larvae spent much of their potential foraging time dangling off the edges of leaves. Often they would climb back up the thread only to be met by a forager on the leaf and drop immediately off the leaf edge again.

2.8 Discussion

Recruitment by *Crematogaster* sp. 1 and 2 colonies to food sources was variable, but all preliminary experiments confirmed that colonies foraged for protein, thereby validating results of the predation and prey density experiments. Dyer (2002) found that at both wet and moist forest sites, predatory *P. clavata* foragers returned mostly with nectar, followed by nothing and then either prey or nest material. Similarly, in nearly every preliminary food trial, *Crematogaster* sp. 1 and 2 colonies recruited the most foragers to the stand that contained sucrose water, followed by the stand that offered protein and finally starch. A consideration regarding the sucrose source is that sucrose water (or nectar) is less manageable in that it must be imbibed as opposed to a single forager carrying prey as a unit (potentially over ten times its body weight), which requires fewer foragers than that required to bring equivalent quantities of 'nectar' to the colony. Even so, sugar provides foragers with readily available energy and *Crematogaster* species have a strong affinity for sugary substances. Thus most species collect nectar and/or are aphidicolous or coccidicolous, while some cultivate societies (herds) of fulgorid, membracid or lycaenid larvae to harvest their secretions (Arnold 1915; Hölldobler & Wilson 1990; Radeghieri 2004; Tanaka *et al*. 2009). Furthermore, plants that provide extra-floral nectaries (EFN's) are protected from herbivores by many ant species requiring sugars and amino acids contained therein (Belt 1874 *In* Becerra 1989; Caroll & Janzen 1973; Fowler & MacGarvin 1985).

During these experiments, foragers consistently recruited less to the starch source in food trials. Similarly, foragers usually recruited less to the controls (1 and 2) than to the agent treatments. This was because the foraging directions were limited to only three. Foragers returning from the less attractive option(s) recruited less actively, via positive recruitment signals (e.g. trail pheromone and/or tactile communication using antennae). Consequently, new foragers that encountered foragers returning from the less appealing option(s) turned back and moved toward the direction(s) from which returning foragers were giving positive recruitment signals. However, a few foragers would continuously visit even the least favoured podiums. Similar results were generated in a computer simulation study by da Silva (2004) which aimed to test the Optimal Foraging Theory (OFT), founded by Emlen (1966), and McArthur and Pianka (1966). da Silva's results seemed to contradict the OFT if one assumes

'optimal' means that foragers should converge on the highest quality food resource. However, the meaning of optimal has not been adequately defined in this context and da Silva (2004) concluded that optimal foraging behaviour is determined by the environment in which the ant colony exists. In nature, food resources are often patchily distributed in both time and space. Thus, it is suggested that foragers should not all be recruited to the same food source. The reason is that if they all foraged in the same direction they would risk missing superior food resources in other directions.

For both the *F. intermedia* and *T. scrupulosa* food trials, *Crematogaster* sp. 1 foragers recruited comparatively more ants per minute to the sucrose source than they did to the agent treatments. However, *Crematogaster* sp. 1 colonies recruited more to *H. laceratalis* larvae than they did to the sucrose source during the preliminary food trials for this agent. This is probably because *H. laceratalis* larvae larger than 10mm required forager co-operation to subdue and to transport. Foragers subdued the larva, and then some dismantled the larva while others imbibed its haemolymph. *Crematogaster* sp. 1 foragers recruited about a third more foragers per minute than they did to either of the hemipteran agent nymphs, which foragers could manage alone. Similarly, *Crematogaster* sp. 2 colonies generally recruited more foragers to the sucrose source than what they did to differing densities of *F. intermedia* or *T. scrupulosa* nymphs. However, *Crematogaster* sp. 2 colonies recruited significantly more foragers to every density of agent offered than what they did to either of the controls (1 or 2).

In a study by Floren *et al*. (2002) to control for a bait artefact, caterpillars were placed on leaves and observed until encountered by an ant. Of the 18 trials performed, in four cases the larvae were grasped by solitary *Polyrachis* sp. foragers, in two cases both the ants and larvae ignored each other, whereas in twelve cases the larvae fled successfully. In the present study, *H. laceratalis* larvae exhibited a defence strategy of wriggling wildly until no longer touched. However, this behaviour often left larvae hanging from silken threads off the edge of the podium or larvae wriggled right off the podium, into the water trap below. Had these laboratory experiments taken place in nature, the larvae that wriggled away would not have drowned but would probably have spent time dangling from their silken threads until they climbed back up and continued feeding, or encountered another forager and dangled again or fell to the

ground/leaves below, where they would also risk predation. In pilot prey acceptability experiments using *Crematogaster* sp. 2, larvae of 16mm length escaped predation but spent most of the experimental time dangling. This equates to massive losses in feeding time which consequently reduces control of lantana and has a disruptive influence on the digestive capabilities of the larvae. When some lepidopteran larvae are unable to feed for many hours, their stomach pH becomes very acidic, digestive bacteria die and they can no longer digest plant material (Schultz 1983a).

2.9 Conclusion

Although these species of *Crematogaster* are considered predaceous, they (and most predatory ant species) appear to harvest sugar more readily than protein. Recruitment of foragers by *Crematogaster* species appears highly variable between colonies and though recruitment rates are indicative of desirability of the food/prey source, interpretation of recruitment rates in chapters III to V should consider several factors, such as: ambient temperature which affects insect movement speed (Crawley 1992), prey size/manageability (i.e. whether prey is manageable by individual foragers or co-operation is necessary), food quality and food quantity because the less food available the fewer foragers are required to harvest it (Harada 2005). For these reasons, recruitment rate is useful but can be difficult to interpret, whereas the removal of prey in a given time (predation rate) may be more informative in that it is absolute. That is, many more foragers were recruited during these experiments than required to handle the available prey and it is assumed that they were searching for alternative food sources. The predation rate represents the exact number of individual agents removed in a given time. The rate of biomass removed is equally informative, as it makes use of the exact number of individuals removed, and their masses, to indicate how much prey biomass the ant colony gained during a particular amount of time. Both the predation rate and the biomass removal rate are also confounded by the factors that make interpreting the recruitment rate difficult. Thus, the agents were chosen for the differences they exhibit, which may help tease apart what makes some insects more susceptible to ant predation than others. Ambient temperature affects

insect movement speed (Crawley 1992), so performing experiments at temperatures within 2°C of one another, was intended to cancel the more subtle effects of varying temperature on the ants' foraging behaviour. Testing two hemipteran agents whose movement speeds differ dramatically, helped to elucidate whether insect mobility affects their susceptibility to predation by ant foragers (chapters III to V). Larvae of the moth *H. laceratalis*, encompass a wide range of body lengths (sizes) and hence should clarify which sized insects are more susceptible to predation by *Crematogaster* sp. 1 and 2 foragers and for which sizes co-operation is necessary (i.e. prey size/manageability). In addition, *F. intermedia* and *T. scrupulosa* nymphs on average gain only 0.3mg and 1mg respectively, whereas *H. laceratalis* larvae on average gain 7mg of biomass by the end of their first instar (Fig. 2.17 to 2.19), making them theoretically the superior quality prey. Agents were offered to *Crematogaster* sp. 2 colonies in increasing densities to investigate the effect, if any, of increased prey quantity on foraging *Crematogaster* sp. 2 ants (chapter IV).

Chapter III

No-choice experiments testing predation by *Crematogaster* sp. 1 on three biological control agents released against *Lantana camara*: *Falconia intermedia*, *Hypena laceratalis* and *Teleonemia scrupulosa*

3 Preface

This chapter focuses on recruitment rates and predation rates of five *Crematogaster* sp. 1 ant colonies on the immature stages of three biological control agent species. Recruitment and predation rates are compared among agents for this ant species and behavioural responses of the agents to *Crematogaster* sp. 1 foragers are discussed.

3.1 Introduction

Certain feeding guilds of herbivores are highly susceptible to predation by ants (Fowler & MacGarvin 1985; Ito & Higashi 1991). Both of these studies found that lepidopteran larvae of the feeding guild categories designated external leaf 'chewers' and leaf rolling 'chewers' were more abundant at sites of low- versus high ant species diversity. Similarly, Fowler & MacGarvin (1985) found that homopteran and heteropteran phloem 'suckers' (excluding ant-tended aphid species), were significantly more abundant at 'ant-poor' (low species diversity) sites. Ito & Higashi (1991) found that lepidopteran larvae inhabiting shoot bases covered by scales and stalk-boring lepidopteran larvae showed no significant difference in abundance between ant-rich and ant-poor sites, whereas acorn-boring lepidopteran larvae were significantly less abundant at ant-rich sites. Ito & Higashi (1991) suggested that the latter larvae were preyed on by ants while moving between acorns. Fowler & MacGarvin (1985) found that a lepidopteran nomadic leaf miner (larvae of which live in a sealed tubular case) did not differ in abundance between ant-rich and ant-poor sites. According to those studies, the agents tested in the present study are all potentially vulnerable to ant predation as they are external, unsheltered leaf feeders. The hemipterans, *Falconia intermedia* and *Teleonemia scrupulosa* are phloem suckers whereas the moth agent, *Hypena laceratalis* is a leaf chewer. The mirid *F. intermedia*

is a relatively new agent that has not established well in South Africa, and Heystek & Olckers (2003) suggest that ant predation may play a role. In contrast with the newer hemipteran agent, the tingid *T. scrupulosa* has been established in South Africa for 50 years and although the nymphs are slow-moving compared to *F. intermedia* nymphs, it is one of the few successful biological control agents for *L. camara* in South Africa (Oosthuizen 1964; Cilliers 1987b, Cilliers & Neser 1991, Baars & Neser 1999; Baars & Heystek 2003; Simelane & Phenye 2005). Levels of ant predation on this agent could be informative for how much ant predation an insect population can withstand provided other factors are favourable. It may be that *T. scrupulosa* has a strategy for evading predation by ants. Like *Prosopidopsylla flava* Burckhardt nymphs, *F. intermedia* and *T. scrupulosa* nymphs are free living and exude no excretions that may reduce predation by ants (van Klinken *et al*. 2003). The native moth agent, *H. laceratalis*, was tested as a representative of the majority of lepidopteran agents used against *L. camara* since *H. laceratalis* larvae are relatively hairless and lack chemical defences (Day *et al*. 2003a). Most lepidopteran lantana agents in South Africa are rare in the field (Baars 2003) whereas *H. laceratalis* can be found across the geographic range of lantana in South Africa (Baars 2003), suggesting that co-adaptation with our native ants may result in lower predation pressure. Although ant predation on lepidopteran agents for *L. camara* has not been measured before this study, the literature lists lepidopteran agent species (for other target weeds) that are preyed on extensively by South African ant species (Hoffmann 1982, Robertson 1985, Kluge 1994, Kluge & Caldwell 1996, Hoffmann *et al*. 1998).

3.1.1 Aims of predation experiments

The aims of these experiments were to determine: 1) whether or not these agents (*H. laceratalis*, *F. intermedia* and *T. scrupulosa*) were removed as food items (as opposed to attacked); 2) how many of the agents offered were removed; 3) at what rate agents were removed; 4) whether *Crematogaster* ants show a preference for any of the agents (as indicated by significantly higher predation rates) and 5) what defences (if any) agents have against ant predation.

3.2 Materials and Methods

Preliminary food trials were performed before every predation trial to ensure that the colonies were harvesting protein (see chapter II; 2.4.1). Predation by *Crematogaster* sp.1 ant colonies on *F. intermedia, T. scrupulosa* and *H. laceratalis* was tested under no-choice conditions (see Chapter V for agent choice experiments).

3.2.1 Experimental design

Predation on biological control agents by *Crematogaster* sp.1 ants was tested by offering foragers: a) agents on a lantana cutting in a petri-dish; b) an empty petri-dish (Control 1) or c) a lantana cutting in a petri-dish (Control 2). The petri-dish provided access for ants and agents to both leaf surfaces by keeping the cuttings vertical. Lantana cuttings consisted of the terminal two leaf pairs. The cut stems were wrapped in moist paper towel to prevent rapid leaf desiccation, which could disturb agent feeding, particularly of the phloem suckers. These experiments were performed in a laboratory in which the ambient temperature was maintained between 20 and 26°C and the relative humidity between 40-60%. Experiments were performed between July and November 2007, although no colonies remained there for the entire duration since they were collected as needed and released once tested. *Crematogaster* sp. 1 colonies were offered 20-30 immature agents. To standardise prey numbers and experiment time for comparison between agents, predation rates (no. agents removed per hour) and percentages of agents removed were calculated (Tables 3.1 - 3.3). Refer to the Methodology (Chapter II, Fig. 2.1 and sections 2.3 and 2.4.3) for details of the experimental set-up and design. As stated in chapter II (2.4.4) only immature agents were tested in this study because immature agents are potentially vulnerable for the longest duration of the life stages.

3.2.2 Statistical analyses

Scatterplots were generated in Statistica to test for significant correlations between: recruitment rates, predation rates and nest volumes. No significant correlations were found between these variables (Chapter II; 2.6) and since predation on *H. laceratalis, F. intermedia, T. scrupulosa* was tested using the same five *Crematogaster* sp.1 colonies, variation in the recruitment and predation rates was considered natural

variation. Therefore, data presented in this chapter were not scaled to a common nest volume of 1L. Scaling the data only becomes meaningful when comparing predation responses between *Crematogaster* sp. 1 and 2, such as the results of preliminary food trials (Chapter II). However, scaled predation rates (to a model 1L nest) are depicted in Tables 3.1-3.3 for comparison of the agent predation rates by *Crematogaster* sp. 1 with those of *Crematogaster* sp. 2 in chapter IV (section 4.3.6).

Analyses were performed using the Kruskal-Wallis by Ranks and Median Test (a non-parametric ANOVA) because these data are non-normally distributed. Kruskal-Wallis ranks the data and then assesses the hypothesis that the different (ranks of the) samples in the comparison were drawn from distributions with the same median or from the same distribution. Kruskal-Wallis is considered a robust test because it ranks the data, which reduces variation and it uses all data points rather than generating means. Note that in graphs, the bars indicate 25% of the data above and below the median quartile (boxes). These are not standard deviation bars as no means are calculated using the Kruskal-Wallis test (Rosner 2000). See chapter II (section 2.6) for details of the Kruskal-Wallis by Ranks and Median Test. The percentages of immature agents removed by each colony were presented in Tables 3.1; 3.2 and 3.3.

3.3 Results

Recruitment rates of some colonies were significantly different from other colonies within each agent predation trial, regardless of whether the recruitment numbers were scaled to nest volume or not.

3.3.1 *Falconia intermedia* predation trials

Although no strong relationship existed between highest recruitment to this agent and highest predation rates, it appears that *Crematogaster* sp.1 colonies recruit to and prey on *F. intermedia* nymphs. On average, 50 % of the available nymphs offered to *Crematogaster* sp.1 colonies were removed as prey by these ant colonies within the experimental time (Table 3.1). Predation rates are presented in this table as unscaled and scaled (divided by nest volume). The latter are for comparison with results of *Crematogaster* sp. 2 predation rates (chapter IV; 4.3.6).

Table 3.1: Predation on *Falconia intermedia* nymphs (20-30) on *Lantana camara* cuttings by *Crematogaster* sp.1 during no-choice laboratory trials.
F.i. = Falconia intermedia nymphs

Colony No.	**Predation rate (#/hr.)**	**Predation rate (#/hr.) /nest vol.**	**Temp. °C**	**% *F.i.* removed**
1	12.6	2.4	23	86
2	16.8	3	24	60
3	13.2	1.8	24 - 25	52
4	0.6	0.6	23 - 24	4
5	10.2	11.4	23	50
Ave.	10.8	3.6		50.4

Colony 3 recruited significantly more foragers per minute (Fig. 3.1) than colony 1 ($p = 0.0012$), colony 2 ($p= 0.0072$) and colony 4 ($p < 0.0001$). Colony 4 also recruited significantly fewer foragers per minute than colony 2 ($p = 0.0346$) and colony 5 ($p = 0.0003$). Although recruitment rates by colony 1 appear medium to low for this agent trial, nearly 86% of the available *F. intermedia* nymphs were removed by colony 1. During the experiment using colony 3, 32% of the *F. intermedia* nymphs drowned in the water trap below the podium, while trying to escape the significantly high density of foraging ants. This may account for the reduced predation rate, despite colony 3 recruiting the highest numbers of foragers for this agent trial. Though colonies 4 and 5 had the same nest volume (0.9L) colony 4 recruited less to the *F. intermedia* treatment and removed only 4% versus the 50% of agents removed by colony 5 (Table 3.1). During this experiment colony 4 foragers were observed chasing *F. intermedia* nymphs. However, they did not recruit actively and very few nymphs were caught and removed. Speed and manoeuvrability were the defence strategies of these nymphs that were visibly disturbed by the presence of ant foragers. Nymphs were never found dead/dying on the podiums and it was concluded that foragers had removed missing *F. intermedia* nymphs as prey items rather than simply attacking them (water traps were checked for drowned agents).

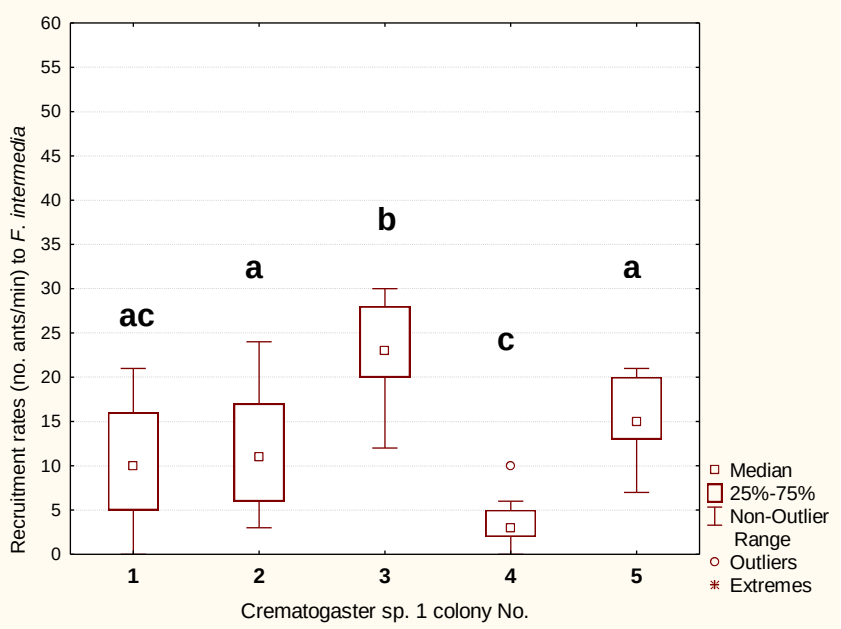

Fig. 3.1: Recruitment rates (no. foragers/min.) of five *Crematogaster* sp.1 colonies to *Falconia intermedia* nymphs on *Lantana camara* cuttings (H = 43.0999, n = 15, p < 0.0001). Letters on the graph denote significant relationships.

3.3.2 *Teleonemia scrupulosa* predation trials using Crematogaster sp. 1

On average, only 9 % of *T. scrupulosa* nymphs were removed by *Crematogaster* sp.1 colonies (Table 3.2). Even so, all colonies recruited foragers to the stand offering *T. scrupulosa* nymphs, and three of the five colonies removed nymphs as prey items. Predation rates were low for *T. scrupulosa* considering these nymphs are gregarious and slow moving. Predation rates scaled to nest volume are presented for comparison with predation on *T. scrupulosa* nymphs by *Crematogaster* sp. 2 (chapter IV; 4.3.6).

Table 3.2: Results of predation on *Teleonemia scrupulosa* nymphs (20-30) on *Lantana camara* cuttings by *Crematogaster* sp.1 ants in no-choice laboratory trials.
T.s. = Teleonemia scrupulosa nymphs

Colony No.	Predation rate (#/hr)	Predation rate (#/hr) /nest vol.	Temp. °C	% *T.s.* removed
1	5.4	1.2	23-24	20
2	0	0	25-24	0
3	3.6	0.6	23	18
4	0	0	23-24	0
5	1.8	2.4	23-24.5	7
Ave.	2.4	0.84		9

Colony 2 was the least active colony, recruiting significantly fewer foragers per minute (Fig. 3.2) than colony 1 ($p < 0.0001$), colony 3 ($p < 0.0001$) and colony 4 ($p = 0.0031$). Colony 5 recruited significantly fewer foragers per minute than did colony 1 ($p = 0.0051$) and colony 3 ($p = 0.0074$). The unusually high recruitment by colony 1 to these nymphs was based on only one recording of 52 foragers recruited to the agent podium. Even so, colony 1 removed 20% of the nymphs offered, while colony 3 removed 18% and colony 5 removed only 7% of the available nymphs. Neither colony 2 nor colony 4 removed any nymphs (Table 3.2). Nymphs killed by *Crematogaster* sp. 1 were never left on the podiums but removed as prey items.

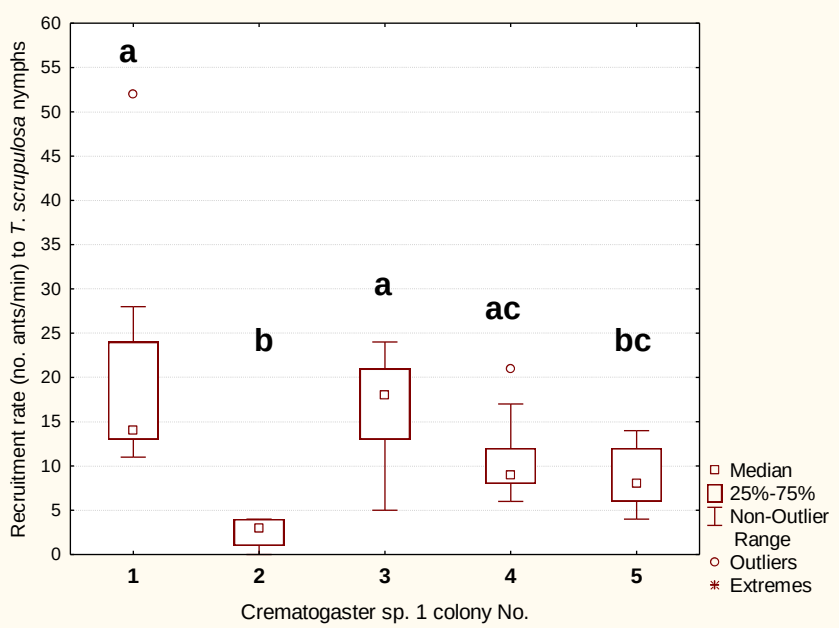

Fig. 3.2: Recruitment rates (no. foragers/min.) of *Crematogaster* sp.1 colonies to *Teleonemia scrupulosa* nymphs on *Lantana camara* cuttings ($H = 54.1789$, $n = 15$, $p < 0.0001$). Letters on the graph denote significant relationships.

3.3.3 *Hypena laceratalis* predation trials

A mass vs. length curve (Chapter II; Fig. 2.19) was produced by weighing 40 larvae ranging in length from 4mm to 26mm. This graph was then used to determine the total biomass of larvae removed by each colony of *Crematogaster* sp.1 during the agent predation experiments (Chapter II; Table 2.2). Forty-one larvae of the size class smaller than 10mm (1st & 2nd instar larvae) were removed by all colonies versus only five larvae larger than 10mm removed by colonies 3 and 4 (Table 2.2). On average, 45% of the available larvae were removed by these colonies. The biomass removed

by each colony was then scaled to that of a model 1L colony nest size. Both actual and scaled predation rates and biomass removal rates can be seen in Table 3.3.

Table 3.3: Results of predation on *Hypena laceratalis* larvae (20-30) on *Lantana camara* cuttings by *Crematogaster* sp.1 colonies during no-choice laboratory trials. *H.l.* = *Hypena laceratalis* larvae

Colony No.	**Predation Rate (#/hr)**	**Predation rate (#/hr) / nest vol.**	**Biomass removal rate (mg/hr)**	**Biomass removed (mg/hr) /nest vol**	**Max. larva Length taken (mm)**	**Temp. °C**	**% *H.l.* taken**
1	12	1.65	13 200	1818	9	25-24	65
2	7.8	0.83	1 800	208	4	23	53
3	7.8	0.83	30 000	2973	15	23-25	56
4	4.2	2.89	22 200	18821	13	23	40
5	1.8	1.24	1 200	916	5	24	9
Ave.	6.6	1.488	**13 680**	4947.2			**44.6**

Due to the great variation in size and hence biomass of *H. laceratalis* larvae, it is particularly informative to compare the predation rate with the rate of biomass removal for this agent. For example, the rates of predation (and percentage larvae removed) were equal for colonies 2 and 3, however colony 3 removed nearly 17 times more caterpillar biomass than did colony 2 (Table 3.3). These colonies (of similar size) differed in foraging behaviour in two ways. The recruitment rate (Fig. 3.3) of colony 2 was significantly lower than colony 3 ($p < 0.0001$), colony 1 ($p < 0.0001$) and colony 5 ($p = 0.0115$). Colony 2 removed only larvae of 4mm and smaller (Chapter II, Table 2.2), whereas colony 3 co-operated to remove (among others), three larvae of 15mm each which resulted in colony 3 having removed the greatest mass of larvae per hour (30 000mg/hr). The foraging behaviours of colonies 4 and 5 (of equal nest size), appear both to contradict and support this pattern, in that the recruitment rate of colony 4 was lower than that of colony 5 and significantly lower than colony 1 ($p < 0.0001$) and colony 3 ($p = 0.0104$). Colony 4 (despite having been offered 15 larvae) removed 40% of *H. laceratalis* larvae offered and among those removed were three larvae of 13mm length which meant that they removed the second largest mass (Table 3.3) of larvae per hour (22 200mg/hr). Colony 5 removed only two small larvae (≤ 5mm) and thus the lowest mass of larvae per hour (1 200mg/hr). Although

recruitment rates of colonies 2 and 4 were not significantly different, it is possible that colony 4 recruited enough foragers to subdue large larvae, while colony 2 did not recruit sufficient foragers at one time to co-operate in subduing large larvae.

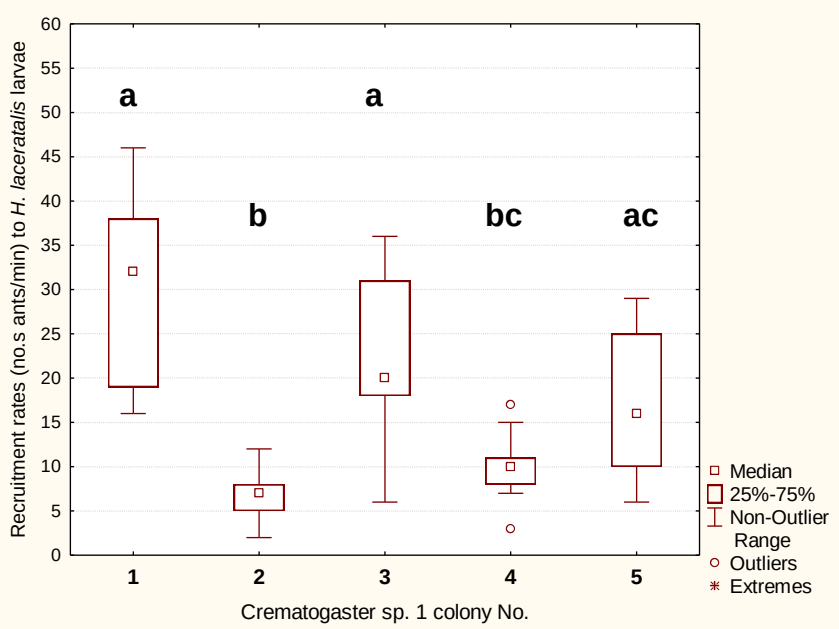

Fig. 3.3: Recruitment rates (No. foragers/min.) of five *Crematogaster* sp.1 colonies to *Hypena laceratalis* larvae of varying sizes on *Lantana camara* cuttings ($H = 46.7395$, $n = 15$, $p < 0.0001$). Letters on the graph denote significant relationships.

Colony 1 maintained the highest recruitment rate for this agent trial and removed the greatest percentage (65%) of larvae offered to them, despite 30% of the larvae having escaped (drowned) during the experiment. Nevertheless, *H. laceratalis* larvae that drowned were not excluded from the results as they did not escape immediately so ant foragers were exposed to them for some time and would have immobilised the larvae were they capable of it. Other than larvae that foragers were busy dismantling when the experiment ended, no larvae were left dead/dying on the podiums or water traps and thus missing *H. laceratalis* larvae were considered removed as prey items.

3.3.4 Comparison of predation on all agents

Predation rates by *Crematogaster* sp.1 colonies did not correlate with nest size or recruitment rates to any of the agent treatments. Recruitment rates by *Crematogaster* sp.1 colonies to *F. intermedia* nymphs and *H. laceratalis* larvae were not significantly different. Although recruitment by *Crematogaster* sp.1 foragers to *F. intermedia* did not differ significantly from recruitment rates to *T. scrupulosa* nymphs (Fig. 3.4), predation rates on *F. intermedia* were significantly higher (p = 0.0486) than on *T. scrupulosa* nymphs (Fig. 3.5), whether or not data were scaled to 1L nests or not.

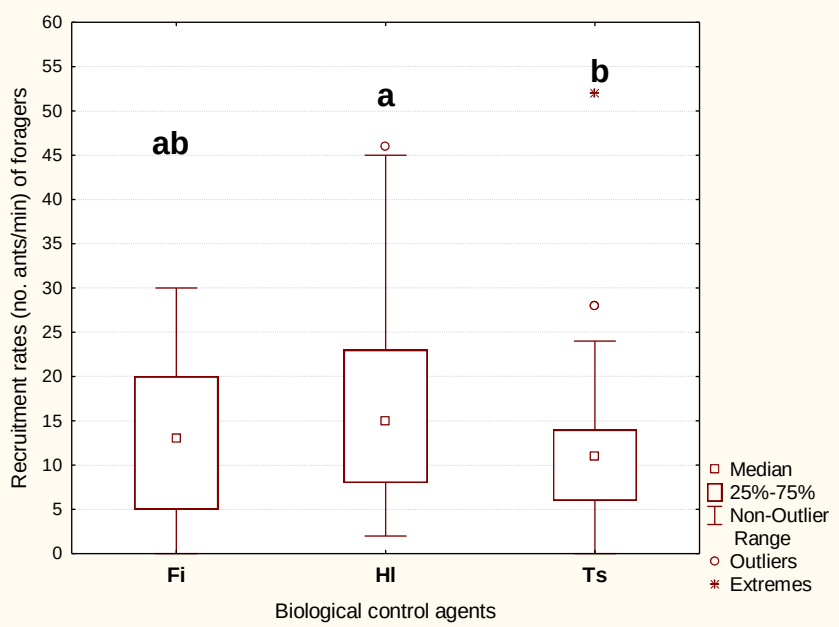

Fig. 3.4: Recruitment by five *Crematogaster* sp.1 colonies to immature biocontrol agents: *F. intermedia* (Fi), *H. laceratalis* (Hl) and *T. scrupulosa* (Ts), on *L. camara* cuttings, during no-choice predation experiments (H = 10.3126, n = 75, p = 0.0058). Letters on the graph denote significant relationships.

Conversely, although significantly more foragers were recruited by colonies to *H. laceratalis* larvae than to *T. scrupulosa* nymphs (p = 0.0043), and a greater number of larvae were removed than *T. scrupulosa* nymphs, overall predation rates on these two agents are not significantly different (Fig. 3.5).

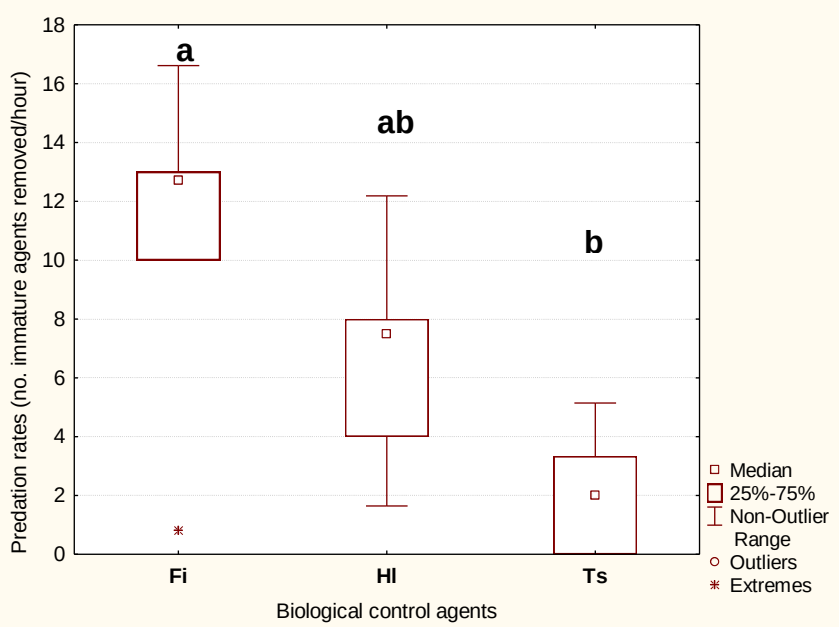

Fig. 3.5: Predation rates by five *Crematogaster* sp.1 colonies on immature *Hypena laceratalis* (Hl), *Falconia intermedia* (Fi) and *Teleonemia scrupulosa* (Ts) on *L. camara* cuttings, during no-choice predation experiments (H = 5.8504, n = 15, p = 0.0537). Letters on the graph denote significant relationships.

Although the colonies exhibited some differences, the proportions in which they removed the respective agents were fairly consistent (Fig. 3.6). Within each agent treatment, predation rates by *Crematogaster* sp.1 colonies 1-5 were never significantly different from one another. Between agent treatments, predation rates by colonies on *F. intermedia* nymphs were not significantly different from predation rates on *H. laceratalis* larvae (Fig. 3.6), whether the data were scaled to 1L nests or not. However, in terms of prey biomass, all colonies removed on average, over than a thousand times more biomass of *H. laceratalis* larvae than either *F. intermedia* or *T. scrupulosa* biomass.

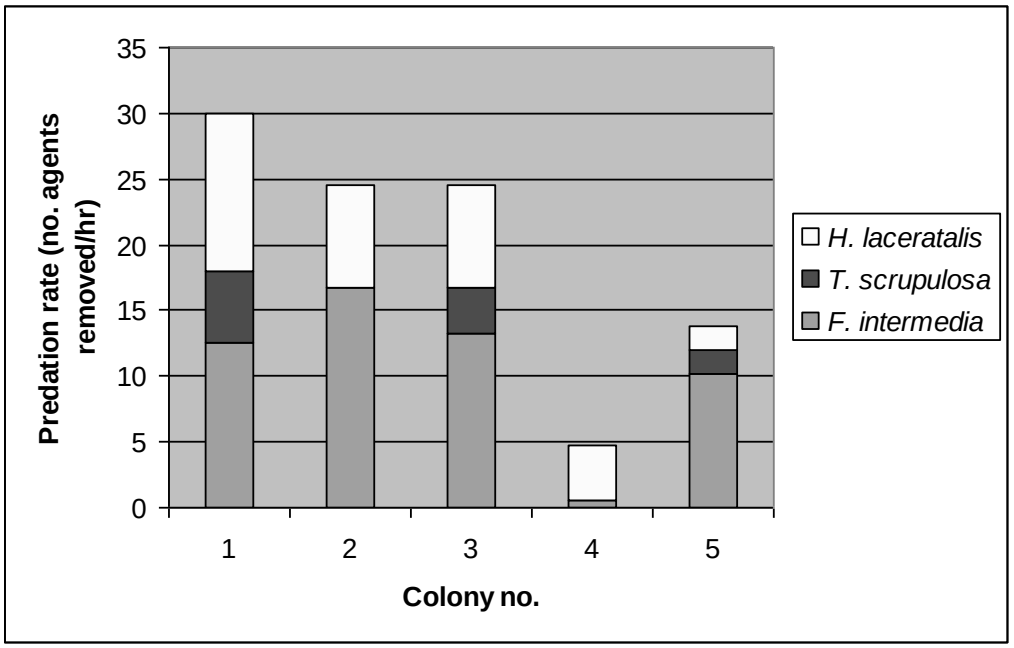

Fig. 3.6: Predation rates (No. agents removed/hour) on lantana biological control agents: *Falconia intermedia*, *Hypena laceratalis* and *Teleonemia scrupulosa* by *Crematogaster* sp.1 colonies (n = 5).

No significant differences existed between the percentages of agents removed. However, whereas the percentages of *T. scrupulosa* removed were not significantly different from zero agents removed, significantly more *F. intermedia* nymphs (n = 20, H = 13.2732, p = 0.0197) and *H. laceratalis* larvae (p = 0.0197) were removed than zero agents.

3.3.5 Behavioural responses to predation

Nymphs of *F. intermedia* are very easily disturbed, darting about if they detect movement near them. They are fast runners and capable of changing direction quickly to escape predators. Ants were able to catch *F. intermedia* nymphs by out-running them over longer distances (such as the length of the leaf surface) or when densities of nymphs and/or foragers on a leaf were high, such that nymphs were no longer able to escape without colliding with another nymph or a forager. In contrast, immature *T. scrupulosa* were not visibly disturbed by *Crematogaster* sp.1 foragers. During the experiments ants were often observed touching stationary *T. scrupulosa* nymphs and then either ran away or simply passed the nymph and continued foraging while nymphs generally remained stationary and continued feeding. The 4th and 5th instar nymphs were observed mobile more frequently than the smallest instars and the

only nymph actually seen removed by *Crematogaster* sp.1 was a mobile 5th instar nymph, though results showed that other instars were also removed. Similarly, *H. laceratalis* larvae generally remained stationary while feeding and digesting, only moving when disturbed. If a larva was touched by a forager, it would flick its posterior segments once and only if a forager persisted in its investigation of the larva would it wriggle wildly in an attempt to knock the ant off. If the larva became overwhelmed by foragers, it would move towards a leave edge from which to dangle on a silken thread, known as 'spinning off' (Fowler & MacGarvin 1985). This defence was more successful for late- than early instar larvae due to their vast size difference which relates to step-length. Once a small larva had moved noticeably, it needed to be close to a leaf edge to survive predation by spinning off. Alternatively, foragers would usually out-run small larvae.

3.4 Discussion

This study has shown that biological control agents for *Lantana camara* are preyed on by South African ant species as suggested by Cilliers & Neser (1991), Baars (2002) and Heystek (2006).

Many ant species, referred to as 'large-eyed ants' because their compound eyes are visible to the naked human eye (*Crematogaster* and *Formica* species), have excellent form vision and very easily detect moving objects (Jander 1957 *In* Hölldobler & Wilson 1990; Ayre 1963; Wehner 1981; Wehner *et al*. 1983). Foragers of such ant species usually do not respond to stationary prey insects but are attracted by the vibrations and visual stimulation of moving prey (Schultz 1982; Bergelson & Lawton 1988; Hölldobler & Wilson 1990). Studies concerned with induced host-plant defences have found that feeding by herbivores induces changes in the leaf cell chemical concentrations and/or composition in the feeding vicinity. This often causes herbivores to move in search of better foliage (nutrient quality) thereby becoming more susceptible to predation (Shultz 1982 & 1983b; Bergelson & Lawton 1988). Freitas and Oliveira (1996) studied ant predation on a species of caterpillar (*E. bechina*), the larvae of which construct frass chains from leaf margins on which to hide when threatened. Results of their study showed that *E. bechina* larvae remained

on their chains when feeding at leaf margins and freeze at the tip of the chain if an ant approaches. After a few attempts at reaching the caterpillar the ant would give up and leave. However, when live termites were stuck to frass chains, their movements encouraged attacking ants which destroyed the chain and obtained the termites. Thus, Freitas and Oliveira (1996) concluded that remaining motionless was probably important for deterring ant foragers. As a means of defence against predators, *H. laceratalis* larvae wriggle wildly when touched and/or 'spin off' leaf edges, while *F. intermedia* nymphs are fast erratic movers. Although *F. intermedia* nymphs are fast-moving making them difficult to catch, their movement itself is probably what identifies these nymphs to ants as prey items (Bergelson & Lawton 1988). In these agent predation trials foragers removed 50 % and 45 % of *F. intermedia* nymphs and *H. laceratalis* larvae respectively, which was significantly more than zero agents removed. Predation rates (no. agents removed per hour) on *F. intermedia* and *H. laceratalis* were comparable, whereas significantly more *F. intermedia* were removed than *T. scrupulosa* nymphs. Larger colonies removed some *T. scrupulosa* nymphs, however the average removal was 9% which was not significantly different from zero agents removed. *T. scrupulosa* nymphs could be considered cryptic in that they predominantly remain motionless in the presence of predators. This appears to be the best defence against ant predation since the fewest *T. scrupulosa* nymphs were removed by *Crematogaster* sp. 1 foragers despite foragers having found and touched (stationary) *T. scrupulosa* nymphs. Bergelson and Lawton (1988) observed identical behaviour in foragers of *Formica lemani* Bondroit, walking past or even over stationary prey, but never ignoring mobile prey.

Results of the *H. laceratalis* predation trials brought to light the importance of analysing data using different units of measurement (i.e. percentage-; number- and biomass removed by colonies). Colonies 2 and 3, of comparable size, removed a similar percentage of larvae offered and had equal predation rates for this agent trial. Yet colony 2 removed only small larvae whereas colony 3 targeted large larvae, had significantly higher recruitment rates and consequently removed nearly 17 times more biomass than did colony 2. It appears that recruitment is paramount and facilitates larger prey size (hence greater biomass) removal. However, the foraging behaviours of colonies 4 and 5 (of equal nest size) are enlightening in that the recruitment rate of colony 5 was greater than that of colony 4, yet colony 5 removed only two small

larvae, while colony 4 removed three large larvae and thereby the second greatest biomass removal overall. This suggests that optimal forager co-operation is more important than high recruitment numbers when large prey is concerned. High recruitment numbers can however assist in greater prey removal if foragers co-operate to remove larger prey while additional foragers individually remove smaller prey (Hölldobler & Wilson 1990).

Overall, colonies recruited slightly more foragers to *H. laceratalis* larvae than to *F. intermedia* nymphs and significantly more to *H. laceratalis* larvae than to *T. scrupulosa* nymphs. The longer body lengths of *H. laceratalis* larvae may have elicited greater recruitment responses by foragers since larvae larger than about 10mm required forager co-operation to immobilise. In *pilot prey acceptability* experiments (Chapter II; 2.4.6), using the temperate *Crematogaster* species 2, it was determined that on average 14 foragers were required to subdue a 12mm long *H. laceratalis* larva in about 13 minutes, while a single forager could remove a 4mm long larva almost immediately (once the ant had the larva in its mandibles). Similarly, while studying the predation habits of a ponerine ant, *Paraponera clavata*, using 909 lepidopteran larvae from 108 species (ranging in mass from 7 to 8900mg) Dyer (2002) found that a positive correlation exists between larval size and time required to subdue the larvae. Prey small enough for an individual worker to subdue was removed but if it was too large, the forager would first try to subdue it and then either return to the foraging trail to recruit or wait to be found by nest mates that would co-operate in carrying the prey back (Franks 1986; Dyer 2002).

Predation rates of these biological control agents are revealing in terms of how many immature agents were removed in time. However, the amount of biomass that each ant colony consumed of the respective agents is indicative of prey quality. Using the average masses of different sized *F. intermedia* and *T. scrupulosa* nymphs (Chapter II; Figures 2.17 & 2.18) a conservative comparison of biomass gained through predation on the agents assumes that only large *F. intermedia* and *T. scrupulosa* nymphs were removed by all colonies. Then the total *F. intermedia* biomass removed by all colonies together amounts to only 18mg. Similarly, total *T. scrupulosa* biomass removed amounts to 15.6mg. In contrast, the lowest biomass of *H. laceratalis* larvae removed by a single *Crematogaster* sp.1 colony was 1150mg, and the total *H.*

laceratalis biomass removed by all colonies together amounted to 100 510mg. Significantly more *H. laceratalis* biomass was removed than either *F. intermedia* or *T. scrupulosa* in comparable time intervals.

Ultimately, ant colonies which removed greater prey mass benefited more than colonies that removed greater numbers of smaller larvae and nymphs. However, a trade-off exists in prey size selection because larger larvae, while providing more biomass, also require more foragers and a longer time to subdue than do smaller prey. Foragers have been shown to use up to seven times more energy when running than when resting (Nielson *et al*. 1982; Lighton *et al*. 1987), indicating that the point of declining returns in energy gain is quickly met (Hölldobler & Wilson 1990). Furthermore, longer times spent foraging (subduing larvae) increase the risk of mortality of foragers by other predators and since the cost of producing replacement workers to forage and defend the colony against attack is high, selection should favour the choice of prey that is manageable in terms of time as opposed to size *per se* (Hölldobler & Wilson 1990).

Pomerinke (1999) saw two arboreal ant species, *Pseudomyrmex gracilis* (Roger) and *Crematogaster ashmeadi* (Mayr) feeding on *P. citrella* in Florida. Similarly, Robertson (1985) found that *Crematogaster* species preyed on small (1st instar) *Cactoblastis cactorum* larvae post hatching and pre-penetration of the cladodes. Other ant species, *Pheidole* sp. and *Anoplolepis steingroveri* preyed on *C. cactorum* larvae post-penetration, while the driver ant, *Dorylus helvolus* caused the most pupal mortality. Freitas and Oliveira (1996) studied the effects of ants as selective agents on an external feeding (leaf chewer) nymphalid butterfly, *Eunica bechina* Talbot. They found 1st and 2nd instar larvae (<6mm) were preyed on significantly more than larger instars. They concluded that susceptibility to ant predation increased with increasing levels of ant visitation and with decreasing larval size. In accordance with those authors (Robertson 1985; Freitas & Oliveira 1996; Xiao *et al*. 2006) results of the present study show that early instar (≤10mm) *H. laceratalis* larvae were removed far more frequently than larvae larger than 10mm (41:5) by all *Crematogaster* sp. 1 colonies. Larger larvae successfully deter foragers by flicking their end segments and spinning off the leaf if out-numbered by ants. However, spinning off equates to a loss of feeding time, and if the thread fails larvae that fall to the ground almost certainly

die because they are vulnerable to predation by other general predators (Fowler & MacGarvin 1985).

The contributions of predation and parasitism to mortality of the citrus leafminer *Phyllocnistis citrella* Stainton (Lepidoptera) populations in Florida were evaluated by ant exclusion and by direct observations of leaf mines in the field (Xiao *et al*. (2006). Survival of *P*. *citrella* was estimated at 11%, with parasitism accounting for slightly less than 30% of real mortality, while predation accounted for slightly less than 60%. Predation, predominantly by ants removing early instars of *P*. *citrella*, accounted for 60% of all deaths by predators and over 30% of all deaths by natural enemies (Huang *et al*. 1989; Pomerinke 1999; Amalin *et al*. 2002; Xiao *et al*. 2006). Unlike the hemipteran agents, lepidopteran agents for lantana suffer parasitism by native parasitoids that have extended their host ranges (Baars 2003). Thus, the combination of parasitism and predation on early instar lepidopteran larvae may be the reason why *H*. *laceratalis* occurs across the geographic range of lantana in South Africa but populations were usually rated by Baars (2003) as occasional.

3.5 Conclusion

Crematogaster sp.1 foragers removed the greatest number of highly mobile *F*. *intermedia* nymphs, followed closely by small *H. laceratalis* larvae (<10mm) and to a far lesser extent by *T. scrupulosa* nymphs. Colonies recruited most actively to *H. laceratalis* larvae and gained, on average, over a thousand times more biomass from preying on *H. laceratalis* larvae than they did from either hemipteran agent. Thus these larvae were most probably the preferred prey choice of these three biological control agent species. Since *T. scrupulosa* nymphs are unable to escape predators by moving, they appear to rely on cryptic behaviour and the presence of alternative prey. It is unlikely that *T. scrupulosa* nymphs are habitually preyed on by ant species unless they attract attention by being mobile, in search of better leaf quality, for example. The flighty escape strategy of *F*. *intermedia* attracts forager attention, so that even if they escape predation, their feeding and ovipositioning is probably interrupted by foraging ant species.

Chapter IV

No-choice experiments testing predation by *Crematogaster* sp. 2 on biological control agents for *Lantana camara*: *Falconia intermedia* and *Teleonemia scrupulosa* at three prey densities

4 Preface

The effect of different prey densities on predation by *Crematogaster* sp. 2 was tested using *Falconia intermedia* Distant (Hemiptera: Miridae) and *Teleonemia scrupulosa* Stål (Hemiptera: Tingidae) *F. intermedia* and *T. scrupulosa* nymphs. Results were compared both within and between agent species at each nymph density and with model predator functional response curves. Prey size preference was investigated for three nymph size classes (small, medium and large) of each agent. Results of the 30 nymph density trials using *Crematogaster* sp. 2 were compared with *Crematogaster* sp. 1 predation results on the same agents (Chapter III). Behavioural responses of the agents to *Crematogaster* sp. 2 foragers are discussed.

4.1 Introduction

Most species of the genus *Crematogaster* are generalist predators (Hölldobler & Wilson 1990). Generalist predators can inflict density-dependent mortality on prey populations (Crawley 1992). By switching to prey on species that are temporarily abundant, the percentage of prey killed can increase with increased prey density. Soloman (*et al*. 1949 *In* Crawley 1992) coined the phrase 'functional response' to describe the manner in which the quantity of prey eaten by a predator can change in accordance with prey density. Several models describe the functional responses of predators to prey density (Crawley 1992). The simplest 'fixed number predation' model (Type I), in which a predator eats a certain number of prey per unit time, irrespective of prey density, leads to a decrease in the percentage of prey population consumed as prey density increases. When prey is scarce, an alternative is the linear 'fixed proportion' functional response. This model is based on the assumption that each predator consumes a fixed proportion of the prey population, such that the

number of prey eaten per predator can increase with increasing prey density, yet the fraction of the prey population consumed remains density-independent. The Type II response, described independently by Holling (1959 *In* Crawley 1992) for shrews and Ivlev (1961 *In* Crawley 1992) for fish, states that when prey are scarce consumption increases with increasing prey density, yet the number of prey a predator can consume in a given time is limited by gut capacity or by the prey-handling time available to the predator. Both equations represent inversely density-dependent predation. Ivlev's equation is appropriate when the predator's gut capacity is the limiting factor, whereas Holling's equation is preferable when handling time can be measured independently and searching time is limited. Density-dependent predation is only represented by the sigmoid functional response (Type III) in which percentage predation increases with prey density to a maximum and then tails off. Many equations describe Type III responses but the most note worthy is one based on the assumption that attack rate varies asymptotically with prey density (Crawley 1992). This describes systems where predators are ineffective at locating prey at low prey densities, but become more successful with increasing prey density to a maximum efficiency when the prey is abundant. The least stable form of the functional responses (Type IV) arises when the efficiency of predator hunting skill decreases at high prey density. Type IV response can occur as a result of 'safety in numbers' as in herd animals or predator confusion by mass scattering of prey (Hamilton 1971 *In* Crawley 1992).

In order to determine whether ant recruitment to- and predation on agents increased with increased prey density, a series of 'prey density experiments' was performed using the agents *F. intermedia* and *T. scrupulosa* and five *Crematogaster* sp. 2 colonies. Larvae of *Hypena laceratalis* Walker (Lepidoptera: Noctuidae) were not included in this study because while nymphs of *F. intermedia* and *T. scrupulosa* are of comparable size, *H. laceratalis* larvae vary substantially in body length (Chapter II; Fig. 2.19). Furthermore, while highly mobile *F. intermedia* and the relatively immobile *T. scrupulosa* differ markedly in behaviour, *H. laceratalis* larvae are relatively immobile when feeding but move wildly as a form of defence making a clear comparison of predator cues impossible.

4.1.1 Aims of prey density experiments

The aims of prey density experiments were: 1) to determine which 'functional response' *Crematogaster* sp. 2 displayed with increasing prey availability; 2) to determine whether foragers would become saturated with agents at a maximum density of 60 nymphs (per two leaf pairs); 3) to test whether less desirable prey becomes more desirable at higher densities; 4) to observe prey (agent) behavioural differences (if any) at varying densities and 5) to determine which (if any) of the hemipteran agent instars are more vulnerable to ant predation, using three nymph classes: small, medium, large.

Since *Crematogaster* sp. 2 are social general predators, the Type I functional response does not apply as ant foragers do not collect fixed proportions of prey. Similarly, colony nutritional requirements limit predation rather than individual gut capacity and though handling time is limited to some extent by the risk of foragers being preyed on by other predators (Hölldobler & Wilson 1990), handling time is not finite *per se* so Type II predation is a less likely predation pattern upon agents by these ants. Thus, it is predicted that predation on agents by predatory species of the genus *Crematogaster* will be determined either by Type III or Type IV functional responses.

4.2 Materials and Methods

Due to availability in the study area and in order to contrast results with those of the sub-tropical *Crematogaster* sp. 1, five *Crematogaster* sp. 2 colonies (A-E) of comparable size were tested in these experiments. Prey density experiments were performed using the same protocol as used for agent predation trials, although the numbers of nymphs offered to the ants were: 10; 30; and 60 nymphs per experiment, and the experimental time was 50-60 minutes. These experiments were performed in an enclosed 10X30m plastic covered tunnel in which the temperature range for the duration of these experiments was between 20°C and 28°C. Ant nests were suspended by string from retort stands. Bunsen-burner stands were used as podiums on which prey-density treatments and controls were made available to the ant nests, via lines of string connecting the nests to the podiums. To prevent the ants from

escaping, all the stands were surrounded by moats of soapy water, which also humidified the air (Chapter II; Fig. 2.1).

For each prey density experiment, nymphs of *F. intermedia* and *T. scrupulosa* were offered in equal proportions from three size classes: small (1st and 2nd instars), medium (3rd instar) and large (4th and 5th instars). The respective body lengths for *F. intermedia* nymphs in each size class were approximately: small (<1mm), medium (1-1.9mm), large (2-2.5mm). Those of *T. scrupulosa* were: small (0.5-1.5mm), medium (2-2.5mm) and large (2.6-3.5mm). All nymphs were weighed according to their size class and their masses (mg) plotted for each species (Chapter II; Fig. 2.17 and 2.18). Average masses of nymphs in the three size classes were determined by dividing the sum of masses for each size class by the number of individuals measured for that size class. Average masses for *F. intermedia* nymphs are: small (0.03mg), medium (0.14mg) and large (0.3mg). Similarly, average masses for *T. scrupulosa* nymphs are: small (0.19mg), medium (0.47mg) and large (1.18mg).

4.2.1 Experimental design

When testing the recruitment to and predation on biological control agents by *Crematogaster* sp. 2 ants, foragers were offered a choice of: a) 10 or 30 or 60 agents on a lantana cutting in a petri-dish (2.5; 7.5 and 15 per two leaf-pairs); b) an empty petri-dish (Control 1) or c) a lantana cutting in a petri-dish (Control 2). The petri-dish provided access for ants and agents to both leaf surfaces by keeping the cuttings vertical. Lantana cuttings consisted of the terminal two leaf pairs of the Whitney farm (33°40'43"S, 26°35'49"E) lantana variety because the more discerning agent, *F. intermedia* accepts this variety (Cilliers 1987a; Urban and Simelane 1999; Baars *et al*. 2003; Heystek 2006). The cut stems were wrapped in moist paper towel to prevent rapid leaf desiccation, which could disturb feeding by these phloem suckers. Refer to Methodology (Chapter II) for details of the experimental design.

4.2.2 Statistical analyses

The nest volumes of *Crematogaster* sp. 2 colonies were similar (1 to 2.6L) and since the same colonies were used for all density trials using both agent species, prey density data presented in this chapter have not been transformed. Recruitment- and

predation rates were scaled to a common nest volume of 1L for comparison of agent predation by *Crematogaster* sp. 1 and 2 colonies. Scaled recruitment rates (Fig. 4.16 & 4.18) and predation rates (Fig. 4.17 & 4.19), are depicted for comparison of the agent predation rates by *Crematogaster* sp. 1 and 2.

Analyses on recruitment rates and predation rates were performed using the Kruskal-Wallis by Ranks and Median Test (a non-parametric ANOVA) because as in Chapter II; III and V these data are non-normally distributed. Similarly, the number of *F. intermedia* and *T. scrupulosa* nymphs belonging to three size classes (small, medium and large) that were removed in each prey density was recorded and as the data was not normally distributed, Kruskal-Wallis was used to test for preferences within prey smaller than 10mm. Kruskal-Wallis ranks the data and then assesses the hypothesis that the different (ranks of the) samples in the comparison were drawn from distributions with the same median or from the same distribution. All data points were analysed using Kruskal-Wallis. Therefore, results for each of the five colonies could be analysed together without the inaccuracy of generating means of means. Graphs present medians and quartiles rather than means, standard errors and standard deviations (Rosner 2000).

4.3 Results

Two hemipteran agent species differing in defence strategy were offered to five colonies of *Crematogaster* sp. 2 to test for functional responses to increasing prey densities. Results displayed here are the range of recruitment and predation rates for all *Crematogaster* sp. 2 colonies. Finally, recruitment and predation rates by *Crematogaster* sp. 1 and 2 are compared for each prey species, *F. intermedia* and *T. scrupulosa*, at a density of 30 nymphs (per two leaf pairs).

4.3.1 *Falconia intermedia* prey density trials

The recruitment responses of *Crematogaster* sp. 2 colonies varied within treatments, but significant recruitment patterns emerged between treatments (Fig. 4.1). Interestingly, significantly more foragers were recruited per minute to the 10 nymph treatment than to the 30 nymph treatment ($p = 0.0218$), while the difference in recruitment response between the 10 and 60 nymph treatments was not statistically significant. Forager recruitment to 60 *F. intermedia* nymphs was significantly greater than to 30 nymphs ($p = 0.0074$).

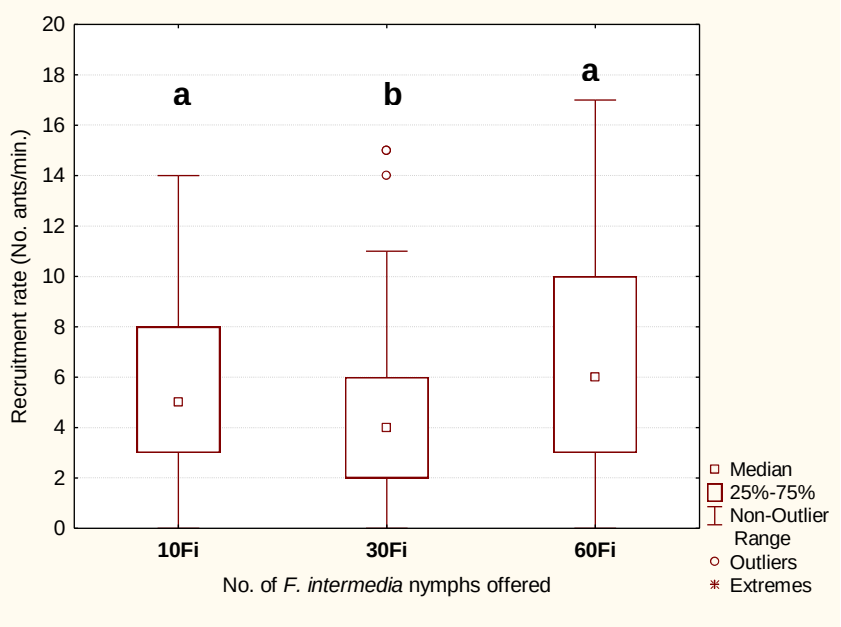

Fig. 4.1: Recruitment rates (No. foragers/min.) of five *Crematogaster* sp. 2 colonies to three densities of *Falconia intermedia* nymphs on *L. camara* cuttings ($n = 75$, $H = 11.0844$, $p = 0.0039$). Letters on graphs denote significant differences.

The recruitment response by foragers provides an indication of how attractive a food source is to the ants, although the predation rate (number of nymphs removed per hour) is perhaps more informative. The Kruskal Wallis nonparametric ranked medians test indicated no significant difference in number of *F. intermedia* removed at 10 versus 30 nymph densities, nor at 30 or 60 nymph availability (Fig. 4.2). However, significantly more nymphs were caught at a density of 60 nymphs (per two leaf pairs) than when only 10 nymphs were available (p = 0.0071).

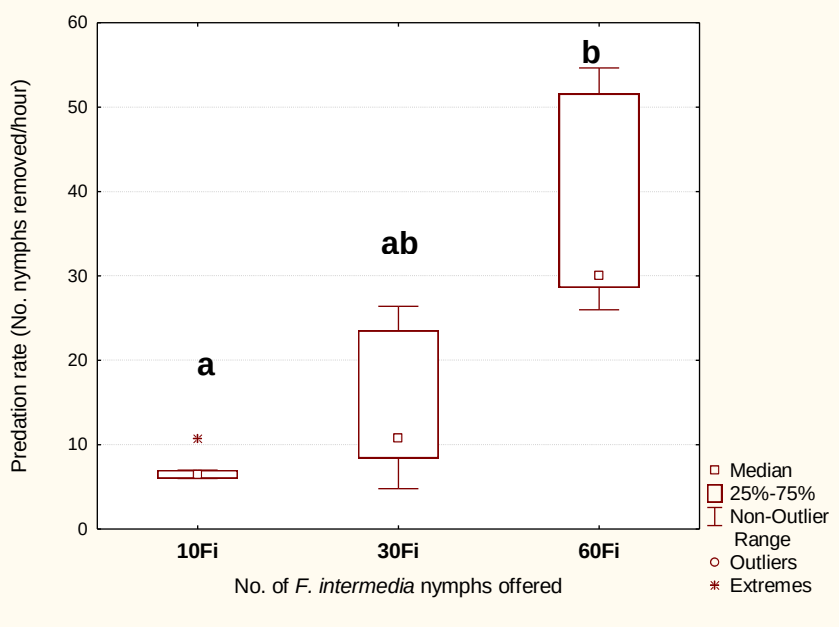

Fig. 4.2: Predation rates of five *Crematogaster* sp. 2 colonies on different densities of *Falconia intermedia* nymphs on *L. camara* cuttings (n = 5, H = 9.6372, p = 0.0081). Letters on graphs denote significant differences.

To meet the requirement of comparing an equal number of variables statistically, the scatterplot (Fig. 4.3) was created using one 'average recruitment' number per experiment (sum of the 15 replicates, divided by 15). This was done because each experiment resulted in only one predation rate per density treatment. The result is a weakly significant correlation between predation rates and forager recruitment ($p = 0.0302$, $r^2 = 0.3129$). This correlation contradicts the above results (Fig. 4.2), illustrating the strength of the Kruskal-Wallis nonparametric test for reporting only highly significant results by ranking the data rather than analysing means of data, as in Fig. 4.3.

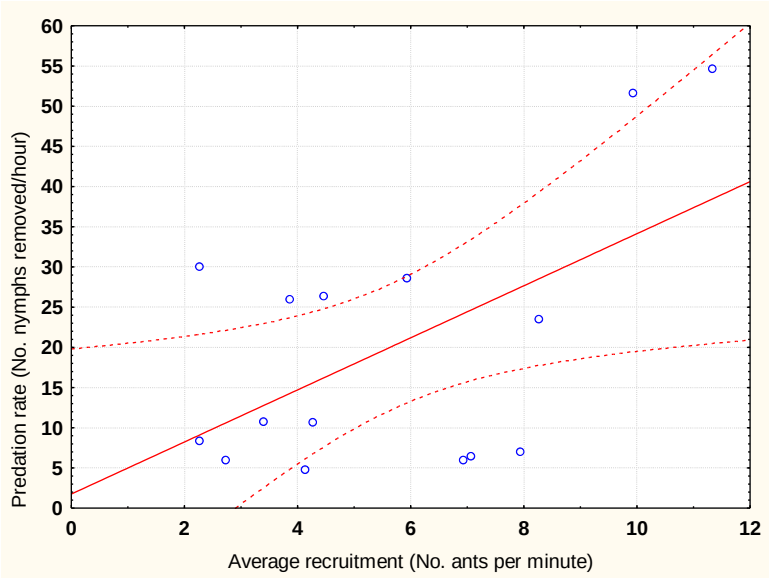

Fig. 4.3: The correlation between predation rate of *F. intermedia* nymphs and average recruitment by *Crematogaster* sp. 2 colonies during prey density experiments in which 10, 30 and 60 nymphs were offered ($r = 0.5594$, $p = 0.0302$, $r^2 = 0.3129$).

4.3.2 *Teleonemia scrupulosa* prey density trials

Results of the *T. scrupulosa* prey density experiments indicated that *Crematogaster* sp. 2 ants recruited similar frequencies of foragers when 10 nymphs or 30 nymphs were offered (Fig. 4.4). However, when 60 nymphs were offered, these colonies recruited significantly more foragers per minute than to 30 nymphs or the 10 *T. scrupulosa* nymph treatment ($p < 0.0001$).

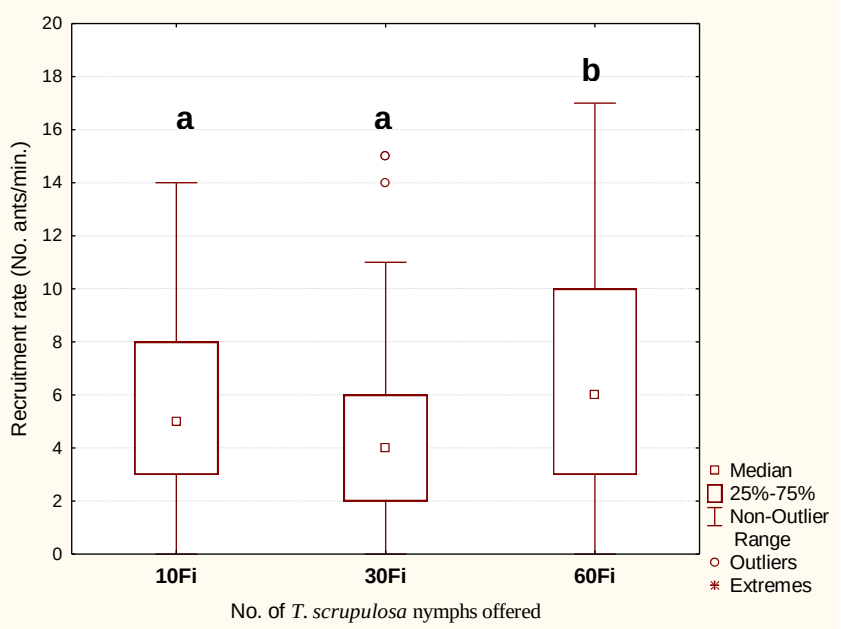

Fig. 4.4: Recruitment rates (No. foragers/min.) of five *Crematogaster* sp. 2 colonies to three densities of *Teleonemia scrupulosa* nymphs on *L. camara* cuttings (n = 75, H = 45.4492, $p < 0.0001$). Letters on graphs denote significant differences.

Significantly more *T. scrupulosa* nymphs were removed at the 60 nymphs (per two leaf pairs) density than when 10 nymphs were available ($p = 0.0362$). Differences in the predation rates at 10 versus 30 and 30 versus 60 densities were not significant (Fig. 4.5). For both *F. intermedia* and *T. scrupulosa* substantially (but not significantly) more nymphs were removed at 60 than 30 nymph densities.

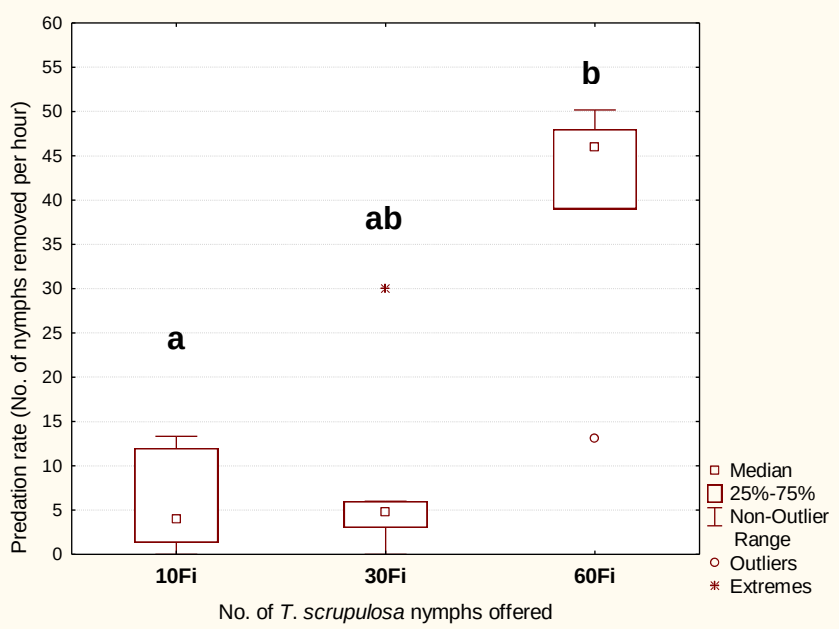

Fig. 4.5: Predation rates of five *Crematogaster* sp. 2 colonies on three densities of *Teleonemia scrupulosa* nymphs on *L. camara* (n = 5, H = 7.9692, p = 0.0186). Letters on graphs denote significant differences.

As in Fig. 4.3, for comparative purposes this scatterplot (Fig. 4.6) was created using one 'average recruitment' number to compare with the predation rates per density treatment. The correlation between predation rates and forager recruitment is significant (p = 0.0012, r^2 = 0.5645).

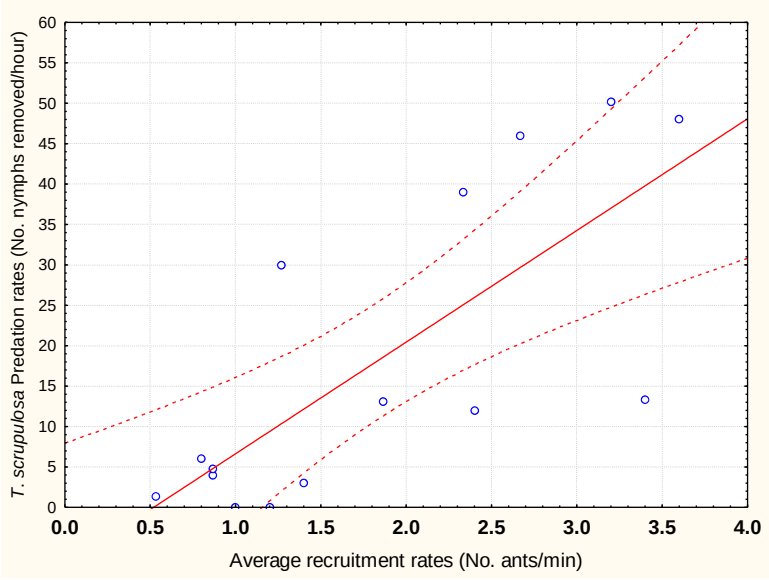

Fig. 4.6: The correlation between predation rate of *T. scrupulosa* nymphs and average recruitment by *Crematogaster* sp. 2 colonies during prey density experiments in which 10, 30 and 60 nymphs were offered (r = 0.7513, p = 0.0012, r^2 = 0.5645).

4.3.3 Comparison of results for agent density trials

Significantly more foragers were recruited to 10, 30 and 60 *F. intermedia* nymphs than to both the 10 and 30 *T. scrupulosa* nymph treatments ($p < 0.0001$ for each density treatment). Similarly, significantly more foragers were recruited to 10 and 60 *F. intermedia* nymphs than to 60 *T. scrupulosa* nymphs ($p < 0.0001$ for both density treatments). However the difference in recruitment between 30 *F. intermedia* nymphs and 60 *T. scrupulosa* was not significant. Significantly more foragers were recruited to the 60 *T. scrupulosa* treatment than to 30 *T. scrupulosa* nymphs ($p = 0.0078$) or 10 *T. scrupulosa* nymphs ($p < 0.0001$). Recruitment rates did not differ significantly between the *F. intermedia* nymph density treatments.

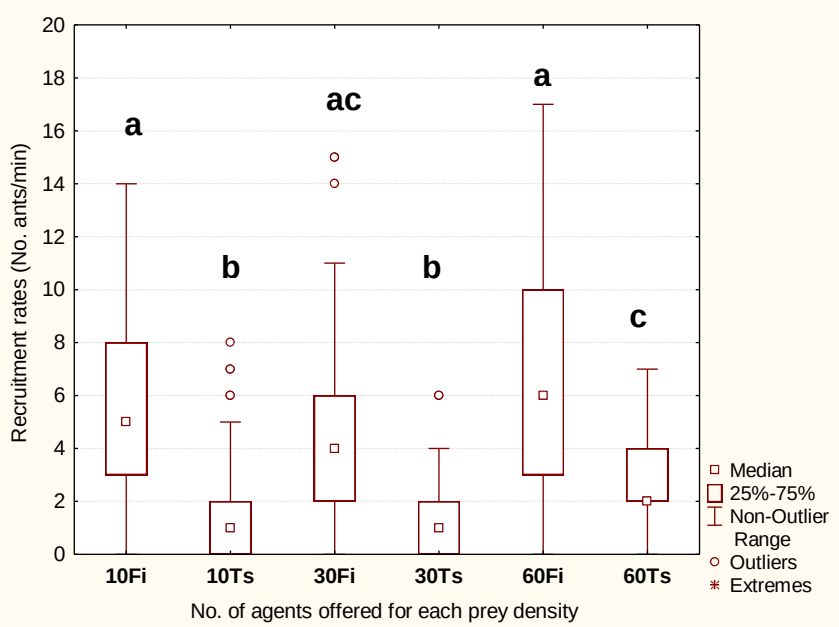

Fig. 4.7: Comparison of recruitment of five *Crematogaster* sp. 2 colonies to three densities (10, 30 and 60) of *F. intermedia* and *T. scrupulosa* nymphs on two *L. camara* leaf pairs in no choice experiments ($n = 75$, $H = 176.9466$, $p < 0.0001$). Letters on graphs denote significant differences.

Despite the significant differences in recruitment rates of ants to the two agents, predation rates were similar. Only the 60 *F. intermedia* density resulted in a significantly higher predation rate than on 10 *T. scrupulosa* nymphs ($p = 0.0484$).

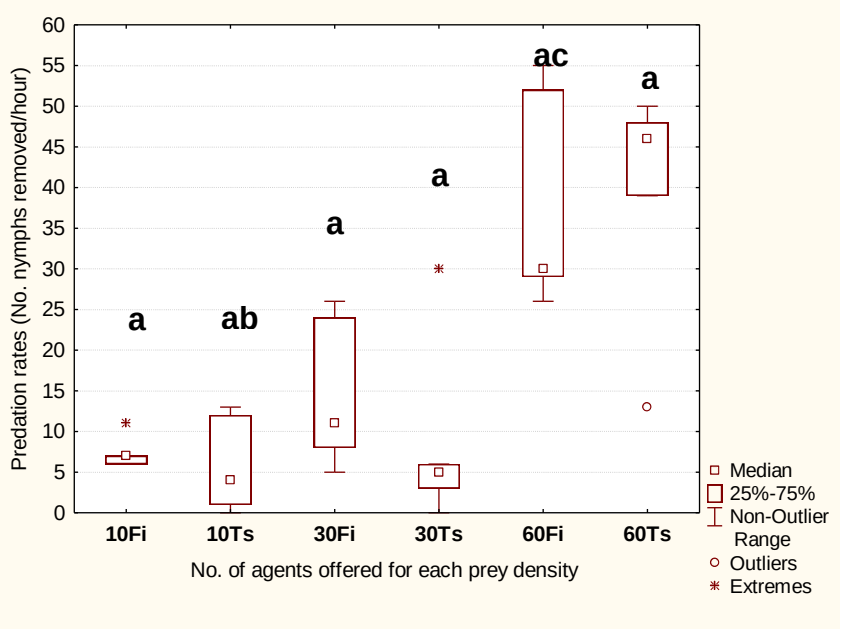

Fig. 4.8: Comparison of predation rates of five *Crematogaster* sp. 2 colonies on three densities (10, 30 and 60) of *F. intermedia* and *T. scrupulosa* nymphs on two *L. camara* leaf pairs in no choice experiments (n = 5, H = 18.5008, p = 0.0024). Letters on graphs denote significant differences.

4.3.4 Predator functional response curves

Both the numbers and percentages of agents consumed at each density tested were plotted against the density of prey available per two leaf pairs (Fig. 4.9). When the numbers of prey removed are plotted against agent density, the curves produced (A & C) are more similar to the Type III sigmoid functional response curve than to the linear, asymptotic or humped curves of Type I, II and IV respectively. However, when the percentage prey consumed is plotted against prey density (B and D), neither agent percentage prey curve resembled any of the model predator functional response curves exactly, as is often the case in nature. Both agent predation curves better resemble, though not closely, either of the non-linear but inverse density-dependent percentage kill functional responses of Type II (with a limit to the amount a predator can eat set by gut capacity or handling time) or Type IV (where the number of prey killed declines at very high densities due to group defence effects). While the percentage of *F. intermedia* nymphs killed was higher at 10 than 60 prey density, the reverse was true for *T. scrupulosa* treatments 10 and 60. Both agent percentage prey curves (B & D) were peculiar in that percentage predation declined from the 10 nymph treatment to the 30 nymph density and then increased again to a maximum

percentage prey removed at 60 in the case of *T. scrupulosa* and a near equal (to the 10 nymph treatment) percentage prey removed in the case of *F. intermedia*. The numbers of nymphs removed in the 60 nymph treatments were significantly greater than in the 10 nymph treatments for both agents and far greater than in the 30 nymph treatments. The decrease in predation rates between the 10 and 30 *F. intermedia* nymph treatment could be a result of the confusion effect that 30 fast-moving *F. intermedia* nymphs have on predators when they dart about. Yet at densities of 60 nymphs, the presence of many foragers (on average 6 foragers per minute, accumulative since some ants remain foraging on the leaves), and more nymphs in a confined space (two leaf-pairs) could mean that nymphs collide with one another or into foragers and are thus more easily caught (Pitcher 1986 *In* Crawley 1992).

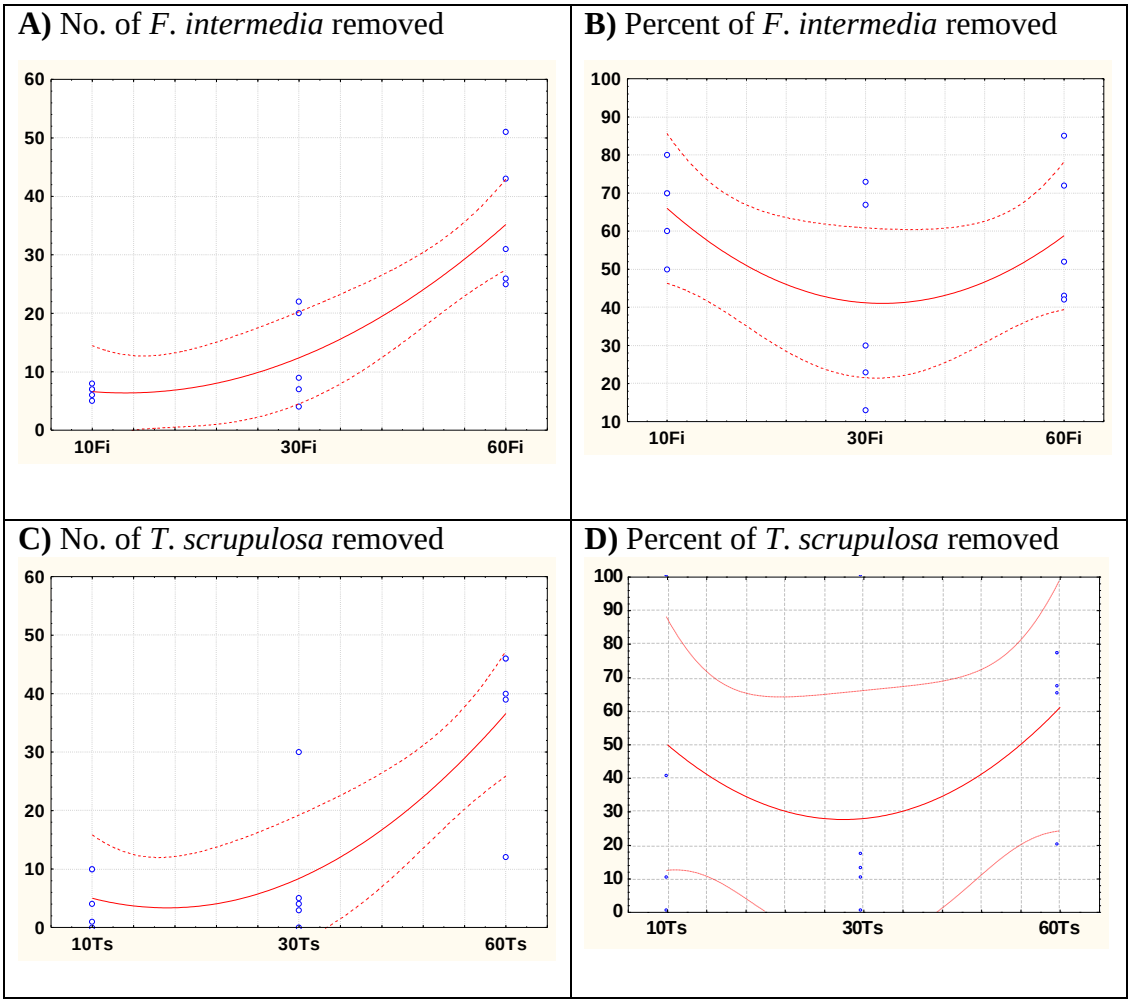

Fig. 4.9: Functional response curves of *Crematogaster* sp. 2 feeding on *F. intermedia* (A & C) and *T. scrupulosa* (B & D) at prey densities of 10, 30 and 60 nymphs per two leaf pairs of *L. camara* cuttings during hour-long experiments.

In the case of *T. scrupulosa* it could be that nymphs did not move much at densities of 30 nymphs and thus were not very appealing but at 60 nymphs per leaf pair, competition for choice feeding positions meant nymphs began to move in search of better leaf quality and once foragers began preying on the nymphs, larger nymphs tried to escape, further attracting forager attention. Forager investigations of *T. scrupulosa* nymphs became more persistent and aggressive once they identified these nymphs as prey items and only well hidden nymphs survived the 60 density experiments. Most of the 10 and 30 nymph density experiments resulted in five or fewer nymphs being removed. Colonies A and D removed all 10 nymphs during the 10 density treatment but this was explained for colony D by the fact that the nymphs were unusually mobile during this experiment. The experiment was performed using new green, hairy leaf pairs (same lantana variety) which seemed to cause the nymphs to move. Another anomaly was when testing colony E all nymphs from the 30 density treatment were removed.

4.3.5 Prey size preference

Results of the Kruskal-Wallis test showed that significantly fewer large *F. intermedia* nymphs were removed when only 10 nymphs were offered than the numbers of small ($p = 0.012$), medium ($p = 0.0026$) and large ($p = 0.0007$) nymphs removed at the 60 nymph density (Fig. 4.10). However no significant differences existed when the comparison was made within density treatments.

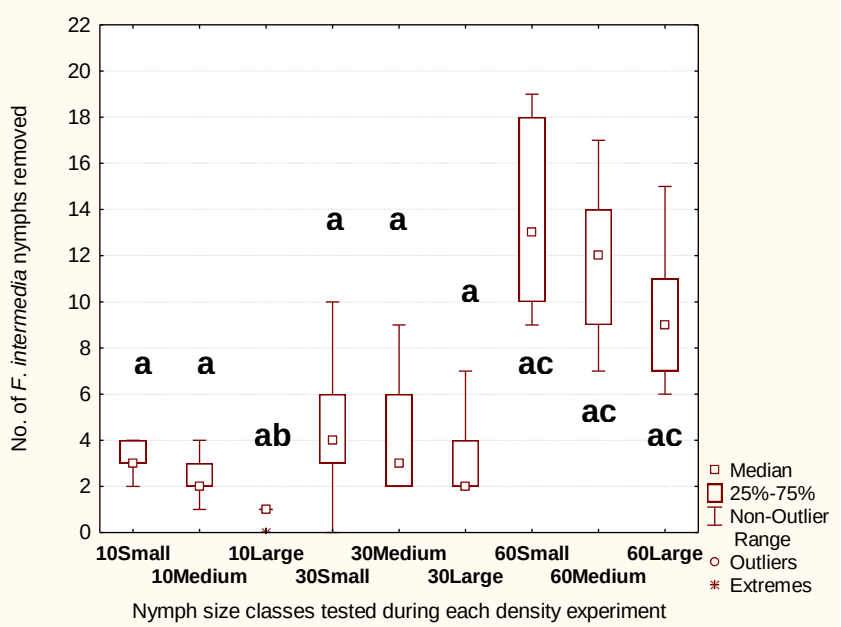

Fig. 4.10: Comparison of predation rates of five *Crematogaster* sp. 2 colonies on different sized *F. intermedia* nymphs at three densities (10; 30; 60) on two *Lantana camara* leaf pairs in no choice experiments (n = 5, H = 32.336, p = 0.0001). Letters on graphs denote significant differences.

Results showed no significant differences between the different sized classes of *T. scrupulosa* nymphs removed by *Crematogaster* sp. 2 both within and between agent density treatments. However, there is significant variation (p = 0.0038) in the numbers of nymphs removed between the density treatments (Fig. 4.11).

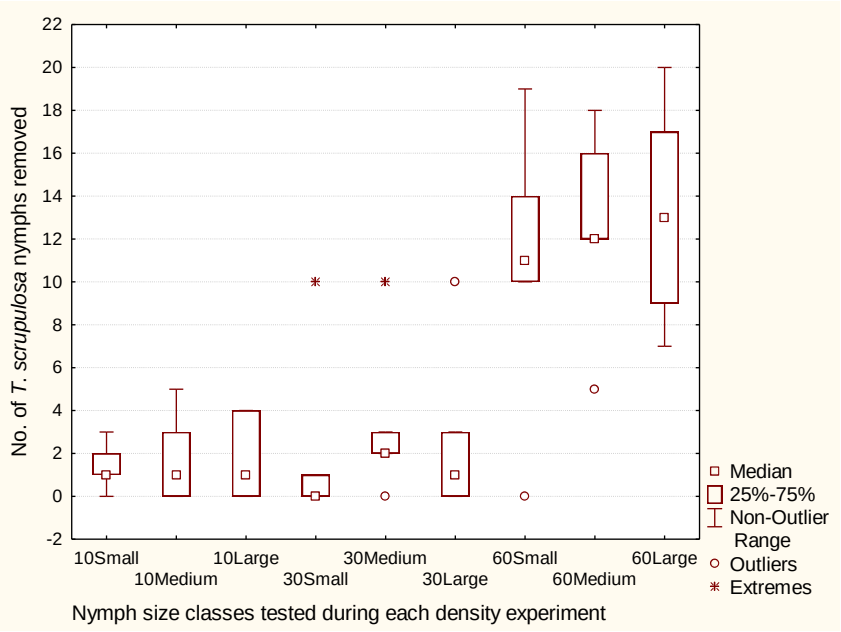

Fig. 4.11: Comparison of predation rates of five *Crematogaster* sp. 2 colonies on different sized *T. scrupulosa* nymphs at three densities (10; 30; 60) on two *Lantana camara* leaf pairs in no choice experiments (n = 5, H = 22.6627, p = 0.0038).

4.3.6 Comparison of agent predation between *Crematogaster* species

Results of these prey density experiments have shown that predation rates at 30 versus 60 nymph densities did not differ significantly for *F. intermedia* (Fig. 4.2) or *T. scrupulosa* (Fig. 4.5) experiments. These results therefore legitimise the comparison of results for the *F. intermedia* predation experiment using *Crematogaster* sp.1 colony 2, even though colony 2 was offered 60 nymphs, while the other four *Crematogaster* sp.1 colonies were offered 30 nymphs. Similarly, these results legitimise comparison of *Crematogaster* sp. 1 and 2 agent data (Fig. 4.12 and 4.13).

Foragers of *Crematogaster* sp. 2 colonies recruited significantly fewer foragers per minute to the *T. scrupulosa* treatment than they did to *F. intermedia* nymphs, or than what *Crematogaster* sp. 1 colonies recruited to either *F. intermedia* or *T. scrupulosa* ($p < 0.0001$ in each case). In addition, *Crematogaster* sp. 1 colonies recruited significantly fewer foragers to the *T. scrupulosa* treatment than *Crematogaster* sp. 2 colonies recruited to *F. intermedia* nymphs ($p < 0.0001$).

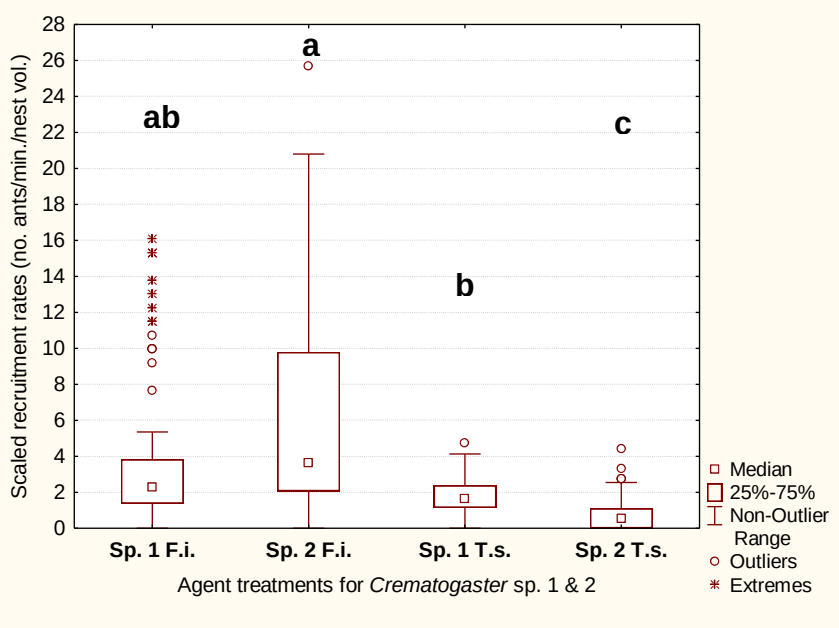

Fig. 4.12: Recruitment rates (scaled to a common nest size of 1L) of five Crematogaster sp. 1 and 2 colonies on *F. intermedia* and *T. scrupulosa* nymphs on *L. camara* cuttings in no choice experiments ($n = 75$, $H = 94.2016$, $p < 0.0001$). Letters on graphs denote significant differences.

Results of the Kruskal-Wallis test showed that *Crematogaster* sp. 2 colonies removed significantly more *F. intermedia* nymphs than *Crematogaster* sp. 1 colonies removed of *T. scrupulosa* nymphs (p = 0.008) in an hour (Fig. 13).

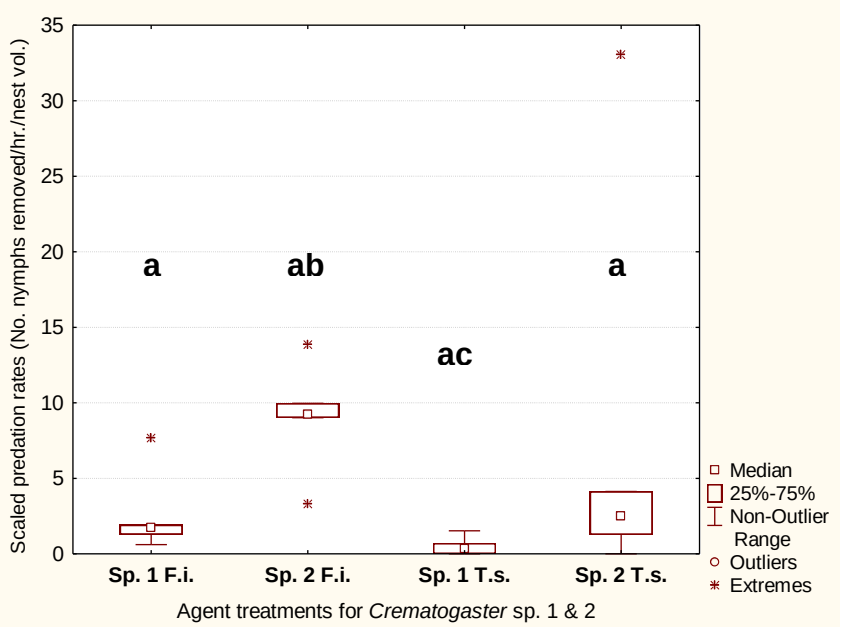

Fig. 4.13: Predation rates (scaled to a common nest size of 1L) of five *Crematogaster* sp. 1 and 2 colonies on *F. intermedia* and *T. scrupulosa* nymphs on two *Lantana camara* leaf pairs in no choice experiments (n = 5, H = 10.4256, p = 0.0153). Letters on graphs denote significant differences.

4.3.7 Behavioural responses of agents to predation

Behaviour exhibited by these agents was similar to their behaviour during the agent predation trials using *Crematogaster* sp. 1 colonies. Nymphs of *F. intermedia* are easily disturbed but their movement probably draws forager attention, identifying them as prey items (Bergelson & Lawton 1988) and *Crematogaster* sp. 2 ants were often able to out-run these nymphs due to their larger step-length. Although *F. intermedia* nymphs are fast moving, it is probably their ability to change direction quickly and dart about that enables them to escape ant predation. When nymphs change direction suddenly, the predator loses sight of the nymph temporarily its trajectory. *Falconia intermedia* nymphs are fast moving and thus difficult to catch but when forager and/or nymph densities on a leaf were high, nymphs could no longer escape without colliding with another nymph or a forager. In contrast, *T. scrupulosa* nymphs generally appeared oblivious to *Crematogaster* sp. 2 foragers. As noted in the agent predation experiments (Chapter III), ants were observed touching stationary

T. scrupulosa nymphs and then leaving them to continue foraging, while nymphs continued feeding. During the 30 nymph density experiment, testing colony C, although about 9 foragers were on the leaf pairs at a time, they did not encounter *T. scrupulosa* nymphs much and did not disturb them when they did. Nymphs were noted as only moving in the absence of ants. No nymphs were removed for this trial or for the 10 density nymph trial which followed, and only 12 nymphs were removed during the 60 density experiment. Similarly, during the 60 (colony C) and 10 (colony E) density treatments, *T. scrupulosa* nymphs were observed quite motionless, and though ants ran past and even over them, they did not attack. During the 30 density treatment, using colony A, ants were seen touching stationary feeding nymphs, sometimes even returning to inspect them, but then left them. A small nymph that had been climbing the string was ignored by foragers because it was stationary at the time. During the 60 density treatment (colony A), a nymph seen walking up the string was ignored by a few foragers but eventually detected and removed. Similarly, during the 30 treatment (colony D) a nymph climbing the string was initially ignored but removed by a subsequent forager. Large (4^{th} and 5^{th} instar) *T. scrupulosa* nymphs were observed being mobile more often than the smallest instars (Cilliers 1987a) probably motivated by a need for larger quantities of good quality phloem. However, there were no significant differences in the numbers of different-sized nymphs removed for either agent within density trials. Observable differences in *T. scrupulosa* behaviour were that at the density of 60 nymphs per two leaf cuttings, these nymphs moved around more, probably in search of suitable feeding areas within the relatively confined space of two leaf pairs. More dramatically however, once foragers had started catching nymphs and were on the leaves in high numbers, *T. scrupulosa* nymphs tried to flee although they were unable to out-run foragers. During the 60 density treatment (colony D), it was noted that one terminal leaf had began to curl due to the high nymph density and this appeared to offer some protection as foragers did not enter the curled up area where the nymphs fed. It was also noted that ants searched predominantly the leaf margins and undersides of the leaves but not the dorsal centres of the leaves. Searching leaf margins possibly makes navigating easier for the ants while undersides of leaves offer protection for the ants from birds and other flying predators.

4.4 Discussion

Prey density experiments were each run for an hour, and none of the five colonies removed every *F. intermedia* nymph during the trials. However, both recruitment and predation rates were significantly higher at the 60 than 30 densities so it is unlikely that colonies were saturated by 60 *F. intermedia* nymphs. The 'confusion effect' refers to reduced attack efficiency experienced by predators when many prey flee simultaneously in different directions (Pitcher 1986 *In* Crawley 1992). This escape tactic appears to hold only for a threshold of prey density as the converse can also be true, in that prey at high densities can themselves become confused and find their escape routes obstructed by their group members (Pitcher 1986 *In* Crawley 1992). Nymphs of *F. intermedia* are fast moving and dart out of the way of predators (*pers. obs.*). However, when space is constrained (i.e. due to high nymph or forager densities per leaf), nymphs do not escape as easily as when there are fewer nymphs and/or foragers per *L. camara* leaf. This is suggested by the fact that at the 60 density these nymphs were caught at significantly higher rates than at a density of 10 nymphs per two leaf pairs, despite the recruitment rates not having differed significantly. Thus it seems that *F. intermedia* nymphs are more difficult to catch as the number of available prey declines. Interestingly, although significantly fewer foragers were recruited to the 30- than to the 10 nymph treatment, predation rates at the 30 nymph treatment were greater (though not significantly) than those of the 10 nymph treatment. This provides support for the trend of higher predation rates at higher densities of either nymphs or foragers ('traffic'). Nymphs were more difficult to catch at lower densities so recruitment was higher at the 10 versus 30 nymph density. At 60 *F. intermedia* nymph density, nymphs were easily caught due to high traffic but recruitment was high in response to a greater quantity of available prey. Of the likely functional responses by *Crematogaster* sp. 2, the sigmoid Type III response fits the curve created by the number of *F. intermedia* nymphs consumed, although the resultant percentage prey removed curve does not resemble the model. Percentage prey killed appears high at low densities and very high densities but low at medium densities. It is likely that percentage kill at low densities (10 nymphs per two leaf pairs) was higher than at 30 density only because recruitment was higher to the 10 density treatment.

The longest established agent in South Africa, *T. scrupulosa*, has been reported as one of the three most effective agents at providing control against *L. camara* (Cilliers & Neser 1991; Baars 2003; Simelane & Phenye 2005). What the three effective agents, *Octotoma scabripennis*, *Uroplata girardi* and *T. scrupulosa* have in common is that they are under low parasitoid pressure and although a few generalist predators prey on them, predation is not heavy enough to reduce their populations (Baars & Neser 1999; Baars & Heystek 2003). Something else that *O. scabripennis* and *T. scrupulosa* have in common is that they are both nearly motionless during the day (Chew *et al*. 2003), which is when most predatory ant species on tropical vegetation forage (Novotny *et al*. 1999). In chapter III it was established that remaining motionless in the presence of predators was a successful strategy for escaping predation (Schultz 1983b; Bergelson & Lawton 1988; Freitas & Oliveira 1996) especially by some ant species (Wehner 1981; Wehner *et al*. 1983; Hölldobler & Wilson 1990).

An observable difference in *T. scrupulosa* behaviour was that at the maximum density of 60 nymphs per two leaf cuttings, these nymphs moved around a little more, probably in search of better feeding areas. Since *T. scrupulosa* nymphs are unable to escape predators by moving, they appear to rely on the presence of other herbivores attracting predator attention. When *T. scrupulosa* do move (e.g. once leaf nutrition is inadequate) they should do so in the absence of predators. Although immobility of *T. scrupulosa* nymphs can be an effective defence strategy at low forager densities, the effectiveness of 'enemy-free space' with regard to the refuges of some prey species, appear rather conditional on the density of the wood ant, *Formica lugubris* Zett. (Ito & Higashi 1991). In areas where *F. lugubris* nests are densely distributed, leaf-miners cannot escape predation (Sato & Higashi 1987) whereas at low *F. lugubris* densities they are almost free from predation (Ito & Higashi 1991). Nymphs of *T. scrupulosa* froze in the presence of foragers (even if they had been moving) unless forager density was high, in which case larger nymphs in particular began to move about. Once foragers had started catching nymphs and were on the leaves in high numbers, *T. scrupulosa* nymphs appeared to be trying to escape although they were not able to out-run foragers.

Crematogaster species employ strategies of foraging that make them highly efficient predators. Foragers participate in group retrieval, enabling them to remove food items

at many times heavier than a solitary forager can carry. A single ant can carry at least ten times its own weight (Traniello 1989). They also forage in large numbers and lay trunk trails (Hölldobler & Wilson 1990), making them better suited to taking advantage of high density food sources than individually foraging species (Davidson 1977). The energy costs of foragers both in energy to run and replace is high, so colonies will not disburse all their foragers continuously unless the food source outweighs the energy cost of retrieving it (Hölldobler & Wilson 1990). The advantage lies in the fact that there are large numbers of foragers that can respond quickly to desirable food sources as indicated by foragers' trails upon discovery. In nature, other ant species frequently make use of *Crematogaster* species trunk trails thereby exploiting the same food source while avoiding attack by *Crematogaster* foragers. Both hemipteran agent species are of a manageable size for individual foragers and didn't require group cooperation, but *F. intermedia* nymphs are far more difficult to catch than *T. scrupulosa* nymphs. This may explain the significantly higher recruitment rates to *F. intermedia* nymphs at each density, although predation rates were comparable for the agents at each density.

The 30 density experiments were performed before the10 *T. scrupulosa* nymph experiments for every colony. It is possible that once foragers had identified *T. scrupulosa* nymphs as prey during the 30 nymph treatment they were then recognised as prey in the 10 nymph treatment. This suggestion is supported by the fact that colonies that did not remove any nymphs during the 30 nymph treatment, did not remove any in the 10 nymph treatment either. However, all colonies removed nymphs during the 60 density treatment.

Comparison of predation on 30 nymph densities of both agents by both ant species (Fig. 4.12) showed that *Crematogaster* sp. 2 colonies generally recruited fewer foragers per minute than did *Crematogaster* sp. 1 colonies to either agent treatment. This is strange considering that *Crematogaster* sp. 2 colonies removed more (though not significantly) of both agents than did *Crematogaster* sp. 1 colonies in an hour (Fig. 4.13). This suggests that *Crematogaster* sp. 2 colonies were better adapted to hunting small hemipterans than *Crematogaster* sp. 1 colonies.

4.5 Conclusion

These prey density experiments reveal that *F. intermedia* nymphs are vulnerable to ant predation, particularly at high nymph densities. Escape by *F. intermedia* nymphs was impeded by high nymph and/or forager densities. Thus it is likely that predation by ant species significantly impacts *F. intermedia* population growth in the field. Predation rates on the agents were similar, but recruitment rates of *Crematogaster* sp. 2 colonies to *F. intermedia* nymphs were significantly higher than to *T. scrupulosa* nymphs for each density of agent tested, because *F. intermedia* are more difficult to catch than nymphs of *T. scrupulosa*. Predation rates on 60 *T. scrupulosa* were higher than the 30 nymph density and significantly higher than predation at the 10 nymph densities. This suggests that while predation in the field may affect *T. scrupulosa* at the high density of 60 nymphs per two leaf pairs, the cryptic escape strategy may serve *T. scrupulosa* better at lower nymph and forager densities.

Chapter V

Ant predation on *Falconia intermedia* or *Teleonemia scrupulosa* nymphs during week-long choice experiments.

5 Preface

This chapter presents results of choice experiments testing the effect of predation by *Crematogaster* sp. 2 ants on the efficacy of *Falconia intermedia* and *Teleonemia scrupulosa* nymphs as biological control agents for *Lantana camara*. Plant parameters were measured to determine the feeding damage produced by the agents on ant-accessed versus ant-excluded plants, and absolute numbers of agents surviving on ant-accessed plants were analysed.

5.1 Introduction

Top-down trophic cascade models predict that species diversity as well as plant biomass can be affected by the number of trophic levels in the ecosystem (Dyer & Letourneau 1999). Food webs differ subtly from trophic cascades in that a food web could involve several natural enemies and several herbivores feeding on a plant, and still be considered a three-level trophic cascade (van Veen *et al*. 2006). Models predict that even- and odd-numbered trophic cascades will exhibit low- and high-plant biomass respectively. These models are often supported by tests performed in aquatic systems but terrestrial systems are believed to be more complex, facilitating buffering and compensation among species (Strong 1988; Dyer & Letourneau 1999; van Veen *et al*. 2006). Studies have shown that top predators in three-level terrestrial systems can positively affect plant biomass (Karhu 1998; Dyer & Letourneau 1999; Dyer 2002; Tanaka *et al*. 2009), while effects of top predators in a four-level terrestrial system manifest more slowly (Dyer & Letourneau 1999). The third trophic level (natural enemies such as predators, parasitoids and pathogens) has been described as an element of plant defence against herbivores. In fact it is possible that for many plants, resistance may only be effective in the presence of natural enemies.

Conversely, resistance can reduce natural enemy effectiveness if, for example, the herbivore can sequester plant defence toxins (Price *et al*. 1980; Shultz 1983a). Other forms of plant resistance are physical adaptations (e.g. pubescence, trichomes, silica, extra floral nectaries) and physiochemical adaptations such as secondary defensive compounds (e.g. resins, toxins, insect hormone analogues) and manipulation of nutrient concentrations in plant parts (Price *et al*. 1980; Shultz 1983a&b; Buckley 1987; Crawley 1989).

Complicating the three-trophic level interaction between plant (weed), herbivore(s) (biological control agents) and ant species is the relationship between many ant species and ant-tended homopteran species. Many ant-tended homopteran species have lost all defence mechanisms exhibited by other homopterans (e.g. waxes and spines) and are solely dependent on ants for protection, transport to desirable feeding sites, hygiene and even parental care (Way 1963; Buckley 1987). In plant-homopteran-ant interactions the ant species which should benefit the plant by preying on the herbivore, is actually enhancing homopteran survival, increasing homopteran populations and therefore promoting plant phloem loss. However, protection offered by ants can exclude not only natural enemies of homopterans but other herbivores too which may result in a net decrease in plant loss (Buckley 1987). Interactions between ant-tended homopterans and ants can be even further complicated by the fact that in some relationships, ants can change from protecting homopterans to preying on them when extra floral nectaries offer better nectar or ants may tend some of the homopteran population while 'culling' others at high population densities (Buckley 1987; Becerra 1989; Cushman 1991). In essence, the presence of a homopteran-ant association on a target weed plant is usually impact herbivorous and parasitoid biological control agents negatively (Price *et al*. 1980; James *et al*. 1997; Martinez-Ferrer *et al*. 2002).

The efficacy of biological control agents is often measured indirectly by the observed herbivory (feeding damage) of agents on the target weed (e.g. Baars 2003; Baars & Heystek 2003). The effect of ant predation on (the survival and efficacy of) biological control agents is a three-level system that can indirectly be denoted by levels of herbivory and plant growth parameters (Robertson 1985). Ant visitation is another common method of measuring potential predation on herbivores by observation; snap-

shot censes often conducted via insecticidal fogging of tree canopies; trapping (e.g. pitfall, bait) and by measuring recruitment rates (numbers of foragers in time) to herbivore feeding sites (Dyer & Letourneau 1999; Novotny *et al*. 1999; Dyer 2002; Floren *et al*. 2002).

5.1.1 Aims of agent choice experiments

The aims of this chapter were to 1) determine whether *Crematogaster* sp. 2 prey on *F. intermedia* and *T. scrupulosa* nymphs under more natural conditions; 2) determine whether *Crematogaster* sp. 2 show a preference for *F. intermedia* or *T. scrupulosa* nymphs; 3) investigate agent defence strategies (survival) at relatively low agent densities, and 4) determine agent efficacy (feeding damage) at relatively low agent densities (0.27 per leaf) in the presence and absence of ants.

Early field experiments were performed using *H. laceratalis* larvae in addition to *F. intermedia* and *T. scrupulosa* nymphs, but it was only possible to use 1st instar larvae to avoid larvae growing to an unmanageable size (for the ants) within the week-long experiment. Thus the time required to coordinate availability of 160 immature agents of each species at the correct life-stages as well as nine similar-sized shrubs was prohibitively long. Furthermore, it was established in Chapter III that small (<10mm) *H. laceratalis* larvae are significantly preyed on by *Crematogaster* sp. 1 and pilot prey acceptability experiments showed that *Crematogaster* sp. 2 also readily prey on *H. laceratalis* larvae of up to 12mm body length (Chapter II).

Crematogaster sp. 1 preyed on *F. intermedia* nymphs significantly more than on *T. scrupulosa* nymphs (Chapter III). Yet in prey density experiments (Chapter IV) both *T. scrupulosa* and *F. intermedia* (especially the latter) nymphs were preyed on by *Crematogaster* sp. 2. In the field, *T. scrupulosa* is a successful agent whereas *F. intermedia* has established at very few localities, making it an ineffective agent thus far. Therefore a comparison is made between the two hemipteran agents without the additional choice of the lepidopteran agent.

5.2 Methods

In previous no-choice experiments (Chapters III and IV) *Crematogaster* sp. colonies were offered one agent species (and controls 1 & 2) on lantana cuttings per hour-long experiment. Choice experiments differ in that *Crematogaster* sp. 2 colonies were offered a choice of 80 *F. intermedia* or 80 *T. scrupulosa* nymphs over a period of a week. Choice experiments were performed in a 10X30m tunnel where the minimum and maximum temperatures were 6°C and 39°C respectively. Day length, temperature and humidity were allowed to vary with ambient conditions, although the cement floor around the experimental set-up was sprayed with water once a day if temperatures exceeded 30°C. Data were captured twice daily (morning and afternoon) at temperatures between 20 and 28°C. Seven potted *L. camara* shrubs having approximately 300 leaves were sprayed with a general insecticide, Chloropyrifos®, three weeks prior to experiments being performed to ensure that the plants were not infested with non-study ant species or herbivores (homopteran species in particular). *Lantana camara* shrubs of the variety from Whitney Farm (33°40'43"S, 26°35'49"E), where *F. intermedia* were collected, were used since *T. scrupulosa* perform well on most varieties (Cilliers 1987a; Urban and Simelane 1999; Baars *et al*. 2003; Heystek 2006).

5.2.1 Experimental design

Seven potted lantana plants were arranged around a *Crematogaster* sp. 2 colony and all except control 3 (uncovered plant without ants) were covered with mosquito net material (fine gauze) such that no flying insects or spiders could infest the study plants (Fig. 5.1). Each plant stood in a plastic tub that had a 10cm band painted with sticky Tanglefoot® to discourage infestation by other arthropod predators. Three plants were attached via string to the ant colony. These plants contained 80 *F. intermedia* nymphs (FA) or 80 *T. scrupulosa* nymphs (TA) or no insects (control 1). The remaining four plants were inaccessible to *Crematogaster* sp. 2 foragers and also contained 80 *F. intermedia* nymphs (F) or 80 *T. scrupulosa* nymphs (T) or no insects (controls 2 and 3). The third control was designed to test for reduced plant growth due to decreased sunlight caused by the gauze. In so doing insect survival and

feeding activity could be compared between ant-excluded plants (F and T) and ant-inoculated plants (FA and TA). Similarly, the damage by each insect species to *L. camara* shrubs could be compared with and between control plant 1 (covered, ants excluded), control plant 2 (covered, with ants) and control 3 (uncovered, ants excluded). Plants received 500ml of water daily and *Crematogaster* sp. 2 colonies were always provided with water and sugar-water on paper towelling. Ant species (of the genera *Pheidole* and *Lepisiota* in particular) readily colonise potted plants, so two days prior to commencement, pots were flooded with diatomaceous earth (Diatomite Dio dust®) dissolved in water, and the surface of the soil around the stem was covered with diatomaceous dust. Diatomaceous earth is an environmentally friendly method of killing organisms that have exoskeletons. The crystalline particles of diatomaceous dust pierce the integument, causing death by dehydration (Chintzoglou *et al*. 2008). Extra precautions were taken because a previous experiment failed due to infestation of a test plant by a species of *Lepisiota*.

Fig. 5.1: Arrangement of no-choice experiments using five *Crematogaster* sp. 2 colonies, showing string walkways connecting nests to ant allowed treatments.

5.2.2 Agent treatments

Plants were inoculated with 1st and 2nd instar nymphs of *F. intermedia* or *T. scrupulosa* so that the nymphs would not mature during the week-long experiment. The number of nymphs per shrub was 80 (0.27 nymphs per leaf) in order to investigate nymph defence at lower densities than in the prey density experiments (2.5; 7.5 and 15 per two leaf-pairs), despite evidence that ants could possibly remove 60 nymphs within a day (Chapter IV). Relatively small plants were used so that insect damage would be easily measurable. Fowler and Macgarvin (1985) suggested it possible that the rate of ant predation on herbivores may be higher during the first few hours after introduction, when herbivores may be more mobile than usual. To allow for this, nymphs were introduced to shrubs one day prior to commencement of the experiments.

5.2.3 Units of measurement

The numbers of agents surviving the week-long experiment were recorded, as well as the percentage feeding activity as measured by assessing the number and proportion of leaves displaying feeding damage. The plant growth parameters measured on each of three labelled branches (per shrub) were length (cm), and numbers of leaf-pairs. Both branch length and numbers of leaf pairs were measured and counted one day prior to commencement of the experiments. Branch lengths and leaf-pairs were recorded again on the last day of the experiment and the differences calculated were then analysed statistically. Numbers of foragers recruited to each of the three treatments (FA; TA and C2) were recorded for 10 repetitions both morning and afternoon for the first five days of the experiment. On the afternoon of the second experiment day, leaves of all shrubs containing nymphs were scouted for five minutes to ensure that an acceptable number of nymphs had survived the inoculation procedure.

5.2.4 Statistical analyses

The numbers of agents surviving on each treatment and numbers of foragers recruited to treatments (recruitment rates) were not normally distributed. Thus Kruskal-Wallis nonparametric ANOVA was performed on these data. Ten replicates of recruitment rates were recorded for each treatment (three treatments per experiment) for each

Crematogaster sp. 2 colony (n = 5) once in the morning and afternoon for five consecutive days. Thus in total 1500 recruitment rates were ranked and compared using the Kruskal-Wallis non-parametric ANOVA (in Statistica). During analysis, the Kruskal-Wallis test determines a median value to indicate which of the recruitment rates per treatment were most often recorded. ANOVAs were performed on the plant parameters (no. of leaf pairs gained and growth of branches). Although Levene's test for homogeneity of variables showed no significant differences within these plant parameters, the data were not normally distributed. For this reason results of the ANOVA were discarded and a non-parametric type of ANOVA was performed instead. Results presented are those obtained using the Kruskal-Wallis non-parametric test.

5.3 Results

The graphs below (Figures 5.2 to 5.4) show results of each treatment (plants 1-7) for all five *Crematogaster* sp. 2 colonies tested. Results of all colonies are grouped according to treatments for better visual representation. However, statistical analyses performed on the data compared all data individually. Thus for both plant parameters (no. of leaf pairs gained and growth of branches), all 35 data points (seven treatment results for five ant colonies) were compared with one another to determine whether there were significant differences.

5.3.1 Insect feeding activity and survival

Significantly fewer *T. scrupulosa* nymphs (TA) survived on ant-inoculated as compared with the ant-excluded (T) shrubs ($p = 0.0373$). By the third morning of these experiments no *T. scrupulosa* nymphs could be found on ant-accessed plants. Many more *F. intermedia* nymphs survived on ant excluded- (F) than on ant-inoculated treatments (FA), although differences were not significant (Fig. 5.2). In addition, a significantly lower percentage of *F. intermedia* nymphs ($p = 0.0228$) and *T. scrupulosa* nymphs ($p = 0.0002$) survived on the shrubs that ants accessed (FA & TA), than the nymph inoculation number (80) at the start of experiments. These results indicate that predation by ants was the cause of the decrease in agent numbers on ant-accessed plants.

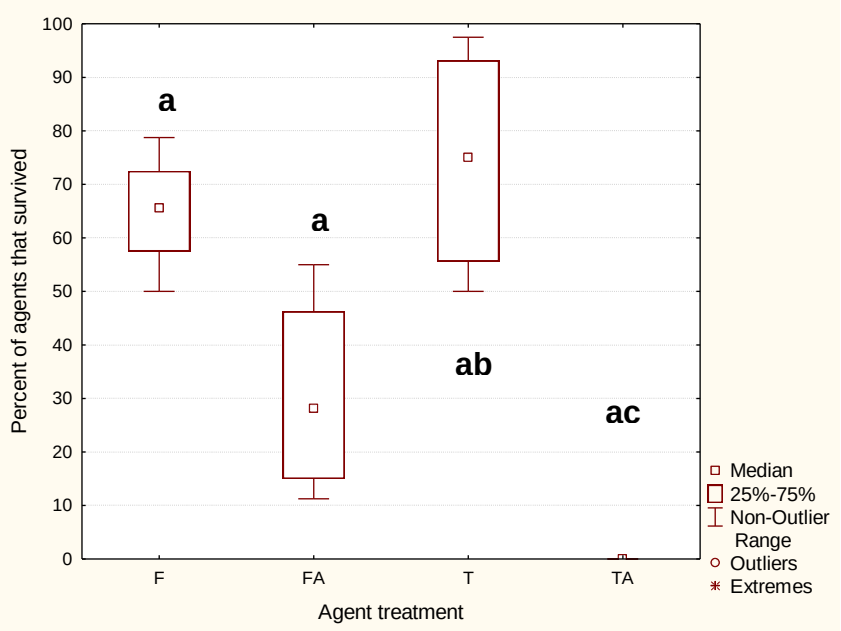

Fig. 5.2: Percentage survival of biological control agents on *L. camara* shrubs in a choice field experiment (n = 5, H = 21.6066, p = 0.0002). F = 80 *F. intermedia* nymphs, ants excluded; FA = 80 *F. intermedia* nymphs, ants inoculated; T = 80 *T. scrupulosa* nymphs, ants excluded; TA = 80 *T. scrupulosa* nymphs, ants inoculated, Control = 80 nymphs at start. Letters on graphs denote significant differences.

The amount of feeding damage incurred by the lantana shrubs in these experiments was fairly low although for all experiments, more feeding damage was observed on ant-excluded than ant-inoculated agent treatments. Results (of the five experiments) for feeding damage by *F. intermedia* nymphs with ants excluded ranged from 5 to 30% and that of *F. intermedia* nymphs with ants present ranged from <5 to10%. Feeding damage by *T. scrupulosa* nymphs without ants ranged from 10 to 30% and that of *T. scrupulosa* nymphs with ants was less than 5%. About 80% of the leaves on the plants with *F. intermedia* nymphs and ants present (FA) had *F. intermedia* frass on them but the damage was very little and highly dispersed. In contrast, damage by *T. scrupulosa* was highly localised and on plants with *T. scrupulosa* nymphs without ants (T) the damage was significant, causing die-back of terminal shoots. These trends held for each of the five experiments. Leaf tips where *T. scrupulosa* nymphs had aggregated (T) were tightly curled and the nymphs concealed within. In contrast, *T. scrupulosa* nymphs on ant-inoculated plants (TA) were all removed by foragers (at high densities) before they could feed enough for the leaves to have curled and offer refuge.

5.3.2 Ant forager recruitment and behaviour

The general trends can be summarized as follows: recruitment rates to all treatments on the first day of the experiments were not significantly different from one another, except that recruitment to *T. scrupulosa* on the first day (am and pm) was significantly higher than recruitment to the control in the afternoon of the first day (Fig. 5.3). In other words, by the afternoon, foragers had determined that the agent treatments were more rewarding than the control treatment. Although foraging on both agent treatments on the first day did not differ significantly, more foragers were recruited to *T. scrupulosa* than *F. intermedia* nymphs. Thereafter, recruitment rates of foragers to all treatments generally did not differ significantly except where noted in Table 5.1.

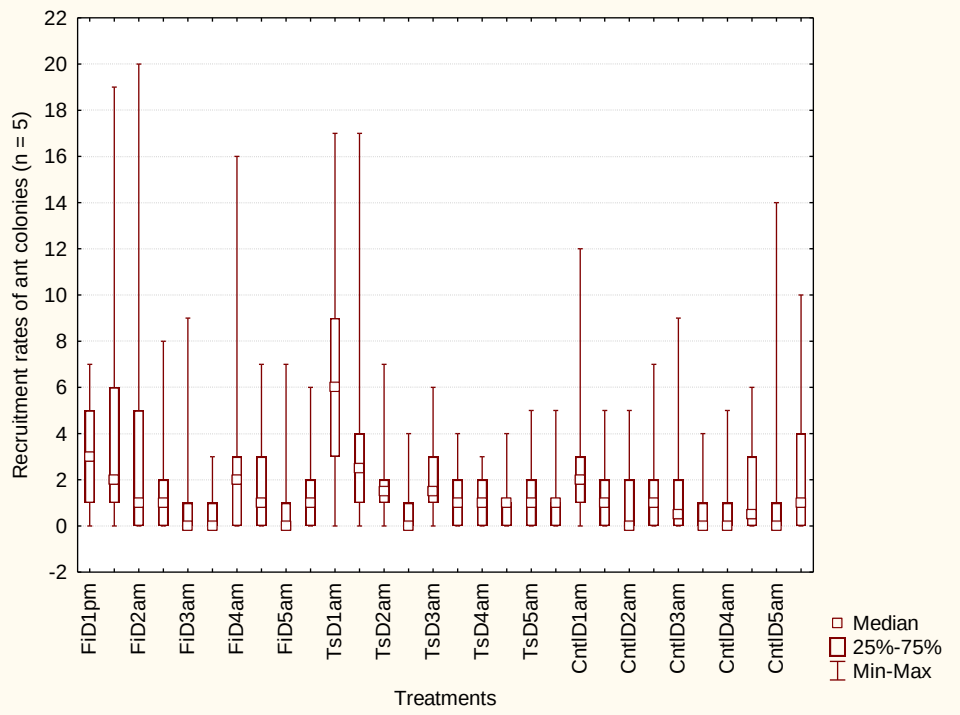

Fig. 5.3: Recruitment rates (No. ants per minute) of five *Crematogaster* sp. 2 colonies to biological control agents on *L. camara* shrubs in choice experiments (n = 10, H = 245.7897, $p < 0.0001$). Fi = 80 *F. intermedia* nymphs, ants inoculated; Ts = 80 *T. scrupulosa* nymphs, ants inoculated; D1 to D5 = the days over which recruitment recorded (am and pm = morning and afternoon).

On the fourth day during one experiment (colony H), ants were observed entirely removing all the black (ripe) *L. camara* berries from the control plant that had many berries compared with the other treatment plants. Some foragers were also seen returning with larvae (ca. 3mm long), found in the berries that were likely to be *Lantanophaga pusillidactyla* (Walker) (Lepidoptera: Pterophoridae). These larvae

probably survived the insecticide treatment because they had already burrowed into flower-heads before the plants were sprayed. When foraging, *Crematogaster* sp. 2 ants tended to visit terminal shoots and reproductive structures (flowers and berries) first, and then search leaves on their way down the plant. At 20°C *Crematogaster* sp. 2 foragers move more slowly than at temperatures higher than 23°C (*pers*. *obs*.). Ants were noted as returning (without prey) in high numbers from the treatments in the morning, while none were going out to the treatments (T = 33°C). This could indicate that ants had been foraging during the night or early morning and were returning to their nest due to the high temperature. Towards the end of the study, foragers were most often recorded as returning from the treatments than going to the treatments. This supports the suggestion that these workers may forage overnight on the foliage they patrol, as do *Crematogaster difformis* in tropical lowland rainforest Borneo (Tanaka *et al*. 2009).

Table 5.1: Significant difference between *Crematogaster* sp. 2 forager recruitment rates (No. ants/min) to three experimental treatments recorded morning and afternoon for five consecutive days (H = 245.7897, n = 5, p < 0.0001).

Recruitment significantly different to:	R.R to *F. intermedia* Day **1** am	R.R.to *F. intermedia* Day **1** pm	R.R. to *T.* scrupulos*a* Day **1** am	R.R. to *T.* scrupulos*a* Day **1** pm	R.R. to control Day **1** am	R.R. to control Day **1** pm
Falconia intermedia	D2pm to D5pm, except D4am & pm	D2pm to D5pm, except D4am & pm	D2am to D5pm	D2pm to D5pm, except D4am	D3pm	No difference, **but control D3 significantly lower than F.i.D4am**
Teleonemia scrupulosa	D2pm; D3pm; D4pm to D5pm	D2pm and D4pm to D5pm	D2am to D5pm	D2pm to D5pm, except D3am	D2pm	**Lower than** D1am and D1pm
Control	D2am and D3am to D5am	D2am and D3am to D5am, except D4pm	D1pm to D5pm	D1pm to D5am	D3pm	No difference

Except where specified in bold, significant differences are to be assumed higher for the treatment column than for the treatment row. R.R. = Recruitment rate, D = day, am = morning and pm = afternoon.

5.3.3 Plant parameters

There were no significant differences between treatments for each experiment for either plant parameter: no. of leaf pairs gained (Fig. 5.4) or the growth of branches (Fig. 5.5). The numbers of leaf pairs gained per branch in one week were not normally distributed because one branch of a control 3 plant gained more leaf pairs than all other plants in this study. However, it was not enough to render the results significantly different from one another.

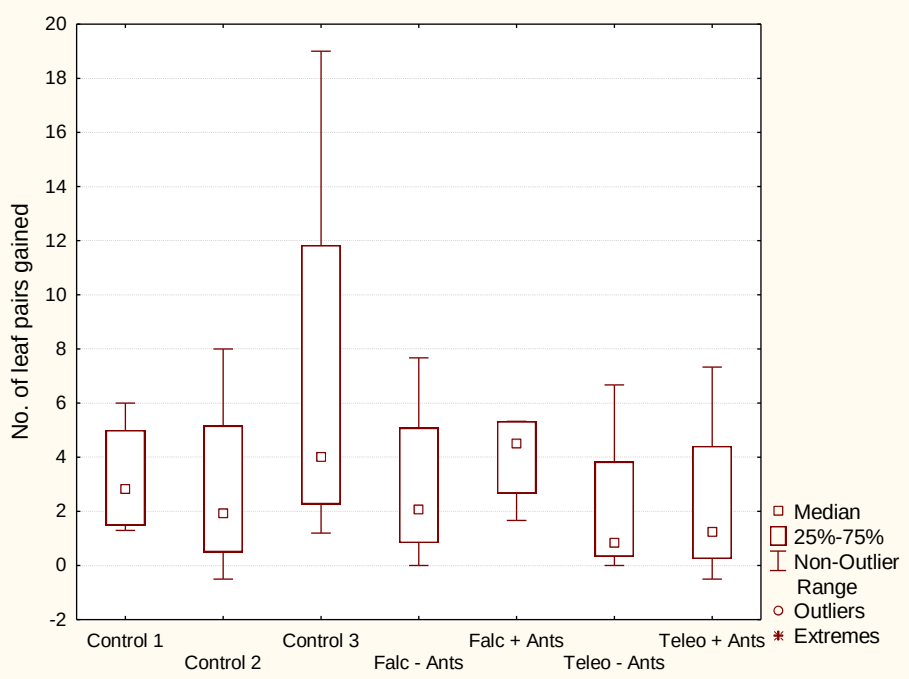

Fig. 5.4: Numbers of leaf pairs grown in one week by *Lantana camara* shrubs as a measure of herbivory by biocontrol agents, *F. intermedia* and *T. scrupulosa* nymphs, when *Crematogaster* ants were included or excluded (n = 5, H = 3.3094, p = 0.7691). Control 1 = plant covered, without ants; Control 2 = plant covered, with ants; Control 3 = plant uncovered, without ants; F = 80 *F. intermedia* nymphs, without ants; FA = 80 *F. intermedia* nymphs, with ants; T = 80 *T. scrupulosa* nymphs, without ants; TA = 80 *T. scrupulosa* nymphs, with ants.

Branches of the uncovered plants (control 3) did not grow significantly longer than those grown under gauze. This means that covering the plants with a fine meshed cloth for a week did not significantly reduce the growth of *L. camara* shrubs. The lengths of branches grown were also not normally distributed. This was because some of the leaf tips had shrunk due to feeding damage by *T. scrupulosa* nymphs, resulting in negative growth rates (Fig. 5.5). Cilliers (1987a) suggested that die-back of internodes may be attributed to leaf drop and plant cell destruction caused by *T. scrupulosa* feeding.

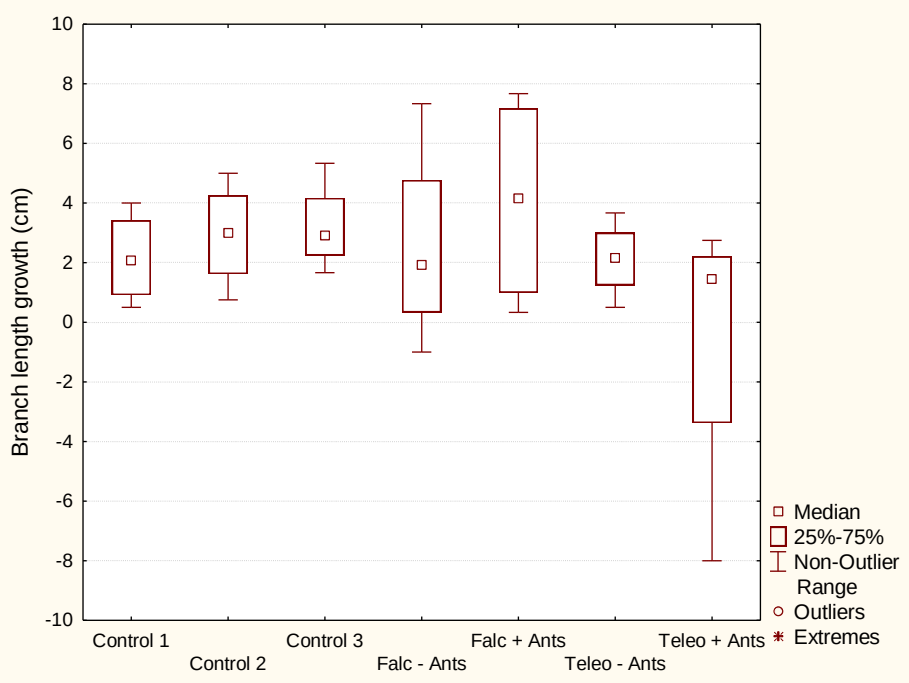

Fig. 5.5: Branch length growth (cm) in one week by *Lantana camara* shrubs as a measure of herbivory by biocontrol agents, *F. intermedia* and *T. scrupulosa* nymphs, when *Crematogaster* ants were included or excluded (n = 5, H = 6.9478, p = 0.3257). Control 1 = plant covered, ants excluded; Control 2 = plant covered, ants inoculated; Control 3 = plant uncovered, without ants; F = 80 *F. intermedia* nymphs, without ants; FA = 80 *F. intermedia* nymphs, with ants; T = 80 *T. scrupulosa* nymphs, without ants; TA = 80 *T. scrupulosa* nymphs, with ants.

5.4 Discussion

Plant growth rates and the allocation of energy reserves to seed production (plant performance) are influenced to some degree by insect herbivory. However, by way of resistance, plants have considerably more influence on herbivore population dynamics than vice-verse (Price *et al*. 1980; Schulz 1988; Crawley 1989; Price 2000). Feeding damage accompanied by *F. intermedia* frass spread throughout the shrubs was indicative of dispersal by these nymphs because they did not remain in any particular area to feed. Upon termination of experiments, leaves were searched for remaining nymphs and those of *F. intermedia* were always found spread over the whole shrub. This may suggest that ants chased these nymphs and prevented them from feeding optimally. In contrast, damage by *T. scrupulosa* was highly localised and on ant-excluded plants the damage was intense, causing leaf tips to curl, which offers nymphs refuge by concealment. Some phloem feeders are able to selectively feed on tissues that contain few or no secondary defence compounds, enabling them to feed in an area even if feeding-induced chemicals have been released (Schulz 1982 & 1983b).

Trophic structure of insect communities is often complex and distinct trophic levels and interactions can be obscured or even absent (Strong 1988; Dyer & Letourneau 1999; van Veen *et al*. 2006). Thus, preventative measures were in place to ensure that non-study arthropod species were not included in these experiments. Special care was taken to exclude honeydew-producing homopterans since their effect on predatory ant species is known to be as dramatic as it is complicated (Buckley 1987; Cushman 1991). Nearly all predaceous ants supplement their diet with sugary substances (Carroll & Janzen 1973; Dyer 2002) which are converted into energy and biomass (Tanaka *et al*. 2009). Both honeydew and pollen from most flowers contain various amino acids – the precursors to protein (Cushman 1991). *Lantana camara* flowers prolifically and can do so all year round where water is not a limiting factor (Day *et al*. 2003a). Flowers in the inflorescences contain nectar (Holm 1977; Stock 2005) and in the absence of honeydew-producing homopterans, foraging ants were highly attracted to the abundant inflorescences of *L*. *camara*. Foragers visited terminal shoots, inflorescences and berries first, before searching older leaves on their way down the plant. Nymphs (and adults) of *T*. *scrupulosa* feed primarily in the terminal regions on flowers and leaves (Cilliers 1987a and *pers*. *obs*.) where ant visitation is high. In contrast, *F*. *intermedia* nymphs feed on most of the leaves on a branch, except the basal (old, nutrient-depleted) leaves. Results indicated that *Crematogaster* sp. 2 colonies recruited comparable densities of foragers to both agent treatments, and differences between the numbers of *F*. *intermedia* and *T*. *scrupulosa* nymphs removed during this study were not significant. However, all 80 *T*. *scrupulosa* nymphs were removed by the morning of the third day, whereas some *F*. *intermedia* nymphs remained on ant-inoculated shrubs at the end of the experiment (day 7). *Crematogaster* sp. 2 foragers required more time to catch *F*. *intermedia* nymphs which had taken advantage of the 'escape space' offered by the whole shrub. Prey density experiments (Chapter IV; 4.3.7), showed that the number of *F*. *intermedia* nymphs caught by *Crematogaster* sp. 2 foragers decreased with decreasing nymph density due to nymphs dodging foragers provided they had space in which to move.

Avoidance of attack was presumed an undesirable characteristic for biological control agents in that it is presumed more effective for species that exist at low population densities, unlikely to affect the weed's population (Smith 2003). These experiments

have shown *T. scrupulosa*'s avoidance of attack by remaining motionless to be relatively ineffective in the absence of other herbivores. In practice, *T. scrupulosa* is one of the most successful agents for *L. camara* worldwide and is far more abundant than *F. intermedia* on *L. camara* in South Africa. If *T. scrupulosa* were preyed on as extensively as they were in this study, this would not be the case. Thus, the presence of other herbivores to detract attention from *T. scrupulosa* nymphs and the indirect protection offered by ants tending honeydew-producing homopterans on lantana, must be essential for the stationary defence strategy to be effective against predatory ants.

Considering the short experiment time and the fact that these study plants were not stressed by other natural enemies during the experiment, the percent feeding damage produced by 80 agent nymphs per ant-excluded shrub was good. A contributing factor was possibly that these shrubs were fertilized weekly, which is known to give some agents an advantage when the plant defence employed is nutrient restriction (Room & Thomas 1985 *In* Crawley 1989). Food quality (mainly nitrogen-content) is a limiting factor for insects and many plants can alter their leaf chemistry in response to herbivory (Dadd 1973; Price *et al*. 1980; Schultz 1983a&b; van Veen *et al*. 2006). The fact that these agents performed fairly well with the addition of fertilizer suggests that nutrient restriction is among *L. camara*'s resistance strategies. Heshula (2005) recorded similar results regarding feeding damage by *F. intermedia* on fertilized *L. camara* shrubs.

5.5 Conclusion

No significant differences were found between treatments for either plant parameter (no. leaf pairs and branch length) measured in these experiments. This too may be attributed to the fact that these *L. camara* shrubs were growing under very good conditions (watered daily and fertilized weekly). Percentage survival of *F. intermedia* nymphs and *T. scrupulosa* nymphs in particular was low on ant-accessed shrubs. So their feeding could not have resulted in much damage, in accordance with the results. In contrast, percentage survival of agents was high where ants were excluded so it is likely that the time scale (one week) was not long enough for the agents to produce more significant feeding damage on those shrubs.

Chapter VI

6 General Discussion

Preface

In this chapter, the major findings of the laboratory (Chapters II, III and IV) and field studies (V) are integrated and discussed in relation to one of the main factors believed to have debilitated the lantana biocontrol programme, namely: suppression of agent populations by predators (Cilliers & Neser 1991; Baars 2002; Heystek 2006). Areas requiring further study are discussed and recommendations are made for future success in the biological control of *Lantana camara*.

6.1 Introduction

Biological control practitioners hope for agent population explosions to spectacularly depress weed populations, although events such as these are rare by design (Price 2000). Insect species prone to eruptive population growth are rare and usually exhibit unsuitable traits for safe use in biological control. Such traits include indiscriminate ovipositioning females and as a result, non-host specific larvae that utilize available food, regardless of nutrient quality. In contrast, specialized insects have evolved a marked preference for vigorous (nutrient rich) plant parts such as shoots, buds, seeds and fruit (Price 2000). High quality food is usually a limiting resource for herbivorous agents because rapid growth is energetically demanding and therefore seasonally brief. In addition, a common plant resistance strategy against herbivores is to reduce food quality by altering the water, nitrogen and/or digestibility reducer (tannins, cellulose) contents in the leaves to deprive herbivores of efficient nutrient uptake (Price *et al*. 1980; Schultz 1983a&b). Reduced food quality results in slower growth rates of herbivores and therefore fewer generations per annum. Thus specialist herbivores are subject to bottom-up regulation by their host plants (Price 2000). However an additional cost of slow herbivore growth rates is that immature agents are vulnerable to parasitism and predation for a longer duration. Top-down regulation of herbivores is not believed to be a major determining factor of indigenous

herbivore populations in temperate ecosystems, but evidence suggests it may be more important in the tropics where ant species are more diverse and abundant (Fowler & MacGarvin 1985; Dyer 2002; Floren *et al*. 2002, Tanaka *et al*. 2009). Similarly, top-down regulation by ant species already in association with a weed can throttle the establishment of biological control agents or be a determining force, suppressing agent populations (Price *et al*. 1980; Goeden & Louda 1976).

6.2 How ant predation affects biological control of *Lantana camara*

Lantana camara produces secondary toxic compounds (e.g. lantadenes A & B) to deter generalist herbivores in addition to physiological herbivore resistance (largely aimed at specialist herbivores), that involve altering leaf nutrient quality (as determined by enhanced agent performance on fertilized plants) and premature leaf drop (Heshula 2005). Since decreased leaf nutrition results in longer developmental times, biological control agents of lantana are subject to both bottom up- and top-down regulation.

In accordance with Franks findings of (1986) and Dyer (2002), time required by *Crematogaster* sp. 2 to subdue different sized *H. laceratalis* larvae is probably a limiting factor determining suitable prey size for ant species (Chapter II). During this study (Chapter III) it was determined that *Crematogaster* sp. 1 prey significantly on *F. intermedia* nymphs and *H. laceratalis* larvae. *Crematogaster* sp.1 foragers showed a significant preference for small *H. laceratalis* larvae (<10mm), in accordance with findings for other lepidopteran species (Pettey 1948; Robertson 1985; Freitas & Oliveira 1996; Floren *et al*. 2002; Xiao *et al*. 2006). Small *H. laceratalis* larvae (9 to 11mm) were demonstrated as providing ten times better returns for energy spent by foraging ants when compared with biomass offered by large *F. intermedia* and *T. scrupulosa* nymphs respectively (Chapter II). Although *F. intermedia* nymphs were preyed on in comparable numbers with *H. laceratalis* larvae, the mirid is not subject to parasitism in South Africa, whereas *H. laceratalis* larvae (and other lepidopteran agents) are parasitized by native parasitoids in South Africa that have extended their host range (Baars 2002, 2003).

Lantana camara does not possess extra floral nectaries (EFNs) but flowers prolifically and varieties in tropical areas flower all year round, thereby consistently supplying nectar to attract natural enemies of herbivorous insect agents (Holm 1977; Stock 2005). Results of preliminary food trials (Chapter II) showed that both *Crematogaster* sp. 1 and 2 recruitment rates were higher to sucrose- than to protein- or starch sources. During choice experiments (Chapter V) higher densities of foragers could be found in lantana inflorescences than those scouting the leaves, and terminal leaves appeared more intensely searched than basal leaves, in accordance with Oliveira (1997). Early instar *H. laceratalis* larvae feed on the upper, newer leaves of *L. camara* (more nutrient-rich but probably containing more digestibility reducers) and feed on lower tougher leaves as they grow (Day 2003a). *Falconia intermedia* nymphs feed preferentially on new leaves but can also be found on older leaves provided the moisture content is sufficient. Many specialist herbivores target vigorous plant parts and since new leaves often grow from terminal shoots in association with flowers, many leaf-feeding agents are susceptible to predation by ant species (Price 2000). By this reasoning, *T. scrupulosa* should be highly vulnerable since this species feeds on terminal shoots as well as flowers themselves (Cilliers 1987a&b). However, *T. scrupulosa* is one of the first agents to have been introduced on lantana and remains one of the three best agents worldwide despite the apparent vulnerability of this undefended, slow-moving hemipteran. During this study (Chapter III) the relatively immobile behaviour of *T. scrupulosa* nymphs (even when touched by ant foragers) was identified as a highly effective cryptic predator avoidance strategy that behaviourally creates enemy-free-space. Several studies (Fowler & MacGarvin 1985; Bergelson & Lawton 1988; Freitas & Oliveira 1996) support the idea that increased herbivore movement increases predation by ant species that more easily detect moving than stationary objects (Ayre 1963; Wehner 1981,1983).

Intraguild predation (IGP) can weaken the effect of natural enemies on herbivores, leading to increased herbivory and decreased plant biomass (Rosenheim *et al*. 1995; Eubanks *et al*. 2002; van Veen *et al*. 2006). This is a positive effect in the context of weed biological control, if for example ants remove parasitized agent larvae, thereby killing the parasitoid species and reducing agent mortality (Rosenheim *et al*. 1995). An example of IGP potentially playing a significant role in the biological control of *L.*

camara is if the presence of honeydew-producing homopterans, such as *Phenacoccus parvus* Morrison (Hemiptera: Pseudococcidae), results in their tending ant species promoting the survival of *T. scrupulosa* by attacking generalist predators. Reported predators of *T. scrupulosa* are spiders (Halaj *et al*. 1997 *In* Karhu 1998), coccinellids, neuropteran larvae, Reduviidae (Fyfe 1937 *In* Cilleirs 1987a), and Lygaeid predatory bugs (Simmonds 1929 *In* Cilleirs 1987a). This would also constitute an example of asymmetrical apparent mutualism (van Veen *et al*. 2006), in which *T. scrupulosa* benefits from the presence of ants indirectly via its association with honeydew-producing homopterans. Honeydew-producing homopterans in turn can benefit asymmetrically by the presence of other herbivorous species (e.g. *F. intermedia* nymphs and lepidopteran larvae) on which their tending ant species can prey. This is of relevance since even mutualistic ant species can switch from tending- to preying on their honeydew-producing homopterans once their intake of honeydew (sugars, organic acids, alcohols, plant hormones, salts, vitamins, amino acids and amides) out-weighs that of protein required for cell building (Becerra 1989; Cushman 1991).

During this study it was noted that *F. intermedia* were never found in high numbers where *P. parvus* occurred and when laboratory cultures became infected with this mealybug, *F. intermedia* cultures rapidly collapsed. In laboratory cultures and at sites where mutualistic ant species are absent, the increased incidence of sooty mold (Bach 1991) and pathogens associated with the accumulation of honeydew on *L. camara* leaves may compromise the success of *F. intermedia*. Accumulated honeydew on leaves can also impede the escape of predators by *F. intermedia* nymphs. Although *T. scrupulosa* should also suffer these effects, nymphs of *T. scrupulosa* move around the leaves far less than *F. intermedia* nymphs and therefore would come into contact with pathogens far less frequently than *F. intermedia* (Schultz 1982). The net result then is that *T. scrupulosa* may benefit from the presence of honeydew-producing homopterans at the cost of *F. intermedia*, which according to van Veen *et al*. (2006) constitutes apparent competition between these two agent species. Even so, that apparent competition does not explain why *F. intermedia* populations are low countrywide, whereas *T. scrupulosa* can also be found in high densities at sites where honeydew-producing homopterans are absent or rare. This is probably due to *T. scrupulosa* nymph's cryptic behaviour of remaining stationary in the presence of predators (Chapter III). Interestingly, the adults of *Octotoma scabripennis* Guérin-

Méneville (Coleoptera: Chrysomelidae), one of the three most successful biocontrol agents for *L. camara* worldwide, also move very little during the day (Chew *et al*. 2003). However, this defence strategy relies heavily on the presence of other herbivores (alternative prey) to attract forager attention away. Similarly, it was determined that this defence strategy is probably density dependent (Chapter IV) in that at very high agent and/or forager density, *T. scrupulosa* nymphs attempted escape. Once they moved, foragers identified them as prey and they were rapidly removed. In addition, each *Crematogaster* sp. 1 colony was offered each agent species only once. These colonies removed significantly high percentages of *F. intermedia* nymphs and *H. laceratalis* larvae but few *T. scrupulosa* nymphs (Chapter III). Whereas, in chapter IV of this study, it was determined that *Crematogaster* sp. 2 foragers preyed equally significantly on *F. intermedia* and *T. scrupulosa* nymphs. It is suggested that because *Crematogaster* sp. 2 colonies were offered each agent three times (at different agent densities), once foragers had identified *T. scrupulosa* as prey, the nymphs were easily captured. Given more time, foragers would probably have removed all nymphs. During the week-long choice experiments (Chapter V) *T. scrupulosa* nymphs were at their most vulnerable, feeding on terminal shoots and flowers (where forager abundance is high) in the absence of honeydew-producing homopterans or other herbivores. *Falconia intermedia* were less vulnerable to ant predation during choice experiments than *T. scrupulosa* nymphs because the mirid can run fast and potentially escape, whereas the tingid nymphs could not out-run ant foragers. It was determined (Chapter IV) that predation on *F. intermedia* is also density dependent in that at high nymph and/or forager densities, escape routes of *F. intermedia* were congested and these nymphs were more easily caught by foragers than at lower nymph densities. This is supported by studies which found that the extent of herbivore predation is influenced by ant forager density (Sato & Higashi 1987; Freitas & Oliveira 1996; Oliveira 1997).

Forager life expectancy is higher in the tunnel than in the field. Thus on occasions when *T. scrupulosa* nymphs in the field are detected moving by foraging ants, because of their movement, this may result in stationary nymphs in the immediate vicinity also being removed. However, these are isolated events and ant foragers are not likely to live long enough to learn to identify stationary *T. scrupulosa* nymphs as prey at another site. In contrast, *F. intermedia* nymphs were quickly recognised as prey and

significantly removed by *Crematogaster* sp. 1 and 2 during these experiments and are likely to elicit a predatory response by ant species in the field. Leston (1973) suggests that insects in tropical environments can be arranged in an 'ant impact' hierarchy in which "flitting" insects, like *F. intermedia* are more affected than "flying" or endophytic species.

Herbivore position on the plant is important regarding enemy search patterns (Price *et al*. 1980). Whereas *T. scrupulosa* nymphs feed on both leaf surfaces, *F. intermedia* nymphs feed primarily on the undersides of lantana leaves (Baars 2002). In accordance with Radeghieri (2004), during this study (chapters III-V) foragers were observed searching the margins and undersides of leaves more thoroughly than the dorsal surfaces. The ants' search pattern is probably selected by predation pressure from flying predators such as dragonflies and birds, whereas the stationary defence mechanism utilised by *T. scrupulosa* nymphs protects them from flying predators.

This study addressed the potential impact of predation by two species of ant on populations of three biological control agents in South Africa. The role of ant predation on lantana biocontrol can only be determined once several other questions are answered. For example, choice experiments involving several co-occurring agents on the same shrubs in the laboratory as well as in the field could be performed to determine how the interaction of agent predation and competition affects control of lantana in the field. Similarly, choice experiments in the laboratory using agents in the presence (and absence) of the honeydew-producing homopteran, *P. parvus* and field observations of agent interactions with this mealy bug species would clarify the extent to which homopteran-tending ants reduce agent efficacy in South Africa.

6.3 Conclusion

In addition to being parasitized by a suite of parasitoids, early instar *H. laceratalis* larvae are preyed on by both *Crematogaster* species tested and significantly by *Crematogaster* sp.1 in South Africa. Even small *H. laceratalis* larvae provided ten times more biomass than the largest hemipteran agents tested, indicating them to be more desirable prey. Based on the literature it is highly probable that other ant

species also prey heavily on a variety of early instar lepidopteran larvae and other lepidopteran agents are attacked by indigenous parasitoids (Baars 2003). Therefore, in agreement with Baars (2002) it is suggested that no more lepidopteran biological control agents be imported for the control of *L. camara*. Although *T. scrupulosa* nymphs were preyed on by *Crematogaster* sp. 1 and 2 in some experiments, it is suggested that they would escape predation in the presence of other herbivores, as in the field. In contrast, *F. intermedia* nymphs were significantly preyed on by both *Crematogaster* species and even if *F. intermedia* can out-run ant foragers, feeding of nymphs and adults as well as ovipositioning of females is probably disturbed by the presence of South Africa's abundant ant assemblage.

Two root-feeding flea beetles, *Longitarsus columbicus columbicus* Harold 1876 and an unidentified *Longitarsus* species of accession no. AcSN 2431 (Chrysomelidae: Alticinae) are promising candidates for biological control of lantana because the family of insects that has resulted in most successful biological control programmes is Coleoptera (Crawley 1989); soil buffers organisms from extreme temperatures and these beetles are capable of diapause to survive cold winters. *Longitarsus columbicus columbicus* feeds externally (in the soil) on root hairs and therefore is likely to suffer mortality caused by soil desiccation and ant predation of pupating larvae (Bateman 1972). Whereas the latter beetle (AcSN 2431) feeds internally on roots and thus should escape desiccation and predation (Baars 2001). A consideration when introducing root feeders is that stress imposed on the plant by their feeding activity can result in high levels of stress hormones and other defence compounds being produced and transported to the leaves, which would negatively impact leaf feeders. However, evidence suggests that addition of foliage feeders does not impact root feeders (Kaplan 2007). In addition, phloem feeders (e.g. *T. scrupulosa*) may escape the release of toxins in response to root feeding because most plants cannot release toxins into the phloem without disrupting normal plant metabolism (Huxley 1986). Another proposed agent is the petiole-galling weevil, *Coelocephalapion camarae* Kissinger (Brentidae), the larvae of which burrow into vascular tissue, disrupting water and nutrient transport to the leaves (Baars *et al*. 2006). This weevil is unlikely to be deterred by the feeding activity of root feeders because it is effectively a phloem feeder. *Coelocephalapion camarae* has a wide geographic distribution and leaf replacement would cause a substantial energy sink from lantana's root reserves. Ten

of the 14 agents imported for the control of lantana in South Africa are leaf-feeding insects. Populations of foliage-feeding biological control agents for *L. camara* are suppressed by climatic factors, physiological defence mechanisms of lantana (e.g. altered leaf nutrient and leaf drop) as well as predation by ant species in South Africa. Therefore, the search for additional agents to control lantana in the future should exclude leaf-feeding agents.

7 References:

Amalin, D.M., Pena, J.E., Duncan, R.E., Browning, H.W. & McSorley. 2002. Natural mortality factors acting on leafminer, *Phyllocnistis citrella*, in lime orchards in South Florida. *BioControl* **47**: 327-347.

Arnold, G. 1915. A monograph of the Formicidae of South Africa. *Annals of the South African Museum* **14**: 1-766.

Ayre, G.L. 1963. Response to movement by *Formica polyctena* Först. *Nature* **199**: 405-406

Baars, J.R. 2001. Biology and laboratory culturing of the root-feeding flea beetle, *Longitarsus columbricus columbricus* Harold, 1876 (Chrysomelidae: Alticinae): A potential natural enemy of Lantana camara L. (Verbenaceae) in South Africa. *Entomotropica* **16**(3): 149-155.

Baars, J. R. 2002. Biological control initiatives against *Lantana camara* L. (Verbenaceae) in South Africa: an assessment of the present status of the programme, and an evaluation of *Coelocephalapion camarae* Kissinger (Coleoptera: Brentidae) and *Falconia intermedia* (Distant) (Heteroptera: Miridae), two new candidate natural enemies for release on the weed. PhD. thesis, Rhodes University, South Africa.

Baars, J.R. 2003. Geographic range, impact and parasitism of lepidopteran species associated with the invasive weed *Lantana camara* in South Africa. *Biological Control* **28**: 293-301.

Baars, J. R. & Neser, S. 1999. Past and present initiatives on the biological control of *Lantana camara* (Verbenaceae) in South Africa. In: Olckers, T. & Hill, M.P. (eds), *Biological Control of weeds in South Africa (1990-1998). African Entomology Memoir* **1**: 21-33.

Baars, J.P., Urban, A.J. and Hill, M.P. 2003. Biology, host range, and risk assessment supporting release in Africa of *Falconia intermedia* (Heteroptera: Miridae), a new biocontrol agent for *Lantana camara*. *Biological Control* **28**: 282-292.

Baars, J.R., Hill, M.P, Heystek, F. & Urban, A.J. 2006. Biology, oviposition preference and impact in quarantine of the petiole-galling weevil, *Coelocephalapion camarae* Kissinger, a promising candidate agent for biological control of *Lantana camara*. *Biological Control* **40**(2): 187-195.

Baars, J. R. and Heystek, F., 2003. Geographical range and impact of five biocontrol agents established on *Lantana camara* in South Africa. *BioControl* **48**: 743-759.

Bach, C.E. 1991. Direct and indirect interactions between ants (*Pheidole megacephala*), scales (*Coccus viridis*) and plants (*Pluchea indica*). *Oecologia* **87**: 233-239.

Bateman, M.A. 1972. The ecology of fruit flies. *Annual Review of Entomology* **17**: 493-518.

Becerra, J.X.I. 1989. Extrafloral nectaries: a defense against ant-Homoptera mutualisms?, *Oikos* **55**(2): 276-280.

Belt, T. 1874. The Naturalist in Nicaragua, Dent and Sons, London.

Bergelson, J.M. & Lawton, J.H. 1988. Does foliage damage influence predation on the insect herbivores of birch? *Ecology* **69**(2): 434-445.

Bhatkar, A. & Whitcomb, W.H. 1970. Artificial diet for rearing various species of ants. *Florida Agricultural Experiment Stations* Journal Series No. 3669.

Boughton, A.J. & Pemberton, R.W. 2008. Efforts to establish a foliage-feeding moth, *Austromusotima camptozonale*, against *Lygodium microphyllum* in Florida, considered in the light of a retrospective review of establishment success of weed biocontrol agents belonging to different arthropod taxa. *Biological Control* **47**: 28-36.

Bownes, A. 2002. The structure of ant communities and their impact on soil-pupating pests in citrus orchards in the Grahamstown area of the Eastern Cape, MSc. Thesis, Rhodes University, South Africa.

Broughton, S. 2000. Review and evaluation of lantana biocontrol programs. *Biological Control* **17**: 272-286.

Buckingham, G.R. 1992. Role of quarantine facilities in Biological Control. *Florida Entomologist* **75**(4): 414-420.

Buckley, R.C. 1987. Interactions involving plants, homoptera and ants. *Annual Review of Ecology and Systematics* **18**: 111-135.

Byrne, M.J.; Currin, S. & Hill, M.P. 2002 The influence of climate on the establishment and success of the biocontrol agent *Gratiana spadicea*, released on *Solanum sisymbrifolium* in South Africa. Biological Control **24**: 128-134.

Carroll, C.R. & Janzen, D.H. 1973. Ecology of foraging by ants. *Annual Review of Ecology and Systematics* **4**: 231-257.

Chew, P., Chew, T. & Chew, S. 2003. *Octotoma scabripennis* - Lantana Biocontrol, Department of Natural Resources and Mines, Queensland.

Chintzoglou, G., Athanassiou, C.G. and Arthur, F.H. 2008. Insecticidal effect of spinosad dust, in combination with diatomaceous earth, against twp stored-grain beetle species. *Journal of Stored Products Research* **44**: 347-353.

Cilliers, C.J. 1987a. Notes on the biology of the established insect natural enemies of *Lantana camara* L. (Verbenaceae) and their seasonal history in South Africa. *Journal of the Entomological Society of Southern Africa* **50**(1): 1-13.

Cilliers, C.J. 1987b. The evaluation of three insect natural enemies for the biological control of the weed *Lantana camara* L. *Journal of the Entomological Society of Southern Africa* **50**(1): 15-34.

Cilliers, C.J. & Neser, S. 1991. Biological control of *Lantana camara* (Verbenaceae) in South Africa. *Agriculture, Ecosystems and Environment* **37**: 57-75.

Cole, B.J. 1983. Assembly of mangrove ant communities: patterns of geographical distribution. *Journal of Animal Ecology* **52**(2): 339-347.

Crawley, M.J. 1989. Insect herbivores and plant population dynamics. *Annual Review of Entomology* **34**: 531-564.

Crawley, M.J. (Ed.) 1992. Natural Enemies: The population biology of predators, parasites and diseases, *Blackwell Scientific publications*, ISBN 0-632-02698-7, pp. 1-576.

Cushman, J.H. Host-plant mediation of insect mutualisms: variable outcomes in herbivore-ant interactions. *Oikos* **61**(1): 138-144.

Dadd, R.H. 1973. Insect nutrition: current developments and metabolic implications. *Annual Review of Entomology* **18**: 381-420.

da Silva, P. 2004. Optimality in ant foraging, BCompSc Honours thesis, Monash University.

Davidson 1977. Foraging ecology and community organisation in desert seed-eating ants. *Ecology* **58**(4): 725-737.

Davis, C.J., Yoshioka, E. and Kageler, D. 1966. Biological control of lantana, prickly pear, and Hamakua pamakani in Hawaii: a review and update, Hawaii XI. *Proceedings of the Hawaiian Entomological Society* **19**(2): 411-431.

Day, M. D., Neser, S. 2000. Factors influencing the biological control of *Lantana camara* in Australia and South Africa. In: N.R. Spencer (ed), *Proceedings of the X International Symposium on biological control of weeds* 4-14 July 1999, Montana State University, Bozeman, USA. pp. 897-908.

Day, M.D., Wiley, C.J., Playford, J. and Zalucki, P. 2003a. Lantana: Current Management Status and Future Prospects, Australian Centre for International Agricultural Research, Canberra, Australia, pp. 128.

Day, M.D., Broughton, S. and Hannan-Jones, M.A. 2003b. Current distribution and status of Lantana camara and its biological control agents in Australia, with recommendations for further biocontrol introductions into other countries, *Biocontrol News and Information* **24**(3): 63-76.

DeBach, P., Fleschner, C.A., Dietrick, E.J. 1951. A biological check method for evaluating effectiveness of entomophagous insects. *Journal of Economic Entomology* **44**: 763-766.

Den Breeÿen, A**.** 2004. Release strategies for the establishment of the leaf spot pathogen, *Mycovellosiella lantanae* var. *lantanae*, on Lantana camara in South Africa. In: Cullen, J.M., Briese, D.T., Kriticos, D.J., Lonsdale, W.M., Morin, L. and Scott, J.K. (eds), *Proceedings of the XI International Symposium on Biological Control of Weeds*. CSIRO Entomology, Canberra, Australia. pp. 386-388.

Dyer, L.A. 2002. A quantification of predation rates, indirect positive effects on plants, and foraging variation of the giant tropical ant, *Paraponera clavata Journal of Insect Science* **2** (18): 1-7.

Dyer, L.A. & Letourneau, D.K. 1999. Trophic cascades in complex terrestrial community. *Proceedings of the National Academy of Science of the United States of America* **96**: 5072-5076.

Emlen, J. M. (1966). The role of time and energy in food preference. *American Naturalist* **100**: 611-617.

Eubanks, M.D. 2002. Estimates of the direct and indirect effects of red imported fire ants on biological control in field crops. *Biological Control* **21**: 35-43.

Floren, A. and Linsenmair, K.E. 1999. Changes in arboreal arthropod communities along a disturbance gradient. *Selbyana* **20**(2): 232-241.

Floren, A., Biun, A. & Linsenmair, K.E. 2002. Arboreal ants as key predators in tropical lowland rain forest trees. *Oecologia* **131**: 137-144.

Fowler, S.V. & Macgarvin, M. 1985. The impact of hairy wood ants, Formica lugubris, on the guild structure of herbivorous insects on birch, *Betula pubescens*. *Journal of Animal Ecology* **54**: 847-855.

Franks, N.R. 1986. Teams in social insects: group retrieval of prey by army ants (*Eciton burchelli*, Hymenoptera: Formicidae). *Behavioral Ecology and Sociobiology* **18**: 425-429.

Freitas, A.V.L. & Oliveira, P.S. 1996. Ants as selective agents on herbivore biology: effects on the behaviour of a non-myrmecophilous butterfly. *Journal of Animal Ecology* **65**: 205-210.

Fyfe, R.V. 1937. The lantana bug, *Teleonemia lantanae* Distant. *Journal of the Council for Scientific and Industrial Research* **10**(3): 181-186.

Gassmann, A. 1996. Classical biological control of weeds with insects: a case for emphasiszing agent demography. In: Moran, V.C., Hoffmann, J.H. (eds). *Proceedings of the IX International Symposium on biological control of weeds*, University of Cape Town, Cape Town. pp. 19-26.

Goeden, R.D. & Louda, S.M. 1976. Biotic interference with insects imported for weed control. *Annual Review of Entomology* **21**: 325-342.

Halaj, J., Ross, D.W. & Moldenke, A.R. 1997. Negative effects of ant foraging on spiders in Douglas-fir canopies. *Oecologia* **109**: 313-322.

Hamilton, W.D. 1971. Geometry for the selfish herd. *Journal of Theoretical Biology* **31**: 295-311.

Harada, Y. 2005. Diel and seasonal patterns of foraging activity in the arboreal ant *Crematogaster matsumurai* Forel. *Entomological Science* **8**(2): 167-172.

Harley, K.L.S. 1973. Biological control of *Lantana* in Australia. In: A.J. Wapshere (ed.). Proceedings of the III International Symposium on Biological Control of Weeds Montpellier, France: pp. 23-29.

Harris, P. 1980. Evaluating biocontrol of weeds projects. In: Del Fosse, E.S. (ed) *Proceedings of the V International Symposium of Biological Control of Weeds*, Brisbane, Australia: 345-353.

Hassell, M.P. 1978. *The dynamics of arthropod predator-prey systems*. Princeton University Press, Princeton.

Hawkins, B.A. 1988. Species diversity in the third and fourth trophic levels: patterns and mechanisms. *Journal of Animal Ecology* **57**: 137-162.

Helms, K.R. & Vinson, S.B. 2002. Widespread association of the invasive ant *Solenopsis invicta* with an invasive mealybug. *Ecology* **83**(9): 2425-2438.

Henderson, L. 2001. *Alien Weeds and Invasive Plants*, Handbook 12. Agricultural Research Council, Plant Protection Research Institute, Pretoria.

Heshula, U. 2005. Establishment and impact of the sap-sucking mired, *Falconia intermedia* (Distant) (Hemiptera: Miridae) on *Lantana camara* (Verbenaceae) varieties in the Eastern Cape Province, South Africa. MSc. Thesis, Zoology and Entomology department of Rhodes University, South Africa.

Heystek, F. 2006. Laboratory and field host utilization by established biological control angents of *Lantana camara* L. in South Africa. MSc. Thesis, Zoology and Entomology department of Rhodes University, South Africa.

Heystek, F. & Olckers, T. 2003. Establishment and impact of *Falconia intermedia* (Hemiptera: Miridae) on Lantana camara (Verbenaceae) in South Africa. In: J. M. Cullen, D.T. Briese, D.J. Kriticos, W.M. Lonsdale, L. Morin and J.K. Scott (eds). *Proceedings of the XI International Symposium on Biological Control of Weeds*, April 27- May 2, 2003. CSIRO, Canberra, Australia. pp. 606.

Hoffmann, J. H. 1982. Evaluation of Tucumania tapiacola Dyar (Lepidoptera: Phycitidae) for the biological control of jointed cactus in South Africa. Unpublished Ph.D. thesis, Rhodes University, South Africa.

Hoffmann, J.H., Moran, V.C. & Zeller, D.A. 1998. Evaluation of *Cactoblastis cactorum* (Lepidoptera: Phycitidae) as a Biological Control Agent of *Opuntia stricta* (Cactaceae) in the Kruger National Park, South Africa. *Biological Control* **12**: 20-24.

Hölldobler B. & Wilson E. 1990. *The ants*, Berlin: Springer Verlag, 732pp.

Holm, L. G., Plucknett, D.L., Pancho, J.V. and Herberger, J.P. 1977. The world's worst weeds. The University Press of Hawaii, Honolulu.

Holling, C.J. 1959. The components of predation as revealed by a study of small mammal predation of European pine sawfly. *Canadian Entomologist* **91**: 293-320.

Horstmann, K. 1975. Zur regulation des beuteeintrages bei Waldameisen (*Formica polyctena* Foerester). *Oecologia* **22**: 57-65.

Horstmann, K. 1977. Waldameisen (*Formica polyctena* Foerster) als abundanzfaktoren für den Massenwechsel des eichenwicklers *Tortrix viridana* L. Z *Angew Entomologie* **82**: 421-235.

Huang, M.L., Lu, Y.S., Qui, Z.S., Zhou, Q.M., Men, Y.J. & Lin, S.J. 1989. Life history of *Phyllocnistis citrella* Stainton, and its occurrence. *Acta Physica Sinica* **16**: 159-162.

Humphries, S.E. and Stanton, J.P. 1992. Weed Assessment in the Wet Tropics Wpröd Heritage Area of north Queensland, Report to The Wet Tropics Management Agency.

Huxley, C.R. 1986. Evolution of benevolent ant-plant relationships. In B. Juniper and T.R.E. Southwood (eds). *Insects and the plant surface*, Edward Arnold, London. pp 257-282.

Ito, F. & Higashi, S. 1991. Variance of ant effects on the different life forms of moth caterpillars. *Journal of Animal Ecology* **60**: 327-334.

Ivlev, V.S. 1961. *Experimental ecology of the feeding of the fishes*. (Translated from Russian by D. Scott). Yale University Press, New Haven.

Jander R. 1957. Die optische richtungsorientierung der rotten waldameise (*Formica rufa* L.). *Zeitschrift ver Vergleichende Physiologie* **40**(2): 162-238.

James, D.G., Stevens, M.M. & O'Malley, K.J. 1997. The impact of foraging ants on populations of *Coccus hesperidium* L. (Hemiptera: Coccidae) and *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) in an Australian citrus grove. *Journal of Applied Entomology* **121**: 257-259.

Johnson, W. T., Lyon, H. H. 1991. Insects that feed on trees and shrubs, Cornell University Press. (2nd ed. Rev.) Ithaca, NY. 560p.

Julien, M.H., Skarratt, B. & Maywald, G.F. 1995. Potential geographic distribution of alligator weed and its biological control by *Agasicles hygrophila*. *Journal of Aquatic Plant Management* **33**: 55-60.

Kaplan, I. 2007. Inducible plant responses linking above- and below ground herbivory: ecological significance and underlying mechanisms. Ph.D, Faculty of the Graduate School of the University of Maryland, College Park, USA. pp. 182.

Karhu, K.J. 1998. Effects of ant exclusion during outbreaks of a defoliator and a sap-sucker on birch. *Ecological Entomology* **23**: 185-194.

Keane, R.M. & Crawley, M.J. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution* **17**(4): 164-170.

Kellerman, T.S., Naude, T.W. and Fourie, N., 1996. The distribution, diagnoses, and estimated economic impact of plant poisonings and mycotoxicoses in South Africa. *Onderstepoort Vetenarian Research* **63**: 65-90.

Kluge, R.L. 1994. Ant predation and the establishment of *Pareuchaetes pseudoinsulata* Rego Barros (Lepidoptera: Arctiidae) for biological control of triffid weed, *Chromolaena odorata* (L.) King & Robinson, in South Africa. *African Entomology*: **2**(1): 71-72.

Kluge, R. L. and Caldwell, P. M. 1996. Failure and frustration of biocontrol of *Chromolaena odorata* in South Africa, In: Prasad U. K., Muniappan, R., Ferrar, P., Aeschliman, J P. and de Foresta H. (eds), *Proceedings of the Third International Workshop on Biological Control and Management of Chromolaena odorata*. Agricultural Experiment Station, University of Guam. pp. 169-173.

Leston, D. 1973. Ants in tropical tree crops, *Proceedings of the Royal Entomological Society of London* **38**(6): 1.

Lighton, J.R.B., Bartholomew, G.A. & Feener, G.H. 1987.Energetics of locomotion and load carriage and a model of the energy cost of foraging in the leaf-cutting ant *Atta colombica* Guér. *Physiological Zoölogy* **60**(5): 524-537.

Marais, C., van Wilgen, B.W. and Stevens, D. 2004. The clearing of invasive alien plants in South Africa: a preliminary assessment of costs and progress. South *African Journal of Science* **100**: 97-103.

Martinez-Ferrer, M.T., Grafton-Cardwell, E.E. and Shorey, H.H. 2002. Disruption of parasitism of the California red scale (Homoptera: Diaspididae) by three ant species (Hymenoptera: Formicidae). *Biological Control* **26**: 279-286.

McArthur, R. H. and Pianka, E. R. (1966). On optimal use of a patchy environment. *American Naturalist* **100**: 603-609.

Mikheyev, A.S. and Tschinkel, W.R. 2004. Nest architecture of the ant *Formica pallidefulva*: structure, costs and rules of excavation. *Insect Sociaux*, **51**: 30-36.

Neser, S. & Cilliers, C.J. 1989. Work towards the biological control of *Lantana camara* perspectives. In: *Proceedings of the 7th International Symposium on Biological Control of Weeds*, Rome, Italy. pp. 363-369.

Nielson, M.G., Jensen, T.F. & Holm-Jesen, I. 1982. Effect of load carriage on the respiratory metabolism of running worker ants of *Camponotus herculeanus* (Formicidae). *Oikos* **39**(2): 137-142.

Novotny, V., Basset, Y., Auga, J., Boen, W., Dal, C., Drozd, P., Kasbal, M., Isua, B., Kutil, R., Manumbor, M. & Molem, K. 1999. Predation risk for herbivorous insects on tropical vegetation: A search for enemy-free space and time. *Australian Journal of Ecology* **24**: 477-483.

Oliveira, P.S. 1997. The ecological function of extrafloral nectaries: herbivore deterrence by visiting ants and reproductive output in *Caryocar brasiliense* (Caryocaraceae). *Functional Ecology* **11**: 323-330.

Oosthuizen, M.J. 1964. The biological control of *Lantana camara* L. in Natal. *Journal of Entomological Society of Southern Africa*. **27**: 3-16.

Palmer, T.M. & Brody, A.K. 2007. Mutualism as reciprocal exploitation: African plant-ants defend foliar but not reproductive structures. *Ecology* **88**(12): 3004-3011.

Pass, M.A. 1986. Current ideas on the pathophysiology and treatment of lantana poisoning of ruminants, 1: Australian Vetenary Journal **63**(6):169-71.

Perkins, R.C.L. and Swezey, O.H. The introduction into Hawaii of insects that attack lantana. Bulletin of the Experiment Station of the Hawaiian Sugar Planters' Association 16: pp. 1-83.

Pettey, F.W. 1948. The biological control of prickly-pear in South Africa. *Science Bulletin*, Department of Agriculture of the Union of South Africa **271**: 1-163.

Pitcher, T.J. 1986. Functions of shoaling behaviour in teleosts. In: Pitcher, T.J. (ed.) *The Behaviour of Teleost Fishes*, Croom Helm, Beckenham. pp. 294-337.

Pomerinke, M.A. 1999. Biological control of citrus leafminer, Phyllocnistis citrella (Lepidoptera: Gracillariidae) in southwest Florida. Ph.D. dissertation, University of Florida, Gainesville, Florida, USA. pp 102.

Price, P.W., Bouton, C.E., Gross, P., McPheron, B.A., Thompson, J.N. & Weis, A.E. 1980. Interactions among three trophic levels: Influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecological Systems* **11**: 41-65.

Price, P.W. 2000. Host plant resource quality, insect herbivores and biocontrol. In: N.R. Spencer (ed), *Proceedings of the X International Symposium on Biological Control of Weeds* 4-14 July 1999, Montana State University , Bozeman, USA, pp. 583-590.

Radeghieri, P. 2004. *Cameraria ohridella* (Lepidoptera Gracillariidae) predation by *Crematogaster scutellaris* (Hymenoptera Formicidae) in Northern Italy (preliminary note). *Bulletin of Insectology* **57**(1): 63-64.

Radunz, L.A.J. 1971. Some aspects of the preferential behaviour of *Teleonemia scrupulosa* Stål, towards its host plant *Lantana camara*, Honours thesis, University of Queensland.

Robertson, H. G. 1985. The ecology of *Cactoblastis cactorum* (Berg) (Lepidoptera: Phycitidae) in relation to its effectiveness as a biological control agent of prickly pear and jointed cactus in South Africa. Unpublished Ph.D. thesis, Rhodes University, South Africa.

Robertson, H.G. & Hoffmann, J.H. 1989. Mortality and life tables on *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) compared on two host plant species. *Bulletin of Entomological Research* **79**: 7-17.

Rosenheim J. A., Kaya H. K., Ehler L. E., Marois J. J. & Jaffee B. A. 1995. Intraguild predation among biological control agents: theory and evidence. *Biological Control* **5**(3): 303-335.

Rosner, B. 2000. Fundamentals of Biostatistics, 5th Ed., Brooks/Cole. ISBN 0-534-37068-3.

Room, P.M. & Thomas, P.A. 1985. Nitrogen and establishment of a beetle for biological control of the floating weed *Salvinia* in Papua New Guinea. *Journal of Applied Ecology* **22**: 139-56.

Sato, H. & Higashi, S. 1987. Bionomics of *Phyllonorycter* (Lepidoptera: Gracillariidae) on *Quercus*. II. Effects on ants. *Ecological Research* **2**: 53-60.

Schultz, J.C. 1982. Many factors influence the evolution of herbivore diets, but plant chemistry is central, *Ecology* **69**: 896-897.

Schultz, J.C. 1983a. Impact of variable plant defensive chemistry on susceptibility of insects to natural enemies. In: Hedin, A. (ed). Plant Resistanct to Insects, American Chemical Society Symposium No. 208, Washington, D.C., USA. pp. 37-54

Schultz, J.C. 1983b. Habitat selection and foraging tactics of caterpillars in heterogeneous trees. In: Denno, R.F. & McClure, M.S. (eds). *Variable plants and herbivores in natural and managed systems*, Academic Press, New York, USA. pp. 61-91.

Schultz, J.C. 1988. Many factors influence the evolution of herbivore diets, but plant chemistry is central. *Ecology* **69**(4): 896-897.

Simelane, D.O. & Phenye, M.S. 2005. Suppression of growth and reproductive capacity of the weed *Lantana camara* (Verbenaceae) by *Ophiomyia camarae* (Diptera: Agromyzidae) and *Teleonemia scrupulosa* (Heteroptera: Tingidae). *Biocontrol Science and Technology* **15**(2): 153-163.

Simmonds, H.W. 1929. The life history of *Teleonemia lantanae*. *Fiji Agricultural Journal* **1**: 36-39.

Smith, L. 2003. Avoiding and exploiting trophic cascading: its role in the selection of weed biological control agents. In: J.M. Cullen, D.T. Briese, D.J. Kriticos, W.M. Lonsdale,

L. Morin and J.K. Scott (eds). *Proceedings of the XI International Symposium on Biological Control of Weeds*, Canberra, Australia. pp. 175-179.

Soloman, S.D.M., Glen, D.M., Kendall, D.A., Milson, N.F. 1976. Predation of overwintering larvae of codling moth (*Cydia pomonella* (L.)) by birds. *Journal of Applied Ecology* **18**: 1-35.

Spies, J.J. 1984. A cytotaxonomic study of *Lantana Camara* (Verbenaceae) from South Africa. *South African Journal of Botany* **3**: 231-250.

Spies, J.J. and Stirton, H. 1982. Meiotic studies of some South African cultivars of *Lantana camara*. *Bothalia* **14**: 101-111.

Spies, J.J., & Du Plessis, H. 1987. Sterile *Lantana camara*: fact or theory. South African *Journal of Plant Soil* **4**: 171-174.

Stirton, C.H., 1977. Some thoughts on the polyploidy complex *Lantana camara* L. (Verbenaceae). In: *Proceedings of the Second National Weeds Conference of South Africa*. A.A, Balkema, Cape Town, **99**: 321-344.

Stock, D. 2005. The Dynamics of *Lantana Camara* (L.) Invasion of Subtropical Rainforest in Southeast Queensland, PhD thesis, Griffith University, Australia. pp. 154.

Strong, D.R. 1988. Special feature. Insect host range. *Ecology* **69**: 885.

Swezey, O.H. 1925. Records of Introduction of Beneficial Insects into the Hawaiian Islands. *The Hawaiian Planters' Record* **29** (4): 369-376.

Tanaka, H.O., Inui Y. & Itioka, T. 2009. Anti-herbivore effects of an ant species, *Crematogaster difformis*, inhabiting myrmecophytic epiphytes in the canopy of a tropical lowland rainforest in Borneo. *Ecological Research* **24**(6): 1393-1397.

Taylor, E.E. 1989. A history of biological control of *Lantana camara* in New South Wales. *Plant Protection Quarterly* **4**: 61-65.

Thomas, S. E. and Ellison, C. A. 2000. In: N.R. Spencer (ed), *Proceedings of the X International Symposium on Biological Control of Weeds* 4-14 July 1999, Montana State University, Bozeman, USA. pp. 97-104.

Traniello, J.F.A. 1989. Foraging strategies of ants. *Annual Review of Entomology* **34**: 191-210.

Tschinkel, W.R. 2005. The nest architecture of the ant. *Camponotus socius*. *Journal of Insect Science* **5**: 9-18.

Urban, A.J. & Simelane, D.O. 1999. Performance of a candidate biological agent, *Falconia intermedia* (Hemiptera: Miridae), on selected biotypes of *Lantana camara* (Verbenaceae). In: Van Rensburg, J.B.J. & J. van den Berg (eds). *Proceedings of the Twelfth Entomological Congress*, Potchefstroom, South Africa. pp. 120.

van Klinken RD, Fichera G, Cordo H. 2003. Targeting biological control across diverse landscapes: the release, establishment and early success of two insects on mesquite (*Prosopis*) in rangeland Australia. *Biological Control* **26**: 8-20.

van Veen, F.J.F., Morris, R.J. & Godfray, H.C.J. 2006. Apparent competition, quantitative food webs, and the structure of phytophagous insect communities. *Annual Review of Entomology* **51**: 187-208.

Way, M.J. 1963. Mutualism between ants and honeydew-producing Homoptera, *Annual Review of Entomology* **8**: 307-344.

Wehner, R. 1981. Spatial vision in arthropods. *In* H. Autrum (ed) *Handbook of sensory physiology*, Springer-Verlag, Heidelberg vol. 7/6C: 287-616.

Wehner, R., Harkness, R.D., Schmid-Hempel 1983. Foraging strategies in individual searching ants *Cataglyphis bicolor* (Hymenoptera: Formicidae). Information processing in animals vol. 1 Gustav Fisher Verlag, New York. pp. 79.

Wheeler, W.M. 1910. *Ants: Their Structure, Development and Behaviour*, Columbia University Press.

Williamson, M. 1996. *Biological Invasions*, Chapman & Hall, London, ISBN 0412591901 (pbk).

Xiao, Y., Qureshi, J.A. & Stansly, P.A. 2006. Contributions of predation and parasitism to mortality of citrus leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) populations in Florida. *Biological Control* **40**: 396-404.

Zalucki, M.P., Day, M.D. and Playford, J. 2007. Will biological control of *Lantana camara* ever succeed? Patterns, processes & prospects. *Biological Control* **42**: 251-261.