

ERRATA

- p. 11 – should read “Figure 1.2” not Figure 2.
- p. 78, -4 line - should read “*Coccus viridis*” not “*Coccus viridus*”
- p. 78, -1 line – “*Pseudococcus citri*” should read “*Pseudococcus citri* (= *Planococcus citri*)”
- p. 79, line 2 - should read “*Coccus viridis*” not “*Coccus viridus*”
- p. 84, para 2, line 4 – “*hesperidium*” should read “*hesperidum*”
- p. 84, para 2, line 7 – “Cockerill” should read “Cockerell”
- p. 87, para 2, line 8 – “*hesperidium*” should read “*hesperidum*”
- p. 87, para 2, line 9 – “*Saissieta*” should read “*Saissetia*”
- Table 4.1 – “*hesperidium*” should read “*hesperidum*”; “*Saissieta*” should read “*Saissetia*”; “*Icerya pruchasi* (Maskell)” should read “*Icerya purchasi* Maskell”
- Table 4.3 – place the following at bottom of table as a subscript. “Asterisks indicate the relative abundance (* = infrequent; ** = occasional; *** = abundant) of scale insect types recorded on host plant species at other locations or which were not a part of the formal survey.”
- Figure 4.5 – place at the end of the figure caption. “*Paratachardina lobata* was never observed to be tended by ants so it was assumed that honeydew production was negligible.”
- p. 145, para 2, last sentence – should read “ant exclusion sites” rather than “control sites”
- p. 146, should read *Coccus viridis*, not *Coccus viridus*; *Pseudococcus citri* should read *Pseudococcus citri* (= *Planococcus citri*)
- p. 169, para 2, line 1-2 - “forest types II, II and III...” should read “forest types I and III, and II and III...”. Table 7.2 should be Table 7.1; Table 7.3 should be Table 7.2.
- p. 195, para 1, line 3 – “*hesperidium*” should read “*hesperidum*”

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**Alien ant invasion on Christmas Island, Indian Ocean:
The role of ant-scale associations in the dynamics
of supercolonies of the yellow crazy ant,
*Anoplolepis gracilipes***

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Thesis submitted for the degree of Doctor of Philosophy
in the School of Biological Sciences,
Monash University, Clayton, VIC.
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“Ecological explosions differ from some of the rest by not making such a loud noise and in taking longer to happen.”

C.S. Elton 1958

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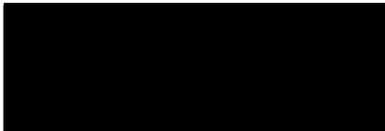
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Declaration

This dissertation does not contain material that has been accepted for the award of any other degree or diploma in any university or institution. Further, to the best of my knowledge, it does not contain material previously published, or written by another person, except where due reference is made in the text.


K.L. Abbott

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Abstract

Biological invasions rank amongst the greatest threats to ecosystem persistence and native biodiversity. Their effects are felt especially on oceanic islands. This thesis explored the dynamics of one of the world's worst invaders, the yellow crazy ant *Anoplolepis gracilipes*, and especially the nature of its association with honeydew-producing scale insects, the cause of 'invasional meltdown' on Christmas Island, a remote tropical oceanic island in the eastern Indian Ocean.

Anoplolepis gracilipes has general attributes that promote invasiveness. A key feature is its unicoloniality and, in rainforest on Christmas Island, *A. gracilipes* formed expansive supercolonies with forager densities sometimes exceeding 20 million ants per hectare. These densities were sustained day and night, 365 days a year, and in some supercolonies for >6 years. Studies of population dynamics of *A. gracilipes* using artificial nests showed that production of queens and males started just before the wet season of each year. Both queens and males were caught in canopy malaise traps, suggesting the occurrence of nuptial flights, which have not been previously recorded for *A. gracilipes*. Area of *A. gracilipes* supercolonies varied from 1 – 787 hectares, where they displaced other ant species and killed native red crabs as supercolony boundaries expanded. Supercolony boundaries were dynamic, with a maximum expansion rate of 163 m/year and maximum contraction rate of 48 m/year. Preference for carbohydrate and protein baits shifted seasonally. Workers recruited in higher numbers to protein during the dry season, between April and October, and carbohydrates between October and April.

Workers of *A. gracilipes* formed 'strong' associations with mostly introduced honeydew-producing scale insects (Hemiptera: Sternorrhyncha: Coccoidea). In a study of 15 sites across three forest types, *A. gracilipes* established associations with potentially eight species of honeydew-producing scale insects, including soft scales (Coccidae) and lac scales (Kerriidae). Outbreaks of scale insects in all three forest types were associated with *A. gracilipes* supercolonies. Averaged over sites and forest types, adult scale insects were 12 times more abundant in supercolonies than in intact forest. This strongly suggests that these adventitious associations form a positive feedback loop, amplifying populations of scale insects and ants over large spatial scales. In the rainforest understorey, experiments excluding *A. gracilipes*

from seedlings showed that ant tending increases the abundance of different life stages of the lac scale insect, *Tachardina aurantiaca* on two host species, *Inocarpus fagifer* and *Tristiropsis acutangula*. Similarly, on *Claoxylon indicum* and *Dysoxylum gaudichaudianum*, which are primarily associated with soft scales (Coccoidae), the abundances of adult females were greater on seedlings with *A. gracilipes*. Abundance of generalist predators of scale insects was significantly greater on seedlings from which *A. gracilipes* had been excluded. These effects of ant association were even more vivid at a forest-wide scale. Elimination of *A. gracilipes* from multi-hectare plots using toxic ant bait in a BACI experiment caused scale insect populations to fall significantly by 68% after 11 weeks, and completely collapse after one year on canopy trees of *I. fagifer* and *Syzygiun nervosum*.

Events that initiate supercolony formation in *A. gracilipes* are poorly understood. Outbreaks of pest insects can be preceded by extreme weather conditions, where stressed plants become susceptible to herbivores due to increased plant nutritional quality or reduced chemical defense. In a field experiment, I tested the combined effect of plant water stress and attendance by *A. gracilipes* on the success of populations of the lac scale, *Tachardina aurantiaca*, on seedlings of rainforest species. After two months, abundance of all life stages increased significantly only in the presence of *A. gracilipes*. However, plant water stress had no significant effect on the abundance of crawlers and adult females. These results indicate that biotic interactions between ants and scale insects may be more important than abiotic factors in the success of *A. gracilipes* and in the formation of supercolonies on Christmas Island.

On Christmas Island, ant-scale association affects a myriad of interactions, many of which may operate similarly on other tropical oceanic islands. Establishment of these adventitious mutualisms following multiple alien species introductions may be pivotal in accelerating population explosions and concomitant impacts of invasive insects. Islands have historically played a central role in our understanding of evolutionary processes and development of ecological theory. These 'model' systems can also sharpen our understanding of biological invasions, as I have shown on Christmas Island. Equally, islands provide small, discrete systems where this increasing understanding can be applied to reduce the impacts of even the most intractable of invaders, like ants.

Chapter One

General Introduction



When Elton (1958) described the potential homogenisation of the earth's biota through the proliferation of exotic species, he may not have envisaged its reality so soon to follow. In the 21st Century, biological invasions (whether through accidental or deliberate introduction) by exotic plant and animal species rank amongst the greatest threats to ecosystem persistence and native biodiversity (Everett 2000; Ferriere *et al.* 2000). From an entirely anthropocentric perspective, biological invasions and the potential for homogenisation of global flora and fauna threaten to erode ecosystems upon which cultural identities are based, resulting in globalisation of culture, loss of distinctiveness and eventual loss of respect for remaining unique environments. The establishment and spread of biological organisms beyond their natural range (biological invasion; Williamson 1996) is not a new phenomena, but increasing human population, associated commerce and advanced transport technology has led to escalating opportunities for movement of species, and rising rates of introduction of exotic species. Detailed investigations into specific invasions and their general underlying mechanisms for success and impacts is key to understanding ultimately how best to manage often catastrophic restructuring of ecosystems.

Effects of ecological explosions are felt especially on oceanic islands where ecosystems appear exceptionally vulnerable to damages caused by exotic plants and animals (Loope & Mueller-Dombois 1989; D'Antonio & Dudley 1995; Simberloff 1995). Original theory (Elton 1958) predicted that a community becomes more resistant to invasion as it increases in diversity and forms highly complex food webs.

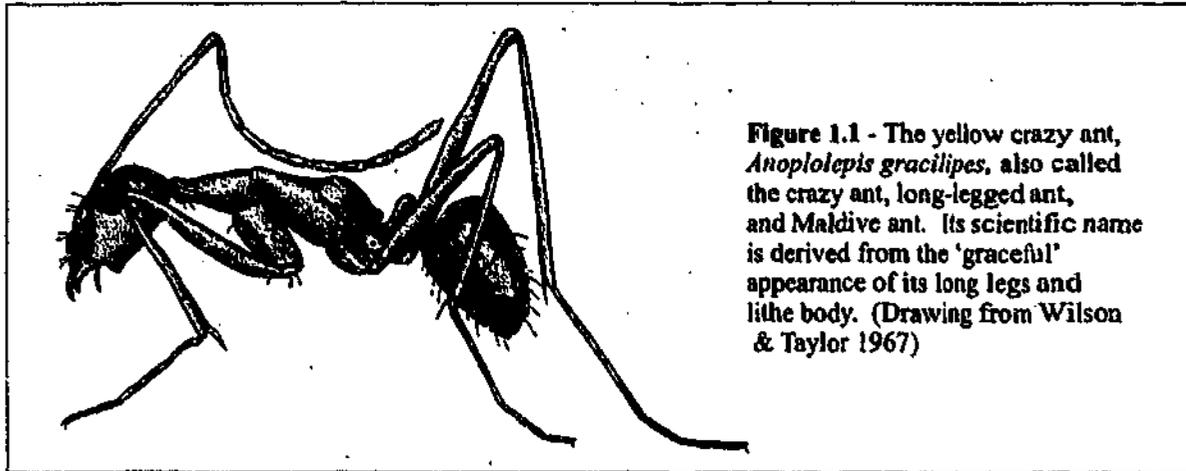
Insular biotas are disharmonic (Wallace 1911), and typically species-poor, even compared to their closest mainland region (MacArthur & Wilson 1967). However, it remains unclear as to whether or not it is general characteristics of the invading species, or some inherent attributes of island communities that explains islands' proposed vulnerability and fragility (Carlquist 1974; Simberloff 1995; Hewitt & Huxel 2002). Their disharmony means islands provide many opportunities to investigate novel interactions between and within species, but it may also reduce 'biotic resistance' (Lake & O'Dowd 1991; Simberloff 1995) and increase their susceptibility to invasion. Introduced species on islands can alter native communities dramatically (Cowie 1992; Meyer & Florence 1996; Fritts & Rodda 1998; O'Dowd *et al.* 2003), and the number of extinctions apparently caused by introduced species is much greater on islands (Atkinson 1989).

It is essential to understand both the key characteristics of invading organisms, and the recipient communities, to help identify the relationship between them, which can differ between stages of an invasion, e.g. availability of individuals, transfer of individuals, establishment of population, and spread (Heger & Trepl 2003). Factors involved in the successful arrival and establishment of a species may be different to those that influence if and when there is a subsequent population explosion. Humans have been moving species around for thousands of years, but these alien species commonly exhibited low population abundances for long periods of time in their introduced range that then boom, and often bust again (Sakai *et al.* 2001; Simberloff & Gibbons 2003). For example, the giant African land snail (*Achatina fulica*), introduced to many Pacific islands (Mead 1979) typically undergoes rapid population expansion followed by a crash (Simberloff & Gibbons 2003), whereas an explosion in abundance of the invasive yellow crazy ant occurred on Christmas Island after a 55 – 65 year lag period.

Ants as invaders

Among invasive organisms, social insects, primarily termites, ants and wasps, are the most potent invaders both on continents and on islands (Moller 1996), and can become pests of natural environments. For example, ants are thought to be the greatest threat to conservation of Pacific Islands, by predation, competition, and creating favourable conditions for other invasive biota (Nishida & Evenhuis 2000). Six species of ants are considered to be highly invasive outside their native range. These are now the most widespread, abundant and damaging of all invasive ants: *Anoplolepis gracilipes* (yellow crazy ant; Fig 1), *Linepithema humile* (Argentine ant), *Pheidole megacephala* (big-headed ant), *Solenopsis invicta* (red imported fire ant), *S. geminata* (tropical fire ant) and *Wasmannia auropunctata* (little fire ant). These species are known as "tramp ants" (Wilson & Taylor 1967; Passera 1994), and have invaded continental areas as well as many oceanic islands, wielding disastrous results around the globe. At high population densities, they displace native ants and invertebrates (Erickson 1971; Haines & Haines 1978b; Porter & Savignaro 1990; Hoffmann *et al.* 1999; Hill *et al.* 2003), impact upon birds and other vertebrates (Allen *et al.* 1998; Jourdan *et al.* 2001; Davis 2002) and become domestic nuisances (Haines & Haines 1978b; Haines *et al.* 1994). They can alter entire ecosystems (O'Dowd *et al.* 2003; Sanders *et al.* 2003) and cause massive financial losses (Lard *et al.* 2001).

They share a suite of general characteristics that afford them super-effective invader status, including high reproductive rates, a broad diet, polygyny (many queens in one nest), polydomy (many nests in one colony), nest site flexibility, colony budding and lack of intraspecific aggression (Moller 1996; Holway *et al.* 1998; Holway *et al.* 2002). These features allow invasive ants to form unicolonial populations, encouraging high local abundances and the domination of entire habitats (O'Dowd *et al.* 2003).



Their impact upon native ecosystems is mainly attributed to numerical dominance (Holway *et al.* 1998). Life history traits, competition, predation and parasitism have typically been at the forefront of hypotheses explaining interactions that limit and regulate populations (Pimm 1989; Watt *et al.* 1990; Adams & Tschinkel 2001), and that may inhibit or promote invasion success and subsequent population explosion (Elton 1958; Pimm 1989; Sakai *et al.* 2001). However, more recently, ecologists recognise positive interactions are at least as important as negative ones and physical forces in determining the distribution and abundance of species (Richardson *et al.* 2000; Stachowicz 2001; Helms & Vinson 2003). It is possible that positive associations between species (mutualisms), in combination with negative interactions give rise to slightly altered models of population and community dynamics (Bruno *et al.* 2003), and are important in the facilitation of introduced exotic species (Simberloff & Von Holle 1999; Stachowicz 2001; O'Dowd *et al.* 2003).

Positive interactions and their role in ant invasions

Positive interactions are ubiquitous (Beamer & Michener 1950; Way 1963; Janzen 1985; Buckley 1987b; Ellison *et al.* 1996; Richardson *et al.* 2000; Bronstein 2001; Stachowicz 2001; Helms & Vinson 2002), but may be unrecognised key processes in the successful invasion by alien species, where impacts by one, or both

partners can be diversified and amplified (Simberloff & Von Holle 1999; Richardson *et al.* 2000; O'Dowd *et al.* 2003). Many introduced plant species rely on mutualisms in new habitats to overcome barriers to establishment, become naturalised, and in some cases, invasive (Richardson *et al.* 2000).

In the majority of cases where ants become pests in horticultural and natural ecosystems, they obtain carbohydrates from extrafloral nectaries (Young 1996; Bluthgen *et al.* 2000) and also form close associations with honeydew-producing Coccoidea (Hemiptera: Sternorrhyncha: Coccoidea; formerly of the suborder Homoptera – Gullan 2001) (Rohrbach *et al.* 1988; Jahn & Beardsley 1994; Dejean & Matileferrero 1996; Ho & Khoo 1997; Helms & Vinson 2002; O'Dowd *et al.* 2003). These associations are often in multi-species complexes (Bristow 1984). It has been hypothesised that tropical rainforest canopy ants, rather than operating as scavengers and predators, derive both carbohydrates and nitrogen primarily from plant and insect exudates (Tobin 1994; 1995; Davidson 1997). In fact, Helms and Vinson (2002) suggested that mutualistic honeydew-producing hemipterans could contribute up to 68% of an invasive ant's energy requirements. However, never has the acquisition of carbohydrates, in the form of insect excretion, been suggested to be the basis for a population explosion of an invasive ant species.

***Anoplolepis gracilipes* as a pest species**

The yellow crazy ant, *A. gracilipes*, is emerging as a pantropical threat to native biodiversity (O'Dowd *et al.* 2003). Although its native range is unknown, humans have facilitated the transglobal spread *A. gracilipes*. It occurs primarily in tropical latitudes, throughout Asia and Pacific and Indian Ocean islands, where it has been receiving increasing attention as an ecological and domestic pest species. Previously, it had been recorded in high densities as a domestic nuisance in the Seychelles during the 1960-70's, where it killed newborn chickens and other domestic animals, and excluded older animals. It affected plants by removing soil from around tree roots, and tending honeydew-producing hemipterans, greatly increasing their populations and causing an

increase in sooty mould on fruit and foliage of trees (Lewis *et al.* 1976; Haines *et al.* 1994). In more recent years, land crabs of the genus *Cardisoma* have been observed to be killed by crazy ants in the Seychelles. The Seychelles' endemic skink *Mabuia seychellensis* disappeared from areas where crazy ants were abundant, sooty terns failed to occupy nesting sites and some chicks of the white tern, *Gygis alba*, were killed by *A. gracilipes* (Feare 1999). The native tree *Pisonia grandis* suffers high densities of coccid scale insects in areas of high crazy ant abundance, to the point of tree death in some instances (Hill *et al.* 2003). On Tokelau, an oceanic atoll system in the Pacific Ocean, *A. gracilipes* has increased in abundance to become a domestic nuisance, and tend honeydew-producing hemipterans on native and introduced vegetation, including tropical fruit trees. Locals have taken to burning vegetation in order to rid ants from certain areas (Lester & Tavite In Press).

A. gracilipes has been observed tending honeydew producing hemipterans wherever it has been recorded (Van der Goot 1916; Way 1953; Wilson & Taylor 1967; Fluker & Beardsley 1970; Greenslade 1971b; 1971a; Haines & Haines 1978; Rao *et al.* 1989; Rao & Veeresh 1991; Young 1996; O'Dowd *et al.* 2003; Lester & Tavite In Press). But the importance placed on insect honeydew as a carbohydrate source in *A. gracilipes*' diet has been inconsistent. Way (1953) declared that honeydew from the aphid *Ceraphis latanie* was the main food source on coconuts in East Africa, but that generally *A. gracilipes* was a scavenger. Greenslade (1971b) observed that *A. gracilipes* was not as dependent on honeydew as other ant species nesting in palms, but Rao *et al.* (1989) reported that the association of *A. gracilipes* with flora was dependent upon the intensity of infestation with various homopterous insects. Furthermore, Haines & Haines (1978a) suggest that, at least in the Seychelles, vegetation type influences ant populations mainly through food supply by providing sugary substances which *A. gracilipes* feeds on directly, or by supporting populations of honeydew-producing insects.

A sudden explosion in abundance of *A. gracilipes* on Christmas Island, Indian Ocean, occurred around 1996, and has resulted in 'invasional meltdown' on the island.

Here, *A. gracilipes* reached extreme densities in rainforest where it eliminates red land crabs and tends honeydew-producing hemipterans. Impacts have dramatically changed the unique ecosystem of the isolated oceanic island (O'Dowd *et al.* 2003).

***Anoplolepis gracilipes* on Christmas Island**

Christmas Island is an elevated oceanic limestone island located at 105° 40'E, 10° 30'S in the eastern Indian Ocean, approximately 360 km south of Java and 1400 km northwest of Australia. It rises to its highest point of 361 m ASL (Murray Hill) in the western part of the island, and covers an area of approximately 135 sq km, of which 74% is covered with broad-leaved, structurally simple rain forest on porous soils.

Introduced to Christmas Island accidentally some time between 1915 and 1934, *A. gracilipes* increased in abundance to form areas in which foraging workers reach incredibly high densities, where they overcome and kill red land crabs (*Gecarcoidea natalis*) and cause a cascade of ecological disruptions (O'Dowd *et al.* 2003). These areas are defined as supercolonies and since 1996 have spread to occupy more than 3000 hectares between 1996 and 2003 (Green *et al.* 2004). The first account of supercolony formation in island rainforest was 1989, in the northeastern section of the island (P.T. Green personal communication), but the occurrence of supercolonies has increased rapidly. Via the elimination of red crabs, *A. gracilipes* indirectly causes increased seedling density, change in seedling composition, and build up of leaf litter on the forest floor. Foraging workers form close associations with honeydew-producing scale insects (Coccoidea) observed at extremely high densities, which promote the build up of sooty mould on rainforest vegetation and possibly cause dieback of some rainforest species (O'Dowd *et al.* 2003). Recent research has shown that the abundance of the ground foraging emerald dove (*Chalcophaps indica*) is significantly reduced in crazy ant supercolonies, and that nesting success of the endemic thrush (*Turdus poliocephalus erythropleurus*) may also be negatively affected (Davis 2002).

Isolated nests of *A. gracilipes* are widespread across the island and present in settled areas, where they are associated with honeydew-producing hemipterans on domestic plants, usually exotics introduced for ornamental purposes. For example, many ornamental cycads sustained populations of soft scale insects that were frequently visited by *A. gracilipes*, and nests were common in the bases of coconut palms and under vegetative debris in backyards (personal observation).

In 1995, supercolonies of *A. gracilipes* were not observed. In September 2002, *A. gracilipes* supercolonies were present in approximately 25% of the island's 10,000 hectares of forest (Green *et al.* 2004). This represents an average rate of increase in area occupied by supercolonies of more than 400 ha per year. In these areas scale insects were ubiquitous, occurring in the understorey on seedlings and saplings of rainforest trees and in trailing into the canopy on the boles of large trees. Given the paucity of canopy tree species on Christmas Island with extrafloral nectaries (Du Puy 1993), scale insects represent the largest source of carbohydrates available for *A. gracilipes*, and may make affordable the high foraging tempo exhibited by *A. gracilipes*. The abundance on both partners in *A. gracilipes* supercolonies on Christmas Island reveals a mutualism operating over huge spatial scales (O'Dowd *et al.* 2003).

Why now?

There are number of hypotheses that attempt to explain the disproportionate abundance of invasive ant species, all equally plausible in explaining the recent explosion in abundance of *A. gracilipes*, and formation of supercolonies on Christmas Island. Most of the more convincing arguments have their basis in genetics, and evidence suggests that a reduction in genetic variation in introduced populations of Argentine ants (*Linepithema humile*) has led to the increased local abundance in the

United States (Holway *et al.* 1998; Tsutsui *et al.* 2000; Tsutsui *et al.* 2001). In addition, the release from natural enemies in their introduced range may lead to larger colony sizes and increased colony densities as a consequence (Porter *et al.* 1997). However, invasive ants may consume resources, such as plant and hemipteran exudates that native ants either fail to exploit or do so less efficiently than invasive ants (Holway *et al.* 2002). The abundance of honeydew-producing scale insects associated with supercolonies of *A. gracilipes* suggests that they may be important in at least the maintenance of crazy ant populations.

Outbreaks of insects are frequently linked to atypical climatic events or stressful conditions (White 1986; Martinat 1987; Mattson & Haack 1987; Atkinson & Nuss 1989; Warkentin *et al.* 1992; McMillin & Wagner 1995), whereby plants subjected to these conditions become more susceptible to herbivorous insects due to the plant's increased nutritional quality and/or reduced concentrations of defensive chemicals (White 1974; Mattson Jr. 1980; White 1984). The Plant Stress Hypothesis (PSH) was developed to describe this phenomenon (White 1984). The distribution of *A. gracilipes* supercolonies on Christmas Island hinted that this might be the case. Extended dry periods may have stressed forest in areas where the soil is shallow and affords low water-holding capacity, leading to adjustments by trees that make them suitable hosts for scale insect proliferation. Sap-sucking insects generally perform better on stressed plants (Koricheva & Larsson 1998), hence the PSH (White 1974; 1984) seemed an appropriate starting point. My study aims to test the general hypothesis that scale insects increase in abundance on more favourable hosts, whose condition is mediated by abiotic factors. Their increase in abundance would subsequently provide the bottom rung of a positive feedback mechanism promoting increased populations of both *A. gracilipes* and scale insects.

Why this thesis?

The invasion of Christmas Island by *A. gracilipes*, and subsequent rapid population explosion, provides an exemplary opportunity to investigate the dynamics of

a specific invasion in an insular ecosystem. Furthermore, *A. gracilipes* remains one of the most understudied of the damaging invasive ant species, and contributions to the ecological knowledge bank on this species are urgent. Relatively high population densities have been recorded in Indonesia (Van der Goot 1916; Baker 1976), Hawaii (de Fluiter 1939), Samoa and Tokelau in the Pacific Ocean (Lester & Tavite In Press), and in the Seychelles (Haines & Haines 1978a; 1978b; Hill *et al.* 2003) and Christmas Island in the Indian Ocean (O'Dowd *et al.* 2003). All studies have indicated the association between *A. gracilipes* and honeydew-producing hemipterans, but up until now, none have suggested that honeydew acquisition and use may be key to their population build up and impacts. The recent formation of supercolonies by crazy ants on Christmas Island has led to synergism in invasive impacts and precipitated invasional 'meltdown' (O'Dowd *et al.* 2003). Understanding the dynamics of *A. gracilipes* supercolonies is crucial in the mitigation of continuous negative impacts on the Christmas Island ecosystem. Furthermore, the mutualism between ants and scale insects highlights the importance of positive interactions in the population dynamics of invasive species.

My thesis aims to demonstrate the significance of honeydew-producing hemipterans in possibly helping facilitate the extreme densities of foraging *A. gracilipes* seen on Christmas Island during the 1990's. This work furthers ideas of Davidson (1997, 1998) and Helms and Vinson (2002, 2003), that mutually beneficial associations between honeydew-producing hemipterans (mealy bugs) and invasive ant species are an important factor in their success.

Layout of this thesis

The *A. gracilipes* supercolony system is multi-trophic, and involves three main players: the ants (*A. gracilipes*), the scale insects (Hemiptera: Sternorrhyncha: Coccoidea), and the rainforest (Mitchell 1985). My thesis is structured according to these players, and first tackles them separately, then in terms of their interactions (Fig. 1.2). It is a collection of standalone investigations, written as extended research papers,

and includes specific details of study sites for each study. There will be unavoidable repetition, however, they are presented in a logical order and are intended to be read in that order in this thesis. Following this general introduction, chapters two through to seven are data chapters, and chapter eight is the general discussion and synthesis of my research.

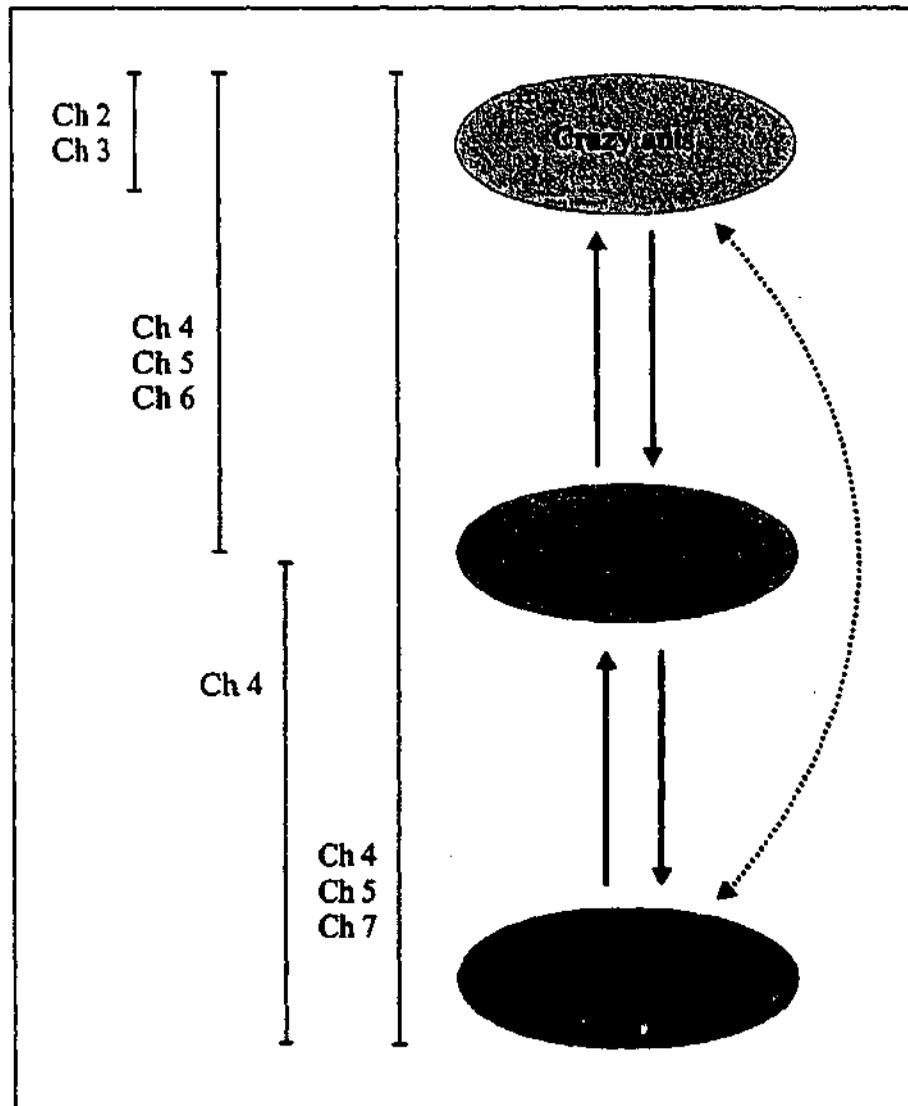


Figure 2 – The layout of data chapters in this thesis in relation to the three main players thought to influence *A. gracilipes* supercolony dynamics. Solid lines indicate direct effects and broken line indirect effects.

Chapters 2 and 3 introduce the basic ecology of the main player - *Anoplolepis gracilipes* in supercolonies on Christmas Island. Chapter 2 addresses the inherent characteristics of the invader. I describe the activity, density and biomass of *A. gracilipes*, investigate intraspecific aggression, the reproductive schedule, and food type preferences of foraging workers.

Understanding, and subsequently preventing the spread of invasive ant species is a crucial management technique for limiting the success of invasive species (Krushelnicky *et al.* 2003; Green *et al.* 2004). Chapter 3 addresses the spatial and temporal dynamics of *A. gracilipes* supercolonies on Christmas Island; how their boundaries move in space and time and how their expansion or contractions affects other ant species richness and red land crab abundance.

Chapter 4 introduces the next player – the scale insects. Very little information prior to this study was available for scale insects on Christmas Island. I provide a list of scale insects on the island and examine their composition across scale family groups and host species. I discuss input and dispersal of mobile crawlers, and establishment of scale insect populations in the context of *A. gracilipes* supercolonies, probable pathways for scale introduction, and implications of their proliferation on Christmas Island, given the inclination of invasive ants to form mutualisms with scale species of economic importance. In addition, I investigate how floristic composition affects the density of all scale insect species and create a 'scale suitability index' to describe the suitability of three main forest types to infestation by scale insects in the presence of *A. gracilipes*.

Chapter 5 and 6 address the mutualism between *A. gracilipes* and scale insects with experimental ant exclusion on two different spatial scales. Chapter 5 is set in the rainforest understorey, where foraging ants are excluded from individual saplings of four species, and populations of scale insects monitored. In chapter 6, I exclude ants from forest plots of between 9-36 hectares and record changes in scale insect populations on three important canopy species. Both experiments test the hypothesis that populations of scale insects will decline significantly in the absence of crazy ants

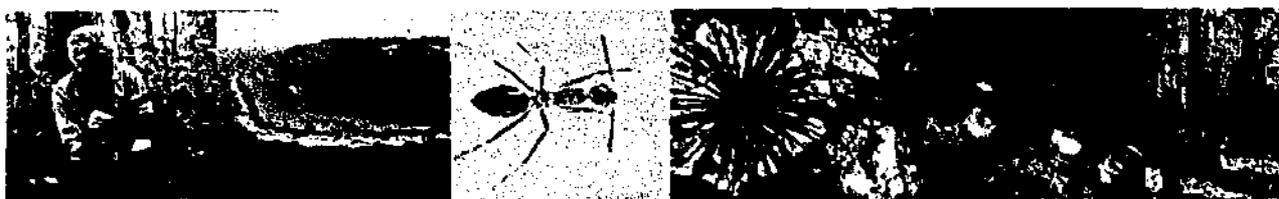
and aim to describe the 'strength' of the mutualism between the crazy ants and honeydew-producing scale insects.

Chapter 7 directly examines the trigger for the formation of *A. gracilipes* supercolonies on Christmas Island. It incorporates a large-scale survey of 'forest water status' and a small-scale field experiment to test the general hypothesis that it is factors affecting scale abundance, rather than ant abundance, that provide the bottom rung of a positive feedback loop possibly leading to supercolony formation. Stressed plants and attendance by *A. gracilipes* are predicted to provide benefits to scale insects mutually exclusive of one another, but their interaction may amplify the effects of both. I test the effect of water-stressed plants and attendance by *A. gracilipes* on the colonisation success and abundance of the lac scale, *Tachardina aurantiaca*, on saplings of three common rainforest species, *Inocarpus fagifer*, *Tristiropsis acutangula* and *Pongamia pinnata*, all of which are infested with lac scale in *A. gracilipes* supercolonies. I predict that the abundance and colonisation success rank order between experimental treatments will be water-stressed/ants > water-stressed/no ants > non water-stressed/ants > non water-stressed/no ants.

Invasion of insular and mainland ecosystems by invasive alien ant species has increased dramatically in the past 10 years. These invasions have both ecological and management implications. However, the roles of positive interactions are often overlooked during the development of research and management programs. In the general discussion (Chapter 8) I focus on the extent to which honeydew-producing hemipterans are associated with invasive ant species in a historic and global context, and the influence they are believed to have on ant populations. Positive exchanges between species in multi-trophic systems can contribute to our understanding of biological invasions and their ecological impacts. I discuss these against a background of negative interactions that have historically dominated theories on the proliferation of exotic species. Lastly, I make suggestions for further research in this area and how it can contribute to successful control programs for invasive ant species particularly in insular ecosystems.

Chapter Two

Anoplolepis gracilipes forms 'carpets of ants' and exhibit general tramp ant characteristics in supercolonies on Christmas Island, Indian Ocean.



Introduction

Invasions by introduced alien ants repeatedly cause ecological disruption affecting both continental and island ecosystems throughout the world (Holway *et al.* 2002). It is often through sheer numerical dominance that invasive ants can achieve superior competitive and interference abilities and recruitment to food sources (Porter & Savignano 1990; Human & Gordon 1996; Holway & Case 2001). Invasive ants sometimes reach abundances exceeding that of all native ant species combined in comparable uninvaded areas (Porter & Savignano 1990; Holway 1998; Hoffmann *et al.* 1999).

The characteristics of invasive ant species (Holway *et al.* 2002) promote the formation of unicolonial populations (Hölldobler & Wilson 1990), within which intraspecific aggression between nests is reduced or absent (Holway *et al.* 1998) and where there are no distinct behavioural boundaries between physically separate nests (Holway *et al.* 2002). These populations are often referred to as 'supercolonies', and several species of invasive ants (e.g. *Linepithema humile*, *Pheidole megacephala* and *Anoplolepis gracilipes*) are described as forming supercolonies (Erickson 1971; Cherix 1980; Hoffmann *et al.* 1999; Tsutsui *et al.* 2000; O'Dowd *et al.* 2003). However, there has been a tendency to define supercolonies with little reference to the activity or density of foraging worker ants. It has been suggested that unicoloniality allows invasive species, such as *Linepithema humile* and *Pheidole megacephala* to attain high

local abundances and dominate entire habitats (Hölldobler & Wilson 1977) Therefore, unicoloniality may be a prerequisite to forming supercolonies, and the term supercolony would infer something about extreme relative densities of ants in a unicolonial population. Intra-specific aggression is typically used as the basis for defining a unicolonial population (Roulston & Silverman 2002). However, once Argentine ants fuse into supercolonies in North America, Holway *et al.* (1998) suggest that worker density becomes the most ecologically meaningful measure of population size. It is foraging workers that are responsible for impacts including biting and stinging humans and animals, displacement of other ant species, overpowering and retrieving prey and the effects of formic acid.

The invasive yellow crazy ant, *Anoplolepis gracilipes* (formerly *Anoplolepis longipes* Jerdon), forms supercolonies on Christmas Island, Indian Ocean, where extremely high densities of ants appear to exhibit little intraspecific aggression and forage on all available surfaces in the field (O'Dowd *et al.* 2003). In supercolonies, incredibly high densities of ants are sustained 24 hours a day, all year round on the ground and in the canopy; adjectives such as 'carpets of ants' and 'seething mass of ants' have been used to describe the phenomenon. In these areas, the density of crazy ants is sufficient to kill red land crabs within 24 hours, resulting in increased leaf litter and increased recruitment of seedlings on the ground (O'Dowd *et al.* 2003).

A. gracilipes was accidentally introduced to Christmas Island probably some time between 1915 and 1934 (O'Dowd *et al.* 1999). The first supercolony was discovered in 1989, and since 1996 supercolonies have rapidly formed and spread across island rainforest. In September 2002, 28% of 10,000 ha of rainforest on Christmas Island was occupied by *A. gracilipes* supercolonies (Green *et al.* 2004). In addition to forming supercolonies on Christmas Island, isolated nests are widely distributed across the island. It is unknown if these nests are genetically related. But even at low densities, crazy ants do not appear to exhibit aggressive behaviour toward conspecifics.

The origin and native range of *A. gracilipes* is unknown, but is most likely tropical Asia or Africa (Wheeler 1910; Wilson & Taylor 1967). However, *A. gracilipes* has been spread by human commerce throughout the tropics, especially in Asia and the Indo-Pacific. It has not only effectively colonised continental areas such as India, Sri Lanka, South Africa, Indonesia, Myanmar, Malaysia and New Guinea, but also a suite of oceanic islands, including Vanuatu, the Seychelles, Mauritius, Reunion, the Cocos (Keeling) Islands, Christmas Island, and Tokelau and Samoa (O'Dowd 2004). *A. gracilipes* has reached high densities in the Seychelles (Lewis *et al.* 1976; Haines & Haines 1978a; Hill *et al.* 2003), where its presence in high densities affected the taxonomic composition of the arthropod community (Hill *et al.* 2003); in urban areas of the Tokelau Islands and Apia, Samoa, high numbers of *A. gracilipes* were caught in pitfall traps in only 24 h (Lester & Tavite in Press); and in India, colonies of *A. gracilipes* were described as multi-queened and without a definite territory or distinct boundary, exhibiting no intraspecific aggression (Rao *et al.* 1991), indicative of a supercolonial population. Aside from these instances, and Christmas Island, *A. gracilipes* has not been reported to have previously formed supercolonies, and has never been recorded to cause ecological impacts as seen on Christmas Island, i.e. the removal of a 'keystone' species (O'Dowd *et al.* 2003).

This chapter describes elements of *A. gracilipes* dynamics in supercolonies on Christmas Island that may aid both in explaining supercolony formation and managing and controlling crazy ants on the island. I have four specific objectives in this chapter:

1. To develop a rapid method for assessing the relative abundance of forager activity in terms of their levels of their activity, and calibrate this parameter with real measures of ant density and biomass of *A. gracilipes* foragers. This should allow rapid and meaningful assessment of ant activity across sites and through time, including diel and seasonal activity.
2. To investigate the level of intraspecific aggression between and within supercolonies, and between isolated nests and supercolonies, to determine

whether any lack of aggression between conspecifics is consistent with uniclonality and my definition of supercolonies.

3. To describe the reproductive schedule of *A. gracilipes* and determine the timing and duration of sexual production, and mating and dispersal events.

4. To document forager preference for carbohydrate and protein baits as a potential indicator of colony status and brood production. It is documented that nitrogen (as amino acids and proteins) is the limiting resource for colony growth and reproduction (Tobin 1995; Davidson 1997), and so a preference for protein may indicate the onset of sexual brood and reproductive stage.

Methods and Materials

The Study System

Christmas Island (105° 40'E, 10° 30'S) is an uplifted oceanic limestone island that lies 360 km south of Java in the north-eastern Indian Ocean. The island is located in the humid tropics, and experiences a monsoonal climate where most of the 2000 mm mean annual rainfall occurs between December and May (Falkland 1986). Presently, approximately 74% of the island is covered with natural vegetation, where three of seven described vegetation types (Mitchell 1985) dominate the primary topographical features of the island, the central plateau and surrounding terrace areas. The vegetation types correspond generally to geological substrate type (Barrie 1967).

This study was conducted between December 2000 and December 2002 in areas of *A. gracilipes* supercolony formation. Supercolonies covered >2500 ha of the island between 2001 and 2003 (Chapter 4). I used eight sites for the majority of data collection in this study, all 50 m x 50 m plots (0.25 ha) in rainforest where all stems >5 cm dbh were mapped and identified (Fig. 2.1; P.T. Green unpublished data). The most common canopy and sub-canopy species occurring in the plots were *Barringtonia racemosa*, *Inocarpus fagifer*, *Pisonia umbellifera*, *Planchonella nitens* and *Syzygium*

nervosum (P.T. Green, unpublished data). Plots were in supercolonies at five sites (ECR, JAM, WCR, WBT & PUM) that had formed recently (<1.5 years old) while supercolonies at three other sites (WAD, VOD & WWB) were approximately 5 years old in 2000 (P.T. Green personal communication). All sites were characterised by the absence of red land crabs and a thick layer of leaf litter. The recently invaded sites were typically carpeted by small seedlings while the older sites had both small and large (~ 2 m tall) seedlings in the understorey.

Ant activity/density calibration

Traditional methods of determining ant abundance (e.g. pitfall trapping and quadrat counts) were impractical for use in *A. gracilipes* supercolonies due to such high densities of ants on the forest floor. Therefore, a rapid field assessment technique for quantifying relative activity of crazy ant foragers on the forest floor was developed. Ant activity was estimated by the number of *A. gracilipes* crossing a 10 cm x 10 cm laminated white card on the ground in 30 seconds (referred to hereafter as 'ants/30 secs'). However, this measure of 'activity' could not be used as a surrogate for ant density unless it consistently correlated with actual density of ants on the forest floor.

To establish whether ant activity was a suitable surrogate for ant density, I made 63 ant activity counts across four different *A. gracilipes* supercolonies near the 0.25 ha plots at each site (9 at WAD, 17 at ECR, 9 at WBT & 28 at WWB). At each point I placed a laminated white card on the ground in an area cleared of litter (diameter 30 cm) and immediately began counting ants using a hand-held counter. Immediately after the count was finished, I placed a circular frame (0.19 m²) over the area and used a HomeLite™ garden vacuum to collect all foraging ants from within the frame. Activity counts spanned the full range of ant activity (0 – 145 ants/30 secs). I limited the calibration exercise to counts up to and including 145 ants/30 secs as I was unable to maintain accuracy in counting at higher activity levels than this. I used linear regression to quantify the relationship between ant activity and actual on ground ant density.

Ant density, biomass and nest entrances in supercolonies

The density (ants/m²) of *A. gracilipes* workers on the forest floor in supercolonies on Christmas Island was estimated by using the regression equation generated in the calibration exercise. The biomass of ants on the forest floor was calculated by multiplying the density by the mean individual weight of an unfed worker ant. For these calculations I collected worker ants with the HomeLite™ garden vacuum, whose gasters were not distended in any way (my subjective sorting) and for my purposes were 'unfed'. Because worker ants are extremely light, I weighed six pooled collections of ants, ranging from 20 - 104 individual workers each, and calculated the overall mean mass of an individual from the average worker mass in each of the six collections.

The density of nests and their microhabitat distribution were determined by concentrations of workers streaming out of a myriad of holes and cracks in the ground, from under logs, rocks and bases of trees. Queens were often found upon excavation of these sites, along with brood. I termed these 'nest entrances', but it is likely that many of these entrances were interconnected either below the surface of the ground or within leaf litter and that each one did not constitute a discrete nest. The excavation of a complete nest was almost impossible, so consequently I quantified entrance density. Entrance density was estimated by counting the number of entrances at three locations on the ground (holes in ground, under rocks/logs, base of a tree/root) and the empty holes in the ground that were potential nesting sites. I did this within five 3 m x 3 m quadrats placed randomly near the 0.25 ha plots (but outside their boundaries) at four sites (ECR, WAD, WWB & WBT). The quadrats were not placed inside the 0.25 ha plots to minimise disturbance for future data collection within the plots. In addition to the quadrats, the presence of nest entrances at the base of trees (dbh >5 cm) in five entire 0.25 ha plots (PUM, JAM, VOD, WWB & WAD) was recorded.

From the quadrat data, I was able to calculate the mean density of nest entrances in use by *A. gracilipes* per site, and also the percentage of holes in the ground utilized by *A. gracilipes* as nesting sites. I used a two-way ANOVA to investigate the effect of site and nest entrance location on the density of nest entrances. From the observations of *A. gracilipes* nests at the base of trees in the 0.25 ha plots, I determined the mean proportion of large rainforest trees that harbour *A. gracilipes* nests.

Ant activity on the forest floor and tree trunks

I recorded diel ant activity on the forest floor every hour for 24 hours on two occasions at two different sites: 21-22 February 2001 at ECR (wet season) and 23-24 August 2001 at WBT (dry season). Each hour, 20 activity counts were made at random locations within the 0.25 ha plots and a mean of those counts was used as the ant activity index for that hour. Activity of *A. gracilipes* on tree trunks was also recorded at WBT in August 2001. Eleven trunks (4 x *Inocarpus fagifera*, 2 x *Dysoxylum gaudichaudianum*, 2 x *Barringtonia racemosa*, 2 x *Celtis timorensis* and 1 x *Ligustrum glomeratum*) >10 cm dbh were chosen and a 10 cm-wide band of white paint sprayed around the trunk at breast height (130 cm above ground). The number of ants in one trail crossing a 10 cm x 10 cm band was recorded for 30 seconds each hour. Relative humidity was measured hourly using a horsehair hygrometer at breast height at ECR, and air temperature at ground level was recorded hourly at WBT. The presence of queens on the surface of the ground was also recorded for 24 hours in August by five-minute visual searches every hour.

Seasonal ant activity was recorded weekly for 21 months commencing on 6 February 2001. Ant activity was recorded in 20 random locations within 0.25 ha plots at ECR and WAD each week. WAD was replaced with WBT in August 2002 after activity at WAD declined below supercolony levels.

Intraspecific aggression

Workers, queens and brood were collected from in situ *A. gracilipes* nests in supercolonies and from isolated nests using a Homelite™ garden vacuum. They were transferred into deep buckets in the lab, and fed with 30% sugar solution. I measured intraspecific aggression with individual workers from within the same supercolony, between supercolonies, between isolated nests, and between isolated nests and supercolonies. In most cases, assays were conducted between 12 and 48 hours after collection from the field. I carried out 10 - 14 trials for each of 13 combinations of nest location. Pairs of ants were placed in flouon-coated 80 ml vials, and the highest level of aggression between the two ants during the interaction was recorded after 10 minutes. I used the mean of these trials for all statistical comparisons.

There are commonly 5 to 7 levels of aggression that are used to assess responses from unfamiliar non-nestmate workers (Hölldobler & Wilson 1990). The assay used here was modified slightly from standard procedure (Holway *et al.* 1998). Interactions were scored as: 0 = *Tolerance*, partner grooming or huddling and exchanging food; 1 = *Antennation*, initial antennation then tolerance; 2 = *Intense antennation* (investigation), rapid mutual antennation; 3 = *Avoidance or flight*, dorsal flexion for chemical defense; 4 = *Aggression*, biting or pulling legs and antennae, repeated back and forth jerking; 5 = *Fight*, prolonged aggression, sparring, charging, biting and chemical defense. Levels 0 - 2 are considered non-aggressive behaviour, and 3 - 5 aggressive (Hölldobler & Wilson 1990). I constructed a frequency histogram to depict the occurrence of aggression in the assays. In addition, I used linear regression to investigate the relationship between the mean level of aggression and distance between nests (Suarez *et al.* 1999).

Reproductive Phenology

I monitored brood production of *A. gracilipes* in six 0.25 ha plots (ECR, JAM, WCR, WBT, PUM & WAD), five 'new' and one 'old' supercolony, respectively, from December 2000 to December 2002. Crazy ants readily colonise artificial domiciles that

can be harvested from the field for observation (Van der Goot 1916; Baker 1976). In each plot, 60 black polypipes (200 mm long, 40 mm diameter), capped at one end and with leaf litter and small amounts of soil inside, were placed on the ground at random grid locations (total grid size 50 m x 50 m, grid points located at 5 m intervals). After two months in the field, I collected five pipes per month from each plot (total of 30 pipes each month) and replaced each collected pipe with a newly marked pipe so that available artificial nest density remained constant. Collected pipes were returned to the lab and placed in the freezer to kill the contents. The following nine life stages were counted: egg masses, queen larvae, other larvae, queen pupae, other pupae, workers, males, dealate queens & alate queens. Monitoring brood production in this manner was based around the assumption that the colonies that establish in them are complete (see Van der Goot 1916). This is the result of a transfer of all brood from colony fragments into the protective environment provided by the pipes. This implies that the composition of the pipe colonies is representative of the more fragmented natural colonies. The only component not accurately represented at the time of sampling is workers (Baker 1976). Rainfall was recorded at Christmas Island airport at 261 m ASL.

The presence of winged queens and males in the canopy may indicate mating flights, the timing and duration of dispersal events, or canopy nest sites. Aerial dispersal of winged stages of *A. gracilipes* was investigated by the use of one canopy malaise trap at each of ECR and WBT on 19 October 2001, prior to the onset of rain on 2 December 2002. Traps were checked weekly until 15 February 2002.

Food Preference Assay

Commencing 15 February 2001, *A. gracilipes* preference for carbohydrate (fig jam) or protein (Black & Gold™ tuna/sardine cat food) was determined in the same 0.25 ha plots that ant activity was recorded (ECR and WAD, then WBT). Five bait stations were placed in each plot in the same position, weekly, for two years. A bait station consisted of a flat plastic feeding platform (10 cm x 8 cm) with a steel peg inserted

through the middle as a stand. A sticky band of Tanglefoot[®] was placed on the peg underneath the platform to restrict access to the feeding platform from the ground, and one teaspoon (approximately 5 g) each of cat food and jam were placed either side of the central peg. A plastic ramp was attached to provide a single access point from which foraging ants climbing onto the platform made a choice between food types. Bait stations were left out for 30 minutes. After that time, the number of *A. gracilipes* at each bait type was recorded. Rain reduced the numbers of ants that were present at the bait, and during heavy rains either baits were washed off the stations or ants did not recruit to the baits at all. Data was not recorded on these occasions. Relative abundance of ants at the two bait types was used to indicate food preference, the assumption being that the more ants recruited, the higher the preference for that food type.

On each occasion preference was determined, I did a one-way ANOVA was used to test for differences in the number of ants recruiting to stations. Bait type the main factor and the number of ants the dependent variable. I present the results of food preference here as a graph showing the ratio of high:low number of ants on each food type.

Results

Ant activity/density calibration

Ant activity was a good representation of actual ant density on the forest floor. There was a positive and significant relationship between ant activity and ant density (Fig. 2.2; $F_{1,51} = 444.291$, $r^2 = 0.897$, $p < 0.001$) where ant density on the ground could be estimated by substituting activity counts for x in the equation: $y = 15.694 x - 21.612$.

Ant density, biomass and nest entrances in supercolonies

The density of *A. gracilipes* workers on the forest floor was extremely high. Using the seasonal activity counts at ECR, WAD and WBT (see below), the mean ant

activity count at any site varied between 6 and 145 ants/30 secs, the equivalent of about 72 ants/m² to 2254 ants/m² (mean 948.7 ± 31.7 SE ants/m²; 0.7 million – 22.5 million ants on the forest floor per hectare). However, the highest density actually measured using the Homelite™ garden vacuum was 7383 ants/m². Foraging *A. gracilipes* workers were by far the most conspicuous organism on any surface in supercolony areas. However, ant activity (and therefore ant density) varied greatly within supercolonies on Christmas Island.

Despite the density of workers being so high, their biomass was comparably low. Unfed ants weighed an average of 0.8214 ± 0.2986 mg. Consequently, the mean biomass of unfed crazy ants on the forest floor was 948.7 x 0.0008214 = 0.779 g/m², and ranged between 0.059 g/m² and 1.851 g/m². Even at the highest densities (and assuming all ants are unfed), the biomass of crazy ants on the forest floor was 7383 x 0.0008214 = 6.064 g/m².

There were three nest entrance locations that were quantifiable on a relatively small scale: holes and cracks in the ground, under logs/rocks and at the base of a tree or near its root system. Ants used holes in the ground for nesting more than any other type, but they only used 72% of available holes in the ground for nesting. The mean density of *A. gracilipes* nest entrances in supercolonies varied significantly between nest types (Table 2.1; F_{3,12}=4.244; p=0.029), but not between sites (Table 2.1; F_{3,12}=0.518, p=0.678). The density of nest entrances was highest at ECR (10.5 nests/m²) and lowest at WAD (2.7 nests/m²). Over 90% (640 of 702) of trees (dbh > 5 cm) observed in 0.25 ha plots had one or more crazy ant nest entrances at their base. Nests were also common inside large fallen and rotting tree trunks, but I did not encounter any within the quadrats in this study. Nests were often found upon felling a dead or dying tree. I uncovered aggregations of workers, pupae and sometimes queens just under leaf litter on the surface of the ground, but it was most likely that these were

temporary shelters and not established nest sites because of the unstable nature of the leaf layer.

Ant activity on the forest floor and tree trunks

Foraging by *A. gracilipes* workers was continuous on every available surface 24 hours a day (Fig. 2.3 & Fig. 2.5). In a 24-hour period at ECR in February 2001, ant activity varied between 13.6 ± 4.3 and 52.2 ± 8.8 ants (mean \pm SE) on the card per 30 seconds. There was a suggestion of two peaks of ant activity, one from mid-morning to mid-afternoon (1130 h and 1500 h) and one in the middle of the night (2200 h and 0200 h). Ant activity was consistently higher at ECR in February than at WBT in August 2001, where ant activity varied between 92.3 ± 8.4 and 135.7 ± 3.4 . However, there was no clear pattern in ant activity over the 24-hour period. Dealate queens were observed walking on the forest floor or on tree trunks and roots during the night only at WBT in August (shaded region, Fig. 2.3).

There was no relationship between activity levels on the forest floor and relative humidity at ECR (Fig. 2.4A; $F_{1,20}=0.921$, $r^2=0.044$, $p=0.349$), or between ant activity on the forest floor and air temperature at ground level at WBT (Fig. 2.4B; $F_{1,17}=3.890$, $r^2=0.186$, $p=0.065$). However, during and immediately after moderate to heavy rains ant activity was noticeably reduced. Crazy ants streamed down tree trunks toward the ground in response to the onset of heavy rain.

Foraging ants were continuously active on tree trunks, in single or multiple trails on larger trees, and haphazardly on all understorey vegetation, but consistent with ant activity on the forest floor, there was no apparent diel pattern (Fig. 2.5A). Ant activity on trunks of *Inocarpus fagifer* was sometimes higher than other species, varying between 29.0 ± 9.3 and 75.3 ± 18.8 ants (mean \pm SE) across a 10 cm x 10 cm section of the trunk, whereas activity was lower on *Barringtonia racemosa*, varying between 4.5 ± 0.5 and 30.5 ± 7.5 ants across a 10 cm x 10 cm section of the trunk (Fig. 2.5B).

Activity of *A. gracilipes* on the forest floor at the older WAD site (approx. 5 yrs old) and new ECR site (<1.5 yrs old) showed distinctively different patterns over an 18-month period. No seasonal pattern in activity occurred at either site. Initial ant activity levels were similar between sites, but diverged after 4 - 5 months of observation. Activity at ECR increased from approximately 45 ants/30 secs to >120 ants/30 secs whereas at this point activity at WAD dropped markedly and maintained levels of activity below what constituted a supercolony for the remainder of the observation period (15 months; Fig. 2.6). Red crabs began to re-establish in the area in early 2002. Ant activity at ECR continued to increase until monitoring ceased in August 2002. Between February 2001 and July 2001, there was no significant relationship between weekly ant activity counts and relative humidity at either ECR ($F_{1,21}=0.173$, $r^2=0.009$, $p=0.683$) or WAD ($F_{1,20}=0.047$, $r^2=0.003$, $p=0.831$).

Intraspecific aggression

Aggression between conspecifics of *A. gracilipes* was not apparent in the field, and in aggression levels in laboratory assays were low in the majority of cases. Even ants from isolated nests (refer Fig. 2.1 for location of isolated nests) did not display any form of intraspecific aggression. Aggression level 1 occurred the most frequently in a total of 136 pair-wise interactions between individual workers (Fig. 2.7). However, I observed 21 aggressive interactions (level 3 and above) between individual workers, and of these eight (6%) involved prolonged fighting. Nevertheless, when these scores were averaged for the nest/supercolony combination, only one pairing showed a mean aggression level of above three (VOD vs WCR; Table 2.2). Furthermore, the ants involved in this aggressive interaction were part of the same larger supercolony and located approximately 1.8 km apart. There was a significant relationship between the individual pair-wise level of aggression and the distance of the nests or supercolonies each of the ants were drawn from ($F_{1,100}=32.905$, $r^2 = 0.248$, $p<0.001$), however, it was not in the expected direction. The relationship suggested that the closer the ants were to one another, the more aggressive they were in 10-minute aggression bioassays.

Reproductive phenology

Egg masses, workers, worker larvae and worker pupae of *A. gracilipes* were present in nests all year round, and adult workers consistently made up over 50% of nest contents (Fig. 2.8a-d). An increase in the number of workers, worker larvae and worker pupae was evident from April – June 2002 (dry season), just after the period of sexual production. In supercolonies, workers were often found in pipes with worker pupae only, and pupae were often found in hollows of litter, or leaves on the ground, covered by a large leaf, where workers tended them. I termed these collections of pupae 'pupae wells', and there was often over 50 pupae per well. The single sexual brood production event commenced just prior to the onset of the wet season, and there was no evidence of further production of queens and males during the year (Fig. 2.8e-h). In all sites, males were detected in natural nests and in pipes in October, but their numbers peaked in November with 35 ± 12 males per pipe (range 3-70, n=6). Production of males was followed by the presence of queen larvae, queen pupae and alate (winged) queens. Although dealate (unwinged) queens were found in all nests throughout the year, their numbers peaked at the end of the 2002 wet season (Fig. 2.8h; mean 29 ± 16 queens per pipe; range 0-98, n=6). Males were not seen in any supercolonies from March through to August (except one male at WBT in April 2002). Single alate queens were found infrequently in nests throughout the year, but it was not determined if these queens had newly emerged or were producing brood.

Alate queens and males were caught in malaise traps >15 m above the ground at both ECR (3 alate queens) and WBT (57 queens and 37 males) at the onset of rains on 18 January and 25 January 2002, respectively.

Food Preference Assay

In supercolonies, *A. gracilipes* workers were on baits almost instantaneously, and generally ants swarmed over the bait stations during placement of them on the ground. The number of ants recruited to carbohydrate baits over a 30 min period ranged from 3.4 ± 2.4 to 84.4 ± 10.5 at WAD, 0.8 ± 0.8 to 101.0 ± 10.4 at ECR and 2.4

± 1.0 to 24.4 ± 5.2 at WBT (mean of 4 or 5 bait stations \pm SE). The number of ants recruited to protein ranged from 0.2 ± 0.2 to 64.4 ± 7.6 at WAD, 0.4 ± 0.4 to 90.6 ± 7.7 at ECR and 2.4 ± 1.6 to 64.8 ± 13.2 at WBT (mean of 4 or 5 bait stations \pm SE).

For the majority of the year (82% of observations) there was no distinct preference for either carbohydrates or protein baits. However, significantly more foraging crazy ants recruited to carbohydrate baits on occasions between October and March, and to protein baits on occasions between March and September in 2001 and April and October 2002 (Fig. 2.9; Table 2.3). Opposite to what was expected, preference for carbohydrates was during some times in the wet season, when sexual brood is produced, and for protein on occasions during the dry season, when there is typically no production of sexual brood.

Discussion

The densities of ground foraging *A. gracilipes* workers in supercolonies on Christmas Island (>20 million ants/ha) are, to my knowledge, the highest density populations of any ant species recorded (Brian 1965; Higashi & Yamauchi 1979; Higashi 1983). These densities were sustained on the ground in individual supercolonies of over 500 hectares, 24 hours a day, 365 days a year and this level of activity was sustained in some instances for up to 6 years (P.T. Green & D.J. O'Dowd personal communication). *A. gracilipes* workers swarmed over everything that entered a supercolony, including crabs, birds and people. Given that in September 2002 supercolonies covered 2378 hectares of Christmas Island rainforest (Green *et al.* 2004), the total mean biomass of crazy ants on the forest floor in supercolonies at that time could possibly have reached over 1600 kg (>16 tonnes).

However, the activity, density and biomass described here are of worker ants in supercolonies on the forest floor only, and represent a massive underestimate of the total population. Vast numbers of workers remained in nests in the ground, trailing into, and in the rainforest canopy, and were not included in the density estimate. When

disturbed, crazy ants displayed erratic, frantic behaviour in a radius often up to 2 m from the disturbance point. Formic acid could be detected from about 50 cm above the forest floor upon continuous disturbance, and foragers would boil up to your waist within 30 seconds in high-density supercolonies.

In the Seychelles, estimates based on maximum forager abundance, nest densities, quadrats and subjective assessments yielded predictions of *A. gracilipes* populations (excluding brood) of 5 million ants/ha in areas of high nest density and up to 10 million ants/ha in areas of maximum abundance (Haines & Haines 1978a). This prediction was also probably an underestimate as no account was taken of foragers and nests in vegetation. Nonetheless, the estimates were based on maximum numbers of foragers and ants in nests (upper 95% limits), unlike this study, where I used means to estimate ant densities. Based on maximum forager activity, the density of *A. gracilipes* workers on the forest floor alone could reach over 70 million ants/ha on Christmas Island. Further work in the Seychelles, on Bird Island, found a mean of 4658 ± 825 individual ants in pitfall traps over three nights (Hill *et al.* 2003), and in urban areas of the Tokelau Islands and Apia, Samoa, a mean of 997 ± 293 (range 57-2040) and 661 ± 115 SE (range 191-1060) respectively, were caught in pitfall traps in only 24 h (Lester & Tavite In Press). Indications are that *A. gracilipes* has the capacity to reach extremely high densities on tropical oceanic islands.

Comparisons of ant, nest and colony density among invasive species, and with native species, are rare in the literature, and difficult to make. This is due largely to variation in colony structure. Six of the most notorious invasive ant species (*Anoplolepis gracilipes*, *Linepithema humile*, *Pheidole megacephala*, *Solenopsis invicta* (polygyne form), *Solenopsis geminata* and *Wasmannia auropunctata*) display unicoloniality in their introduced range (Haines & Haines 1978a; Obin & Vander Meer 1989; Hoffmann *et al.* 1999; Giraud *et al.* 2002), where the polygynous forms (and monogynous form for *S. invicta*) reach high local abundances. However, the failure for invasive ant species to maintain an identifiable and exclusive colony structure throughout their geographical distribution makes it often impossible even to compare

ant, nest and colony densities within a species itself. For example, colony density and biomass of the red imported fire ant, *S. invicta*, can be estimated from mound number and mound volume (Porter *et al.* 1992; Tschinkel *et al.* 1995), but data on a spatially discrete colony can give a misleading picture of population dynamics of the species as a whole.

Numerical advantages have allowed *A. gracilipes* to dominate the Christmas Island ecosystem. Three main hypotheses have been proposed as an explanation for the disproportionate abundance of invasive ants in their introduced range. First, the enemy release hypothesis predicts that escape from coevolved natural enemies and competitors may help [organisms] achieve large colony sizes and increased colony densities as a consequence (Buren 1983; Williamson 1996; Crawley 1997). Second, reduced intraspecific aggression and subsequent allocation of energy and workers to tasks other than colony defence may assist in the creation of unicolonial populations and formation of supercolonies (Holway *et al.* 1998; Tsutsui *et al.* 2000). Third, the increased efficiency compared to native species, with which invasive ants exploit resources (mainly food resources) may lead to their high abundance. It appears that these three mechanisms operate simultaneously in generating the abundance of *A. gracilipes* on Christmas Island.

Because crazy ants are introduced, there are no coevolved natural enemies, and the only predator of *A. gracilipes* I observed anywhere in three years was the Christmas Island thrush (*Turdus poliocephalus erythropleurus*) and white eye (*Zosterops natalis*) that removed individual ants from their bodies and nearby stems whilst perching in supercolonies. Nothing is known about natural enemies in its native range; however, its native range has never been defined.

Intraspecific aggression was virtually non-existent in the field and very low in laboratory assays within and between supercolonies on Christmas Island. The only aggressive pair-wise interaction was between ants belonging to the same supercolony, albeit 1.8 km apart, which suggests that within large supercolonies there may be morphological or chemical variation of individual workers to elicit aggression (Tsutsui

et al. 2001; Giraud *et al.* 2002). Smaller, perhaps genetically distinct supercolonies may have fused to form the larger supercolonies observed between 2000 and 2003. An explanation for this could be multiple introductions of *A. gracilipes* over time, resulting in genetically distinct populations that successfully dispersed across the island. However, low levels of intraspecific aggression between workers from distant supercolonies suggest that this was not the case, and that genetic variability among *A. gracilipes* on Christmas Island is likely to be low. They cooperate in finding food and are likely to tend each other's broods instead of allocating their energy to aggression (Tsutsui *et al.* 2000). Similarly, Fluker and Beardsley (1970) concluded that compared with the big-headed ant (*Pheidole megacephala*) and Argentine ants (*Linepithema humile*), *A. gracilipes* was the least aggressive ant in Hawaii. But the toxic spray from its abdomen provided a more effective defense than fighting in the other two species.

Fights are common during intraspecific interactions in ants (Crowell 1968; Gordon & Kulig 1996; Adams 1998). However, the lack of intraspecific aggression is a key feature of invasive, unicolonial ants and affords them the advantage of allocating resources, usually spent on defending territories, to growth, maintenance and reproduction (Tsutsui *et al.* 2001). Argentine ants in non-aggressive colony pairs produce more than three times the amount of brood and support twice the number of workers than aggressive colony pairs (Holway *et al.* 1998). It has also been established that lack of genetic variation within unicolonial populations of invasive Argentine ants (*Linepithema humile*) in California has improved their abilities to compete with local ants for habitat and food resources.

At supercolony densities on Christmas Island, *A. gracilipes* have two major immediate and obvious impacts, both of which can be viewed as resource exploitation: the acquisition of protein from the red land crab, *Gecarcoidea natalis*, and the promotion of scale insect populations on vegetation within supercolonies from which they acquire carbohydrate-rich honeydew (O'Dowd *et al.* 2003). Rainforest on the island supports a mean biomass of red land crabs, *Gecarcoidea natalis*, of 114 g/m² (wet mass) (Green 1997). Even at an average density of 1554 ants/m², *A. gracilipes*

kill and eliminate red crabs from the rainforest. Therefore, 1.276 g/m² of *A. gracilipes* workers can overcome and kill approximately 114 g/m² crabs on Christmas Island, over 100 times their own biomass. Despite the pulse of protein at particular times, for example during their annual migration to the ocean for spawning (Hicks 1985), in the Seychelles high densities of *A. gracilipes* occur in the absence of land crabs or alternative abundant proteinaceous food source, and on Christmas Island supercolonies of *A. gracilipes* persist for years in the absence of land crabs and during times of low invertebrate abundance (Green *et al.* 1999).

Continuous activity of foraging workers with expanded and translucent gasters on all trunks of both understorey and canopy trees suggests continual sequestering of carbohydrates from honeydew-producing scale insects in the canopy, where densities of adults lac scale insects (Kerriidae) reach over 500 adult females/m twig (see Chapters 4 & 6) and constitute a stable carbohydrate source (Sundstrom 1993). Access to this abundant carbohydrate-rich food resource may help maintain the high foraging tempo of *A. gracilipes* in supercolonies, and the further monopolisation of resources (Davidson 1997; 1998). The utilisation of hemipteran honeydew has been implicated in the success of other invasive ant species (Helms & Vinson 2002; Helms & Vinson 2003) and the diversification and amplification of impacts caused by *A. gracilipes* on Christmas Island (O'Dowd *et al.* 2003)

Reproductive Phenology and Food Preference

The reproductive phenology of *A. gracilipes* on Christmas Island concurs with patterns observed in Indonesia (Van der Goot 1916), the Solomon Islands (Greenslade 1971; 1971b), Papua New Guinea (Baker 1976), the Seychelles (Haines & Haines 1978a) and in India (Rao & Veeresh 1991b) in that the production of sexual brood is dependent upon the onset of rains (but see Fig. 2.10 for detail). Despite alate queen being present (albeit in low numbers) throughout the year on Christmas Island, sexual brood was only produced over one period annually. Queen larvae and pupae were present in nests, in some cases, for up to two months preceding the first rains until mid-

late wet season. The wet season on Christmas Island usually occurs from December through to May, but during this study the wet seasons in both 2000-01 and 2001-02 commenced in January and were considered finished by April (2001 & 2002 respectively; Fig. 2.8). Male *A. gracilipes* appeared up to three months before the onset of rain, in October, coinciding with a shift in bait preference on some occasions from proteinaceous baits by workers in the field to carbohydrates. Queen brood (larvae and pupae) were produced soon after, in November, corresponding with the transition to a significant preference for carbohydrate baits on several occasions. Both alate and dealate queens were most abundant during the period where a preference for carbohydrates was recorded. Previous observations suggest there may be up to 360 queens in one *A. gracilipes* nest, but most nests observed in the Seychelles contained between 27 and 56 queens (based on 95% CI; Haines & Haines 1978). On Christmas Island, I observed upward of 1000 queens in one nest (approx. 1 cubic metre in volume). This may prove to be an exception to the rule, but "mega-nests" appear to be relatively common in supercolonies in/under large dead logs on the ground. If one inseminated *A. gracilipes* queen were able to found a nest alone, through colony budding or flight, overcrowding of queens in a "mega-nest" might trigger dispersal of inseminated queens and facilitate rapid spread of *A. gracilipes* supercolonies (see Chapter 3 for rate of spread).

Although bait preference was significant for carbohydrate or protein on only 19% of occasions, it is unclear if this pattern of bait preference was driven by colony demands or by the availability of a specific food type in the environment, since the abundance of invertebrates and other proteinaceous sources and brood phenology was not recorded simultaneously. Honeydew (carbohydrate) is necessary for the upkeep of workers, whereas protein is used for brood production and must also be supplied to the queens to sustain oviposition (Brian 1973). However, a preference for protein was shown during the driest months of 2001, when invertebrate abundance is at its lowest (Green *et al.* 1999), and sexual brood is not produced. This result is in direct contradiction to that of (Stein *et al.* 1990) study of the bait preference exhibited by the

red imported fire ant (*Solenopsis invicta*). Their study supported the hypothesis of greater recruitment to protein sources during periods of colony brood reproduction.

This difference may be due to *A. gracilipes* being able to flexibly change their foraging tactics according to changing resource abundance and distribution in order to optimize foraging efficiency or intake (Carroll & Janzen 1973; Hölldobler & Lumsden 1980; Traniello 1989; Hölldobler & Wilson 1990; Nonacs & Dill 1990). Several authors have inferred that the availability of food resources may have a substantial, if not the greatest, influence on *A. gracilipes* population size (Greenslade 1971; Baker 1976; Lewis *et al.* 1976; Haines & Haines 1978b; 1978a; Haines *et al.* 1994). Greenslade (1971a) found that rainfall increased the abundance of proteinaceous prey and decreased foraging by *A. gracilipes* in coconut palms harbouring honeydew producers, and he interpreted this as a shift from gathering honeydew to searching for prey in the ground layer. He concluded that this increased abundance in prey coupled with the increased taking of prey by *A. gracilipes*, was adequate to explain the seasonality of *A. gracilipes* in the Solomon Islands. The opposite was seen on Christmas Island: increased recruitment to a bait type was associated with its dearth in the environment. Just as the abundance of invertebrates is lowest during the dry season, the wet season may reduce the availability of honeydew to ants in the canopy. Rain can wash away honeydew before ants have an opportunity to remove it. Viewed in this way, the recruitment to bait types in this study may not be a preference as such, but a measure of *foraging effort*, a more qualitative behavioural trait that has been used to assess the availability of resources to ants (Kay 2002).

The abundance of a resource may often reveal little about its availability, that is, the amount available to a consumer (Kay 2002). If a food type is scarce in the environment, then an increased foraging effort may be required to obtain necessary amounts of it; more foragers are allocated to a particular food type when it is rare. Further to this idea, (Sundstrom 1993) demonstrated that red wood ants, *Formica truncorum*, considered an unpredictable protein resource qualitatively different from a stable sugar resource. Two separate groups of foragers recruited to the different

resources (baits), while a 'reserve' group of foragers contributed to higher recruitment rates at the two types of bait. Of course, further experimentation would elucidate this possibility for *A. gracilipes*. But it seems likely that with such high numbers of foragers available, it would be optimal for ants to be task-persistent for the type of resource they collect, saving time and energy on switching tasks. The relatively weak seasonal variation in carbohydrate:protein recruitment ratios in *A. gracilipes* indicates that, over time, it is most likely either an intrinsic requirement for a particular resource, or its availability in the environment that drives their foraging effort and recruitment to that resource.

Mating flights of *A. gracilipes* have not been previously documented (Rao *et al.* 1991, Haines *et al.* 1994), but Dammerman (1929) suspected that mating flights could occur. The simultaneous capture of relatively high numbers of alate queens and males on Christmas Island suggests that mating flights do occur at the onset of rains, the main advantage being an increased rate of spread. Alate queens were observed at and around fluorescent light sources (and generally lit areas) in settled areas for up to three nights following the first rains of the wet season in January 2001 and 2002 on Christmas Island, whereas no dealates were observed at the same light sources. At one light trap placed in the field for 3 hours in December 2001, no queens were caught, however, several were seen flying around the area. Furthermore, when a sticky Tanglefoot™ barrier was placed around the trunks of the Tahitian Chestnut, *Inocarpus fagifer*, dealate queens were captured above the barrier, indicating they were nesting in the canopy of the tree. If nests were abundant in the canopy, then an alate queen produced there may choose to fly to a new nesting site, rather than nest in the same tree, where sites may already be saturated.

Therefore, *A. gracilipes* is capable of both colony budding and colony fission, the later which is typically seen in monogynous ant species (Bourke & Franks 1995). In combination, colony budding and colony fission are advantageous, and may provide adaptive benefits, for several reasons. Colony budding results in colony buds that are close together in space, often founded by already mated queens. This can lead to

polydomous colonies (having multiple nest sites), increased kin recognition and cooperation, fusion of colony boundaries and ultimately the formation of supercolonies (Hölldobler & Wilson 1990; Passera 1994; Bourke & Franks 1995). In contrast, colony fission results in fully independent daughter colonies (Pamilo 1991), usually at greater distances than those produced by colony budding. This affords *A. gracilipes* greater dispersal powers, and the ability to disperse by either method may even allow them to switch between modes dependent upon colony status or environmental conditions.

The characteristics that contribute to the success of *A. gracilipes* are widespread among invasive ants species. This study has established the reproductive phenology of *A. gracilipes* on Christmas Island, and confirmed this dynamic is widespread in areas where *A. gracilipes* is found. It appears that polygyny, combined with low intraspecific aggression and a high reproductive rate (among other factors) has contributed to extraordinary densities of workers on the forest floor. *A. gracilipes* shows flexible nesting habits and dispersal abilities that may provide them with an adaptive advantage for the colonisation of, and proliferation in new habitats.

Anoplolepis gracilipes remains one of the most understudied invasive ant species. However, negative impacts associated with high densities of crazy ants have been felt on Indian and Pacific oceanic islands. This study contributes to the bank of ecological knowledge on *A. gracilipes*, but has also documented massive increases in local abundance on Christmas Island, which ultimately led to complete ecosystem disruption (O'Dowd *et al.* 2003). Given its continuing impacts in the Seychelles (Lewis *et al.* 1976, Haines & Haines 1978a, 1978b, Hill *et al.* 2003) and increasing association with honeydew-producing scale insects on tropical oceanic islands (O'Dowd *et al.* 2003, Lester & Tavite In Press), this species represents a threat to many tropical ecosystems. Its abundance and association with honeydew-producing insects should be monitored in tropical regions to help mitigate potentially detrimental effects to the host environment.

Table 2.1 - (a) Mean density of nests at different locations, and (b) percentage of rainforest trees (dbh >5 cm) in *A. gracilipes* supercolonies on Christmas Island, Indian Ocean. Nests at the base of trees censused in 0.25 ha plots, and all other nest types quantified using quadrat counts.

(a)						
Mean nest entrances/m ² (\pm SE; n=5)						
Nest entrance location	ECR	WAD	WWB	WBT	Mean	
Holes in ground	9.2 \pm 1.3	1.6 \pm 0.2	1.7 \pm 0.3	5.1 \pm 1.6	4.4 \pm 1.8	
Under log/rock	0.3 \pm 0.2	0.8 \pm 0.2	0.6 \pm 0.3	0.6 \pm 0.1	0.6 \pm 0.1	
Base of tree/root (dbh <5 cm)	0.9 \pm 0.2	0.3 \pm 0.1	0.6 \pm 0.1	0.5 \pm 0.2	0.6 \pm 0.1	
Total used nests	10.5 \pm 1.5	2.7 \pm 0.1	2.9 \pm 0.4	6.2 \pm 1.7	5.6 \pm 1.8	
Empty hole in ground	0.9 \pm 0.3	1.1 \pm 0.5	1.3 \pm 0.3	1.2 \pm 0.4	1.1 \pm 0.1	
% holes in use	89% (466)	60% (124)	57% (138)	81% (285)	72% \pm 7.8	

(b)						
Percentage of trees in 0.25 ha with nest entrances						
	PUM	WAD	WWB	JAM	VOD	Mean %
Base of trees (dbh >5 cm)	90% (174)	91% (92)	95% (80)	93% (227)	86% (129)	91% \pm 1.5

Table 2.2 – Mean intraspecific aggression scores from within isolated nests and supercolonies, between isolated nests and supercolonies, and between supercolonies. Numbers in parentheses represent the distance between the two collection sites. Asterisk indicates that the two collection points are located within the same supercolony.

	IN ^a 1	IN 2	WAD	SH	WCR	WBT	WWB	HV	VOD
IN 1									
IN 2	2.4 ± 0.2 (7.4)	1.0 ± 0.0 (0.0)							
WAD									
SH			1.5 ± 0.1 (11.3)						
WCR					1.0 ± 0.2 (0.0)				
WBT					2.4 ± 0.3 (1.0)	0.8 ± 0.4 (0.0)			
WWB	1.5 ± 0.2 (7.0)								
HV			0.8 ± 0.2 (7.3)	0.5 ± 0.2 (8.1)					
VOD		1.2 ± 0.1 (5.5)			3.3 ± 0.3 (1.8*)	2.4 ± 0.4 (0.8)			1.1 ± 0.1 (0.0)

^a - IN: isolated nest.

Table 2.3 (following page) – One-way ANOVA results for the test of bait preference at each site separately. The main factor was food type and the number of ants recruited to that type of food after 30 mins the dependent variable. Only significant results presented for clarity. The discrepancy in the degrees of freedom is due to using either 4 or 5 bait stations on each occasion.

Date/Site	Source	SS	df	MS	F-ratio	p-value
17.8.2001						
ECR	Bait	2664.500	1	2664.500	31.501	0.001
	Error	507.500	6	84.583		
30.8.2001						
ECR	Bait	3570.125	1	3570.125	20.290	0.004
	Error	1055.750	6	175.958		
WAD	Bait	1378.125	1	1378.125	34.779	0.001
	Error	237.750	6	39.625		
16.11.2001						
WAD	Bait	2016.125	1	2016.125	26.718	0.002
	Error	452.750	6	75.458		
23.11.2001						
WAD	Bait	231.125	1	231.125	7.121	0.037
	Error	194.750	6	32.458		
30.11.2001						
ECR	Bait	1512.500	1	1512.500	17.621	0.006
	Error	515.000	6	85.833		
8.2.2002						
ECR	Bait	9453.125	1	9453.125	30.190	0.002
	Error	1878.750	6	313.125		
19.4.2002						
ECR	Bait	7452.900	1	7452.900	58.022	<0.001
	Error	1027.600	8	128.450		
24.6.2002						
ECR	Bait	1849.600	1	1849.600	9.851	0.014
	Error	1502.000	8	187.750		
1.7.2002						
ECR	Bait	4494.400	1	4494.400	20.341	0.002
	Error	1767.600	8	220.950		
15.7.2002						
ECR	Bait	1060.900	1	1060.900	15.210	0.005
	Error	558.000	8	69.750		
19.8.2002						
WBT	Bait	1488.400	1	1488.400	9.188	0.016
	Error	1296.000	8	162.000		
2.9.2002						
ECR	Bait	2371.600	1	2371.600	23.992	0.001
	Error	790.800	8	98.850		
WBT	Bait	2280.100	1	2280.100	7.786	0.024
	Error	2342.800	8	292.850		
9.9.2002						
WBT	Bait	1984.500	1	1984.500	13.416	0.011
	Error	887.500	6	147.917		
30.9.2002						
ECR	Bait	3042.000	1	3042.000	24.287	0.003
	Error	751.500	6	125.250		
28.10.2002						
ECR	Bait	4536.900	1	4536.900	17.558	0.003
	Error	2067.200	8	258.400		
5.11.2002						
ECR	Bait	3240.000	1	3240.000	8.400	0.020
	Error	3085.600	8	385.700		

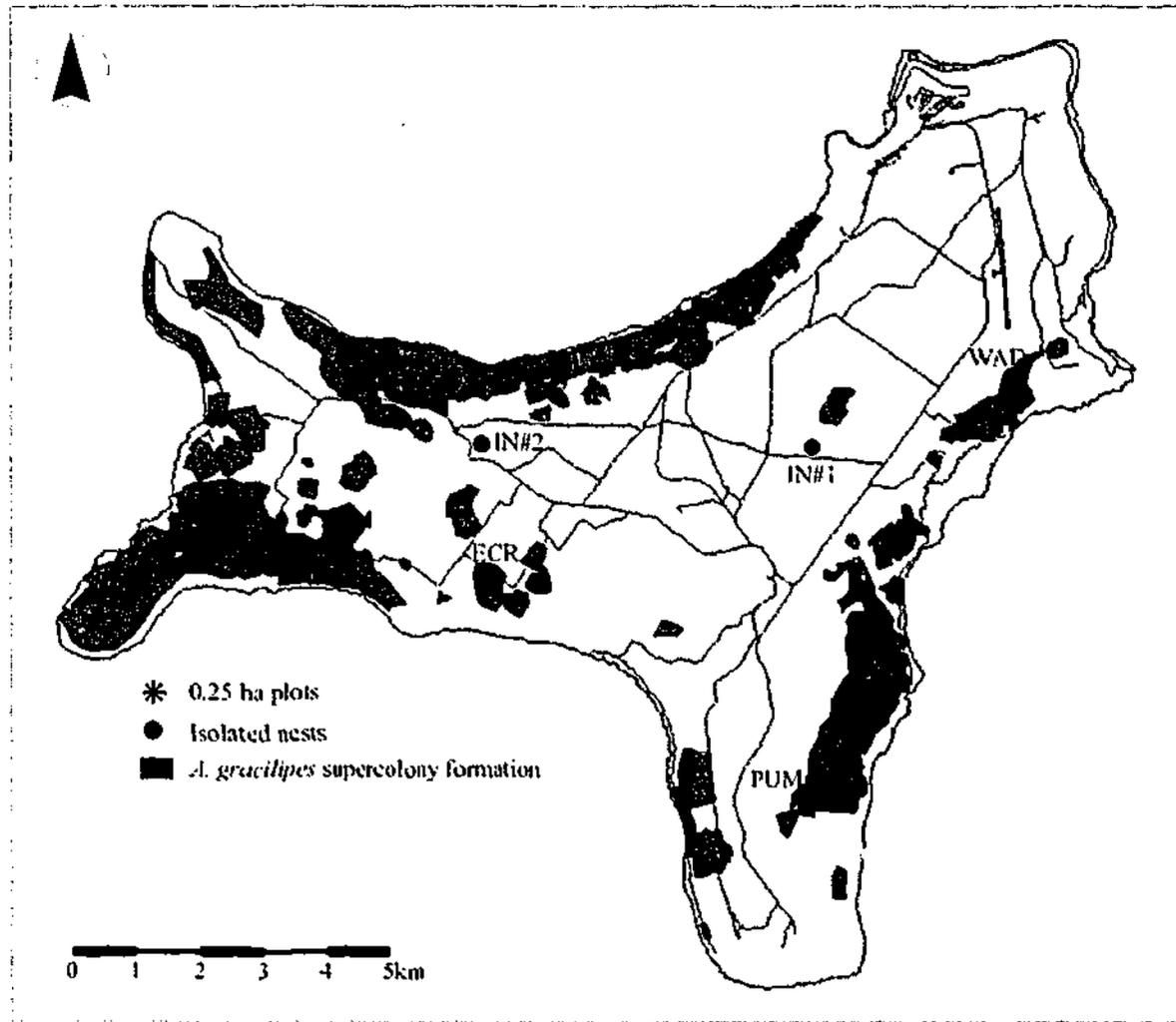


Figure 2.1 – Location of 0.25 ha *A. gracilipes* supercolony sites (ECR, WAD, PUM, JAM, WCR, VOD & WBT) referred to in this chapter, as well as isolated nests (IN#1 & IN#2) used in intraspecific aggression assays. WAD, WWB & VOD were approximately 5 years old in 2000, and all others approximately 1.5 years old.

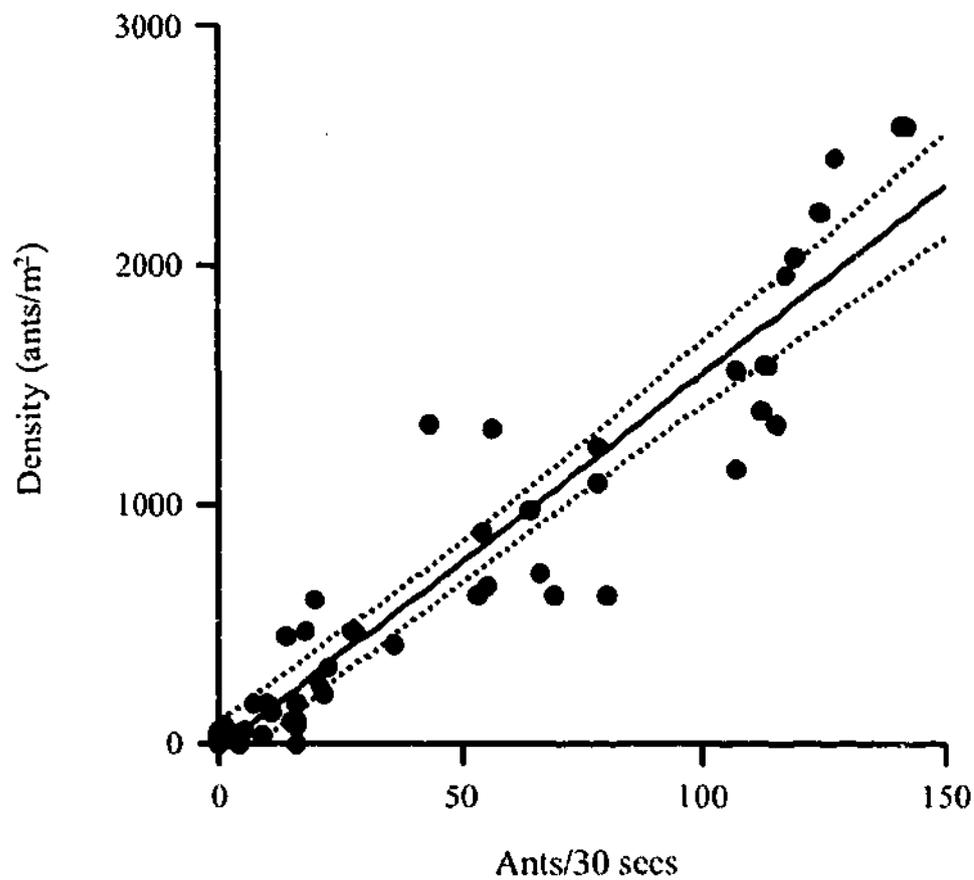


Figure 2.2 – Density of ground foraging crazy ants in relation to ant activity on cards, as explained by the number of ants crossing a 10 cm x 10 cm white card in 30 seconds (ant activity counts). Points represent individual activity counts and D-vac suction samples taken across a range of ant densities (within and outside supercolonies). Equation of the line: $y = 15.694x - 21.612$ and dotted lines are 95% confidence intervals.

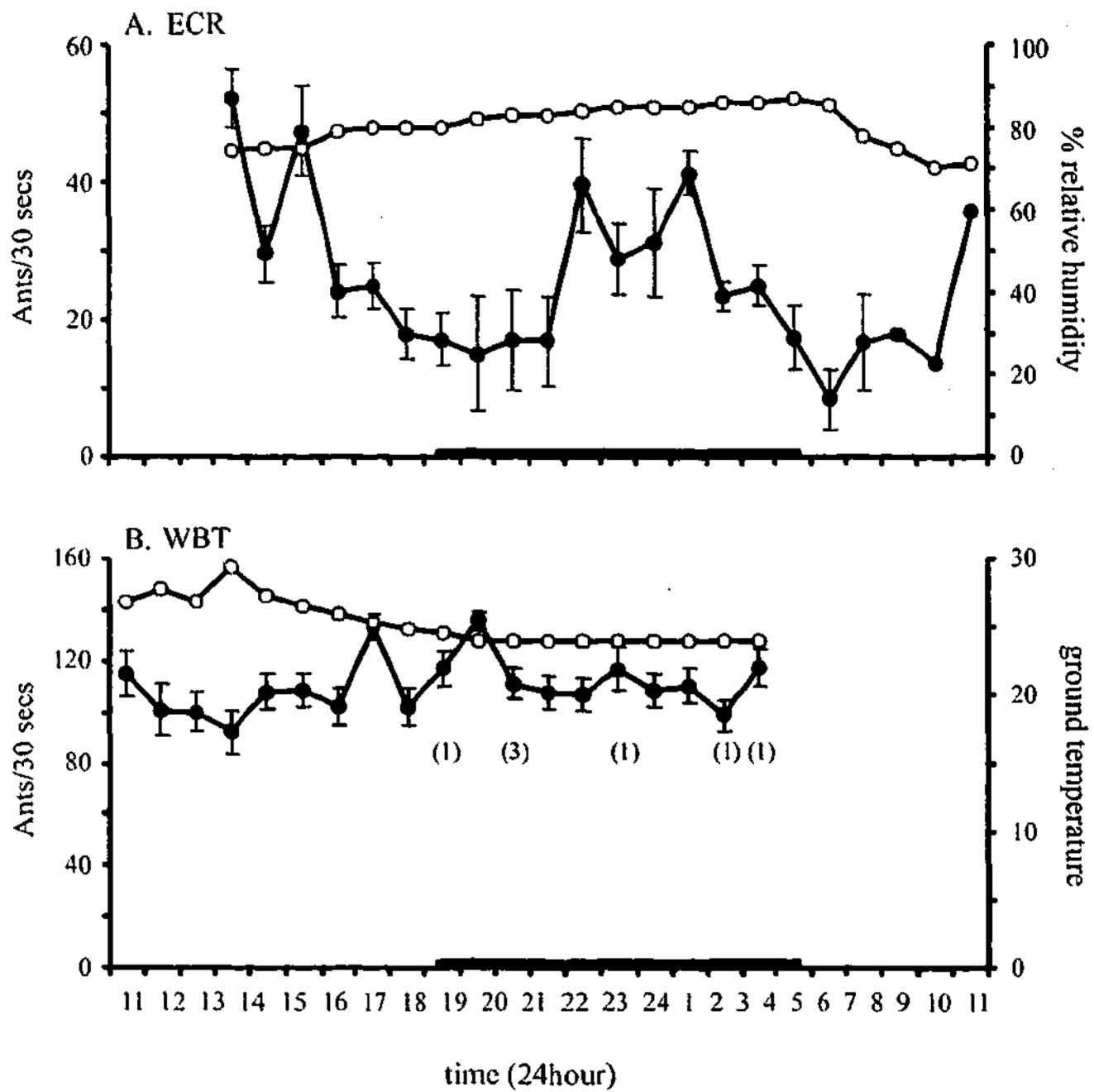


Figure 2.3 – Diel activity of ground foraging workers at (a) ECR, 21-22 February 2001 (24 hours), and (b) WBT, 23-24 August 2001 (18 hours). Each point represents a mean of 20 random counts in a 0.25 ha plot every hour (\pm SE). ●: ant activity; ○: A. %RH, B. ground temperature ($^{\circ}$ C). The heavy black bar on the x-axis represents nighttime. Numbers in parentheses indicate the number of dealate queens observed walking in the open in 5-minute searches each hour.

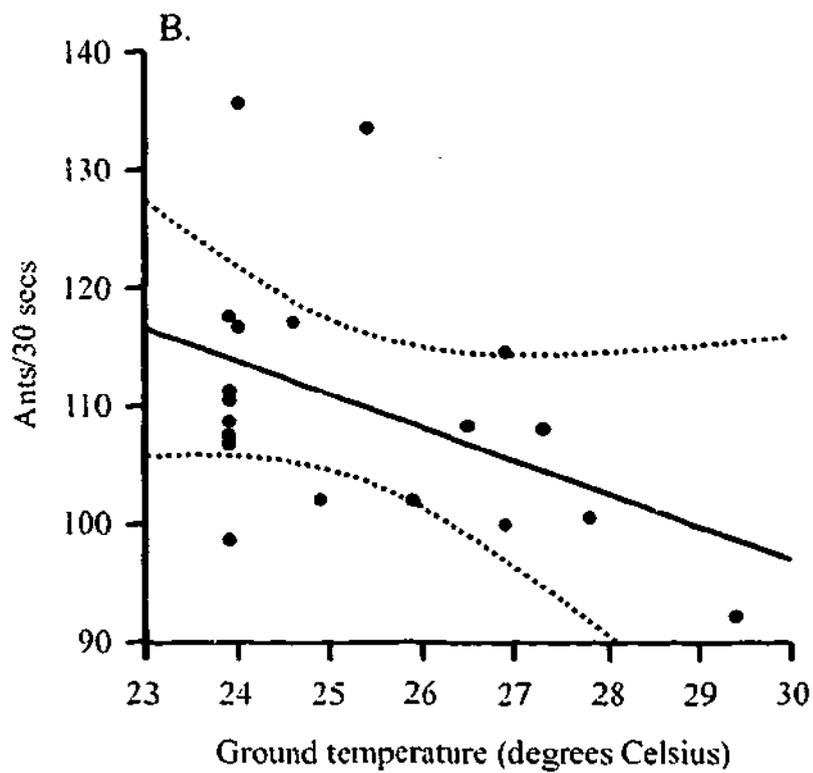
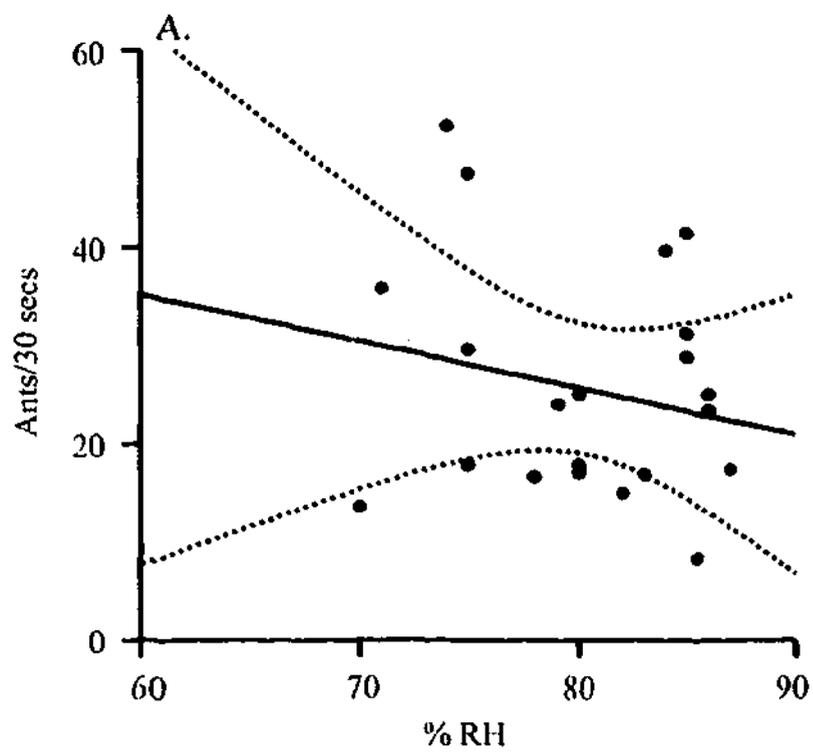


Figure 2.4 - Relationship between ant activity levels and (a) %RH at ECR in February 2001, and ant activity and (b) ground temperature at WBT in August 2001. Each point represents an individual %RH or ground temperature reading associated with the mean ant activity of 20 activity counts at the site on the hour.

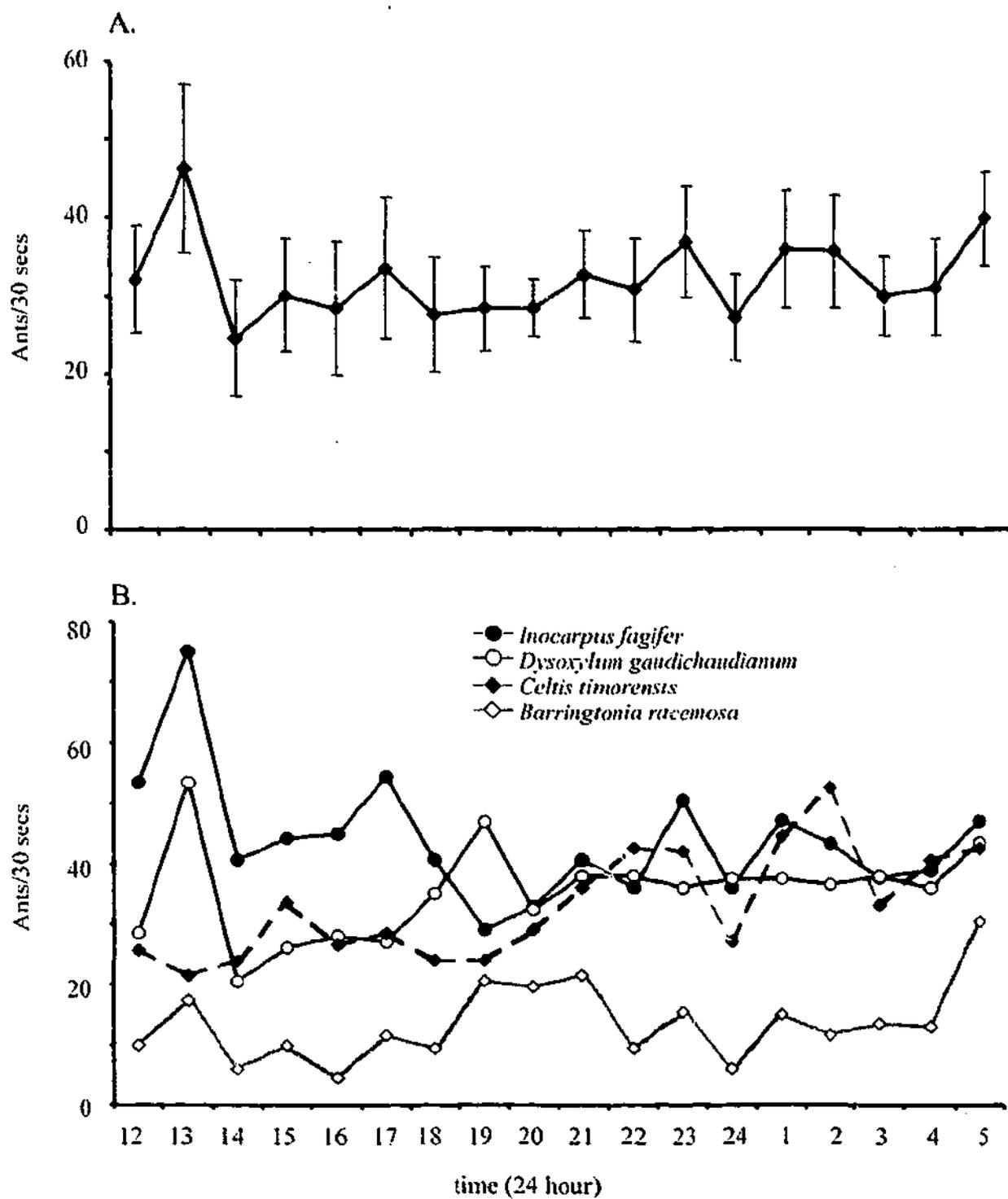


Figure 2.5 – Activity of trunk foraging workers at WBT, 23-24 August 2001 (18 hours). (A.) all tree species combined, where each point represents a mean activity from 11 individual trees (\pm SE), and (B.) species separated. *Inocarpus fagifer*, *Dysoxylum gaudichaudianum*, *Celtis timorensis* and *Barringtonia racemosa*. Error bars omitted on graph B. for clarity.

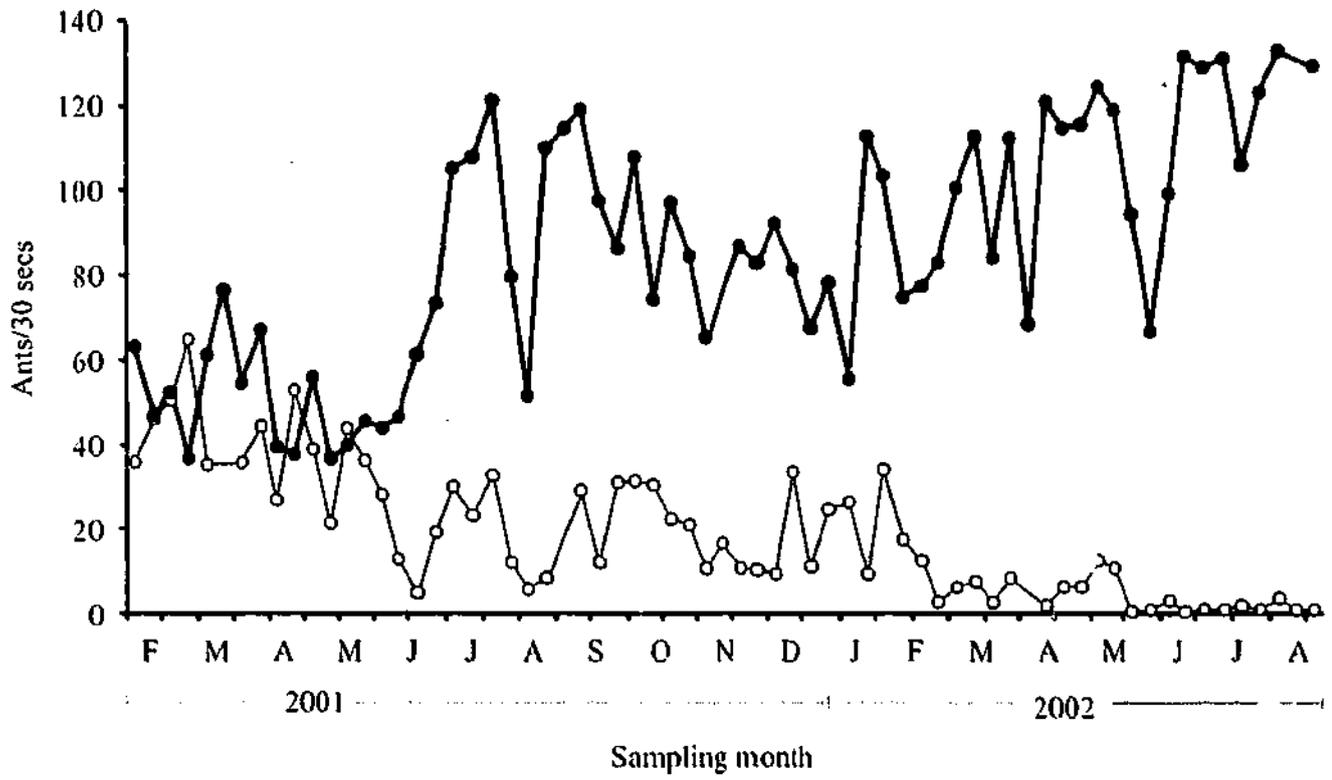


Figure 2.6 - Seasonal activity of ground foraging workers at ECR (●) and WAD (○). Each point on the line is the mean of 20 ant activity counts made weekly in a quarter hectare plot. Error bars omitted for clarity.

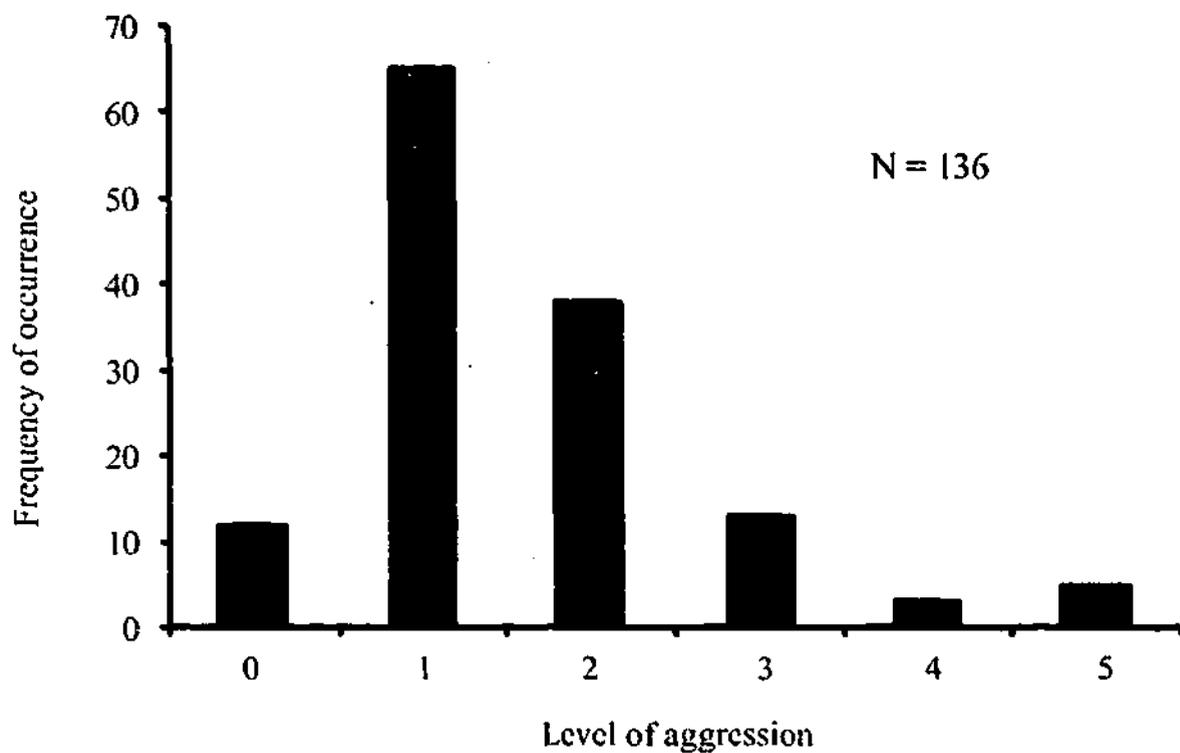
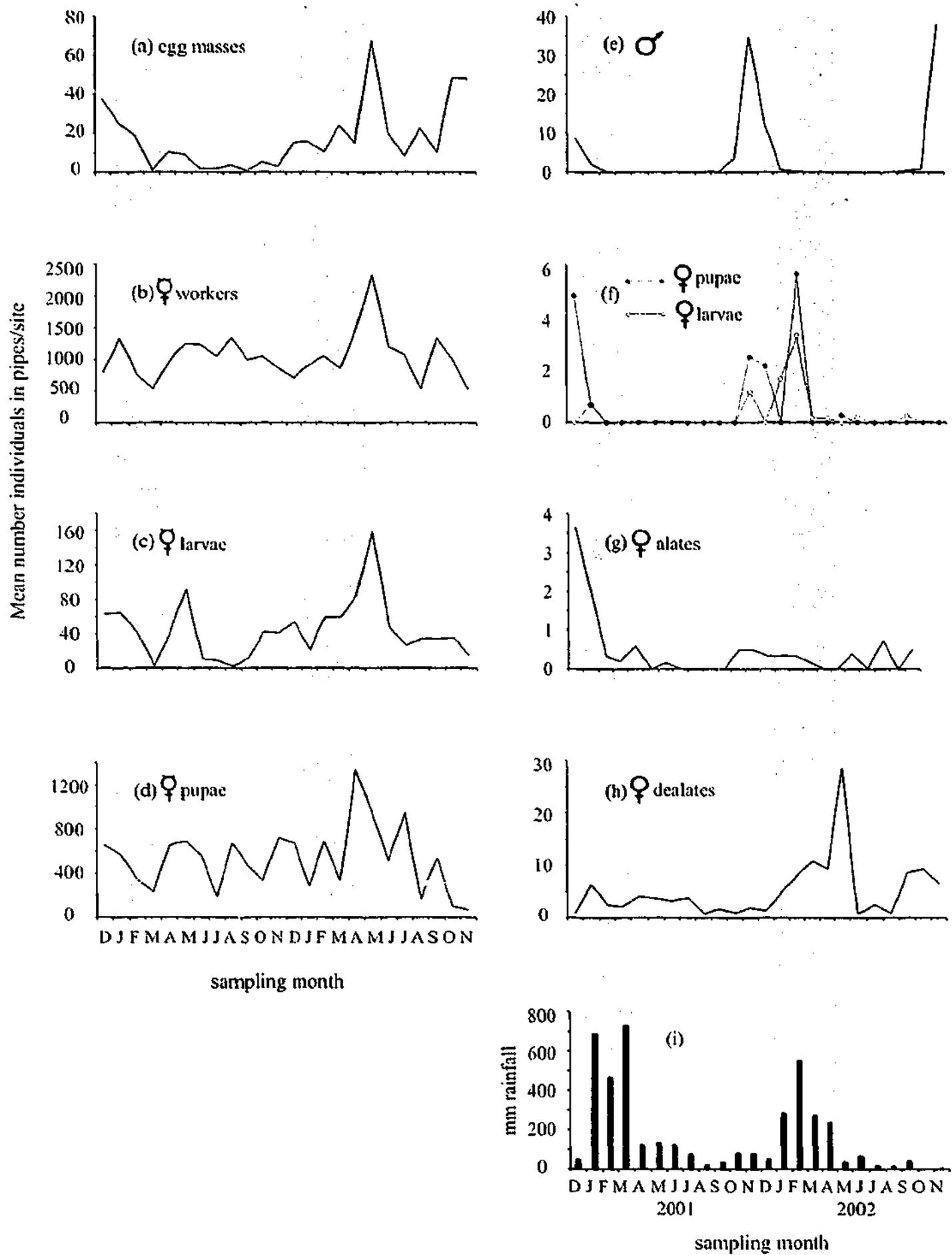
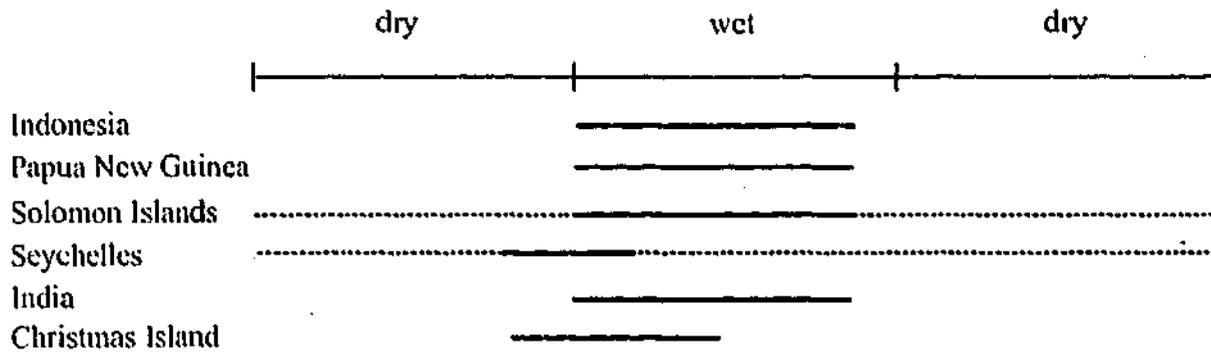


Figure 2.7 – Frequency of the level of aggression between pair-wise interactions of individual *A. gracilipes* in aggression assays.

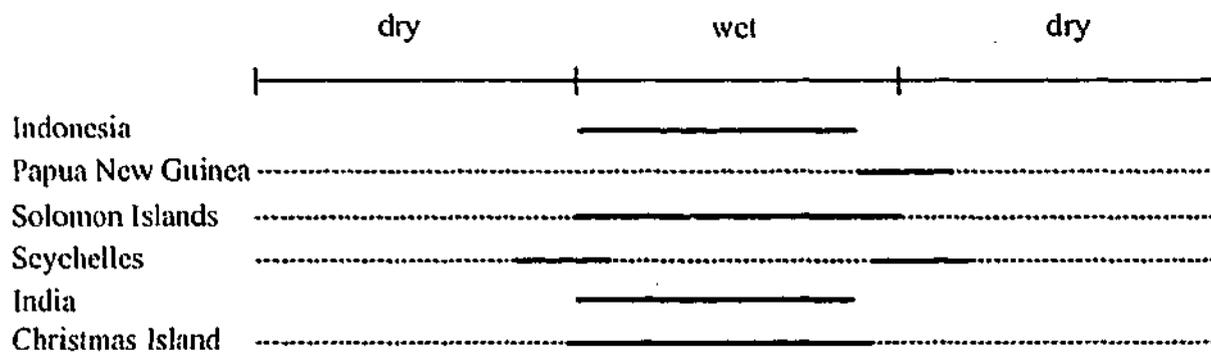
Figure 2.8 (following page) – Brood production in *Anoplolepis gracilipes* on Christmas Island between December 2000 and November 2002. Monthly values are the mean individuals of that life stage per site (n=6). Monthly rainfall for those months in which the study was carried out was taken from Bureau of Meteorology data, recorded at the airport on Christmas Island. Shaded regions indicate rainy months.



A. Males



B. Alate queens



C. Dealate queens

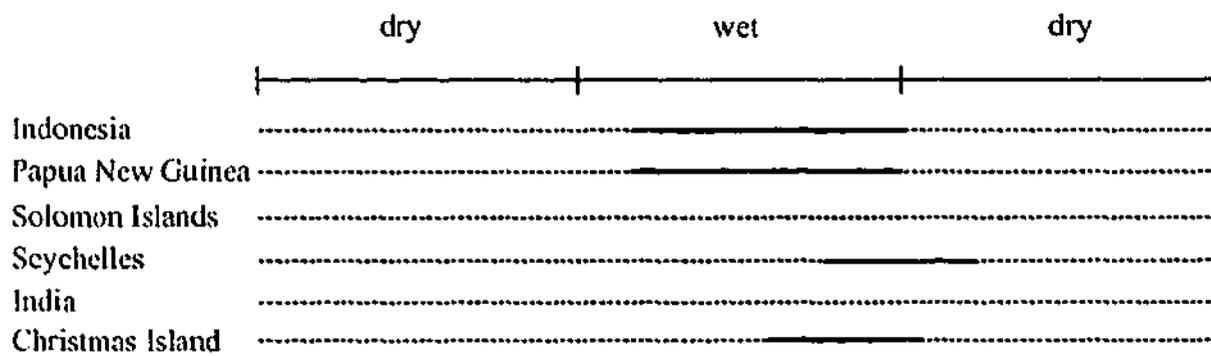


Figure 2.10 – A comparison of *A. gracilipes* male, alate and dealate queen phenology between five studies in various locations. Heavy lines indicate the time when most individuals were recorded, and dotted lines indicate the presence of low number of individuals. The wet season is shown in grey and dry season either side.

Sources: Indonesia – Van der Goot 1916; Papua New Guinea – Baker 1975; Solomon Islands – Greenslade 1971; Seychelles – Haines & Haines 1978; India – Rao & Vereesh 1991; Christmas Island – this study.

Chapter Three

Spatial and temporal dynamics of *Anoplolepis gracilipes* supercolonies on Christmas Island, Indian Ocean.



Introduction

Introduced species are foremost among the fundamental causes of global biodiversity loss (Vitousek *et al.* 1996; Mack *et al.* 2000) and can be economically expensive (Leung *et al.* 2002). Key to their management is an understanding of the scope of an invasion, the rate of proliferation, and consequently the rate at which invaded habitats become degraded. The area occupied by alien species can determine the magnitude of a management plan and subsequent control program, while their rate of spread can dictate the urgency with which such plans are implemented. Given immediate conservation threats and time constraints in developing control programs to mitigate impact, (Holway *et al.* 2002; Leung *et al.* 2002; Krushelnycky *et al.* 2003), it is not surprising that the spatial dynamics, the tempo of invasion, and its effects are key issues in invasion biology today. This knowledge is especially vital if action aims to prevent impacts rather than respond to them.

Several of the world's worst invasive species are ants (Lowe *et al.* 2000). Given their wide, and increasing geographic range, elevated local abundance, and potential to disrupt ecosystems, invasive ants represent a significant conservation concern (Holway *et al.* 2002) and threat to agricultural and tourism industries (Vander Meer *et al.* 1990; Jahn & Beardsley 1994). The Argentine ant (*Linepithema humile*) competitively displaces native ant species wherever it is introduced (Erickson 1971; Bond & Slingsby 1984; Human & Gordon 1996; Holway 1998a; Holway 1999) and

invasion fronts have been reported to expand at rates of between 0 – 250 m per year (Holway 1998b; Suarez *et al.* 2001). The red imported fire ant (*Solenopsis invicta*) decimated native fauna as it spread across the southeastern United States (Allen *et al.* 1995; Vinson 1997; Allen *et al.* 1998). In monsoonal Australia, high abundance of the big-headed ant (*Pheidole megacephala*) corresponded with a 42 – 85% decrease in the abundance of other native invertebrates (Hoffmann *et al.* 1999).

The process of spread by invasive ant species has been described through mathematical modelling (Shigesada & Kawasaki 1997), which depicts spread by diffusion (local movements often through colony budding) and long-distance jumps (human-mediated dispersal). Flight dispersal events by winged females represent a more regional (middle-distance) scale of movement. The relative contributions of these processes to the rate of spread for most invasive ant species is unclear (but see Suarez *et al.* 2001). Nonetheless, the majority of front expansions occur on a local scale via a diffusion-like process (Holway 1998b, Hoffmann *et al.* 1999), while introduction of new propagules to more distant areas illustrates the process of middle and long-distance jump dispersal (Suarez *et al.* 2001). The tendency for invasive ants to form unicolonial populations where large numbers of ants occupy expansive areas (Holway *et al.* 1998; Giraud *et al.* 2002; O'Dowd *et al.* 2003) offers an opportunity to study processes such as spread by diffusion and middle-distance dispersal.

There have been several studies documenting the spatial dynamics of supercolonies of unicolonial ant species. These have primarily investigated the area occupied by the colony/supercolony (Erickson 1971; Cherix 1980; Way *et al.* 1997), the rate of spread or advancement of boundaries (Porter & Savignano 1990; Holway 1998b; Krushelnycky *et al.* 2003), densities of nests or colonies (Haines & Haines 1978a; Porter *et al.* 1992; Tschinkel *et al.* 1995; McIver *et al.* 1997; Adams & Tschinkel 2001), and more recently the genetic relationship among workers and queens (Passera *et al.* 2001) and among kin in unicolonial populations (Chapuisat *et al.* 1997; Tsutsui *et al.* 2000; Giraud *et al.* 2002).

The invasive yellow crazy ant, *Anoplolepis gracilipes*, threatens insular ecosystems in both the Indian and Pacific Oceans (Haines & Haines 1978; Haines *et al.* 1994; O'Dowd *et al.* 2003; Lester & Tavite In Press). On Christmas Island it forms supercolonies, within which the density of ants on the forest floor is >2000 ants/m² (>20 million ants/ha; Chapter 2). Ants in supercolonies exhibit no intraspecific aggression and sustain extremely high densities of workers on all surfaces in rainforest. Supercolonies lack distinct boundaries, where ants at low densities can be observed hundreds of metres beyond the main high-density region (cf. O'Dowd *et al.* 2003). The formation of supercolonies by *A. gracilipes* on Christmas Island is a relatively new phenomenon: the first supercolony was observed in 1989, but since 1996 supercolonies have progressively increased in number and area to infest 28% (>2500 hectares) of rainforest on Christmas Island by September 2002 (Green *et al.* 2004). In these areas, crazy ants rapidly eliminate the red land crab, *Gecarcoidea natalis* (Brachyura: Gecarcinidae), changing the composition and structure of the forest (O'Dowd *et al.* 2003), resulting in major ecosystem disruption, and providing favourable conditions for secondary invasions (Green *et al.* 2001).

The rate of spread of *A. gracilipes* supercolonies has been reported at between 0.3 – 1.1 metres per day in the Seychelles (Haines & Haines 1978a). Impacts associated with high densities of crazy ants include displacement of native spiders in Hawaii (Gillespie & Reimer 1993) and domestic animals in the Seychelles (Haines & Haines 1978). The Seychelles' endemic skink disappeared from areas where *A. gracilipes* was abundant, and sooty terns failed to nest, and some chicks were killed by crazy ants (Feare 1999). On Christmas Island, red crabs are killed within 48 hours (O'Dowd *et al.* 1999), the abundance of the ground foraging emerald dove (*Chalcophaps indica*) is significantly reduced in crazy ant supercolonies, and nesting success of the endemic Christmas Island thrush (*Turdus poliocephalus erythropneurus*) might also be negatively affected by *A. gracilipes* (Davis 2002). These impacts have been observed within high densities of *A. gracilipes*, but have not examined the speed with which impacts occur at invasion fronts, and across the diffuse boundaries that *A.*

gracilipes exhibits. In this chapter I examine the spatial dynamics of *A. gracilipes* supercolonies on Christmas Island, Indian Ocean, and the impacts dynamic boundaries have on local ant fauna and red crabs.

I investigate the distribution and spatial scale of *A. gracilipes* supercolony formation in an island-wide context, and document the rate of movement of supercolony boundaries, including expansion and contraction. I investigate the nature of supercolony boundary transition zones between high-density supercolonies and intact rainforest that often extend hundreds of metres beyond the main supercolony. Finally, I report impacts of expanding invasion fronts, on the species richness of the local ant fauna, red crabs and crab burrows.

Methods and Materials

The Study System

This study was conducted on Christmas Island (105° 40'E, 10° 30'S), an isolated oceanic limestone island that lies 360 km south of Java in the northeastern Indian Ocean. The island is located in the humid tropics, and experiences a monsoonal climate where most of the annual 2000 mm rainfall occurs between December and May (Falkland 1986). Approximately 74% of the island is covered with natural vegetation, where three of seven described vegetation types (Mitchell 1985) dominate both plateau and terrace topography, and correspond generally to geological substrate type (Barrie 1967). Data for this study were collected between December 2000 and December 2002. Supercolonies were defined as having *A. gracilipes* activity sufficient to kill red crabs, and between 2001 and 2003 these areas covered >2500 ha of the island (Green 2003).

Distribution of A. gracilipes supercolonies on Christmas Island

The mapping of *A. gracilipes* supercolonies on Christmas Island occurred in stages. Initially, P.T. Green, D.J. O'Dowd and P.S. Lake noticed an increase in the

abundance of *A. gracilipes* in research plots in 1996, and gathered information regarding their distribution. This was done primarily through anecdotal evidence from Christmas Island residents and recording their presence/absence along 4WD tracks and along accessible rainforest tracks (P.T. Green, personal communication). The distribution of *A. gracilipes* was formalised in April 2001 with an island-wide survey, undertaken by Parks Australia and Monash University. In that survey, 972 survey points distributed in a regular grid pattern across the entire island were visited and assessed for crazy ant activity using card counts (see Chapter 2). These data formed the basis for subsequent detailed mapping of boundaries between the survey points. I combined this information to produce a map of the total area occupied by supercolonies of *A. gracilipes* between 1995 and 2003. These areas did not include transition zones. Here I present the range in supercolony sizes and the frequency of occurrence of each size class, as well as their distribution across substrates.

Boundary movement and transition zones

The movement of 13 *A. gracilipes* supercolony boundaries was documented over a period ranging between 10 to 20 months. In November 2000, boundaries were chosen to include different supercolonies that were relatively easy to access, and representing a range of substrates and topographical situations, including gentle soil slopes, scree slopes (small limestone pieces) and steep limestone cliffs. Boundaries of supercolonies were rarely sharp, and ant density near the edges of supercolonies typically declined from very high to very low or zero over a distance of 10s or 100s of metres. I called the point along a linear transect (running perpendicular to the boundary from the interior of a supercolony to "ant-free" forest) at which crazy ant activity began to decline the "boundary". The extended zone of declining ant activity thereafter was the "transition zone".

I placed a marker peg at a point where I perceived there to be a marked drop in ant activity as a reference point, and established a transect perpendicular to the

boundary that crossed from supercolony densities through the transition zone and into intact forest where no crazy ants were present. This line constituted the boundary transect for subsequent observations (Fig. 3.1). I recorded crazy ant activity on the forest floor using a white laminated 10 cm x 10 cm card placed at 10 m intervals along the transects until, beyond the supercolony, ants were absent for at least 25 m, and into the supercolony until ant activity was consistently >50 ants/30 secs for 30 metres and dead crabs were detected. Observations were made every three months, weather permitting (conditions were sometimes dangerous in wet weather, and *A. gracilipes* activity declines in wet conditions; Chapter 2). As supercolony boundaries expanded and ant activity increased beyond the supercolony, I expanded the transect and continued to record ant activity until ants were absent for at least 25 m, following the ants outward from supercolony densities. Given the transition zone varied in width between and within boundaries over time, the displacement of a supercolony boundary was calculated as the total distance along the transect between an initial and final ant activity count of 50 ants/30 secs.

In addition to crazy ant activity on the forest floor, I recorded variables that allowed me to assess impacts at 10 m intervals. These included the number of other ant species observed in a one minute visual search of 1 m² quadrat around the point of activity, the number of red crab burrows that had been invaded by *A. gracilipes*, as well as intact red crab burrows and the number of dead crabs in a 2 m x 6 m quadrat around the point of activity (Fig. 3.1). I looked for a correlation between the *A. gracilipes* activity on the forest floor and other ant species richness (using raw data) and analysed the shape of the relationship between the two variables using nonlinear regression on raw data. I analysed the relationship between ant activity and dead crabs and invaded and intact crab burrows using linear regression on raw data (SYSTAT ver. 10).

Results

Distribution of A. gracilipes supercolonies on Christmas Island

Since 1995, *A. gracilipes* supercolonies have occupied over 3000 ha of rainforest on Christmas Island (Fig. 3.2). Approximately 34 supercolonies have formed between 1989 (first supercolony discovered) and 2003, ranging in size across nearly three orders of magnitude from 0.9 – 787 hectares (Fig. 3.3). They are irregular in shape, often with extensions ‘fingering’ outward from the main supercolony. Given their recent discovery and irregularly timed formation, regions of *A. gracilipes* in supercolonies and low density have formed a dynamic mosaic across the entire island since 1989, but lack of accurate distributional data before 2001 prevents further analysis.

Results from the island-wide survey demonstrated that forest types I, II & III occurred over 32.4%, 41.0% and 25.7% of the grid points surveyed across the island, respectively. However, of all supercolony points in forest, 10% were in forest type I, 54.7% were in type II and 35.3% were in type III. This suggests that *A. gracilipes* formed supercolonies more frequently than expected in forest types II and III, considering the area of rainforest available for supercolony formation. Furthermore, 78.8% of all supercolonies were below 200 m ASL (P.T. Green unpublished data), most of which is considered ‘terrace’ forest.

Most supercolonies have occurred primarily on the terraces, around the edges of the island, associated with shallow soils characterised by relatively low moisture retention (Barrie 1967), but, larger supercolonies spanned all three substrate and forest types. These forest types included a variety of substrate types, from soil, through to soil interspersed with limestone pinnacles, to areas completely covered by limestone. The limestone slopes separating the terraces are often devoid of soil. I often observed nests within pockets and cracks on limestone cliff faces, and foragers were active on the surface of the rocks. There were nine discrete supercolonies located in plateau rainforest on relatively deeper soil, but all three of the largest supercolonies (448, 778 & 787 ha), and presumably the oldest, were primarily located on the terraces and were

distributed along the north coast, east coast and in the southwestern corner of the island, ranging in elevation from 20 m (terrace above the water) to >160 m ASL.

Boundary movement and transition zones

The rate of movement of supercolony boundaries was extremely variable on Christmas Island. All 13 observed boundaries were dynamic; nine out of 13 boundaries advanced over a period of between 10 and 20 months of observation (Table 3.1). The highest rate of expansion was 150 m in 11 months (0.50 m/day), and the slowest expanding boundary advanced 20 m in 20 months (0.03 m/day). The greatest contraction of a boundary was 80 m in 20 months (0.13 m/day), however, there was a net expansion of supercolony boundaries during the observation period (Fig. 3.4).

Transition zones spanned one, two and three substrate types. There appeared to be no pattern of movement associated with age of the supercolony, nor was there any discernable pattern whereby ants expanded or contracted into or out of particular substrate types. However, 11 out of 13 boundaries moved across substrate boundaries during the period of observation. Figure 3.5 illustrates the spatial patterns of foraging ants along the transects, which ran perpendicular to the invasion front. Over time, boundaries expanded, contracted or fused with adjacent supercolonies (Fig. 3.5A-C respectively), which were usually expanding as well. Boundaries were rarely sharp, and crazy ant activity decreased gradually as observation points along the transects approached ant-free rainforest. The mean length of transition zones between supercolony levels of crazy ants and undetectable crazy ants ranged from 46 ± 11 m to 153 ± 19 m (mean \pm SE) (Fig. 3.5D).

Within transition zones, where densities of ants were relatively low compared to within the main supercolony, *A. gracilipes* co-existed with other ant species as well as red crabs, *Gecarcoidea natalis*. As the activity of *A. gracilipes* increased to supercolony levels at the initial boundary peg (i.e. as boundaries expanded), other ant species richness declined (Fig. 3.6). The relationship between *A. gracilipes* activity and other species richness can best be described with an exponential decay model,

where crazy ant activity explains only 46% of the variation within other ant species richness (Fig. 3.7; nonlinear curve estimation, SYSTAT ver.10; $r^2=0.46$). Where there was only one other species of ant present in supercolonies, 81% of the time it was *Technomyrmex albipes*, which also appeared along the transition zone into intact forest. Other ant species occasionally encountered in supercolony densities of *A. gracilipes* were *Paratrechina longicornis*, *Pheidole* sp., *Tetramorium* sp., *Monomorium* sp. and *Tapinoma melanocephalum*, all species distributed widely throughout the tropics. Species most commonly found in transition zones, but not in supercolony densities were *P. longicornis*, *Pheidole* sp., *Brachyponera christmasi*. Toward intact forest, where *A. gracilipes* activity was very low, *Camponotus maculatus* and *Odontomachus simillimus* became more abundant.

As *A. gracilipes* activity increased at the initial boundary peg (i.e. as boundaries expanded) the number of intact crab burrows declined and the number of burrows used by ants as nesting site increased. After being occupied by ants for a period of time, crab burrows lost their structural integrity and collapsed, resulting in a total absence of crab burrows within supercolonies that had been active for about 12 months or more (Fig. 3.8). Generally, dead crabs increased and live crabs decreased as *A. gracilipes* activity intensified. However, it depended upon crab movement through supercolony areas (which was higher during crab migration at the onset of rains usually in Nov./Dec.) and the initial density of red crabs in the area. Nonetheless, at the initial boundary peg, there was a significant relationship between ant activity and the number of dead crabs observed at that point on expanding boundaries only over time (Fig. 3.9; $F_{1,51}=31.113$, $r^2=0.379$, $p<0.001$). As the density of crazy ants increased, they killed resident red crabs.

Discussion

The original entry point of *A. gracilipes* into Christmas Island was almost certainly in the northeast of the island. Steep cliffs surround most of the island, and there have only ever been two operational ports (Flying Fish Cove and Waterfall Bay; Fig. 3.2), both in the northeast and both in use in the early part of last century when the species is thought to have reached the island. The first supercolony was discovered approximately half way between these two sites in 1989 (P.T. Green, personal communication). Since then, crazy ants have reached virtually every corner of the island, but most supercolonies have formed in the western half.

The spread of *A. gracilipes* on an island-wide scale over the past 55 - 65 years cannot be attributed to budding from one introduced nest/colony. Even at the most rapid rate of spread by budding observed in this study (163.2 m/year), colonisation of the entire island by this method would be improbable. There are three methods of dispersal that, combined, have more likely aided the spread of *A. gracilipes* across Christmas Island. First, humans might have been responsible for the movement of propagules throughout the island, especially during the early 1900's, when ants would have hitched rides during exploration of the island and when railways and roads were under construction (Gibson-Hill 1949). This represents a likely explanation for colonisation of the south-eastern and south-western sections of Christmas Island. Second, *A. gracilipes* also spreads naturally from focal colonies in two ways; colony budding, and winged dispersal by queens to uninfested areas where they may start a colony of their own. Queens can walk on foot, accompanied by workers to a new nesting site, up to 3.2 m from their natal nest (Rao *et al.* 1991). However, it is aerial dispersal that may help explain *A. gracilipes*' establishment across Christmas Island, and account for isolated nests (see Chapter 2 for flying queens).

In 2000, supercolonies of *A. gracilipes* were located primarily on the periphery of the island (see Fig 3.2), in 'terrace' forest, dominated by forest types II and III (Mitchell 1985), characterised by shallow soils and generally drier forests containing more deciduous species with a more open canopy (Gillison 1989; Du Puy 1993). In

contrast, the central 'plateau' of Christmas Island is dominated by forest type I, which typically has deep soils with an improved water supply, and a tall, closed canopy. However, discrete, isolated and relatively small (5 – 25 ha) supercolonies increased in abundance on the plateau between 2000 and 2002. They most probably originated from the aerial dispersal of winged queens (Chapter 2), as these isolated supercolonies were often greater than 400 m from a larger supercolony.

The colonisation of the entire island by *A. gracilipes* is distinct from the formation of supercolonies in recent years. It represents an early stage of the invasion process (the engagement of available propagules with a vector, transfer to recipient location and establishment in recipient location), whereas the formation of supercolonies is the final stage of an invasion process - increase in abundance to cause ecological and human impacts. The increase in abundance of *A. gracilipes* has occurred rapidly on Christmas Island, but after a considerable lag period (approximately 55 – 65 years). The phenomenon of a lag-time is well acknowledged for some invasions (Crooks & Soulé 1999; Simberloff & Gibbons 2003), and they can be caused by the inherent nature of population growth and range expansion of a species, and also prolonged due to environmental factors related to improving ecological conditions for the organism (Crooks & Soulé 1999). Physical conditions often set clear limits on the distribution of organisms (Karatayev *et al.* 1998; Bale 2002; Bruneau 2002), and, mediated by abiotic factors, have been shown to drive the increase in abundance of pest insects in north America (Swetnam & Lynch 1993; Williams & Liebhold 1995). Abiotic factors have also been shown to be prime determinants in the rate of invasion of the Argentine ant, *Linepithema humile*, where stream flow (i.e. availability of water) was the only highly significant predictor of invasion rate (Holway 1998b). The environment on the edges of Christmas Island might provide an indication of favourable conditions for the formation of supercolonies, mainly relating to abiotic factors. A hypothesis is developed in the following chapters, based on the spatial distribution and associated abiotic characteristics of those areas (see Chapter 7).

Since 1996, supercolonies of *A. gracilipes* have formed in a temporally asynchronous manner across the island. Some supercolonies increased in activity, while others were declining (Chapter 2; ECR increased >2-fold in activity level while WAD declined almost to zero). Declines in *A. gracilipes* abundance have been reported elsewhere. In Indonesia, a decline in the production of worker brood, and subsequent population crash occurred in a cacao plantation, which Baker (1976) attributed to "instability of the population induced by the adoption and retention of an abnormal number of dealate queens". The mechanism has not been verified, but the population decline continued until no *A. gracilipes* colonies were found at the site 2 years later. *A. gracilipes* (then *Anoplolepis longipes*) had been recorded in Indonesia almost 50 years previously (Dammerman 1929). Wilson and Taylor (1967) described *A. gracilipes* as the dominant ant in disturbed habitats of Melanesia and Micronesia, confirmed by Greenslade (1971a, 1971b & 1972). Yet no subsequent observations have described their abundance in those areas. In addition, Hill *et al.* (2003) reported that control measures and/or natural population processes have led to a reduction in *A. gracilipes* problems in the Seychelles over the past 20 – 30 years, despite it having spread to nine islands in the Central Seychelles (unpublished report cited in Hill *et al.* 2003). On Christmas Island, the supercolony at WAD (Chapter 2) declined in activity after approximately 6 years, along with two other sites where *A. gracilipes* had been observed in high densities for >5 years (P.T. Green unpublished data).

On a local scale, the rate of movement of supercolony fronts on Christmas Island represents diffusion by budding and a highly variable rate of movement. The rate of movement is within the range of Argentine ants, *Linepithema humile*, (Suarez *et al.* 2001), but greater than *Pheidole megacephala* whose primary dispersal method is also colony budding (Hoffmann *et al.* 1999). Invasion fronts of *A. gracilipes* supercolonies on Christmas Island expanded at a maximum rate of 0.5 m/day (163.2 m/year), and contracted at a maximum rate of 0.1 m/day (48 m/year). However, Haines & Haines (1978a) reported rapid spread of an invasion front in the Seychelles of 1.1

m/day (402 m/year), and Rao *et al.* (1991) found that during colony budding in India, queens walked a maximum of 3.2 metres from their original colony. In addition to the rate of spread of *A. gracilipes* supercolonies being highly variable, only sections of an entire boundary moved (expanded or contracted), and where and when it occurred was highly unpredictable. Contrary to the expectation that supercolonies would expand while, or just after queens were being produced, there was actually a net contraction of supercolony boundaries during sexual brood production (Chapter 2 for reproductive schedule). Krushelnycky *et al.* (2003) suggest that dispersal (and hence boundary expansion) of Argentine ants in Hawaii appears to occur when worker production in the colony approaches its peak and total ant abundance is high, but this did not seem to be the case for *A. gracilipes* on Christmas Island. It is more likely that the contraction of *A. gracilipes* supercolonies during wet periods was due to increased rainfall during the time when sexual brood was produced, which depresses forager activity, especially in areas of low ant density. Conversely, reduced rainfall during the long dry seasons experienced between 2000 and 2003 may allow for increased time outside the nest for workers and queens, which may include travelling to new nest sites.

The variation in the measured rate of movement may also be associated with extended transition zones. Transition zones of over 180 metres wide were observed, and often made detection of the invasion front difficult because of its diffuse nature (Haines & Haines 1978a, cf. Argentine ant invasion fronts; Erickson 1971, Holway 1998b). Within transition zones ant activity dropped gradually, and the length of these zones varied greatly over time. It is through budding that supercolonies on Christmas Island are able to 'finger' outward from the main colony and form lower density transition zones, but extended and diffuse boundaries are not consistent with the hypothesis of range expansion by budding alone (Hölldobler & Wilson 1990). I observed single queens walking in the open accompanied by workers, both in supercolony areas and transition zones. On one occasion I observed over 100 dealate queens under a small log 50 m beyond the transition zone, having not seen a worker

since the edge of the transition zone. Whether they walked or flew (then shed their wings) is a mystery.

Although it is not typically done, it is important to make the distinction between expansion and contraction of invasive ant colony boundaries, as the mechanisms by which the colony expands and contracts may be completely different. It may be their biotic interactions with other species (Human & Gordon 1996) that confer them superior abilities and cause the boundary to expand into uninfested areas, but abiotic factors that force activity levels to decline and boundaries to contract (Hoffmann *et al.* 1999). Biotic interactions encountered as *A. gracilipes* supercolony boundaries expanded into intact rainforest could be categorised as competition, predation and mutualism. Potential competitors for *A. gracilipes* on Christmas Island included other ant species, predators included birds and other invertebrates, and scale insects served as mutualists. This study did not assess the relative importance of each of these factors in determining the rate of invasion, however, it appears that crazy ants are clearly good competitors, given the reduced ant species richness in areas of *A. gracilipes* supercolonies. Competition and predation confer on a community a 'biotic resistance' (Elton 1958; Lake & O'Dowd 1991), but mutualisms involving animal-mediated pollination and seed dispersal often facilitate invasions (Richardson *et al.* 2000). The role of mutualists as 'biotic facilitators' of invasive ant success is only just starting to emerge in the literature (Simberloff & Von Holle 1999; Helms & Vinson 2002; Helms & Vinson 2003).

In Hawaii, *A. gracilipes*, *L. humile* and *P. megacephala* were mutually exclusive of one another where each species was dominant (Fluker & Beardsley 1970), and in Zanzibar, *A. gracilipes* (*A. longipes*) displaced *Oecophylla longinoda* where they competed for honeydew in the crowns on coconut palms (Way 1953). Furthermore, Haines & Haines (1978b) found that where *A. gracilipes* was co-dominant, the other ants present were *Technomyrmex albipes*, *Tapinoma melanocephalum* and *Pheidole punctulata* (*T. albipes* was the most abundant). The displacement of other ant species by an invasive ant species is common (Fluker & Beardsley 1970; Bond & Slingsby

1984; Lubin 1984; Ward 1987; Camilo & Philips Jr. 1990; Human & Gordon 1996; Holway 1999).

In this study, *A. gracilipes* displaced other ant species as their activity increased and boundaries expanded, except for *Technomyrmex albipes* and *Paratrechina longipes*, which I commonly found co-existing with *A. gracilipes* in supercolony areas. In contrast, *A. gracilipes* seemed to displace *P. longicornis* on islands of Tokelau in the Pacific (Lester & Tavite In press). I did not test the underlying mechanism by which *A. gracilipes* dominated those habitats, but the co-existing ant species were predominantly small in size, and either able to inhabit small interstitial spaces and run between crazy ant foragers or avoid excessive encounters with *A. gracilipes* by remaining under organic matter on the forest floor. Foragers of *A. gracilipes* monopolised baits in supercolonies (Marr 2003), which indicates that sheer abundance may confer them superior competitive abilities in those areas. It is unclear whether there are any native ant species on Christmas Island, so competition is occurring between *A. gracilipes* and populations of introduced species.

Management implications

The negative impacts caused by supercolonies of *A. gracilipes* on Christmas Island have been rapid (O'Dowd *et al.* 2003). Control and ongoing management of supercolonies (by reducing ant density) is crucial to the continued existence of the endemic red land crab, *Gecarcoidea natalis*, and the preservation of Christmas Island rainforest and its inhabitants. Waiting for a possible natural decline of high-density populations would be detrimental to the natural ecosystem. The spatial mosaic of varying sized *A. gracilipes* supercolonies combined with the temporally asynchronous nature of suspected "boom and bust" cycles of supercolonies, presents challenges for managers. Techniques for the control or eradication of invasive species can take years to develop. Where it is unfeasible to do so, containment via the application of toxic baits at invasion fronts may provide temporary abatement of invasion (Krushelnicky *et al.* 2003). Control of over 2500 ha of *A. gracilipes* supercolonies on Christmas Island

was achieved in September 2002 by the aerial distribution of toxic bait (Green *et al.* 2004), but since then, numerous small supercolonies have formed in those areas not targeted in 2002. With the knowledge that *A. gracilipes* supercolonies may establish at any time, successful control of *A. gracilipes* supercolonies on Christmas Island depends upon long-term management strategies. Surveillance, continued control operations and stable sources of long-term funding are vital for effective implementation of management prescriptions. Furthermore, research on what causes "booms and busts" of *A. gracilipes* supercolonies might better inform management for use in predictions of the invasion. I consider hypotheses for "booms" in Chapter 7.

Table 3.1 – Attributes of 13 supercolony boundaries on Christmas Island. Boundaries are divided by main substrate type and observed for varying lengths of time. The mean transition zone length is calculated from the length of the transition zones at each time of observation (no. of obs. in brackets). Three letter codes are abbreviations for site names.

Boundary	Months observed	Total displacement (m)	Rate of movement (m/yr)	Mean length transition zone (m ± SE)
<i>Soil</i>				
WBT(west)	20	120	72.0	46 ± 11 (8)
WBT(east)	20	20	12.0	71 ± 10 (8)
WCR	11	90	98.4	114 ± 17 (5)
ECR(east)	16	120	90.0	97 ± 8 (7)
ECR(west)	20	90	54.0	56 ± 6 (6)
SH	10	40	48.0	55 ± 9 (4)
<i>Scree slope</i>				
WCR(north)	20	20	12.0	89 ± 11 (7)
JAM	11	150	163.2	78 ± 17 (5)
ECR(north)	15	-20	-15.6	98 ± 8 (6)
PUM(north)	11	30	32.4	53 ± 14 (4)
PUM(south)	11	-30	-32.4	110 ± 15 (4)
<i>Steep soil & scree slope</i>				
HV	11	-10	-10.8	153 ± 19 (4)
WAD	20	-80	-48.0	105 ± 18 (8)
Mean		41.6 ± 18.9	53 ± 12	86.5 ± 8.5

Table 3.2 - Reported rates of expansion and contraction from direct observations at the colony front of invasive ant species.

Species	Rate of movement		Source
		(m/yr)	
<i>Anoplolepis gracilipes</i>	Expansion	12 - 402	Haines & Haines 1978, Rao <i>et al.</i> , 1991; This study
	Contraction	11 - 48	
<i>Linepithema humile</i>	Expansion	0 - 270	Cited in Suarez <i>et al.</i> , 2001
	Contraction		
<i>Pheidole megacephala</i>	Expansion	20 - 40	Hoffman <i>et al.</i> , 1999
	Contraction	0 - 12	
<i>Solenopsis invicta</i>	Expansion	10 - 40	Porter <i>et al.</i> , 1988
	Contraction		
<i>Wasmannia auropunctata</i>	Expansion	170 - 500	Lubin 1984, 1985
	Contraction		

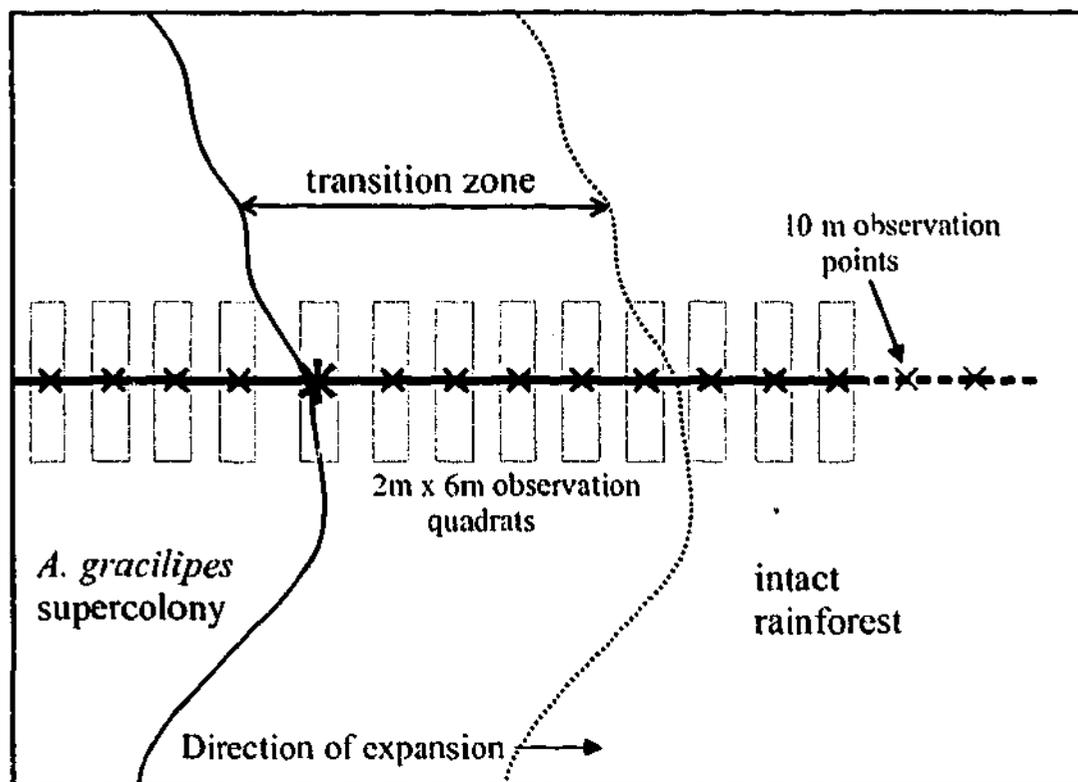


Figure 3.1 – Arrangement of transects perpendicular to *A. gracilipes* supercolony boundary, extending into intact rainforest. The larger asterisk is the initial marker peg. The 2m x 6m and 1 m² observation quadrats were centred on each 10 m-interval point (X) where *A. gracilipes* activity was recorded. As boundaries expanded (toward the right in figure) I extended the transect to reflect the change in boundary position.

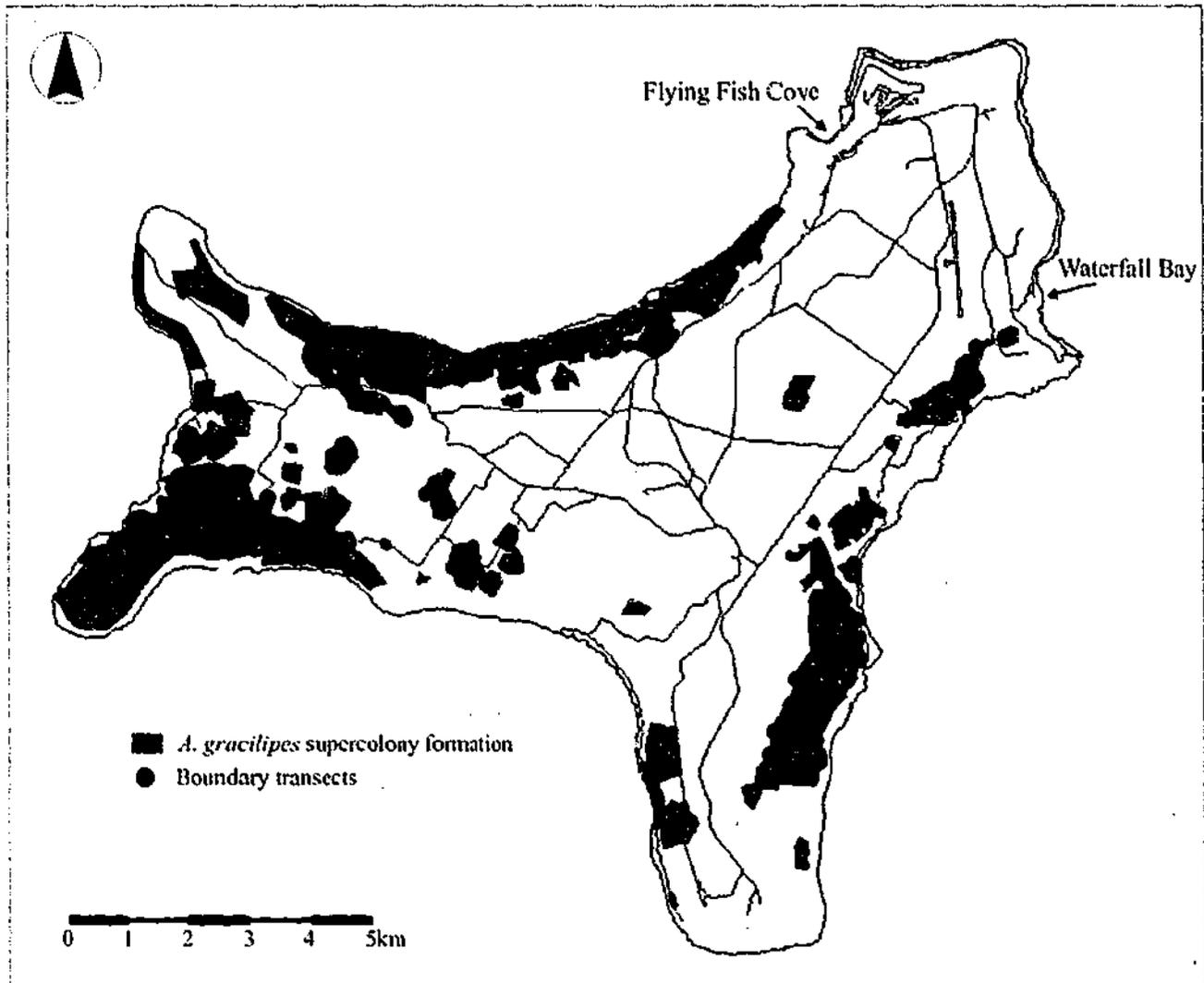


Figure 3.2 – The estimated total area occupied by *A. gracilipes* supercolonies between 1989 and end 2002 (>3000 hectares) on Christmas Island, Indian Ocean, showing location of boundary transects (●) Areas in which the density of *A. gracilipes* was not sufficient to kill red crabs (below supercolony levels) are not on this map, but occur across the island. Flying Fish Cove and Waterfall Bay are the only ports used over the past century.

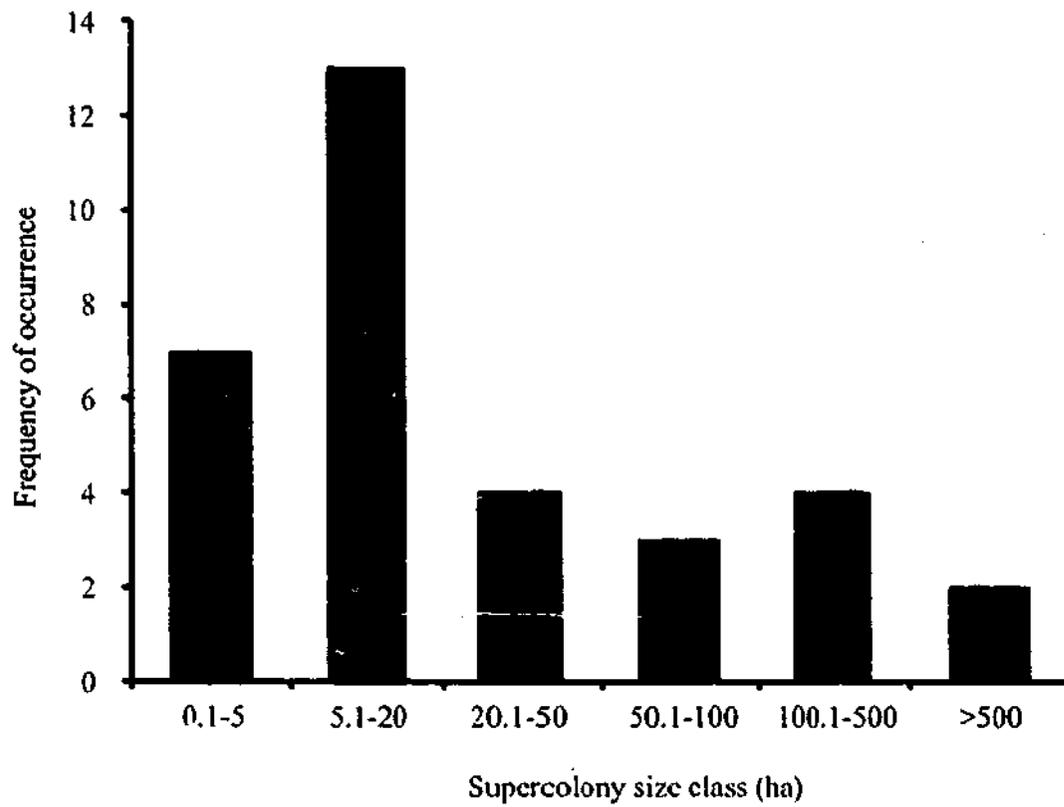


Figure 3.3 – Range of supercolony areas (ha) on Christmas Island. Supercolonies described here are those shaded in Figure 3.2.

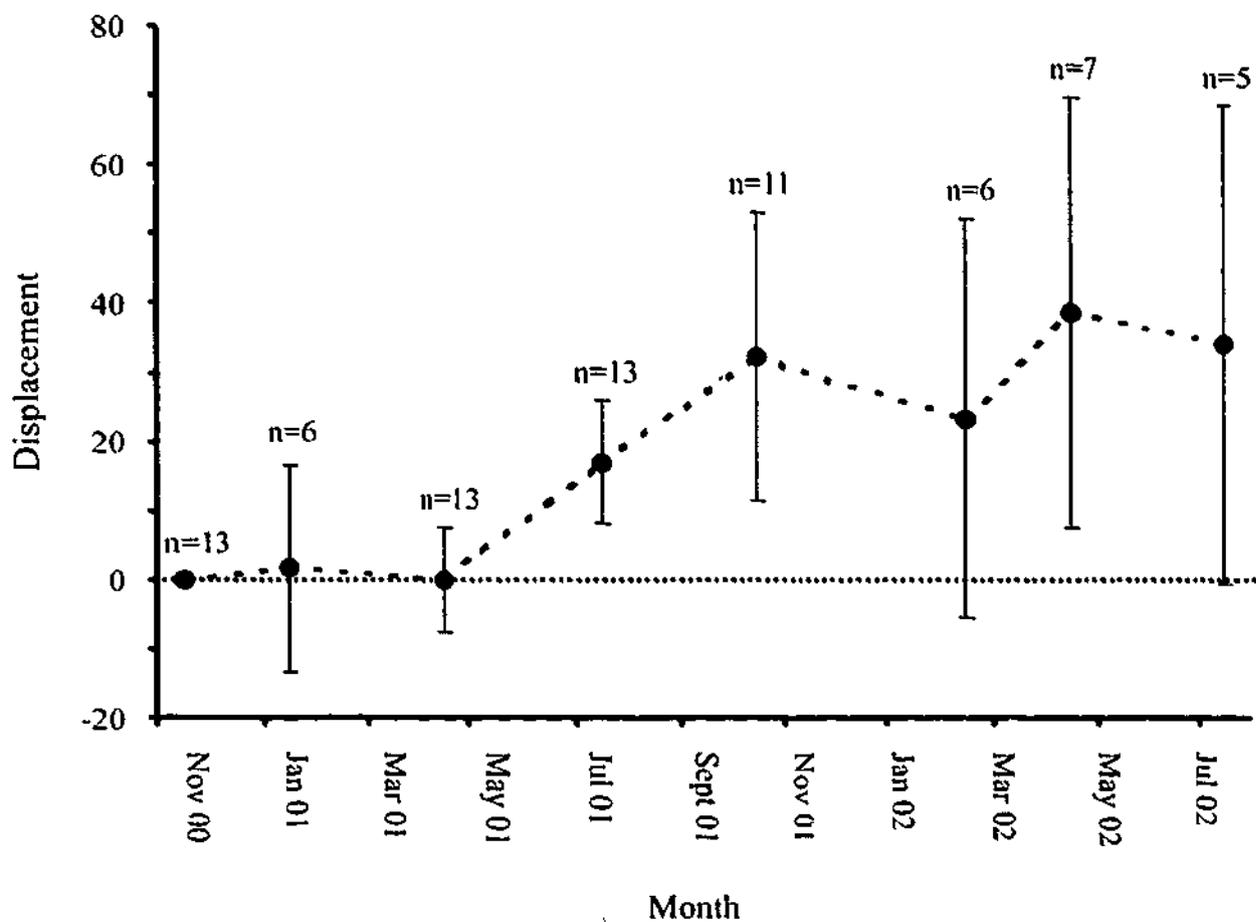


Figure 3.4 – Average (\pm SE) displacement (in relation to first observation) of supercolony densities of *A. gracilipes* along transects between November 2000 and July 2002. Positive displacement = boundary expansion, negative displacement = boundary contraction. Dotted line indicates zero net displacement.

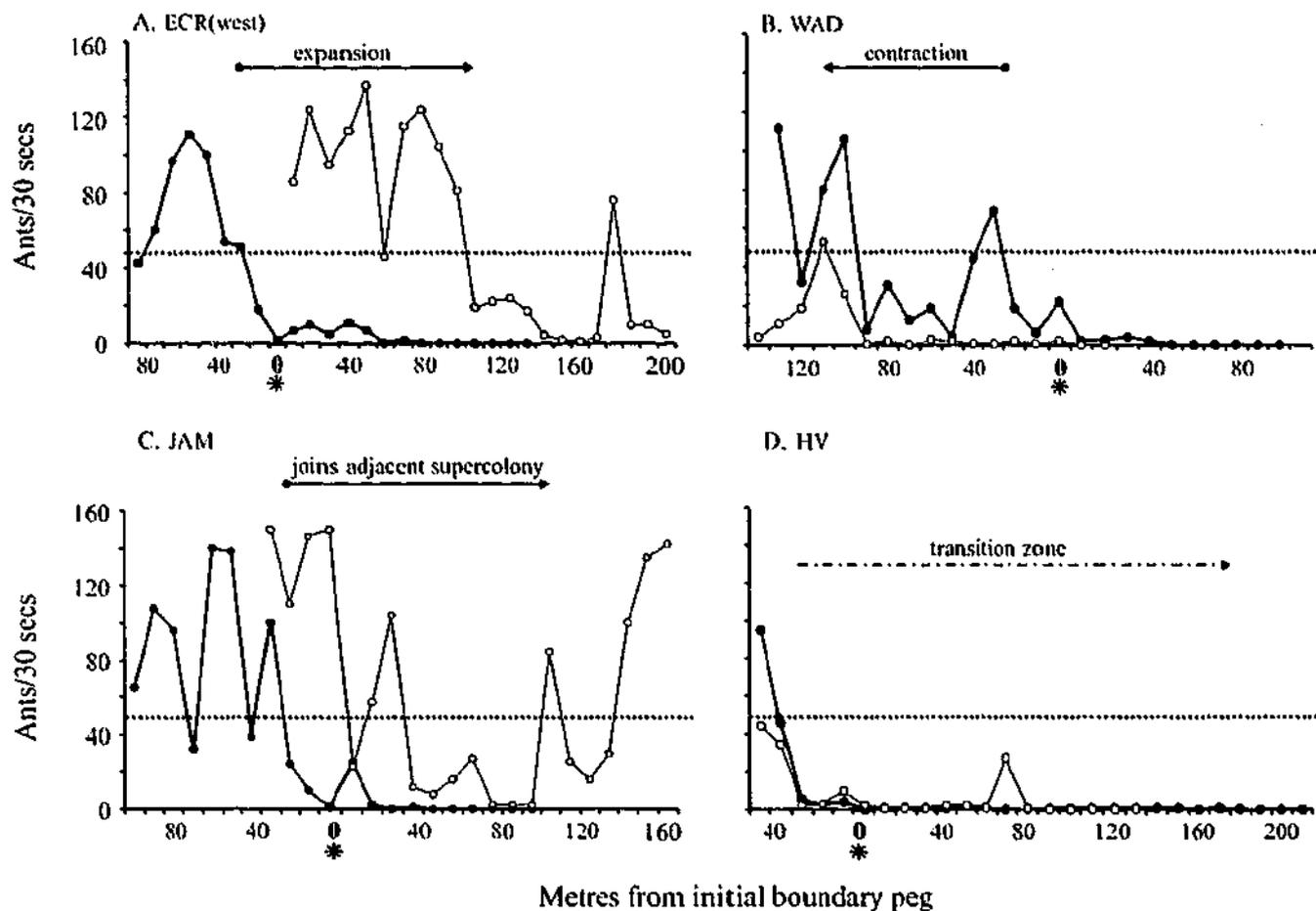


Figure 3.5 - Examples of supercolony boundary expansion (A, left to right), contraction (B, right to left) and amalgamation with a nearby supercolony (C). In each example, ant activity (ants/30 s) is shown for stations spaced 10 m apart along a transect oriented at right angles to a supercolony boundary, for both the initial observation (●; November 2000 all sites,) and last observation (○; July 2002 for ECR (west) and WAD, October 2001 for JAM and HV). The dotted line indicates crazy ant activity at supercolony levels, where worker density and activity is sufficient to kill red land crabs. The transition zone begins below the point, and (D) is an example of a long transition zone.

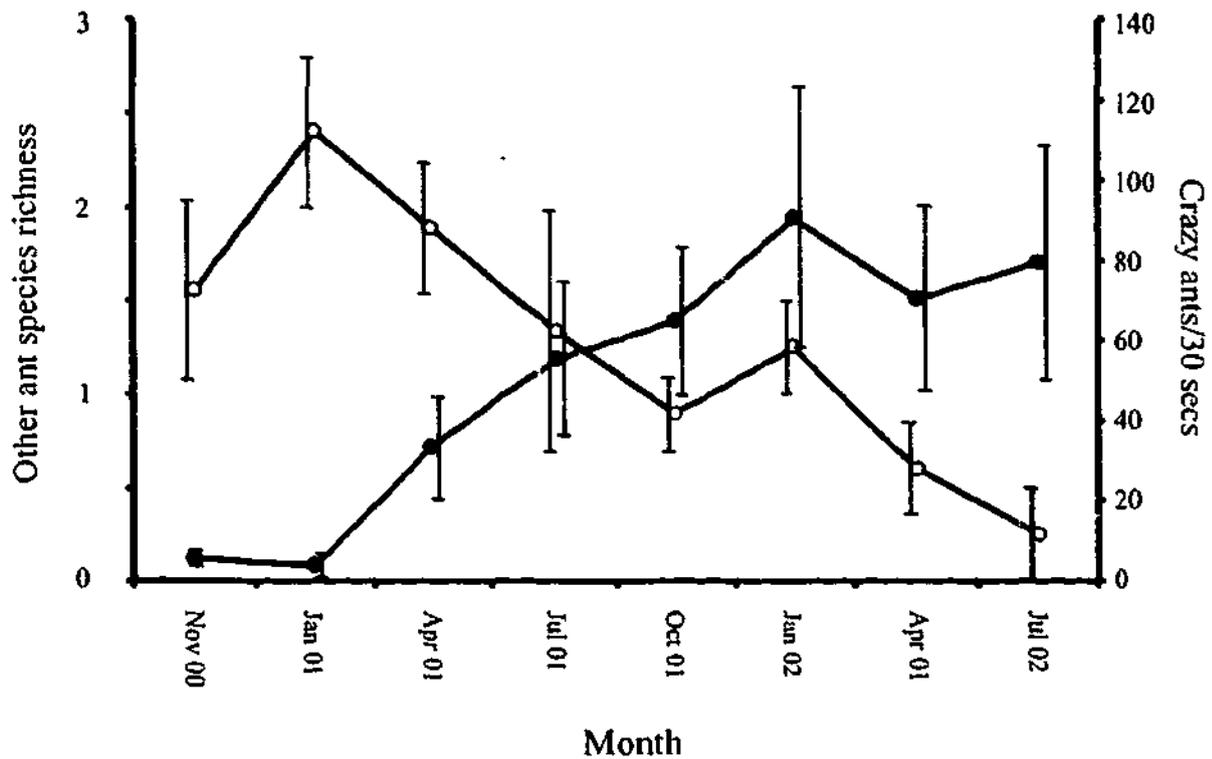


Figure 3.6 – Mean crazy ant activity \pm SE (●) at the initial boundary peg over time, and mean number of ant species \pm SE other than *A. gracilipes* (○) recorded in a 12m² quadrat at the initial boundary peg during a 1-minute visual search (\pm SE). Data from expanding boundaries only. Note the disparity in the magnitude of the error bars due to the different scales on each axis.

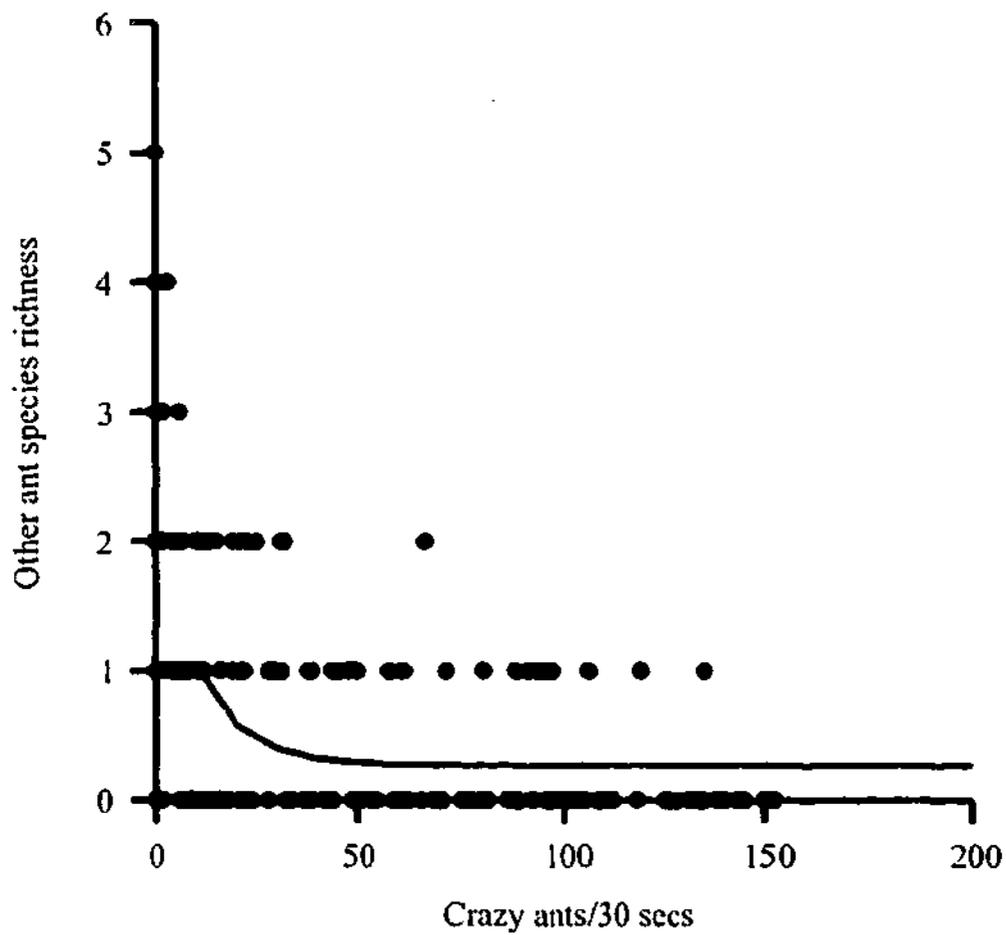


Figure 3.7 – Relationship between crazy ant activity and other ant species richness at the initial boundary peg on the first survey time (November 2000). As crazy ant activity increased, the abundance of other ant species decreased in a manner best described by an exponential decay model. Data from all transect points from 13 transects. N=321. Equation of the line is $y = 0.27 + 1.84^(-x/11.39)$

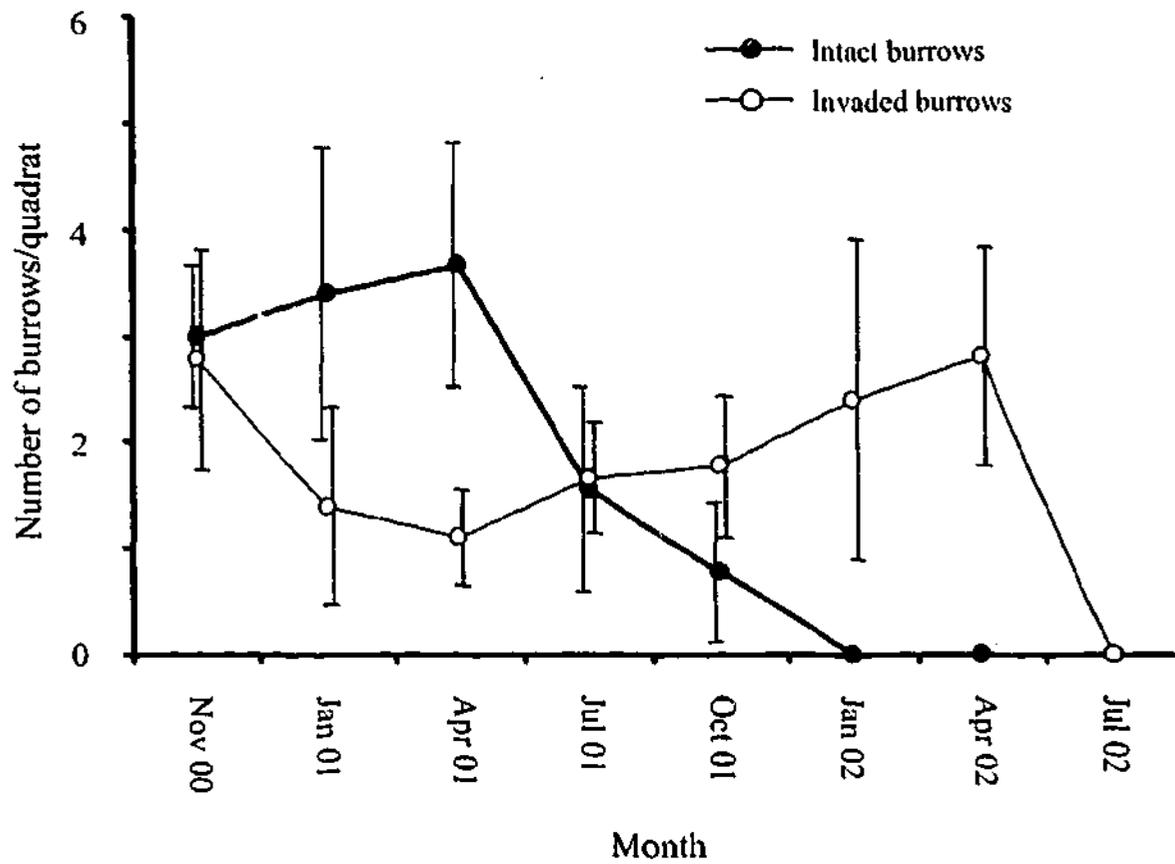


Figure 3.8 – The mean number of intact and ant invaded red crab burrows (\pm SE) in a 12m² quadrat at the initial boundary peg over time. Data from expanding boundaries only.

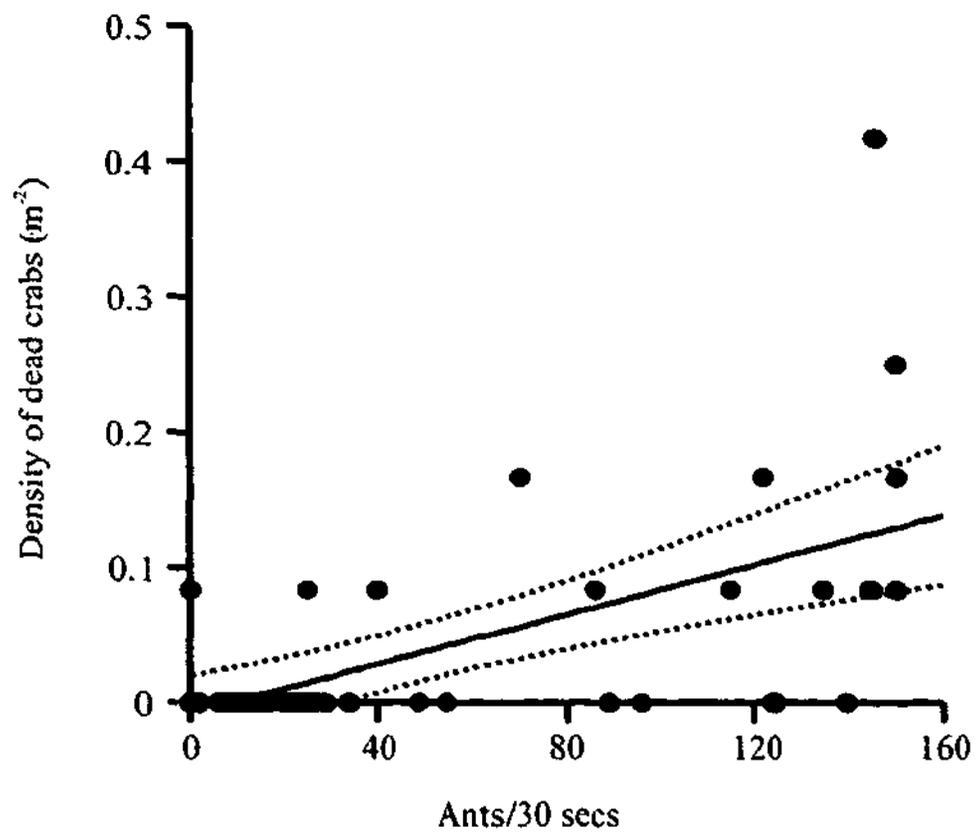


Figure 3.9 – Relationship between ant activity and the density of dead crabs observed at the initial boundary peg over time. The density of dead red crabs increased as *A. gracilipes* activity increased. $N = 54$.

Chapter Four

Scale insects (Hemiptera: Sternorrhyncha: Coccoidea) on Christmas Island, Indian Ocean: Species, distribution and association with forest type and *Anoplolepis gracilipes* supercolonies



Introduction

Hemipterans are small, cryptic components of the ecosystem, yet they are ubiquitous and often damaging to plants (Buckley 1987a; Kosztarab & Kozár 1988; Ben-Dov & Hodgson 1997). Destructive species frequently go undetected until they increase in abundance and cause significant damage in agriculture, horticulture and forestry (Buckley 1987a), as well as natural systems (Beggs 2001; O'Dowd *et al.* 2003). In particular, scale insects (Hemiptera: Sternorrhyncha: Coccoidea; formerly of the suborder Homoptera – Gullan 2001) are known to cause damage to citrus, tropical fruit, pine plantations and natural forests (Haines & Haines 1978b; Haines *et al.* 1994; Ben-Dov & Hodgson 1997; James *et al.* 1997; Grafton-Cardwell *et al.* 2000), and the worldwide economic loss attributed to all scale insects, including the cost of control, has been estimated to be US\$5 billion annually (Kosztarab & Kozár, 1988). Of the 840 native Australian species, 30 are significant pests and have been the subject of extensive research into their biological control (Waterhouse & Sands 2001).

Scale insects frequently form mutualistic associations with social insects, especially ants (Nixon 1951; Buckley 1987b; Itioka & Inoue 1996; Helms & Vinson 2002; O'Dowd *et al.* 2003). Ants dominate arthropod faunas in tropical rainforest canopies (Erwin 1983; Stork 1988; Tobin 1991), and it is thought that large populations of long-lived ants might be sustained by an abundance of dietary carbohydrate and

demand little protein (Tobin 1994; 1995; Davidson 1998; Davidson *et al.* 2003). Honeydew from scale insects is composed principally of carbohydrate, and is thought to be a key dietary component for many ant species, especially canopy ants in tropical rainforest (Bluthgen & Fiedler 2002). As such, scale insects may be key drivers of population growth in ants and other social insects (Beggs 2001). Conversely, ants are also key drivers of scale insect populations. Among other services, ants protect scale insects against natural enemies, they remove excess honeydew that might otherwise asphyxiate adult females, and tend larval stages (Boucher *et al.* 1982; Cushman & Addicott 1989; Bach 1991; Gullan 1997).

Although scale insects alone can have strong direct effects on their hosts, it is typically via their association with ants that scales reach damaging densities and have the greatest impacts (Van der Goot 1916; de Fluiter 1939; Elton 1958; Haines & Haines 1978a; 1978b; Helms & Vinson 2002). Adult females extract phloem sap from their hosts (Buckley 1983), and their excretion of honeydew facilitates the build-up of sooty moulds on leaves (Bach 1991, Gullan 1997), inhibiting photosynthesis (Wood *et al.* 1988). Sometimes, populations of scales and ants become extreme and cause the death of their host plants (O'Dowd *et al.* 2003, P. Lester personal communication).

Mutualisms between scale insects and ants appear to be facultative, and associations readily form under a variety of circumstances. Even outside of their native ranges, the most widespread and notorious invasive ant species, *Linepithema humile* (Argentine ant), *Pheidole megacephala* (big-headed ant), *Solenopsis* spp. (fire ant) and *Anoplolepis gracilipes* (yellow crazy ant) attend scale insects and other hemipterans (Nixon 1951; Samways *et al.* 1982; Helms & Vinson 2002; O'Dowd *et al.* 2003) often enhancing the pest status of the coccids they attend. In particular, outbreaks of scale insects have often been reported in the presence of *A. gracilipes*. For example in Java, the scale insect *Coccus viridus* flourished in the presence of *A. gracilipes*, with an average of 1,057 scales per coffee bush, compared to 70 on ant-free bushes (Van der Goot 1916). Similarly, de Fluiter (1939) observed that the severity of infestations of the scale insect *Pseudococcus citri* in coffee plantations in Java was positively related

to the abundance of *A. gracilipes*. In the Seychelles, *Ceroplastes rubens* was 20 - 160 times more abundant and *Coccus viridus* 5 - 50 times more abundant at sites where *A. gracilipes* was the dominant ant (Haines & Haines 1978b). Most recently, outbreaks of scale insects, especially the lac scale *Tachardina aurantiaca* and soft scale *Coccus celatus*, have been reported from Christmas Island (Indian Ocean) in association with invasion and supercolony formation by *A. gracilipes* (O'Dowd *et al.* 2003). Coccids have become a outstanding feature of Christmas Island rainforest since the proliferation of *A. gracilipes* on Christmas Island in the mid 1990s; incredibly high densities of *T. aurantiaca* (Sternorrhyncha: Coccoidea: Keriidae) have even been implicated in the dieback of the forest canopy species *Inocarpus fagifer* (O'Dowd *et al.* 2003; P.T. Green unpublished data).

In the first half of this study, I make a detailed investigation of the scale insects of Christmas Island, which prior to this study, were extremely poorly known. First, I list all species of scale insects currently known for Christmas Island, using my own observations and building on those of previous surveys. Second, I record the densities of scale insects within and outside of crazy ant supercolonies, and examine composition across scale family groups and host species. Third, I list host plant species for main species groups; and fourth, discuss input and dispersal of mobile crawlers (specifically *Tachardina aurantiaca*) and establishment of scale insect populations in the context of *A. gracilipes* supercolonies. In the second half of this study, I use this information, in conjunction with surveys of host plant abundance, to test the hypothesis that the propensity of crazy ant supercolonies to form in certain forest types and not others (see Chapter 2) is largely a consequence of variation between these types in their "suitability" as hosts for outbreaks of scale insects. As key mutualists of *A. gracilipes*, scale insects and the factors determining their distribution relative to forest type may be key determinants of the incidence and distribution of crazy ant supercolonies on Christmas Island.

Methods and Materials

The Study System

The study was conducted between January 2001 and February 2003 on Christmas Island (105° 40'E, 10° 30'S), an elevated oceanic limestone island situated 360 km south of Java in the northeastern Indian Ocean. The island is in the humid tropics, and experiences a monsoonal climate where most of the annual 2000 mm rainfall occurs between December and May (Falkland 1986). Approximately 74% of the island is covered with natural vegetation, 96% of which is composed of three forest types (Fig. 4.1; Mitchell 1985). Forest type I is described as tall closed forest on deep soil, typically located above 160 m in elevation and with an average canopy height of 34 m. Forest type II is closed forest over shallower soils, usually found above 130 m in elevation (including some terraces), with an average canopy height of 28 m. Forest type III is closed forest, mostly on a substrate of limestone pinnacles and rocks. Although found at a range of altitudes across the island, it is predominantly located on the lower slopes and coastal terraces. The average canopy height is 22 m. Forest types II & III are drier and have a greater proportion of deciduous species than forest type I. Mitchell (1985) provided a map detailing the distribution of these forest types across the island, and I used the digitised version of this map in the Christmas Island GIS when selecting sites for this study.

Scale insect species, abundance and host breadth

An island-wide survey formed the main part of data collection, and was designed to record the species of scale insects, their abundance, composition and host breadth. The survey was stratified by forest type and invasion status, with five sites in each of three forest types both in areas of supercolony formation by *A. gracilipes*, and intact, uninvaded forest ($N = 5 \times 3 \times 2 = 30$ sites; Fig. 4.2). At each site, I sampled for scale insects on those tree species that made up 80% of stems typically found in each forest type. To determine which plant species characterised each forest type, I conducted plant surveys in the five sites of each forest type where *A. gracilipes*

supercolonies were absent (15 sites), plus two additional non-supercolony sites of each forest type used as part of the island-wide survey for forest water stress (see Chapter 7; $15 + 6 = 21$ sites in total). At each site, I laid out a transect tape and recorded the abundance of trees ≥ 10 cm dbh by their species identity, within 20 m either side of the tape. I moved along the transect counting trees until I had identified 200 individuals at each site. Tree counts were pooled across seven replicate sites within forest types, and those species comprising 80% of the pooled total for each forest type were sampled for scale insects at 5 invaded and 5 uninvaded sites in each forest type.

In all 30 sites I counted individual scale insects on at least one, and usually three individuals of each species at each site, and on each individual tree, one branch was sampled from between 3 – 5 m above ground level using pole pruners. On each branch, I counted scale insects on three 20 cm sections from three randomly chosen twigs. Where twigs were not long enough to sample 20 cm sections, 10 cm sections were used. In each 20 cm section, the number of adult female scale insects was recorded for all species. All soft scale species were eventually lumped into one category, as they are difficult to identify for non-specialists in the field (P.J. Gullan personal communication). Males were not recorded for any species because they are short-lived, and spend a relatively brief time encased and feeding upon the plant. Once they have moulted into adults they are not important to ants. Because each scale species is found on different parts of the plant, the number of adult scale insects on five randomly chosen leaves was also recorded. Due to their dearth and failure to produce honeydew that benefits ants directly, Diaspidids were deleted from the data set; however, *Paratachardina* was included because, although it does not appear to produce visible honeydew, they occurred at high frequency and hence have potential to cause an impact upon their hosts (Howard & Pemberton In Press). Determination of host species range for the main scale groups was based mostly on the island-wide survey described above, but also incorporated the results of haphazard searches during general fieldwork. A plant was considered to be a host species when adult females were established on its branches or leaves.

Scale crawler input and dispersal

The mobile crawlers are the primary mode of dispersal for scale insects, and preliminary observations revealed bright red mobile crawlers were present on almost every individual tree/shrub/herb in the rainforest understory in supercolonies, often in the absence of adult females. Both *Tachardina aurantiaca* and *Paratachardina lobata* produce bright red crawlers, impossible to distinguish in the field. However, given the overwhelming abundance of *Tachardina* compared to *Paratachardina* in the canopy, it was probable that most crawlers were *Tachardina*, and so I recorded them as one species. It appeared that due to the abundance of adult lac scales in the canopy, crawlers were literally raining down from the canopy onto the vegetation below, and continued throughout the year, indicating continuous crawler production.

I quantified this crawler input using 0.45 m² sheets of lab bench protector strung between two tree trunks that caught crawlers as they fell from the canopy ('hammocks'; Fig. 4.3). The upper surface of the sheets was slightly furry, which provided crawlers with something to hold onto, and the underneath was lined with plastic, making them durable and almost water resistant. I suspended six hammocks in variety of both supercolony and non-supercolony sites around the island, in the dry seasons of both 2001 and 2002. Hammocks were spaced at least 10 m apart within sites, and left out over two nights (approximately 48 hours). Crawler input ('rain') from the canopy was recorded as the number of crawlers intercepted by the hammocks per square metre per day. To determine whether crawler input was related to *A. gracilipes* activity on the ground, I conducted crazy ant activity counts (using method described in Chapter 2) at the same sites and time as the hammocks were collected. I used a linear regression to analyse log(x+1)-transformed data (to normalise residuals; SYSTAT ver.10) to determine the relationship between ant activity and crawler input, and to predict crawler input from ant activity.

The "suitability" of forest types as hosts for scale insects outbreaks

Supercolonies of crazy ants may form in forest types II and III more often than expected because their particular mixes of plant species, and the densities at which these species occur, are more favourable to outbreaks of scale insects than the composition of plant hosts occurring in forest type I. I tested this idea by generating a "Scale Suitability Index" (hereafter, SSI) for each of the seven plant sampling sites in uninvaded forest within each forest type (above), and then testing for significant differences in SSI between forest types I, II and III. The SSI is a composite measure, and takes into account host plant identity, the abundance of honeydew-producing scale insects typically found on these host plants within crazy ant supercolonies, and the stem density of the host plants themselves in uninvaded forest. In essence, the SSI is one measure of a site's potential to host an outbreak of honeydew-producing scale insects. The index is calculated for uninvaded sites only, because tree mortality due to scale insects and crazy ants in supercolonies could have biased the data for host tree abundance.

For each site in each forest type ($n = 7$), I multiplied the density of stems of each plant species (stems/ha) by that species mean scale density in crazy ant supercolonies, as determined from the survey above. Stem density for each host species could be calculated from the floristic surveys at each site, because I also measured the area in which I sampled the 200 trees. I then summed this number across all host species at a site, divided it through by 1000 (simply to make it more manageable), to calculate the SSI for each site. I used a one-way ANOVA (forest type as the main factor and 'scale suitability index' as the dependent variable) to test the hypothesis that there was a significant difference in scale insect suitability between forest types.

Verification of forest types

The preceding analyses are based on the assumption that the floristic composition of the forest types was significantly different, as originally described in

qualitative terms by Mitchell (1985). I used the floristic surveys at the 21 uninvaded sites to verify that there were indeed statistically significant differences in floristic composition between the forest types in my study, using non-metric Multi-Dimensional Scaling (nMDS), followed by Analysis of Similarities (ANOSIM; Primer™; (Clarke & Warwick 1994).

Results

Scale insect species, abundance and host breadth

In addition to the six species of Coccoidea previously recorded on Christmas Island, I confirmed the presence of eight additional species that inhabit the rainforest and settled areas on Christmas Island. They included five Coccidae; *Coccus hesperidum* Linnaeus, *Mitviscutulus mangiferae* (Green), *Ceroplastes ceriferus* (Fabricius), *Ceroplastes destructor* Newstead, and *Saissetia coffeae* (Walker); two Diaspididae, *Hemiberlesia palmae* (Cockerill) and *Lindingaspis* sp., and one Kerriidae, *Paratachardina lobata* (Chamberlin) (Table 4.1). Fifteen of the sixteen species were associated with *A. gracilipes* supercolonies were they were tended almost exclusively by crazy ants, but in uninvaded rainforest honeydew-producing Coccoidea were often tended by other ant species including *Camponotus maculatus*, *Tapinoma melanocephalum* and *Pheidole* spp. Only *Icerya purchasi* (cottony cushion scale) was found exclusively in the settled areas of the island, mainly on roses and other exotic garden species.

Scale insects were significantly more abundant in *A. gracilipes* supercolonies than in non-supercolony sites in all three forest types (Fig. 4.4). The mean density of scale insects varied between 2.6 and 8.7 insects/m stem in non-supercolony sites, but from 58.8 to 74.7 insects/m stem in supercolony sites. The differences in scale density were highly significant between supercolony and non-supercolony sites (Table 4.2; Invasion status $p < 0.001$), but not between forest types (Table 4.2; Forest type $p = 0.755$). Within supercolonies, adult lac scales (Kerriidae: 2 species) comprised 52%

of all adult scales recorded, soft scales 44% (Coccidae: ~ 7 species), and non-honeydew producing armoured scales (Diaspididae: ~ 4 species) 4% (Fig. 4.5). *Tachardina* alone made up 28% of all adult female scale insects recorded. Honeydew-producing scales insects (*Tachardina* + Coccidae) comprised more than 72% of all scale insects counted in supercolonies.

Within supercolonies, host plant species differed in their susceptibility to attack by scale insects (Fig. 4.6). Irrespective of forest type, species such as *Inocarpus*, *Pongamia*, *Dysoxylum*, and *Claoxylon* consistently played host to outbreak densities of scale insects, while others, such as *Leea*, *Ochrosia*, *Guettarda* and *Hernandia* were rarely attacked. Further, plant species differed in the suites of scale insect species they hosted, and most species experiencing heavy scale infestations could be classified as either kerrid or coccid hosts (Table 4.3). For example, *Inocarpus*, *Tristiropsis* and *Pongamia* hosted mainly *Tachardina* and *Paratachardina* (both Kerriidae), whereas *Claoxylon* and *Dysoxylum* hosted Coccidae almost exclusively. Of the seven host species that sustained mean densities of scale insects of above 100 individuals/m stem in *A. gracilipes* supercolonies, five of them hosted mainly lac scales, and *Tachardina* in particular.

I found 39 native rainforest and introduced species that were host to scale insects on Christmas Island, including canopy emergents, canopy and sub-canopy trees, and understorey shrubs, herbs and vines (Table 4.3). Only three species, *Syzygium nervosum*, *Inocarpus fagifer* and *Barringtonia racemosa* (all natives) were host to all three families of scale insect (Kerriidae, Coccidae & Diaspididae), and adult female scale insects were found in the highest densities on canopy and sub-canopy trees. Lac scales exhibited the widest native host range of all scale insect species, *Tachardina* found on 29 native plant species and *Paratachardina* on 16; ~ seven species of soft scale together found on 22 native hosts, and Diaspidids on seven hosts.

Scale crawler input and dispersal

Crawlers of lac scales were present on every understorey plant species, regardless of whether adult female lac scales had established on the plant or not; crawlers constituted 62% of the total individuals encountered during the survey (61,224 individuals in *A. gracilipes* supercolonies), and often appeared in densities of >200/m twig. Both species of lac scale (*Tachardina* and *Paratachardina*) have bright red crawlers and were impossible to distinguish in the field. However, given the difference in adult female abundance between these species (see Table 4.3), at least 75% of the crawlers I encountered on the scale hammocks may have been *Tachardina*.

In supercolonies, crawler input ranged from 4.7 - 3100 crawlers/m²/day (mean 194.4 ± 34.4 SE). Because scale hammocks were only out during the dry season, I was unable to detect any seasonality in crawler input from the canopy. However, there were always new crawlers on understorey vegetation, and I continually observed gravid females of *Tachardina*, indicating a constant production of crawlers by lac scale. This indicates that *Tachardina* is multivoltine on Christmas Island. There was a positive and significant relationship between crazy ant activity on the forest floor (quantified using activity cards; see Chapter 2) and the density of crawlers from the canopy per day (Fig 4.7; $F_{1,21}=158.161$, $r^2=0.88$, $p<0.001$).

The "suitability" of forest types as hosts for scale insects outbreaks

There were no significant differences in the SSI between forest types I, II & III (Fig. 4.8, Table 4.4; One-way ANOVA $F=2.567$, $p=0.106$).

Verification of forest types

There were no significant differences between the forest types in their relative species composition of stems ≥ 10 cm DBH (Fig. 4.4 & 4.5). The nMDS ordination showed no clear separation of sites according to forest type, confirmed by the non-significant ANOSIM analysis (Global R = 0.029, ANOSIM $p=0.266$). However, sites

did appear to fall out according to increasing elevation along the x axis of the nMDS plot.

Discussion

Scale insects are able to colonise novel habitats through the dispersal of their highly mobile first instar crawlers, even over extremely long distances (Willard 1974; Hill 1980; Peck 1994; Roque & Causton 1999). However, the majority of species found in this survey have probably been introduced accidentally to Christmas Island over more than a century, since first settlement in 1888. For 12 of the 14 species listed in Table 4.2, their status as introduced by humans can be strongly inferred from their areas of origin, and/or their current worldwide distribution. Many are important agricultural pests in Australia and elsewhere. For example, *Coccus hesperidum*, *Ceroplastes destructor* and *Saissetia coffeae* are all pests in Australia (Waterhouse & Sands 2001), *Ceroplastes ceriferus* is a pest in Japan and North America, and *Paratachardina lobata* is a serious, emerging pest in Florida (Pemberton 2003; Howard & Pemberton In Press). The status of only two species on Christmas Island is unclear; the native range and current distribution of *Lindingaspis* sp. (Diaspididae) are both unknown, whereas it is not known how *Tachardina aurantiaca* (Kerriidae) arrived on Christmas Island (P.J. Gullan, personal communication).

The timing of the introduction to Christmas Island of the newly recorded species is not known. Scale insects were not reported in the first comprehensive account of the fauna of the island (Andrews 1900), and were first reported by Campbell as recently as 1964. He recorded just four species; *T. aurantiaca*, *I. purchasi*, *A. destructor* and *P. pentagona*. *Paratachardina lobata* and soft scales (Coccidae) have only been recorded in recent years (Bellis *et al.* 2000; O'Dowd *et al.* 2003). It is tempting to speculate that the rate of species introductions to the island has escalated over the last few decades, but given their cryptic nature, and the fact that this is the first comprehensive survey for scale insects on the island, makes this uncertain. In any

case, the local population has been importing both food and ornamental plants ever since the island was settled, making it almost certain that many of the species reported here have been present on the island for a long time. Most species recorded in this study had broad host plant ranges. In addition to the 29 host species found on Christmas Island, *Tachardina* has been recorded from 10 other hosts from six families (Veilleux *et al.* 2001). *Paratachardina* has recently been found on more than 200 hosts from 55 families in south Florida (Howard *et al.* 2002; Pemberton 2003), and all seven species of Coccidae are known plant pests with an extensive combined host range, including important fruit and vegetable crops (Ben-Dov & Hodgson 1997).

Outbreaks of scale insects in all forest types were clearly associated with *A. gracilipes* supercolonies. Averaged over sites and forest types, adult scale insects were 12 times more abundant in supercolonies, than in uninfested forest. This is consistent with other reports of elevated scale insect abundance in the presence of high densities of *A. gracilipes* (Haines and Haines 1978a; Lester and Tavite In Press). Undoubtedly, the "nannying" of scale crawlers, and removal of honeydew by *A. gracilipes* have been key factors in the outbreak of scale insects over extremely large areas of otherwise intact forest on Christmas Island (see Chapters 5, 6 and 7). In addition, crazy ants may also protect their scale insect mutualists from natural enemies. Aggressive ant species provide better protection for some species of Coccids (Hanks & Sadof 1990; Buckley & Gullan 1991), and the erratic, frenzied and aggressive behaviour of *A. gracilipes* has been exploited in the past in biological control programs (Entwistle 1972; Room 1973). I rarely observed parasitism of scale insects at any of my sites in crazy ant supercolonies.

The lac scale *Tachardina aurantiaca* comprised the majority (28%) of all scale insects recorded in supercolonies, and on host plants such as *Inocarpus* and *Pongamia*, reached densities of several hundred adults per metre of stem. At these densities, adult females literally encased twigs for their entire length (Fig. 4.11), and whole canopies were infested with scale insects and the crazy ants that tended them. The impact of *Tachardina* on their host trees can be considerable; *Inocarpus* trees in crazy ant

supercolonies experienced significantly higher rates of canopy dieback and mortality, and lower rates of reproduction and seedling recruitment, than trees in uninvaded control sites (P.T. Green unpublished data). Even in non-supercolony sites, the density of adult *Tachardina* females on *Inocarpus* could exceed 80 adults/m. Here, I frequently observed the ants *Camponotus maculatus*, *Tapinoma melanocephalum* and *Pheidole* sp. tending adult scale insects. Interaction with these ant species outside of supercolonies only rarely produced *Tachardina* outbreaks similar in scope and impact to that seen within supercolonies but on a much smaller scale (i.e. on 1-5 trees); at two sites not included in this study, I noted canopy dieback in *Inocarpus* trees tended by large numbers of *Camponotus* that appeared to be nesting in the trees themselves.

Bright red mobile lac scale crawlers (~1 mm long) were present on every surface in *A. gracilipes* supercolonies. Given the relative abundance of *Tachardina* and *Paratachardina* adults, most crawlers were probably of the former species. Variation in the abundance of *A. gracilipes* explained much of the variation in the density of crawlers falling from the canopy (88%), and the mechanism for this is fairly clear. The abundance of lac scale crawlers is obviously correlated with their abundance of adults, which in turn is largely determined by the activities of crazy ants (Chapters 5, 6 & 7). However, elsewhere the abundance crawlers is also influenced by the rate of crawler emergence, temperature and light thresholds (Taylor 1963; Willard 1972; Hill 1980), and wind direction and velocity (Willard 1976), and these factors may also be important on Christmas Island. As described above, the density of adult female lac scales in the canopy, especially *Tachardina*, was sufficiently high in most instances to prevent the settlement of crawlers in the immediate vicinity of their sites of emergence. This would increase their searching time for a settlement position, and may make them more susceptible to being picked up by air movement, creating a high-density inoculum of crawlers to settle on the first obstacle that they encounter. This inoculum explains the presence of crawlers on all understorey vegetation, even in the absence of adult female scales of the same species. Subsequent to landing on a plant, factors that would

enable establishment of the crawlers include host quality and suitability, microclimate conditions and the presence of predators.

Forest types and "suitability" as hosts for scale insect outbreaks

Plant species on Christmas Island differed greatly in the susceptibility to infestation by scale insects (Fig. 4.6). The disproportionately high occurrence of *A. gracilipes* supercolonies in forest types II and III could have been linked to the varying mix of host species in the respective forest types, as measured by the SSI. However, I tested, and rejected, this hypothesis for one fundamental reason. Floristic composition did not differ between forest types, and therefore does not appear to be a determinant of scale insect outbreaks in these forest types, and subsequently of supercolony formation by *A. gracilipes* (see Chapter 7).

Why didn't forest types differ in their SSI? One reason is that floristic composition of trees ≥ 10 cm only did not differ significantly between forest types. For the ant-scale interaction, it is in trees of this size where most of the action happens, so this size class is the right one to investigate. However, the fact that my analysis did not reveal significant differences between forest types is probably a consequence of how the types were defined initially. Many forest classification schemes are based on either species associations, or structural/life form attributes (Webb *et al.* 1974). Mitchell's scheme was neither – he based his on geological substrate. My experience on Christmas Island suggests that relative species abundances are determined to some degree by the substrate, but these differences are not large enough, or consistent enough, for the forest types defined by Mitchell (1985; and based on geology) to differ significantly in their floristics. What it does mean, however, is that all rainforest on Christmas Island is 'suitable' for the colonisation of honeydew-producing scale insects at outbreak densities seen in *A. gracilipes* supercolonies, and that supercolonies can potentially form in any forest type.

Scale insect honeydew as the primary source of energy for A. gracilipes on Christmas Island

One would expect that the honeydew supplied to *A. gracilipes* by scale insect populations at these extreme densities in the canopy would be unlimited, given the constant production of crawlers, profusion of hosts for ongoing adult scale establishment and production of honeydew by the most abundant scale insect species. Studies in New Zealand have shown that honeydew produced by the endemic scale insects *Ultracoelostoma assimile* (Maskell) and *U. brittani* is consumed by birds, lizards and invertebrates (Thomas *et al.* 1990; Beggs 2001), as well as social insects (*Vespula* spp.). The kaka (*Nestor meridionalis meridionalis*), a native parrot, can obtain their daily energy requirement by feeding on honeydew for about three hours (Beggs & Wilson 1991). Furthermore, although highly variable, peak densities of wasps that utilise the honeydew can be up to 370 wasps.m⁻² of tree trunk, and these densities reduced the standing crop of honeydew in *Nothofagus* forests by more than 99% for four months in late summer and autumn (Moller *et al.* 1991). Beggs (2001) suggests that the impact on honeydew removal by introduced invasive ants may be greater than wasps since they are present throughout the year, and often reach extreme densities.

Given the scarcity of extrafloral nectaries in canopy species on Christmas Island, scale insects represent the biggest source of carbohydrates available for *A. gracilipes*, and may make affordable the high foraging tempo exhibited by *A. gracilipes*. Greenslade (1972) suggests that *A. gracilipes* and local hemipteran fauna are mutually adapted in areas where the ant is native (he assumes Africa), and honeydew is the major source of food. In an mutualistic ant-lycaenid system (Cushman *et al.* 1994) suggest that if recruitment to honeydew sources represents an increasing investment in resource acquisition for ants in general (Hölldobler & Wilson 1990), then the honeydew-producing insects not only benefit ant colonies, but may be among the highest quality resources available to them. The abundance on both partners in *A.*

gracilipes supercolonies clearly reveals a successful mutualism operating over huge spatial scales.

It has been hypothesised that tropical rainforest canopy ants, rather than operating as scavengers and predators, derive both carbohydrates and nitrogen primarily from plant and insect exudates (Tobin 1994; 1995; Davidson 1997). Davidson *et al.* (2003), in explaining the disproportionate abundance of ants in tropical lowland rainforest, found that many arboreal ant species from Peru and Brunei obtain little nitrogen through predation and scavenging, and that microsymbionts of ants and their hemipteran partners might play roles in the nutrition of species that specialise in N-poor exudates. This idea adds another trophic level to the positive interactions that may facilitate the abundance of honeydew-feeding ants, and involves plants, sap-sucking hemipterans, microsymbionts and ants. However, it also emphasises the importance of positive associations in complex multi-trophic systems. Whereas the associations between tropical rainforest canopy ants, their hemipteran partners and microsymbionts may have evolved over time, Helms and Vinson (2002) point out that the association between introduced *Solenopsis invicta* and the introduced invasive mealybug *Antoninoides graminis* in the southeast United States is fortuitous and not the result of a long-term coevolution between species. Similarly, the mutualism between *A. gracilipes* and scale insects on Christmas Island would also seem fortuitous, given that many of the scale insects now present on Christmas Island evolved in areas far removed from the hypothesised native range of *A. gracilipes*. This system represents such a facultative mutualism, which are being increasingly recognised as key drivers of many invasions worldwide (Simberloff & Von Holle 1999; Richardson *et al.* 2000; Helms & Vinson 2002; Helms & Vinson 2003; O'Dowd *et al.* 2003).

Table 4.1 – General attributes of Coccoidea recorded on Christmas Island, Indian Ocean.

Family/ Species	Common Name	Honeydew producer	Habitat ^ψ	Origins	Current worldwide distribution ^φ
Coccidae					
<i>Coccus celatus</i> De lotto		+	Forest	Native to Africa ^a	Tropical regions
* <i>Coccus hesperidum</i> Linnaeus	Brown soft scale	+	Forest	Native to ?Sth Africa ^b	Cosmopolitan
* <i>Milviscutulus mangiferae</i> (Green)	Mango shield scale	+	Forest	Unknown	Worldwide in tropics
* <i>Ceroplastes ceriferus</i> (Fabricus)	Indian wax scale	+	Forest	Native to Sth America ^b	Cosmopolitan
* <i>Ceroplastes destructor</i> Newstead	White wax scale	+	Forest	Native to Africa ^b	Australasia, Africa, Mexico
<i>Saissetia ?oleae</i> (Olivier)	Black scale	+	Settlement/ forest	Native to Africa ^b	Cosmopolitan
* <i>Saissetia coffeae</i> (Walker)	Hemishperical scale	+	Settlement/ forest	Native to Africa ^b	Tropical Old & new World
Diaspididae					
<i>Aspidiotus destructor</i> (Signoret)	Coconut scale	-	Settlement/ forest	Unknown	Tropical & sub-tropical regions
<i>Pseudaulacaspis pentagona</i> (Gossard?)	White peach scale		Settlement/ forest	Native to China ^c	Cosmopolitan
* <i>Hemiberlesia palmarum</i> (Cockerell)	Tropical palm scale	-	Forest	?	Cosmopolitan
* <i>Lindingaspis</i> sp.	?	-	Forest/ settlement	?	?
Kerriidae					
<i>Tachardina aurantiaca</i> (Cockerell)	Yellow lac scale	+	Forest/ settlement	SE Asia (?)	Tropical Old & New World
* <i>Paratachardina lobata</i> (Chamberlin)	Bishop's hat lac scale	+	Forest	Native to India & Sri Lanka ^d	Tropical Old & New World
Margarodidae					
<i>Icerya purchasi</i> (Maskell)	Cottony cushion scale	-	Settlement	Native to Australia ^e	Cosmopolitan

Notes & Sources:

ψ – Habitat occurring first is the primary habitat species is found on Christmas Island. * - Indicates those species that I have added to the list during this study.

φ – 'Cosmopolitan' refers to distribution including Africa, Australasia, mainland Asia, North and South America and Europe.

a – Williams (1982)

b – Waterhouse & Sands (2001)

c – Gossard (1901)

d – Pemberton (2003)

e – Maskell (1878)

Table 4.2. Two-way ANOVA showing the effect of forest type and invasion status by crazy ants on the density of scale insects. N = 5 for each treatment combination.

Source of variation	SS	df	MS	F-ratio	p-value
Invasion Status	0.040	1	26837.4	35.82	< 0.001
Forest type	0.159	2	213.2	0.28	0.755
Type x Status	0.000	2	182.8	0.24	0.785
Error	0.512	24	749.2		

Table 4.3 – The densities (number/m of stem) of each scale insect type on host species in *A. gracilipes* supercolonies. Table also indicates the presence or absence of extrafloral nectaries.

Plant type & Family	Species	<i>Tachardina aurantiaca</i>	<i>Paratachardina lobata</i>	<i>Coccidae</i>	<i>Diaspididae</i>	No. scale species/host	Extrafloral nectaries?
Emergent canopy trees							
Myrtaceae	<i>Syzygium nervosum</i>	0.06 ± 0.06	55.62 ± 9.86	28.12 ± 12.48	*	4	
Sapotaceae	<i>Planchonella nitida</i>	0.76 ± 0.41	2.50 ± 1.45	35.16 ± 11.20		3	
Hernandiaceae	<i>Hernandia ovigera</i>	4.26 ± 3.63	7.22 ± 3.95		*	3	
Canopy trees							
Fabaceae	<i>Inocarpus fagifer</i>	120.21 ± 22.36	8.26 ± 3.11	*	*	4	
Lecythidaceae	<i>Barringtonia racemosa</i>	1.52 ± 0.80	10.28 ± 3.76	0.67 ± 0.26	*	4	
Combretaceae	<i>Terminalia catappa</i>	66.67 ± 29.77	11.25 ± 7.25	4.53 ± 4.05		3	✓
Moraceae	<i>Ficus microcarpa</i>	27.96 ± 14.07	9.57 ± 3.15	4.56 ± 4.03		3	
Lauraceae	<i>Cryptocarya nitens</i>	*	51.60 ± 13.51			2	
Sapindaceae	<i>Tristiropsis acutangula</i>	81.94 ± 19.32	17.75 ± 7.32			2	
Meliaceae	<i>Dysoxylum gaudichaudianum</i>	*		174.56 ± 65.57		2	
Fabaceae	<i>Pongamia pinnata</i>	234.58 ± 96.25				1	
Fabaceae	<i>Erythrina variegata</i>	*				1	✓
Euphorbiaceae	<i>Macaranga tanarius</i>	*				1	✓
Ulmaceae	<i>Celtis timorensis</i>	2.47 ± 1.30	78.38 ± 20.20	0.08 ± 0.08		1	
Clusiaceae	<i>Calophyllum inophyllum</i>				*	1	
Rubiaceae	<i>Guetterda speciosa</i>			0.03 ± 0.03		1	
Urticaceae	<i>Dendrocnide peltata</i> var. <i>murrayana</i>	7.78 ± 0.00		1.67 ± 0.00		1	
Tiliaceae	<i>Berrya cordifolia</i>					0	

Plant type & Family	Species	<i>Tachardina aurantiaca</i>	<i>Paratachardina lobata</i>	<i>Coccidae</i>	<i>Diaspididae</i>	No. scale species/host	Extrafloral nectaries?
Sub-canopy trees							
Euphorbiaceae	<i>Claoxylon indicum</i>	3.73 ± 1.49		128.60 ± 31.26		2	✓
Leeaceae	<i>Leea angulata</i>		1.44 ± 1.12			1	
Sterculiaceae	<i>Kleinhovia hospita</i>	127.08 ± 91.25				1	
Caesalpiniaceae	<i>Cynometra ramiflora</i>	*				1	
Urticaceae	<i>Dendrocnide sinuata</i>	1.17 ± 1.17	0.56 ± 0.56	4.93 ± 4.46		2	
Apocynaceae	<i>Ochrosia ackeringae</i>			17.40 ± 8.76	*	2	
Moraceae	<i>Ficus saxophila</i>	*				1	
Nyctaginaceae	<i>Pisonia unbellifera</i>			4.89 ± 3.22		1	
Pittosporaceae	<i>Pittosporum ferrugineum</i>			*		1	
Rutaceae	<i>Acronychia trifoliolata</i> var. <i>trifoliata</i>	*				1	
Oleaceae	<i>Ligustrum glomeratum</i>					0	
Meliaceae	<i>Melia azedarach</i>					0	
Shrubs, vines and herbs							
Rubiaceae	<i>Aidia aff. racemosa</i>	***	**	***		3	
Myrsinaceae	<i>Ardisia colorata</i>	*	*	*		3	
Euphorbiaceae	<i>Callicarpa longifolia</i>	*		*		2	✓
Sapindaceae	<i>Allophylus cobbe</i>	83.33 ± 1.67	9.17 ± 2.50	34.17 ± 30.83		2	
Moraceae	<i>Maclura cochinchinensis</i>	*		*		2	
Boraginaceae	<i>Carmona retusa</i>	*				1	
Euphorbiaceae	<i>Croton caudatus</i>	*				1	
Euphorbiaceae	<i>Alchornea rugosa</i>		*			1	
Orchidaceae	<i>Corymborkis veratrifolia</i> var. <i>veratrifolia</i>			*		1	
Asclepiadaceae	<i>Hoya aldrichii</i>				*	1	

Plant type & Family	Species	<i>Tachardina aurantiaca</i>	<i>Paratachardina lobata</i>	<i>Coccidae</i>	<i>Diaspididae</i>	No. scale species/ host	Extrafloral nectaries?
<i>Lamiaceae</i>	<i>Leucas flaccida</i>	*				1	
<i>Araliaceae</i>	<i>Schefflera elliptica</i>			*		1	
<i>Celastraceae</i>	<i>Celastrus paniculatus</i>					0	
<i>Combretaceae</i>	<i>Combretum acuminatum</i>					0	
<i>Menispermaceae</i>	<i>Pachygone ovata</i>					0	
Total host species		29	16	22	7		

Table 4.4 – One-way ANOVA statistics showing no significant effect of forest type on the scale suitability index. Forest type is the main factor and scale suitability index the dependent variable.

Source of variation	SS	df	MS	F-ratio	p-value
Forest type	234.978	2	117.489	2.567	0.106
Error	777.992	17	45.764		

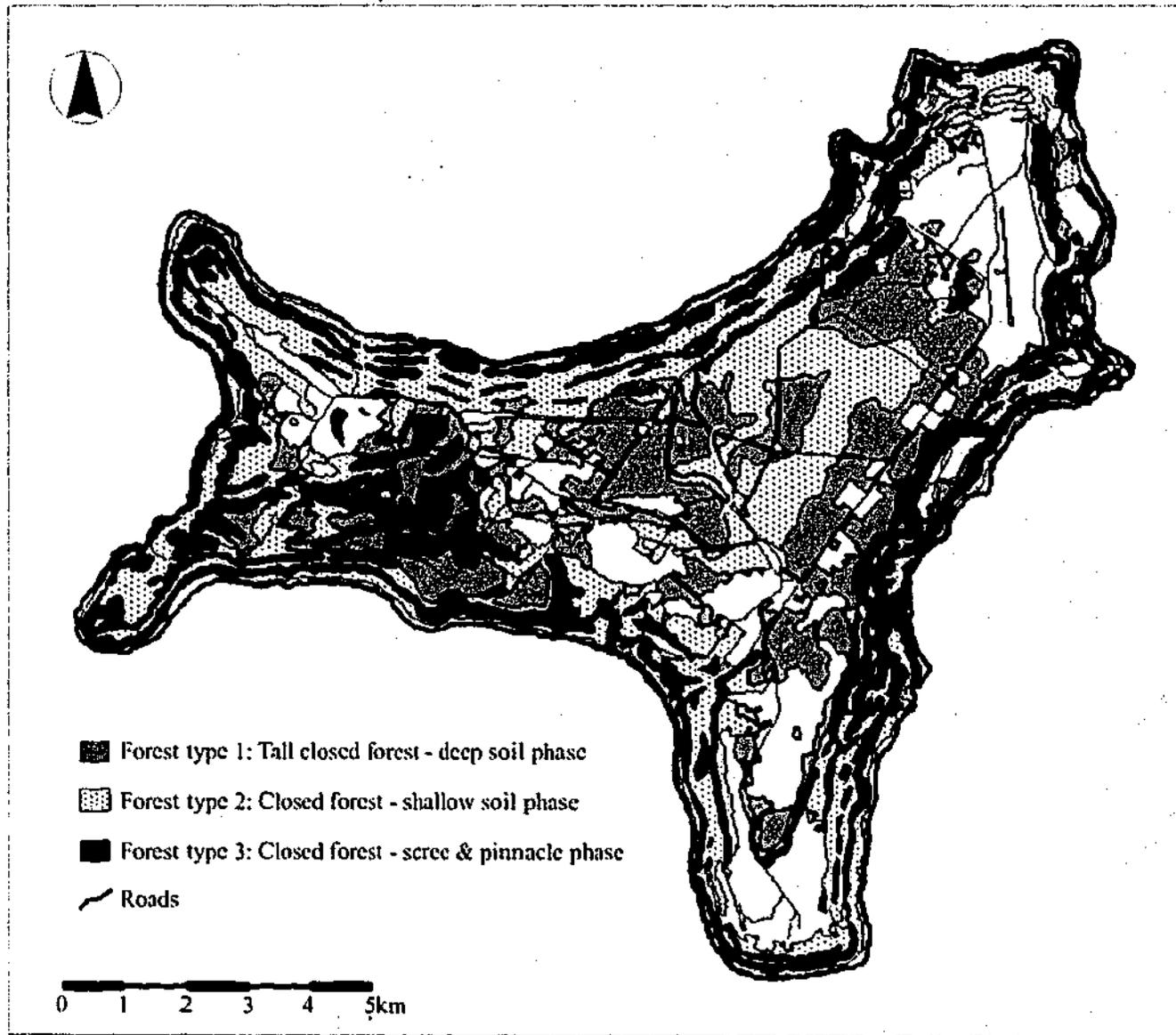


Figure 4.1 – Distribution of the tree main forest types according to Mitchell (1985).
Open areas are clearings mined for phosphate.

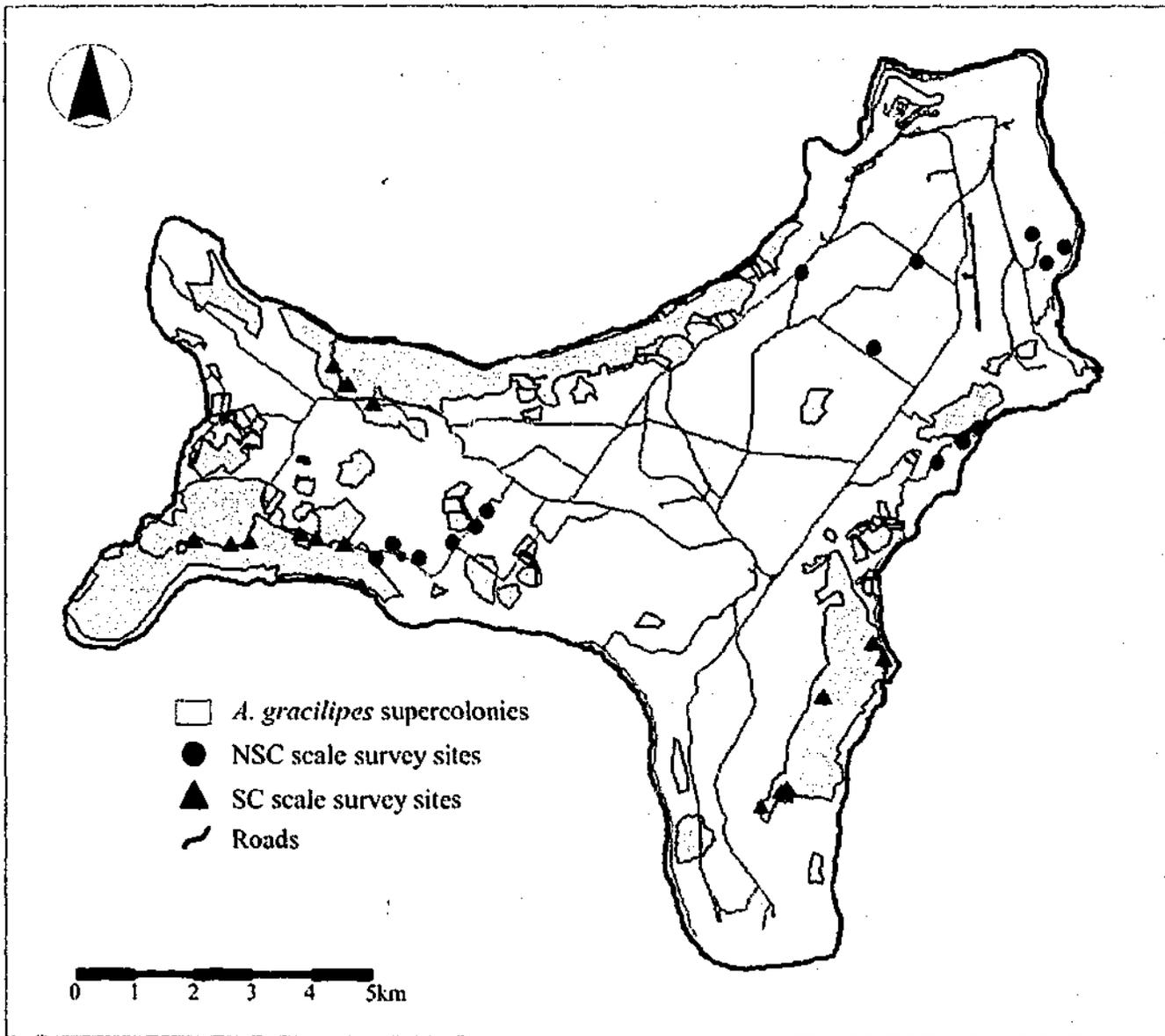


Figure 4.2 – Locations of sampling sites in *A. gracilipes* supercolony (SC) and non-supercolony (NSC) areas, indicating five clusters of sites in forest types I, II & III for each treatment.



Figure 4.3 – Scale “hammock” strung between tree trunks in rainforest to catch lac scale insect crawlers from the canopy. Crawlers remained on the upper furry side of the hammocks, enabling accurate counts.

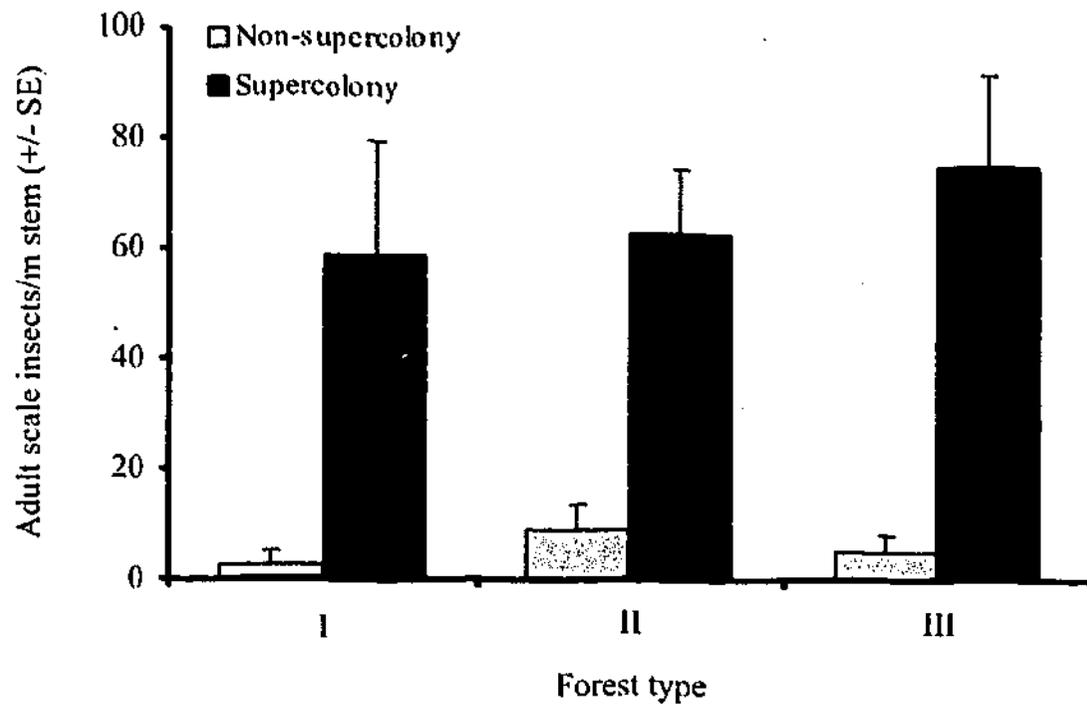


Figure 4.4 - The mean density of adult scale insects per metre of stem by forest type and invasion status (supercolony vs non-supercolony).

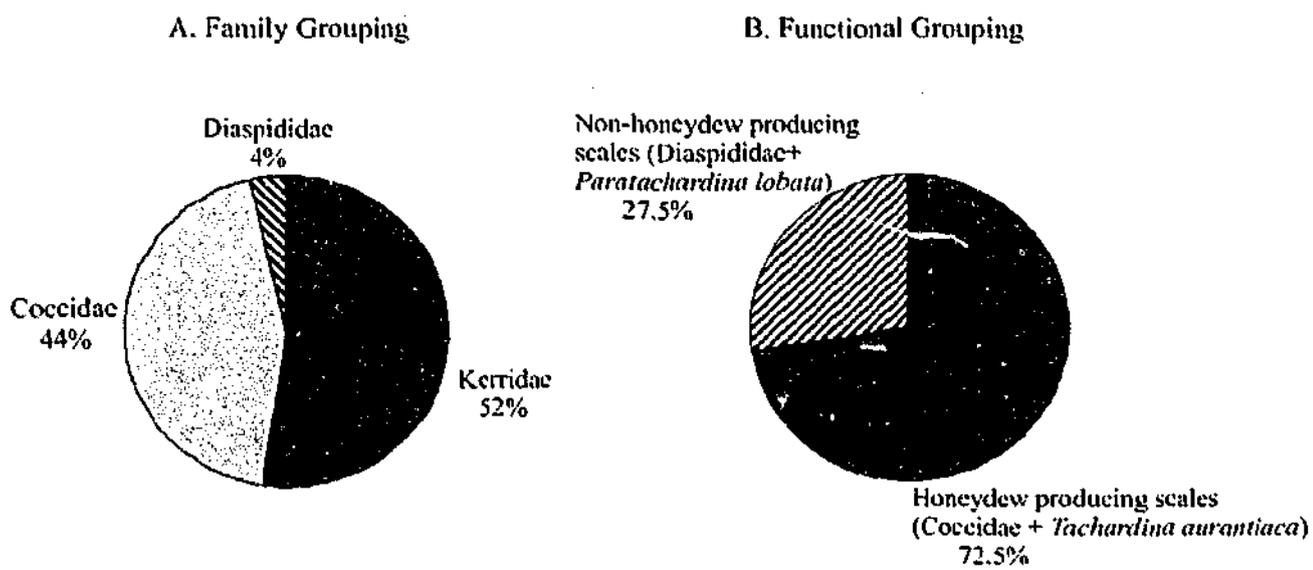
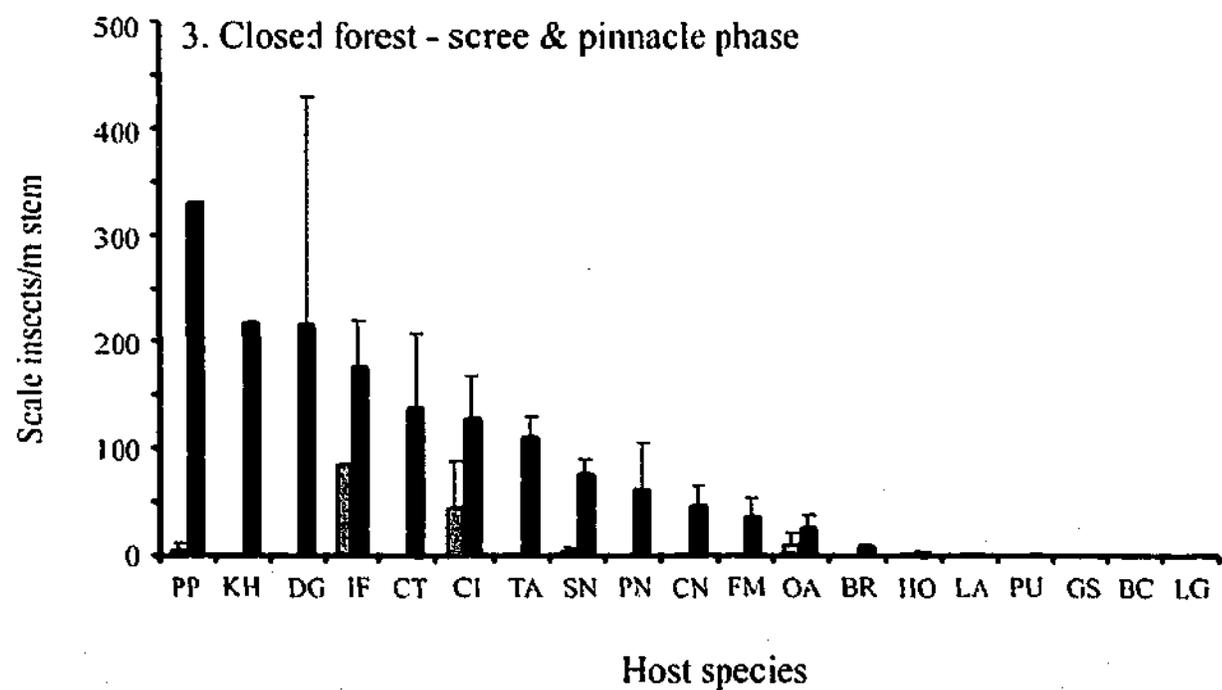
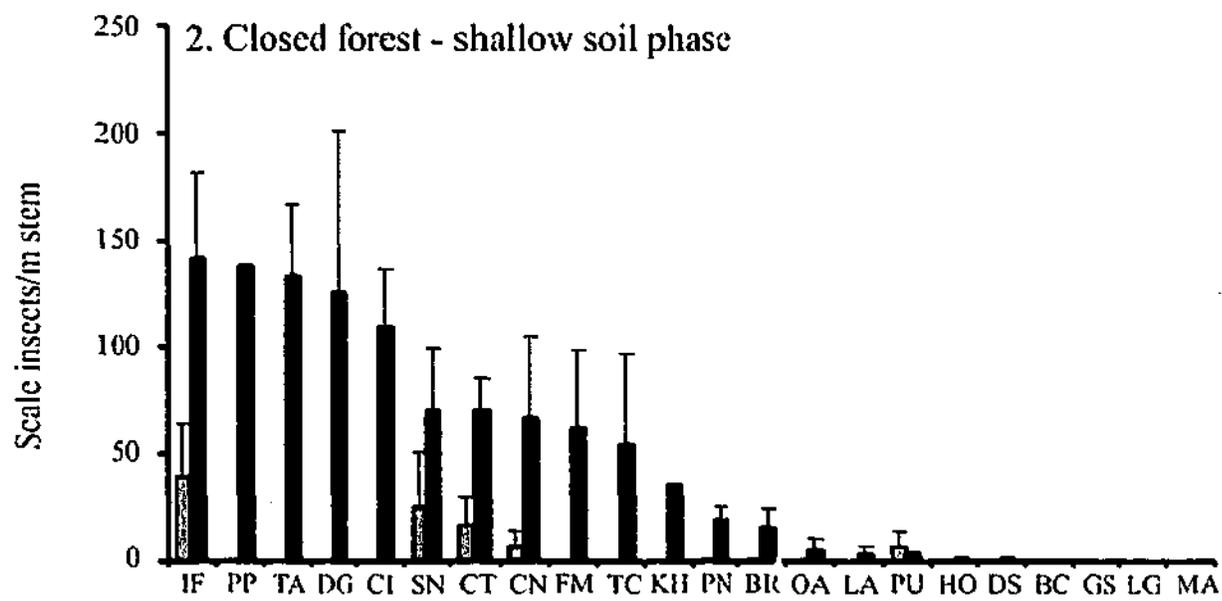
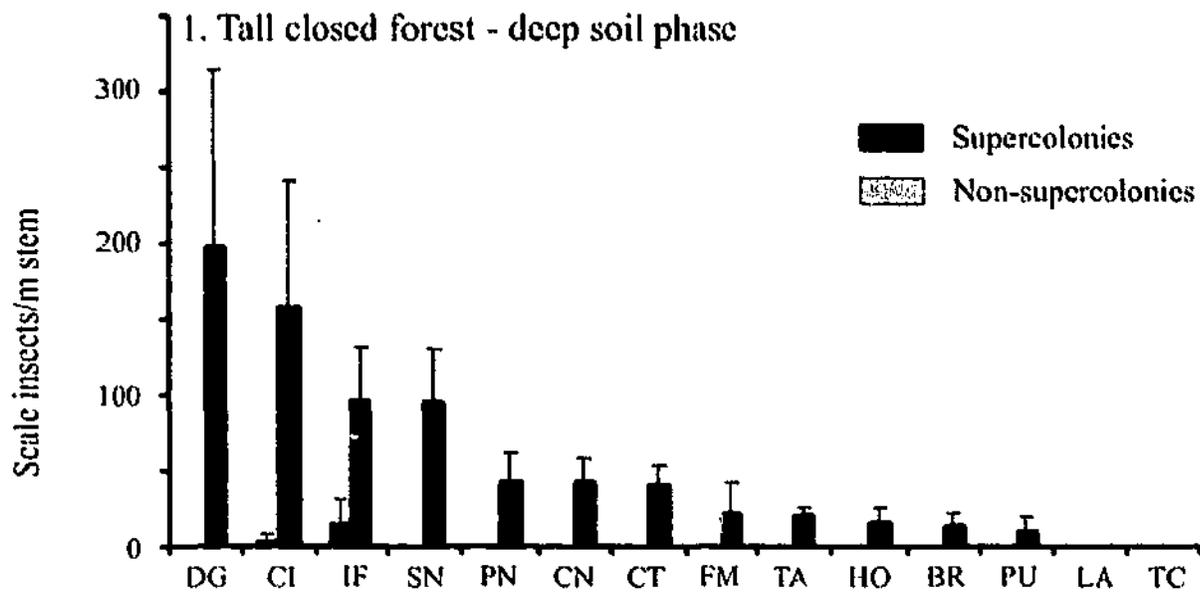


Figure 4.5 – Proportion of a total of 22348 female adult scale insects in *A. gracilipes* supercolonies surveyed in rainforest, Christmas Island: by family grouping (A.) and functional grouping (B.). *Paratachardina lobata* comprised 24% of the 52% Kerriidae.

Figure 4.6 (following page) – The mean density (\pm SE) of all adult female scale insect species combined (excluding Diaspididae) on native hosts within *A. gracilipes* supercolonies (black bars) and in non-supercolony sites (grey bars). Columns with no error bar indicate only one sample was taken.

Codes for plant species: AC: *Allophylus cobbe*, BC: *Berrya cordifolia*, BR: *Barringtonia racemosa*, CI: *Claoxylon indicum*, CN: *Cryptocraya nitens*, CT: *Celtis timorensis*, DG: *Dysoxylum gaudichaudianum*, DS: *Dendrocnides sinuata*, DP: *Dendrocnides peltata*, FM: *Ficus microcarpa*, GS: *Guettarda speciosa*, HO: *Hernandia ovigera*, IF: *Inocarpus fagifer*, KH: *Kleinhovia hospita*, LA: *Leea angulata*, LG: *Ligustrum globulatum*, MA: *Melia azedarach*, OA: *Ochrosia ackeringae*, PU: *Pisonia umbellifera*, PN: *Planchonella nitida*, PP: *Pongamia pinnata*, SN: *Syzygium nervosum*, TA: *Tristiropsis acutangula*, TC: *Terminalia catappa*.



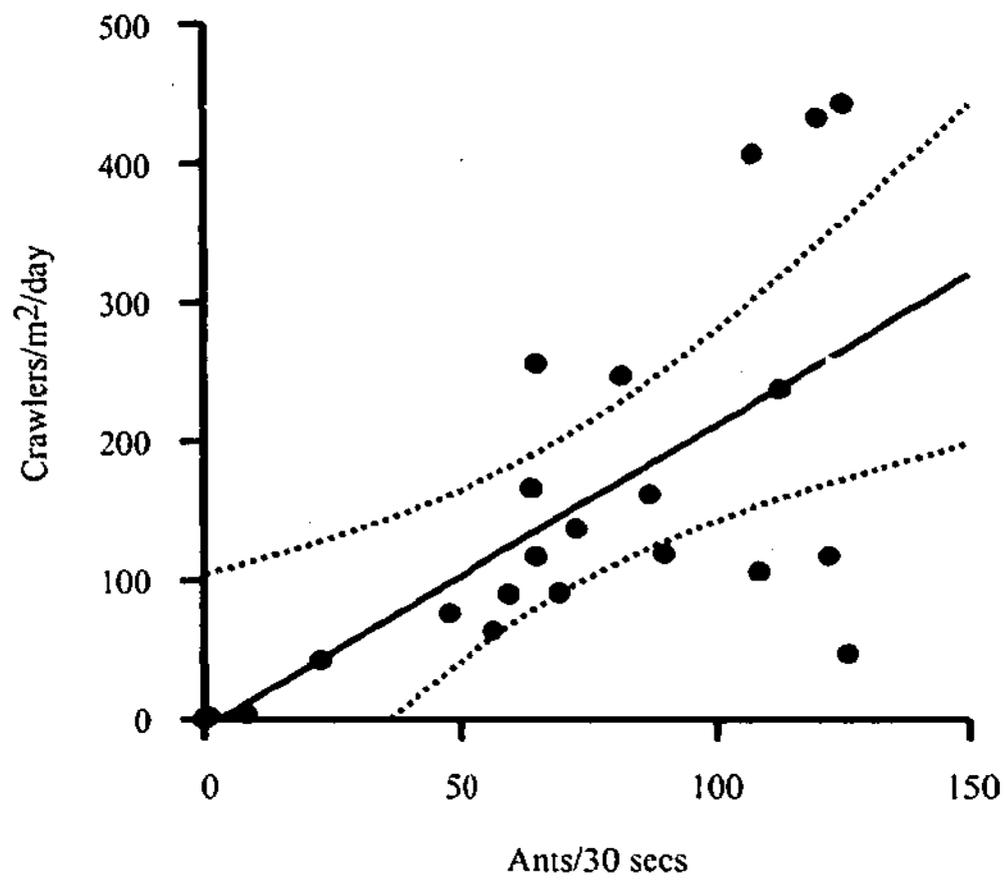


Figure 4.7 – Relationship between ant activity on the forest floor and crawler input as measured by the number of crawlers falling from the canopy/m²/day. As *A. gracilipes* activity increased, so did the density of the crawler inoculum.

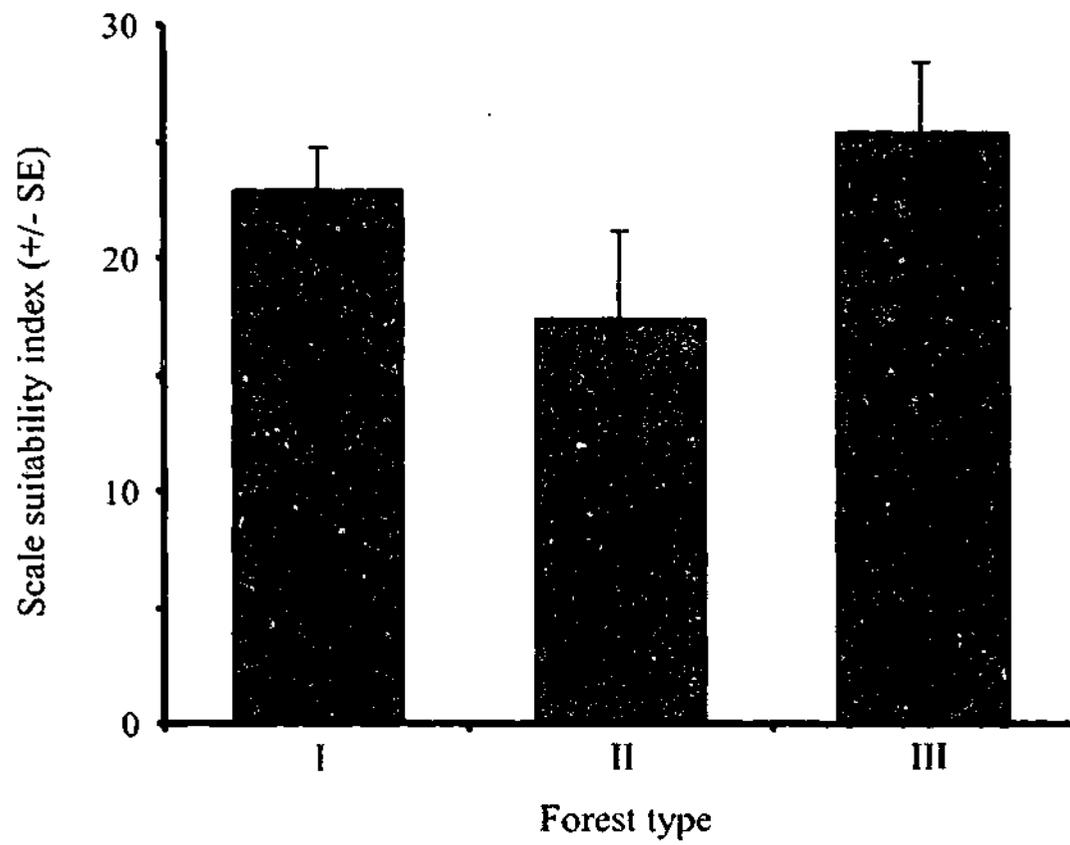


Figure 4.8 – Scale suitability index by forest type. The index is a composite number representing the suitability of a forest type to support honeydew-producing scale insects in the presence of *A. gracilipes*. It takes into account the mean density of all scale insect species on each host species in *A. gracilipes* supercolony areas and the stem density of those hosts in intact forest.

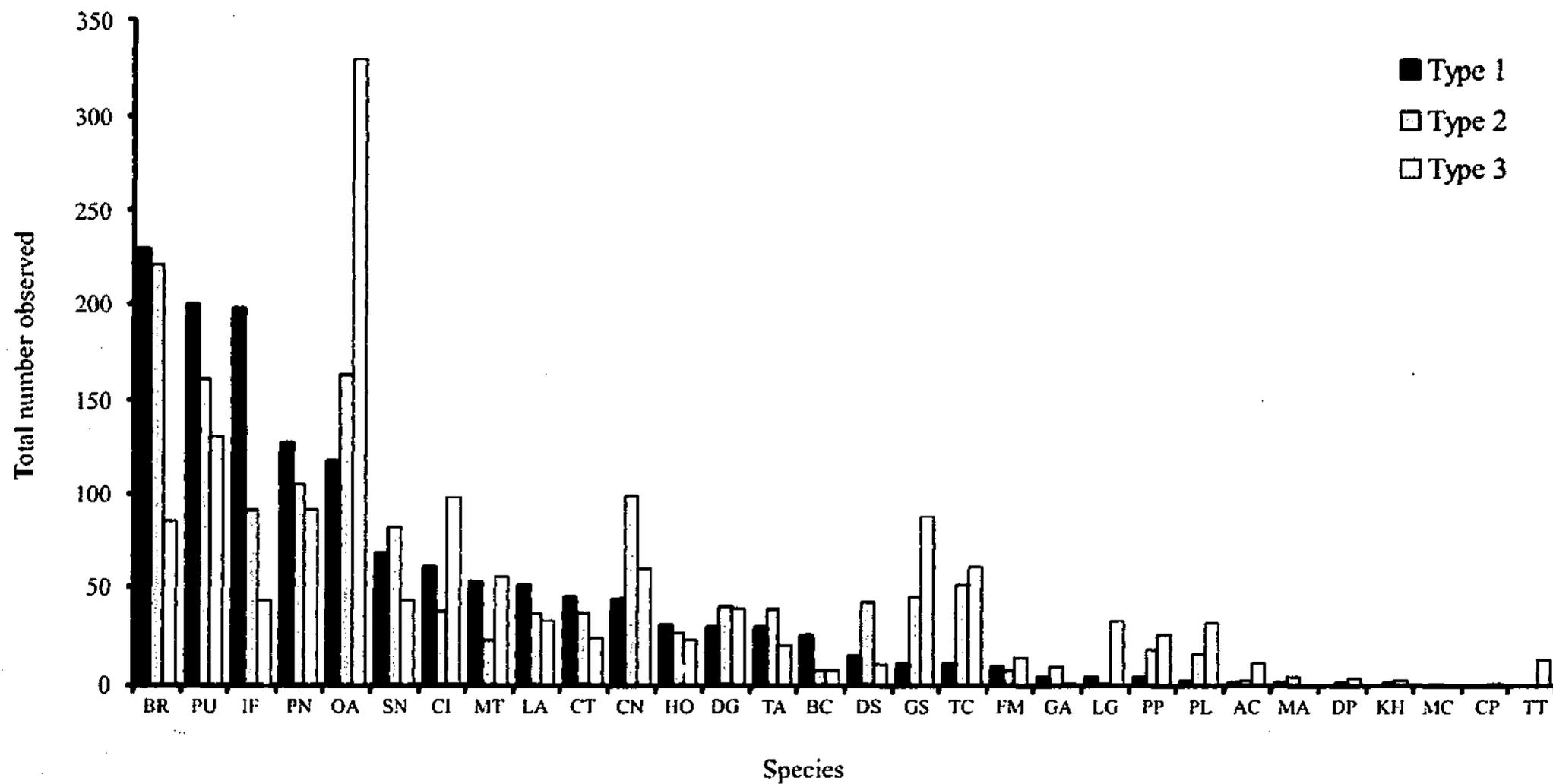


Figure 4.9 – Total number of each plant species observed in each forest type. Species codes as for Fig. 4.6.

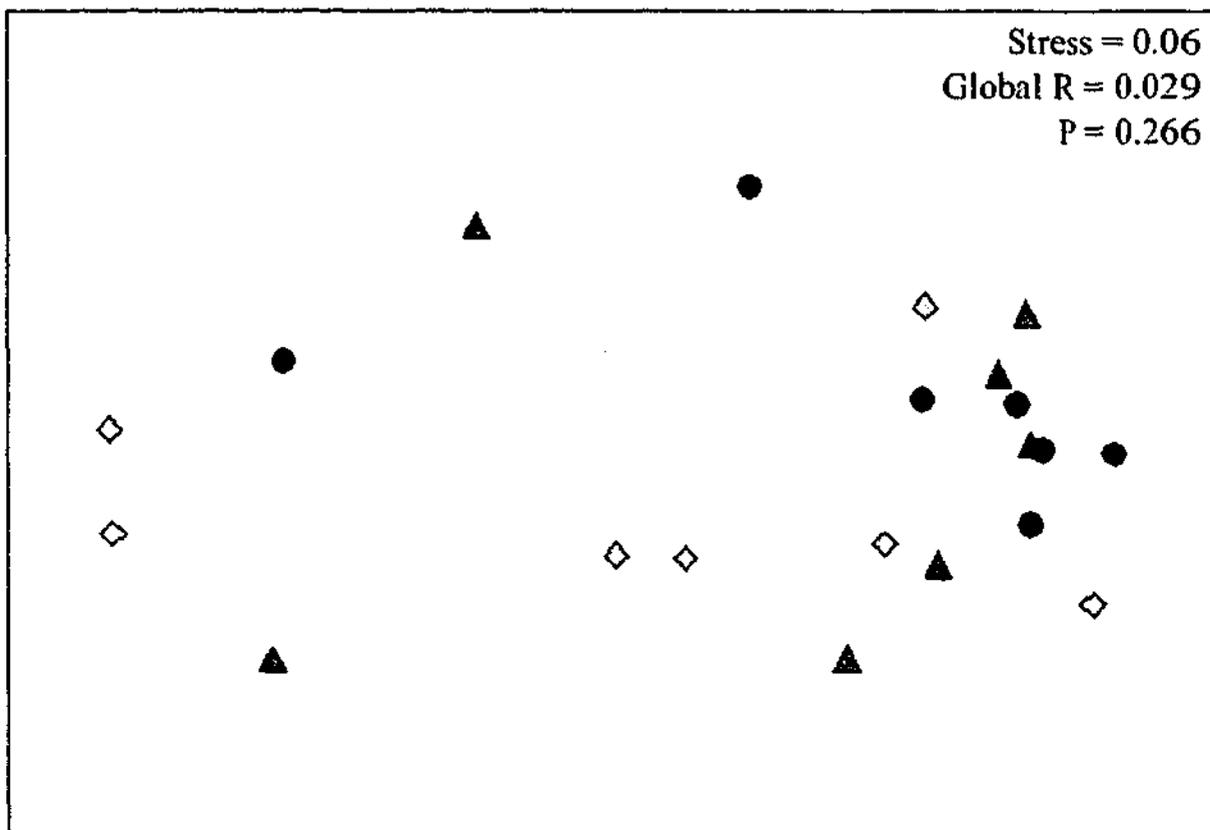


Figure 4.10 – Non-metric multidimensional scaling of floristic composition of forest types, using $\log(x+1)$ -transformed data. ● – forest type I; ▲ - forest type II; ◇ - forest type III. N=7 for each forest type.

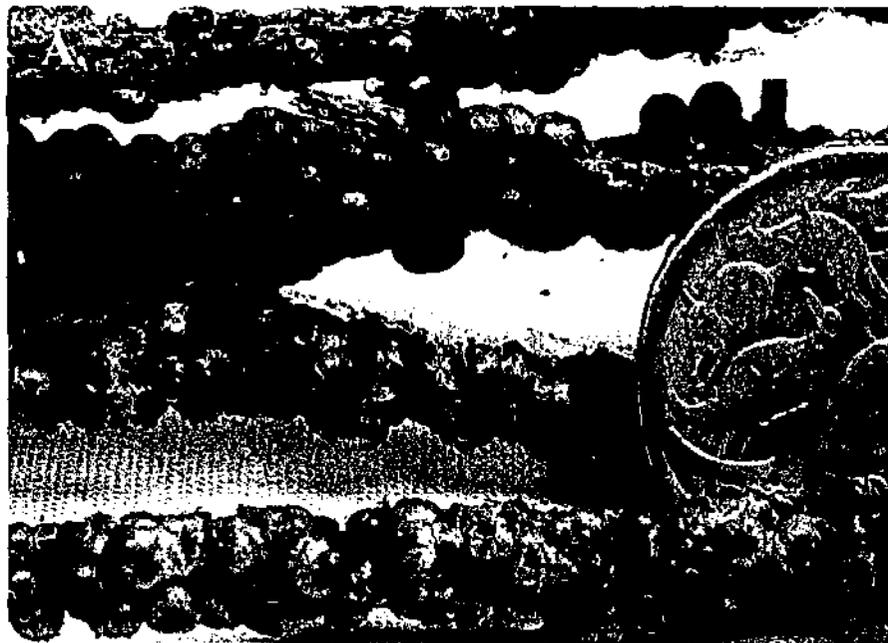


Figure 4.11 – Examples of the densities of *Tachardina aurantiaca* on *Inocarpus fagifer*. Top photo (A.) shows an AU\$1 coin for scale, and includes dead adult female scale insects, those starting to accumulate fungus and healthy adult females. The bottom photo (B.) indicates the densities of healthy adult female *Tachardina* in the presence of *A. gracilipes* in the field.

Chapter Five

Yellow crazy ants, *Anoplolepis gracilipes*, provide differential benefits to scale insect life stages in a multi-species complex in the rainforest understorey.



Introduction

The presence, and often the significance, of mutualisms in ecology has been widely acknowledged (Flanders 1951; Way 1963; Janzen 1985; Buckley 1987b; Richardson *et al.* 2000; Bronstein 2001; Helms & Vinson 2002; 2003). It has been suggested that every organism on earth is probably involved in at least one mutualism during its lifetime (Janzen 1985). Mutualisms may be key processes in the successful invasion by alien species, where impacts by one, or both partners are diversified and amplified (Simberloff & Von Holle 1999; Richardson *et al.* 2000; O'Dowd *et al.* 2003), but considering their ubiquity, positive interactions in general have not been given the attention they deserve in invasion literature (Richardson *et al.* 2000; Helms & Vinson 2002; Holway *et al.* 2002; Helms & Vinson 2003) and in ecological theory in general (Stachowicz 2001; Bruno *et al.* 2003).

Invasive ants have a propensity to form close associations with hemipteran plant pests, confirmed as mutually beneficial interactions (Helms & Vinson 2002; Holway *et al.* 2002), often in multi-species complexes (Bristow 1984). Because of their extensive distribution and escalating rates of introduction, invasive ants represent a significant threat to natural and agricultural ecosystems, largely due to their capacity to displace invertebrates, disrupt native ant communities (Erickson 1971; Porter & Savignano 1990; Cole *et al.* 1992; Lee *et al.* 1994; Human & Gordon 1996; Holway 1998;

Hoffmann *et al.* 1999) and, thereby, to possibly disrupt the role of keystone species within ecosystems (O'Dowd *et al.* 2003). Mutualistic associations are frequently facultative and non-specific (Addicott 1978; Buckley 1987a), and need not arise through a coevolutionary history (Simberloff & Von Holle 1999). Many ant-hemipteran associations, in particular, are formed in one, or both partner's introduced range (Helms & Vinson 2002, O'Dowd *et al.* 2003). Their associations are generally facultative mutualisms, where each partner is not necessarily wholly dependent upon the other, but both members benefit by the presence of one another. Essentially, aphids, membracids, coccids and other insects provide excretory honeydew to the ants, primarily as a source of carbohydrates and amino acids, in return for protection from predators and increased sanitation by removing honeydew, which might otherwise asphyxiate them (Nixon 1951; Bach 1991; Gullan 1997). Associations often involve several species, and families of both ants and hemipterans (Bristow 1984; Addicott 1986; Bishop & Bristow 2001), and interactions between different species can vary in the efficiency with which they provide benefits for their mutualists (Addicott 1979; Bristow 1984) and subsequently influence population growth (Gullan 1997; Itioka & Inoue 1999). In some instances, the presence of certain species of mutualist ants is critical for the population growth of their hemipteran partners (Itioka & Inoue 1999).

On Christmas Island, the introduced yellow crazy ant, *Anoplolepis gracilipes*, forms mutualisms with scale insects in areas of supercolony formation. In September 2002, *A. gracilipes* supercolonies were present in approximately 25% of the island's 10,000 hectares of forest (Green *et al.* 2004) and in these areas the density of scale insects was 16-fold higher than those areas where crazy ants were absent (Chapter 4, this thesis). The association between crazy ants and scale insects was ubiquitous in the understorey on seedlings and saplings of rainforest trees where crazy ants were by far the most abundant tenders of hemipterans. In some supercolonies, the increase in seedling density in the understorey provided scale insects with 30 times the number of individual seedlings on which to establish populations (O'Dowd *et al.* 2003). In addition, the trunks of forest trees supported large numbers of crazy ant traffic to the

canopy, suggesting that the ants gained substantial benefit in the canopy of these trees – honeydew from scale insects on a grand spatial scale.

Many small-scale ant exclusion experiments have shown that, usually, hemipteran populations escalate in the presence of ant attendants (de Fluiter 1939; Way 1953; Bach 1991; Helms & Vinson 2002) and that sometimes they decline in the absence of ants (Jutsum *et al.* 1981; Bristow 1984). I was interested in the population response of scale insects due to the attendance of *A. gracilipes* in supercolonies, on understorey vegetation, and a snapshot of the cascade of effects that result from the association between invasive ants in extreme densities and honeydew-producing scale insects.

One of the most commonly cited, and tested, mechanisms responsible for the positive influence ants exert on scale insects is the protection from natural enemies (Bach 1991; James *et al.* 1997; Morales 2000). I observed no parasitism of any scale species on Christmas Island, in supercolonies or intact rainforest. Furthermore, mobile newly emerged crawlers are the only life stage vulnerable to predation by generalist invertebrate predators. I was interested in whether generalist invertebrate predators were displaced from *A. gracilipes* supercolony areas, essentially releasing crawlers from pre-establishment predation. If *A. gracilipes* were able to displace generalist predators, presumably it would include herbivores (Buckley 1983; Bach 1991), in which case their displacement from the plant may benefit the host plant (Room & Smith 1975; Messina 1981).

In addition to the benefit ants may confer the host plant by removing herbivores, the removal of excess honeydew from adult scale insects by ants would decrease the probability of the build up of sooty moulds (Capnodiaceae), and prevent reduction in photosynthesis (Haleem 1984; Wood *et al.* 1988) and even plant death resulting from the combination of pathogenic effects of the scale insects plus the effect of the sooty moulds (Mibey 1997; Vranjic 1997). The presence of sooty moulds is always a significant addition to the damage caused by the associated insect pests (Mibey 1997).

In this chapter I investigate the dynamics of the *A. gracilipes*-scale mutualism across a suite of host species, on a relatively small-scale, looking at the effect of ants on scale insects in the understorey where I could experimentally exclude *A. gracilipes* from individual saplings. I tested three hypotheses.

1. That the abundance of all distinguishable life stages of scale insects on four species of host saplings would be higher on plants accessible to *A. gracilipes* than those where *A. gracilipes* was excluded.
2. That the number of generalist predators on individual saplings would be lower on plants accessible to *A. gracilipes* than where *A. gracilipes* was excluded.
3. That relative plant growth, as a coarse measure of plant performance, would be greater on plants accessible to *A. gracilipes* than where *A. gracilipes* was excluded.

Methods and Materials

The Study Site

This study was conducted on Christmas Island (105° 40'E, 10° 30'S), an oceanic limestone island that lies 360 km south of Java in the northeastern Indian Ocean. The island is located in the humid tropics, and experiences a monsoonal climate where most of the annual 2000 mm rainfall occurs between December and May (Falkland 1986). Presently, approximately 74% of the island is covered with natural vegetation, where three of seven described forest types (Mitchell 1985) dominate both plateau and terrace areas, which correspond generally to geological substrate type (Barrie 1967). The first supercolony of the yellow crazy ant, *A. gracilipes*, was observed in 1989, where ants were in densities sufficient to kill the red land crab, *Gecarcoidea natalis* (Brachyura: Gecarcinidae). Since then supercolonies have established across the island, occupying areas of between ~5 ha – 900 ha. Data for this ant exclusion experiment were collected between November 2000 and September 2001

at one site (WAD) in the northeastern section of Christmas Island, in an *A. gracilipes* supercolony, where ant activity was sufficient to kill red crabs.

Effect of A. gracilipes on scale insect life stages on saplings

In November 2000, 14, 11, 15 & 11 pairs of saplings from *Inocarpus fagifer*, *Tristiropsis acutangula*, *Claoxylon indicum* and *Dysoxylum gaudichaudianum*, respectively, were tagged for monitoring of different scale insect life stages at WAD. This supercolony of crazy ants was estimated to be approximately five years old (P.T. Green, personal communication). The species were chosen to represent a range of species that hosted lac scales, *Tachardina aurantiaca*, and soft scales, Coccidae. They ranged in height from 1.30 – 3.50 m. Each pair of saplings was located within three metres of each other, and one sapling in each pair was assigned the ant exclusion treatment and one the control treatment, allowing ants free access. Saplings assigned the exclusion treatment had all ants removed by hand, and foraging ants were excluded with a band of Tanglefoot[®] applied onto a sleeve at the base of the plant stem that was checked regularly to ensure continued efficacy (Fig. 5.1). To supplement the efficacy of Tanglefoot[®] barriers, I clipped surrounding vegetation back to eliminate any connections to the experimental saplings. During the experiment it was impossible to prevent ants from falling from the canopy onto plants and individual crazy ants were sometimes found on ant exclusion saplings.

I commenced sampling immediately upon ant removal and treatment application, on December 5 2000 (day 0), and subsequent samples were taken on days 17, 36, 66, 84, 175 & 280 for *Inocarpus*, up to day 175 for *Tristiropsis*, day 84 for *Claoxylon* and *Dysoxylum*. To estimate scale insect density, I haphazardly chose three branches on each sapling, where I counted individual scale insects in a marked 5 cm section of stem for the duration of the experiment. The mean numbers of individual scale insects of each life stage from the three stems were used in analysis; sapling pairs were used as replicates.

Hosts were more susceptible to infestation by different scale species; so different species were recorded on the various hosts. *Inocarpus* and *Tristiropsis* were colonised primarily by the lac scale, *Tachardina aurantiaca*, and *Claoxylon* and *Dysoxylum* were colonised by a suite of indistinguishable soft scales in the family Coccidae. Individual crawlers of soft scales were difficult to count, but once they became sessile and produced white waxy spiracles as 2nd instars they were easier to see. Each host species also had distinctly different architecture and hence places for scales to colonise. On *Inocarpus* I counted individual crawlers, newly sessile 2nd instars, adult females, and males of *Tachardina* in 5 cm sections at the terminus of three randomly chosen stems where there were multiple stems, and 5 cm sections along the stem where there was only one main stem. On *Tristiropsis* I counted crawlers, newly sessile 2nd instars, adults females, and males of *Tachardina* in 5 cm sections of three randomly chosen compound leaf petioles, beginning directly adjacent to the main stem. On *Claoxylon* I counted newly sessile 2nd instars and adult female soft scales in 5 cm sections at the terminus of three randomly chosen stems, and on *Dysoxylum* I counted newly sessile 2nd instars and adult female soft scales in 5 cm sections of three randomly chosen compound leaf petioles directly adjacent to the main stem.

Crazy ant activity at the site was recorded on each scale insect sampling day ($n=20$), which was the number of ants that crossed a white 10 cm x 10 cm laminated card in 30 seconds as the activity index (refer to Chapter 2 for calibration with density).

Because of the unidirectional flow of time, errors are not independent among times within treatment level time periods, or between them (Green 1993). Data for each species were analysed separately using one-way repeated measures ANOVA (SYSTAT ver. 10) because numbers of scales on different sampling dates were not independent. The total number of scales in 15 cm section (3 x 5 cm sections) on each sapling was used as a replicate for analysis. The ANOVAs tested for effects of treatment (control vs. ant exclusion), sampling date, and an interaction between treatment and sampling date. The first sampling date for all species was excluded from the repeated measures test as it refers to the initial conditions of the experiment. Data

from the initial sampling date were analysed separately to confirm the similarity in the number of scale insects on control and ant exclusion saplings at the beginning of the experiment. Results for lac scales and soft scales are presented separately as they preferentially colonised different host species.

Effect of A. gracilipes on generalist predators and herbivores on saplings

I investigated the effect of *A. gracilipes* presence on the abundance of generalist predators and herbivores, including spiders, neuropteran larvae and lepidopteran larvae. I tested the hypothesis that ants would deter generalist predators from those plants on which they are present, one mechanism whereby ants have been shown to indirectly benefit homopterans. On saplings of all species, I counted the total number of predators and herbivores on the entire plant on each sampling occasion, day 0, 17, 36, 66, 84, 177 & 280. I used the mean number of individuals per sapling to test for a difference between control and ant exclusion saplings. Data for each species were analysed separately using one-way repeated measures ANOVA (SYSTAT ver. 10) where ant exclusion treatment was the main factor, the number of predators per plant the dependent variable and time the repeated measures factor.

Effect of A. gracilipes on host saplings

I investigated the effect of *A. gracilipes* on plant growth (as measured by % height increase) for all four species, and the proportion of leaves colonised with sooty mould for saplings of *Inocarpus*, *Tristiropsis* and *Dysoxylum* in the understorey. For each sapling, relative height increase was used as a coarse indicator of plant performance, and the proportion of leaves with visible sooty mould (Capnodiaceae) was recorded as a measure of potential for loss of plant fitness. Sooty moulds generally either covered the entire adaxial surface of the leaves, or were absent. I rarely encountered leaves with only a proportion of the leaf with sooty mould. I measured the height of each *Inocarpus*, *Tristiropsis*, *Claoxylon* and *Dysoxylum* sapling on day 0 and day 66 of sampling, and also day 280 for *Inocarpus* and *Tristiropsis*. On all saplings, I

counted the total number of leaves at each sampling date and the number of leaves that had visible sooty mould on their adaxial surface. Plant height data were analysed using a one-way ANOVA (SYSTAT ver. 10), to test the effect of the presence of *A. gracilipes* on plant growth for each species, which was measured as the percentage of initial sapling height 66 days after ant exclusion for *Claoxylon* and *Dysoxylum* and 280 days after ant exclusion for *Inocarpus* and *Tristiropsis*. A one-way ANOVA was used to test the effect of the presence of *A. gracilipes* on the absolute change in proportion of leaves with sooty mould on day 0, and day 36.

Results

Effect of A. gracilipes on scale insect life stages on saplings

On the initial sampling day, upon imposing the ant exclusion treatment, scale densities did not differ between control and ant exclusion plant for any life stage of lac scales (*Tachardina*) on *Inocarpus* and *Tristiropsis* (Fig. 5.2, Table 5.1), or for life stages of soft scales on *Claoxylon* and *Dysoxylum* (Fig. 5.3, Table 5.1). I found no soft scales of any species on *Inocarpus* or *Tristiropsis* throughout the experiment, but encountered a few adult female lac scales on two *Claoxylon* saplings (which are not included in the data for analysis). Despite the presence of adult female lac scales only on particular species, their bright red crawlers were present on all understorey vegetation in the experimental area.

Inocarpus: At the start of the experiment *Inocarpus* saplings had a mean of 114.4 ± 11.0 and 104.9 ± 12.1 crawlers/15 cm of stem (mean \pm SE) on ant exclusion and control saplings respectively, but these numbers had declined by 88% and 58%, respectively, by the end of the experimental period. On *Inocarpus* I detected a significant positive effect of ant exclusion and time on the number of crawlers per sapling, but no significant interaction between these two factors (Table 5.2; Fig. 5.2A). This result indicates that, even though the magnitude of the treatment effect changed

through time, there were consistently more crawlers on control saplings than ant exclusion plants. The presence of ants also significantly positively affected the number of 2nd instars on *Inocarpus* (Table 5.2; Fig. 5.2B), and a highly significant interaction between ant exclusion and time indicates that the treatment effect changed through time. Similarly, ant exclusion had a significant impact on the numbers of honeydew producing adult female lac scales on *Inocarpus*, although the magnitude of the effect changed during the experiment (Table 5.2; Fig. 5.2C). The number of adult females on ant exclusion plants declined upon ant exclusion, from 7.1 ± 3.0 to 2.1 ± 1.0 individuals/15 cm of stem (mean \pm SE) at the end of the experiment. Numbers of adult females on control plants increased for a month to 17.8 ± 4.5 individuals/15 cm of stem (mean \pm SE), but then steadily declined to a mean of 3.9 ± 1.2 individuals/15 cm of stem (mean \pm SE). There were consistently more male lac scales on *Inocarpus* ant exclusion saplings than controls (Table 5.2; Fig. 5.2D), where the number of males declined after ant exclusion, but males on plants with ants increased for a month then declined. After 280 days of observation, all life stages of lac scales were present on *Inocarpus*.

***Tristiropsis*:** Saplings maintained fewer numbers of all life stages than *Inocarpus*, which were affected differently by ant exclusion throughout the experiment (Fig. 5.2E-I). After 175 days all lac scale life stages, except crawlers, on both control and ant exclusion *Tristiropsis* saplings had been reduced to zero. There was no significant effect of ant exclusion on the numbers of crawlers or 2nd instars on *Tristiropsis* (Table 5.2; Fig. 5.2F & G). For adult females and males, however, despite there being no main treatment effect detected statistically, the significant interaction between ant exclusion and time indicates that there was a treatment effect at some time during the experiment. Further analysis of data at separate sampling dates revealed this difference was on sampling days 66 & 84, and 36, 66 & 84 for adult females and males respectively. In contrast to *Inocarpus*, only *Tachardina* crawlers were present at the end of the experiment. There were no adult females present on plants; therefore these

crawlers must have come from the crawler 'rain' from the canopy (see Chapter 4 for definition and density of crawler 'rain').

During the experiment, several ant exclusion saplings of both *Inocarpus* and *Tristiropsis* were found with *A. gracilipes* present (Fig. 5.2E & J). In all cases except one, single ants only were found on ant exclusion plants, whereas control plants always had more than one ant present.

Claoxylon & Dysoxylum: The number of 2nd instars of soft scales (Coccidae) showed remarkably similar patterns through time on both control and ant exclusion saplings of *Claoxylon* and *Dysoxylum* (Table 5.3; Fig. 5.3A & D). The number of 2nd instars declined immediately upon ant exclusion but remained higher on control plants for both *Claoxylon* and *Dysoxylum*. This was a statistically significant effect of ant exclusion on 2nd instars for both *Claoxylon* and *Dysoxylum* (Table 5.3). The response by adult female soft scales to ant exclusion on *Claoxylon* was similar to that of 2nd instars. There was a significant effect of ant exclusion on adult female soft scales on *Claoxylon*, where the non-significant interaction term from the repeated measures ANOVA indicates that number of adult female scales was consistently higher on control plants than on ant exclusion plants (Fig. 5.3B). The variation in the number of adult females on all *Dysoxylum* saplings was relatively high throughout the experiment (Fig. 5.3E). The non-significant treatment effect, and slightly significant interaction term demonstrates no consistent effect of ant exclusion on the number of adult female soft scales on *Dysoxylum* over the course of the experiment (Table 5.3).

Effect of A. gracilipes on generalist predators and herbivores on saplings

Protection from predators may be only one mechanism by which ants directly benefit scale insects. Upon application of Tanglefoot® to experimental saplings, the numbers of generalist arthropod predators on saplings were statistically similar for all species (Fig. 5.4; $F_{1,26}=0.000$, $p=1.000$; $F_{1,28}=0.054$, $p=0.818$; $F_{1,20}=0.060$, $p=0.810$ for *Inocarpus* *Claoxylon* & *Dysoxylum* respectively; *Tristiropsis* all zero on first sampling

date). However, over the following three months (84 days), there was a significant difference in the number of arthropods, primarily predators, on control and ant exclusion saplings for all plant species (Table 5.4). Generalist predator numbers increased by at least 4-fold on *Inocarpus*, *Tristiropsis*, *Claoxylon* and *Dysoxylum* saplings when *A. gracilipes* were excluded, whereas the number of generalist predators on saplings allowed access by ants remained relatively low.

I observed seven groups of other arthropods on the experimental saplings, including predators (spiders & lacewing larvae), herbivores (lepidopteran larvae), beetles (primarily chrysomelids), cockroaches, praying mantises and millipedes. However, spiders were by far the most abundant, comprising >80% of all observations, and in most cases >90%, over the experimental period, whereas herbivores made up <1% of observations. Other invertebrates were most common on *Inocarpus*, followed by *Tristiropsis*, *Claoxylon* and *Dysoxylum* (Table 5.5).

Effect of A. gracilipes on host saplings

Tristiropsis grew faster than any other species, increasing in height by 37.4% and 23.5% on ant exclusion and control saplings respectively. *Claoxylon* and *Dysoxylum* grew at similar rates, *Claoxylon* increasing in height by 7.9% and 9.8%, and *Dysoxylum* by 5.9% and 11% on ant exclusion and control saplings respectively. *Inocarpus* was the slowest growing species, ant exclusion saplings increasing in height by 4.4% and controls by 3.3%. However, I found no significant effect of ant exclusion on the growth (as measured by % height increase) of any of the four species, *Inocarpus*, *Tristiropsis*, *Claoxylon* or *Dysoxylum* (Fig 5.5; $F_{1,26}=0.618$, $p=0.439$; $F_{1,17}=3.144$, $p=0.094$; $F_{1,28}=0.443$, $p=0.511$; $F_{1,20}=1.983$, $p=0.174$ respectively).

Nor was there a significant effect of *A. gracilipes* on the proportion of leaves that had visible sooty mould on their adaxial surface on *Inocarpus*, *Tristiropsis* or *Claoxylon* (Fig 5.6; $F_{1,26}=0.718$, $p=0.405$; $F_{1,20}=0.001$, $p=0.970$; $F_{1,28}=2.333$, $p=0.138$ respectively). Despite no effect of *A. gracilipes* on the level of sooty mould accumulation on saplings, the proportion of leaves on all *Tristiropsis* saplings that had

visible sooty mould on their surface increased by approximately 30% in the first five weeks of the experiment, while the proportion of leaves on all *Claoxylon* saplings that sooty mould decreased by approximately 15%.

Discussion

This study has shown that, in the rainforest understorey, attendance by the yellow crazy ant, *Anoplolepis gracilipes*, provides differential advantages for distinct life stages of the lac scale, *Tachardina aurantiaca*, within and between two rainforest host species, *Inocarpus fagifer* and *Tristiropsis acutangula*. Furthermore, on *Claoxylon indicum* and *Dysoxylum gaudichaudianum*, where the suite of scale species is entirely different, attendance by *A. gracilipes* has markedly different effects on only the adult female scales between species. To my knowledge, this is the first study to investigate these mutualistic interactions with as many associated species.

Many species of ants readily associate with several species of honeydew producing hemipterans (Way 1963; Greenslade 1972; Haines & Haines 1978; Bronstein 1994; Bishop & Bristow 2001; Helms & Vinson 2002; O'Dowd *et al.* 2003). It was Addicott (1979) who first proposed that the differential benefits provided by ants to various hemipteran species might be a critical element in determining the success of individual species (but see Bristow 1984). However, the population response of hemipterans to the attendance of ants has primarily been tested on adults (de Fluiter 1939; Way 1963), two life stages; either "small" and "large" scale insects (e.g. Bach 1991) and "nymphs" and "adults" of a membracid (Carroll & Janzen 1973; Cushman & Whitham 1989), or whole colonies (Bristow 1984, Helms & Vinson 2002). Because of the inherent difficulties in identifying and counting the often miniscule life stages of hemipteran species, it has been unclear in the past how distinct life stages are directly affected by the presence of ants. However, I provide evidence here that distinct life stages of different scale insects are affected differently to one another within host species, and also between host species.

The disparity in the success of crawlers on individual species in the absence of ants might be explained by host plant-mediated effects on their survival, establishment and growth (Mattson Jr. 1980; Breton & Addicott 1992; White 1993; Tikkanen *et al.* 2000; Lower *et al.* 2003). On *Tristiropsis*, at the end of the experimental period (175 days/25 weeks) all life stages of *Tachardina* had decreased to zero, except crawlers, even on control saplings where ants were allowed access to scale insects. That *Inocarpus* was able to maintain adult females in the absence of *A. gracilipes* but with crawler recruitment 'rain' from the canopy, and *Tristiropsis* was not, suggests that *Tristiropsis* may not be of requisite quality to sustain scale insect populations. Only the cessation of the crawler 'rain' from the rainforest canopy would remove the inoculum for establishment on understorey vegetation, and force scale populations in the understorey to become dependent on local recruitment. This is precisely the situation in areas of low *A. gracilipes* abundance on Christmas Island. I have shown here that in the absence of *A. gracilipes* in supercolony densities, populations of honeydew-producing scale insects decline, are unlikely to persist, and may eventually collapse.

It has been proposed that host plant quality mediates mutualistic systems (see Breton & Addicott 1992), especially between ants and honeydew producing hemipterans, as usually associations are facultative and non-specific (Addicott 1978). Host plant quality can be measured in numerous ways; water stress of a host can increase the amount of readily available and assimilable nitrogen in the tissues and thus increase the chance of survival of insects feeding on those tissues (White 1984; 1993), and plant height has been used to provide an indirect measure of plant quality (Breton & Addicott 1992). But it is generally acknowledged that host plant quality can help determine an associated insect's population dynamics (White 1984; Mattson & Haack 1987; Larsson 1989; White 1993; Eubanks & Denno 1999).

Claoxylon and *Dysoxylum* sustained numbers of soft scales similar to lac scales on *Inocarpus* and *Tristiropsis* respectively. However, after only 84 days (12 weeks) into the experiment populations of soft scale had plummeted to almost zero on all saplings. This significant decline in numbers of soft scale on both *Claoxylon* and

Dysoxylum was accompanied by an approximately 20% drop in *A. gracilipes* activity at the site, suggesting that soft scales may be more sensitive to a decrease in ant abundance, and presumably tending density and efficiency. The peak in adult females soft scales on *Dysoxylum* five weeks after ant exclusion was extremely variable within the treatment, which makes interpretation difficult. But since populations of soft scales declined on both *Claoxylon* and *Dysoxylum* at similar rates over the experimental period, it is unlikely that the quality of the host influenced their numbers, and more likely that it was *A. gracilipes* driving their abundance patterns. It is documented that different aphid species produce honeydew that varies in composition, even when reared on clones of the same host plant species (Volkl *et al.* 1999). It is thought that species-specific differences in their ability to transform the ingested phloem is responsible for this result (Volkl *et al.* 1999). However, the difference in quality and quantity of aphid honeydew significantly affected the presence and intensity of tending by the ant *Lasius niger*. I did not measure the quality or quantity of honeydew produced by lac and soft scale insects in this study, but it may have been qualitative or quantitative nutritional effects of honeydew produced by soft scales that influenced attendance intensity by *A. gracilipes* and led to the decline in the abundance of soft scales. Considering the multi-species associations in *A. gracilipes* supercolonies, investigation into this possibility is warranted.

Inocarpus and *Claoxylon* maintained populations of scales for the duration of the experiment on both ant exclusion and control saplings, whereas scales on *Tristiropsis* and *Dysoxylum* declined to almost zero, yet all four species sustain some of the highest densities of scale insects in the canopy of large trees in *A. gracilipes* supercolony areas. Host quality may become more important in the maintenance of scale populations than the attendance by *A. gracilipes* when ants are in low numbers, for example after day 100 of the experiment, when the effect of ant attendance on scale populations was reduced for all species.

These differential benefits must be interpreted taking into account two important factors. First, the supercolony within which the experiment was conducted

was in natural decline, which added an unanticipated complication in that *A. gracilipes* activity did not remain constant on control saplings. The overwhelming pattern on all species of sapling was the decline in number of all life stages during the experimental period, concurrent with this decline in *A. gracilipes* activity at the site around day 100 of the experiment. Even on saplings where ants were allowed access the numbers of scales insects reflected this decline in crazy ant activity. The first drop in crazy ant activity was after one month of observation, but after 84 days activity dropped markedly, to levels that no longer constituted a supercolony (See Chapter 2 for expansion on supercolony definition). The reason for decline in ant activity was not known.

The second complication in interpreting the data is due to the continual input of lac scale crawlers from the canopy. This could have had the effect of elevating the number of crawlers observed on saplings, and provided an increased recruitment opportunity for lac scales on all species of sapling, independent of the effect of ants. Bright red mobile crawlers of the lac scale (presumably *Tachardina aurantiaca*, as I observed very few of the other lac scale on Christmas Island, *Paratachardina lobata*, at this site) were present on all saplings for the duration of the experiment, with the exception of *Dysoxylum*, where crawlers were present on all saplings for the first three sampling dates, then absent on >50% of saplings after that.

The ant exclusion experiment carried out here was effectively within the spatial confines of a larger-scale association between an *A. gracilipes* supercolony, scale insects and plants. The supercolony within which this experiment was conducted was approximately 105.8 ha in size. In supercolonies *A. gracilipes* was always associated with scale insects on plants; so all three partners were interacting over a much larger spatial scale than just the experimental site. The consequences of this are clear when explaining the presence of the mobile crawler stage of the lac scale, *Tachardina*, but not so obvious for other life stages. I observed bright red mobile crawlers on virtually all understorey vegetation for the first three sampling dates, after which they became less frequent, but still present. The persistence of crawlers on understorey vegetation,

including experimental saplings indicates that the input of crawlers from a constant source in the canopy provides a considerable number of crawlers for recruitment that can maintain small populations scales in sects on some species but not on others.

In this study there was no effect of ant exclusion on the proportion of leaves that had sooty mould on their surfaces, but over a 36-day period the proportion of leaves of *Tristiropsis* with sooty mould increased by approximately 40% on both ant exclusion and control saplings, while on *Claoxylon* they decreased by approximately 20%. On *Tristiropsis*, this increase was due to an accumulation of sooty mould on all saplings, but the decrease in proportion of leaves with sooty mould on *Claoxylon* was the result of a leaf flush which increased the total number of leaves without them accumulating sooty mould in that time.

The absence of an ant effect on the extent of sooty mould accumulation on leaves suggests that, despite the removal of hemipteran honeydew by *A. gracilipes* in the canopy, sufficient sugar-laden substances reach the understorey to aid the colonisation of sooty moulds. This study commenced at the onset of the wet season, and ant activity declines in wet conditions. The decrease in ant activity combined with rain washing the honeydew from scales in the canopy onto saplings below may help explain the build up of sooty mould on all understorey vegetation.

While numbers of scale insects on both ant exclusion and control saplings declined over the course of the experiment, the number of generalist predators (mainly spiders) increased dramatically on ant exclusion saplings, and only slightly on control saplings. This was true for all species except for *Tristiropsis*, where predator numbers on control plants increased at the end of the experimental period. This result suggests that it was not just *A. gracilipes* exclusion that allowed predators to increase, but low ant numbers in general. Predators were released from *A. gracilipes* encounter on both exclusion and control plants, where the release from ants was far greater on exclusion plants from the outset and was less dramatic on control plants, as ant activity at the site declined slowly over time. *A. gracilipes* has been implicated in the exclusion of native spiders from native and disturbed forest in Hawaii (Gillespie & Reimer 1993).

However, in addition to the release from ant encounter, resources such as honeydew from scales that is not taken by ants could attract predators to ant-free plants (Bluthgen *et al.* 2000). I did not measure the abundance of generalist predators on understory saplings in non-supercolony areas, and consequently did not know if the number of predators on experimental exclusion saplings were comparable to non-supercolony levels, or if they were elevated simply because ant exclusion saplings represented an 'ant-free island' in the sea of *A. gracilipes*. The simultaneous increase in predators and decrease in scale insects on ant-exclusion saplings suggests that escape from predators may be one mechanism driving the success of scale insects in the rainforest understory in *A. gracilipes* supercolonies. However, spiders and lacewing larvae primarily target mobile crawlers as prey, and considering the continual input of crawlers from the canopy, reduced adult reproductive rate and survival due to decreasing number of attendant ants was also responsible for the drop in numbers.

In summary, here I have presented further evidence for an invasive ant providing benefits for multiple honeydew-producing scale insect species. Different scale insect life stages gain differential advantages from the presence of *A. gracilipes* within hosts and between them in a multi-species complex in the understory of Christmas Island. In addition, I have documented the decline of scale insect populations simultaneous to a decline in *A. gracilipes* activity, and the implications for the mutualism between *A. gracilipes* and scale insects occurring at a large spatial scale on different host species, including continual recruitment of crawlers and the possibility of host plant effects on scale insect populations at low ant density. The presence of ants did not affect host plant height, or the proportion of leaves with visible sooty mould, but on ant exclusion saplings, the number of predators significantly increased.

Table 5.1 – ANOVA table of initial conditions of sapling ant-exclusion experiment, indicating no significant difference in the number of any life stages on *Inocarpus*, *Tristiropsis*, *Claoxylon* and *Dysoxylum*. Statistics derived from separate one-way ANOVAs on SYSTAT ver. 10.

Source of variation	Crawlers				2nd instars				Adult females				Males			
	MS	df	F-ratio	p-value	MS	df	F-ratio	p-value	MS	df	F-ratio	p-value	MS	df	F-ratio	p-value
<i>Inocarpus</i>	631.750	1	0.338	0.566	104.143	1	0.324	0.574	222.893	1	1.100	0.304	234.321	1	0.461	0.503
Error	1869.959	26			321.676	26			202.541	26			507.761	26		
<i>Tristiropsis</i>	169.136	1	0.205	0.656	222.727	1	0.413	0.528	33.136	1	1.219	0.283	6.545	1	1.506	0.234
Error	826.373	20			539.182	20			27.182	20			4.345	20		
<i>Claoxylon</i>					607.500	1	0.596	0.447	56.033	1	0.382	0.542				
Error					1019.567	28			146.748	28						
<i>Dysoxylum</i>					710.227	1	0.178	0.677	26.182	1	0.923	0.348				
Error					3983.518	20			28.355	20						

Table 5.2 – Effect of *A. gracilipes* on four different life stages of lac scales (*Tachardina aurantiaca*) on *Inocarpus* and *Tristiropsis*. ANOVA statistics derived from separate one-way repeated measures analysis for each species, where the first sampling date is not included in analysis, as it refers to the initial conditions of the experiment, and control and treatment states were all statistically similar.

Source of variation	Crawlers				2nd instars				Adult females				Males			
	MS	df	F-ratio	p-value	MS	df	F-ratio	p-value	MS	df	F-ratio	p-value ^Φ	MS	df	F-ratio	p-value ^Φ
<i>Inocarpus</i>																
<i>Between subjects</i>																
Treatment	11957.720	1	10.926	0.003	1141.929	1	6.412	0.018	8.846	1	40.577	<0.001	5.161	1	13.966	0.001
Error	1093.925	26			178.086	26			0.218	26			0.370	26		
<i>Within subjects</i>																
Time	30105.815	5	34.780	<0.001	2718.895	5	25.772	0.000	1.736	5	12.561	<0.001	3.937	5	32.092	<0.001
Treatment x time	611.706	5	0.707	0.619	433.914	5	4.113	0.002	0.436	5	3.154	0.010	0.243	5	1.982	0.085
Error	865.609	130			105.497	130			0.138	130			0.123	130		
<i>Tristiropsis</i>																
<i>Between subjects</i>																
Treatment	78.627	1	0.097	0.097	275.236	1	0.998	0.330	0.206	1	0.342	0.565	0.902	1	2.060	0.167
Error	812.835	20			275.802	20			0.603	20			0.438	20		
<i>Within subjects</i>																
Time	4680.309	4	10.412	<0.001	992.491	4	6.765	<0.001	0.235	3	4.375	0.007	0.549	3	8.296	<0.001
Treatment x time	33.264	4	0.074	0.990	220.918	4	1.506	0.208	0.222	3	4.123	0.010	0.208	3	3.149	0.031
Error	449.516	80			146.700	80			0.054	60			0.066	60		

Φ - Data analysed using log-transformed values

Table 5.3 - Effect of *A. gracilipes* on two different life stages of soft scales (Coccidae) on *Cloaxylon* and *Dysoxylum*. ANOVA statistics derived from separate one-way repeated measures analysis for each species. The first sampling date has been deleted from analysis, as it refers to the initial conditions of the experiment and control and treatment states were statistically similar.

Source of variation	2nd instars				Adult females			
	MS	df	F-ratio	p-value	MS	df	F-ratio	p-value ^Φ
<i>Cloaxylon</i>								
<i>Between subjects</i>								
Treatment	3286.533	1	14.476	0.001	2.472	1	8.676	0.006
Error	227.040	28			0.285	28		
<i>Within subjects</i>	3792.456	3	32.564	<0.001	2.452	3	41.165	0.000
Time	949.133	3	8.150	<0.001	0.076	3	1.267	0.291
Treatment x time	116.461	84			0.060	84		
Error								
<i>Dysoxylum</i>								
<i>Between subjects</i>								
Treatment	8190.920	1	7.177	0.014	169.136	1	0.593	0.450
Error	1141.289	20			285.241	20		
<i>Within subjects</i>								
Time	8815.708	3	13.236	<0.001	361.227	3	2.706	0.053
Treatment x time	2436.860	3	3.659	0.017	57.894	3	0.434	0.730
Error	666.034	60			133.511	60		

Φ - Data analysed using log-transformed values

Table 5.4 – Effect of *A. gracilipes* on generalist predators on *Inocarpus* (A), *Tristiropsis* (B), *Claoxylon* (C) and *Dysoxylum* (D) saplings in the understorey of the rainforest. ANOVA statistics derived from separate one-way repeated measures analysis for each species.

Source of variation	MS	df	F-ratio	p-value
<i>Inocarpus</i>				
<i>Between subjects</i>				
Treatment	1243.149	1	55.951	<0.001
Error	22.218	26		
<i>Within subjects</i>				
Time	158.554	5	28.658	<0.001
Treatment x time	80.563	5	14.561	<0.001
Error	5.533	130		
<i>Tristiropsis</i>				
<i>Between subjects</i>				
Treatment	193.782	1	23.322	<0.001
Error	8.309	20		
<i>Within subjects</i>				
Time	107.623	4	32.126	<0.001
Treatment x time	30.77	4	0.919	0.457
Error	3.350	80		
<i>Claoxylon</i>				
<i>Between subjects</i>				
Treatment	156.408	1	40.240	<0.001
Error	3.887	28		
<i>Within subjects</i>				
Time	16.586	3	7.984	<0.001
Treatment x time	3.164	3	1.523	0.214
Error	2.077	84		
<i>Dysoxylum</i>				
<i>Between subjects</i>				
Treatment	118.227	1	25.500	<0.001
Error	4.636	20		
<i>Within subjects</i>				
Time	11.530	3	4.557	0.006
Treatment x time	0.197	3	0.078	0.972
Error	2.530	60		

Table 5.5 – Proportion of total general predators and herbivores on control and ant exclusion saplings of *Inocarpus*, *Tristiropsis*, *Claoxylon* and *Dysoxylum* in the rainforest understorey.

	Feeding guild	<i>Inocarpus</i>	<i>Tristiropsis</i>	<i>Claoxylon</i>	<i>Dysoxylum</i>
<i>Arachnida</i>	General predator	0.94	0.95	0.90	0.81
<i>Neuroptera</i>	Larvae general predators	0.04	<0.01	0.03	0.1
<i>Coleoptera</i>	Predators, herbivores	0.00	0.01	<0.01	0.05
<i>Blattodea</i>	Scavengers	<0.01	<0.01	<0.01	0.00
<i>Mantodea</i>	Predators	0.01	<0.01	0.05	0.03
<i>Diplopoda</i>	Detritivores	<0.01	0.00	0.00	0.00
<i>Lepidoptera</i>	Larvae herbivorous	<0.01	0.02	0.01	0.01
Total individuals		619	422	277	96



Figure 5.1 – Band of Tanglefoot® on sapling stem to exclude *A. gracilipes*. Tanglefoot® was placed onto a waterproof paper sleeve fastened securely to the stem at a height of between 10 – 80 cm above the ground.

Tachardina aurantiaca

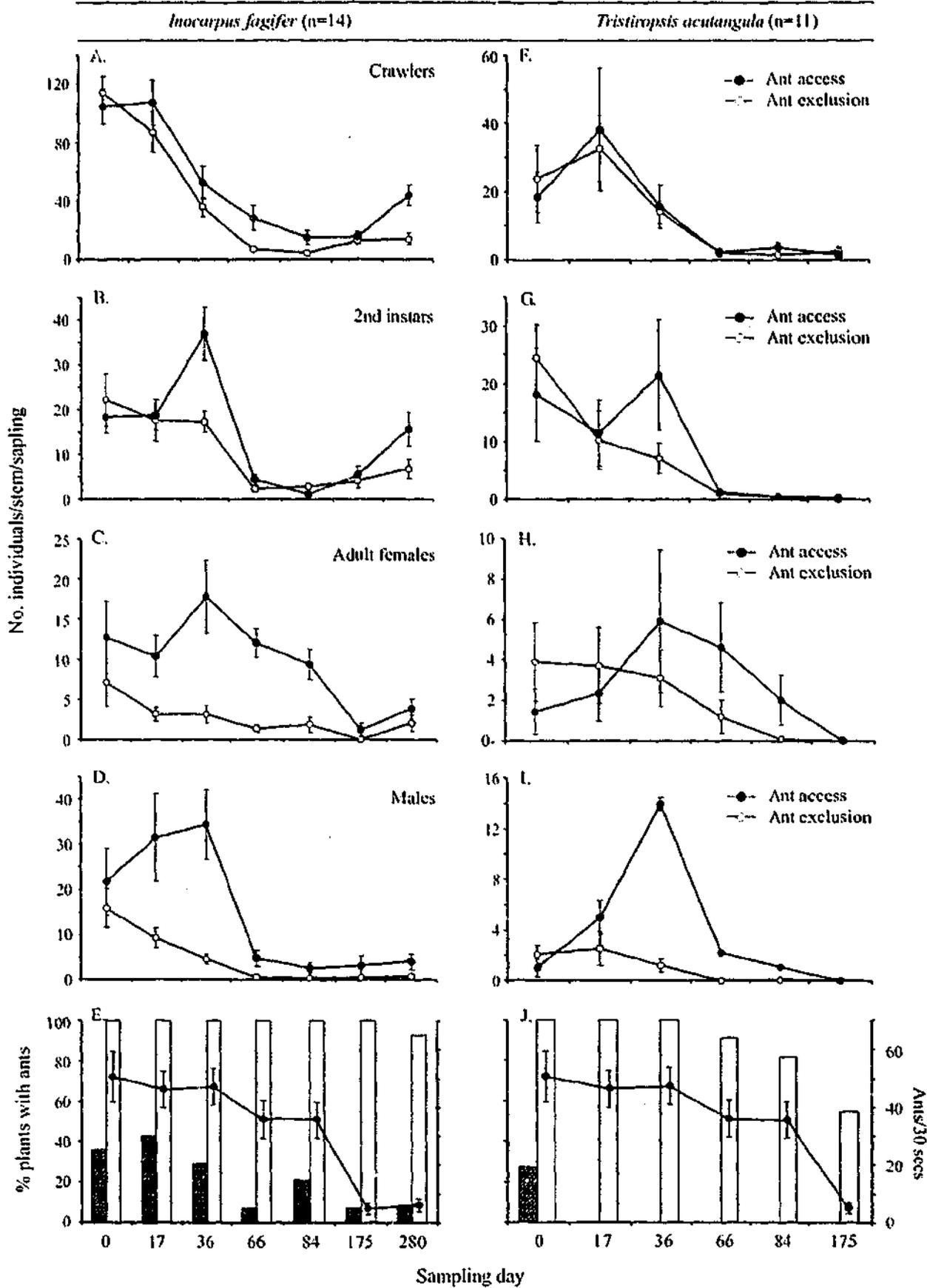


Figure 5.2 - Mean number of individuals (\pm SE) from different life stages of *Tachardina aurantiaca* on *Inocarpus fagifer* (A - D) and *Tristiropsis acutangula* (F - I) where ants were present and excluded. Graphs E & J are ant activity on the forest floor at each sampling time (line), and the percentage of plants in each treatment that had ants present at the time of observation (bars; ant access open bars, ant exclusion filled bars). Note difference in y-axis scales on all graphs. In all cases of ants present on the ant exclusion treatment, there was one ant present only.

Coccidae

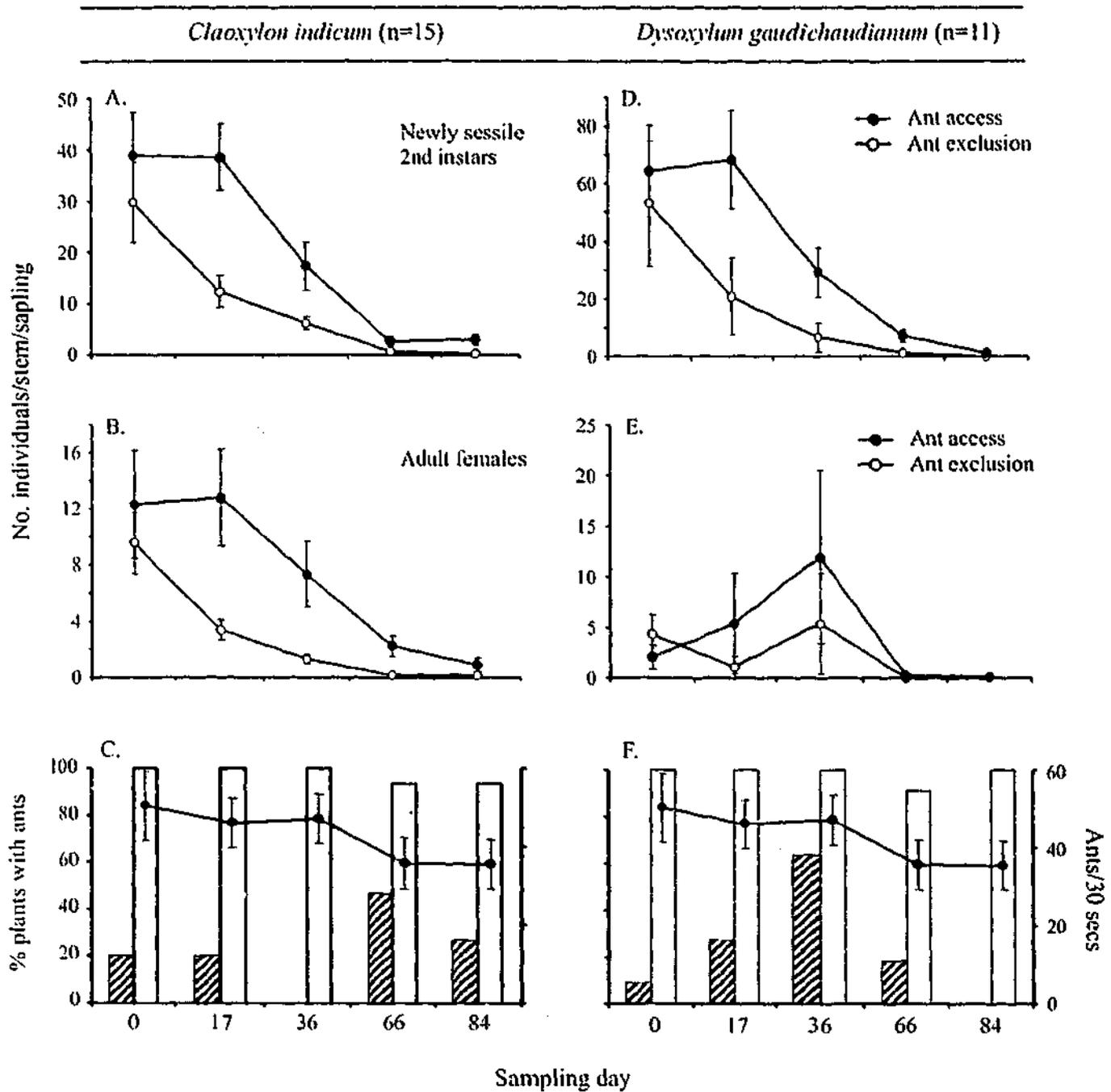


Figure 5.3 – Mean number of individuals (\pm SE) from different life stages of soft scales (Coccidae) on *Claoxylon indicum* (A & B) and *Dysoxylum gaudichaudianum* (D & E). Also presented is the activity of *A. gracilipes* on the forest floor at each sampling time (C & F, lines), and the percentage of plants in each treatment that had ants present (C & F; ant access open bars, ant exclusion hatched bars), and. Note difference in y-axis scales on all graphs. Where plants in the ant exclusion had ants present, there was only one ant observed in all cases.

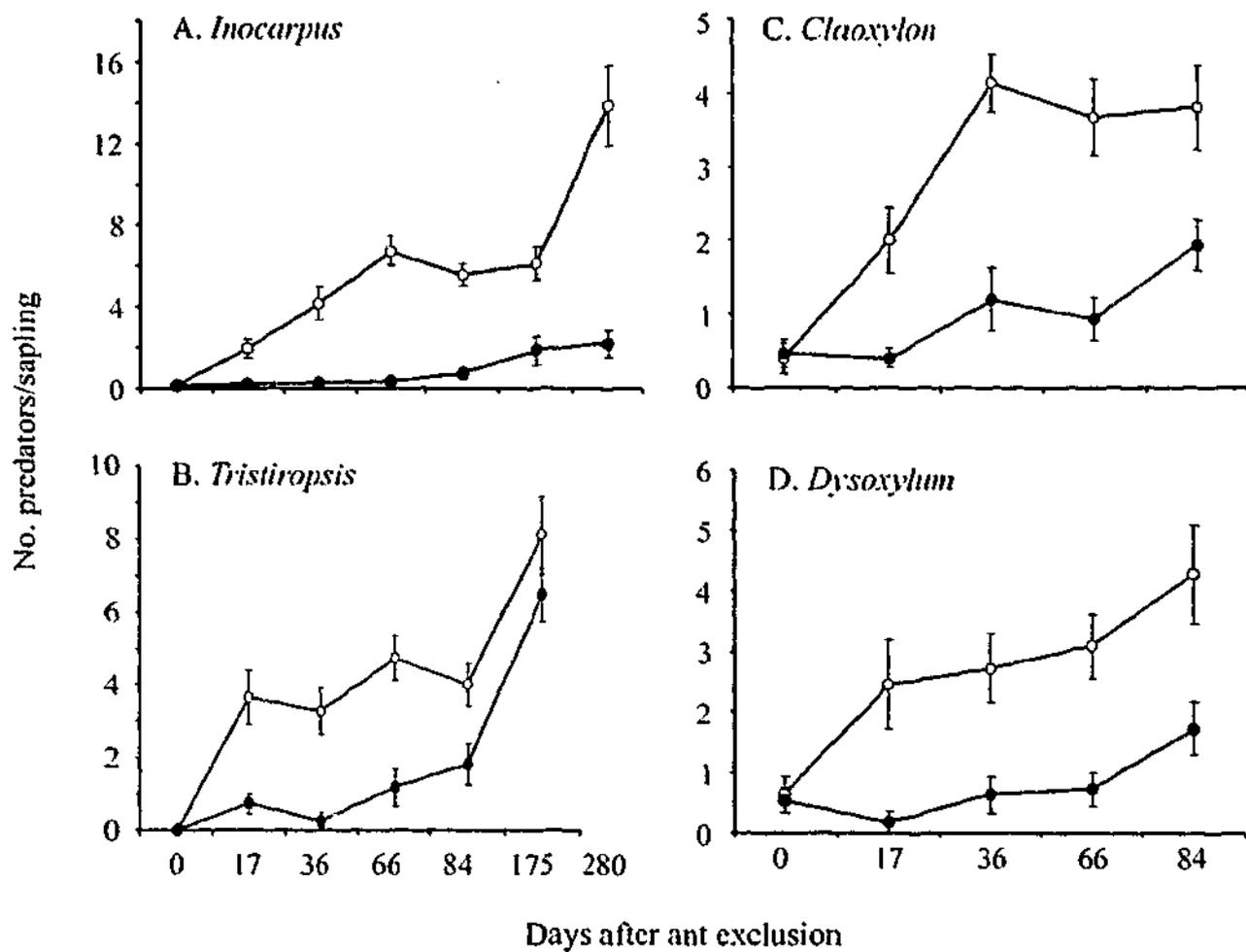


Figure 5.4 – The mean number of generalist predators (\pm SE) on saplings for *Inocarpus*, *Tristiropsis*, *Claoxylon* and *Dysoxylum* in the understorey at each sampling time. Note similar x-axis time scales, but different y-axis scales.

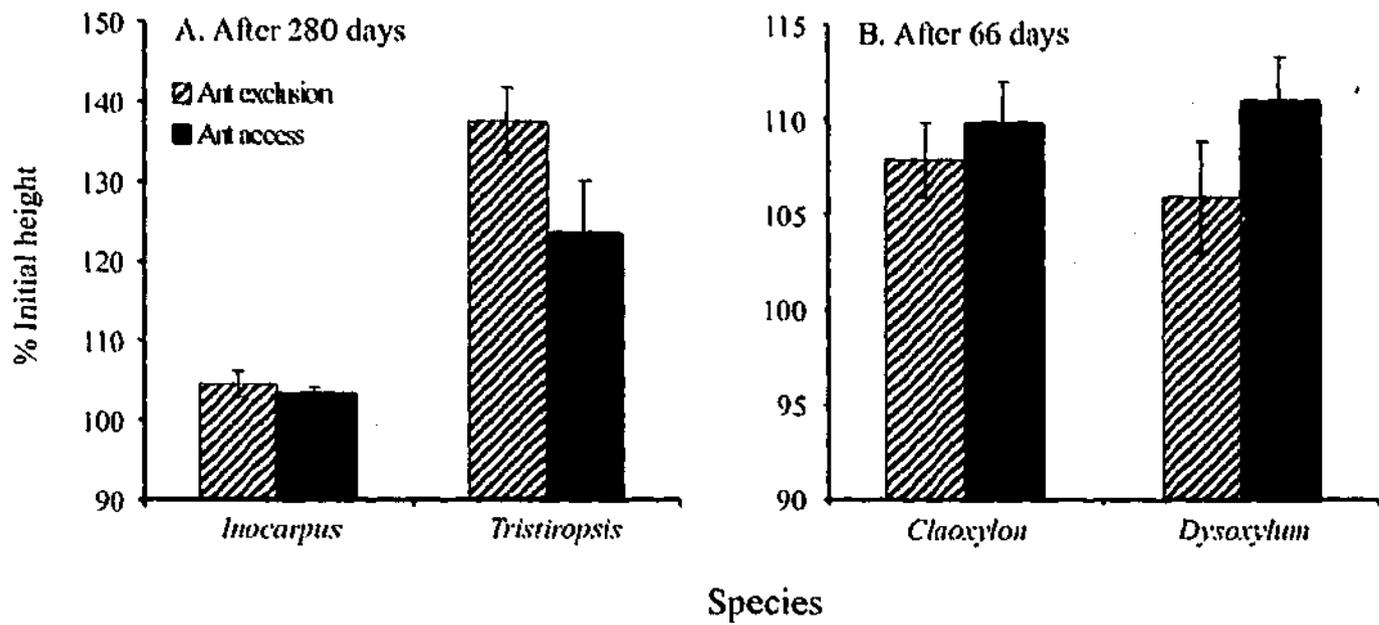


Figure 5.5 – Effect of *A. gracilipes* on the growth of *Inocarpus* (n=14), *Tristiropsis* (n=11), *Claoxylon* (n=15) and *Dysoxylum* (n=11) control & ant exclusion saplings in rainforest understorey at WAD on Christmas Island, as measured by the percentage of initial height of each sapling.

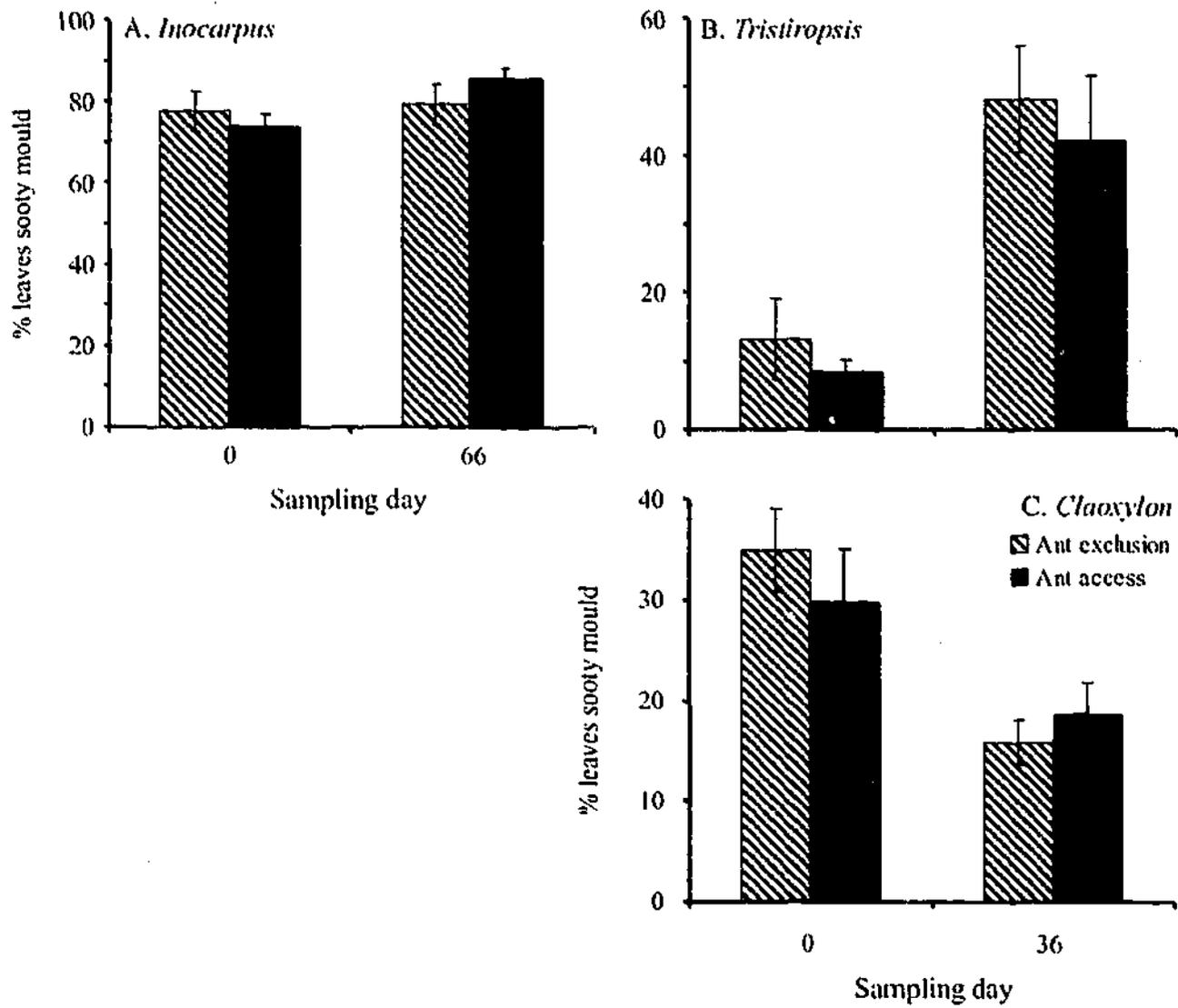


Figure 5.6 – Proportion of leaves with visible sooty mould (\pm SE) on *Inocarpus* (A.), *Tristiropsis* (B.) and *Claoxylon* (C.) upon ant exclusion and on day 66 for *Inocarpus* and day 36 for *Tristiropsis* and *Claoxylon*.

Chapter Six

Large-scale experimental analysis of a mutualism: the 'strength' of the *A. gracilipes*-scale association in the rainforest canopy on Christmas Island, Indian Ocean.



Introduction

Up until about 20 years ago, most mutualistic interactions were described purely in terms of that which was exchanged (Boucher *et al.* 1982). Since then, studies have examined, experimentally, the dynamics of both members of a mutualism, and demonstrated increased fitness and/or population growth rates by one or both species within an interaction (Buckley 1987; Cushman & Whitham 1989; Bach 1991; Cushman *et al.* 1994; Richardson *et al.* 2000), the decline of a partner upon removal of the other (Grant & Moran 1986; Bach 1991), and changes in partner populations due to alternation of mutualistic species (Itioka & Inoue 1999).

Our understanding of multi-trophic associations and their dynamics, however, is directly linked to the scale at which we make our ecological observations (Pandolfi 2002). Mutualisms between honeydew-producing hemipterans and ants have often been addressed on relatively small spatial scales. It is generally for practical and logistical reasons that larger scale experiments are rarely found in the literature, even when the mutualisms occur on large spatial scales in nature, which they often do. Populations of associated hymenopterans and hemipterans have been shown to cover thousands of hectares (Helms & Vinson 2002; O'Dowd *et al.* 2003). On Christmas Island, Indian Ocean, the invasive alien yellow crazy ant, *Anoplolepis gracilipes*, forms multi-queened supercolonies that can occupy areas of over 700 hectares, within which

extremely high densities of honeydew-producing scale insects (Coccoidea) are found. In supercolonies, foraging *A. gracilipes* on the forest floor exist in densities of >2000 ants m^{-2} (Chapter 3), resulting in the decimation of populations of endemic red land crabs.

Because of their extensive distribution and escalating rates of introduction, invasive ants represent a significant threat to natural and agricultural ecosystems. This is largely due to their tendency to form close associations with hemipteran plant pests (Holway *et al.* 2002; Helms & Vinson 2003), their capacity to displace native invertebrates (Porter & Savignano 1990; Hoffmann *et al.* 1999), their ability to become a keystone species (Risch & Carr 1982), or to disrupt the role of keystone species within ecosystems (O'Dowd *et al.* 2003). Associations between hemipterans and their attendant ants have been identified world-wide, and the benefits that each partner receives has been documented comprehensively in many systems, from commercial citrus orchards (Flanders 1951; Waterhouse & Sands 2001) to tropical rainforests (Bluthgen *et al.* 2000). These associations are generally accepted as facultative mutualisms, where each partner is not necessarily wholly dependent upon the other, but both members benefit in the presence of one another. Essentially, aphids, membracids, coccids and other insects provide excretory honeydew as a source of carbohydrates and amino acids to the ants in return for protection from predators, increased sanitation by removing honeydew which may otherwise asphyxiate them or cause build up of fungus and moulds (Nixon 1951; Bach 1991; Gullan 1997).

The first supercolony was reported in 1989 (P.T. Green personal communication), but since 1995, similar high-density areas of *A. gracilipes* increased around the island, concurrent with increased seedling densities, dead endemic land crabs and large scale insect populations in infested areas (O'Dowd *et al.* 2003). In September 2002, approximately 25% of the island's 10,000 hectares of rainforest was infested with *A. gracilipes* supercolonies (Green *et al.* 2004). In those areas, the density of scale insects was 16-fold higher than those areas where crazy ants were absent (Chapter 4). The association between crazy ants and scale insects was observed

in the understorey on seedlings and saplings of rainforest trees. However, the trunks of canopy trees supported large numbers of crazy ant traffic to the canopy, suggesting that the ants gained substantial benefit from the canopy of these trees – honeydew from scale insects on a grand spatial scale. Some rainforest trees such as the native Tahitian chestnut, *Inocarpus fagifer*, were particularly susceptible to infestation by the lac scale, *Tachardina aurantiaca*, and continually maintained high numbers of both ants and scale insects.

Given the impacts of the crazy ant, control of crazy ant supercolonies was given priority by Parks Australia, the federal agency responsible for natural resource management on Christmas Island (CINP 2002). The effectiveness of a control program was suspected to be linked to the crash of their scale mutualists. Many ant exclusion experiments have shown that, usually, scale populations escalate in the presence of ant attendants (Way 1953; Bach 1991) and that sometimes they decline in the absence of ants (Jutsum *et al.* 1981; Bristow 1982). However, there is a continuum of ‘strengths’ of facultative mutualisms (that do not necessarily fall into the categories of obligate or facultative *per se*); that is, a range of how dependent each partner of the mutualism is upon the other. If the mutualism is relatively ‘weak’, upon removal of one partner the other may persist at similar densities to what it does in the presence of its mutualist. But if the mutualism were ‘strong’, the removal of one partner should result in immediate and dramatic population decline of its mutualist. This description must also be put in context of positive interactions between conspecifics and heterospecifics; that outcomes of an interaction are dependent upon environmental conditions (Bronstein 1994). My referral to the interaction between *A. gracilipes* and scale insect mutualists is based on an assumption that wherever they are associated on Christmas Island the outcomes are positive, i.e. always at the positive end of the continuum of outcomes of interactions between species.

The relative strength of the mutualism between crazy ants and scale insects has direct implications for management of crazy ants on Christmas Island, and the control program that was undertaken jointly by Parks Australia North and Monash University

between 2000 and 2003. If the mutualism was 'weak', then the control program may need to target scale insects as well as ants, or require annual treatment of ants to stop supercolony reformation, dramatically increasing the cost of the program. Furthermore, persistence of scale insects in Christmas Island rainforest would afford an opportunity for mobile scale crawlers to colonise neighbouring forest and establish the bottom rung of a positive feedback loop, promoting populations of both crazy ants and scale insects in intact forest, or for other ant species to replace *A. gracilipes* as attendants of scale insects. If the mutualism was 'strong' and scale insect populations collapsed upon ant exclusion, a single control treatment may be sufficient to dissolve the mutualism and halt the persistence of supercolonies on Christmas Island.

In this study, I investigated the effect of *A. gracilipes* on scale insects on a forest-wide scale, in the canopy of three common rainforest species. I excluded *A. gracilipes* from areas between 12 - 25 ha and assessed the densities of lac scales (*Tachardina aurantiaca*) and soft scales (Coccidae) before and after the elimination of ants from the forest floor from control and impact sites. I was able to do this in conjunction with routine baiting of *A. gracilipes* supercolonies by Parks Australia North. I evaluate the implications of this mutualism for management of *A. gracilipes* supercolonies on Christmas Island, Indian Ocean.

Methods and Materials

The Study Sites

Christmas Island is an elevated oceanic island of 135 km², which supports a broad-leaved, structurally simple tropical rainforest. Approximately 74% of the island is covered with natural vegetation, where three of seven described forest types (Mitchell 1985), which correspond generally to geological substrate type (Barrie 1967), dominate both plateau and terrace areas. The island lies 360 km south of Java in the

humid tropics, and experiences a monsoonal climate where most of the annual 2000 mm rainfall occurs between December and May (Falkland 1986).

Supercolonies of *A. gracilipes* rapidly increased in size and frequency across the island starting in 1996, where ants were in densities sufficient to kill the red land crab, *Gecarcoidea natalis* (Brachyura: Gecarcinidae). Data for this study were collected between November 2000 and September 2001, in areas of *A. gracilipes* supercolony formation. Between 2001 and 2003 these areas covered >2500 ha of the island (Fig. 6.1). They were characterised by the absence of red crabs, increased seedling density and a thick carpet of leaf litter. Other ant species were reduced in areas of supercolony formation (Chapter 3).

Effect of A. gracilipes on scale insects in the canopy of the rainforest

I used a Before-After-Control-Impact (BACI) design, consisting of two treatment levels (control & ant exclusion), three times (before ant exclusion, 11 wks after ant exclusion & 12 months after ant exclusion), and three replicate site pairs in *A. gracilipes* supercolonies where one of each pair was assigned the ant exclusion treatment and one used as a control (Fig. 6.1). It was important to assume, and subsequently confirm, that all treatment sites did not differ from supercolony sites before the ant exclusion occurred. Environmental heterogeneity can coincide with control versus impact location contrasts, and hamper interpretation of the data (Green 1993). Therefore, the paired samples were located as close as logistically possible in space and time, with three weeks between the first and last sample taken on all six sites.

The three treatment sites were 15.0 ha, 9.1 ha and 35.6 ha supercolonies, located in forest type I (see Chapter 4). Control sites were also located in forest type I, which typically has a tall canopy (mean 34 m), and is characterised by deep soils with good moisture retention. The dominant canopy species in the sites were *Syzygium nervosum*, *Planchonella nitida*, *Dysoxylum gaudichaudianum*, *Barringtonia racemosa*, *Inocarpus fagifer*, *Tristiropsis acutangula*, *Pisonia umbellifera*, *Claoxylon indicum* and *Ochrosia ackerangae*.

To exclude crazy ants from the three treatment sites, granulated Presto™ bait was distributed by hand, spreading a standard measure per five steps in a search and rescue type sweep, where 5 – 14 persons walked 8 – 10 m apart. The concentration of active ingredient (Fipronil) in the granulated bait was 0.01%, and the mean baiting rate in the field was 5.18 kg/ha (S. Comport, personal communication).

In all sites, at three sampling times, I measured *A. gracilipes* activity on the forest floor using white 10 cm x 10 cm laminated activity cards (see Chapter 2) over three days. Twenty activity counts were carried out on each day and the mean used for analysis. I also recorded ant activity on tree trunks with a trail activity scoring system of 1=low activity, 2=medium, and 3=high activity. I scored each *A. gracilipes* trail on a trunk and the resulting trunk activity index for an individual tree was the number of trails multiplied by their activity rating. To quantify these trail intensities, I vacuumed ants for 30 seconds from 35 trails (one trail per trunk) that I had visually scored and counted the ants in the sample. Low activity had a mean of 39.2 ants/30 seconds (n=10; range 21 – 76), medium activity had a mean of 131.4 ants/30 seconds (n=13; range 70 – 228), and high activity had a mean of 283.9 ants/30 seconds (n=12; range 166 – 502).

I sampled the density of adult scale insects in the canopy of three canopy species, *Inocarpus fagifer*, *Syzygium nervosum* and *Barringtonia racemosa*. I retrieved one branch from >10 m on live trees of each species at each site, using a pump action shotgun. Individual trees were chosen so that branch retrieval would be relatively simple. From each branch, I randomly selected five stems, and in a 20 cm section on each stem counted live adult females, and dead adults where I was able to distinguish them. Counts made on each of the 20 cm sections were combined for each tree, and the total number of adult females in one-metre sections from five trees used in analysis.

Furthermore, in each site I sampled the mobile crawler stages of *Tachardina* before ant exclusion and 11 wks after ant exclusion. I placed six scale 'hammocks' (0.45 m² catching sheets; see Chapter 4) in a 0.25 ha area for approximately 48 hours,

after which I counted individual bright red crawlers on each sheet. The mean number of crawlers/m²/day (crawler input) was used in analysis.

Analysis

The effect of Presto™ ant bait on the activity of *A. gracilipes* on the forest floor and tree trunks, and the effect of *A. gracilipes* on scale insects in the canopy and on crawler 'rain' from the canopy, were analysed using one-way repeated measures ANOVAs, using bait application as the main factor and time (before, 11 wks after baiting & 12 months after baiting) as the repeated measures factor. Site pairs (n=3) were used as replicates in all analyses. All graphs are presented using raw data.

Results

Effect of A. gracilipes on scale insects in the canopy of the rainforest

The densities of adult female scale insects in the canopy of the various tree species varied considerably (Table 6.1). *Inocarpus* sustained high densities of the lac scale *Tachardina aurantiaca* (mean 117.3 ± 11.5 SE adult females/m of stem at control sites) and no soft scale (Coccidae) across the three site pairs; *Syzygium* hosted relatively lower densities of soft scale (mean 27.0 ± 3.3 individuals/m of stem at control sites) and the rare individual *Tachardina*; *Barringtonia* was host to lower numbers of both types of scale insect (4.5 ± 1.7 and 6.5 ± 4.6 individuals/m of stem for *Tachardina* and Coccids respectively).

The distribution of Presto™ ant bait had a significant and negative effect on *A. gracilipes* activity at all ant exclusion sites, resulting in the death of foraging workers on the forest floor and on tree trunks within three days of distribution (Tables 6.2 & 6.3). There was 98% decrease in activity on the forest floor (Fig. 6.2) and 99.8% reduction in activity on tree trunks (Fig. 6.3) 11 weeks after the distribution of bait. Twelve months after ant exclusion, total elimination of foraging crazy ants had been achieved at the ant exclusion sites, whereas crazy ant activity at control sites remained

high for the duration of the experiment. Trunk ant traffic was consistently higher on *Inocarpus* than *Syzygium* at the control sites, and traffic on both species remained higher than trunk traffic on *Barringtonia* for the 12-month period.

Eleven weeks after ant exclusion with Presto™ ant bait there was a 67% decline in adult lac scales, *Tachardina*, in the canopy of *Inocarpus*, 100% decline in adult Coccidae in the canopy of *Syzygium* and 100% decline in both *Tachardina* and Coccid scales in the canopy of *Barringtonia* (Fig. 6.4). Twelve months after ant exclusion, populations of all scale insects (*Tachardina* in the canopy of *Inocarpus*, Coccidae in the canopy of *Syzygium*, and both types in *Barringtonia*) had collapsed, and this was a significant effect of ant exclusion for both *Inocarpus* and *Syzygium*, but not *Barringtonia* (Table 6.4). Furthermore, there was no sign of live scale insects on any species after 12 months in any control sites and there were visible signs of canopy recovery, for example new leaves and scale-free new shoots.

In *A. gracilipes* supercolonies, daily density input of the mobile crawlers of the lac scale *Tachardina aurantiaca* ranged from 11 crawlers/m²/day to 30 crawlers/m²/day. However, 11 weeks after ants were excluded crawler input was reduced by 98% at all ant exclusion sites while control sites were unaffected (Fig. 6.5; and see Table 6.5 for ANOVA results). Twelve months after ant exclusion, lac scale crawler input from the canopy had ceased altogether.

Discussion

The 'strength' of the mutualism between the crazy ant, *Anoplolepis gracilipes*, and scale insects in the canopy of Christmas Island rainforest was such that on a forest scale, within 12 months of ant exclusion, scale insect populations collapsed, despite their initial density. This could be considered complete dissolution of a mutualism, as all associated species (*A. gracilipes*, lac scales and soft scales, and host plant species) had ceased to positively interact in the experimental areas. Dissolution was achieved at a forest scale by means of eliminating foraging crazy ants on the forest floor and from

tree trunks by baiting with Presto™ toxic ant bait. Furthermore, the interruption to crawler input from local sources means that, at this spatial scale, populations of scale insects were unable to establish, and eventually collapsed.

To my knowledge, this is the first study to experimentally exclude ants on a forest-wide scale (but see James *et al.* 1997; 1998), and describe the association between ants and honeydew-producing hemipterans at this scale. No other studies have found a complete collapse of a hemipteran mutualist partner, but instead, have found varying degrees of reliance on ants for population growth, from no effect (Clarke & Debarr 1996) to a highly significant effect (Addicott 1979; Bristow 1984; Bach 1991; James *et al.* 1997; Helms & Vinson 2002). This study demonstrated complete dissolution of a mutualism across a multi-species complex; three host plant species, two types of scale insects (lac scales and soft scales) and *A. gracilipes*, and this occurred irrespective of the densities of scale insects in the canopy. In Java, the scale insect *Coccus viridus* flourished in the presence of *A. gracilipes*, with an average of 1,057 scales per coffee bush, compared to 70 on ant-free bushes (Van der Goot 1916). Similarly, (de Fluiter 1939) described that the severity of infestations of the scale insect *Pseudococcus citri* in coffee plantations in Java was positively related to the abundance of *A. gracilipes*. In plantations where the ant was absent, scale outbreaks were only sporadic. In the Seychelles, *Ceroplastes rubens* was 20 - 160 times more abundant and *Coccus viridus* 5 - 30 times more abundant at sites where *A. gracilipes* was the dominant ant (Haines *et al.* 1994). In all these systems, other species of ants were present but appeared not to contribute to the high numbers of scale insects.

Supercolonies of *A. gracilipes* cause a rapid, catastrophic shift in the rainforest ecosystem on Christmas Island, through the removal of red land crabs and their association with scale insects in the canopy (O'Dowd *et al.* 2003). Given the negative effects of scale insects on host plants (Gullan 1997, P.T. Green unpublished data), and that the mutualism between ants and scales can be dissolved by removal of *A. gracilipes*, release from impacts of the mutualism would be larger on *Inocarpus*, where densities of lac scales are highest and impact greatest, than on *Syzygium* or

Barringtonia, where lower densities of scale insects appear not to be causing harm to the plant. The disruption to the mutualism at the forest scale has several positive implications for the management of crazy ant supercolonies on Christmas Island.

First, because one application of toxic bait controlled two pests simultaneously, albeit over a period of 12 months, the elimination of scale insects via alternative means was unnecessary to provide complete control. Second, the crazy ants, through collapse of scale insects in the canopy, had lost a substantial and important part of their diet, and therefore were unable to sustain nests at all. Re-baiting of those supercolonies used in this study was unnecessary, and therefore the control of two pests considerably less expensive. Third, the mobile crawler stage of *Tachardina* had been eliminated 11 weeks after baiting, removing the potential for mobile scale crawlers to colonise neighbouring forest and initiate crazy ant and scale insect population build up in intact forest.

Furthermore, twelve months after baiting, other species of ants had failed to cause an increase in the abundance of *Tachardina* in the canopy and establish an energy source for crazy ants. This is possibly due to a time lag in the re-colonisation of these areas by other species of ants, or perhaps the decreased efficiency with which other, less aggressive, ant species tend scale insects (Buckley & Gullan 1991). Crazy ants in supercolony densities appear to exclude or inhibit other species of ants (Chapter 3), and if alternate territories had already been established elsewhere by displaced ant species, then colonisation of crazy ant supercolony territory by other species post-baiting would take time. Alternatively, crazy ants appear to be more 'efficient', or aggressive in tending *Tachardina* other species of ants, with perhaps the exception of a large *Camponotus* spp.(?*maculatus*). In the absence of crazy ants, the lag time for colonisation combined with inefficient tending of scales by other ant species would most likely be the reason for the lack of *Tachardina* re-infestation.

This result differs from the small-scale ant exclusion experiment (Chapter 5) in that the populations of both partners declined to practically zero, whereas on saplings in the rainforest understorey, *Tachardina* populations remained on the plants even after

almost nine months of ant exclusion. Considering the input of mobile crawlers from the canopy in the small-scale experiment, they are not strictly comparable. However, investigating the association between *A. gracilipes* and scale insects on only one of these spatial scales would lead to different conclusions about its 'strength'. On the small spatial scale, the effect of ant exclusion was not as dramatic as on the large, and would lead one to conclude that scale insects were not completely dependent upon *A. gracilipes* for their proliferation. The large-scale ant exclusion would lead one to conclude that scale insects on Christmas Island are almost completely dependent upon tending by *A. gracilipes* to reach the densities seen in supercolonies (Chapter 4).

Stachowicz (2001) challenged ecologists to develop an understanding of how environmental conditions, as well as morphological and chemical characteristics of mutualism participants cause shifts along the continuum of 'strengths' or outcomes of this interaction (see Bronstein 1994 for further ideas on this). I propose that spatial scale may significantly influence our understanding of mutualisms, and that the challenge is conducting experiments that reflect the spatial scale at which associations between species occur in nature.

Table 6.1 – Mean (\pm SE) number of adult female *Tachardina* and *Coccidae* found in the canopy of experimental trees at three control and three ant exclusion plots.

	Before ant exclusion		11 wks after exclusion		12 mo after exclusion	
	<i>Tachardina</i>	<i>Coccidae</i>	<i>Tachardina</i>	<i>Coccidae</i>	<i>Tachardina</i>	<i>Coccidae</i>
<i>Inocarpus</i>						
Control (n=3)	115.3 \pm 3.2	0	98.4 \pm 38.8	0	138.2 \pm 28.2	0
Ant exclusion (n=3)	98.7 \pm 14.5	0	32.9 \pm 8.2	0	0	0
<i>Syzygium</i>						
Control (n=3)	0.2 \pm 0.2	23.6 \pm 10.6	0.3 \pm 0.3	23.2 \pm 10.0	1 \pm 1.0	33.7 \pm 2.8
Ant exclusion (n=3)	0		0	0	0	0
<i>Barringtonia</i>						
Control (n=3)	5.2 \pm 3.2	15.5 \pm 9.9	7.1 \pm 7.0	0.7 \pm 0.3	1.2 \pm 1.1	3.4 \pm 2.8
Ant exclusion (n=2)	0.9 \pm 0.7	9.7 \pm 9.1	0	0	0	0

Table 6.2 – The effect of Presto™ ant bait on *A. gracilipes* activity on the forest floor. Statistics derived from one-way repeated measures ANOVA where baiting was the main treatment factor and times were before baiting, 11 wks after baiting and 12 months after baiting.

Source of variation	df	MS	F-ratio	p-value
Treatment	1	19269.462	55.832	0.002
Error	4	345.134		
Time	2	1432.761	8.005	0.012
Treatment x time	2	2942.603	16.441	0.001
Error	8	178.983		

Table 6.3 – Effect of Presto™ ant bait on *A. gracilipes* activity on tree trunk of *Inocarpus*, *Syzygium* and *Barringtonia*. Statistics derived from one-way repeated measures ANOVA, where ant exclusion (via baiting with Presto™) is the main factor, and times are before baiting, 11 wks after baiting and 12 mo after baiting.

	<i>Inocarpus</i>				<i>Syzygium</i>				<i>Barringtonia</i>			
	df	MS	F-ratio	p-value ^Φ	df	MS	F-ratio	p-value ^Φ	df	MS	F-ratio	p-value
Ant activity on tree trunks												
<i>Between subjects</i>												
Treatment	1	2.497	230.569	<0.001	1	10.173	76.815	0.001	1	17.689	1.988	0.253
Error	4	0.011			4	0.132			3	8.896		
<i>Within subjects</i>												
Time	2	0.611	69.845	<0.001	2	1.788	14.907	0.002	2	18.834	3.114	0.118
Treatment x time	2	0.491	56.132	<0.001	2	1.920	16.010	0.002	2	16.706	2.762	0.141
Error	8	0.009			8	0.120			6	6.048		

^Φ Indicates statistics generated from (log₁₀+1) transformed data

Table 6.4 – The effect of *A. gracilipes* on adult lac scales (*Tachardina aurantiaca*) and soft scales (Coccidae) in the canopies of *Inocarpus*, *Syzygium* and *Barringtonia*. Statistics derived from one-way repeated measures ANOVA, where ant exclusion (via baiting with Presto™) is the main factor, and times are before baiting, 11 wks after baiting and 12 mo after baiting.

	<i>Inocarpus</i>				<i>Syzygium</i>				<i>Barringtonia</i>			
	df	MS	F-ratio	p-value [Ⓞ]	df	MS	F-ratio	p-value [Ⓞ]	df	MS	F-ratio	p-value [Ⓞ]
<i>Tachardina aurantiaca</i>												
<i>Between subjects</i>												
Treatment	1	3.467	841.754	<0.001	1	63.840	0.778	0.433	1	63.840	0.778	0.433
Error	4	0.004			3	82.067			3	82.067		
<i>Within subjects</i>												
Time	2	1.461	39.929	<0.001	2	12.016	0.621	0.503	2	12.016	0.621	0.503
Treatment x time	2	1.790	48.945	<0.001	2	10.456	0.540	0.533	2	10.456	0.540	0.533
Error	8	0.037			6	19.364			6	19.364		
					df	MS	F-ratio	p-value [Ⓞ]	df	MS	F-ratio	p-value [Ⓞ]
<i>Coccidae</i>												
<i>Between subjects</i>												
Treatment					1	4.815	33.066	0.005	1	39.336	0.466	0.544
Error					4	0.146			3	84.392		
<i>Within subjects</i>												
Time					2	0.215	2.474	0.146	2	215.125	2.347	0.177
Treatment x time					2	0.586	6.739	0.019	2	7.971	0.087	0.918
Error					8	0.087			6	91.678		

[Ⓞ] Indicates statistics generated from (log₁₀+1) transformed data

Table 6.5 - The effect of *A. gracilipes* on the input of lac scale crawler 'rain' from the canopy as measured by crawler hammocks strung between trees. Statistics derived from raw data analysed with a one-way repeated measures ANOVA.

Source of variation	df	MS	F-ratio	p-value
<i>Between subjects</i>				
Treatment	1	2.446	82.962	0.001
Error	4	0.029		
<i>Within subjects</i>				
Time	1	2.044	57.160	0.002
Treatment x time	1	2.301	64.337	0.001
Error	4	0.036		

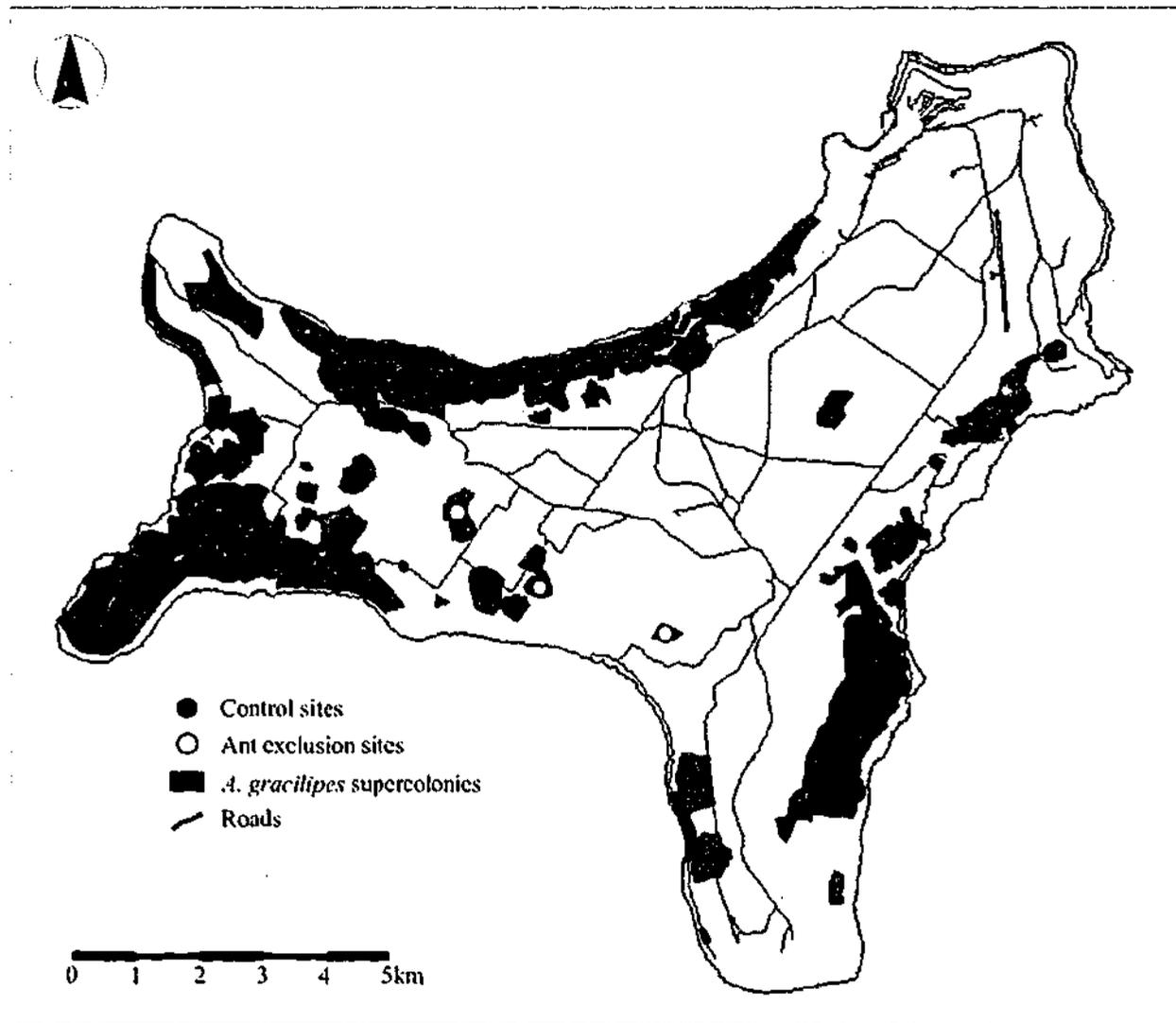


Figure 6.1 – Total area occupied by *A. gracilipes* supercolonies between 2000 and 2003, and location of control and ant exclusion sites for large-scale ant exclusion experiment on Christmas Island, Indian Ocean.

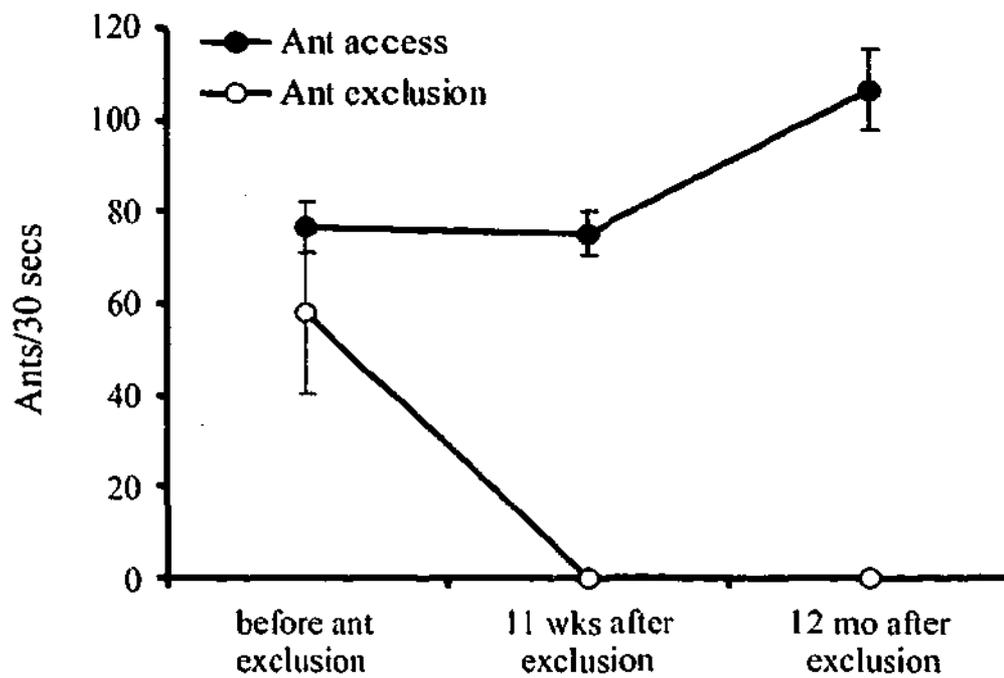


Figure 6.2 - The effect of Presto™ ant bait on *A. gracilipes* activity on the forest floor. Closed circles: control sites, open circles: ant exclusion sites, at three sampling times. All data points are means ($n=3$) \pm SE. Trunk activity score is the mean trunk activity score measured on each trunk ($n=5$) at every site each time, averaged across sites. Note different y-axis scales.

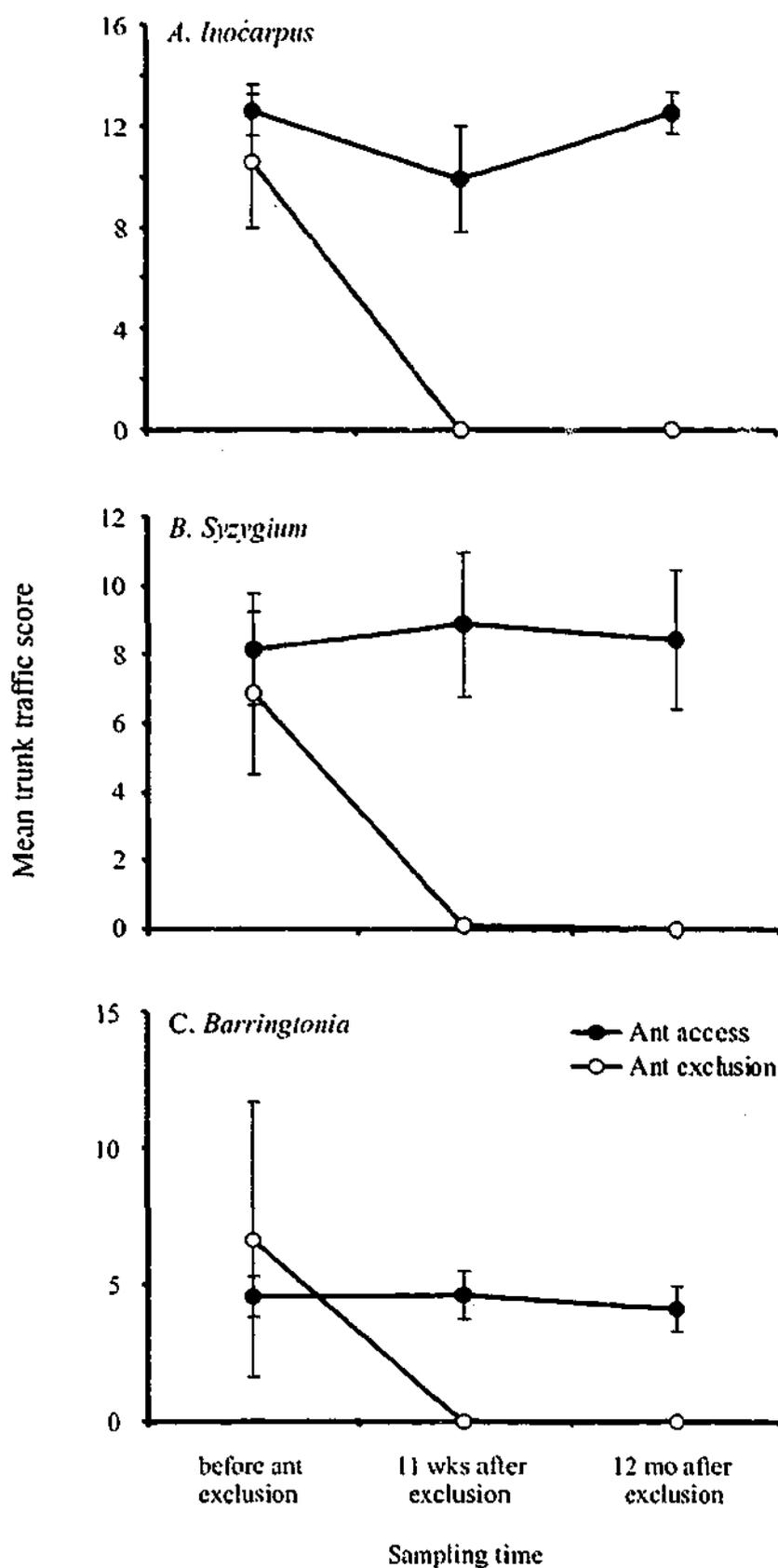


Figure 6.3 – The effect of Presto™ ant bait on *A. gracilipes* activity on trunks of *Inocarpus* (A), *Syzygium* (B) and *Barringtonia* (C). Closed circles: control sites, open circles: ant exclusion sites. All data points are means ($n=3$) \pm SE, except for ant exclusion points for *Barringtonia* ($n=2$). Trunk activity score is the mean trunk activity score measured on each trunk ($n=5$) at every site each time, averaged across sites. Note different y-axis scales.

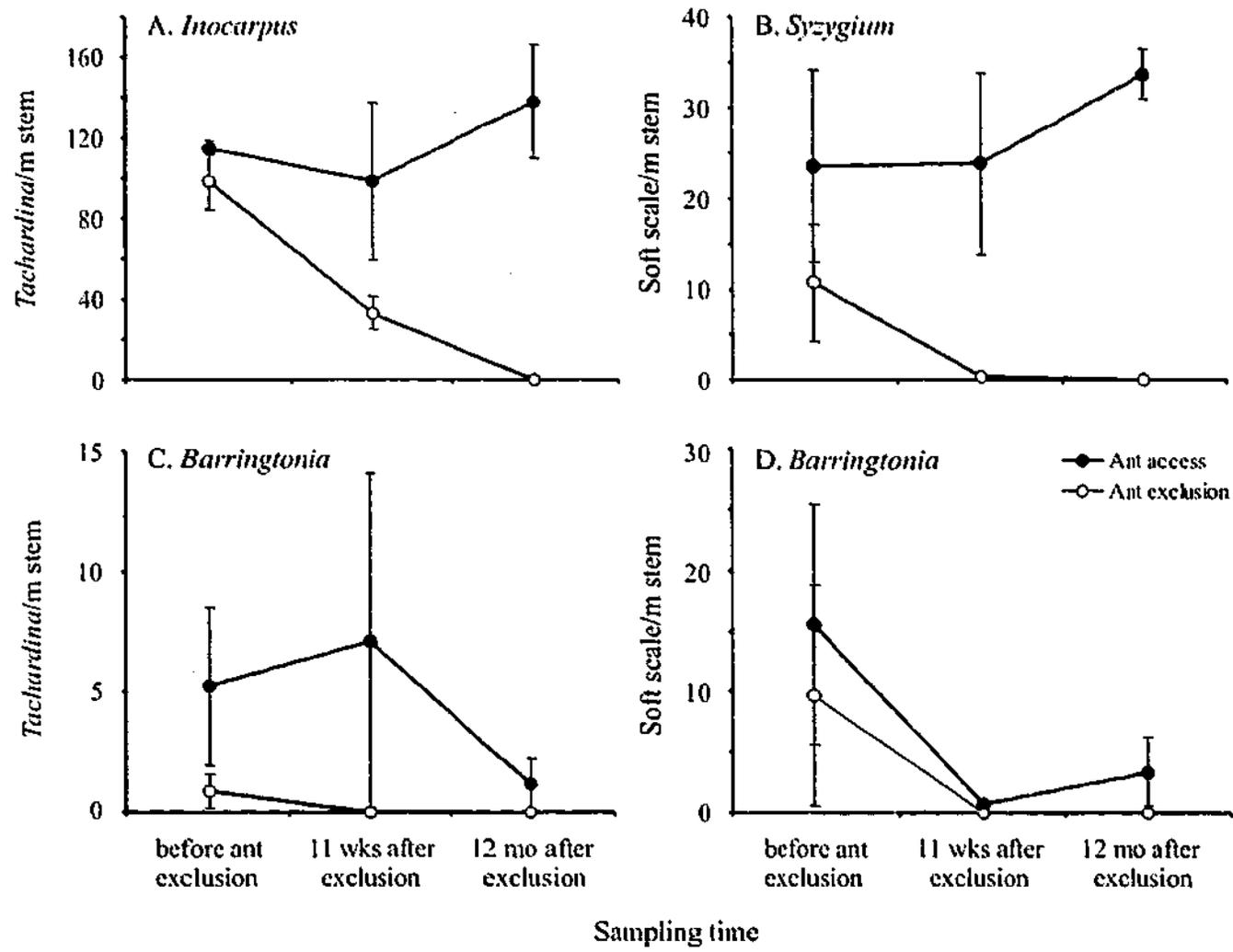


Figure 6.4 – The effect of *A. gracilipes* exclusion on adult female lac scales, *Tachardina aurantiaca*, on *Inocarpus* (A), adult female soft scales, Coccidae, on *Syzygium* (B), and both types of scale insect on *Barringtonia*. Closed circles: control sites, open circles: ant exclusion sites. All data points are means (n-3) \pm SE. Note different y-axis scales.

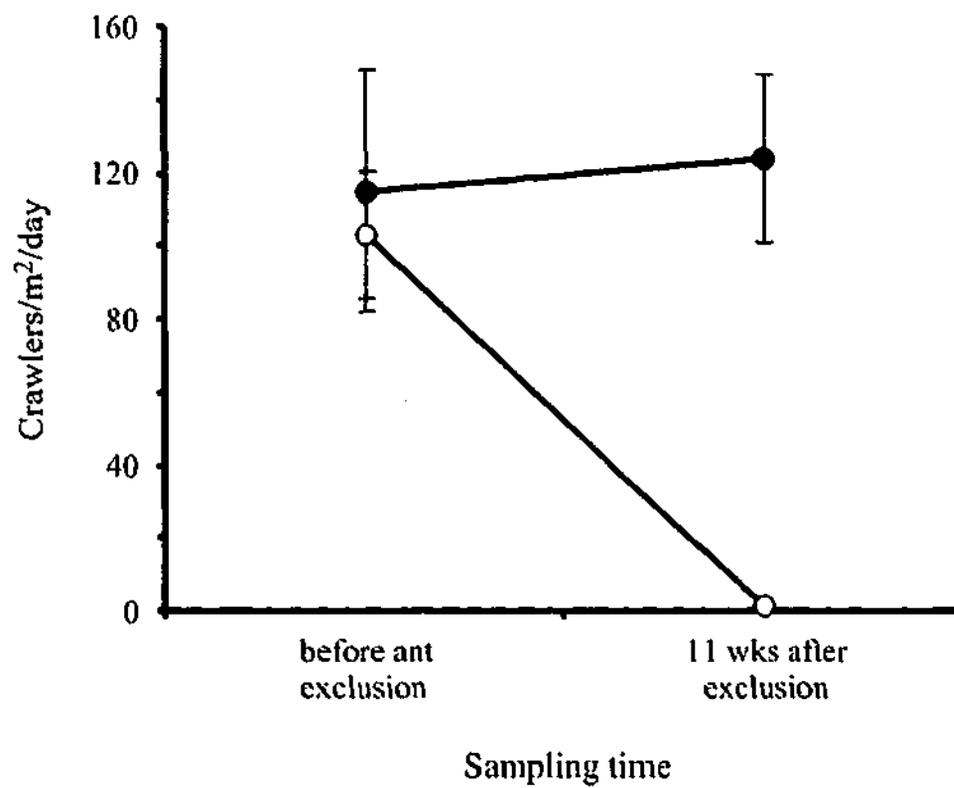


Figure 6.5 - Effect of *A. gracilipes* on lac scale crawler input from the canopy of the rainforest on Christmas Island. Closed circles: control sites, open circles: ant exclusion sites. Data points are means ($n=3$) \pm SE.

Chapter Seven

Crazy ants and water stress as facilitators of scale insect outbreaks on Christmas Island.



Introduction

A sudden increase in abundance of an invasive species may be triggered by the abiotic environment, biotic elements of the target community, characteristics of the invader, and interactions between these variables (Ehrlich 1986; Orians 1986; Lodge 1993; Rejmanek & Richardson 1996). Research has sought to explain general characteristics of invaders and, independently, their target communities (Loope & Mueller-Dombois 1989; Pimm 1989; Simberloff 1989; Case 1990), and indeed, there appear to be some general characteristics of invaders, and of invaded ecosystems, which improve the chances of successful invasion. However, we have been notoriously unsuccessful in predicting the success or spread of introduced species, almost certainly a result of our tendency to focus on only characteristics of the invader, or independently, abiotic conditions of invaded habitats; rarely both, or their relationship to each other.

Insect outbreaks worldwide are often preceded by atypical weather conditions, particularly drought, precipitation or unusually extreme temperatures (White 1984; 1986; Martinat 1987; Mattson & Haack 1987; Watt *et al.* 1990). Some of the best-known examples of insect outbreaks documented as driven by abiotic factors include gypsy moths (*Lymantria dispar*), mountain pine beetle (*Dendroctonus ponderosae*), forest tent caterpillar (*Malacosoma disstria*) and spruce budworm (*Choristoneura*

fumiferana) in the northern hemisphere, where synchronous outbreaks of all these species are thought to be due to high spatial correlation in weather variables (Peltonen *et al.* 2002).

Two central hypotheses have emerged to explain mechanisms whereby abiotic conditions can increase insect populations on host plants, the Plant Stress Hypothesis (PSH; White 1984) and the Plant Vigour Hypothesis (PVH; Price 1991). The PSH predicts that stressed plants are more suitable hosts for insect herbivores due to increased nutritional value (mainly free amino acids and assimilable nitrogen) and/or the reduction of defensive compounds (White 1984; Coley *et al.* 1985; White 1993). It is also based on the premise that different types of abiotic stresses (e.g. water, herbivory, physical damage, disease and pollution) affect the plant in a similar manner. The hypothesis as it stands has received much experimental attention, but lacks empirical support (Larsson 1989; Koricheva & Larsson 1998; Inbar *et al.* 2001). However, insect feeding guild has perhaps the greatest influence on the effect of plant stress on insect performance, and in general, sucking insects (e.g. scale insects) have performed better on stressed plants than on vigorous ones (Larsson 1989; Koricheva & Larsson 1998). The PVH, in contrast, predicts that vigorous young growth on plants provides the most nutritious source of food for the survival and growth of early instars (Price 1991).

Organisms outside their native range can also increase in abundance in response to biotic interactions; release from natural enemies, and predation and competition pressures (Williamson 1996; Crawley 1997; Keane & Crawley 2002), but also via mutually beneficial interactions with other species in a community (Callaway & Walker 1997; Richardson *et al.* 2000; Stachowicz 2001; Bruno *et al.* 2003). In fact, mutualisms are important to the persistence of many species in a natural communities (Bond & Slingsby 1984; Bond 1994; Christian 2001; Schenk & Mahall 2002), and in combination with favourable abiotic conditions, should further increase the abundance of each partner. Wagner and Kurina (1997) demonstrated that *Hemiargus isola* butterflies use *Formica perpilosa* ants as oviposition cues, and butterflies laid twice as

many eggs on plants with ants than without. But further, the butterflies also laid more eggs on watered plants than unwatered ones.

Mutualistic associations between either native and introduced species or pairs of introduced species are common in terrestrial and aquatic environments, and can favour certain invasions (Simberloff & Von Holle 1999; Richardson *et al.* 2000; Ricciardi 2001), and provide the basis for positive feedback loops promoting population increases of both partners. For example, in 1958, Elton described the association (which he called a 'closed chain of symbiosis') between the Argentine ant, *Linepithema humile*, and the Asian red scale, *Aonidiella aurantii* in Californian citrus orchards. The interaction resulted in positive population responses of both partners (although only the population impact of ants on scale was reported), but negative impacts on the citrus trees. On Christmas Island, I have shown that honeydew-producing scale insect populations decline significantly in the absence of the introduced invasive ant *Anoplolepis gracilipes*, and that in *A. gracilipes* supercolonies densities of scale insects are 16-fold greater than in non-supercolony areas (Chapter 4). The population build up of these two partners (although I have measured only the scale insect's population response to the ants) have resulted in synergistic effects to amplify impacts, leading to 'invasional meltdown' on Christmas Island (O'Dowd *et al.* 2003).

The island-wide distribution of *A. gracilipes* supercolonies may provide clues to a potential abiotic trigger to supercolony formation. The island-wide survey (see Chapter 4) indicated that supercolonies form significantly more often in forest types II and III, and significantly less often in forest type I, than expected, based on the relative coverage of these forest types across the island. Forest types II and III account for 67% of all forest on the island, but 90% of survey points at which supercolonies were detected were in these forest types, and although forest type I accounts for 32% of all forest on the island, only 10% of survey points at which supercolonies were detected were in this forest type. The propensity for supercolonies to form in forest types II and III largely explains the fact that most *A. gracilipes* supercolonies (79%) have been detected at elevations below 200 m ASL (55% of forest), on the periphery of the island.

Sixty-five percent of all forest types II and III occur below this elevation, and these types comprise 80% of all forest below 200 m ASL.

Among the many differences between these forest types, two are important for an examination of the factors leading to supercolony formation; floristic composition and soil depth. In Chapter 4 I tested, and rejected, the hypothesis that floristic composition and "scale suitability" varied between the three main forest types. Further, I concluded that floristic differences alone were insufficient to explain the supercolony formation in forest types II and III, supposedly as a consequence of these forest types being more suitable than forest type I to promote outbreaks of scale insects.

However, one clear difference between these forest types is soil depth (in fact, Mitchell [1985] based his vegetation map almost exclusively on Barrie's (1967) geological map, itself a broad reflection of soil depth and substrate type). Forest type I typically grows where soil is deep (usually several metres), with rare to infrequent limestone outcropping. Forest type II grows in areas where the soil is much shallower and where limestone outcrops are more frequent, while forest type III grows on almost pure limestone, with virtually no surface soil. Both forest types II and III comprise more deciduous species than type I. The water-holding capacity of the substrate is of critical importance to plant water status (Painter 1966; Holtzer *et al.* 1988; Borchert 1994), and many authors (Mitchell 1975; 1985; Gillison 1989; Du Puy 1993) have noted that forest growing on shallow soils in types II and III on Christmas Island is noticeably "drier" than that occurring in on the deeper soils of forest type I.

In this chapter, I test the hypothesis the idea that a key trigger to crazy ant supercolony formation on Christmas Island is plant stress induced by low water availability. As explained above, the PSH posits that stressed plants are more suitable hosts for insect herbivore than unstressed plants, and that sap-sucking insects receive positive benefits from stressed plants more often than insects in other feeding guilds. If so, this may explain the tendency of *A. gracilipes* supercolonies to have formed in the "drier" forest types on Christmas Island. Greater plant stress in forest types II and III may have promoted elevated rates of population increase in scale insects. Then,

positive feedback loops with *A. gracilipes* caused population increases of both ants and scales to a threshold where *A. gracilipes* colonies fused into supercolonies. Through surveys and experiments, I test three specific hypotheses;

1. That the water status of forest types II and III is lower (more stressed) than forest type I
2. That water-stressed plants are more suitable hosts for the lac scale *Tachardina aurantiaca* than unstressed plants (the PSH hypothesis)
3. That the presence of *A. gracilipes* can amplify the effects of water stress in promoting *Tachardina* abundance.

Methods and Materials

The study system

Christmas Island (105° 40'E, 10° 30'S) is an elevated oceanic limestone island, located 360 km south of Java in the northeastern Indian Ocean, and covers an area of approximately 135 sq km. The interior of the island is a slightly undulating plateau, ranging in height from approximately 160 m ASL to its highest point (Murray Hill) at 361 m ASL. This plateau is surrounded by a series of inland cliffs and terraces, which terminate in sheer sea cliffs, often undercut, that encircle the island. The island has been inhabited for about 106 years, and the economy is based around mining phosphate from guano deposits found mainly on the central plateau areas. Presently, approximately 74% of the island is covered with natural vegetation, where three of seven described vegetation types (Mitchell 1985, but see Chapter 4 this thesis) dominate both plateau and terrace areas.

The first supercolony of the yellow crazy ant, *A. gracilipes*, was observed in 1989, where ants were in densities sufficient to kill the endemic red land crab, *Gecarcoidea natalis* (Brachyura: Gecarcinidae). Since then supercolonies have spread across the island, occupying individual areas of between ~5 – 900 ha (Chapter 3). Data

for this study were collected between July 2002 and September 2002 in areas of *A. gracilipes* supercolony formation and intact forest. Supercolonies were defined as having *A. gracilipes* activity sufficient to kill red crabs, and between 2001 and 2003 these areas covered >2500 ha of the island (Chapter 2 & 3).

Hypothesis 1 – island-wide survey for differences in water status between forest types

I tested the hypothesis that forest types II and III were significantly more water-stressed than forest type I by measuring mean water potential at 15 sites (five each of I, II and III) across the island (Fig. 7.1 for forest types, refer to Fig. 4.2, Chapter 4 for sampling sites in each forest type). At each site, I obtained leafy twigs from between two and five randomly selected individuals of those species that made up 80% of all stems ≥ 10 cm dbh at those sites (Appendix 1). An extendable pole pruner was used to collect these samples from the lower branches of trees ≤ 10 cm dbh, or from large saplings. The number of species in each forest type varied - there were at least six species in each forest type, but only three common to all types (and not present at all sites). All sampling took place at dawn over a 50-day period, from 2 September to 22 October 2002. During this time a total of 41.2 mm of rain was recorded, with 27 mm falling on 6 September. I did not proceed with sampling if moisture was visible on leaves, as it can influence the water potential reading by the leaves taking in residual water. Consequently, even light rain interrupted the sampling schedule over the sampling period.

The water potential of two leaves or shoots per branch was measured with a portable pressure chamber in the field at the time of collection (Scholander *et al.* 1965). Total water potential was measured as the pressure (MPa) necessary to force water from the cut end of the petiole or shoot. Where leaf water potential was measured, petioles were cut at an angle to ensure an even surface to visualise water droplets. However, leaf-bearing shoots of most species were more suitable for the pressure

chamber. In this case, the twig was cut to ensure a long enough section to be inserted into the pressure chamber.

For determination of "forest water status", I first calculated a mean value for water potential of each species at each site (mean of two leaves/shoots per individual, averaged across individuals within species), and then multiplied the positive water potential value (water potential is actually a negative value; -MPa) by the stem density of the species at that site and divided that number by 10 to make it more manageable. I then averaged these values for those species that made up 80% of canopy trees surveyed to obtain a site value for mean "forest water status". Hence, the more positive the value, the drier the forest type. For analysis I used sites as replicates, and tested for differences in "forest water status" between forest types with a one-way ANOVA (SYSTAT ver.10), where forest type was the main factor and "forest water status" the dependent variable.

At each site, I also took measurements of soil moisture in the top 7 cm of soil, using a ThetaProbe (Delta-T Devices). This device measures volumetric soil moisture content (θ) by responding to changes in the apparent dielectric constant of moist soil. The changes are converted into a dc voltage in mV, which is then converted to soil moisture content using a linearisation table and soil type parameters. Soil moisture was recorded at 20 randomly chosen locations within each site, and the mean of these readings was used as a site replicate for analyses. I analysed the effect of forest type on soil moisture with a one-way ANOVA using forest type as the main factor and soil moisture as the dependent variable.

Hypotheses 2 & 3; manipulative field test of the PSH and ant tending hypotheses for Tachardina abundance

I tested the combined effects of *A. gracilipes* attendance (biotic effect) and water stress (abiotic effect) on the colonisation and survival of *Tachardina* populations in a manipulative field experiment. This experiment was conducted in an *A. gracilipes* supercolony, next to the WBT site (see Chapter 3 for site location). This site was

chosen because it had very high densities of ants, and was relatively flat and accessible for transporting water and plants.

In March 2002, approximately 70 seedlings each of three species (*Inocarpus fagifer*, *Pongamia pinnata* and *Tristiropsis acutangula*) were carefully removed from the forest, and transplanted into 5-litre plastic pots using forest topsoil as the potting medium. I chose these species because they were highly susceptible to colonisation by *Tachardina*, and *Tachardina* made up >25% of all scale insects in *A. gracilipes* supercolonies (Chapter 4). All plants were held under nursery conditions and watered daily for 16 weeks. On 2 July 2002, c. 48 healthy seedlings of each species were placed in the field. At this time seedlings of *Inocarpus* were 63 ± 2 cm tall (mean \pm SE; range 43 – 89 cm), seedlings of *Pongamia* were 70 ± 2 cm (range 50 - 100 cm), and seedlings of *Tristiropsis* were 45 ± 2 cm (range 18 - 64 cm).

The plants were arranged in a randomised block design consisting of six blocks, each comprising 8 seedlings of each species (24 plants in each block). Four treatments were applied in each block: 1) water stressed plants with ants excluded, 2) water stressed plants with ant access, 3) non-stressed plants with ants excluded, and 4) non-stressed plants with ant access. There were $n=2$ plants per species per treatment in each block. One plant was used to track the numerical response of *Tachardina* to these treatments, while the other was used as a “sacrificial” plant to destructively sample and measure water potential. Within species and blocks, plants were randomly assigned to either the experimental or sacrificial groups. Within the experimental plants, there was no significant difference in plant height within a species, between blocks or treatments for *Inocarpus* and *Pongamia* (one-way ANOVA for block and treatment separately; *Inocarpus*: $p=0.325$, $p=0.338$; *Pongamia*: $p=0.251$, $p=0.252$ respectively). However, there was a significant difference in the height of *Tristiropsis* plants between blocks but not treatments ($p=0.016$, $p=0.778$ respectively). Post hoc tests using Tukey’s method revealed that the difference between blocks was due to one very small *Tristiropsis* plant, which I was obliged to use.

The treatments were imposed as follows. At the start of the experiment, every plant was given one litre of water. Plants in the water-stress treatments were then left for seven days without water, while the non-stressed plants were watered three times with 300 ml. Thereafter, water stressed plants were watered once per week with 300 ml and non-stressed plants watered every second day with 300 ml for the duration of the experiment. Ants were excluded from seedlings on 10 July 2002 by applying a band of Tanglefoot[®] band to the bases of their stems. Pots were placed on small circles of nursery grade plastic to prevent roots from reaching the surrounding soil.

All plants were free of scale insects when first placed in the field. *Tachardina* crawlers were allowed to accumulate on the plants for three days thereafter, at which time I erected a shelter over the plants in each block. This shelter was constructed from bamboo corner poles, covered with translucent plastic sheeting (200 μm thickness). These shelters were necessary to exclude rain for maintenance of the water stress treatment, and to minimise the "swamping" effect of continual input of scale crawlers from the canopy.

Verification of treatments

I verified that the watering regime produced stress treatment differences in three ways. First, I used half of the individuals in each species in each block as "sacrificial" plants on which to destructively measure either leaf (*Pongamia* and *Tristiropsis*) or shoot (*Inocarpus*) water potential. These plants were sampled on two occasions, 9 August (day 29) and 30 August 2002 (day 50). Commencing at dawn, I measured water potential using the Scholander pressure chamber at the experimental site. I used a two-way ANOVA to test the effect of watering regime and ant access on the water status of the plants, where water and ants were the main factors, and water potential the dependent variable. In this analysis, $n=6$ for each treatment (one plant per species per block, x 6 blocks).

Second, the soil moisture of all potted plants was recorded on 30 August (day 50 of experiment) using the ThetaProbe (see above). The soil moisture of both

experimental and sacrificial plants was measured, and averaged between plants ($n=2$ in each species/treatment combination) to calculate a block replicate for analysis. I used a one-way ANOVA to determine the difference in mean % soil moisture between the two treatment types (stressed vs non-stressed). Data were not available to test for a difference between ant access treatments. I expected that a difference in the moisture content of the soil would translate into a difference in water potential of the plants. Third, I recorded plant mortality in each treatment over the course of the experiment.

I verified the ant exclusion treatment by noting the presence or absence of *A. gracilipes* on plants at each sampling time.

Effect of water stress and ants on Tachardina populations

Tachardina crawlers, 2nd instar and adult females were counted on experimental plants in each block (i.e. 1 plant per species per treatment per block, or $n=6$ plants per species per treatment for the whole experiment). The first counts of scale insects were made on 11 July 2002, and subsequently on days 15 (26 July), 33 (13 August), 49 (29 August) and 67 (16 September) of the experiment. On each occasion I counted the number of *Tachardina* crawlers, 2nd instars and adults present in three randomly chosen 5 cm sections of the plant, which I tagged to enable me to follow the same sections of stem throughout the experiment. For analysis, I combined these 5 cm sections and used the mean number of individuals per stem (15 cm) per plant.

Because scale insects established on plants over time, crawlers were present at the start, but 2nd instars were not observed until the second observation period (day 15), and adults were not observed until day 49 of the experiment. Therefore, I was able to examine the effect of watering regime and *A. gracilipes* on *Tachardina* crawlers and 2nd instars over time. I used a two-way repeated measures ANOVA to do this, using water and ants as the main factors and the mean number of individuals/stem/plant as the dependent variable. However, I used a two-way ANOVA to test the effect of watering regime and ants on the number of adult female *Tachardina* that had colonised the plants on the last sampling day only (16 September, day 67).

Results

Hypothesis 1 – island-wide survey for differences in water status between forest types

Mean forest water status, as measured by dawn water potentials and stem density, did not differ between forest types (Table 7.2a, Fig 7.2a). However, mean forest water status of forest type III was almost double that of forest types I and II. When viewed by species, there were no consistent patterns in water potential across forest types (Fig. 7.3). The most negative water potential was for *Berrya cordifolia* in forest type II (-4 Mpa), and the least negative was *Leea acutangula* in forest type III (-0.375), both species that do not support populations of scale insects. Four tree species exhibited water potentials that became more negative from forest type I to III: *Cryptocarya nitens*, *Dysoxylum gaudichaudianum*, *Inocarpus fagifer* and *Ochrosia ackeringae*. Due to lack of replication I could not formally analyse the variation in all four species. However, *Cryptocarya nitens* showed significantly different water potentials between forest types (one-way ANOVA; $F_{2,6} = 8.075$, $p = 0.02$) whereas *Dysoxylum gaudichaudianum* did not ($F_{2,6} = 2.974$, $p = 0.127$). Only *Tristiropsis acutangula* showed the reverse of what was expected (water stress forest type I > type II > type III), but I could not formally analyse this pattern.

There was a significant difference in % soil moisture between forest types II, II and III (Table 7.2b, Fig. 7.2b). Forest type I had a mean % soil moisture of 11.1 ± 1.5 SE, forest type II 8.9 ± 1.4 and forest type III 5.8 ± 0.4 . Post Hoc analysis using Tukeys method revealed that forest type III was significantly different from both I and II, which were not significantly different from each other.

Hypotheses 2 & 3 - manipulative field test of the PSH and ant tending hypotheses for Tachardina abundance

Verification of treatments

The watering regime imposed upon the experimental and sacrificial plants had a significant effect on the soil moisture, water potential and mortality of the plants. Soil moisture was significantly lower in stressed plants (mean 17.7 ± 0.7 SE) than in non-

stressed plants (mean 30.4 ± 0.3 SE; Table 7.2A. & B.). The leaf/shoot water potential ($\Psi_{\text{leaf}} / \Psi_{\text{shoot}}$) of stressed *Tristiropsis* plants was significantly different on day 29 (9 August 2002), but for *Inocarpus* and *Pongamia* there was no significant difference in water potential between treatments. However, on day 50 (30 August 2002), all species showed significantly higher water potentials in the stressed treatments (Fig. 7.2, Table 7.4). Additionally, at the end of the experiment (day 67; 16 Sept 2002), plant mortality was highest in the water-stressed/ant access treatment, followed by non-stressed/ant access, water stressed/ant exclusion, with no mortality in the control treatment, non-stressed/ant exclusion (Table 7.5).

The Tanglefoot[®] ant exclusion barrier appeared to be 100% successful. I visited the site every second day to water plants, check barriers, and remove any ants on exclusion treatments. Consequently, on the sampling days I never encountered *A. gracilipes* on plants assigned the ant exclusion treatment.

Effect of water stress and ants on Tachardina populations

After three days exposure in the field, there was no significant difference in the number of *Tachardina* crawlers on plants between species (mean 80.9 ± 3.5 SE crawlers/seedling, $n=24$ for each species; $F_{2,69}=0.237$, $p=0.790$; Fig. 7.6A, 7.7A, 7.8A). Second instar *Tachardina* were first observed on plants on day 15 and adult females on day 49.

There were highly significant, positive effects of *A. gracilipes* access, but no effect of watering regime, on the density of all life stages of *Tachardina* on all host species. Crawlers, 2nd instars and adult females all followed a similar pattern on the three host species.

Inocarpus: The presence of ants positively affected all *Tachardina* life stages on *Inocarpus*, whereas watering regime had no effect at all (Fig. 7.6, Table 7.5). The number of crawlers decreased over time on all treatments, but more rapidly on plants without ants than where ants had access. Over the 67-day period, there were consistently more *Tachardina* crawlers on plants where ants had access than on plants

without ants, except for between days 49 and 67 of the experiment. During this time there was an increase in the number of crawlers on water-stressed/ant access plants and a decrease in the number of crawlers on non-stressed/ant access plants. Second instars colonised plants in all treatments, however, the number of 2nd instars increased to day 33 more rapidly on plants where ants had access than where ants were excluded. Over the four sampling days that 2nd instars were recorded, there were always significantly more on plants with ants than those without. However, watering regime did not affect the number of 2nd instars at all. Similarly, adult females colonised plants in all treatments, but attendance of *A. gracilipes* significantly increased colonisation success, and the number of adult females increased more rapidly over a 34-day period on plants where ants had access than where they were excluded, whereas watering regime had no significant effect on colonisation success of adult females. There was more than a 5-fold difference in the average density of adult females on plants where ants had access than where they were excluded. After 67 days, there was no significant effect of watering regime on the density of any life stage, crawlers, 2nd instars and adult females.

***Pongamia*:** The response of all life stages of *Tachardina* to *A. gracilipes* access and watering regime on *Pongamia* was similar to those on *Inocarpus*. Ant access significantly and positively affected the number of crawlers, 2nd instars and adult female *Tachardina* on *Pongamia* over the 67-day experimental period, whereas watering regime did not affect *Tachardina* numbers at all, nor was there any interaction between the two factors (Fig. 7.7, Table 7.6). Interestingly, between days 49 and 67 of the experiment, *Tachardina* crawlers on *Pongamia* showed the same pattern as on *Inocarpus* - there was an increase in the number of crawlers on water-stressed/ant access plants and a decrease in the number of crawlers on non-stressed/ant access plants. After 67 days, there was more than a 4-fold difference in the average density of adult females on plants where ants had access than where they were excluded and no significant effect of watering regime on the density of any life stage, crawlers, 2nd instars and adult females.

***Tristiropsis*:** Although 2nd instars and adult females were less abundant on *Tristiropsis* compared to *Inocarpus* and *Pongamia*, they exhibited a significant positive response to *A. gracilipes* access, but not to the watering regime (Fig. 7.8, Table 7.7). Similar to both *Inocarpus* and *Pongamia*, *Tachardina* crawlers on *Tristiropsis* decreased in numbers over 67 days, but more rapidly on plants without ants than on plants where ants were allowed access. Second instars colonised plants in all treatments, but more rapidly on plants with ants than without. Adult female *Tachardina* responded significantly to ants, and there was a significant difference in the number of adults on plants with ants that on those without. However, after 67 days, the greatest difference in numbers of adults was between plants in the non-stressed/ant access and non-stressed/ant exclusion treatments (mean 34.5 ± 7.4 and 2.3 ± 1.1 respectively). Within the water-stressed treatment, plants with ants had a mean of 18.3 ± 5.8 and those without ants a mean of 12.0 ± 8.2 .

Discussion

I found no experimental evidence in support of the Plant Stress Hypothesis in its original form (White 1974; 1984). Results indicated no difference in the number of adult female lac scales between water-stressed and non water-stressed plants, despite plant water potential being significantly more negative the majority of the time in the water-stressed treatments (but see further discussion on water-stressing plants below). However, attendance by yellow crazy ants, *Anoplolepis gracilipes*, resulted in significantly greater numbers of crawlers, 2nd instars and honeydew-producing adult females of the lac scale *Tachardina aurantiaca* on three host species. After 67 days, densities of adult females were, on average, 4-fold greater on plants where ants were allowed access compared to on plants where ants were excluded, indicating greater colonisation success in the presence of *A. gracilipes*.

I experimentally quantified the combined effects of two potentially beneficial factors (water stress and ant attendance) on scale insects over three trophic levels

(plant, sap-sucker, ant tenders). Field experiments at this level of complexity are scarce in the literature. It has even been suggested that the fear of experimental failure at higher levels of complexity has led to papers of little relevance for the understanding intricate interactions and behaviours of, and between, species (Cörner 1994). However, a similarly designed experiment documented that the butterflies *Hemiargus isola* use *Formica perpilosa* ants as oviposition cues, laying more eggs on plants with ants than on those without ants. In addition, butterflies laid more eggs on plants that had been supplemented with water than on those that were unwatered, but ants also increased the number of inflorescences on a plant, and it was thought that the butterflies responded to an increased number of inflorescences on watered plants, rather than to tissue water content itself (Wagner & Kurina 1997). Their result indicates a clear interaction between biotic and abiotic factors in explaining the abundance of eggs laid by *H. isola* and its subsequent success. However, since water stress may directly influence plant quality for sap-sucking scale insects, I have demonstrated that attendance by *A. gracilipes* (biotic factor) is more important than plant stress (abiotic factor) in facilitating colonisation of crawlers, and determining subsequent abundance of adult female lac scale insects on three rainforest host species.

Scale insects at the densities seen on Christmas Island are a pest in their own right. Decline in tree vigour and canopy dieback of several rainforest species that sustain high densities of *Tachardina*, have been observed in *A. gracilipes* supercolonies (P.T. Green unpublished data), indicating that the escalation of both scale insects and *A. gracilipes* is detrimental for forest integrity. Not only does *A. gracilipes* eliminate red crabs, causing increased seedling density and altered composition, as well as a thick carpet of leaf litter (O'Dowd *et al.* 2003). Ants indirectly cause the decline in canopy species, and subsequently more canopy gaps, which can affect forest dynamics considerably (Denslow 1996; Green 1996; Ostertag 1998; Svenning 2000; Blundell & Peart 2001; Busing & Brokaw 2002).

Mechanisms by which *A. gracilipes* increased numbers of scale insects include protection from predators (Chapter 5), and benefits other than protection from

parasitoids, as I observed no parasitism of any scale insect species on Christmas Island. An important benefit to adult female *Tachardina* was removal of honeydew (Flanders 1951; Buckley 1987; Bach 1991; Gullan 1997). On plants where *A. gracilipes* was excluded, adult females quickly became covered in their own excreted honeydew, and fungus and moulds appeared to asphyxiate them. Furthermore, crawlers were caught in the honeydew that was covering adults, unable to free themselves to establish on scale-free space (Fig. 7.8).

Water stressed forests, as direct promoters of honeydew-producing scale insects, appear not to have produced the spatial distribution of *A. gracilipes* supercolony formation on Christmas Island. Historically, *A. gracilipes* supercolonies formed around the edges of the island, in drier terrace forests. This led to the hypothesis that abiotic stresses caused drier forests to become suitable hosts for honeydew-producing scale insects (White 1984; Mattson & Haack 1987; Holtzer *et al.* 1988), whose populations increased and provided the bottom rung of the positive feedback mechanism promoting increased abundance of both scale insects and *A. gracilipes*. But now, 15 years after the first supercolony was discovered, and eight years after the progressive increase in their size and occurrence, supercolonies are becoming progressively more common in forest type I where the soils are deeper and moisture availability to rainforest plants is increased (Barrie 1967; Mitchell 1985; Falkland 1986). Forest type, whether described by floristics, scale suitability or water status, appears not to be the driver for the formation of *A. gracilipes* supercolonies, and the disproportionate frequency with which *A. gracilipes* form supercolonies in forest types II and III may require an alternative explanation.

Water, however, is commonly considered to be the most important environmental factor affecting the growth and distribution of trees (Hinckley *et al.* 1991). Despite the mechanisms of its action being poorly understood, seasonal water stress is likely to determine the timing of phenological events in tropical dry forests (Murphy & Lugo 1986). Leaf shedding usually follows increased water stress, then many trees rehydrate after leaf shedding and rehydration always precedes flowering or

flushing (Borchert 1980; Reich & Borchert 1984; Borchert 1994). Furthermore, site-dependent differences in soil moisture availability, not seasonal rainfall, is the prime environmental cause of variation in tree water status, phenology, and distribution of tree species in Costa Rica lowland dry forests (Borchert 1994). The terraces of Christmas Island harbour typically drier forests, with many more deciduous species compared with the higher plateau forests (Du Puy 1993). This indicates a higher rate of leaf and new stem growth and turnover in response to seasonal water stress. Indirectly then, water stress of the forest may provide increased time with new shoots and space on new shoots per annum for scale insect crawlers to establish, and proliferate. The terraces may be the location for an alternative to the PSH: the Plant Vigour Hypothesis (PVH; Price 1991), which states that herbivorous insects will prefer and perform better on rapidly growing (vigorous) plants, especially when juvenile (or larval) feeding and development is closely associated with host plant growth processes.

Stem dieback caused by extreme densities of scale insects depleting translocated resources in non-deciduous forests is a major cause of mortality for sessile insects that preferentially colonise stems (Vranjic & Ash 1997). Stem death also reduces the potential colonisation space available for crawlers to establish and subsequent generations of scale insects to persist. Therefore, increased growth and turnover of shoots and leaves in deciduous forests may provide more time and space for the colonisation of scale insect crawlers, production of honeydew-producing adults and persistence of a carbohydrate resource for *A. gracilipes* to exploit. Unfortunately, the PVH does not readily explain why scale insects, and subsequently *A. gracilipes*, did not gain a foothold earlier in the deep soil plateau forests but are doing so now.

The formation of *A. gracilipes* supercolonies on Christmas Island may not be a spatial issue, whereby the abiotic environment 'selected' for the rapid increase in abundance of ants at specific locations. Instead, *A. gracilipes* supercolonies may be a temporal condition, the culmination of incremental population increases over a long period of time, taking advantage of their inherent characteristics including polygyny, broad nesting habits, broad diet, close association with honeydew-producing scale

insects and lack of intraspecific aggression. This implies that any population of *A. gracilipes*, given sufficient time over which incremental increases in abundance occurs, may reach a threshold whereby they fuse into supercolonies and, in the presence of adequate food (primarily honeydew-producing insects), are able to dominate an ecosystem.

This raises a critical question about the spatial distribution on supercolonies on Christmas Island. Why they did first appear in lower elevations, on terraces and in type II and types III forests? Was it because they had been present in those areas for longer than on the plateau? Historically, considering human traffic has been most prevalent in the plateau areas, it is doubtful. But extended lag times between introduction of a species and when they become invasive are not uncommon (Simberloff 2003); it is the reason behind the majority of lags that is not understood. This study suggests that it may be the effects of a mutually beneficial interaction between species that leads to the proliferation of populations of both partners, but also highlights the need for further research into the mechanisms behind lag times before population explosions in invasive species.

In both the large-scale water status survey and the small-scale water stress experiment there are methodological issues, which must be taken into account when interpreting the results. First, controlled experimental induction of water stress in the field is notoriously difficult, and the degree of plant stress can affect the response of insects (Inbar *et al.* 2001). Larsson (1989) predicted that on plants under moderate stress, phloem feeders may perform better, but may be negatively affected under more severe stress. The plants in the water-stressed treatments in this study had significantly more negative water potentials than those plants in the non-stressed treatment after 50 days of the imposed watering regime. Indeed, plants that were watered less often sometimes looked as if they were almost at wilting point, and in the absence of *A. gracilipes*, water-stressed plants died (Table 7.4). However, the plant mortality data suggest that in combination with water stress, *A. gracilipes* in supercolony densities (and associated increase in scale insect abundance) can cause plant death.

Second, if population increases of both *A. gracilipes* and scale insects have been incremental, and the initial state of each population very small, it may have been very subtle changes in the environment up to 40 years ago that provided biologically meaningful conditions to amplify the positive feedback mechanism of *A. gracilipes* and scale insects. My island-wide survey looked at rather coarse differences in 'forest water status' between forest types and may not have been sensitive enough to identify differences, adaptations or adjustments of individual species that may have been responsible for driving scale insect populations.

Table 7.1 – One-way ANOVA statistics showing no significant effect of forest type on (a) 'forest water status' and (b) soil moisture in the top 7 cm of soil at those sites used to assess forest water potential. Forest type is the main factor and forest water potential the soil moisture the dependent variables for each analysis respectively.

A.

Source of variation	SS	df	MS	F-ratio	p-value
Forest type	321359.613	2	160679.807	1.315	0.305
Error	1466406.257	12	122200.521		

B.

Source of variation	SS	df	MS	F-ratio	p-value
Forest type	70.450	2	35.225	4.820	0.029
Error	87.689	12	7.307		

Table 7.2 – Effect of watering regime and ant access on the water potential of seedlings of each species on two occasions (day 29 & day 50) during the water stress experiment at WBT. ANOVA statistics derived from a two-way ANOVA carried out separately for each time for each species.

Source of variation	Day 29 (9 August)					Day 50 (30 August)				
	SS	df	MS	F-ratio	p-value	SS	df	MS	F-ratio	p-value
<i>Inocarpus fagifer</i>										
Water	0.040	1	0.040	1.474	0.240	0.375	1	0.375	4.624	0.046
Ants	0.159	1	0.159	5.894	0.025	0.149	1	0.149	1.840	0.193
Water x Ants	0.000	1	0.000	0.000	1.000	0.085	1	0.085	1.052	0.319
Error	0.512	19	0.027			1.377	17	0.081		
<i>Pongamia pinnata</i>										
Water	0.000	1	0.000	0.021	0.887	2.042	1	2.042	8.163	0.010
Ants	0.000	1	0.000	0.021	0.887	0.007	1	0.007	0.027	0.872
Water x Ants	0.034	1	0.034	1.688	0.209	0.120	1	0.120	0.481	0.496
Error	0.400	20	0.020			5.003	20	0.250		
<i>Tristiropsis acutangula</i>										
Water	1.628	1	1.628	6.673	0.018	1.984	1	1.984	4.554	0.045
Ants	0.008	1	0.008	0.035	0.854	0.010	1	0.010	0.024	0.879
Water x Ants	0.055	1	0.055	0.226	0.640	0.010	1	0.010	0.024	0.879
Error	4.878	20	0.244			8.712	20	0.436		

Table 7.2 – Effect of watering regime and ant access on the water potential of seedlings of each species on two occasions (day 29 & day 50) during the water stress experiment at WBT. ANOVA statistics derived from a two-way ANOVA carried out separately for each time for each species.

Source of variation	Day 29 (9 August)					Day 50 (30 August)				
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<i>Inocarpus fagifer</i>										
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Ants	0.159	1	0.159	5.894	0.025	0.149	1	0.149	1.840	0.193
Water x Ants	0.000	1	0.000	0.000	1.000	0.085	1	0.085	1.052	0.319
Error	0.512	19	0.027			1.377	17	0.081		
<i>Pongamia pinnata</i>										
Water	0.000	1	0.000	0.021	0.887	2.042	1	2.042	8.163	0.010
Ants	0.000	1	0.000	0.021	0.887	0.007	1	0.007	0.027	0.872
Water x Ants	0.034	1	0.034	1.688	0.209	0.120	1	0.120	0.481	0.496
Error	0.400	20	0.020			5.003	20	0.250		
<i>Tristiropsis acutangula</i>										
Water	1.628	1	1.628	6.673	0.018	1.984	1	1.984	4.554	0.045
Ants	0.008	1	0.008	0.035	0.854	0.010	1	0.010	0.024	0.879
Water x Ants	0.055	1	0.055	0.226	0.640	0.010	1	0.010	0.024	0.879
Error	4.878	20	0.244			8.712	20	0.436		

Table 7.3 – A. Mean soil moisture of potted plants in water stress experiment at WBT. Both the stressed and non-stressed treatments included an ant access and ant exclusion treatment, however, they were combined as they received the same watering regime. Only the watering regime between stressed and non-stressed plants varied.

B. One-way ANOVA statistics showing a significant difference between soil moisture of potted plants at WBT.

A.

Stressed (n=6)	Non-stressed (n=6)
17.7 ± 0.7	30.4 ± 0.3

B.

Source of variation	SS	df	MS	F-ratio	p-value
Treatment	482.078	1	482.078	300.804	<0.001
Error	16.026	10	1.603		

Table 7.4 - Plant mortality of experimental plants at the end of the water stress experiment, 16 September 2002. N=6 for each species (18 total) for each treatment at start of experiment.

	Stressed		Non-stressed	
	Ant access	Ant exclusion	Ant access	Ant exclusion
<i>Inocarpus fagifer</i>	3	0	0	0
<i>Pongamia pinnata</i>	2	2	2	0
<i>Tristiropsis acutangula</i>	4	0	3	0
Total	9	2	5	0

Table 7.5 – Results for *Inocarpus fagifer*: a two-way repeated measures ANOVA showing the effect of watering regime and *A. gracilipes* on the abundance of (A.) *Tachardina* crawlers from day 0 – day 67 and (B.) *Tachardina* 2nd instars from day 15 – day 67. Two-way ANOVA showing effect of watering regime and ants on (C.) adult female *Tachardina* on day 67 of the experiment, 16 September 2002.

Source of variation	SS	df	MS	F-ratio	p-value
A. Crawlers					
<i>Between subjects:</i>					
Water	117.927	1	117.927	0.154	0.699
Ants	23839.207	1	23839.207	31.184	<0.001
Water x Ants	70.727	1	70.727	0.093	0.765
Error	12996.167	17	764.480		
<i>Within subjects:</i>					
Time	26386.227	4	6596.557	19.731	<0.001
Time x water	838.173	4	209.543	0.627	0.645
Time x ants	4762.893	4	1190.723	3.562	0.011
Time x water x ants	237.507	4	59.377	0.178	0.949
Error	22733.667	68	334.319		
B. 2nd instars					
<i>Between subjects:</i>					
Water	806.008	1	806.008	1.067	0.316
Ants	31460.408	1	31460.408	41.639	<0.001
Water x Ants	686.408	1	686.408	0.908	0.354
Error	12844.292	17	755.547		
<i>Within subjects:</i>					
Time	20126.625	3	6708.875	20.047	<0.001
Time x water	425.625	3	141.875	0.424	0.737
Time x ants	4749.092	3	1583.031	4.730	0.005
Time x water x ants	498.825	3	166.275	0.497	0.686
Error	17067.208	51	334.651		
C. Adults					
Water	12.033	1	12.033	0.048	0.829
Ants	16193.633	1	16193.633	64.630	<0.001
Water x Ants	0.300	1	0.300	0.001	0.973
Error	4259.500	17	250.559		

Table 7.6 – Results for *Pongamia pinnata*: a two-way repeated measures ANOVA showing the effect of watering regime and *A. gracillipes* on the abundance of (A.) *Tachardina* crawlers from day 0 – day 67 and (B.) *Tachardina* 2nd instars from day 15 – day 67. Two-way ANOVA showing effect of watering regime and ants on (C.) adult female *Tachardina* on day 67 of the experiment, 16 September 2002.

Source of variation	SS	df	MS	F-ratio	p-value
A. Crawlers					
<i>Between subjects:</i>					
Water	346.188	1	346.188	0.563	0.465
Ants	3840.097	1	3840.097	6.250	0.025
Water x Ants	149.388	1	149.388	0.243	0.630
Error	8601.267	14	614.376		
<i>Within subjects:</i>					
Time	37969.873	4	9492.468	17.327	<0.001
Time x water	723.600	4	180.900	0.330	0.857
Time x ants	645.873	4	161.468	0.295	0.880
Time x water x ants	4020.400	4	1005.100	1.835	0.135
Error	30678.900	56	547.837		
B. 2nd instars					
<i>Between subjects:</i>					
Water	486.835	1	486.835	0.623	0.443
Ants	9923.335	1	9923.335	12.695	0.003
Water x Ants	187.926	1	187.926	0.240	0.632
Error	10943.563	14	781.683		
<i>Within subjects:</i>					
Time	20789.157	3	6929.719	10.816	<0.001
Time x water	911.294	3	303.765	0.474	0.702
Time x ants	7842.157	3	2614.052	4.080	0.012
Time x water x ants	1411.384	3	470.461	0.734	0.537
Error	26908.354	42	640.675		
C. Adults					
Water	367.500	1	367.500	1.070	0.316
Ants	16945.633	1	16945.633	49.344	<0.001
Water x Ants	86.700	1	86.700	0.252	0.622
Error	5494.667	16	343.417		

Table 7.7 – Results for *Tristiropsis acutangula*: a two-way repeated measures ANOVA showing the effect of watering regime and *A. gracilipes* on the abundance of (A.) *Tachardina* crawlers from day 0 – day 67 and (B.) *Tachardina* 2nd instars from day 15 – day 67. Two-way ANOVA showing effect of watering regime and ants on (C.) adult female *Tachardina* on day 67 of the experiment, 16 September 2002.

Source of variation	SS	df	MS	F-ratio	p-value
A. Crawlers					
<i>Between subjects:</i>					
Water	1608.022	1	1608.022	0.757	0.399
Ants	22579.200	1	22579.200	10.626	0.006
Water x Ants	164.356	1	164.356	0.077	0.785
Error	29749.133	14	2124.938		
<i>Within subjects:</i>					
Time	14886.633	4	3721.658	11.099	<0.001
Time x water	2923.478	4	730.869	2.180	0.083
Time x ants	4934.411	4	1233.603	3.679	0.010
Time x water x ants	1169.922	4	292.481	0.872	0.486
Error	18777.533	56	335.313		
B. 2nd instars					
<i>Between subjects:</i>					
Water	25.000	1	25.000	0.033	0.858
Ants	11953.778	1	11953.778	15.774	0.001
Water x Ants	1248.444	1	1248.444	1.647	0.220
Error	10609.167	14	757.798		
<i>Within subjects:</i>					
Time	10642.889	3	3547.630	28.351	<0.001
Time x water	478.889	3	159.630	1.276	0.295
Time x ants	3539.667	3	1179.889	9.429	<0.001
Time x water x ants	225.000	3	75.000	0.599	0.619
Error	5255.500	42	125.131		
C. Adults					
Water	42.250	1	42.250	0.259	0.618
Ants	1482.250	1	1482.250	9.104	0.009
Water x Ants	667.361	1	667.361	4.099	0.062
Error	2279.500	14	162.821		

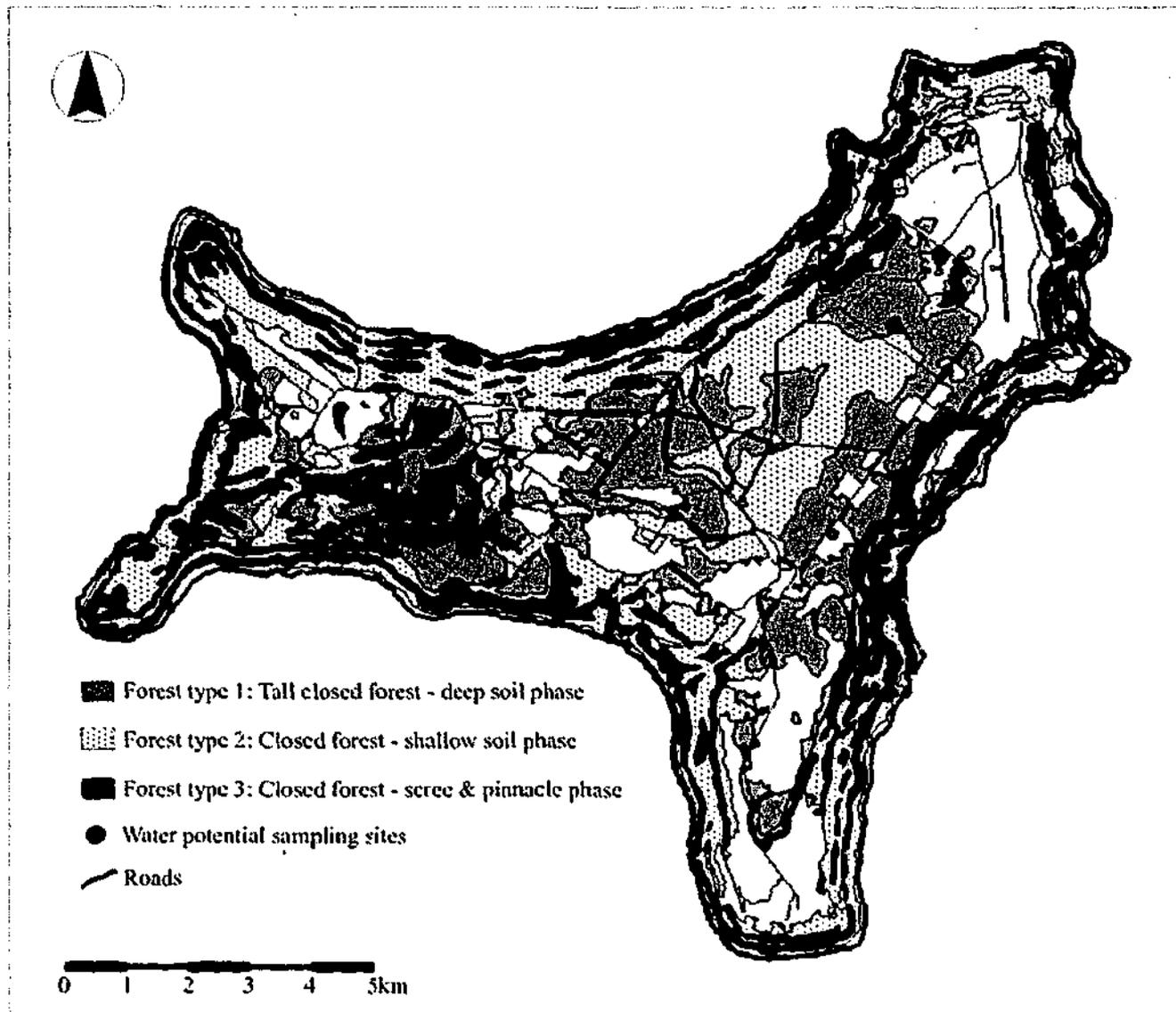


Figure 7.1 – Christmas Island with forest types and sites where water potential of forest types was measured using the pressure chamber. N=5 sites for each forest type.

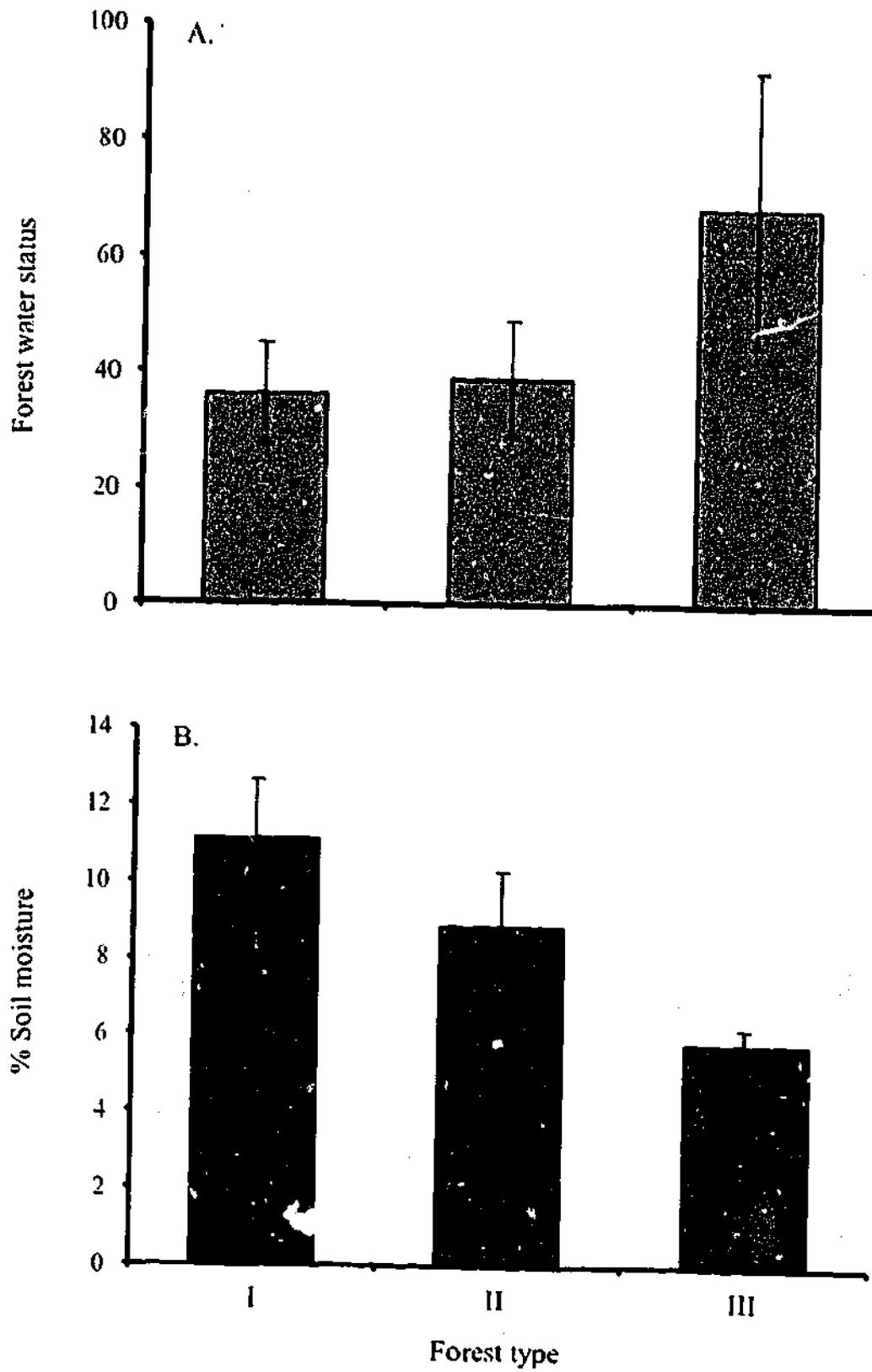


Figure 7.2 – (A.) Forest water status index, and (B.) % soil moisture of forest types I, II & III. The higher the forest water status, the more negative the water potential and the drier the forest type.

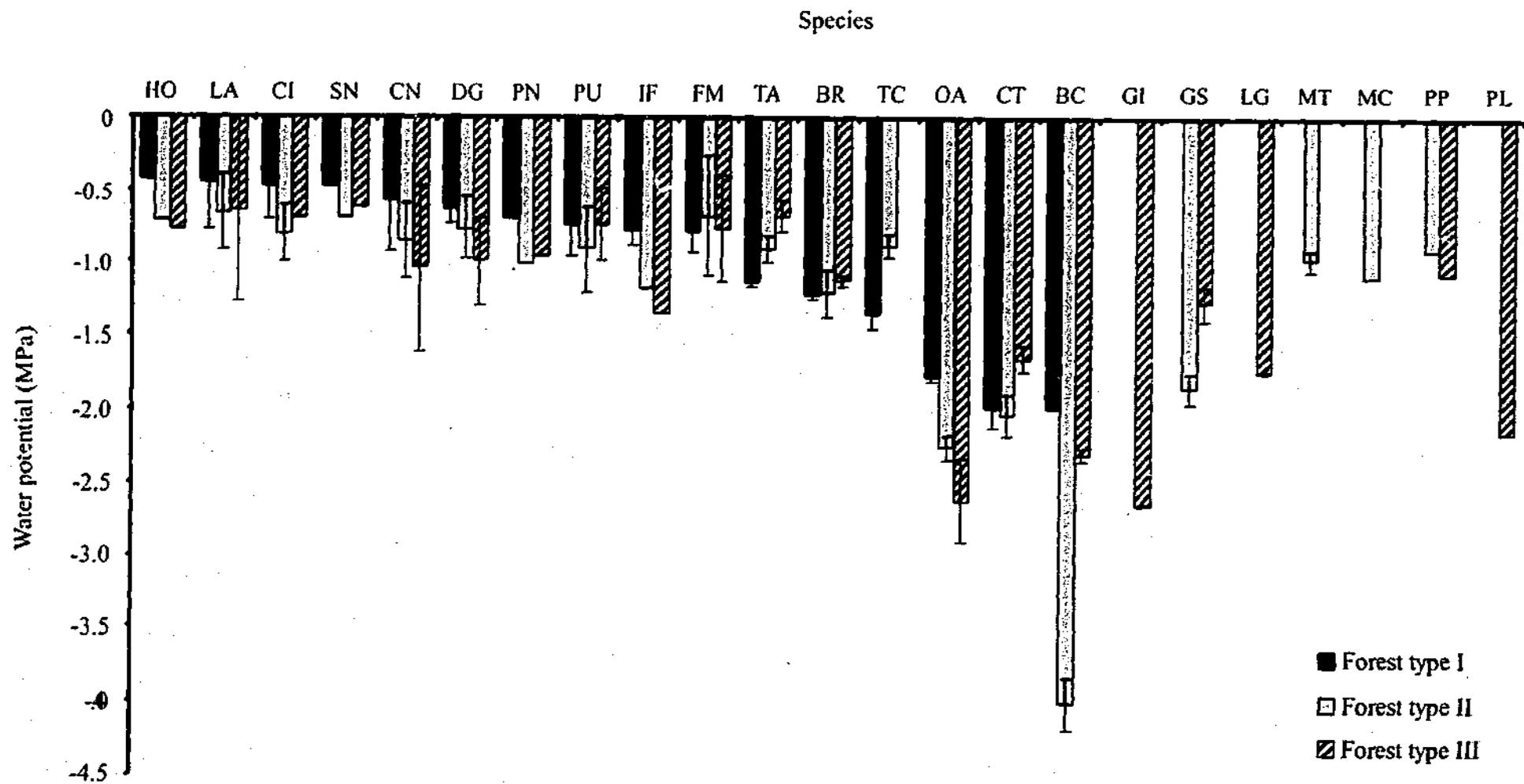


Figure 7.3 – Water potential of all species measured using the Scholander pressure chamber in the island-wide survey. N varies for each species. Codes for plant species: BC: *Berrya cordifolia*, BR: *Barringtonia racemosa*, CI: *Claoxylon indicum*, CN: *Cryptocraya nitens*, CT: *Celtis timorensis*, DG: *Dysoxylum gaudichaudianum*, FM: *Ficus microcarpa*, GI: *Grewia insularis*, GS: *Guettarda speciosa*, HO: *Hernandia ovigera*, IF: *Inocarpus fagifer*, LA: *Leea angulata*, LG: *Ligustrum globulatum*, MT: *Macaranga tanarius*, MA: *Melia azedarach*, MC: *Muntingia calabura*, OA: *Ochrosia ackeringae*, PU: *Pisonia umbellifera*, PN: *Planchonella nitida*, PP: *Pongamia pinnata*, SN: *Syzygium nervosum*, TA: *Tristiropsis acutangula*, TC: *Terminalia catappa*.

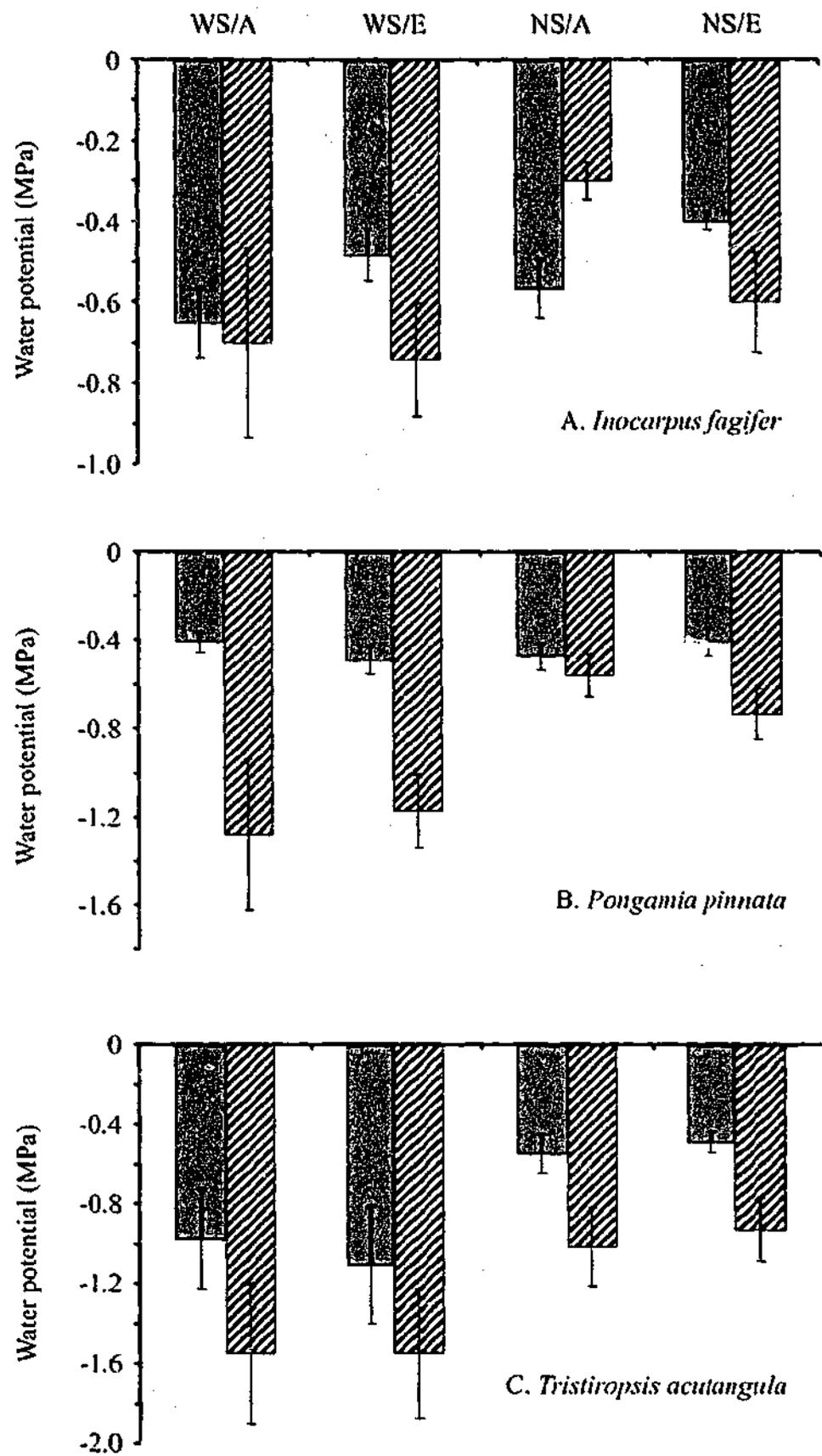


Figure 7.4 – The mean (\pm SE) water potential of plants from three species in pots in the field on two occasions. Closed bars: Day 29 (9 August 2002), hatched bars: Day 50 (30 August 2002). Note the differences in scales on the y-axes.

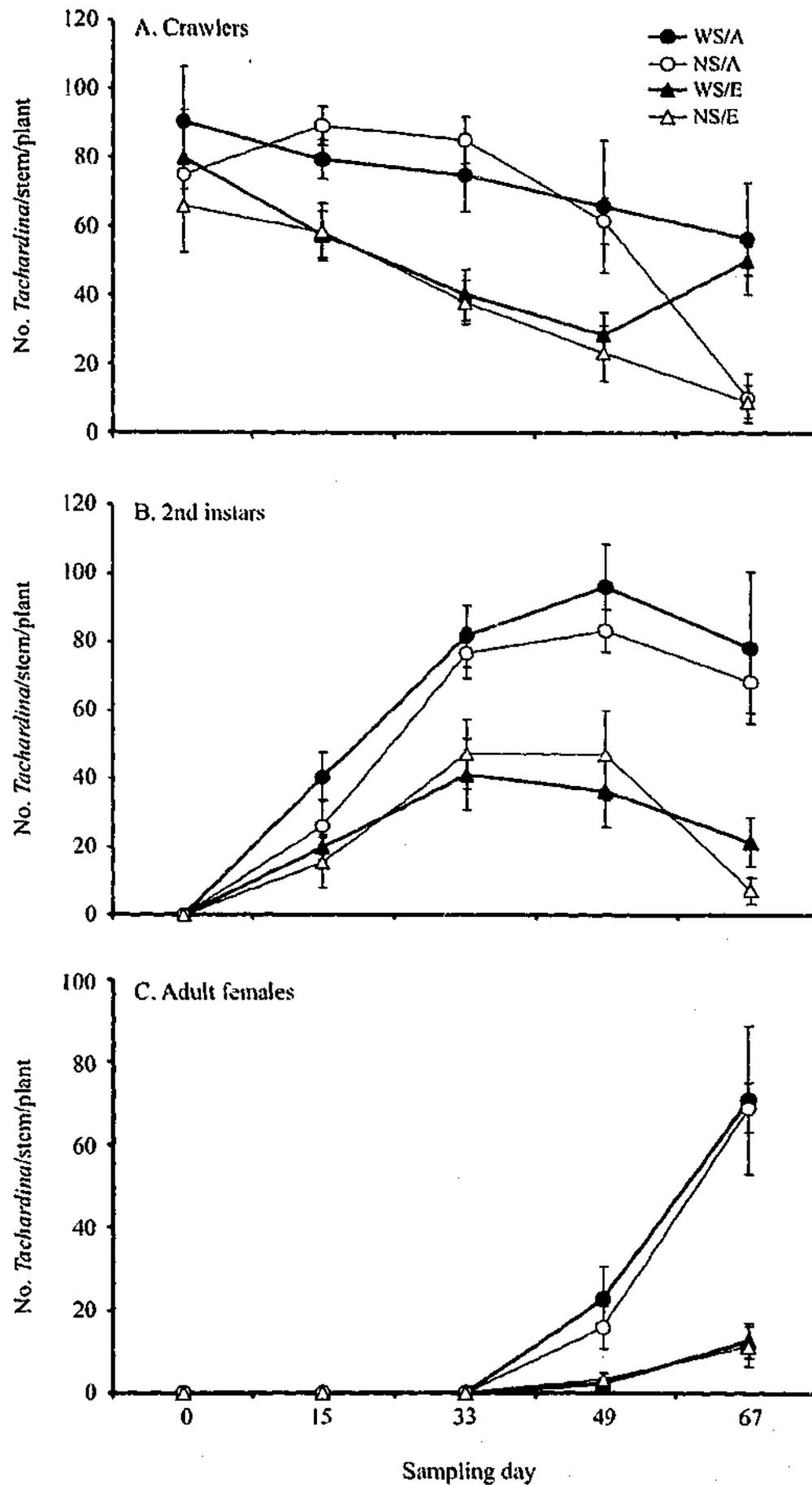


Figure 7.5 – Effect of ants and watering regime on crawlers, 2nd instars and adult female *Tachardina aurantiaca* on *Inocarpus fagifer* at WBT over the course of the experiment. Note the different scales on the y-axes. Day 0 is 11 July 2002.

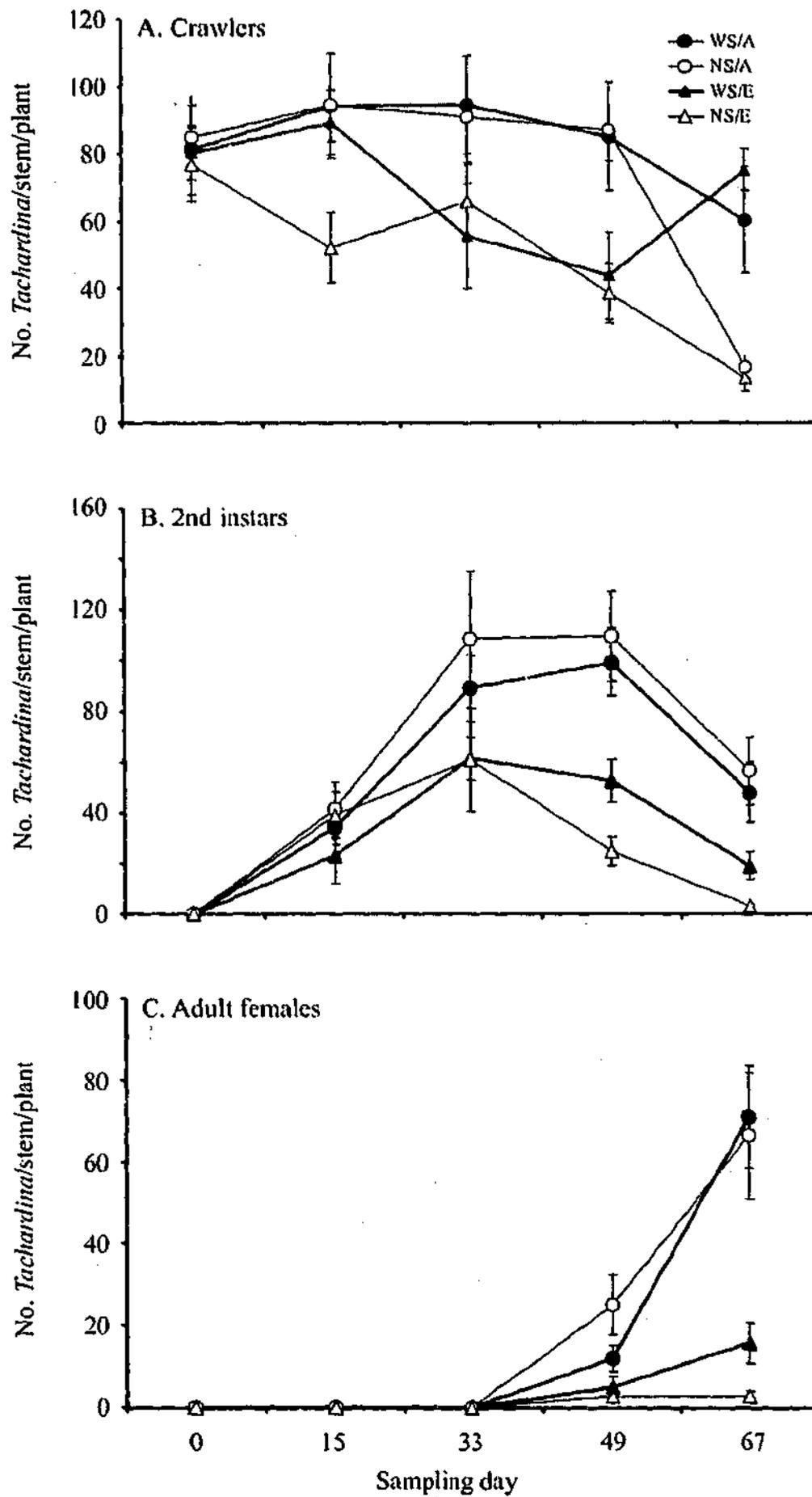


Figure 7.6 - Effect of ants and watering regime on crawlers, 2nd instars and adult female *Tachardina aurantiaca* on *Pongamia pinnata* at WBT over the course of the experiment. Note the different scales of the y-axes. Day 0 is 11 July 2002.

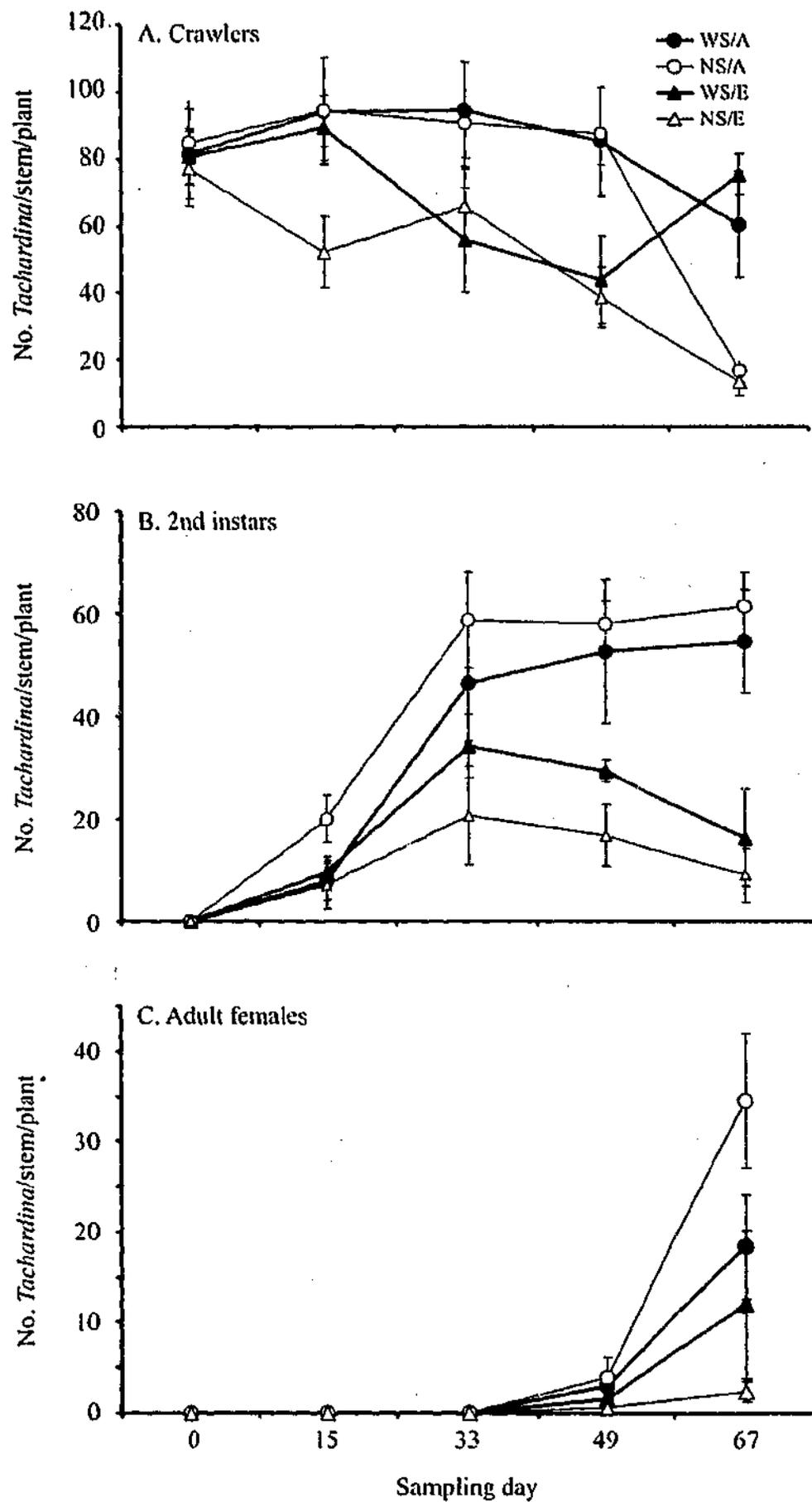


Figure 7.7 - Effect of ants and watering regime on crawlers, 2nd instars and adult female *Tachardina aurantiaca* on *Tristiropsis acutangula* at WBT over the course of the experiment. Note the different scales on the y-axes. Day 0 is 11 July 2002.



Figure 7.8 – *Tachardina aurantiaca* adult females covered in mould (A.) and accumulated honeydew (B.), in which crawlers get caught and are unable to settle.

Chapter Eight

General discussion and synthesis

This thesis has documented the dynamics of supercolonies of the yellow crazy ant, *Anoplolepis gracilipes*, the cause of 'invasional meltdown' in rainforest on Christmas Island, a remote tropical oceanic island in the eastern Indian Ocean (O'Dowd *et al.* 2003). I have shown that mutualisms between this ant and scale insects may be a key driver in the formation of ant supercolonies, their rapid expansion, and resulting invasive impacts. The yellow crazy ant possesses inherent general characteristics that promote the formation of unicolonial populations. Equipped with this characteristic prior to arrival, unicoloniality was a pre-requisite for the formation of expansive supercolonies, where densities of foraging workers on the forest floor alone reach more than 20 million ants/ha on Christmas Island (potentially the highest recorded density of any ant species; Chapter 2). Lack of intraspecific aggression associated with unicoloniality is likely to have contributed to higher foraging rates and rapid increases in abundance and distribution since 1996. Individual supercolonies occupied areas of between 1 – 787 hectares (Chapter 3), where they displaced other ant species and killed native red crabs as supercolony boundaries expanded (Chapter 3).

However, it is their 'strong' association with honeydew-producing scale insects that forms the basis of a positive feedback loop, amplifying populations of scale insects and ants over large spatial scales (Chapter 4, 5 & 6). I suggest that the provision of energy-rich honeydew by scale insects makes affordable the high foraging intensity and tempo exhibited by *A. gracilipes* on Christmas Island (Tobin 1994; 1995; Davidson 1997; 1998; Davidson *et al.* 2003), and increased provision of food to the colony (whether it be proteinaceous or carbohydrate-based). In short, more scale insects means more food, hence more ants.

The mutualism between *A. gracilipes* and honeydew-producing scale insects was such that populations of scales declined in the understorey following ant exclusion on individual saplings (Chapter 5) and collapsed almost completely after ant exclusion from mature canopy trees on a forest-wide spatial scale (~9–35 ha; Chapter 6). This suggests that it is the scale insects' association with *A. gracilipes*, rather than the quality of their host plants *per se* that is driving the abundance of both partners. Results from a manipulative field experiment, where I tested the effect of both biotic factors (ants) and abiotic factors (water stress) on scale insect colonisation and success, confirmed that indeed, biotic interactions with *A. gracilipes* significantly affected scale insects, whereas host quality via an abiotic stress (albeit only one) did not affect scale populations at all.

The overwhelming influence of this positive interaction on populations of scale insects and *A. gracilipes* in Christmas Island rainforest reinforces the need to incorporate mutualisms into the ecological theory of population and community dynamics. Although frequently acknowledged as important interactions, mutualisms have rarely been incorporated into the theoretical framework of modern ecology. This paints an incomplete, often misleading picture of our understanding of the structure and organisation of ecological systems (Simberloff & Von Holle 1999; Bruno *et al.* 2003). For example, the availability of preferred honeydew-producing insects (mutualists) has a strong influence on the vigour of green tree ant colonies in Australia (Bluthgen & Fiedler 2002). This study on Christmas Island has confirmed that positive interactions also play a pivotal role in structuring communities following invasion and proliferation of introduced species (Simberloff & Von Holle 1999; Richardson *et al.* 2000; Helms & Vinson 2002; Helms & Vinson 2003).

My thesis has made an important contribution to the knowledge base for *Anoplolepis gracilipes*, one of the world's worst invaders. I consider the most important contribution to ecology this thesis has made is the demonstration of a 'strong' mutualism between an introduced ant and mostly introduced scale insect species, *A. gracilipes*

proliferating to form supercolonies that dominate and dramatically alter an insular tropical rainforest ecosystem (O'Dowd *et al.* 2003). *A. gracilipes* has also been documented to associate with the soft scales *Coccus hesperidum* on Tokelau in the Pacific Ocean (Lester & Tavite In Press), with *C. viridis* in the Seychelles and in Indonesia (Van der Goot 1916; Haines & Haines 1978), and with *C. celatus* in Papua New Guinea (Williams 1982). However, the association with the other species on Christmas Island, e.g. *Tachardina aurantiaca*, is novel, but not unexpected. The mutualism appeared to override effects indirectly mediated by abiotic conditions on scale insect success, and explained the significantly greater colonisation and survival of scale insects in Christmas Island rainforest. Furthermore, the mutualism between *A. gracilipes* and scale insect species on Christmas Island is adventive, without any specific coevolutionary history (cf. Simberloff & Von Holle 1999).

Below I discuss the extent to which honeydew-producing hemipterans are associated with invasive ant species in a historic and global context, and the influence they are believed to have on ant populations. Inherent and shared general characteristics of 'tramp' ants contribute to their ability to form unicolonial populations and persist at high densities (Passera 1994; Moller 1996; Holway *et al.* 2002) and need no further elaboration here. However, I will evaluate the importance of biotic interactions in the proliferation of invasive ant species, and how positive exchanges between species in multi-trophic systems contribute to our understanding of biological invasions and their ecological impacts.

In light of the increasing economic costs of controlling ant species that have already caused damage (Pimentel *et al.* 2000), lack of ecological research into what are considered high-risk invasive ant species hinders our abilities to predict their invasion and mitigate their devastating impacts on natural systems (Holway *et al.* 2002). I discuss here the implications of my findings for management of *A. gracilipes* and of other invasive ant

species, and suggest directions for future research and management considerations that may help mitigate the devastating ecological impacts of invasive ant species.

Associations of invasive ants with honeydew-producing insects

All species of invasive ants have been recorded in association with honeydew-producing insects (Elton 1958; Buckley & Gullan 1991; Wetterer *et al.* 1999; Helms & Vinson 2002; Holway *et al.* 2002; Helms & Vinson 2003). Holway *et al.* (2002) listed 83 instances where invasive ant species affected invertebrates either positively or negatively, and 24% of those were interactions with honeydew-producing hemipterans, whereby the presence of ants increased their abundance, or removed or interfered with natural enemies. All other invertebrates were either directly or indirectly negatively affected by the invasive ant species. The yellow crazy ant forms mutualisms with soft scales (Coccidae), lac scales (Kerriidae), mealybugs (Pseudococcidae), aphids (Aphididae) and cushion scale insects (Margarodidae), all of which produce honeydew. It also associates with armoured scale insects (Diaspididae), which do not produce honeydew but are likely to benefit indirectly by the presence of *A. gracilipes* when they share host plants with honeydew-producing hemipterans, via the reduction of natural enemies. Argentine ants (*Linepithema humile*) are associated with scale insect and mealybug outbreaks (Elton 1958; Samways *et al.* 1982); honeydew was an order of magnitude more important than other items as food for the little fire ant (*Wasmannia auropunctata* (Clark *et al.* 1982), and the red imported fire ant (*Solenopsis invicta*) facilitates populations of an invasive mealybug in Texas (Helms & Vinson 2002; Helms & Vinson 2003).

These ant-scale associations are largely adventive, as are the interactions between *A. gracilipes* and scale insects on Christmas Island. They have arisen independently of coevolutionary histories or association in other locations around the world. The ability of *A. gracilipes* to form these adventive mutualisms emphasises the risk of population explosions in areas where *A. gracilipes* co-exists with honeydew hemipterans.

The positive association between ants and honeydew-producing insects directly and indirectly affects a myriad of interactions (Simberloff & Von Holle 1999). Many of these associations are similar on other tropical oceanic islands (e.g. Seychelles, Solomon Islands and Tokelau) and mainland areas (India), but I have presented them graphically here in the context of Christmas Island (Fig. 8.1). Not only does *A. gracilipes* extirpate native red land crabs, which alters the composition and structure of the rainforest considerably (O'Dowd *et al.* 2003). Between plants, honeydew-producing insects and ants, effects can even be viewed in context of further mutualisms and symbioses, which might be key to the harnessing of essential amino acids from lower trophic sources by ants. For example, symbiotic bacteria in the gut of aphids enable the synthesis of essential amino acids from plant sap that would otherwise be unavailable to the aphids (Douglas 1993; 1994). The nitrogen in plant sap comprises free amino acids, dominated by nonessential amino acids. To sequester sufficient essential amino acids, scale insects on Christmas Island presumably require bacteria to help them overcome this dietary shortfall. Further up the trophic chain, bacteria in the guts of some ants might enable them to efficiently extract amino acids and nitrogen from honeydew excreted from insects feeding on plants (Van *et al.* 2002; Davidson *et al.* 2003), which may allow them to augment their nitrogen budget through their putative "nitrogen-recycling symbionts". Through these symbiotic associations, both sap-sucking insects and ants may gain access to complex metabolic capabilities that afford them benefits at the population level. However, detailed analysis of these multi-trophic systems and their implications for the success of invasive ants are still lacking.

Ants, plant stress and scale insects

Stressed trees may not be the cause of the scale insect outbreaks, but they appear to be a result. I found that *A. gracilipes* in supercolonies significantly enhanced honey-producing scale insect populations irrespective of host plant water potential. Despite there being a distinct lack of support for the Plant Stress Hypothesis, sap-sucking insects

typically do better than other insect guilds on stressed plants (Koricheva & Larsson 1998). Once populations of scale insects reach high densities, the insects themselves stress the trees, and saplings can die (Chapter 7) and mature canopy trees significantly decline in health (P.T. Green unpublished data). That high densities of sap-sucking insects cause a decline in tree health has been documented extensively (Waterhouse & Sands 2001). Scale insects affect plants by depleting translocated resources, but also by significantly reducing root growth (Vranjic & Ash 1997). In combination with infestation by scale insects, nesting at the base of trees by *A. gracilipes* may contribute to a reduction of soil from around their base, and breakage of fine roots of rainforest trees near the surface of the soil that take up surface moisture.

After almost six years at supercolony densities, *A. gracilipes* activity at several sites on Christmas Island naturally declined to a point that no longer constituted a supercolony (P.T. Green unpublished data). The canopy at these sites appeared to have been affected by dieback, and shoot death of large trees was common. There were many dead scale insects on the remaining twigs and branches of canopy and understorey vegetation, which reduced space for colonisation of subsequent generations of scale insects. Populations of *A. gracilipes* at these sites had eliminated red land crabs in previous years. It appeared that *A. gracilipes* had used up all its resources and populations 'busted' accordingly. Cyclical population 'booms' and 'busts' appear not uncommon in introduced populations (see Simberloff & Gibbons 2004). However, the mechanisms responsible for the lag times, and subsequent busts, are unclear. The possibility of over-exploitation of resources might be one explanation for the natural decline in *A. gracilipes* populations at these sites, but clearly more investigation is required.

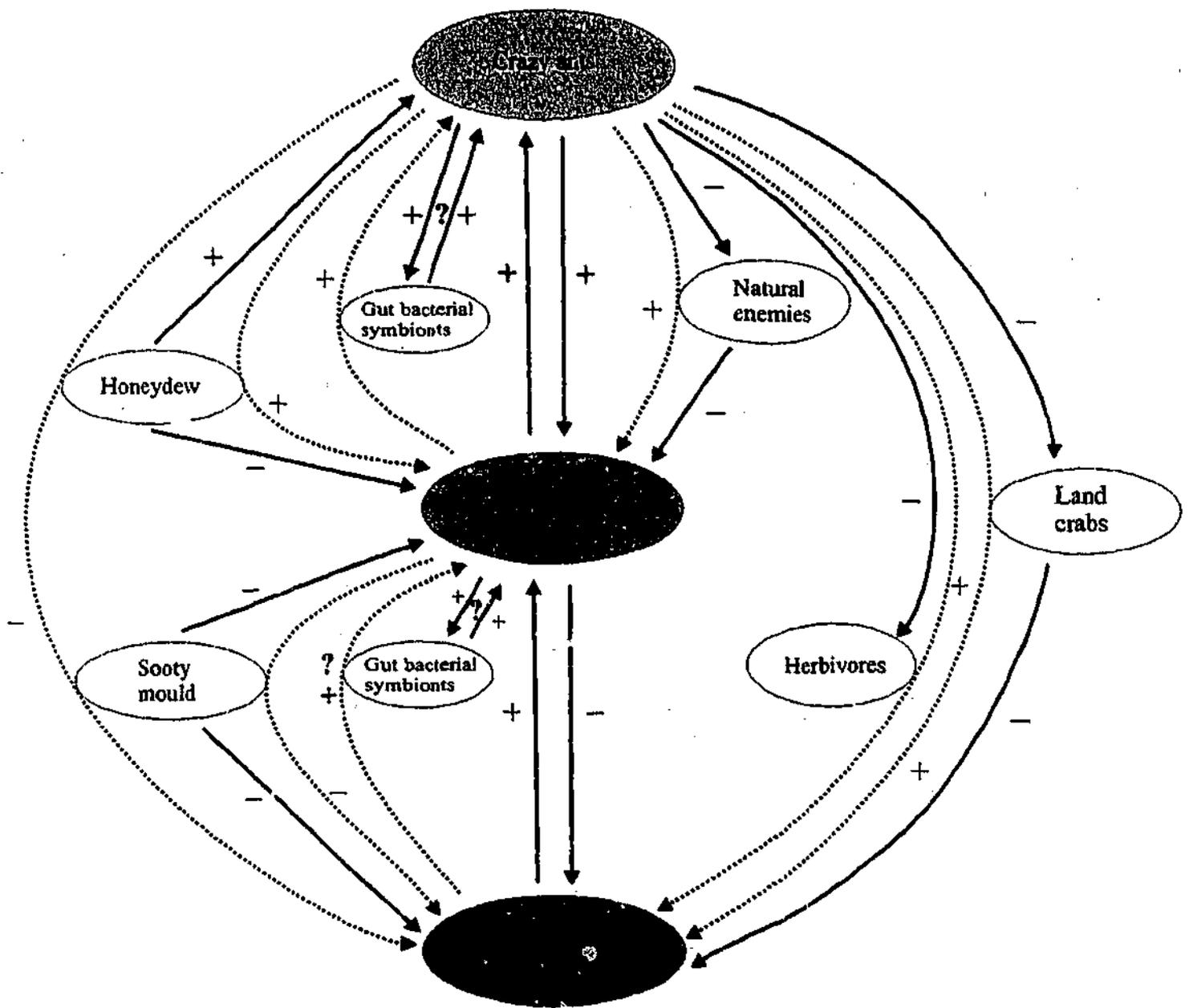


Figure 8.1 – The multi-trophic system in which the yellow crazy ant, *A. gracilipes*, plays a pivotal role. Solid line indicates direct effects, and dotted lines indirect effects.

Management of invasive ant species in insular ecosystems

Specific, case-by-case, ecological research (as opposed to using "general rules" for all invasive species, or even invasive ant species) is essential to effective management of a particular alien invasive species. For example, the extent to which communities are invaded by Argentine ants in southern California depends primarily on features of the physical environment (abiotic factors; Holway *et al.* 2002), and it is other invertebrates that mainly suffer when Argentine ants invade (Cole *et al.* 1992; De Kock *et al.* 1992; Holway 1998; Kennedy 1998; Wetterer *et al.* 2001). However, in contrast, my study highlights biotic interactions as key in the invasion success of *A. gracilipes*. These differences are important, because they highlight that, even within a taxonomic group (ants), management strategies are context-dependent with different priorities in different locations.

Clearly, preventing the introduction of an invasive species is the most desirable and cost-effective way to mitigate the potential impacts of damaging species anywhere. However, it has been suggested that only a very small fraction, perhaps 0.1%, of introduced species become invasive pests (Williamson & Fitter 1996). Once established, early detection of a potentially damaging population is essential in determining whether eradication is feasible. At this point, specific information on the population biology of the species can be crucial to the success of the program (Simberloff 2003). If it forms expansive populations, where eradication is not possible, control is the only means by which to reduce detrimental interference with native systems. Despite there being many reports of successful and complete eradication of invasive species (Mack *et al.* 2000; Simberloff 2001; 2003), ongoing control of species already established outside their natural range are much more common, where impacts are already great (Dahlsten 1986; Abedrabbo 1994; Haines *et al.* 1994; Hooper *et al.* 1998; Green *et al.* 2004). Given the increasing rate of biological invasions (Levine & D'Antonio 2003) and speed at which economic and ecological impacts can emerge (Leung *et al.* 2002; O'Dowd *et al.* 2003), the construction of a predictive framework for the prevention of invasions is a key goal (Lodge

1993; Vermeij 1996; Tsutsui & Suarez 2003). However, the development of such a framework relies heavily on, and will continue to rely upon case studies of specific invasions. Taxonomic-specific characteristics and mechanisms are critical for a reliable framework to be relevant. Yellow crazy ants spread by both colony budding and aerial dispersal (Chapters 2 & 3), whereas Argentine ants spread through colony budding alone. A monitoring program for these two species might differ in that surveys are conducted further from a focal colony of crazy ants than of Argentine ants, given the higher probability that they will have spread aurally to a more distant location.

Ecological research contributes to this emerging predictive framework, and specifically to control operations of invasive ant species. Results of my study have highlighted some important aspects of *A. gracilipes* ecology that have proven valuable considerations for management agencies attempting to control *A. gracilipes* on Christmas Island. The most significant of these is experimental evidence for a 'strong' mutualism between *A. gracilipes* and honeydew-producing scale insects (Chapters 4,5 & 6), which might be exploited in the continued control of *A. gracilipes*.

Given that ongoing area-wide suppression is a difficult management policy to sustain in a natural area and remote location with uncertain funding and poor infrastructure, direct control of scale insects, via the introduction of host-specific natural enemies from their area of origin or from closely related species, could provide an alternative. Natural enemies might reduce scale insect densities, which in turn, could markedly reduce honeydew available to *A. gracilipes*, resulting in a decline in their abundance and impact (O'Dowd & Green 2004). The effectiveness of this control strategy for *A. gracilipes* is based on the assumption that supercolony formation depends heavily on its strong mutualistic association with the scale insects. By reducing the density of targeted scale insects, *A. gracilipes* would be denied carbohydrates and nutrients from the honeydew, thereby decreasing ant densities and associated impacts (discussed further in Chapter 6). To my knowledge, this novel, indirect control method for invasive ants has not

been attempted before, perhaps due to logistical and practical reasons. However, the fact that all invasive ant species are associated with honeydew-producing insects implies that direct control of their mutualists could be employed over landscape scales to suppress invasive ant species, without eliminating native or local ants. This is true only if the technique is developed with sound safety protocols, without the non-target impacts frequently associated with long-term suppression programs using chemical control (O'Dowd and Green 2004). For example, interference with parasitoids by ants tending scale insects may reduce their efficacy when ants are at high densities. However, by the time ants reach damaging densities, direct poisoning of the ants themselves might be the most effective means by which to immediately halt their impacts in localised areas. Coupled with ongoing reduction of mutualistic hemipteran partners through biological control, these methods could constitute the bulk of invasive ant control programs, especially on oceanic islands, where the risk of biological control agents colonising proximate regions is low.

Additionally, this study has contributed to control efforts of *A. gracilipes* on Christmas Island by provision of information in three other instances. First, confirmation of the timing and duration of sexual reproduction. Second, and related to brood production, that *A. gracilipes* may prefer, or recruit to, different food sources at varying intensities throughout the year. Third, the documentation of dynamic boundaries and transition zones surrounding supercolonies. The bait developed for effective control of *A. gracilipes* on Christmas Island is a proteinaceous fishmeal formulation, with Fipronil as the active ingredient (Green *et al.* 2004). Foraging workers showed a preference for protein on occasions during the dry season, between April and October (Chapter 2), and sexual brood (males and queens) was produced after this, between October and April (Chapter 2). Consequently, deployment of toxic bait was considered most effective during the dry season, when foraging ants were more likely to recruit to granules of protein. Existing queens and males were killed via transfer of the bait from workers, but sexual

brood produced after the elimination of workers would have a significantly lower chance of reproduction and survival without workers to support the colony. The dry season was also advantageous for baiting due to desirable conditions for fieldwork and a low probability that bait would be wet, or the toxicant hydrolysed by rain. Mapping supercolony boundaries for toxic bait application was based on densities of *A. gracilipes* that were able to kill red land crabs, and to some degree was somewhat subjective (Green *et al.* 2004). However, I documented that *A. gracilipes* occurred in low abundance for distances of up to 200 m beyond supercolony boundaries, which has been useful in locating areas to return to for monitoring and follow-up baiting efforts.

Directions for future research

Rarely has a terrestrial mutualism been validated from the perspective of both partners (but see Cushman & Beattie 1991). Despite the association between ants and honeydew-producing insects being widespread and documented extensively, the population response of ants to association with scale insects remains untested, primarily due to logistical and practical constraints. I would argue that this is a high priority, and might be achieved through novel use of systemic insecticides that reduce scale insect populations, and help eliminate honeydew from ant's diets. Without honeydew as a major carbohydrate source, I would hypothesise that the abundance of foraging ants would decline.

However, sufficient evidence exists to move beyond research that simply aims to validate the mutualism between invasive ants and honeydew-producing insects. Considering the complexity of their interactions and impacts, specific mechanisms that allow ants to use resources and dominate ecosystems might be one new focus. Details on the quantity and quality of honeydew are available (Douglas 1993; Fischer & Shingleton 2001; Fischer *et al.* 2002), but this aspect of the mutualism may be crucial in identifying specific mechanisms whereby ants gain the benefits that allow such rapid population

proliferation. Are invasive ant species more 'efficient' at extracting honeydew from these insects than native ants? How important are symbiotic gut bacteria in allowing ants to extract essential amino acids? More research is needed into how much of the ants' energetic requirements are being met by the sequestration of honeydew. Do invasive ants preferentially tend insects that produce more honeydew? How much honeydew do individual workers retain, and how much is passed onto the colony? Questions like these will aid in the understanding of just how invasive ants use honeydew to fuel their high tempo foraging and increase in abundance.

Another remaining challenge is the documentation of population responses of invasive ants to the presence of scale insects, at an appropriate spatial scale to that seen in nature. Despite there being no single "correct" spatial scale on which to describe populations or ecosystems (Levin 1992 and references therein), I have shown here that the 'strength' of the mutualism between *A. gracilipes* and scale insects can be conditional on the spatial scale at which it is examined. This is also relevant to invasive species management since control is often context-dependent and requires specific case-by-case ecological information. We should attempt to study the spatial scale of interest, and of most relevance, to a specific invasion.

Concluding comments

Alien invasive species are among the most significant drivers of environmental change worldwide. Their presence constrains environmental conservation, economic growth, and sustainable development, but may also contribute to social instability and economic hardship. Costs to society greatly exceed those that can be measured in currency. They include impacts on infrastructure, food and water shortages, environmental degradation, loss of biodiversity, increased rates and severity of natural disasters, illness, and lost lives. Nowhere is this better illustrated than on oceanic islands (Reaser & Meyerson 2003).

Historically, island ecosystems have contributed significantly to our understanding of evolutionary, ecological and biogeographical theory (e.g. Darwin 1860; MacArthur & Wilson 1967; Carlquist 1974), yet are threatened by the plethora of biological introductions and their associated impacts (Haines & Haines 1978; Clark *et al.* 1982; Reimer 1994; Simberloff 1995; Meyer & Florence 1996; Jourdan 1997; Fritts & Rodda 1998; Feare 1999; Mack *et al.* 2000; O'Dowd *et al.* 2003). Islands represent a haven for invasive ants. Rarely do oceanic islands possess a native ant fauna (Wilson & Taylor 1967). More than three quarters of reports of *A. gracilipes* associated with high densities of scale insects have been on islands (Van der Goot 1916; de Fluiter 1939; Haines & Haines 1978; Williams 1982; Carver *et al.* 1993; O'Dowd *et al.* 2003; Lester & Tavite In Press). Considering the widespread association of *A. gracilipes* with outbreak levels of scale insects, I would argue that the knowledge gained from this study in terms of the ant-scale mutualism is applicable on other islands where *A. gracilipes* (and other invasive ants) are associated with high numbers of honeydew-producing insects. However, this study serves as a warning, that tropical island ecosystems where *A. gracilipes* is present are at risk of eventually being dominated by high densities of foraging workers over time, and biodiversity and forest integrity suffering as a consequence. In fact, the biological integrity of Palmyra Atoll in the Pacific Ocean is currently under threat by the big-headed ant, *Pheidole megacephala*, whose associations with honeydew-producing hemipterans are causing decline in tree health and in many cases, tree death (P.J. Lester personal communication).

Christmas Island remains relatively intact compared to most other tropical oceanic islands (e.g. Hawaiian Islands and populated Pacific islands). The island has clearly benefited from the contribution of scientific research to the management of the ecosystem in the past few decades. But it is only prevention of further introductions and rapid intervention to halt damaging impacts by resident invasive species that will maintain its integrity and biological uniqueness. Islands provide us with natural laboratories;

microcosms for helping us understand how humans have transformed, and will continue to transform the biotic make-up of our planet. However, islands – like Christmas Island – provide more than just model systems for improving our understanding of invasions. They also provide relatively small and discrete 'demonstration' sites for improving our abilities to respond to biological invasions, giving us hope that we can hold, and maybe even turn back, the tide of biological invasions.

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Appendix 1 (following page) - Total number of stems of each species recorded in my floristics survey at 7 sites of each forest type, I, II & III. Only genus is displayed in the table for practical purposes. Full species names are as follows: *Allophyllus cobbe*, *Berrya cordifolia*, *Barringtonia racemosa*, *Carica papaya*, *Claoxylon indicum*, *Cryptocraya nitens*, *Celtis timorensis*, *Dendrocnides sinuata*, *Dendrocnides peltata*, *Dysoxylum gaudichaudianum*, *Ficus microcarpa*, *Guettarda speciosa*, *Gyrocarpus americana*, *Hernandia ovigera*, *Inocarpus fagifer*, *Leea angulata*, *Ligustrum globulatum*, *Macaranga tanarius*, *Melia azedarach*, *Morinda citrifolia*, *Ochrostia ackeringae*, *Pisonia umbellifera*, *Planchonella nitida*, *Pongamia pinnata*, *Premna* Luc, *Syzygium nervosum*, *Terminalia catappa*, *Trema Tristiropsis acutangula*.

Species	Forest type I							Forest type II							Forest type						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
<i>Allophylus</i>	0	0	0	0	0	0	2	0	0	0	0	3	0	0	0	3	0	6	0	1	3
<i>Barringtonia</i>	40	49	18	37	27	58	0	42	54	0	75	11	0	38	9	63	14	0	0	0	0
<i>Berrya</i>	0	0	0	0	0	9	18	0	0	6	0	0	8	0	0	0	0	2	0	1	5
<i>Carica</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>Celtis</i>	0	0	0	5	6	16	18	0	3	3	1	1	5	23	0	7	3	10	4	0	1
<i>Claoxylon</i>	15	3	31	9	2	1	0	11	2	2	1	22	0	0	73	7	13	3	2	0	0
<i>Cryptocarya</i>	14	14	7	6	3	0	0	22	22	22	15	18	0	0	8	6	22	7	17	0	0
<i>D. sinuata</i>	0	8	5	1	2	0	0	8	1	20	4	4	2	3	0	2	7	0	2	0	0
<i>D. peltata</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	4
<i>Dysoxylum</i>	5	8	5	3	3	5	1	5	5	9	2	16	1	2	3	11	1	8	16	0	0
<i>F. microcarpa</i>	0	0	0	0	1	0	9	0	0	1	1	0	3	3	0	2	3	6	2	2	0
<i>Guetlarda</i>	0	0	5	0	0	0	7	0	0	5	0	3	3	34	1	1	16	30	30	7	3
<i>Gyrocarpus</i>	0	0	0	0	0	0	5	0	0	0	0	0	9	1	0	0	0	0	0	1	0
<i>Hernandia</i>	5	10	12	3	1	0	0	3	1	11	0	13	0	0	5	0	16	3	0	0	0
<i>Inocarpus</i>	22	22	27	31	32	44	0	29	40	2	17	3	0	0	1	37	5	0	0	0	0
<i>Leea</i>	0	4	0	12	13	5	17	1	4	0	7	8	1	16	2	6	3	14	6	1	1
<i>Ligustrum</i>	0	0	0	3	2	0	0	0	0	0	0	1	0	0	2	0	0	22	9	0	0
<i>Kleinhovia</i>	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	2
<i>Macaranga</i>	17	9	1	3	3	8	9	5	2	0	0	6	0	11	36	1	1	3	7	4	4
<i>Melia</i>	2	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0
<i>Morinda</i>	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
<i>Ochrosia</i>	0	0	1	0	3	19	95	0	0	19	0	0	128	16	0	0	11	32	22	116	148
<i>Pisona</i>	42	33	22	47	30	15	1	27	18	48	24	35	8	1	25	5	38	20	43	0	0
<i>Planchonella</i>	24	26	25	26	25	0	2	23	17	20	27	17	0	1	18	24	22	15	12	0	0
<i>Pongamia</i>	0	0	1	0	2	0	2	0	0	3	0	7	6	3	0	0	4	4	6	6	7
<i>Premna</i>	0	0	0	0	0	0	3	0	0	0	0	1	15	1	0	0	0	7	7	9	9
<i>Syzygium</i>	12	12	17	8	14	6	0	10	16	21	19	17	0	0	15	8	11	3	6	0	0
<i>Terminalia</i>	0	0	0	0	1	5	6	4	6	2	3	2	2	32	0	11	0	3	3	38	6
<i>Trema</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	1
<i>Tristropis</i>	2	2	10	6	10	0	0	10	8	6	1	12	1	1	1	3	10	1	6	0	0