# The invasive ant *Pheidole megacephala* on an oceanic island: impact, control and community-level response to management

by

René Gaigher

Dissertation presented for the degree of Doctor of Philosophy in the Faculty of AgriSciences

at Stellenbosch University
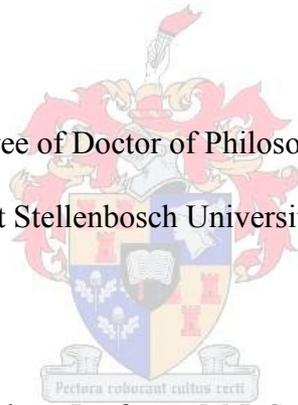
Supervisor: Professor M.J. Samways

March 2013

Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: March 2013

The following chapters have been accepted or submitted for publication in journals:

Chapter 2: Gaigher, R., Samways, M.J.[1], Henwood, J.[2] & Jolliffe, K.[3] 2011. Impact of a mutualism between an invasive ant and honeydew-producing insects on a functionally important tree on a tropical island. Biological Invasions 13, 1717-1721.

Chapter 3: Gaigher, R., Samways, M.J., Jolliffe, K.G. & Jolliffe, S.M.[4] 2012. Precision control of an invasive ant on an ecologically sensitive tropical island: a principle with wide applicability. Ecological Applications 22, 1405-1412.

Chapter 4: Gaigher, R., Samways, M.J. 2013. Strategic management of an invasive ant-scale mutualism enables recovery of a threatened tropical tree species. Biotropica 45, 128-134.

Chapter 5: Gaigher, R., Samways, M.J., Van Noort, S.[5] Saving a tropical ecosystem from a destructive ant-scale mutualism with support from a diverse natural enemy complex. Under review Biological Invasions.

Abstract

Invasive species are among the most important global conservation threats. Their management is one of the key conservation challenges that will have to be addressed in the next few decades. The study of real invasions and their management in natural ecosystems provides an opportunity to gain important information on theoretical and applied aspects of biological invasions.

This project focuses on the broader ecological context of invasive ant management in an ecologically sensitive island habitat. The thesis has three main components: 1) assessing the role of the invasive ant *Pheidole megacephala* in the ecosystem and evaluating its threat to the system, 2) evaluating a low-impact management program for the ant, and 3) using a community-level approach to assess ecosystem response to ant removal.

The ant occupied almost 30% of the island's total land area and reached extremely high densities in some areas. The ant was associated with exotic hemipteran scale insects through trophobiotic mutualisms that facilitated high ant and hemipteran abundances. The highly destructive scale insect *Pulvinaria urbicola* was among the hemipterans that benefited from ant attendance. High levels of hemipteran feeding resulted in dieback of functionally important and threatened native *Pisonia* trees, which represented a significant threat to the forest ecosystem.

A management program was initiated in response to this threat, consisting of baiting with selective hydramethylnon-based bait delivered in bait stations, accompanied by detailed pre-and post-baiting monitoring. The method was highly effective at suppressing the ants, whilst preventing bait uptake by non-target organisms. It was also cost-effective and adaptable to ant density in the field, but was only effective over short distances. The method may be applicable to other sensitive environments with similar challenges.

After ant control, the ant-scale mutualism was decoupled and the *Pu. urbicola* population collapsed. There were variable responses in different taxa to the removal of these highly abundant exotic species, the most important of which was the recovery in *Pisonia* trees. Shoot condition and foliage density improved and there was a decrease in sooty mold. Herbivory on *Pisonia* increased due to recovery of native canopy herbivores, but the overall impact was far less than that of the exotic hemipterans.

Soil surface arthropods, a group that may have been vulnerable to the treatment method, were unaffected by baiting. Instead, they increased significantly after ant removal, confirming the ant's impact on other arthropods. Other ant diversity and non-ant arthropod abundance increased post-baiting, including the endemic ant *Pheidole flavens farquharensis* and some functionally important insects such as the Indian cockroach.

Natural enemies that interacted predictably with the mutualists were influenced by management. Predators of hemipterans increased significantly after ant removal and were instrumental in the scale population collapse, whereas parasitoids of hemipterans that benefited from the mutualism declined. Additionally, groups that were unrelated to the mutualism were indirectly influenced by management. The natural enemy assemblage as a whole showed recovery to pre-invasion conditions.

The study shows how widely interconnected and influential the ant was in the ecosystem. It highlights the threat of the species in natural systems as well as the complex responses following invasive ant removal. Yet, it also demonstrates the potential to safely and effectively manage the species, thereby raising the opportunity for ecosystem recovery.

4

Opsomming

Indringerspesies is van die belangrikste globale bedreigings vir natuurbewaring. Hulle bestuur is van die grootste bewaringsuitdagings wat in die volgende paar dekades aangespreek moet word. Die studie van werklike invalle en hul bestuur in natuurlike ekosisteme bied 'n geleentheid om belangrike inligting te verkry oor teoretiese en toegepaste aspekte van biologiese indringing.

Hierdie projek fokus op die breër ekologiese konteks van uitheemse mier bestuur in 'n ekologies sensitiewe eiland habitat. Die tesis het drie hoofkomponente: 1) die beoordeling van die rol van die indringer mier *Pheidole megacephala* in die ekosisteem en evaluering van sy bedreiging vir die sisteem, 2) die evaluering van 'n lae-impak bestuursprogram vir die mier, en 3) die gebruik van 'n gemeenskaps-vlak benadering om ekosisteem reaksie op mierverwydering te assesseer.

Die mier het byna 30% van die totale landoppervlak van die eiland beslaan en het in party areas baie hoë digthede bereik. Die mier was geassosieer met uitheemse dopluis spesies in mutualismes wat hoë mier en dopluis getalle gefasiliteer het. Die hoogs beskadigende dopluis *Pulvinaria urbicola* was een van die spesies wat bevoordeel is deur die mutualisme. Hoë vlakke van dopluis voeding het die terugsterwe van funksioneel belangrike, bedreigde inheemse *Pisonia* bome veroorsaak, wat 'n groot bedreiging vir die ekosisteem verteenwoordig het.

'n Bestuursprogram is geïmplimenteer as gevolg van hierdie bedreiging, wat bestaan het uit selektiewe hidrametielnoon-gebaseerde lokaas wat in die veld geplaas is in lokaashouers, vergesel deur intensiewe monitering voor en na lokaasplasing. Die metode was hoogs effektief in die onderdrukking van die miere en het lokaasinname deur nie-teiken organismes verhoed. Dit was ook koste-effektief en aanpasbaar volgens mierdigtheid in die

5

veld, maar was slegs effektief oor kort afstande. Die metode mag van toepassing wees in ander sensitiewe omgewings met soortgelyke uitdagings.

Na mierbeheer is die mier-dopluis mutualisme ontkoppel en die *Pu. urbicola* bevolking het drasties verminder. Daar was verskillende reaksies in verskillende taxa tot die verwydering van die oorvloedryke eksotiese spesies, maar die belangrikste reaksie was die herstel van *Pisonia* bome. Spruittoestand en blaardigtheid het verbeter en daar was 'n afname in roetskimmel. Herbivorie op *Pisonia* het toegeneem as gevolg van 'n herstel in inheemse herbivore, maar die algehele impak was veel minder as dié van die eksotiese dopluis.

Grondoppervlak gelidpotiges, 'n groep wat kwesbaar kon wees vir die behandelingsmetode, was onaangeraak deur die lokaas, maar het beduidend na mierverwydering vermeerder. Mierdiversiteit het vermeerder en die Seychelles endemiese mier *Pheidole flavens farquharensis* is hervestig. Ander gelidpotiges het ook vermeerder, insluitend funksioneel belangrike spesies soos die Indiese kakkerlak.

Natuurlike vyande wat geassosieer was met die mutualiste is beïnvloed deur die mierbestuur. Predatore van dopluis het beduidend toegeneem na mierverwydering en was hoogs betrokke by die vermindering van dopluis, terwyl parasiete van dopluis, wat voordeel getrek het uit die mutualisme, gedaal het. Daarbenewens is groepe wat onverwant was aan die mutualisme indirek beïnvloed deur mierbestuur. Die algehele natuurlike vyand gemeenskap het herstel na pre-indringing toestand.

Die studie toon hoe wydverbind en invloedryk die mier was in die ekosisteem. Dit beklemtoon die bedreiging van die spesies in natuurlike stelsels asook die komplekse reaksies wat uitheemse mierverwydering volg. Tog demonstreer dit die potensiaal om die spesies veilig en doeltreffend te bestuur, en sodoende die geleentheid vir ekosisteemherstel te skep.

6

Acknowledgements

I would like to thank my supervisor Michael Samways for setting me up with this stimulating project and for his involvement in all facets of the project. His enthusiasm for conservation biology has greatly influenced this research.

The ant baiting campaign would not have been possible without the assistance of Cousine Island conservation staff Kevin and San-Marie Jolliffe who spent many hours in the field baiting ants and discussing the project. Jock Henwood was involved in initiating the ant management program and together with Janine Henwood, Des and Kim Nel, and Cousine Island support staff, provided significant logistical support. Fred Keeley, patron of Cousine Island, is thanked for the opportunity to carry out the research on his property and for his support of our work.

I am indebted to various people who assisted with arthropod identifications including Simon van Noort (wasps), Ansie Dippenaar-Schoeman (spiders), Justin Gerlach (beetles and leafhoppers), Penny Gullan (scale insects), Ben Hoffmann (ants), Brian Fisher (ants), Ian Millar (scale insects), Gerhard Prinsloo (wasps), Henk Geertsema (moths) and Mervyn Mansell (flies). Daan Nel provided assistance with statistical analyses and Kim Stoltz kindly advised on baiting methodology. Thanks to Jarred Knapp, Andrew Perkins and Archie Sasa for constructing the bait stations. Adam Johnson, Colleen Louw and Marlene Isaacs provided significant technical and logistical support throughout the project. Also, thanks to my friends and colleagues at the department for their great feedback and support.

The project was funded by the DST-NRF Centre of Excellence for Invasion Biology (C•I•B) and the Working for Water Programme through their collaborative research project

on "Integrated Management of Invasive Alien Species", and Stellenbosch University. Cousine Island provided financial support for the ant baiting campaign.

Finally, a special thanks to my family whose continued support has made it possible for me to reach this milestone.

Table of Contents

List of Figures

12

List of Tables

1. General introduction

The current state of invasive species management

Biological invasions are widely recognized as being among the most significant drivers of global environmental change (Mack *et al.* 2000; Vitousek *et al.* 1996) and are an important focus of international conservation concern (Lowe *et al.* 2000). Invasive species have contributed to ecological damage through biodiversity loss, environmental degradation and disruption of ecosystem function and biogeochemical processes (Mack *et al.* 2000; Mooney & Cleland 2001). Socioeconomic impacts from biotic invasions have also been widespread, with adverse effects on agriculture, forestry, fisheries and human health (Reaser *et al.* 2007). With increasing international trade and improved transport technologies it is expected that the rate at which species are moved around the world will continue to increase (Hulme 2009; Levine & D'Antonio 2003). This increased human-aided dispersal, together with the interacting forces of habitat transformation and climate change, is predicted to intensify the impacts of biological invasions (Reaser *et al.* 2007).

The management of invasive species is considered to be one of the greatest conservation challenges that will have to be addressed in the next few decades (Allendorf & Lundquist 2003; Vitousek *et al.* 1996). Invasive species control, and particularly eradication, has previously been considered to be unachievable, too expensive and risky to native biota in natural systems (Lester 2008; Simberloff 2001). But this view is currently being challenged by a rapidly growing field of invasive species management. Due to substantial recent advances in management techniques and control options, as well as in our understanding of the requirements for successful management programs, it has become possible to effectively and safely manage many types of invasive species (Clout & Veitch 2002; Gentz 2009; Hoffmann *et al.* 2010). Within this emergent field, there is enormous scope for fundamental

and applied research that will ultimately improve our ability to deal with exotic species. In this thesis, I attempt to address a number of issues in this field that require greater attention, highlighted in the following sections.

The broader ecological context of invasive species management

In general, management of invasive species has focused simply on the reduction of the target species population levels (Caut *et al.* 2009). But it is becoming increasingly obvious that the ecological context of invasive species management is much more complex than previously thought (Zavaleta *et al.* 2001). Invaded communities consist of numerous interacting networks and feedback loops (Buckley 2008) and therefore the consequences of management may be unpredictable (Hulme 2006). The perceived role of the invader in the system and expected consequences of alien removal can be confounded by previously unrecognized factors such as diverse interactions of the invasive species with native species (e.g. Caut *et al.* 2008), interactions with other exotic species (e.g. Bergstrom *et al.* 2009; Caut *et al.* 2009) and complex indirect effects (White *et al.* 2006).

In many cases, alien removal has resulted in ecological recovery (Cook 2003; Hoffmann 2010), but it has also caused unexpected trophic cascades (Bergstrom *et al.* 2009; Courchamp *et al.* 2003; Zavaleta *et al.* 2001) and secondary invasions (Plentovich *et al.* 2010a). In addition, the management actions themselves may have negative ecological consequences (Messing & Wright 2006; Plentovich *et al.* 2010b). These are important possibilities to consider when designing management programs. Studies that have focused on the complexities of real ecosystems have contributed significantly to our understanding of these complex effects of management (Buckley 2008). Additional research that takes a whole-ecosystem approach to invasive species removal may help improve our ability to more accurately predict management outcomes (Zavaleta *et al.* 2001).

16

The need to integrate research and management

Eradication projects have generally been underrepresented in conservation literature. Invasive species management is mostly performed by management teams, and the results often remain unpublished (Simberloff 2009), making it less visible in invasion research. However, these management projects provide unique opportunities for revealing the role of invasive species in invaded ecosystems. It has been proposed that, where possible, eradication programs should be regarded as large-scale ecological experiments that can elucidate invasive species impact, behaviour and population dynamics in a way that is much more conclusive than correlative studies (Courchamp & Caut 2006; Myers *et al.* 2000; Zavaleta *et al.* 2001).

The integration of management programs with research and reporting may also improve dissemination of information on management techniques and broader management strategies (Caut *et al.* 2009). This improved knowledge exchange may increase the rate at which control methods are refined and will also provide practitioners with a more sound scientific backing (Donlan *et al.* 2003).

Island invasion and the study system

Island ecosystems are ideal study systems for addressing the abovementioned issues. Because of their discrete and isolated nature, and their relatively small size and biological simplicity, they provide excellent opportunities for investigating ecological processes, such as biological invasions, against a simpler background (Wardle 2002). They are suitable for manipulative experiments, particularly invasive species removals, as they are more manageable than continental habitats (Wardle 2002; Reaser *et al.* 2007). Additionally, islands are of significant importance to global biodiversity, yet they are particularly vulnerable to impacts from biological invasions (Lane 2010; New 2008; Samways *et al.* 2010a).

17

This study takes advantage of an invasive species management issue that needed to be addressed on a small island in the Seychelles archipelago, Cousine Island. This conservation island has undergone extensive restoration since the 1970's (Samways *et al.* 2010a; Samways *et al.* 2010b). It is currently believed to closely resemble its natural state and sustains populations of many endemic species, contributing substantially to the overall conservation value of the archipelago (Samways *et al.* 2010a; Samways *et al.* 2010b).

The invasive big-headed ant *Pheidole megacephala*[1] was first recorded on the island in the 1980's and has recently come under scrutiny because of a noticeable increase in its population size. This species has been listed by the IUCN as one of the five worst invasive ants globally (Lowe *et al.* 2000) and its impacts on continental and island ecosystems have been well-documented (Callan & Majer 2009; Heterick 1997; Hoffmann *et al.* 1999; Hoffmann & Parr 2007; Krushelnycky & Gillespie 2008; Vanderwoude *et al.* 2000).

As part of the ongoing conservation efforts on the island, this project was initiated to assess the role of this potentially destructive species in the system and to develop and carry out a management program for the ant. We used the opportunity to also investigate the ant's broader interactions in the system, to demonstrate the community-wide consequences of alien removal and to provide a detailed evaluation of a potential control method. The aim was to contribute to fundamental knowledge on invasive species community interactions, but also to

---

[1] In certain regions the taxonomic boundaries among *Ph. megacephala*, its subspecies and closely related species are not clear-cut (Wetterer 2007), but the population on Cousine Island has recently been confirmed as *Ph. megacephala*. In addition to morphological confirmation (B.L.Fisher pers. comm., B.D.Hoffmann pers. comm.), CO1 barcoding has verified that populations identified as *Ph. megacephala* throughout the Western Indian Ocean islands are conspecific (B.L.Fisher, unpublished data), including those from Mauritius, which is the type locality for the species (Fabricius 1793).

provide practical information that can support management decisions of the species on the island and in other natural ecosystems.

Thesis objectives and outline

The themes of the thesis are 1) the role of the invasive ant in the ecosystem and its threat to conservation of the island ecosystem (Chapter 2), 2) evaluation of a potential control method in an ecologically sensitive environment (Chapter 3) and 3) the response of various functionally important groups to the control of the invasive ant (Chapters 4 & 5). In the following section, I briefly describe the objectives of each chapter in the context of the main themes of the thesis.

Chapter 2: Impact of a mutualism between an invasive ant and honeydew-producing insects on a functionally important tree on a tropical island. Trophobiotic mutualisms between ants and honeydew-producing hemipteran insects have been described as keystone interactions that can be highly influential within arthropod food webs (Styrsky & Eubanks 2007). In this chapter, I investigate such an association of *Ph. megacephala* with two exotic hemipteran scale insects that dominate the canopy assemblage on the native host tree *Pisonia grandis*. Evidence of trophobiosis and frequent co-occurrence between the ant and scale insects on the island, as well as reports of severe *Pisonia* tree damage by such mutualisms on other islands (Handler *et al.* 2007; O'Neill *et al.* 1997; Smith *et al.* 2004), prompted this investigation into the risk of the mutualism to the ecosystem. I estimate mutualist densities on host trees, assess the strength of the association between the mutualists and evaluate the effect of the mutualism on *Pisonia* trees, with the overall aim of informing pest management decisions.

Chapter 3: Precision control of an invasive ant on an ecologically sensitive tropical island: a principle with wide applicability. A management program for *Ph.*

megacephala was initiated in response to the risk assessment. In Chapter 3 I describe the management technique that was used to control high *Ph. megacephala* densities on the island, whilst minimizing risk to non-target species and avoiding adverse environmental effects. Selective formicidal bait was delivered in custom-made bait stations, targeting areas of high ant activity and adapting application rates to small-scale ant densities. I assess the efficacy of the method for suppressing high ant densities and evaluate its advantages and disadvantages, including estimates of costs and labour. To detect any impact on non-target organisms, I also monitor other soil-surface arthropods, the group most likely to be affected by the baiting. The overall aim was to provide the most comprehensive assessment possible of a method that can be applied within other ecologically sensitive habitats.

Chapter 4: Strategic management of an invasive ant-scale mutualism enables recovery of a threatened tropical tree species. Area-wide ant suppression was expected to disrupt the mutualism, which would result in scale insect population decline and a decrease in pressure on the host trees. However, in complex natural systems, the net effects of these mutualisms on host plant fitness can be extremely variable, making the outcome of ant removal unpredictable (Rosumek *et al.* 2009). In Chapter 4, I monitor the response of the scale population to ant suppression, as well as that of the rest of the herbivore assemblage, a guild that could play a significant role in host plant fitness. *Pisonia* tree condition was also monitored throughout the survey period to assess the overall effect of the management approach on the key species threatened by the mutualism. The primary aim was to determine whether damage to the *Pisonia* could be sufficiently reversed by low-intensity management, whilst taking into account the response of the broader herbivore assemblage.

Chapter 5: Diverse natural enemy responses to the managed collapse of a destructive ant-scale mutualism. Displacement and mortality of hemipteran natural enemies by tending ants can interfere significantly with biological control of hemipteran pests (Kaplan

& Eubanks 2005; Mgocheki & Addison 2009). The manipulation of the ant population provided the opportunity to investigate the interactions of the exotic mutualists within a diverse natural enemy assemblage, which was the general aim of Chapter 5. The monitoring of natural enemies that specialize on hemipterans enabled an assessment of the extent of *Ph. megacephala* interference with hemipteran pest regulation. Additionally, the monitoring of natural enemy groups external to the mutualism revealed interactions within the broader predator and parasitoid assemblage.

In the final chapter (Chapter 6) I discuss the most important overall findings in the context of the thesis themes, focusing specifically on the relevance of our results to invasive species management.

References

Allendorf, F. W. & Lundquist, L. L. 2003. Introduction: population biology, evolution and control of invasive species. *Conservation Biology* 17, 24-30.

Bergstrom, D. M., Lucieer, A., Kiefer, K., Wasley, J., Belbin, L., Pedersen, T. K., & Chown, S. L. 2009. Indirect effects of invasive species removal devastate World Heritage Island. *Journal of Applied Ecology* 46, 73-81.

Buckley, Y. M. 2008. The role of research for integrated management of invasive species, invaded landscapes and communities. *Journal of Applied Ecology* 45, 397-402.

Callan, S. K. & Majer, J. D. 2009. Impacts of an incursion of African Big-headed ants, *Pheidole megacephala* (Fabricius), in urban bushland in Perth, Western Australia. *Pacific Conservation Biology* 15, 102-115.

21

Caut, S., Angulo, E., & Courchamp, F. 2009. Avoiding surprise effects on Surprise Island: alien species control in a multitrophic level perspective. *Biological Invasions* 11, 1689-1703.

Caut, S., Angulo, E., & Courchamp, F. 2008. Dietary shift of an invasive predator: rats, seabirds and sea turtles. *Journal of Applied Ecology* 45, 428-437.

Clout, M. N. & Veitch, C. R. 2002. Turning the tide of biological invasions: the potential for eradicating invasive species. In: *Turning the tide: the eradication of invasive species.* Proceedings of the international conference on eradication of island invasives, eds. Veitch, C.R. & Clout, M.N. pp. 1-3. Occasional paper of the IUCN Species Survival Commission no. 27.

Cook, J. L. 2003. Conservation of biodiversity in an area impacted by the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae). *Biodiversity and Conservation* 12, 187-195.

Courchamp, F. & Caut, S. 2006. Use of biological invasions and their control to study the dynamics of interacting populations. In: *Conceptual ecology and invasion biology: reciprocal approaches to nature,* eds. M. W. Cadotte, S. M. McMahon, & T. Fukami, pp. 243-269. Springer, Dordrecht, Netherlands.

Courchamp, F., Chapuis, J. & Pascal, M. 2003. Mammal invaders on islands: impact, control and control impact. Biological Reviews 78, 347-383.

Donlan, C. J., Tershy, B. R., Campbell, K., & Cruz, F. 2003. Research for requiems: the need for more collaborative action in eradication of invasive species. *Conservation Biology* 17, 1850-1851.

Fabricius, J. C. 1793. Entomologia systematic emendata et aucta. *Secundum classes, ordines, genera, species, adjectis synonimis, locis observationibus, descriptionibus.* Tome 2. C. G. Proft, Hafnia

Gentz, M. C. 2009. A review of chemical control options for invasive social insects in island ecosystems. *Journal of Applied Entomology* 133, 229-235.

Handler, A. T., Gruner, D. S., Haines, W. P., Lange, M. W., & Kaneshiro, K. Y. 2007. Arthropod Surveys on Palmyra Atoll, Line Islands, and Insights into the Decline of the Native Tree *Pisonia* grandis (Nyctaginaceae). *Pacific Science* 61, 485-502.

Heterick, B. E. 1997. The interaction between the coastal brown ant, Pheidole megacephala (Fabricius), and other invertebrate fauna of Mt Coot-tha (Brisbane, Australia). *Australian Journal of Ecology* 22, 218-221.

Hoffmann, B. D. 2010. Ecological restoration following the local eradication of an invasive ant in northern Australia. *Biological Invasions* 12, 959-969.

Hoffmann, B. D., Andersen, A. N., & Hill, G. J. E. 1999. Impact of an introduced ant on native rain forest invertebrates: *Pheidole megacephala* in monsoonal Australia. *Oecologia* 120, 595-604.

Hoffmann, B. D. & Parr, C. L. 2007. An invasion revisited: the African big-headed ant (*Pheidole megacephala*) in northern Australia. *Biological Invasions* 10, 1171-1181.

Hoffmann, B. D., Abbott, K. L., & Davis, P. 2010. Invasive ant management. In *Ant Ecology*, eds. L. Lach, C. L. Parr, & K. L. Abbott, pp. 287-304. Oxford University Press, New York, USA.

Hulme, P. E. 2006. Beyond control: wider implications for the management of biological invasions. *Journal of Applied Ecology* 43, 835-847.

Hulme, P. E. 2009. Trade, transport and trouble: managing invasive species pathways in an era of globalization. *Journal of Applied Ecology* 46, 10-18.

Kaplan, I. & Eubanks, M. D. 2005. Aphids alter the community-wide impact of fire ants. *Ecology* 86, 1640-1649.

Kruschelnicky, P. D. & Gillespie, R. G. 2008. Compositional and functional stability of arthropod comunities in the face of ant invasion. *Ecological Applications* 18, 1547-1562.

Lane, D. J. W. 2010. Tropical islands biodiversity crisis. *Biodiversity and Conservation* 19, 313-316.

Lester, P. J. 2008. Integrated pest management: an under-utilized tool for conservation and the management of invasive ants and their mutualistic Hemiptera in the Pacific. *Pacific Conservation Biology* 14, 246-247.

Levine, J. M. & D'Antonio, C. M. 2003. Forecasting Biological Invasions with Increasing International Trade. *Conservation Biology* 17, 322-326.

Lowe, S., Browne, M., Boudjelas, S., & De Poorter, M. 2000, *100 of the world's worst invasive alien species: a selection from the global invasive species database*, The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN).

Mack, R. N., Simberloff, D., Lonsdale, W. M., Evans, H., Clout, M. N., & Bazzazz, F. A. 2000. Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications* 10, 689-710.

Messing, R. H. & Wright, M. G. 2006. Biological control of invasive species: solution or pollution? *Frontiers in Ecology and the Environment* 4, 132–140.

Mgocheki, N. & Addison, P. 2009. Interference of ants (Hymenoptera: Formicidae) with biological control of the vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae). *Biological Control* 49, 180-185.

Mooney, H. A. & Cleland, E. E. 2001. The evolutionary impact of invasive species. *PNAS* 98, 5446-5451.

Myers, J. H., Simberloff, D., Kuris, A. M., & Carey, J. R. 2000. Eradication revisited: dealing with exotic species. *Trends in Ecology and Evolution* 15, 316-320.

New, T. R. 2008. Insect conservation on islands: setting the scene and defining the needs. *Journal of Insect Conservation* 12, 197-204.

O'Neill, P., Olds, J., & Elder, R. 1997. *Report on investigations of* Pulvinaria urbicola *infestations of* Pisonia grandis *forests, and masked and brown booby populations in the Coral Sea, 25 Nov-18 Dec 1997*.

Plentovich, S., Eijzenga, J., Eijzenga, H., & Smith, D. 2010a. Indirect effects of ant eradication efforts on offshore islets in the Hawaiian Archipelago. *Biological Invasions* 13, 545-557.

Plentovich, S., Swenson, C., Reimer, N. J., Richardson, M., & Garon, N. 2010b. The effects of hydramethylnon on the tropical fire ant, Solenopsis geminata (Hymenoptera:

Formicidae), and non-target arthropods on Spit Island, Midway Atoll, Hawaii. *Journal of Insect Conservation* 14, 459-465.

Reaser, J. K., Meyerson, L. A., Cronk, Q., De Poorter, M., Eldrege, L. G., Green, E., Kairo, M., Latasi, P., Mack, R. N., Mauremootoo, J., O'Dowd, D. J., Orapa, W., Sastroutomo, S., Saunders, A., Shine, C., Thrainsson, S., & Vaiutu, L. 2007. Ecological and socioeconomic impacts of invasive alien species in island ecosystems. *Environmental Conservation* 34, 1-14.

Rosumek, F. B., Silveira, F. A. O., De S Neves, F., De U Barbosa, N. P., Diniz, L., Oki, Y., Pezzini, F., Fernandes, G. W., & Cornelissen, T. 2009. Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. *Oecologia* 160, 537-549.

Samways, M. J., Hitchins, P. M., Bourquin, O., & Henwood, J. 2010a. Restoration of a tropical island: Cousine Island, Seychelles. *Biodiversity and Conservation* 19, 425-434.

Samways, M. J., Hitchins, P. M., Bourquin, O., & Henwood, J. 2010b. *Tropical island recovery: Cousine Island, Seychelles* Wiley-Blackwell, Chichester.

Simberloff, D. 2001. Eradication of island invasives: practical actions and results achieved. *Trends in Ecology and Evolution* 16, 273-274.

Simberloff, D. 2009. We can eliminate invasions or live with them. Successful management projects. *Biological Invasions* 11, 149-157.

Smith, D., Papacek, D., Hallam, M., & Smith, J. 2004. Biological control of *Pulvinaria urbicola* (Cockerell) (Homoptera:Coccidae) in a *Pisonia grandis* forest on North East Herald Cay in the Coral Sea. *General and Applied Entomology* 33, 61-68.

Styrsky, J. D. & Eubanks, M. D. 2007. Ecological consequences of interactions between ants and honeydew-producing insects. *Proceedings of the Royal Society, B-Series* 274, 151-164.

Vanderwoude, C., Lobry De Bruin, L. A., & House, A. P. N. 2000. Response of an open-forest ant community to invasion by the introduced ant, *Pheidole megacephala. Austral Ecology* 25, 253-259.

Vitousek, P. M., D'Antonio, C. M., Loope, L. L., & Westbrooks, R. 1996. Biological invasions as global environmental change. *American Scientist* 84, 468-478.

Wardle, D. A. 2002. Islands as model systems for understanding how species affect ecosystem properties. *Journal of Biogeography* 29, 583-591.

Wetterer, J. K. 2007. Biology and Impacts of Pacific Island Invasive Species. 3. The African Big-Headed Ant, *Pheidole megacephala* (Hymenoptera: Formicidae)1. *Pacific Science* 61, 437-456.

White, E. M., Wilson, J. C., & Clarke, A. R. 2006. Biotic indirect effects: a neglected concept in invasion biology. *Diversity and Distributions* 12, 443-455.

Zavaleta, E. S., Hobbs, R. J., & Mooney, H. A. 2001. Viewing invasive species removal in a whole-ecosystem context. *Trends in Ecology and Evolution* 16, 454-459.

2. Impact of a mutualism between an invasive ant and honeydew-producing insects on a functionally important tree on a tropical island

Abstract

Mutualisms between invasive ants and honeydew-producing Hemiptera have the potential to result in unusually high population levels of both partners, with subsequent major changes to ecosystem composition and dynamics. We assessed the relationship between the invasive ant, *Pheidole megacephala*, and its hemipteran mutualists, *Dysmicoccus* sp. and *Pulvinaria urbicola*, on Cousine Island, Seychelles. We also assessed the impacts of the mutualism on the condition of the hemipteran host plant, *Pisonia grandis*, a native and functionally important tree species. There was a strong positive relationship between *Ph. megacephala* activity and hemipteran abundance, and the exclusion of ants from *Pi. grandis* resulted in a significant decline in *Pu. urbicola* abundance. High abundance of the mutualists was strongly associated with damage to the *Pi. grandis* forest. This indicates that the mutualism is contributing to the massive increase in the population levels of the mutualist species, and is intensifying their impacts on the island. The widespread trophobiosis and its associated high densities of mutualists pose serious threats to the ecosystem, highlighting the need to control the ant and associated hemipteran populations.

Introduction

Positive interactions between introduced species can be instrumental in their establishment and population growth (Abbott & Green 2007; Simberloff & Von Holle 1999) and can be major determinants of their invasion success (Helms & Vinson 2003). Trophobiosis between ants and honeydew-producing Hemiptera is a prime example of a positive interaction that can facilitate the success of the species involved (Helms & Vinson 2003; Holway *et al.* 2002). These mutualisms involve the protection and sanitation of Hemiptera in return for honeydew, a carbohydrate-rich byproduct of hemipteran metabolism, as a food source for the ants (Hölldobler & Wilson 1990).

Studies on invasive ant-hemipteran mutualisms on islands have demonstrated that these positive interactions can lead to unusually high densities of the mutualists (Abbott & Green 2007). This can cause serious damage to the native communities (Handler *et al.* 2007; Hill *et al.* 2003; Smith *et al.* 2004), sometimes affecting multiple trophic levels (O'Dowd *et al.* 2003).

On Cousine Island, Seychelles, high densities of the invasive African big-headed ant, *Pheidole megacephala,* in association with alien hemipteran insects, have been observed in certain parts of the island. Furthermore, where hemipteran densities are high, several indigenous tree species in the forest exhibit symptoms of hemipteran damage. Among these trees is *Pisonia grandis*, a functionally important species. It is a significant nesting tree for the Lesser Noddy (*Anous tenuirostris*) and White Tern (*Gygis alba*) seabirds, which supply important endemic species such as skinks (*Mabuya* spp.) with dropped food items and the soil with nutrients (Samways *et al.* 2010a). It is uncertain whether the hemipteran damage is the result of direct feeding or the transmission of disease, but it manifests as leaf distortion

29

and shoot dieback, which can seriously affect photosynthetic ability. This association is of concern for this highly protected and restored island (Samways *et al.* 2010b), considering the damage that trophobiotic relationships have caused to forests on other islands (Handler *et al.* 2007; O'Neill *et al.* 1997; Smith *et al.* 2004). As small islands are particularly vulnerable to invasive species, there is great urgency to determine the risk of this relationship and to manage it accordingly. In the Seychelles, where alien ants and Hemiptera are widely distributed yet understudied, an evaluation of the impact of these species is much needed. My aim therefore was to determine the strength of the association between *Ph. megacephala* and its hemipteran mutualists and to appraise the impact of high densities of the mutualists on *Pi. grandis* tree condition.

Methods

Cousine Island is a small (27 ha) granitic island situated in the Seychelles archipelago at 4°20'41"S and 55°38'44"E (Fig. 2.1). Hemipteran species tended by *Ph. megacephala* included *Dysmicoccus* sp. and *Pulvinaria urbicola,* both of which are alien, cosmopolitan species (Ben-Dov 2006). These were the only two hemipterans recorded on the indigenous tree *Pi. grandis*, but they were widespread and abundant and seemed to be increasingly affecting tree condition. My study therefore focused on this ant/hemipteran/tree interaction for quantitative assessments.

Forty 10 m x 10 m sampling units (SU) were selected to include areas with various levels of *Ph. megacephala* activity. Within each SU, we measured *Ph. megacephala* activity, assessed the abundance of *Dysmicoccus* sp. and *Pu. urbicola,* and assessed leaf damage to *Pi. grandis* trees. Each SU was sampled once in the period 23 Sept-1 Oct 2008, and again during 6 Oct-15 Oct 2008. Ant activity was quantified by measuring ant traffic along *Pi. grandis* tree

trunks with a diameter of >20 cm. The tree with highest ant activity within the SU was selected to standardize counts between SUs. The number of ants in the high density zone moving in one direction across a 4 cm line was counted for 30 sec. The mean of three counts at different heights on the trunk was used for analyses. All ant traffic assessments were done between 06:30 and 10:00 for comparability, as ant activity can vary considerably at different times of day. Hemiptera abundance was quantified by assigning abundance scores to ten shoots per SU. Scores were assigned as follows: 0 = no hemipterans, 1 = 1-10 hemipterans, 2 = one cluster of more than 10 hemipterans, 3 = numerous clusters of more than 10 hemipterans, 4 = extensive clusters of hemipterans. These categories were chosen subjectively, based on previous observations on the variation in hemipteran abundance on *Pi. grandis* on the island. For leaf damage assessments, ten shoots per SU were each assigned a leaf damage score based on distortion and dieback. Damage was assigned as follows: 0 = leaves are undamaged with no distortion, 1 = 25% of leaves with slight distortion, 2 = 50% of leaves distorted, 3 = 75% of leaves distorted, 4 = 100% of leaves distorted and severe shoot dieback.

An experiment was also conducted, in May-July 2010, to determine the effect of *Ph. megacephala* tending on the survival of *Pu. urbicola*. Eight *Pi. grandis* trees with high levels of *Pu. urbicola* were selected in 1 ha of high *Ph. megacephala* activity, where ant workers were actively tending hemipterans. Two branches were selected per tree and three random leaves were selected per branch. *Pu. urbicola* individuals were counted on the underside of leaves using a 10x hand lens. Ants were then excluded from one branch per tree by applying commercial vehicle grease around the base of each branch. Trees were checked periodically to ensure that ants did not gain access to hemipterans on greased branches. Hemipteran abundance was reassessed on the same branches six weeks later.

The assessment data from SUs were pooled for the two sampling periods in 2008 and averages of SU's were used in analyses. To assess the relationship between *Ph. megacephala* activity, hemipteran abundance and leaf damage, Spearman rank correlations were performed on these parameters. Non-parametric tests were used as the assessment data did not satisfy parametric assumptions. To determine if the exclusion of *Ph. megacephala* from *Pi. grandis* leaves had an effect on *Pu. urbicola* abundance, a general linear model was used on the parametric experimental data set. Repeated measures ANOVA was performed in two directions on *Pu. urbicola* abundance data, as abundance on ant excluded and control branches on the same tree were correlated. Bonferroni post-hoc tests were performed to detect significant pairwise differences.

Results

Ph. megacephala activity was strongly positively correlated with *Dysmicoccus* sp. abundance (r_s = 0.67, P < 0.05) and *Pu. urbicola* abundance (r_s = 0.79, P < 0.05) (Fig. 2.2). The abundance of both hemipterans was also positively correlated with damage to *Pi. grandis* (*Dysmicoccus* sp. r_s = 0.92, P < 0.05) (*Pu. urbicola* r_s = 0.49, P < 0.05) (Fig. 2.3). *Ph. megacephala* activity had a significant positive relationship to *Pi. grandis* leaf damage (r_s = 0.67, P < 0.05) (Fig. 2.4).

There was a significant effect of *Ph. megacephala* exclusion on *Pu. urbicola* abundance ($F_{1,7}$ = 7.99, P = 0.026) (Fig. 2.5). On ant excluded branches, *Pu. urbicola* declined significantly from a mean of 133.45 ± 24.45 individuals per leaf to 35.50 ± 12.21 individuals per leaf (P = 0.016). On control branches, *Pu. urbicola* declined slightly, but non-significantly from 131.29 ± 23.19 individuals per leaf to 119.83 ± 40.14 individuals per leaf.

Discussion

These results show that the mutualisms contribute to an increase in the abundance of *Ph. megacephala* and the hemipteran species *Dysmicoccus* sp. and *Pu. urbicola*, and intensify their impacts on *Pi. grandis* trees on the island. The mechanism whereby the mutualism benefits both groups has yet to be tested for in this system. However, *Ph. megacephala* is known to disrupt the searching behaviour of natural enemies of hemipterans (Gonzalez-Hernandez *et al.* 1999), to fend off predators (Bach 1991), and to prevent the accumulation of excess honeydew (Bach 1991), thereby lowering the mortality rate of its hemipteran mutualists. The decline of *Pu. urbicola* in the absence of *Ph. megacephala* in our exclusion experiment supports the assumption that the ants sustain high population densities of the hemipterans on Cousine.

Few studies have tested empirically how these mutualisms promote the growth of ant populations (Lach *et al.* 2009). However, it has been demonstrated that carbohydrates can fuel foraging activities (Davidson 1998) and can increase *Ph. megacephala* worker survival (Lach *et al.* 2009), thereby favouring ant population growth. Helms and Vinson (2008) provide evidence of significantly higher population growth of invasive fire ants reared on a diet of honeydew and insect prey, as opposed to insect prey only. In addition, correlative evidence for increased ant densities in the presence of hemipteran mutualists is ample (e.g. Abbott & Green 2007; Helms & Vinson 2003; O'Dowd *et al.* 2003), and our data are consistent with these findings.

The effects of ant-hemipteran mutualisms on host plant health can vary considerably between different systems (Styrsky & Eubanks 2009). In this study, the mutualism led to a decline in *Pi. grandis* condition. This is of considerable importance, as the impact of the *Ph. megacephala*-hemipteran mutualism on the *Pi. grandis* forest is a phenomenon that mirrors the situation on other island ecosystems, many of which have undergone rapid forest decline.

33

On the Palmyra Atoll in the Pacific Line Islands, high densities of *Pu. urbicola,* tended by *Ph. megacephala,* caused defoliation and death of a large number of *Pi. grandis* trees, with a 30% loss of forested area between 2002 and 2005 (Handler *et al.* 2007). Exactly the same ant-hemipteran association led to an 87% loss of *Pi. grandis* forest on Tryon Island in the Capricorn Cays (O'Neill *et al.* 1997). On the Coringa Islet in the Coringa-Herald National Nature Reserve, *Pi. grandis* forests were completely destroyed by *Ph. megacephala*-driven scale outbreaks, considerably altering vegetation structure (Smith *et al.* 2004). *Pi. grandis* forests are structurally and functionally very important to these island ecosystems, providing nesting and roosting habitat for a range of seabirds, regulating understorey vegetation structure and as a valuable source of organic material (Handler *et al.* 2007; Smith *et al.* 2004). As *Pi. grandis* stands are rare and declining in their native range (Kay *et al.* 2003), and as they are threatened in the Seychelles (Samways *et al.* 2010b), their conservation is of great importance.

My study affirms that the control of these invasive insects should be of the highest priority. The species surveyed on Cousine are distributed widely throughout the tropics and occur on many of the Western Indian Ocean islands (Fisher & Snelling 2010; Ben-Dov 2006). Yet, little research has been done on their impact in these regions. Increased documentation of the impacts of these invasives will help raise awareness of the threat that these species pose to island ecosystems in general, and emphasizes the urgent need to integrate their control into conservation management strategies.

References

Abbott, K.L. & Green, P.T. 2007. Collapse of an ant-scale mutualism in a rainforest on Christmas Island. *Oikos* 116,1238-1246.

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

Ben-Dov, Y. 2006. ScaleNet: A database of scale insects of the world. http://www.sel.barc.usda.gov/scalenet/scalenet.htm. Accessed 5 August 2010.

Davidson, D.W. 1998. Resource discovery versus resource domination in ants: a functional mechanism for breaking the trade-off. *Ecological Entomology* 23:484-490.

Fisher, B.L. & Snelling, R.R. 2010. Seychelles ants. http://www.antweb.org/seychelles.jsp. Accessed 30 July 2010.

González-Hernández, H., Johnson, M.W. & Reimer, N.J. 1999. Impact of *Pheidole megacephala* (F.) (Hymenoptera: Formicidae) on the biological control of *Dysmicoccus brevipes* (Cockerell) (Homoptera: Pseudococcidae). *Biological Control* 15, 145-152.

Handler, A.T., Gruner, D.S., Haines, W.P., Lange, M.W. & Kaneshiro, K.Y. 2007. Arthropod surveys on Palmyra Atoll, Line Islands, and insights into the decline of the native tree *Pisonia grandis* (Nyctaginaceae). *Pacific Science* 61, 485-502.

Helms, K.R. & Vinson, S.B. 2003. Apparent facilitation of an invasive mealybug by an invasive ant. *Insectes Sociaux* 50, 403-404.

Helms, K.R. & Vinson, S.B. 2008. Plant resources and colony growth in an invasive ant: the importance of honeydew-producing Hemiptera in carbohydrate transfer across trophic levels. *Environmental Entomology* 37, 487-493.

Hill, M., Holm, K., Vel, T., Shah, N.J. & Matyot, P. 2003. Impact of the introduced yellow crazy ant *Anoplolepis gracilipes* on Bird Island, Seychelles. *Biodiversity and Conservation* 12, 1969-1984.

Hölldobler, B. & Wilson, E.O. 1990. The Ants. Belknap Press, Cambridge.

Holway, D.A., Lach, L., Suarez, A.V., Tsutsui, N.D. & Case, T.J. 2002. The causes and consequenses of ant invasions. *Annual Review of Ecology and Systematics* 33, 181-233.

Kay, A., Olds, J. & Elder, R. 2003. The impact and distribution of the soft scale *Pulvinaria urbicola* in the *Pisonia grandis* forests of the Capricornia Cays national parks 1993–2002. Report on the scale insect and vegetation monitoring program on Tryon Island and the scale insect surveys on other Capricornia cays. Queensland Parks and Wildlife Service Internal Report.

Lach, L., Hobbs, R.J. & Majer, J.D. 2009. Herbivory-induced extrafloral nectar increases native and invasive ant worker survival. *Population Ecology* 51, 237-243.

O'Dowd, D.J., Green, P.T. & Lake, P.S. 2003. Invasional meltdown on an oceanic island. *Ecology Letters* 6: 812-817.

O'Neill, P., Olds, J. & Elder, R. 1997. Report on investigations of *Pulvinaria urbicola* infestations of *Pisonia grandis* forests, and masked and brown booby populations in the Coral Sea, 25 November to 18 December 1997, Environment Australia Report.

Samways, M.J., Hitchins, P.M., Bourquin, O. & Henwood, J. 2010a. *Tropical island recovery: Cousine Island, Seychelles*. Wiley-Blackwell, Chichester, UK.

Samways, M.J., Hitchins, P.M., Bourquin, O. & Henwood J. 2010b. Restoration of a tropical island: Cousine Island, Seychelles. *Biodiversity and Conservation* 19, 425-434.

Simberloff, D. & Von Holle, B. 1999. Positive interactions of nonindigenous species: invasional meltdown? *Biological Invasions* 1, 21-32.

Smith, D., Papacek, D., Hallam, M. & Smith, J. 2004. Biological control of *Pulvinaria urbicola* (Cockerell) (Homoptera:Coccidae) in a *Pisonia grandis* forest on North East Herald Cay in the Coral Sea. *General and Applied Entomology* 33, 61-68.

Styrsky, J.D. & Eubanks, M.D. 2007. Ecological consequences of interactions between ants and honeydew-producing insects. *Proceedings of the Royal Society of London B-Series Biology* 274, 151-164.
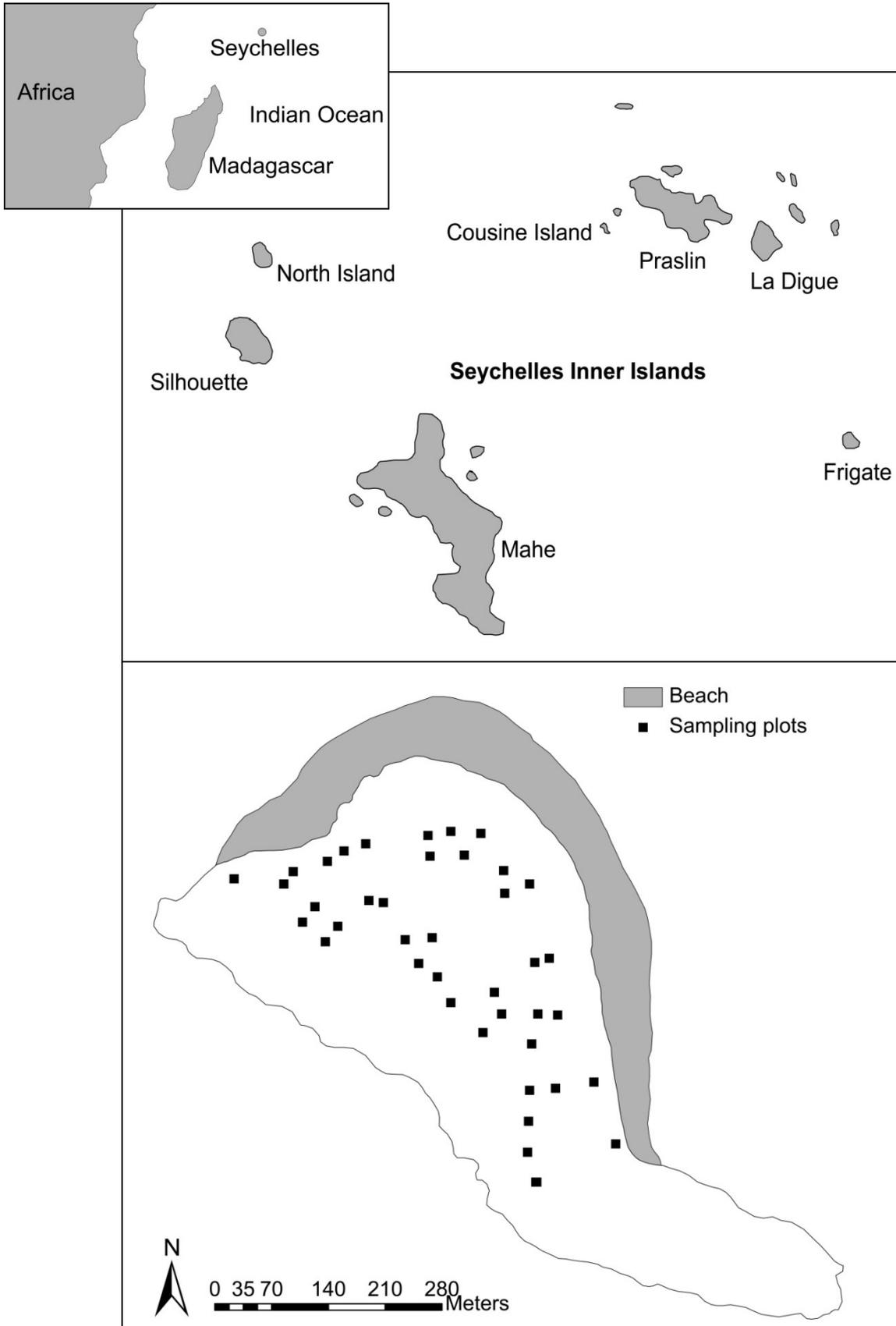
Figure 2.1. Location of the study site, Cousine Island, and layout of sampling plots.
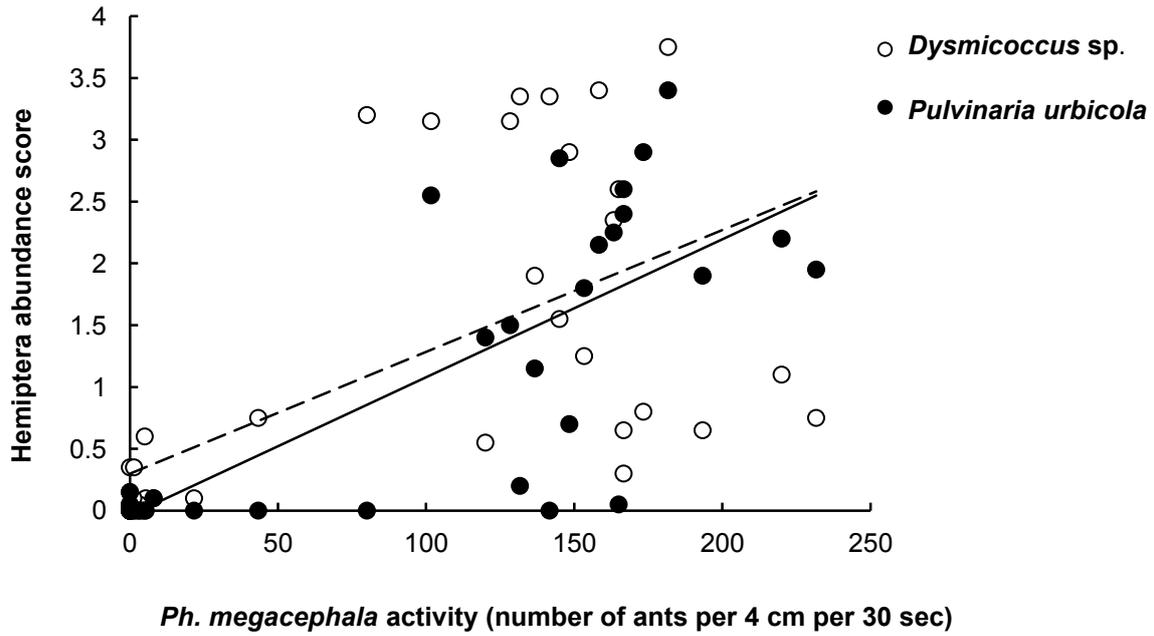
Figure 2.2. Correlation of *Pheidole megacephala* activity and *Pulvinaria urbicola/Dysmicoccus* sp. abundance scores. The solid trendline represents the correlation between *Ph. megacephala* and *Pu. urbicola*, while the dotted line represents the *Ph. megacephala* and *Dysmicoccus* sp. correlation

Figure 2.3. Correlation of *Pulvinaria urbicola/Dysmicoccus* sp. abundance and *Pisonia grandis* leaf damage scores. The solid trendline represents the correlation of *Pu. urbicola* and leaf damage scores, while the dotted line represents *Dysmicoccus* sp. and leaf damage score correlation

40
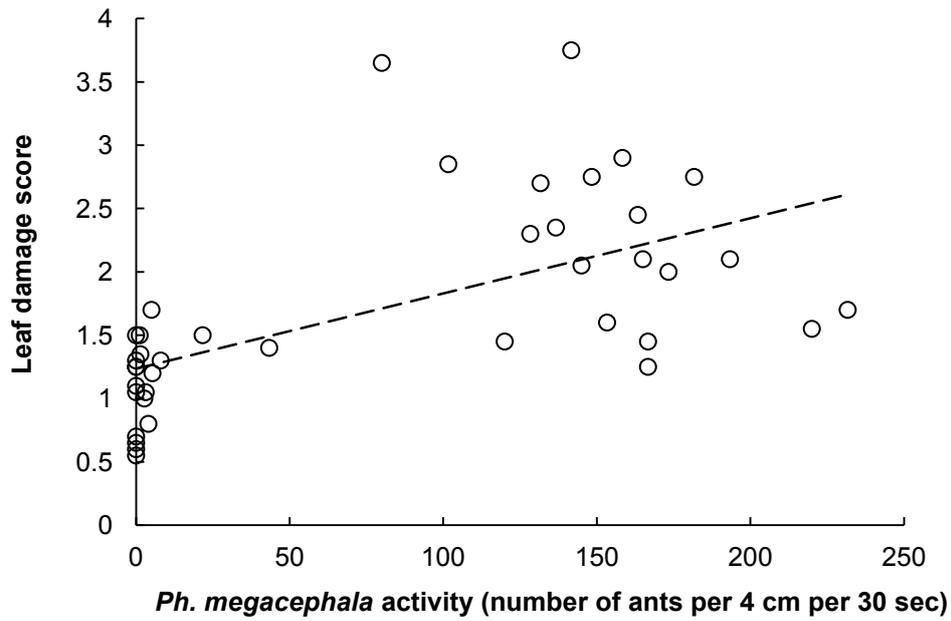
Figure 2.4. Correlation of *Pheidole megacephala* activity and *Pisonia grandis* leaf damage scores. The dotted trendline represents the correlation of *Ph. megacephala* activity and leaf damage scores.
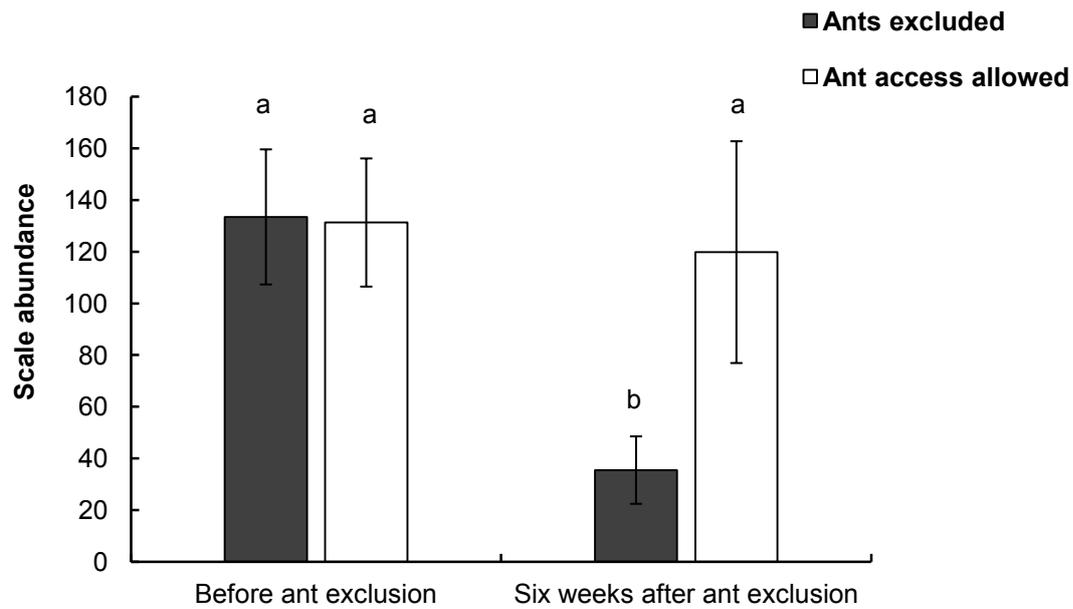
Figure 2.5. The effect of ant exclusion on mean *Pulvinaria urbicola* abundance (± S.E.) on *Pisonia grandis* leaves. Means with letters in common are not significantly different at P < 0.05.

3. Precision control of an invasive ant on an ecologically sensitive tropical island: a principle with wide applicability

Abstract

Effective management of invasive ants is an important priority for many conservation programs, but can be difficult to achieve, especially within ecologically sensitive habitats. This study assesses the efficacy and non-target risk of a precision ant baiting method aiming to reduce a population of the invasive big-headed ant *Pheidole megacephala* on a tropical island of great conservation value. Area-wide application of formicidal bait, delivered in bait stations, resulted in the rapid decline of 8 ha of *Ph. megacephala*. Effective suppression remained throughout the succeeding 11-month monitoring period. I detected no negative effects of baiting on non-target arthropods. Indeed, abundance and species richness of non-target ants and abundance of other soil-surface arthropods increased significantly after *Ph. megacephala* suppression. This bait station method minimized bait exposure to non-target organisms and was cost-effective and adaptable to target species density. However, it was only effective over short distances and required thorough bait placement. This method would therefore be most appropriate for localized *Ph. megacephala* infestations where the prevention of non-target impacts is essential. The methodology used here would be applicable to other sensitive tropical environments.

Introduction

Management of invasive species is essential for the conservation of ecosystems (Zavaleta *et al.* 2001) but can be extremely challenging (Myers *et al.* 2000). Social invasive insects, such as ants, are among the species causing the most widespread ecological damage (Holway *et al.* 2002; New 2008), but are especially difficult to control (Holway *et al.* 2002; Gentz 2009). The development of management strategies for well-established invasive ants can be time-consuming and costly (Williams *et al.* 2001) and control programs for some species have had limited success (Silverman & Brightwell 2008). Management is further complicated in sensitive habitats where environmental repercussions of management practices have to be taken into account (Gentz 2009). The possibility of non-target impacts and accumulation of toxins in the environment is a considerable risk in fragile or protected habitats, and ecosystem-wide effects can be unpredictable (Plentovich *et al.* 2010*a*; 2010*b*).

The development of highly selective insecticides, with precise mechanisms of action and greatly reduced environmental risk, provides an opportunity to manage invasive ants in areas of high conservation value (Gentz 2009). Several studies have demonstrated that selective formicidal bait can be used to locally eradicate invasive ants, with most successes reported for smaller, isolated infestations (Abedrabbo 1994; Hoffmann & O'Connor 2004; Causton *et al.* 2005; Plentovich *et al.* 2009; Hoffmann 2011). Formicidal bait has also been used to reduce population levels (Cook 2003) and to limit range expansion of invasive ants (Krushelnycky *et al.* 2004). Different methodologies were used according to local conditions and the species involved. Results of the treatments have been varied, with recovery of native species in some cases (Cook 2003; Hoffmann 2010). However, in other cases non-target and indirect effects (Plentovich *et al.* 2010*a*; 2010*b*) or post-treatment recovery of the target species occurred (Plentovich *et al.* 2009). Clearly we need more information on the efficacy,

44

costs/benefits and risks of different strategies to refine and develop control methodologies (Simberloff 2009; Hoffmann *et al.* 2010; Hoffmann 2011).

Cousine Island, Seychelles, is of major conservation significance to the archipelago, as it sustains populations of many endemic and threatened species (Samways *et al.* 2010*a*). Unfortunately, also present is the highly invasive big-headed ant *Pheidole megacephala*, which is notorious for impacting native ecosystems (Hoffmann *et al.* 1999; Holway *et al.* 2002; Wetterer 2007; Krushelnycky & Gillespie 2008). In recent years, this ant has significantly impacted parts of this island ecosystem (Gaigher *et al.* 2011), thereby posing a major threat to some significant biota. Effective control of the species has since become a priority for island management (Samways *et al.* 2010*a*), with the greatest challenge being minimising non-target impacts on the large number of endemic species within this small and ecologically sensitive environment. Here I present an evaluation of the efficacy and non-target impacts of a precision baiting method recently used to control *Ph. megacephala* on Cousine Island.

Methods

Delineation of treatment area

Cousine Island is a 27 ha granitic island in the Seychelles, 4°20'41"S, 55°38'44"E. A pre-treatment survey of *Ph. megacephala* population levels was conducted in May-June 2010 to demarcate the treatment area. Ant activity, defined as the number of ants moving in one direction across a 4 cm horizontal section of a trunk foraging trail in 30 seconds, was recorded across the island on haphazardly selected trees and mapped using a GPS. We sampled 494 trees, which provided sufficient detail to detect fine-scale variation in population levels. Activity levels were categorized as absent (no ants per 4 cm per 30 sec),

low (1-25 ants), medium (26-50 ants) or high (> 50 ants). High and medium ant densities on Cousine were associated with direct impacts on other fauna (pers. obs.) and indirect impacts on the native forest (Gaigher *et al.* 2011), but no impacts were obvious at low densities. Because the treatment aimed not for eradication, but for the suppression of the overall population to low activity levels which result in no observable ecological impact, the treatment area was a single 8 ha area with medium and high ant activity (Fig. 3.1).

Treatment

Treatment was conducted between 15 June and 23 July 2010 using the commercial formicidal bait Siege (also known as Amdro). Siege granules consist of maize grits, soybean oil and the active ingredient hydramethylnon, a slow-acting metabolic inhibitor, which is dispersed among workers within colonies by communal feeding (Gentz 2009; Bacey 2011). Siege is highly effective at controlling *Ph. megacephala* in agricultural (Samways 1986; Zerhusen & Rashid 1992; Taniguchi *et al.* 2005; Arakaki *et al.* 2009) and natural systems (Hoffmann & O'Connor 2004; Plentovich *et al.* 2010*a*). Siege is also of low toxicity thereby presenting minimal risk to most non-target terrestrial organisms, except for scavenging arthropods that may ingest the bait (Stanley 2004). Risk of environmental contamination is minimal, as hydramethylnon degrades rapidly in sunlight and water (Apperson *et al.* 1982; Vander Meer *et al.* 1982).

The bait was distributed inside bait stations (Fig. 3.2) (Grout 2008) to provide the best likelihood of avoiding non-target impacts, as well as to prolong bait efficacy by limiting bait exposure to sunlight and water. These stations allowed ant access, but excluded most non-target species. The stations were 200 mm long pieces of plastic irrigation tubing, 15 mm diameter, sealed at the ends, with two 7 mm holes drilled into the sides for ant access. Each station held 10 g of bait and stations were placed at the base of trees with *Ph. megacephala*

activity. Station density was adapted to *Ph. megacephala*density, with overall bait coverage being 4 kg/ha. We used a higher dosage than the recommended 2.5 kg/ha, because of the exceptionally high densities of ants throughout the area. Stations were collected after one week, while simultaneously inspecting for persisting colonies which were subsequently baited with new bait stations.

Data collection

To document the short-term *Ph. megacephala* response to the treatment, we recorded ant activity in ten locations in the treated area on four days in the week before treatment, daily after treatment until the ants were suppressed to low activity levels after one week, and once a week for five weeks after suppression.

To test for longer-term effects of baiting on *Ph. megacephala* and non-target arthropods, pairs of pitfall traps were placed in forty random locations, twenty within the baited area and twenty within the unbaited area[2]. Traps within each pair were 1 m apart and each location was separated by at least 10 m. Each pitfall trap was a 50 ml test tube with a 2.5 cm diameter, half filled with water and a drop of detergent. Traps were left open for two days during each survey, which was undertaken two weeks before baiting, two weeks after baiting, four months after baiting and 11 months after baiting. Abundance data of soil-surface arthropods were recorded. Ants were identified to species level and other arthropods to order, and sorted into morphospecies. Voucher specimens are in the Stellenbosch University Entomological Museum.

---

[2] Before the baiting program, the baited and unbaited areas differed in *Ph. megacephala* density, possibly due to the ant being associated with greater disturbance in the north. Therefore plots in unbaited areas were not true control sites. However, this study design allowed the detection of variation that was unrelated to baiting, such as natural population fluctuation caused by external environmental conditions, by comparing the relative change over time between baited and unbaited areas.

To detect localized *Ph. megacephala* resurgence outside the permanent sampling locations, we conducted island-wide surveys four and 11 months after treatment (in October 2010 and May 2011 respectively), when activity levels were recorded, categorized and mapped as in the pre-treatment survey. The purpose of the October 2010 survey was to detect resurgence mainly within the treated area and included 149 trees in the treated and adjacent areas. In May 2011, I aimed to resurvey the entire island and sampled 290 trees across the island. Sampling intensity was lower than in the initial survey as we determined that a lower sampling effort would be sufficient to detect resurgence, based on initial survey results.

Statistical analysis

To determine the short-term response of *Ph. megacephala* to treatment, one-way analysis of variance (ANOVA) was performed with Bonferroni corrected post-hoc pairwise comparisons. As data did not satisfy parametric assumptions, they were square root transformed prior to analysis (Townend 2002). Generalized Estimating Equations (GEEs) were used to test for longer-term effects of baiting on *Ph. megacephala* abundance, and the abundance and species richness of other ants and non-ant arthropods. GEEs extend the generalized linear model algorithm to account for correlated observations, in this case the repeated measurements per plot (Liang & Zeger 1986). "Plot" was specified as the subject variable in the model, and "time" and "treatment" as within-subject variables, with the important term in the analysis being the "time by treatment" interaction, which indicates whether there is change over time as a result of treatment. This analysis examines the relative change in baited and unbaited areas, and thus accounts for external ecological influences on response variables that are unrelated to baiting. I used a log-link function and negative binomial distribution for all data sets and performed bootstrap post-hoc multiple comparisons to detect pairwise differences. Analyses were done in Statistica 10 (Statsoft 2003) and SPSS 19 (SPSS Inc. 2010). All graphs are presented using raw data.

Results

Short-term Ph. megacephala *response to baiting*

Ph. megacephala activity was significantly reduced within a week after treatment from a mean of 62 ± 11 ants to a mean of 1 ± 1 ant ($F_{13, 126}$ = 42.19, P < 0.0001). Activity remained suppressed below four ants per 4 cm per 30 sec in these plots for the duration of the five week survey.

Longer-term effects of baiting on Ph. megacephala *and non-target arthropods*

Baiting caused a significant longer-term decline in *Ph. megacephala* abundance from a mean of 145 ± 21 ants per plot to fewer than 12 ants per plot for the rest of the 11-month study period (Wald Chi-square = 170.4, P < 0.0001) (Fig. 3.3; Table 3.1) while *Ph. megacephala* abundance in unbaited plots remained unchanged (P > 0.05) (Fig. 3.3).

We detected no negative effect of baiting on any of the non-target arthropods, but instead a positive effect of *Ph. megacephala* removal. Abundance of other ants in baited plots was significantly influenced by baiting (Wald Chi-square = 16.7, P < 0.005) and increased from 1 ± 1 ant before baiting to 12 ± 3 ants after 11 months. Abundance in unbaited plots also increased over time, although less so than in baited plots (Fig. 3.4a; Table 3.1). Baiting significantly influenced species richness of other ants (Wald Chi-square = 21.1, P < 0.0001) (Fig. 3.4b; Table 3.1). Ant species richness in baited plots increased from 0.1 ± 0.1 species per plot to 2.1 ± 0.3 species per plot, whereas ant species richness in unbaited plots remained unchanged (P > 0.05) (Fig. 3.4b). The composition of the ant assemblage in baited plots also changed after baiting. Before baiting, the ant assemblage was dominated by *Ph. megacephala* (99.6%), with only *Brachymyrmex cordemoyi* sympatric (Table 3.2). Assemblages in unbaited plots throughout the study period consisted of a greater diversity of species including other introduced species and the Seychelles endemic *Pheidole flavens*

49

farquharensis (Table 3.2). The diversity of ants in baited plots steadily increased after baiting (Table 3.2), and 11 months after treatment, assemblages in baited plots consisted of the tramp ants *Ph. megacephala* (47.0%), *B. cordemoyi* (7.0%), *Tetramorium simillimum* (1.1%), *Paratrechina longicornis* (2.5%), *Paratrechina bourbonica* (8.1%), *Tapinoma melanocephalum* (1.3%), *Plagiolepis alluaudi* (0.4%), *Cardiocondyla emeryi* (6.2%), *Camponotus maculatus* (0.9%) and the endemic *P. flavens farquharensis* (25.5%) (Table 3.2).

Non-ant arthropods in pitfall traps included cockroaches, isopods, mites, spiders, springtails, beetles, centipedes, millipedes, true bugs and pseudoscorpions, with 94% of the total number of arthropods trapped being represented by one species of alien cockroach *Pycnoscelus indicus* and two species of unidentified isopods. Non-ant arthropod abundance was significantly influenced by baiting (Wald Chi-square = 21.4, P < 0.0001) (Fig 3.5a; Table 3.1), increasing from 47 ± 9 individuals to 219 ± 35 individuals after 11 months, corresponding with no change in unbaited plots (P > 0.05) (Fig. 3.5a). The effect of baiting on non-ant arthropod species richness was non-significant (Wald Chi-square = 1.4, P = 0.70) (Fig. 3.5b; Table 3.1).

Island-wide Ph. megacephala *activity surveys*

Four months after baiting, we recorded 79% *Ph. megacephala* absences, 19% low activity, 2% medium activity and 0% high activity in the treated area (n = 107). Untreated areas had 51% *Ph. megacephala* absences, 46% low activity, 2% medium activity and 0% high activity observations (n = 42) (Fig. 3.6). Eleven months after baiting, treated areas had 67% *Ph. megacephala* absences, 31% low activity, 2.5% medium activity, and 0% high activity observations (n = 134) (Fig. 3.7). Untreated areas had 49% absences, 37 % low activity, 15 % medium activity and 0% high activity observations (n = 156).

Hours worked and costs of the treatment

A total of 322 hours were worked during the treatment of the 8 ha area. This included construction of the bait stations (82 hours), filling stations with bait (49 hours), deploying them in the field (83 hours), collecting empty bait stations (60 hours) and all pre- and post-treatment surveys (48 hours). A total of $ 2616.40 US was spent on materials used during treatment of the 8 ha area and included the cost of Siege used in treatment ($ 1922.73 US), shipping costs ($ 450.79 US) and materials for bait stations ($ 242.87 US).

Discussion

Efficacy of the treatment

The treatment was effective at reaching the conservation goal of suppressing the 8 ha *Ph. megacephala* infestation to innocuous levels. Area-wide application of Siege in bait stations resulted in rapid decline of *Ph. megacephala* density, with effective suppression after one week. This decline in ant density is significant, as the population levels of the ant on the island had been continuously high over many preceding years (Samways *et al.* 2010a; Samways *et al.* 2010b). Suppression lasted for the duration of the 11-month post-treatment monitoring period. Population levels were still low throughout the treated area at the end of the study and only very localized spot treatments have since been required where isolated nests recovered to maintain suppression.

The biology of *Ph. megacephala* most likely contributed to the efficacy of the treatment. Silverman and Brightwell (2008) emphasize three traits of most invasive ants that make them well-suited for management attempts: 1) dispersal through budding, which results in clear colony boundaries, 2) flexible diet to ensure acceptance of the bait, and 3) rapid recruitment to, and monopolizing of, food resources, which ensures spread of the toxicant through the colony. For all of these factors, *Ph. megacephala* fits the description (Holway *et*

al. 2002; Wetterer 2007), making it susceptible to control measures and an ideal candidate species for management (Hoffmann & O'Connor 2004; Hoffmann 2010).

As in other effective *Ph. megacephala* management programs (Hoffmann & O'Connor 2004; Plentovich *et al.* 2010*a*; Hoffmann 2011), effective suppression was aided by the small dimensions of the infestation. The small area allowed focused treatment in locations of high ant density, which increased the possibility of achieving complete coverage in these areas. The isolation of the island also ruled out the possibility of re-introduction which has caused resurgence after treatment in other studies (Apperson *et al.* 1982; Samways 1986; Cook 2003).

Effect of baiting on non-target arthropods

To fully evaluate the outcomes of control methods, information on non-target effects is essential, especially in natural habitats. Although hydramethylnon-based baits are reported to be highly specific (Stanley 2004; Bacey 2011) there have been reports of impacts of broadcasting on non-target arthropods (Plentovich *et al.* 2010*a*; 2010*b*). Cousine is home to a rich endemic litter fauna (Kelly & Samways 2003) and a threatened keystone detritivore, the Seychelles giant millipede (*Sechelleptus seychellarum*) (Lawrence & Samways 2003). Such smaller organisms may have been vulnerable to baiting despite their physical exclusion by bait stations. The lack of non-target effects observed here suggests that the ecological costs of treatment are insignificant, and lends support for the use of this treatment method in sensitive habitats.

The significant increase in non-target ants and other soil-surface arthropods following *Ph. megacephala* control indicates that there is potential for the arthropod community to recover following *Ph. megacephala* management. Consequences of *Ph. megacephala* control in other tropical ecosystems have been varied. In northern Australia, *Ph. megacephala*

52

eradication resulted in recovery of the native ant assemblage (Hoffmann 2010). However, its eradication in Hawaii resulted in subsequent invasion by *Anoplolepis gracilipes*, the impact of which was considered to be worse than that of *Ph. megacephala* (Plentovich *et al.* 2010*a*). In our study, both native and exotic species benefitted from *Ph. megacephala* control, but none of the exotics are considered to be aggressive invaders (Dorow 1996; Samways *et al.* 2010*b*) and some such as *P. indicus* are functionally important naturalized components of the ecosystem (Samways *et al.* 2010*b*). Overall, the system appears to have benefitted substantially from *Ph. megacephala* suppression.

Benefits and disadvantages of the bait station method

Bait stations have been used to control ants in agricultural systems (Taniguchi *et al.* 2003; Taniguchi *et al.* 2005; Arakaki *et al.* 2009) and to selectively exclude ants from tropical forest canopies (Klimes *et al.* 2011). But they have never been used for invasive ant management in natural habitats. For this environment, it proved to be a very effective application method.

The main advantage of this precision baiting method is the reduced opportunity for bait uptake by non-target organisms. I have observed cockroach mortality during previous small scale broadcasting trials on Cousine, as well as ingestion of exposed bait by endemic taxa. Due to the risk of non-target effects of broadcasting, I considered it essential to avoid exposure of these species to the bait, and bait stations provided the opportunity to do so.

In areas with variable *Ph. megacephala* levels, the bait stations were ideal, as they allowed focused bait placement and control over small scale application rates, which is less achievable with broadcasting. A drawback of the localized influence of the stations is that they were only effective over short distances (up to 5 m), making thorough bait placement necessary. This was in contrast to the 15 meter influence of bait stations used by Taniguchi *et*

al. (2003) in pineapple fields and the ability of *Ph. megacephala* to detect bait stations from 12 m away in orchards (Grout 2008). The complex vegetation and terrain of the island, compared to these agricultural systems may have influenced the distance over which the bait stations were effective. Additionally, it is likely that the island with its large proportion of suitable nesting habitat was able to support a higher ant nest density compared to agricultural land. This would have influenced the rate of bait uptake and increased the need for higher station density on the island.

Approximately $350 US was spent per ha on materials used in the treatment and 41 hours were worked per ha. These estimates include only material and personnel costs for the treatment phase in the field and do not include time spent preparing for field trips, laboratory work or overhead costs, which may contribute significantly to the overall costs. The total cost of eradicating a 21 ha infestation of *Wasmannia auropunctata* from Marchena Island was $13 680 US per hectare (Causten *et al.* 2005). The cost of the program on Cousine was more comparable to that of the eradication of *Ph. megacephala* (30 ha) and *Solenopsis geminata* (3 ha) from Kakadu National Park at approximately $900 US per hectare, which was considered to be very cost-effective (Hoffmann & O'Connor 2004).

Conclusion

The precision bait station method was suitable for use on a small tropical island, as it effectively controlled high *Ph. megacephala* densities with no observed non-target effects. The method used in this study is surely applicable within other sensitive tropical environments threatened by this species, particularly undisturbed habitats and protected areas. This study demonstrates that the innovative use of low-tech, low cost methods can be effective in achieving invasive ant management goals and I hope that it will stimulate further research on selective low impact control methods.

References

Abbott, K.L., & Green, P.T.. 2007. Collapse of an ant-scale mutualism in a rainforest on Christmas Island. *Oikos* 116, 1238-1246.

Abedrabbo, S. 1994. Control of the little fire ant *Wasmannia auropunctata*, on Santa Fe Island in the Galàpagos Islands. Pages 219-227 in D.F. Williams, editor. *Exotic ants: biology, impact, and control of introduced species*. Westview Studies in Insect Biology, Boulder, Colorado.

Apperson, C.S., Leidy, R.B. & Powell, E.E. 1982. Effects of Amdro on the red imported fire ant (Hymenoptera: Formicidae) and some nontarget ant species and persistence of Amdro on a pasture habitat in North Carolina. *Journal of Economic Entomology* 77, 1012-1018.

Arakaki, A., Taniguchi, G., Arce, R., Sugano, J. & Fukuda, S. 2009. Control of the big-headed ant, *Pheidole megacephala* (Hymenoptera, Formicidae), in coffee fields using bait stations containing Amdro (hydramethylnon*). Sociobiology* 52, 403-410.

Bacey, J. Environmental fate of hydramethylnon. Department of Pesticide Regulation, Sacramento, California. Available from:

www.cdpr.ca.gov/docs/emon/pubs/fatememo/hydmthn.pdf. (accessed June 2011)

Causton, C.E., Sevilla, C.R. & Porter, S.D. 2005. Eradication of the little fire ant, *Wasmannia auropunctata* (Hymenoptera:Formicidae), from Marchena Island, Galápagos: on the edge of success? *Florida Entomologist* 88, 159-168.

Cook, J.L. 2003. Conservation of biodiversity in an area impacted by the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae). *Biodiversity Conservation* 12, 187-195.

Dorow, W.H.O. 1996. Review and bibliography of the ants of the Seychelles (Hymenoptera: Formicidae*). Journal of African Zoology* 110, 72-95.

Gaigher, R., Samways, M.J., Henwood, J. & Jolliffe, K. 2011. Impact of a mutualism between an invasive ant and honeydew-producing insects on a functionally important tree on a tropical island. *Biological Invasions* 13, 1717-1721.

Gentz, M.C. 2009. A review of chemical control options for invasive social insects in island ecosystems. *Journal of Applied Entomology* 133, 229-235.

Grout, T.G. 2008. An inexpensive ant bait station. *South African Fruit Journal* Oct/Nov.

Hoffmann, B.D. 2010. Ecological restoration following the local eradication of an invasive ant in northern Australia. *Biological Invasions* 12, 959-969.

Hoffmann, B.D. 2011. Eradication of populations of an invasive ant in northern Australia: successes, failures and lessons for management. *Biodiversity and Conservation* 20, 3267-3278.

Hoffmann, B.D., Abbott, K.L. & Davis, P. 2010. Invasive ant management. Pages 287-304 in L. Lach, C.L. Parr and K.L. Abbott, editors. *Ant Ecology*. Oxford University Press, New York, USA.

Hoffmann, B.D., Andersen, A.N. & Hill, G.J.E. 1999. Impact of an introduced ant on native rain forest invertebrates: *Pheidole megacephala* in monsoonal Australia. *Oecologia* 120, 595-604.

Hoffmann, B.D., & O'Connor, S. 2004. Eradication of two exotic ants from Kakadu National Park. *Ecological Management and Restoration* 5, 98-105.

Holway, D.A., Lach, L., Suarez, A.V., Tsutsui, N.D., & Case, T.J. 2002. The causes and consequences of ant invasions. *Annual Review of Ecology and Systematics* 33, 181-233.

Kelly, J.A., & Samways, M.J. 2003. Diversity and conservation of forest-floor arthropods on a small Seychelles island. *Biodiversity and Conservation* 12, 1813.

Klimes, P., Janda, M., Ibalim, S., Kua, J. & Novotny, V. 2011. Experimental suppression of ants foraging on rainforest vegetation in New Guinea: testing methods for a whole-forest manipulation of insect communities. *Ecological Entomology* 36, 94-103.

Krushelnycky, P.D., & Gillespie, R.G. 2008. Compositional and functional stability of arthropod communities in the face of ant invasion. *Ecological Applications* 18, 1547-1562.

Krushelnycky, P.D., Loope, L.L. & Joe, S.M. 2004. Limiting spread of a unicolonial invasive insect and characterization of seasonal patterns of range expansion. *Biological Invasions* 6, 47-57.

Lawrence, J.M., & Samways, M.J. 2003. Litter breakdown by the Seychelles giant millipede and the conservation of soil processes on Cousine Island, Seychelles. *Biological Conservation* 113, 125-132.

Myers, J.H., Simberloff, D., Kuris, A.M., & Carey, J.R. 2000. Eradication revisited: dealing with exotic species. *Trends in Ecology and Evolution* 15, 316-320.

New, T.R. 2008. Insect conservation on islands: setting the scene and defining the needs. *Journal of Insect Conservation* 12, 197-204.

Plentovich, S., Eijzenga, J., Eijzenga, H. & Smith, D. 2010a. Indirect effects of ant eradication efforts on offshore islets in the Hawaiian Archipelago. *Biological Invasions* 13, 545-557.

Plentovich, S., Hebshi, A. & Conant, S. 2009. Detrimental effects of two widespread invasive ant species on weight and survival of colonial nesting seabirds in the Hawaiian Islands. *Biological Invasions* 11, 289-298.

Plentovich, S., Swenson, C., Reimer, N.J., Richardson, M. & Garon, N. 2010b. The effects of hydramethylnon on the tropical fire ant, *Solenopsis geminata* (Hymenoptera: Formicidae), and non-target arthropods on Spit Island, Midway Atoll, Hawaii. *Journal of Insect Conservation* 14, 459-465.

Samways, M.J. 1986. Appraisal of the proprietary bait 'Amdro' for control of ants in southern African citrus. *Citrus and Subtropical Fruit Journal* 621, 14-17.

Samways, M.J., Hitchins, P.M., Bourquin, O. & Henwood, J. 2010a. Restoration of a tropical island: Cousine Island, Seychelles. *Biodiversity and Conservation* 19, 425-434.

Samways, M.J., Hitchins, P.M., Bourquin, O. & Henwood, J. 2010b. *Tropical island recovery: Cousine Island, Seychelles*. Wiley-Blackwell, Oxford, UK.

Silverman, J., & Brightwell, R.J. 2008. The Argentine ant: challenges in managing an invasive unicolonial pest. *Annual Review of Entomology* 53, 231-252.

Simberloff, D. 2009. We can eliminate invasions or live with them. Successful management projects. *Biological Invasions* 11, 149-157.

Stanley, M.C. 2004. Review of the efficacy of baits used for ant control and eradication. Landcare Research Contract Report: LC0405/044, Ministry of Agriculture and Forestry, Wellington, New Zealand.

Statsoft Inc. 2003. *Statistica* (Data analysis software system) Statsoft Inc. Tulsa, USA.

Taniguchi, G.Y., Ota, A.K., Kawate, M.K., Rohrbach, K.G. & Uchida, G.K. 2003. Preliminary field tests on the suitability of Amdro™ and Distance™ in ant bait container for control of the big-headed ant, *Pheidole megacephala* (Hymenoptera: Formicidae). *Proceedings of the Hawaiian Entomological Society* 36, 129-133.

Taniguchi, G.Y., Thompson, T. & Sipes, B. 2005. Control of the big-headed ant, *Pheidole megacephala*, (Hymenoptera, Formicidae) in pineapple cultivation using Amdro in bait stations. *Sociobiology* 45, 1-7.

Townend, J. 2002. *Practical statistics for environmental and biological scientists*. Wiley, West Sussex, UK.

Vander Meer, R.K., Williams, D.F. & Lofgren, C.S. 1982. Degradation of the toxicant AC 217,300 in Amdro imported fire ant bait under field conditions. *Journal of Agricultural and Food Chemistry* 30, 145-148.

Wetterer, J.K. 2007. Biology and impacts of Pacific Island invasive species. 3. The African big-headed ant, *Pheidole megacephala* (Hymenoptera: Formicidae). *Pacific Science* 61, 437-456.

Williams, D.F., Collins, H.L. & Oi, D.H. 2001. The red imported fire ant (Hymenoptera: Formicidae): An historical perspective of treatment programs and the development of chemical baits for control. *American Entomologist* 47, 146-159.

Zavaleta, E.S., Hobbs, R.J. & Mooney, H.A. 2001. Viewing invasive species removal in a whole-ecosystem context. *Trends in Ecology and Evolution* 16, 454-459.

Zerhusen, D., & Rashid, M. 1992. Control of the bigheaded ant *Pheidole megacephala* Mayr. (Hyrn., Forrnicidae) with the fire ant bait 'AMDRO' and its secondary effect on the population of the African weaver ant *Oecophylla longinoda* Latreille (Hyrn., Forrnicidae). *Journal of Applied Entomology* 133, 258-264.

Table 3.1. Effect of baiting on *Ph. megacephala* abundance, and abundance and species richness of other ants and non-ant arthropods. Statistics derived from Generalized Estimating Equations

Response variables	df	Wald Chi-square	P
Ph. megacephala abundance			
Treatment	1	25.9	< 0.0001
Time	3	117.4	< 0.0001
Time x Treatment	3	170.4	< 0.0001
Other ant abundance			
Treatment	1	35.1	< 0.0001
Time	3	50.1	< 0.0001
Time x Treatment	3	16.7	< 0.005
Ant species richness (excluding *P. megacephala*)			
Treatment	1	41.6	< 0.0001
Time	3	42.2	< 0.0001
Time x Treatment	3	21.1	< 0.0001
Non-ant arthropod abundance			
Treatment	1	203.1	< 0.0001
Time	3	49.1	< 0.0001
Time x Treatment	3	21.4	< 0.0001
Non-ant arthropod species richness			
Treatment	1	73.9	< 0.0001
Time	3	18.0	< 0.0001
Time x Treatment	3	1.4	0.70

Table 3.2. Total abundance of each ant species sampled in baited and unbaited plots before and after baiting

Species name	Status	Baited plots				Unbaited plots			
		14 d before baiting	14 d after baiting	120 d after baiting	330 d after baiting	14 d before baiting	14 d after baiting	120 d after baiting	330 d after baiting
Brachymyrmex cordemoyi	Exotic	11	10	42	33	8	6	11	10
Camponotus maculatus	Exotic	0	0	0	4	0	0	1	0
Cardiocondyla emeryi	Exotic	0	0	20	29	0	0	0	1
Monomorium floricola	Exotic	0	0	0	0	7	1	8	0
Monomorium seychellense	Exotic	0	0	0	0	0	0	3	1
Odontomachus simillimus	Exotic	0	0	0	0	113	116	69	110
Paratrechina bourbonica	Exotic	0	0	0	38	1	0	2	12
Paratrechina longicornis	Exotic	0	0	0	12	4	0	3	33
Pheidole flavens farquharensis	Native	0	0	0	120	7	1	14	2
Pheidole megacephala	Exotic	2897	107	17	221	24	27	37	17
Plagiolepis allaudi	Exotic	0	0	0	2	0	2	0	1
Strumigenys emmae	Exotic	0	2	0	0	0	1	1	0
Tapinoma melanocephalum	Exotic	0	2	0	6	0	0	0	75
Technomyrmex albipes	Exotic	0	0	0	0	0	0	0	3
Tetramorium simillimum	Exotic	0	0	0	5	21	11	69	72
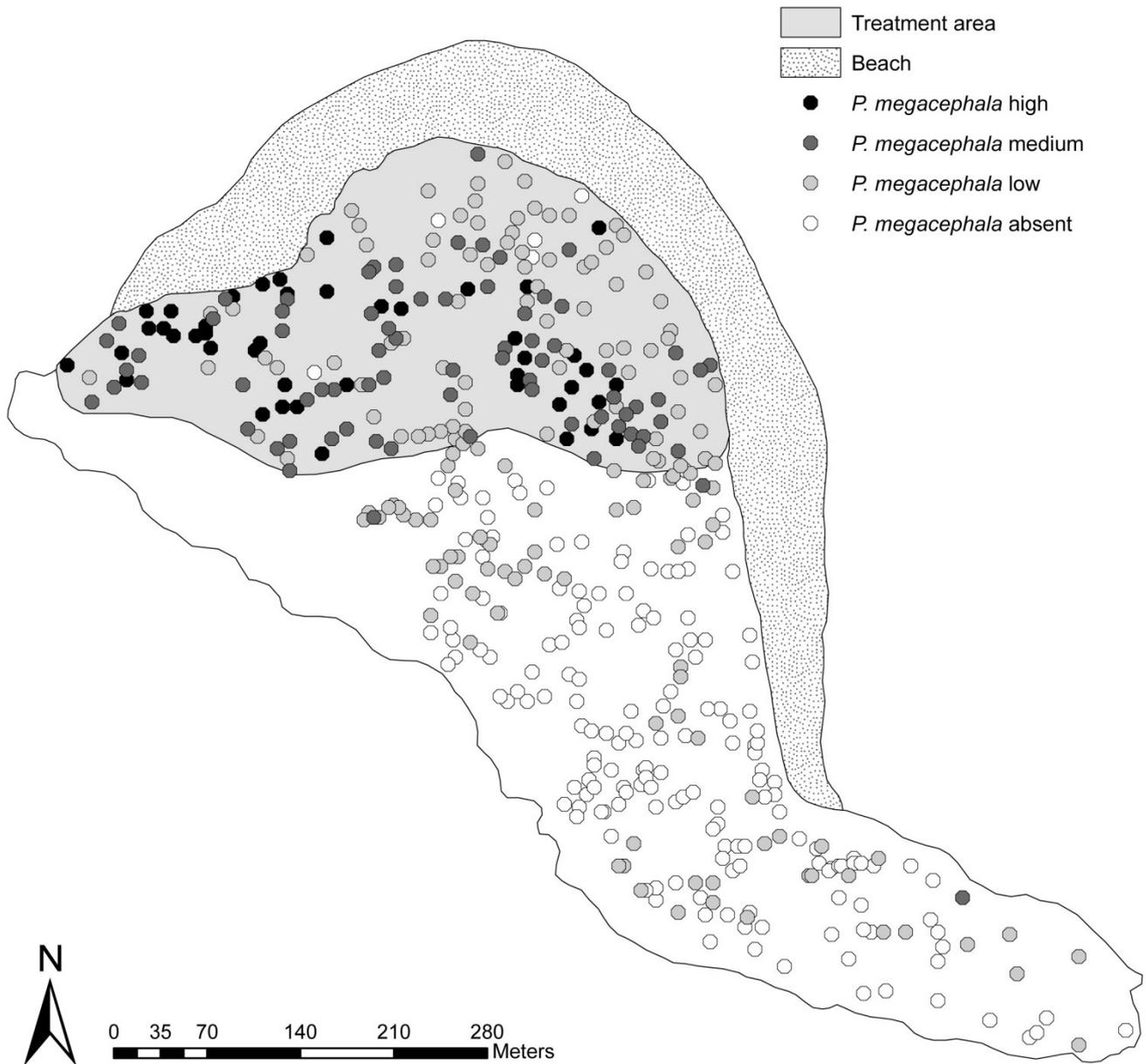
Figure 3.1. *Pheidole megacephala* distribution and activity levels on Cousine Island, Seychelles in June 2010 before ant bait application

Figure 3.2. Example of the bait stations used to deliver bait in the ant baiting program. The arrows indicate the 7 mm diameter holes through which ants gain access to the bait.
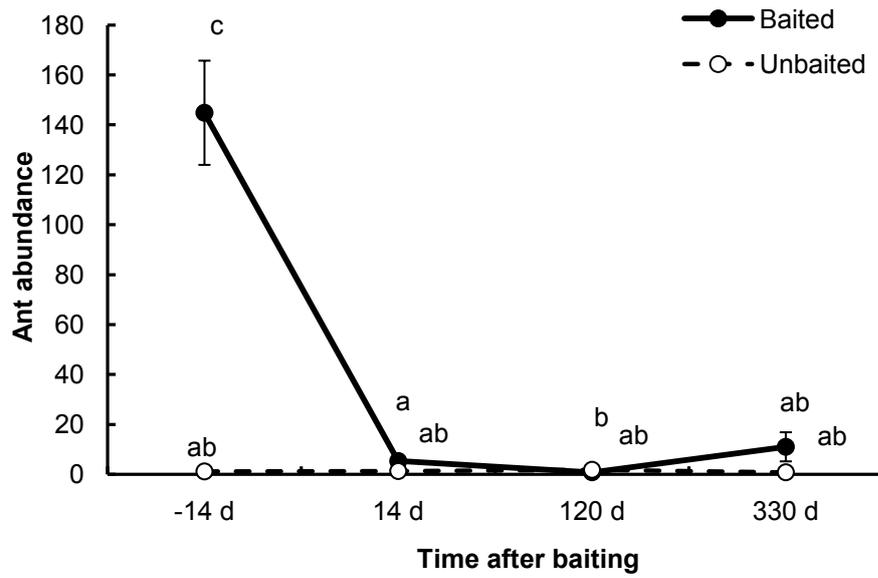
Figure 3.3. Mean *Ph. megacephala* abundance (± S.E.) in baited and unbaited plots before and after baiting. Means with letters in common are not significantly different at P < 0.05.
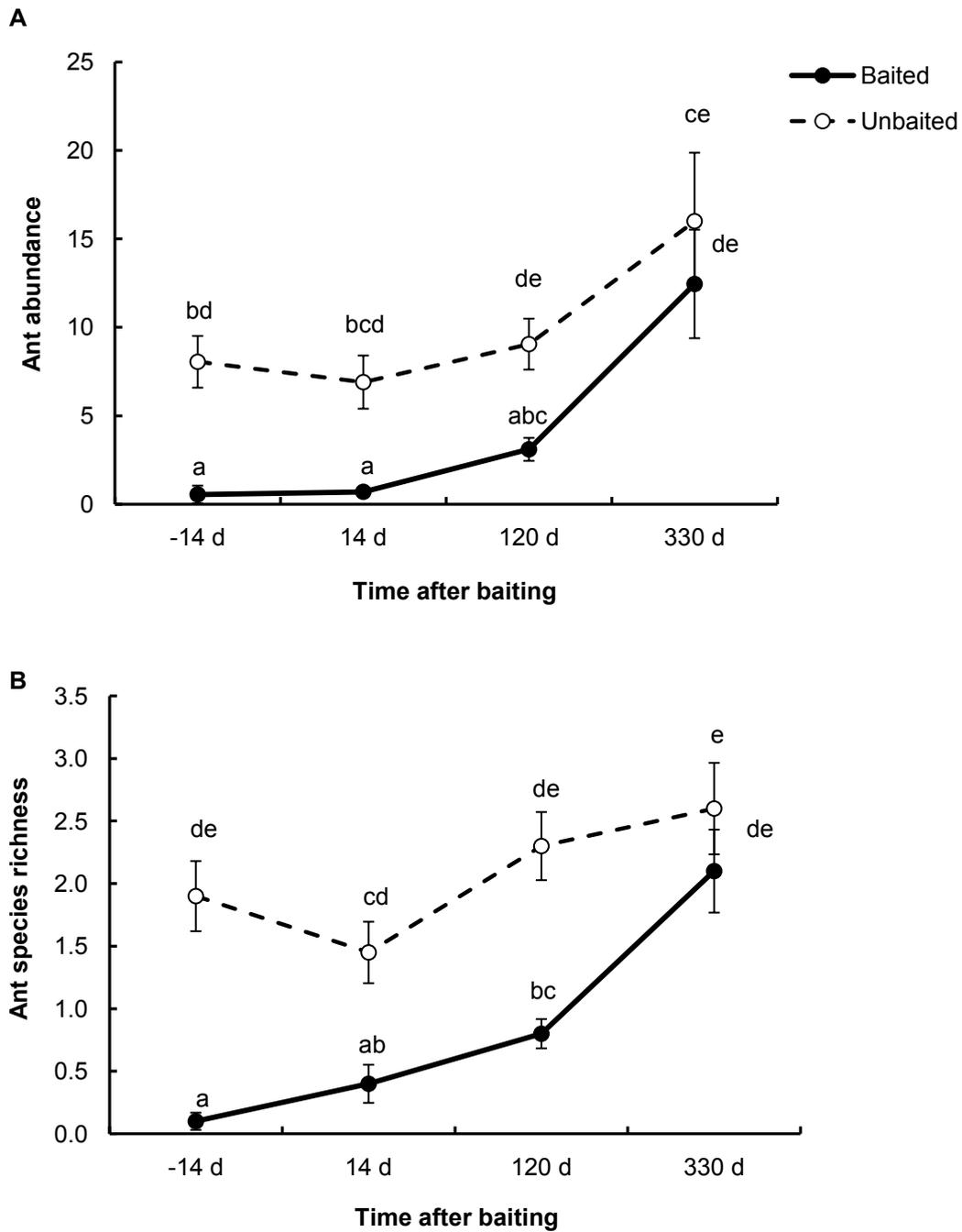
Figure 3.4. Mean ant a) abundance (± S.E.) and b) species richness (± S.E.) in baited and unbaited plots before and after baiting (excluding *P. megacephala*). Means with letters in common are not significantly different at P < 0.05.
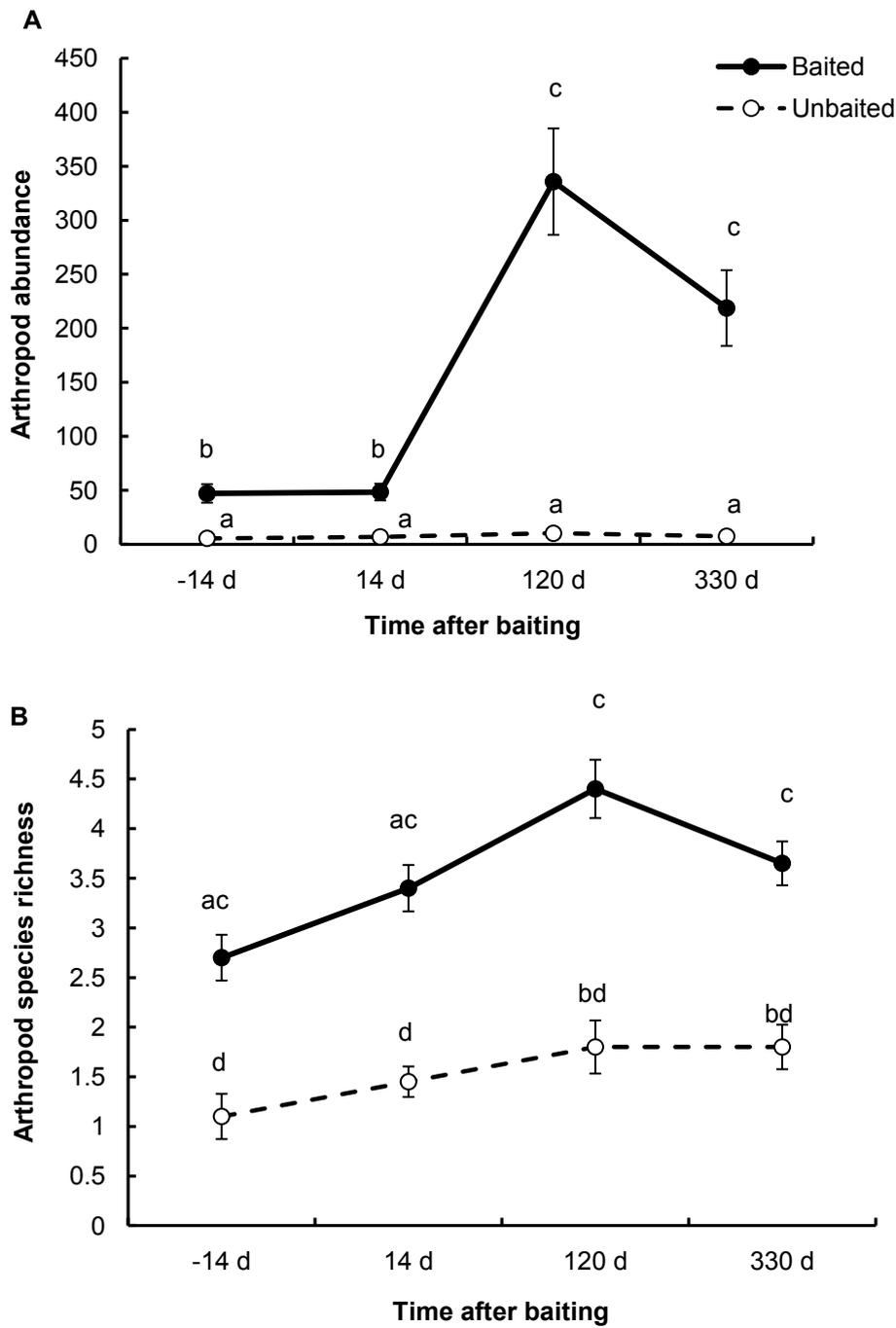
Figure 3.5. Mean non-ant arthropod a) abundance (± S.E.) and b) species richness (± S.E.) in baited and unbaited plots before and after baiting. Means with letters in common are not significantly different at P < 0.05.
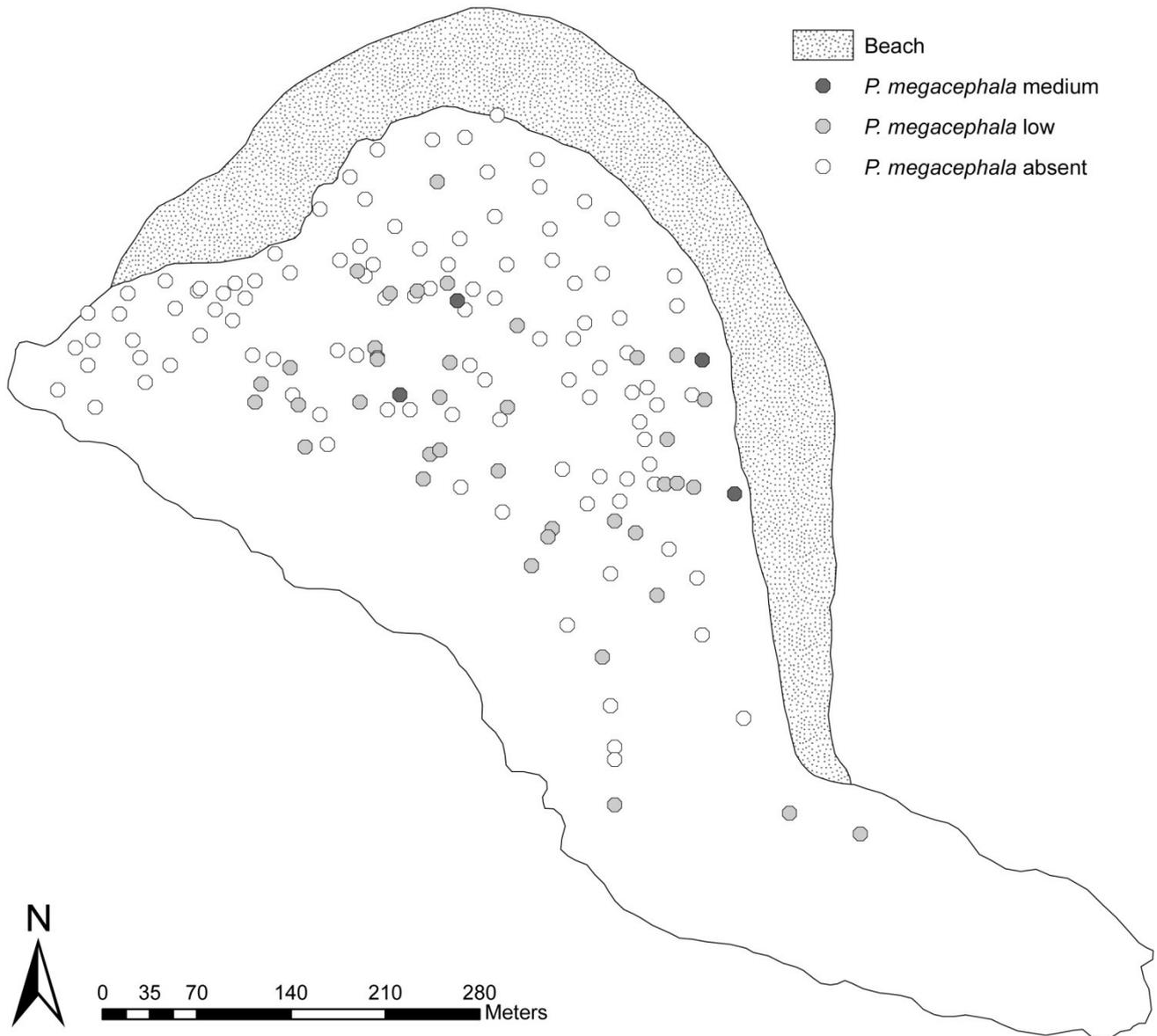
Figure 3.6. *Pheidole megacephala* distribution and activity levels on Cousine Island, Seychelles in October 2010 four months (120 d) after ant bait application.
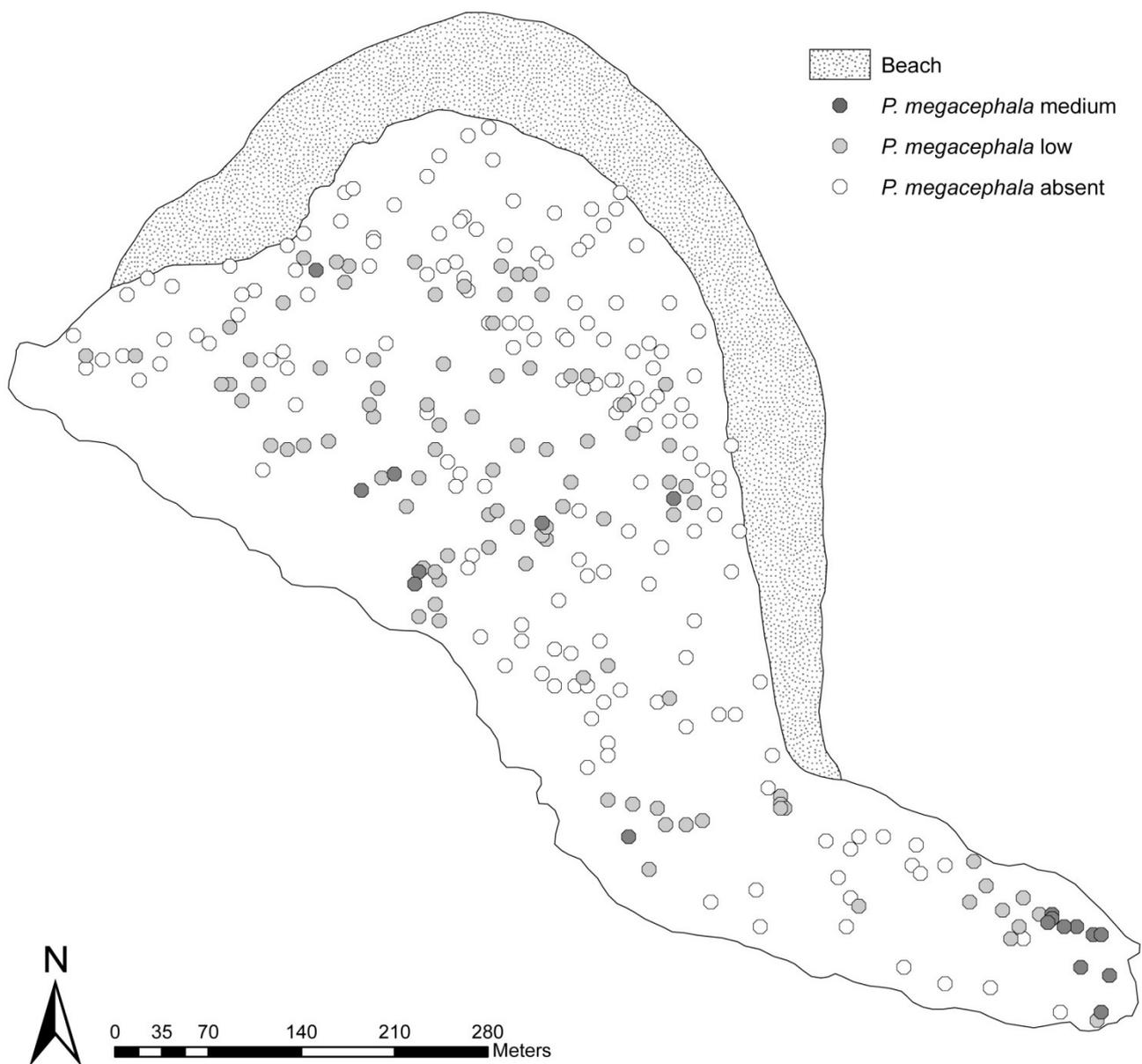
Figure 3.7. *Pheidole megacephala* distribution and activity levels on Cousine Island, Seychelles in May 2011 eleven months (330 d) after ant bait application.

4. Strategic management of an invasive ant-scale mutualism enables recovery of a threatened tropical tree species

Abstract

Mutualisms between invasive ants and honeydew-producing insects can have widespread negative effects on natural ecosystems. This is becoming an increasingly serious problem worldwide, causing certain ecosystems to change radically. Management of these abundant and influential mutualistic species is essential if the host ecosystem is to recover to its former non-invaded status. This negative effect is particularly prevalent on some tropical islands, including Cousine Island, Seychelles. On this island, the invasive ant *Pheidole megacephala* has caused serious indirect damage to the threatened native *Pisonia grandis* trees via a mutualism with an invasive scale insect, *Pulvinaria urbicola*. I aimed to suppress the ant, thereby decoupling the mutualism and enabling recovery of the *Pisonia* trees. We treated all areas where ant pressure was high with selective formicidal bait, which was deployed in custom-made bait stations designed to avoid risk of treatment to endemic fauna. In the treated area, ant foraging activity was reduced by 93 percent and was followed by a 100 percent reduction in scale insect density. However, abundance of endemic herbivorous insects and herbivorous activity increased significantly after the decline in mutualistic species densities. Despite the native herbivore increase, there was considerable overall improvement in *Pisonia* shoot condition and an observed increase in foliage density. My results demonstrate the benefit of strategic management of highly mutualistic alien species to the native *Pisonia* trees. It also supports the idea that area-wide suppression is a feasible alternative to eradication for achieving positive conservation management at the level of the forest ecosystem.

Introduction

Trophobiotic interactions, where ants protect hemipteran scale insects in return for carbohydrate-rich honeydew, are common in arthropod communities and can have broad ecological effects (Styrsky & Eubanks 2007), often with important implications for conservation (Lach 2003). Although the outcomes of these mutualisms are variable and unpredictable (Rosumek *et al.* 2009), in many ecosystems it has resulted in outbreaks of scale insects (Ness & Bronstein 2004; Styrsky & Eubanks 2007), which can significantly affect host plant fitness through increased phloem consumption, sooty mold accumulation and transmission of phytopathogens (Delabie 2001). The effect of the association can be intensified considerably when invasive species are involved (Lach 2003). Key characteristics of invasive ants, such as their numerical and behavioural dominance in introduced habitats (Holway *et al.* 2002), and their ability to monopolize honeydew resources (Gibb & Cunningham 2009; Paris & Espadaler 2009) make them highly efficient mutualistic partners of scale insects. In addition, non-native scale insects can be extremely invasive, and they can have detrimental effects on native ecosystems where they become abundant and widespread (O'Dowd *et al.* 2003; Smith *et al.* 2004; Abbott & Green 2007).

Pisonia grandis ('*Pisonia*') forest is a threatened forest type which is currently under pressure from trophobiotic relationships between alien invertebrates (Smith *et al.* 2004; Handler *et al.* 2007). *Pisonia* trees on oceanic islands have been heavily damaged by outbreaks of the West Indian coccid *Pulvinaria urbicola* tended by invasive ants, including *Pheidole megacephala*, *Anoplolepis gracilipes* and *Tetramorium bicarinatum* (Hill *et al.* 2003; Handler *et al.* 2007; Greenslade 2010). *Pisonia* damage resulting from this ant-scale association has occurred on numerous islands throughout the Pacific and Indian Oceans (O'Neill *et al.* 1997; Hill *et al.* 2003; Smith *et al.* 2004; Handler *et al.* 2007; Gaigher *et al.* 2011). Consequences of the mutualism include tree death, leaf loss, leaf distortion and sooty

71

mold build-up (O'Neill *et al.* 1997; Handler *et al.* 2007; Gaigher *et al.* 2011) and in severe cases, it has resulted in the complete loss of large forested areas (Smith *et al.* 2004). This is significant, as *Pisonia* forest has a history of destruction throughout its declining native range (Walker 1991; Kay *et al.* 2003). Furthermore, the *Pisonia* tree is of great functional importance to the ecology of tropical islands in particular. In the Seychelles, this tree is used by many seabirds, including the Lesser noddy (*Anous tenuirostris*), White tern (*Gygis alba*) and Black noddy (*A. minitus*) for nesting habitat and as a source of nesting material (Walker 1991; Samways *et al.* 2010b). Endemic insectivorous birds such as the Seychelles warbler (*Acrocephalus sechellensis*) and the Seychelles fody (*Foudia sechellensis*) commonly forage in *Pisonia* canopies, as they support high densities of invertebrate prey (Bathe &Bathe 1982; Komdeur 1994). *Pisonia* trees also produce a peat-like humic soil which is rich in phosphate and uncommon on oceanic islands (Walker 1991).

Conservation of this species is essential to ecosystem functioning of these islands, but there have been few attempts to alleviate the pressure from the ant-scale mutualisms (but see Smith & Papacek 2001; Smith *et al.* 2001; Smith & Papacek 2002; Smith *et al.* 2004). Management plans designed specifically to limit the densities of these invasive species on islands are urgently needed (Handler *et al.* 2007; Lester 2008). However, there is a lack of information on the effects of such management attempts on the rest of the ecosystem, particularly on native host plant fitness (Styrsky & Eubanks 2007), making it difficult to predict the outcomes.

On Cousine Island, an island of conservation significance in the Seychelles (Samways *et al.* 2010a), *Pisonia* forest was heavily damaged by a *Ph. megacephala-Pu. urbicola* mutualism and required urgent management action, involving controlled baiting with a pesticide (Gaigher *et al.* 2012). A complete eradication attempt for these two alien insects was not considered to be feasible on the island, as this would have required intensive

pesticide use, with associated risks to endemic and already threatened taxa. In response, this study evaluates whether area-wide suppression of the ant population levels using a strategic and targeted baiting methodology is an adequate alternative approach for managing the effect of the species on the *Pisonia* trees. Specifically, I assess whether the suppression of the ant population levels results in a decline in the scale density, thereby decoupling the mutualism, with the aim of bringing both mutualists down to low and innocuous levels. As other herbivorous arthropods are generally known to interact closely with ant-scale mutualists (Rosumek *et al.* 2009), I also monitor how herbivores other than *Pu. urbicola* are affected by the management of the invasive mutualists. Finally, I monitor the overall effect of the treatment on the *Pisonia* tree condition and evaluate whether this management approach is sufficient to reverse the damage to the tree species.

Methods

Study site

Cousine Island is a 27 ha granitic island in the Seychelles archipelago at 4°20'41"S and 55°38'44"E. Vegetation on the island is mostly indigenous forest dominated by *P. grandis*, *Ficus reflexa*, *F. lutea, Euphorbia pyrifolia* and *Pandanus balfourii* (Samways *et al.* 2010b). The mean monthly temperature for the study period (May 2010-May 2011) was 27.5 ± 0.2°C, with a minimum of 22.2°C and a maximum of 33.2°C. Mean monthly rainfall was 92.3 ± 28.3 mm and mean humidity was 87.3 ± 1.1 percent relative humidity. *Ph. megacephala* occurred in very high densities in a continuous area of 8 ha on the north hill and northern plain (Fig. 1), and was closely associated with high densities of *Pu. urbicola* in these areas (Gaigher *et al.* 2011). The ants occurred in low densities throughout most of the rest of the island, but with low scale densities and no noticeable scale damage to the trees.

Treatment

The 8-ha area of high ant density was treated with a single application of the commercial hydramethylnon-based formicidal bait, Siege® in June/July 2010, the middle of the dry season (Gaigher *et al.* 2012). Bait was deployed in plastic bait stations (Grout 2008) that were 200 mm long with 15 mm internal diameter and two 7 mm holes drilled in the side. The holes allowed ant access to the bait, while reducing the opportunity for bait uptake by non-target organisms of conservation significance, such as the Seychelles giant millipede (*Sechelleptus seychellarum*), skinks (*Mabuya seychellensis* and *M. wrightii*) and some endemic birds, especially the Seychelles magpie robin (*Copsychus sechellarum*), the Seychelles fody (*Foudia sechellarum*), and the Seychelles warbler (*Acrocephalus sechellensis*). Each bait station held 10 g of bait. Bait stations were placed at tree bases where ant nest holes were concentrated. Station placement was adapted to ant density in the field, but was applied at an average dosage of 4 kg per ha. Spent bait stations were collected after one week.

Data collection

Forty 10 m x 10 m permanent monitoring plots were selected on the island. 20 plots were within the 8-ha baited area and 20 were outside the baited area. As the entire area of high mutualist densities was treated in response to management requirements, the treated and control plots differed in mutualist species abundance and forest condition at the start of the study. However, as we were interested in the relative response to treatment over time and not the difference between baited and unbaited plots per se, this design allowed us to detect variation unrelated to baiting in the system.

Within each plot, estimates of ant foraging activity, scale insect density, other herbivore abundance and *Pisonia* condition were made 2 weeks before baiting, 2 weeks after baiting, 4 months after baiting, and 11 months after baiting. An additional herbivore survey

was conducted 1 month after baiting, as this group was expected to respond strongly in the short period following ant suppression.

Ant foraging activity was defined as the number of ants moving in one direction across a 4 cm-wide section in the middle of a trunk trail for 30 sec. An average of three estimates per plot at each time was used in analyses. *Pisonia* trees of > 20 cm diameter at breast height were used in surveys and all activity surveys were conducted between 0630 h and 1000 h.

For scale insect density estimates, we estimated the percentage cover of mature female individuals on the midrib and primary veins on the ventral surface of *Pisonia* leaves. For each plot, three random shoots were selected and estimates were made on three leaves per shoot. To standardize leaf selection, the three leaves were always the smallest leaves of three fully developed leaf pairs starting from the apical point.

Herbivores other than *Pu. urbicola* were monitored at each survey time using one 8 cm × 20 cm yellow sticky trap per plot hung in the lower canopy to sample mobile species. Traps were collected after three days. For less mobile herbivores, the lower canopies of *Pisonia* trees were inspected for 5 minutes per plot per survey, and all herbivores were recorded. Herbivores that could not be identified in the field were collected with an aspirator and identified in the laboratory. Data from yellow sticky traps and direct surveys were combined.

Pisonia condition was recorded by assigning subjective condition scores to five random shoots per plot at each survey. Previous observations indicated that high feeding by scale insects resulted in shoot distortion and dieback (Gaigher *et al.* 2011). Based on the level of distortion and dieback, scores of 0-4 were assigned to each shoot as follows: 0 = all leaves distorted and severe shoot dieback, 1 = ~75 percent of leaves distorted, 2 = ~50 percent of

leaves distorted, 3 = ~25 percent of leaves with slight distortion, 4 = leaves are undamaged and smooth with no distortion. Early *Pisonia* leaf size was also estimated during each survey, as early leaves on highly infested trees were underdeveloped (R. Gaigher pers. obs.). Length and width of three leaves on each of five random shoots per plot were measured. To standardize leaf selection, the three leaves were always the smallest leaf per pair of three fully developed leaf pairs starting from the apical point. Means of all leaf size measurements per plot were used in analyses. Damage to *Pisonia* caused by other herbivores was assessed at each survey. The total percentage leaf surface-area damaged by herbivory was estimated for each of five shoots per plot to obtain mean percentage damage per plot.

Statistical analyses

All data sets were analyzed in SPSS 19 (SPSS Inc. 2010) using Generalized Estimating Equations that extend the generalized linear model algorithm to account for correlated observations, in this case the repeated measurements per plot (Liang & Zeger 1986). "Plot" was specified as the subject variable in the model, and "time" and "treatment" as within-subject variables, with the important term in the analysis being the "time by treatment" interaction, which indicates whether there is change over time as a result of treatment. This analysis examines the relative change in baited and unbaited areas, and thus accounts for external ecological influences on response variables that are unrelated to baiting. An identity link function was used for normally distributed leaf size data. A log link function was used for Poisson distributed ant activity, scale density, herbivore abundance and herbivory data, and a cumulative logit link function was used for multinomially distributed shoot condition data (McCullagh & Nelder 1989). Bonferroni corrected post-hoc multiple comparisons were performed to detect pairwise differences in the normally distributed data set and bootstrap post-hoc multiple comparisons were performed in data sets where the response was not normally distributed. All graphs are presented using raw data.

76

Results

Baiting had a significant effect on *Ph. megacephala* foraging activity on *Pisonia* trees (Wald Chi-square = 156.1, P < 0.0001; Fig. 4.1a; Table 1). Mean ant activity declined from 59 ± 5 ants per 4 cm per 30 sec (with a maximum estimate of 90 ants per 4 cm per 30 sec) to zero within two weeks and remained below 0.5 ± 0.3 ants per 4 cm per 30 sec for the duration of the 11-month monitoring period. Ant foraging activity on *Pisonia* in unbaited plots remained at a level below 5 ants per 4 cm per 30 sec for the entire study period (Fig. 4.1a).

Pu. urbicola density was significantly influenced by baiting (Wald Chi-square = 20.9, P < 0.0001; Fig. 4.1b; Table 1). Two weeks after baiting, it was not significantly different from pre-treatment levels, but declined from a mean of 21.3 ± 3.8 percentage cover in the first post-treatment survey (with a maximum estimate of 90% cover) to zero after four months. Scale density remained at negligible levels in unbaited plots (Fig. 4.1b).

Baiting significantly influenced other herbivore abundance in baited plots over time relative to unbaited plots (Wald Chi-square = 29.9, P < 0.0001; Fig. 4.1c; Table 1). Herbivore abundance in baited plots increased significantly from a mean of 6 ± 1 individuals per plot before baiting to 44 ± 6 individuals 11 months after baiting. Herbivores that increased after baiting included Hemiptera, Lepidoptera and Orthoptera, but the greatest increase was observed for two species, *Osaka relata* (Hemiptera: Fulgoridae) and *Epicroesa* sp. (Lepidoptera: Heliodinidae), which represented 84 percent of the total number of individuals over the entire study period. There was no significant change in herbivore abundance in unbaited plots (Fig. 4.1c).

Baiting had a significant effect on *Pisonia* shoot condition over time (Wald Chi-square = 15.5, P < 0.005; Fig. 4.2a; Table 1), but not on early leaf size (Wald Chi-square = 2.4, P = 0.49; Fig. 4.2b; Table 1). *Pisonia* shoot condition improved in baited plots relative to

unbaited plots, from a mean score of 3.0 ± 0.1 before baiting to 3.6 ± 0.3 eleven months after baiting, whereas unbaited plots were not significantly different from pre-treatment levels at the end of the study period (Fig. 4.2a). Herbivore damage on *Pisonia* leaves was significantly influenced by baiting (Wald Chi-square = 35.1, P < 0.0001; Fig. 4.2c; Table 1). In baited plots, percentage herbivory increased from a mean of 1.6 ± 0.5 percent before baiting to 5.0 ± 0.5 percent 11 months after baiting. Percentage herbivory in unbaited plots declined from a mean of 8.8 ± 1.0 percent before baiting to 4.8 ± 0.6 percent 11 months after baiting (Fig. 4.2c).

Photographs comparing an area of high mutualist densities before baiting in May 2010 and 12 months after baiting in May 2011 (Fig. 4.3a-b), illustrate the precipitous decline in sooty mold and increase in foliage density on *Pisonia* after baiting which was evident throughout the treated area (R. Gaigher & M.J. Samways pers. obs.). Figures 4.4a-b compare another plot in the treated area before baiting in May 2010, early in the dry season, and four months after baiting in October 2010, at the end of the dry season, indicating substantial recovery of *Pisonia* shoots after baiting, despite dry conditions.

Discussion

Effect of ant suppression on the mutualism

The outcome of this strategic management approach using bait stations and a highly specific bait was immensely successful. The ant population crashed, and so did that of the mutualist scale insect. In turn, this led to recovery of *Pisonia* trees in the baited area, of which the majority were in poor condition before baiting. The significant decline in the scale population following ant suppression shows that high scale densities cannot be maintained without similarly high densities of its mutualistic ant partner, and that area-wide suppression of the ant was sufficient to decouple the mutualism. These results are consistent with those of a

study on Christmas Island, where high densities of alien scale insects were reduced to negligible levels following *A. gracilipes* ant control through baiting (Abbott & Green 2007).

Neither of the mutualists here recovered during the 11-month post-treatment monitoring period. Low levels of the ant still remained throughout the treated area, but at these low levels they were not associated with intensive scale tending (Gaigher *et al.* 2011). Furthermore, no scale insects were recorded during the 4-month and 11-month surveys. The lack of this highly abundant source of carbohydrate-rich honeydew in the system after treatment is likely to impede ant resurgence. Access to hemipteran honeydew contributes significantly to the ecological dominance of invasive ants, as it fuels ant aggression and activity, enabling greater resource discovery and defense, and increased colony performance (Davidson 1998; Grover *et al.* 2007; Helms & Vinson 2008). The removal of this important food source therefore greatly reduces the ant's competitive advantage. However, it will be essential to continue to monitor the mutualists, so as to be alert to any resurgence in their populations.

Effect of mutualist management on host plant condition

High levels of the alien mutualists were closely associated with leaf distortion and shoot dieback of *Pisonia* trees (Gaigher *et al.* 2011). In other ecosystems, *P. urbicola*-invasive ant mutualisms were also related to leaf loss, reduced leaf size and death of *Pisonia* (Hill *et al.* 2003; Handler *et al.* 2007). We therefore expected leaf size and shoot condition to respond positively to management of the mutualism. Leaf size was unaffected by baiting, but shoot condition showed a consistent improvement in baited areas. In addition, there was a substantial improvement in overall *Pisonia* tree condition, evident in the observed decline in sooty mold and increase in foliage density. The positive effect of our management program on *Pisonia* condition is consistent with results from the *Pu. urbicola* scale management

program in the Coringa-Herald National Nature Reserve, Australia, where outbreaks were managed through a combination of biological control and invasive ant suppression. This resulted in the prevention of further loss of *Pisonia* forest (Smith *et al.* 2004).

Other herbivores in the ecosystem can also influence host plant fitness, and the monitoring of their response to the decline in mutualist densities enables a more complete assessment of management outcomes. Hemipteran-tending ants can reduce abundance and diversity of unattended herbivorous arthropods on plants through predation and displacement (Kaplan & Eubanks 2005; Styrsky & Eubanks 2007; Rosumek *et al.* 2009). Ant removal may thus lead to increased population levels of other herbivores, with a corresponding increase in herbivore damage (e.g. Floate & Whitham 1994; Wimp & Whitham 2001). While herbivore abundance and herbivory increased after ant suppression on our island, overall condition of *Pisonia* improved despite this increase. The net effect of removal of strong mutualists on host plant condition depends upon the densities and pest status of the tended hemipterans relative to other herbivores in the system (Lach 2003; Kaplan & Eubanks 2005). Here, the herbivores that responded most to ant suppression, *O. relata* and *Epicroesa* sp., are both Seychelles endemics (Gerlach & Matyot 2006; Holzinger *et al.* 2008) and their increase was moderate compared to pre-treatment scale densities. Their effect on *Pisonia* was therefore far less than that of the alien scale herbivore, while the increase in the population levels of these island endemics is an added benefit for conservation.

Management implications

Pisonia forests are threatened in many regions by similar associations as the one studied here, and it is encouraging that there are effective options for managing this threat to the forest. The main objective here was to reduce the stress imposed on *Pisonia* trees by the mutualism, and we demonstrate that this can be achieved through targeted suppression of one of the

mutualists. This supports the idea that complete eradication may not be necessary to achieve land management goals (Lach 2003). Area-wide suppression, with the aim of reducing the long-term negative effect of the invasive species may be a suitable alternative when eradication is not feasible or too risky (Myers *et al.* 2000; Hulme 2006).

This method may be applicable to other environments with similar challenges. However, there are some limitations associated with the study design that need to be considered when making inferences based on these results. As is common in studies of biological invasions (Krushelnycky & Gillespie 2008; Hoffmann 2010), the invasion itself was not replicated and samples are therefore not from independent treatments (Hulbert 1984). I aimed to account for the lack of independence by sampling throughout the greatest possible extent of the invaded area and by having the maximum possible distance between sampling plots (> 10 m). Results from this study were clear and dramatic, but as the treatment area here only represents the range of local conditions, the response to baiting may differ in other environments.

Furthermore, the outcomes of the management program may be highly dependent on ecological context. Results from this study and previous work on the island (Gaigher *et al.* 2012) indicate that various native and naturalized exotic species, including trees, canopy herbivores, ants and epigaeic arthropods, benefited from the management program. However, the broader effects of mutualist suppression in other systems may vary depending on factors such as the strength of the mutualism, the presence and diet breadth of natural enemies, the influence of other herbivores on host plant fitness and the occurrence of potentially worse invasive species that may replace the managed alien species. An understanding of the role and interactions of the managed species within the ecosystem is essential for effective application of such management programs (Zavaleta *et al.* 2001).

References

Abbott, K. L. & Green, P. T. 2007. Collapse of an ant-scale mutualism in a rainforest on Christmas Island. *Oikos* 116, 1238-1246.

Bathe, H. & Bathe, G. 1982. Feeding studies of three endemic land birds, *Acrocephalus sechellensis* (Seychelles Brush Warbler), *Foudia sechellarum* (Seychelles Fody) and *Nectarinia dussumieri* (Seychelles Sunbird) on Cousin Island, Seychelles, with implications for their conservation through vegetation management. International Council for Bird Preservation, Cambridge, UK.

Davidson, D. W. 1998. Resource discovery versus resource domination in ants: a functional mechanism for breaking the trade-off. *Ecological Entomology* 23, 484-490.

Delabie, J. H. C. 2001. Trophobiosis between Formicidae and Hemiptera (Sternorrhyncha and Auchenorrhyncha): an overview. *Neotropical Entomology* 30, 501-516.

Floate, K. D. & Whitham, T. G. 1994. Aphid-ant interaction reduces chrysomelid herbivory in a cottonwood hybrid zone. *Oecologia* 97, 215-221.

Gaigher, R., Samways, M. J., Henwood, J. & Jolliffe, K. 2011. Impact of a mutualism between an invasive ant and honeydew-producing insects on a functionally important tree on a tropical island. *Biological Invasions* 13, 1717-1721.

Gaigher, R., Samways, M. J., Jolliffe, K. G. & Jolliffe, S. 2012 Precision control of an invasive ant on an ecologically sensitive tropical island: a principle with wide applicability. *Ecological Applications* 22, 1405–1412.

Gibb, H. & Cunningham, S. A. 2009. Does the availability of arboreal honeydew determine the prevalence of ecologically dominant ants in restored habitats? *Insectes Sociaux.* 56, 405-412.

Greenslade, P. 2010. Did alien ants initiate a population explosion of a coccoid plant pest on an islet in the Coral Sea? *Journal of Insect Conservation* 14, 419-421.

Grout, T. 2008. An inexpensive bait station. *South African Fruit Journal*. Oct/Nov: 15.

Grover, C. D., Kay, A. D., Monson, J. A., Marsh, T. C. & Holway, D. A. 2007. Linking nutrition and behavioural dominance: carbohydrate scarcity limits aggression and activity in Argentine ants. *Proceedings of the Royal Society B-Series* 274, 2951-2957.

Handler, A. T., Gruner, D. S., Haines, W. P., Lange, M. W. & Kaneshiro, K. Y. 2007. Arthropod surveys on Palmyra Atoll, Line Islands, and insights into the decline of the native tree *Pisonia grandis* (Nyctaginaceae). *Pacific Science* 61, 485-502.

Helms, K. R. & Vinson, S. B. 2008. Plant resources and colony growth in an invasive ant: the importance of honeydew-producing Hemiptera in carbohydrate transfer across trophic levels. Environmental Entomology 37, 487-493.

Hill, M., Holm, K., Vel, T., Shah, N. J. & Matyot, P. 2003. Impact of the introduced yellow crazy ant *Anoplolepis gracilipes* on Bird Island, Seychelles. *Biodiversity and Conservation* 12, 1969-1984.

Hoffmann, B.D. 2010. Ecological restoration following the local eradication of an invasive ant in northern Australia. *Biological Invasions* 12, 959-969.

Holway, D. A., Lach, L., Suarez, A. V., Tsutsui, N. D. & Case, T. J. 2002. The causes and consequences of ant invasions. *Annual Review of Ecology and Systematics* 33, 181-233.

Hulbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. *Ecological Monographs* 54, 187-211.

Hulme, P. E. 2006. Beyond control: wider implications for the management of biological invasions. *Journal of Applied Ecology* 43, 835-847.

Kaplan, I. & Eubanks, M. D. 2005. Aphids alter the community-wide impact of fire ants. *Ecology* 86, 1640-1649.

Kay, A., Olds, J. & Elder, R. 2003. The impact and distribution of the soft scale *Pulvinaria urbicola* in the *Pisonia grandis* forests of the Capricornia Cays national parks 1993–2002. Report on the scale insect and vegetation monitoring program on Tryon Island and the scale insect surveys on other Capricornia cays.

Komdeur, J. 1994. Conserving the Seychelles Warbler *Acrocephalus sechellensis* by translocation from Cousin Island to the islands of Aride and Cousine. *Biological Conservation* 67, 143-152.

Krushelnycky, P.D. & Gillespie, R.G. 2008. Compositional and functional stability of arthropod communities in the face of ant invasions. *Ecological Applications* 18, 1547-1562.

Lach, L. 2003. Invasive ants: unwanted partners in ant-plant interactions? *Annals of the Missouri Botanical Gardens* 90, 91-108.

Lester, P. J. 2008. Integrated pest management: an under-utilized tool for conservation and the management of invasive ants and their mutualistic Hemiptera in the Pacific. *Pacific Conservation Biology* 14. 246-247.

Liang, K. & Zeger, S.L. 1986. Longitudinal data analysis using generalized linear models. *Biometrika* 73, 13-22.

McCullagh, P. & Nelder, J.A. 1989. *Generalized linear models.* Chapman and Hall, London, UK.

Myers, J. H., Simberloff, D., Kuris, A. M. & Carey, J. R. 2000. Eradication revisited: dealing with exotic species. *Trends in Ecology and Evolution* 15, 316-320.

Ness, J. H. & Bronstein, J. L. 2004. The effects of invasive ants on prospective ant mutualists. *Biological Invasions* 6, 445-461.

O'Dowd, D. J., Green, P. T. & Lake, P. S. 2003. Invasional meltdown on an oceanic island. *Ecology Letters* 6, 812-817.

O'Neill, P., Olds, J. & Elder, R. 1997. Report on investigations of *Pulvinaria urbicola* infestations of *Pisonia grandis* forests, and masked and brown booby populations in the Coral Sea, 25 Nov-18 Dec 1997.

Paris, C. I. & Espadaler, X. 2009. Honeydew collection by the invasive garden ant *Lasius neglectus* versus the native ant *L. grandis. Arthropod-Plant Interactions* 3, 75-85.

Rosumek, F. B., Silveira, F. A. O., De S Neves, F., De U Barbosa, N. P., Diniz, L., Oki, Y., Pezzini, F., Fernandes, G. W. & Cornelissen, T. 2009. Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. *Oecologia* 160, 537-549.

Samways, M. J., Hitchins, P. M., Bourquin, O. & Henwood, J. 2010a. Restoration of a tropical island: Cousine Island, Seychelles. *Biodiversity and Conservation* 19, 425-434.

Samways, M. J., Hitchins, P. M., Bourquin, O. & Henwood, J. 2010b. *Tropical island recovery: Cousine Island, Seychelles*. Wiley-Blackwell, Oxford.

Smith, D. & Papacek, D. 2001. Report on visit to the Coringa - Herald Nature Reserve 30 July - 10 August, 2001 with regard to the releasing of parasitoids and ladybird predators of the pest scale *Pulvinaria urbicola* on *Pisonia grandis*. Report to Environment Australia.

Smith, D. & Papacek, D. 2002. Report on visit to the Coringa – Herald Nature Reserve and SE Magdelaine Cay, 15-22 March, 2002 with regard to the releasing of parasitoids and ladybird predators of the pest scale *Pulvinaria urbicola* on *Pisonia grandis* and the assessment of biocontrol options for hawkmoths. Report to Environment Australia.

Smith, D., Papacek, D., Hallam, M. & Smith, J. 2004. Biological control of *Pulvinaria urbicola* (Cockerell) (Homoptera:Coccidae) in a *Pisonia grandis* forest on North East Herald Cay in the Coral Sea. *General and Applied Entomology* 33, 61-68.

Smith, D., Papacek, D. & Smith, J. 2001. Report on visit to the Coringa - Herald Nature Reserve 17-21 December 2001 with regard to the releasing of parasitoids and ladybird predators of the pest scale *Pulvinaria urbicola* on *Pisonia grandis*. Report to Environment Australia.

SPSS Inc. 2010. *SPSS for Windows*, Release 19.0.0. SPSS Inc, Chicago, USA.

Styrsky, J. D. & Eubanks, M. D. 2007. Ecological consequences of interactions between ants and honeydew-producing insects. *Proceedings of the Royal Society B-Series* 274, 151-164.

Walker, T. A. 1991. *Pisonia* islands of the Great Barrier Reef part 1. The distribution, abundance and dispersal by seabirds of *Pisonia grandis*. *Atoll Research Bulletin* 350, 1-23.

Wimp, G. M. & Whitham, T. G. 2001. Biodiversity consequences of predation and host plant hybridization on an aphid-ant mutualism. *Ecology* 82, 440-452.

Zavaleta, E. S., Hobbs, R. J. & Mooney, H. A. 2001. Viewing invasive species removal in a whole-ecosystem context. *Trends in Ecology and Evolution* 16, 454-459.

Table 4.1. Effect of baiting on ant activity, herbivore abundance and host plant condition. Statistics derived from Generalized Estimating Equations.

Response variables	df	Wald Chi-square	*P*
1. Ant activity			
Ph. megacephala foraging activity on *P. grandis*			
Treatment	1	6.91	< 0.05
Time	3	107.5	< 0.0001
Time × Treatment	3	156.1	< 0.0001
2. Herbivore abundance			
Pu. urbicola percentage cover on *P. grandis*			
Treatment	1	16.1	< 0.0001
Time	3	38.9	< 0.0001
Time × Treatment	3	20.9	< 0.0001
Other herbivore abundance			
Treatment	1	13.7	< 0.0001
Time	4	129.9	< 0.0001
Time × Treatment	4	29.9	< 0.0001
3. Host plant condition			
Condition score of *P. grandis* shoots			
Treatment	1	0.04	0.84
Time	3	38.3	< 0.0001
Time × Treatment	3	15.5	<0.005
Early leaf size of *P. grandis*			
Treatment	1	2.3	0.13
Time	3	8.3	0.04
Time × Treatment	3	2.4	0.49
% Herbivore damage to *P. grandis*			
Treatment	1	33.1	< 0.0001
Time	3	12.7	< 0.05
Time × Treatment	3	35.1	< 0.0001
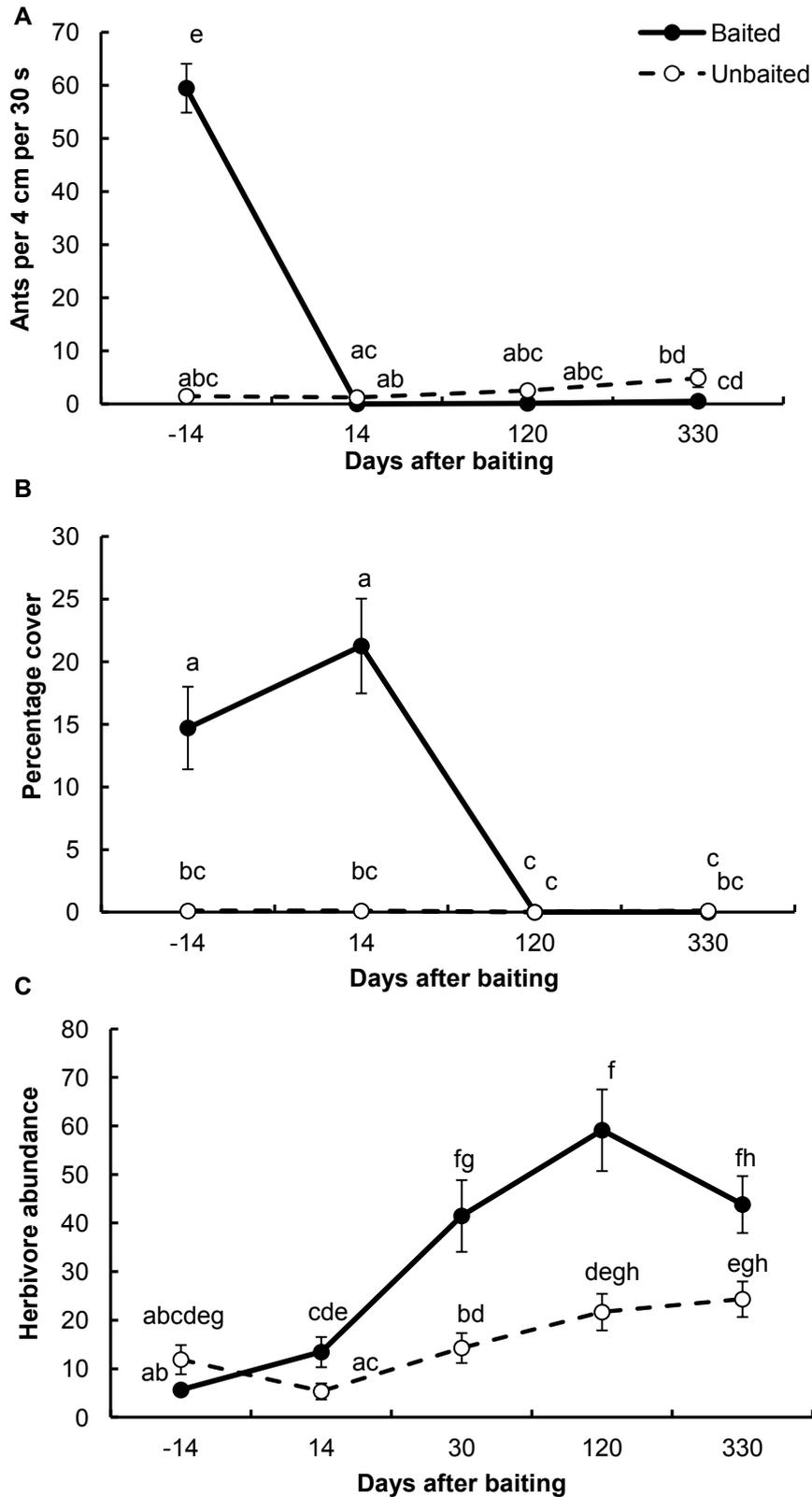
Figure 4.1. Mean a) *Pheidole megacephala* ant foraging activity, b) *Pulvinaria urbicola* scale percentage cover and c) herbivore abundance (excluding *P. urbicola*) ± S.E. on *Pisonia grandis* trees in baited and unbaited plots before and after baiting. Means with letters in common are not significantly different at *P* < 0.05.
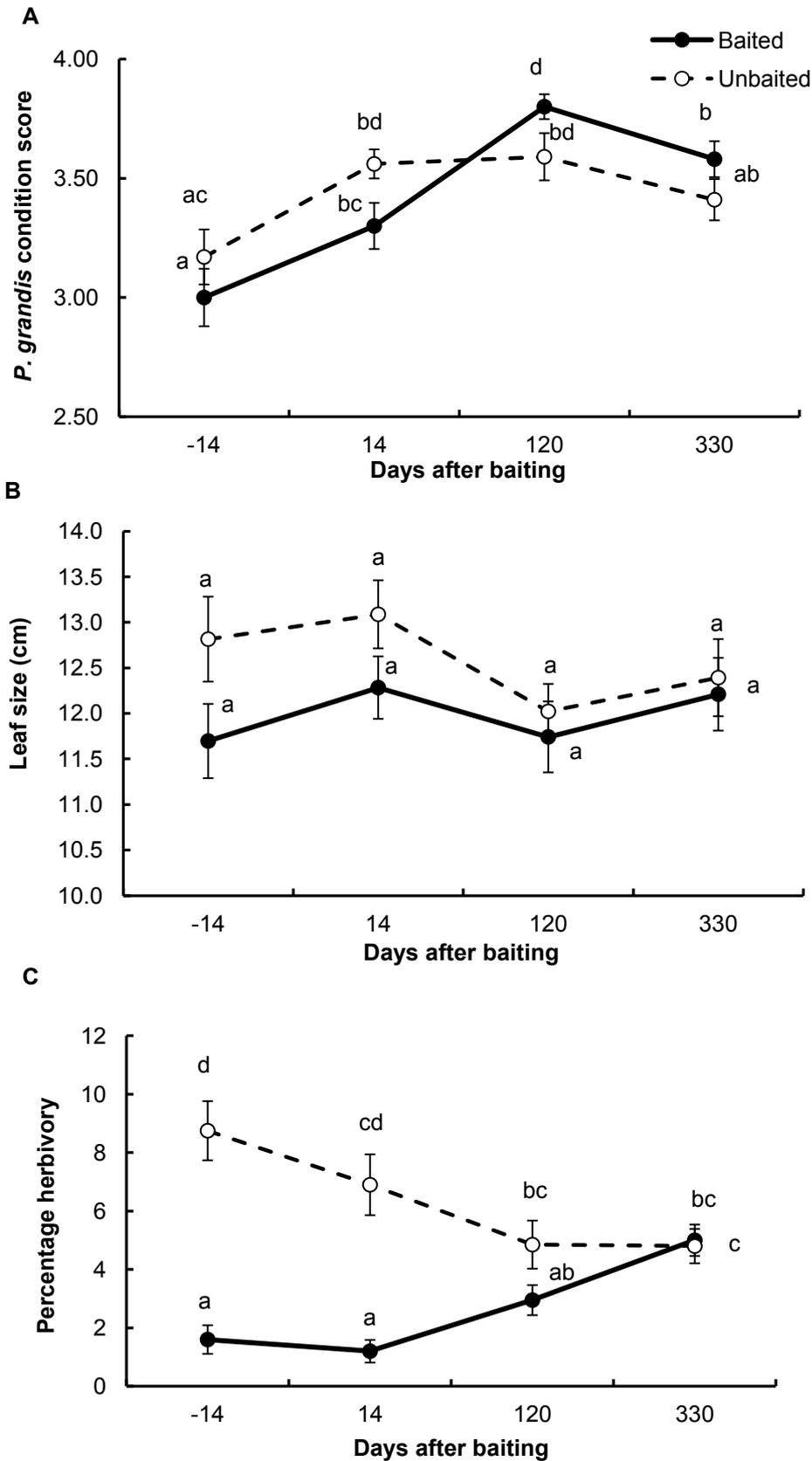
Figure 4.2. Mean *Pisonia grandis* a) shoot condition score ± S.E. and b) early leaf size ± S.E. and c) percentage herbivore damage ± S.E. in baited and unbaited plots before and after baiting. Means with letters in common are not significantly different at *P* < 0.05.

Figure 4.3. *Pisonia grandis* a) before baiting in May 2010 and b) eleven months (330 d) after baiting in May 2011 in an area of high *Pheidole megacephala* and *Pulvinaria urbicola* densities indicating a decrease in sooty mold and an increase in foliage density.
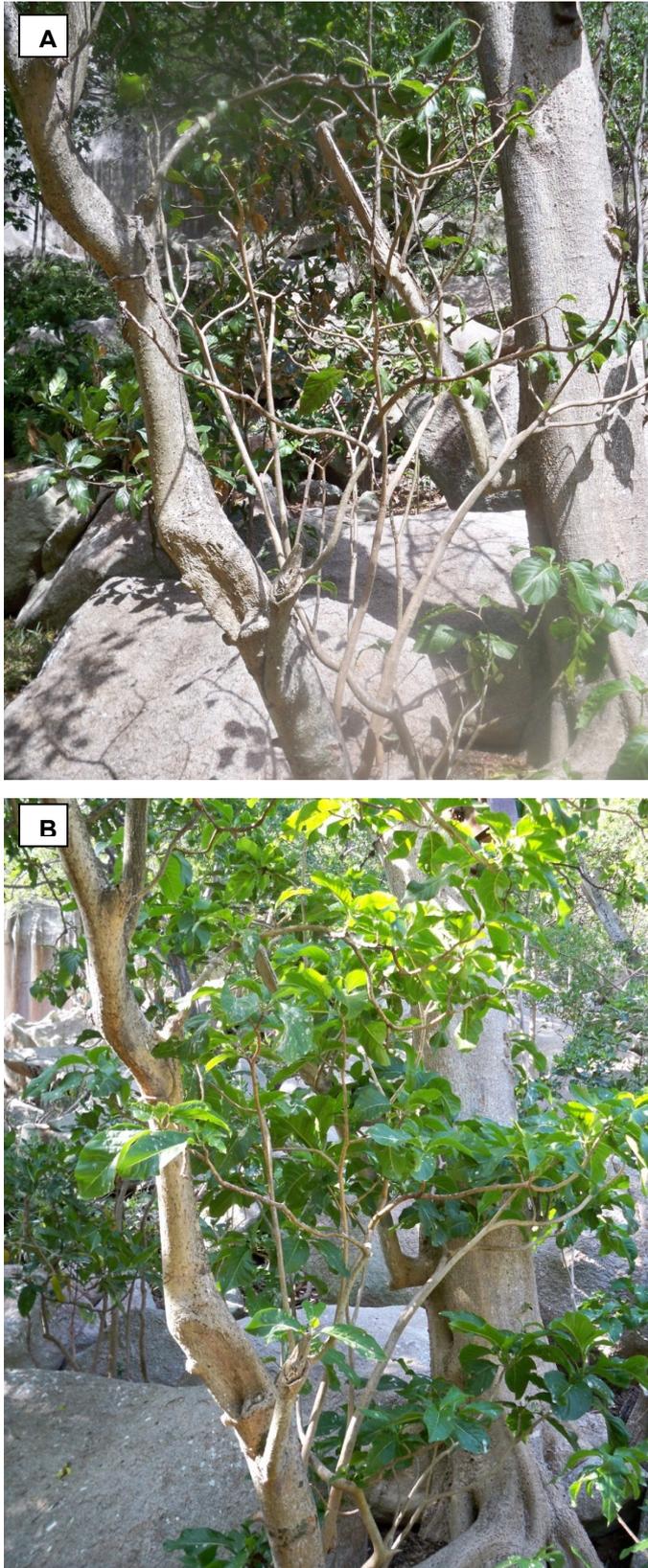
Figure 4.4. *Pisonia grandis* a) before baiting in May 2010 and b) four months (120 d) after baiting in October 2011 in an area of high *Pheidole megacephala* and *Pulvinaria urbicola* densities indicating the increase in foliage density.

5. Saving a tropical ecosystem from a destructive ant-scale mutualism with support from a diverse natural enemy complex

Abstract

Tending ants can disrupt the biological control of hemipteran pests by interfering with natural enemies, which can have far-reaching effects within arthropod assemblages. I examine here the interference of an invasive ant *Pheidole megacephala* with the pest regulation of an exotic hemipteran scale insect *Pulvinaria urbicola* in a natural tropical ecosystem. I assess the response of the overall natural enemy assemblage and various functional guilds to the controlled disruption of the mutualism through ant suppression. Natural enemy abundance and species richness increased significantly after ant suppression, with varying responses among the different functional guilds. Primary parasitoids were able to coexist with tending ants, but could not regulate high scale densities alone. A significant increase in predators of hemipterans was largely responsible for the collapse of the scale population. Guilds external to the mutualism were also affected, with primary parasitoids of various non-hemipteran taxa increasing at the end of the survey, which contributed significantly to the recovery of the assemblage to pre-invasion assemblage structure. My results highlight the pervasive effects of the tending of hemipterans by an invasive ant on a functionally important arthropod group, but also illustrate the potential to restore these natural enemies through management of the exotic mutualists.

Introduction

Ants and honeydew-producing hemipteran insects are commonly associated through trophobiotic mutualisms, where hemipterans provide ants with food in return for tending services and protection from natural enemies (Delabie 2001). Natural enemy exclusion, harassment and mortality by tending ants can interfere significantly with the biological control of hemipteran populations (Eubanks *et al.* 2002; Kaplan & Eubanks 2002; Mgocheki & Addison 2009) and can have important consequences for both plant protection and conservation (Styrsky & Eubanks 2007).

Research on the disruption of biocontrol by ants has typically focused on interactions between the ant, hemipteran pest and pertinent natural enemy species (Cardinale et al 2003; James *et al.* 1999; Letourneau & Andow 1999). However, most biological control systems involve diverse assemblages of natural enemies (Snyder & Ives 2003) and there is increasing evidence for the effect of the mutualism on the broader natural enemy community. Recent studies have highlighted the impact of honeydew-seeking ants on suites of beneficial arthropods (Eubanks *et al.* 2002; Kaplan & Eubanks 2002; James *et al.* 1999; Kaplan & Eubanks 2005) and have demonstrated the variable responses of different natural enemies to ant aggression (Daane *et al.* 2007; Völkl 1992; Völkl & Mackauer 1993). There has also been focus on interactions among natural enemies in relation to ant tending and its consequences for pest regulation (Kaneko 2003; Kaneko 2007; Liere & Perfecto 2008). These mutualisms are clearly enmeshed in complex, interconnected assemblages and the dynamics within these interaction webs can be variable, with important implications for pest management in both agricultural and natural systems.

Here I adopt a community approach to assess the pest regulation of an exotic scale insect, *Pulvinaria urbicola* (Homoptera: Coccidae), within a complex natural ecosystem. The study was conducted on Cousine Island, a small island in the Seychelles that has undergone

94

conservation restoration. The scale insect occurred in extremely high densities in parts of the native forest, facilitated greatly by its mutualism with the invasive ant *Pheidole megacephala,* and was responsible for damage to native trees throughout the high density area (Gaigher *et al.* 2011). Biological control of *Pu. urbicola* can be achieved by various natural enemy species including the parasitoid wasps *Coccophagus ceroplastae, Euryischomyia flavithorax* and *Metaphycus luteolus,* the coccinelid *Cryptolaemus montrouzieri* and scale pathogen *Verticillium lecanii* (Smith et al 2004). But, it is often released from natural enemies in its introduced range, and in association with aggressive, effective mutualist partners like *Ph. megacephala*, it can reach outbreak densities (Gaigher *et al.* 2011; Handler *et al.* 2007; O'Neill *et al.* 1997).

In response to concerns about the impact of the mutualism on the native forest, a management program was initiated consisting of a toxic ant baiting campaign (Gaigher *et al.* 2012) which resulted in disruption of the mutualism and a significant decline in scale insect density shortly after ant control (Gaigher & Samways 2012). This study documents the concurrent response of the natural enemy assemblage to the decoupling of the mutualism. The aim is to 1) assess the effect of ant interference with scale biocontrol by examining the mechanisms responsible for the scale decline after ant suppression, and 2) to investigate the interactions of the mutualists within the broader natural enemy assemblage.

Methods

Study design

Cousine Island is a 27 ha granitic island in the Seychelles archipelago at 4°20'41"S and 55°38'44"E. *Ph. megacephala* and its mutualistic soft scale *Pu. urbicola* occurred at low densities throughout most of the island, but at very high densities in a continuous 8 ha area in

95

the northern parts of the island. The ants were suppressed to insignificant levels in this area through toxic baiting in May 2010 (methods described in Gaigher *et al.* 2012), which resulted in decoupling of the mutualism and a significant decline in scale insect density (Gaigher & Samways 2012).

Forty permanent monitoring sites were selected on the island, 20 of which were within the 8-ha baited area and 20 were outside the baited area. At each site, estimates of natural enemy abundances were made two weeks before baiting, two weeks after baiting, one month after baiting, four months after baiting, and 11 months after baiting.

Natural enemies were monitored at each survey time using one 8 × 20 cm yellow sticky trap hung in the lower canopy at each monitoring site. Traps were collected after three days. For less mobile species, the lower tree canopies in a 10 × 10 m area were inspected for 5 min per site per survey, and all predators and parasitoids were recorded. Species that could not be identified in the field were collected with an aspirator and identified in the laboratory. Data from yellow sticky traps and direct surveys were combined.

To supplement our data on parasitoids, we collected parasitized scales opportunistically throughout the survey period. Scales were placed in rearing boxes (10 × 10 × 15 cm) and adult parasitoids were collected as soon as they emerged into the attached test tubes (50 ml, 2.5 cm diameter).

Specimens were identified to the lowest taxonomic level possible. Species that could be assigned with certainty to functional guilds based on their level of identification were grouped appropriately into primary parasitoids, secondary parasitoids and predators, and were further assigned to groups based on their hosts or prey (Table 5.1). A few parasitoids could be identified only to family level and were assigned to a separate primary or secondary parasitoid group, as their exact functional guild was uncertain. This group was excluded from

analyses assessing guild responses. Parasitoid specimens were deposited in the Iziko South African Museum, predatory beetles in the Stellenbosch University Entomological Museum and spiders in the South African Arachnid Collection.

Data analyses

To determine whether there was a significant response in 1) overall natural enemy abundance and species richness, and 2) abundance of the different functional guilds to the disruption of the mutualism, Generalized Estimating Equations (GEEs) were done in SPSS 19 (SPSS Inc. 2010). GEE's extend the generalized linear model algorithm to account for correlated repeated measurements (Liang & Zeger 1986). 'Plot' was specified as the subject variable in the model, and 'time' and 'treatment' as within-subject variables, with the important term in the analysis being the 'time by treatment' interaction, which indicates whether there is change over time as a result of treatment. This analysis examines the relative change in baited and unbaited areas, and thus accounts for external ecological influences on response variables that are unrelated to baiting. A Poisson distribution and log link function was specified for all models (McCullagh & Nelder 1989). Bootstrap pairwise comparisons were performed to account for non-normal response.

Non-metric multivariate analyses were done in Primer 5.2.9 (Clarke & Gorley 2001) so as to investigate the effect of mutualism disruption on the natural enemy assemblage structure. Data were pooled for each treatment (baited or unbaited) per time, and a similarity matrix was constructed using the Bray-Curtis similarity measure based on $\log(x+1)$ transformed abundance data. Patterns in natural enemy assemblages among groupings were then graphically represented using non-metric multidimensional scaling (nMDS) ordination plots (Clarke & Warwick 2001).

To test for significant differences in natural enemy assemblages among groupings, one-way analyses of similarities (ANOSIM) were performed, and similarity percentage

analyses (SIMPER) were performed to detect the species that contributed most to differences between groupings of interest. The ratio of the average dissimilarity among groupings (Dis) and the associated standard deviation (SD) indicates how consistently a species contributes to differences between groupings. Species with a high Dis/SD ratio are considered to be key discriminating species (Clarke & Warwick 2001) and therefore species with a ratio >1 were analysed further. Relative abundances for each of the discriminating species were displayed by superimposing bubble plots on the nMDS ordination plot to indicate the relative contribution of those species to ordination patterns.

Results

Forty-six natural enemy species in 40 genera and17 families were recorded during the survey (Table 5.1). Thirty-four of these species were parasitoid wasps and included 26 species of primary parasitoids, four species of secondary parasitoids and four primary or secondary parasitoids. 12 predator species were recorded. Within these groups, almost a third of all species parasitize or prey on hemipterans, whereas the others specialize on various non-hemipteran taxa or are generalist natural enemies (Table 5.1). An additional six species that occurred as singletons were recorded, but were excluded from analyses and further discussion to focus on responses of great biological significance. Parasitoid species that were also reared from scales included *Metaphycus* sp. 1, *Aprostocetus* sp. 1, *Anicetus* sp. 1, *Aphycus* sp. 1, *Cheiloneurus cyanonotus* and *Marietta leopardina* (Table 5.1). The first four species are primary scale parasitoids and the last two are secondary parasitoids.

There was a significant response in natural enemy abundance (Wald Chi-square = 11.97, P = 0.02) and species richness (Wald Chi-square = 46.52, P < 0.0001) to the disruption of the mutualism (Fig. 5.1, Table 5.2). In baited areas, overall abundance increased significantly after baiting and then decreased to pre-baiting levels at the end of the survey 11

months after baiting, with two peaks in abundance at two weeks and four months after baiting. Natural enemy species richness increased steadily to four months after baiting in baited areas and then declined to pre-baiting levels 11 months after baiting (Fig. 5.1). There was fluctuation in abundance and richness in unbaited areas, but much less pronounced than in baited areas, with both showing a maximum at four months after baiting (Fig. 5.1).

Primary parasitoid abundance showed a significant response to baiting (Wald Chi-square = 19.54, P = 0.001), including groups with hemipteran (Wald Chi-square = 55.12, P < 0.0001), and non-hemipteran hosts (Wald Chi-square = 38.13, P < 0.0001) (Fig. 5.2a-b, Table 5.3). Primary parasitoids with hemipteran hosts were highest pre-baiting and declined to low levels four months after baiting (Fig. 5.2a), whereas those with non-hemipteran hosts increased after baiting and showed a peak in abundance at four months after baiting (Fig. 5.2b). Overall predator abundance was significantly influenced by baiting (Wald Chi-square = 88.85, P < 0.001). Predators specializing on Hemiptera showed a significant response (Wald Chi-square = 38.62, P < 0.0001), but not generalist predators (Wald Chi-square = 5.66, P = 0.23) (Fig 5.2c-d, Table 5.3). Predators with hemipteran prey increased after baiting with maximum abundance one month after baiting, and declined to pre-baiting levels at the end of the survey (Fig. 5.2c). Generalist predator abundance fluctuated in both treatments (Fig. 5.2d). Response in secondary parasitoids was non-significant (Wald Chi-square = 4.05, P = 0.40), but abundance was significantly higher in baited areas one month after baiting (Fig. 5.2e).

Natural enemy assemblage structure differed significantly among treatments and times (Global R=0.48, P < 0.001; Fig. 5.3, Table 5.4). Baited areas early in the survey (BT1-BT3) were different from all other groupings (R range=0.45-0.90; Fig. 5.3, Table 5.4), whereas baited areas later in the survey (BT4 & BT5) resembled unbaited areas more closely (R range=0.18-0.74) than early baited areas (R range=0.62-0.90).

We report SIMPER results only for species discriminating between BT1 and UT1 to highlight differences between baited and unbaited areas pre-baiting, and between BTU1 and BTU5 to highlight how the baited areas changed over time. Key discriminating species between BT1 and UT1 were Encyrtidae Genus B sp.1, *Aphycus* sp. 1, *Palpoteleia* sp. 1, *Spalangia* sp. 1 and *Phlyctenolotis scotti* (Fig. 5.4, Table 5.5). All except for *P. scotti* also accounted for most of the differences between BT1 and BT5, and also included *Synopeas* sp. 1 (Fig. 5.4, Table 5). Encyrtidae Genus B sp. 1 and *Aphycus* sp. 1 (usually associated with Hemiptera) were most abundant in the early baited plots (BT1-3) whereas the other four species (parasitoids and predator of various taxa) increased in later baited areas (BT4-5).

Discussion

Mechanism of hemipteran decline

Management of the mutualism was effective due to the presence of a remarkable abundance of natural enemies on the island. After the tending ants were suppressed, there was a great increase in natural enemy abundance and richness that corresponded with the rapid, area-wide decline of the scale population. These results are consistent with other studies that have shown that ant suppression can enhance the biological control of hemipterans (Daane *et al.* 2007; Del-Klaro & Oliveira 2000; Queiroz & Oliveira; Renault *et al.* 2005; Vanek & Potter 2010).

It is unlikely that all of the natural enemies were involved in scale regulation, but for many we are certain of their role in *Pu. urbicola* control. Six of the 34 parasitoid species recorded are primary scale parasitoids (Noyes 2012; Scholtz & Holm 2008). Of these, the genera *Moranila*, *Coccophagus*, *Anicetus*, *Aphycus* and *Metaphycus* all include economically important species that have been introduced for control of agricultural soft scale pests (Myers *et al.* 1989). *Anicetus* sp. 1, *Aphycus* sp. 1 and *Metaphycus s*p. 1, as well as *Aprostocetus* sp. 1

were also reared from *Pu. urbicola* in this study. Additionally, scale insects are the main prey for three of the 12 predators recorded; *Chilocorus nigritus*, *Cryptolaemus montrouzieri* and *Sticholotis madagassa*. All three coccinellids are voracious scale and mealybug predators that are widely used in biocontrol programs (Jalali & Singh 1989; Kaur & Virk 2012; Samways & Wilson 1988). These results suggest that the interference of the ants with the top-down control of the herbivore pest was strong and pervasive, and enabled the scale to reach damaging levels, even in the presence of a diverse natural enemy assemblage.

Ant interference with natural enemies is well documented (Renault *et al.* 2005; Majerus *et al.* 2006; Suzuki & Ide 2008). However, ant attendance can have varying effects on different natural enemies (Daane *et al.* 2007; Völkl & Mackauer 1993) and may also mediate interactions among them (Kaneko 2007; Kaneko 2002), making the effects of ant suppression unpredictable. This is apparent from the diverse responses of the different guilds involved with the mutualism on the island. Primary parasitoids of hemipterans were at their highest abundance before baiting despite high ant densities, and declined after baiting, whereas predators of hemipterans increased to their highest abundances one month after ant suppression.

Many parasitoids have adaptations that allow them to persist in the presence of ants (Daane *et al.* 2007; Bartlett 1961), including species in some of the genera recorded here e.g. *Coccophagus* sp. (Bartlett 1961) and *Metaphycus* sp. (Barzman & Daane 2001). These species often select ant-tended hemipteran colonies that provide them with enemy-free space where they are protected from intraguild predation and hyperparasitism (Völkl 1992; Barzman & Daane 2001). Pre-baiting ant attendance seemed to promote high primary parasitoid densities in this way. Yet clearly, this guild alone was not effective at reducing high scale densities.

The scale population collapsed with the increase in hemipteran-feeding predators one month after baiting. Other multi-taxa studies have indicated that increased predator diversity can enhance pest suppression (Cardinale *et al.* 2003; Colfer & Rosenheim 2001; Costamagna 2008). But predator identity also seems to be a key determinant of the outcome, as the occurrence of species with high per capita feeding rates can have disproportionately large effects on pest control within multi-taxa systems (Chalcraft & Resetarits 2003; Denoth *et al.* 2002; Straub & Snyder 2006). Our findings are consistent with these ideas. 96% of the scale predator abundance here was *C. nigritus*, a species with a very high feeding rate that was successfully introduced to the Seychelles for biocontrol of scale on coconut palm (Samways & Wilson 1988). This species operates well in combination with parasitoids, as it suppresses hemipterans that escape parasitism at high densities, but is less effective when prey is scarce (Samways 1984; Samways 1988). Primary parasitoids declined with the declining scale population, but remained in the area at low densities, suggesting that there was potential for an additive effect of the predators and parasitoids on pest suppression in the absence of the ants.

Interactions with the broader natural enemy assemblage

The natural enemy assemblage as a whole showed a significant response to mutualism disruption. Assemblages in the baited areas changed over time to resemble those in the unbaited areas towards the end of the survey, suggesting a return to an assemblage structure more similar to pre-invasion conditions. Both the guild and assemblage analyses indicated that mutualism disruption influenced not only natural enemies involved in the mutualism, but also affected groups external to the mutualism.

Primary parasitoids with various taxa as hosts increased in abundance over time, and four of the key discriminating species between invaded and uninvaded areas were species that

parasitize or prey on various non-hemipteran taxa. Previous studies on this system indicated that the abundance of many soil-surface and canopy arthropods increased after the baiting program (Gaigher *et al.* 2012; Gaigher & Samways 2012), and it is likely that the increase in these natural enemies was in response to the recovery of potential hosts and prey. These results support the argument that ant tending of hemipterans can have far-reaching effects in ecosystems (Styrsky & Eubanks 2007; Grover *et al.* 2008), as the effects of the mutualism carried across trophic levels, influencing various guilds within this functionally important assemblage.

Conservation implications

The great variety of natural enemies is noteworthy considering the island's small size and the isolation of the Seychelles archipelago. Other islands with similar environmental conditions and pest species have required introductions of biocontrol agents in conjunction with ant control to reduce *Pu. urbicola* densities (Smith *et al.* 2004; Smith & Papacek 2002). The persistence of natural enemies in the environment can increase the options for managing hemipteran pests, and is promising for future pest management in the Seychelles. Cousine supports five other scale species in addition to the dominant *Pu. urbicola* (Gaigher and Samways unpublished data), and many of these species and other coccids have been implicated in damage to native trees on other Seychelles islands (Haines & Haines 1978; Hill *et al.* 2003; Hill & Newbery 1982). It is encouraging that with targeted and careful management of the highly destructive ant-hemipteran mutualism, this complex of natural enemies can be re-established to continue to maintain the scale at a low population level where natural ecosystems are no longer seeing a major ecological regime shift.

References

Bartlett. B. R. 1961. The influence of ants upon parasites, predators, and scale insects. *Annals of the Entomological Society of America* 54, 543-551.

Barzman, M. S. & Daane, K. M. 2001. Host-handling behaviours in parasitoids of the black scale: a case for ant-mediated evolution. Journal of Animal Ecology 70, 237-247.

Cardinale, B. J., Harvey, C. T., Gross, K., & Ives, A. R. 2003. Biodiversity and biocontrol: emergent impacts of a multi-enemy assemblage on pest suppression and crop yield in an agroecosystem. *Ecology Letters* 6, 857-865.

Chalcraft, D. R., & Resetarits, W. J. 2003. Predator identity and ecological impacts: functional redundancy or functional diversity? *Ecology* 84, 2407–2418.

Clarke, K. R. & Gorley, R. N. 2001. PRIMER v5: User manual/tutorial, PRIMER-E, Plymouth UK.

Clarke, K. R. & Warwick, R. M. 2001. *Change in marine communities: An approach to statistical analysis and interpretation.* PRIMER-E, Plymouth, UK.

Colfer, R. G. & Rosenheim, J. A. 2001. Predation on immature parasitoids and its impact on aphid suppression. *Oecologia* 126, 292-304.

Costamagna, A. C., Landis, D. A., & Brewer, M. J. 2008. The role of natural enemy guilds in *Aphis glycines* suppression. *Biological Control* 45, 368-379.

Daane, K. M., Sime, K. R., Fallon, J., & Cooper, M. L. 2007. Impacts of Argentine ants on mealybugs and their natural enemies in California's coastal vineyards. *Ecological Entomol*ogy 32, 583-596.

Delabie, J. H. C. 2001. Trophobiosis between Formicidae and Hemiptera (Sternorrhyncha and Auchenorrhyncha): an Overview. *Neotropical Entomology* 30, 501-516.

Del-Claro, K. & Oliveira, P. S. 2000 Conditional outcomes in a neotropical treehopper-ant association: temporal and species-specific variation in ant protection and homopteran fecundity. *Oecologia* 124, 156-165.

Denoth, M., Frid, L. & Myers, J. H. 2002. Multiple agents in biological control: improving the odds? *Biological Control* 24, 20-30.

Eubanks, M. D., Blackwell, S. A., Parrish, C. J., Delamar, Z. D., & Hull-Sanders, H. 2002. Intraguild predation of beneficial arthropods by red imported fire ants in cotton. *Environmental Entomology* 31, 1168-1174.

Gaigher, R., Samways, M. J., Jolliffe, K. G., & Jolliffe, S. 2012. Precision control of an invasive ant on an ecologically sensitive tropical island: a principle with wide applicability. *Ecological Applications* 22, 1405-1412.

Gaigher, R. & Samways M. J. 2012. Strategic management of an invasive ant-scale mutualism enables recovery of a threatened tropical tree species. *Biotropica* In press. (DOI: 10.1111/j.1744-7429.2012.00898.x).

Gaigher, R., Samways, M. J., Henwood, J. & Jolliffe, K. 2011. Impact of a mutualism between an invasive ant and honeydew-producing insects on a functionally important tree on a tropical island. Biological Invasions 13, 1717-1721.

Grover, C. D., Dayton, K. C., Menke, S. B., & Holway, D. A. 2008. Effects of aphids on foliar foraging by Argentine ants and the resulting effects on other arthropods. *Ecological Entomol*ogy 33, 101-106.

Haines, I. H. & Haines, J. B. 1978. Pest status of the crazy ant, *Anoplolepis longipes* (Jerdon)(Hymenoptera:Formicidae), in the Seychelles. *Bulletin of Entomological Research* 68, 627-638.

Handler, A. T., Gruner, D. S., Haines, W. P., Lange, M. W., & Kaneshiro, K. Y. 2007 Arthropod surveys on Palmyra Atoll, Line Islands, and insights into the decline of the native tree *Pisonia grandis* (Nyctaginaceae). *Pacific Science* 61, 485-502.

Hill, M., Holm, K., Vel, T., Shah, N. J. & Matyot, P. 2003. Impact of the introduced yellow crazy ant *Anoplolepis gracilipes* on Bird Island, Seychelles. *Biodiversity and Conservation* 12, 1969-1984.

Hill, M. G. & Newbery, D. M. 1982. An analysis of the orgins and affinities of the coccid fauna (Coccoidea; Homoptera) of Western Indian Ocean islands, with special reference to Aldabra Atoll. *Journal of Biogeography* 9, 223-229.

Hübner G & Völkl, W. 1996. Behavioral strategies of aphid hyperparasitoids to escape aggression by honeydew-collecting ants. *Journal of Insect Behaviour* 9, 143-157.

Jalali, S. K. & Singh, S. P. 1989. Biotic potential of three coccinellid predators on various diaspine hosts. *Journal of Biological Control* 3, 20-23.

James, D. G., Stevens, M. M., O'Malley, K. J. & Faulder, R. J. 1999. Ant foraging reduces the abundance of beneficial and incidental arthropods in citrus canopies. *Biological Control* 14, 121-126.

Kaneko, S. 2007. Predator and parasitoid attacking ant-attended aphids: effects of predator presence and attending ant species on emerging parasitoid numbers. *Ecological Research* 22, 451-458.

Kaneko, S. 2003. Different impacts of two species of aphid-attending ants with different aggressiveness on the number of emerging adults of the aphid's primary parasitoid and hyperparasitoids. *Ecological Research* 18, 199-212.

Kaneko, S. 2002. Aphid-attending ants increase the number of emerging adults of the aphid's primary parasitoid and hyperparasitoids by repelling intraguild predators. *Entomological Science* 5, 131-146.

Kaplan, I. & Eubanks, M. D. 2005. Aphids alter the community-wide impact of fire ants. *Ecology* 86, 1640-1649.

Kaplan, I. & Eubanks, M. D. 2002. Disruption of cotton aphid (Homoptera: Aphididae)—natural enemy dynamics by red imported fire ants (Hymenoptera: Formicidae). *Environmental Entomology* 31, 1175-1183.

Kaur, H. & Vink, J. S. 2012. Feeding potential of *Cryptolaemus montrouzieri* against the mealybug *Phenacoccus solenopsis*. *Phytoparasitica* 40, 2131- 2136.

Letourneau, D. K. & Andow, D. A. 1999. Natural enemy food webs. *Ecological Applications* 9, 363-364.

Liang, K. & Zeger, S. L. 1986. Longitudinal data analysis using generalized linear models. *Biometrika* 73, 13-22.

Liere, H. & Perfecto, Y. 2008. Cheating on a mutualism: indirect benefits of ant attendance to a coccidophagous coccinellid. *Environmental Entomology* 37, 143-149.

Majerus, M. E. N., Sloggett, J. J., Godeau, J. F., & Hemptinne, J. L. 2006. Interactions between ants and aphidophagous and coccidophagous ladybirds. *Population Ecology* 49, 15-27.

McCullagh, P. & Nelder, J. A. 1989. *Generalized linear models*. Chapman and Hall, London, UK.

Mgocheki, N. & Addison, P. 2009. Interference of ants (Hymenoptera: Formicidae) with biological control of the vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae). *Biological Control* 49, 180-185.

Myers, J. H., Higgins, C. & Kovacs, E. 1989. How many insect species are necessary for the biological control of insects? Environmental Entomology 18, 541-547.

Noyes, J. S. 2012. Universal Chalcidoidea Database. World Wide Web electronic publication. http://www.nhm.ac.uk/chalcidoids. Accessed August 2012.

O'Neill, P., Olds, J. & Elder, R. 1997. Report on investigations of *Pulvinaria urbicola* infestations of *Pisonia grandis* forests, and masked and brown booby populations in the Coral Sea, 25 Nov-18 Dec 1997. Environment Australia Report.

Queiroz, J. M. & Oliveira, P. S. 2001. Tending ants protect honeydew-producing whiteflies (Homoptera: Aleyrodidae). *Environmental Entomology* 30, 295-297.

Renault, C. K., Buffa, L. M., & Delfino, M. A. 2005. An aphid-ant interaction: effects on different trophic levels. *Ecological Ressearch* 20, 71-74.

Samways, M. J. 1988. A pictorial model of the impact of natural enemies on the population growth rate of the scale insect *Aonidiella aurantii*. *South African Journal of Science* 84, 270-272.

Samways, M. J. & Wilson, S. J. 1988. Aspects of the feeding behaviour of *Chilocorus nigritus* (F.) (Col., Coccinellidae) relative to its effectiveness as a biocontrol agent. *Journal of Applied Entomology* 106, 177-182.

Samways, M. J. 1984. Biology and economic value of the scale predator *Chilocorus nigritus* (F.) (Coccinellidae). *Biocontrol News and Information* 5, 91-105.

Scholtz, C. H. & Holm, E. 2008. *Insects of Southern Africa*. Protea Book House, Pretoria.

Smith, D., Papacek, D., Hallam, M., & Smith, J. 2004. Biological control of *Pulvinaria urbicola* (Cockerell) (Homoptera:Coccidae) in a *Pisonia grandis* forest on North East Herald Cay in the Coral Sea. *General and Applied Entomology* 33, 61-68.

Smith, D. & Papacek, D. 2002. Report On Visit to the Coringa – Herald Nature Reserve and SE Magdelaine Cay, 15-22 March, 2002 with regard to the releasing of parasitoids and ladybird predators of the pest scale *Pulvinaria urbicola* on *Pisonia grandis* and the assessment of biocontrol options for hawkmoths. Environment Australia Report.

Snyder, W. E. & Ives, A. R. 2003. Interactions between specialist and generalist natural enemies: parasitoids, predators and pea aphid biocontrol. *Ecology* 84, 94-107.

SPSS Inc. 2010. *SPSS for Windows*, Release 19.0.0. SPSS Inc, Chicago, USA.

Straub, C. S. & Snyder, W. E. 2006. Species identity dominates the relationship between predator biodiversity and herbivore suppression. *Ecology* 87, 277-282.

Styrsky, J. D. & Eubanks, M. D. 2007. Ecological consequences of interactions between ants and honeydew-producing insects. *Proceedings of the Royal Society B-Series* 274, 151-164.

Suzuki, N. & Ide, T. 2008. The foraging behaviors of larvae of the ladybird beetle, *Coccinella septempunctata* L., (Coleoptera: Coccinellidae) towards ant-tended and non-ant-tended aphids. *Ecological Research* 23, 371-378.

Vanek, S. J. & Potter, D. A. 2010 Ant-exclusion to promote biological control of soft scales (Hemiptera: Coccidae) on woody landscape plants. *Environmental Entomology* 39, 1829-1837.

Völkl, W. 1992. Aphids or their parasitoids: Who actually benefits from ant-attendance? *Journal of Animal Ecology* 61, 273-281.

Völkl, W. & Mackauer, M. 1993. Interactions of ants attending *Aphis fabae* spp. *cirsiiacanthoidis* on thistles and foraging parasitoid wasps. *Journal of Insect Behaviour* 6, 301-312.

Table 5.1. Natural enemies recorded during the survey on Cousine Island May 2010-May 2011. Species with asterisks were also reared from the dominant scale insect *Pulvinaria urbicola*. Guild abbreviations: Prim=Primary parasitoid, Sec=Secondary parasitoid, Prim or sec=Primary or secondary parasitoid, Pred=Predator.

Family	Species	Guild	Host/prey
Parasitoids			
Aphelinidae	*Coccophagus* sp. 1	Prim	Hemiptera
	Marietta leopardina*	Sec	Hemiptera
Bethylidae	Genus A sp. 1	Prim	Various taxa
	Genus B sp. 1	Prim	Various taxa
	Genus C sp. 1	Prim	Coleoptera
Braconidae	*Chelonus* sp. 1	Prim	Lepidoptera
	Genus A sp. 1	Prim	Lepidoptera
	Genus B sp. 1	Prim	Various taxa
Ceraphronidae	*Ceraphron* sp. 1 & 2	Sec	Various taxa
Chalcididae	*Brachymeria* sp. 1	Prim or Sec	Various taxa
	Brachymeria sp. 2	Prim or Sec	Various taxa
	Hockeria sp. 1	Prim	Lepidoptera
Encyrtidae	*Anicetus* sp. 1*	Prim	Hemiptera
	Aphycus sp. 1*	Prim	Hemiptera
	Cheiloneurus cyanonotus*	Sec	Hemiptera
	Cheiloneurus sp. 2	Sec	Hemiptera
	Genus A sp. 1	Prim or Sec	Various taxa
	Homalolytus sp. 1	Prim	Coleoptera
	Metaphycus sp. 1*	Prim	Hemiptera
Eulophidae	*Aprostocetus* sp. 1*	Prim	Hemiptera
	Pediobius sp. 1	Prim	Various taxa
	Sympiesis sp. 1	Prim	Various taxa
Eupelmidae	*Eupelmus* sp. 1	Prim or Sec	Various taxa
Figitidae	*Ganaspis* sp. 1 & 2	Prim	Diptera
Mymaridae	*Gonatocerus* sp. 1	Prim	Hemiptera
Platygastridae	*Gryon* sp. 1	Prim	Various taxa
	Gryon sp. 2	Prim	Various taxa

111

	Palpoteleia sp. 1	Prim	Various taxa
	Synopeas sp. 1	Prim	Diptera
	Synopeas sp. 2	Prim	Diptera
Pteromalidae	*Moranila* sp. 1	Prim	Hemiptera
	Spalangia sp. 1	Prim	Diptera
	Spalangia sp. 2	Prim	Diptera
	Sycoscapter sp. 1	Prim	Hymenoptera

Beetles

Coccinellidae	*Chilocorus nigritus*	Pred	Hemiptera
	Cryptolaemus montrouzeiri	Pred	Hemiptera
	Phlyctenolotis scotti	Pred	Various taxa
	Stethorus cf. aethiops	Pred	Various taxa
	Sticholotis madagassa	Pred	Hemiptera

Spiders

Araneidae	*Neoscona subfusca*	Pred	Various taxa
Salticidae	*Heliophanus* sp. 1	Pred	Various taxa
	Heliophanus sp. 2	Pred	Various taxa
	Myrmarachne constricta	Pred	Various taxa
Theridiidae	*Theridion* sp. 1	Pred	Various taxa
Uloboridae	*Uloborus* sp. 1	Pred	Various taxa
	Undetermined sp. 1	Pred	Various taxa

112

Table 5.2. The effect of mutualism disruption on the overall natural enemy abundance and species richness. Statistics derived from Generalized Estimating Equations.

Response variables	df	Wald's chi-square	P
Natural enemy abundance			
Treatment	1	32.61	< 0.0001
Time	4	31.05	< 0.0001
Time x Treatment	4	11.97	0.02
Natural enemy species richness			
Treatment	1	43.78	< 0.0001
Time	4	77.07	< 0.0001
Time x Treatment	4	46.52	< 0.0001

113

Table 5.3. The effect of mutualism disruption on abundance of natural enemy feeding guilds. Groups not listed did not have sufficient data at all survey periods to carry out the analyses. Statistics derived from Generalized Estimating Equations.

Response variables	df	Wald's chi-square	P
Primary parasitoid abundance			
Treatment	1	40.92	< 0.0001
Time	4	282.00	< 0.0001
Time x Treatment	4	19.54	0.001
Host: Hemipterans			
Treatment	1	56.33	< 0.0001
Time	4	33.08	< 0.0001
Time x Treatment	4	55.12	< 0.0001
Host: Various taxa			
Treatment	1	2.75	0.98
Time	4	217.64	< 0.0001
Time x Treatment	4	38.13	< 0.0001
Predator abundance			
Treatment	1	8.04	0.005
Time	4	20.58	< 0.0001
Time x Treatment	4	88.85	< 0.001
Prey: Hemipterans			
Treatment	1	12.86	< 0.001
Time	4	37.01	< 0.0001
Time x Treatment	4	38.62	< 0.0001
Prey: Various taxa			
Treatment	1	2.05	0.15
Time	4	23.86	< 0.0001
Time x Treatment	4	5.66	0.23
Secondary parasitoid abundance			
Treatment	1	10.37	0.001
Time	4	29.86	< 0.0001
Time x Treatment	4	4.05	0.40

Table 5.4. R-statistics derived from ANOSIM indicating similarities in natural enemy assemblage structure among baited and unbaited areas at different times after baiting (BT1-BT5=baited plots, time 1-5, UT1-UT5=unbaited plots, time 1-5). Values closer to 0 indicate greater similarity and values closer to 1 indicate greater differences. R-values in bold are statistically significant at P < 0.001. The low significance level was due to Bonferroni correction for multiple comparisons.

	BT1	BT2	BT3	BT4	BT5	UT1	UT2	UT3	UT4
BT2	**0.19**								
BT3	**0.46**	**0.26**							
BT4	**0.81**	**0.83**	**0.90**						
BT5	**0.62**	**0.67**	**0.81**	**0.48**					
UT1	**0.57**	**0.57**	**0.65**	**0.68**	**0.44**				
UT2	**0.63**	**0.52**	**0.60**	**0.74**	**0.51**	0.02			
UT3	**0.61**	**0.48**	**0.52**	**0.64**	**0.41**	0.08	-0.04		
UT4	**0.65**	**0.84**	**0.91**	**0.64**	**0.40**	**0.63**	**0.71**	**0.64**	
UT5	**0.35**	**0.45**	**0.57**	**0.20**	**0.18**	**0.22**	**0.27**	**0.20**	**0.34**

Table 5.5. Results from SIMPER analyses showing relative mean abundances of key discriminating species (as indicated by Dis/SD>1) and their contributions to dissimilarities between pre-baiting baited and unbaited sites (BT1 and UT1) and baited sites at the start and end of the survey (BT1 and BT5).

|	Mean abundance		Dis/SD	% Contribution to dissimilarity	Cumulative % dissimilarity
Average dissimilarity = 79.19%	**BT1**	**UT1**			
Encyrtidae Genus B sp. 1	12.65	5.35	1.44	15.86	15.86
Aphycus sp. 1	16.2	0.2	1.27	14.76	30.62
Palpoteleia sp. 1	0.3	6	1.49	13.95	44.57
Spalangia sp. 1	2.15	0.95	1.12	7.8	52.36
Phlyctenolotis scotti	0.1	0.8	1.03	4.68	63.14
Average dissimilarity=62.4%	**BT1**	**BT5**			
Palpoteleia sp. 1	0.3	9.2	2.34	17.55	17.55
Spalangia sp. 1	2.15	9.85	1.67	13.81	31.37
Aphycus sp. 1	16.2	0.55	1.2	13.37	44.74
Encyrtidae Genus B sp. 1	12.65	15.75	1.17	9.41	54.15
Synopeas sp. 1	0	0.9	1.25	5.01	59.16
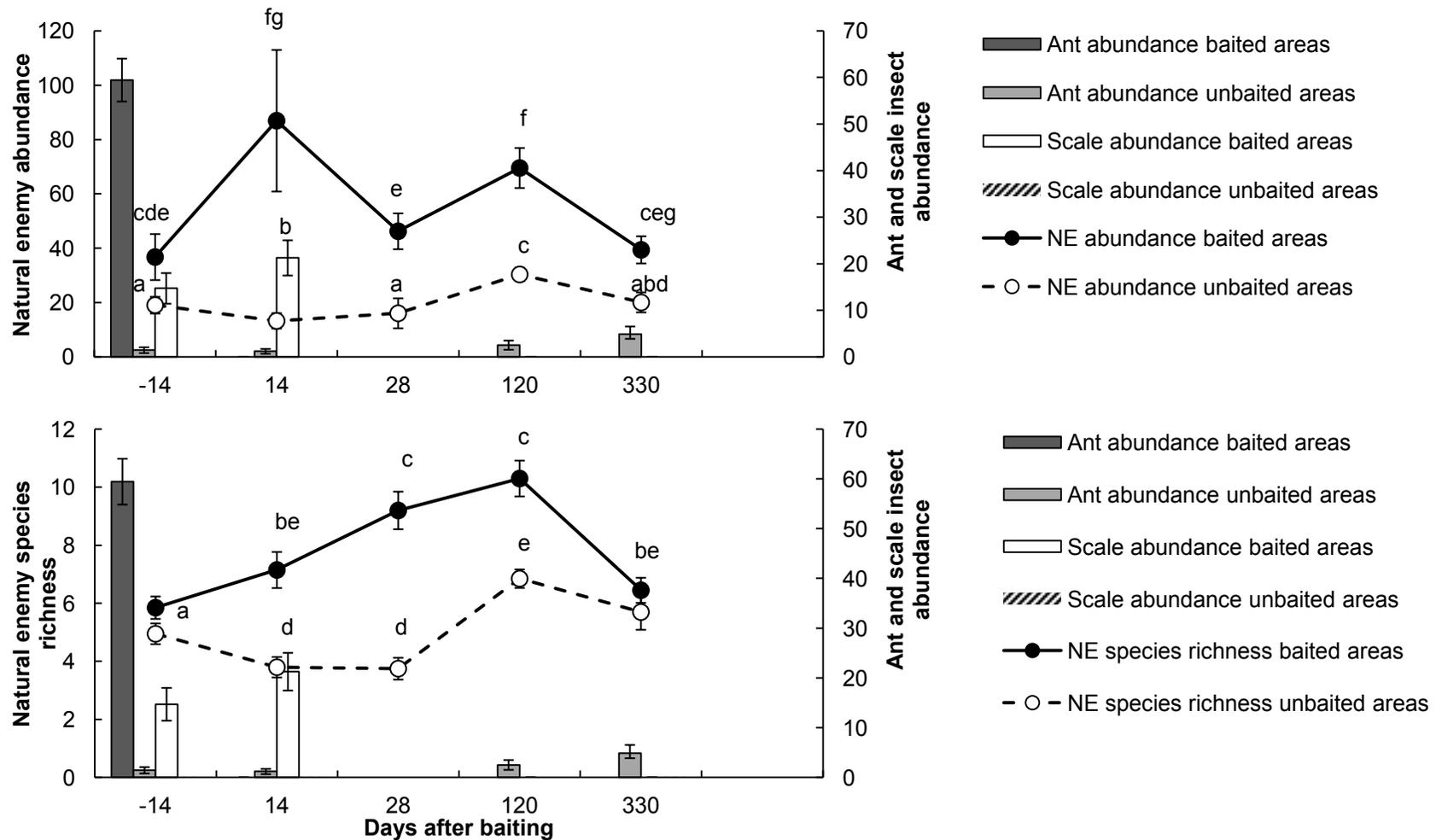
Figure 5.1. Natural enemy (NE) a) abundance and b) species richness, as well as ant and scale abundance (± S.E.) in baited and unbaited areas before and after mutualism disruption. Treatment date is indicated by the arrow. Natural enemy means with letters in common are not significantly different at $P < 0.05$. Ant and scale data were obtained from Gaigher & Samways (2012). Ant and scale abundance was not assessed at 28 days after baiting.
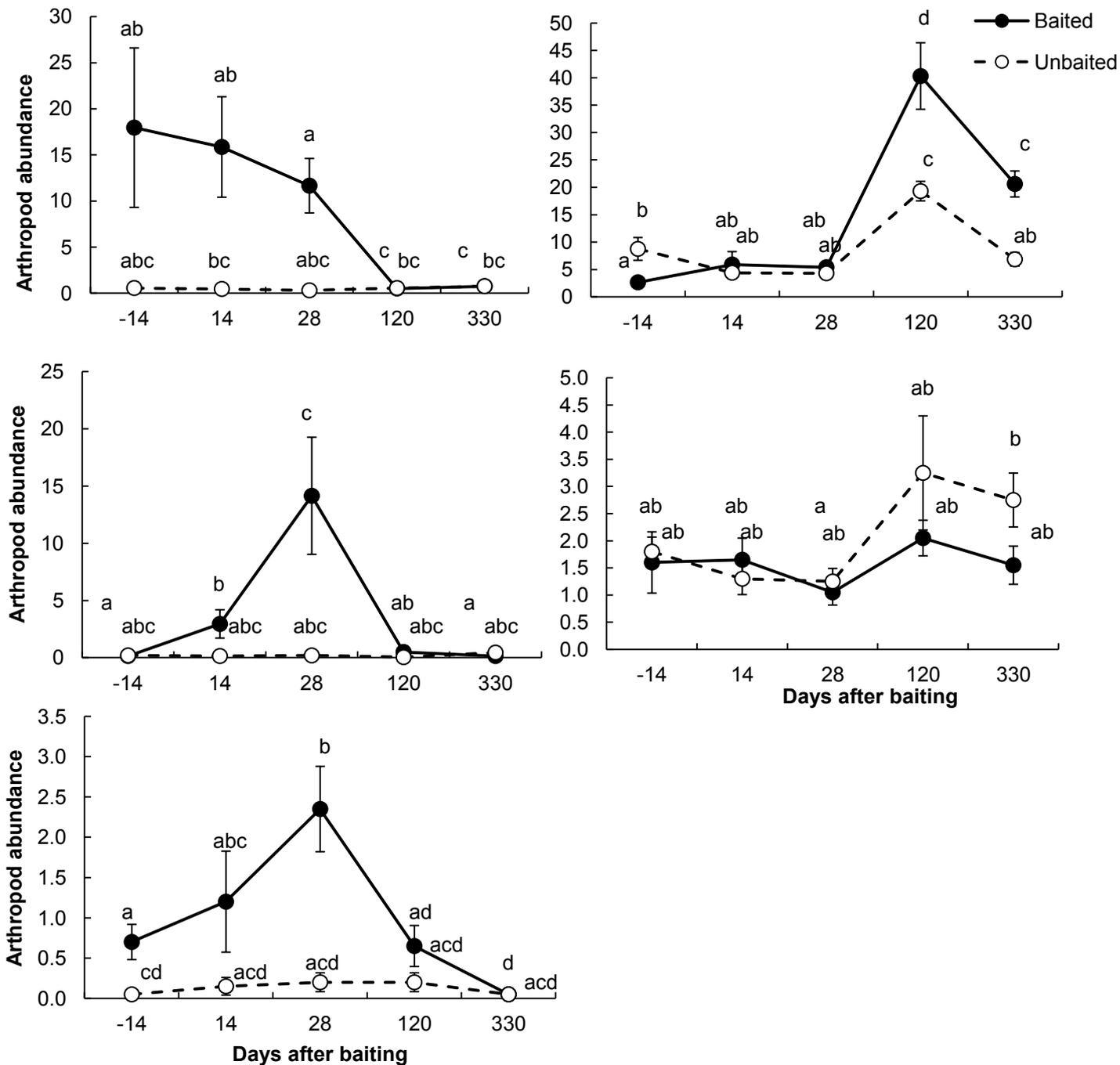
Figure 5.2. The abundance of different functional guilds before and after mutualism disruption in baited and unbaited areas. a) primary parasitoids with hemipteran hosts b) primary parasitoids with various hosts, c) predators with hemipteran prey, d) generalist predators and e) secondary parasitoids. Means with letters in common are not significantly different at P < 0.05. Groups not shown did not have sufficient data at all survey periods to carry out the analyses.
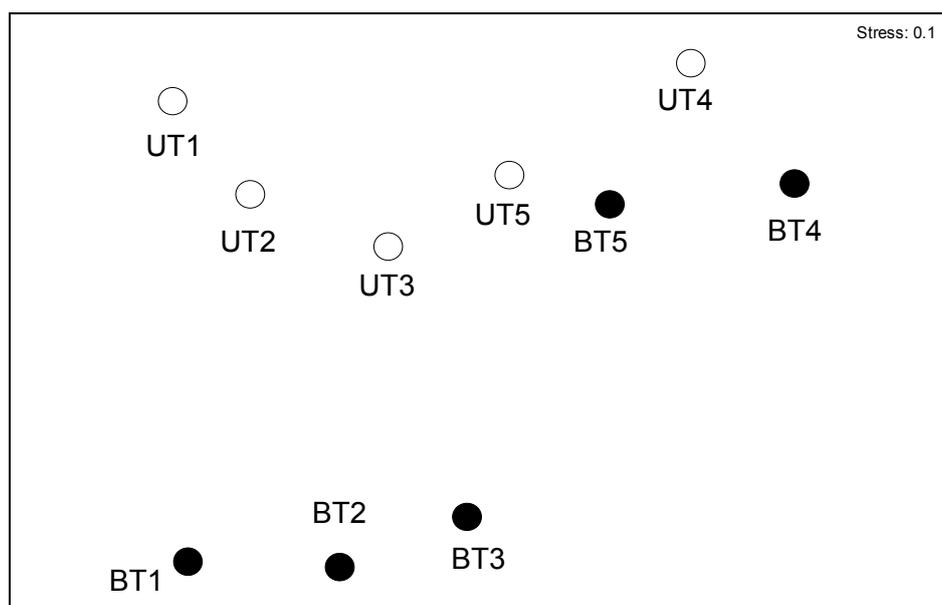
Figure 5.3. nMDS ordination plot of time and treatment groupings (UT1-UT5 = unbaited plots, time 1-5, BT1-BT5=baited plots, time 1-5) based on log(x+1) transformed abundance data.
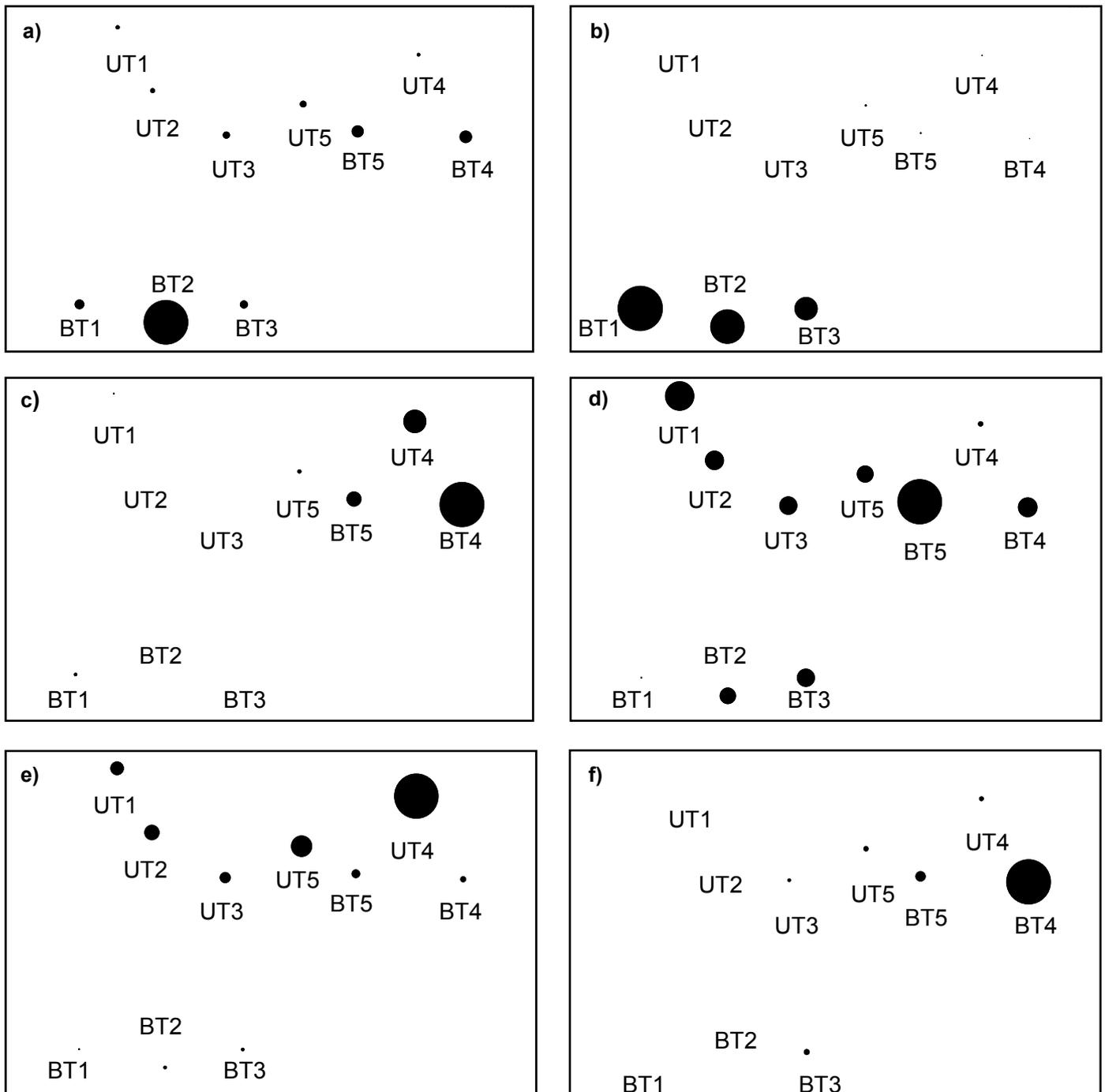
Figure 5.4. Abundances of key discriminating species a) Encyrtidae genus B sp. 1, b) *Aphycus* sp. 1, c) *Spalangia* sp. 1, d) *Palpoteleia* sp. 1, e) *Phlyctenolotis scotti*, f) *Synopeas* sp. 1, accounting for most of the variation between baited and unbaited groupings, as well as pre- and post-baited groupings, superimposed onto the nMDS ordination of the groupings. Bubble size represents abundance. (BT1-BT5=baited plots, time 1-5, UT1-UT5=unbaited plots, time 1-5).

6. **General discussion**

Invasive ant management and ecosystem restoration

One of the key findings of this project is the potential for successful management of *Ph. megacephala*. It was possible to effectively manage extremely high ant densities with limited resources and personnel, even within a highly sensitive and complex environment (Chapter 3). It is very fortunate that the species is so susceptible to hydramethylnon-based baits (Hoffmann 2011), considering the difficulty in developing effective measures against other invasive ants (Silverman & Brightwell 2008; Williams *et al.* 2001), while presenting a great opportunity for mitigating the impacts of an important invasive species.

There have been a number of successful eradications of *Ph. megacephala* infestations ranging in size and occurring in various different habitat types and disturbance regimes, with all of them using similar bait broadcasting methods (Hoffmann & O'Connor 2004; Hoffmann 2011; Hoffmann *et al.* 2010; Plentovich *et al.* 2010). The evaluation of the bait station application method (Chapter 3) showed that this can be a useful alternative method in conservation areas where the prevention of non-target effects is essential. This is significant for some of the *Ph. megacephala*-infested Seychelles islands, many of which have reintroduced threatened species, where even a low level of risk to native species is considered unacceptable.

The method proved to be effective, low-cost and environmentally safe, although its ability to completely eliminate an infestation has not yet been established (Chapter 3). This project was limited to a single system and three-year time frame, and additional testing will be necessary to establish how well this method performs at a larger scale. Further experimentation with the bait station method is currently underway on other Seychelles

islands (Adam *et al.* 2012), as well as ongoing area-wide control of the ant on Cousine Island, and these programs may provide additional information on its efficacy.

In addition to the reduction of target species density, post-control recovery of the native community is considered an important measure of management success (Caut *et al.* 2009). This study demonstrates that there is potential for ecosystem recovery after *Ph. megacephala* removal, even within a relatively short time span. *Pisonia* tree recovery after ant suppression was considered to be the most significant positive outcome of the program (Chapter 4). The cascading effect of the ant via its mutualist that ultimately affected the forest trees had the potential to significantly alter the ecosystem, both structurally and functionally. Similar exotic species associations have had massively disruptive effects in other systems that were not managed early enough e.g. large-scale dieback and death of trees on Christmas Island due to *Anoplolepis gracilipes*-scale insect mutualisms (O'Dowd *et al.* 2003) and near-complete loss of *Pisonia* forest due to *Pulvinaria urbicola-Ph. megacephala* mutualisms in the Capricornia Cays (O'Neill *et al.* 1997). Considering the pre-baiting condition of *Pisonia* trees in the ant-infested area on Cousine, it seems reasonable to assume that management intervention prevented significant damage to this important component of the forest ecosystem.

Additionally, many arthropod groups recovered in response to ant management, including soil-surface arthropods (Chapter 3), canopy herbivores (Chapter 4) and various parasitoids and predators (Chapter 5). Species that benefitted from ant control included Seychelles endemics, such as the ant *Pheidole flavens farquharensis* and the hemipterans *Osaka relata* and *Epicroesa* sp., as well as functionally important non-native species such as the cockroach *Pycnocelus indicus* (an important food item of the Seychelles Magpie Robin) and the coccinellid *Chilocorus nigritus* (an important biocontrol agent of scales)(Chapters 3-

5). This emphasizes the conservation benefits of ant control on the island, from both a biodiversity and ecosystem functioning perspective.

Community interactions of the invasive ant

Another recurring finding was how widely interconnected the invasive ant was within the invaded ecosystem. Fig. 6.1 represents the role of *Ph. megacephala*in the arthropod food web before and after baiting (from Chapters 2-5), and illustrates the major groups that were influenced by ant suppression. Effects of the ant on specific groups have been demonstrated before e.g. on other ants (Burwell *et al.* 2012; Callan & Majer 2009), invertebrate prey (Dejean *et al.* 2007), mutualist scale insects (Bach 1991) and their natural enemies (González-Hernández, *et al.* 1999). However, this is the first time that such a diversity of interactions has been shown for the species in a single system.

These results demonstrate the range of effects of the ant on the island and suggest that its effects on recipient communities may be more extensive than previously thought. This is an important consideration for risk assessment of the species. Although *Ph. megacephala* is regarded as a serious pest (Lowe *et al.* 2000), it has received little attention compared to other invasive ants such as *Solenopsis invicta* and *Linepithema humile* (Holway *et al.* 2002). A greater research and management focus has been recommended for *Ph. megacephala* (Hoffmann & Parr 2007; Hoffmann 2011; Holway *et al.* 2002). In Chapter 2, I also emphasize the need for additional research on the species, especially those highlighting its threat to natural ecosystems. In addition to this, research that demonstrates its diverse roles may support more accurate assessments of the risk of the species in new environments.

The ant's most influential interaction on the island was its mutualism with hemipteran scale insects, particularly the destructive *Pu. urbicola*. Results from Chapter 2 and 4 suggest that the mutualism facilitated extremely high ant and hemipteran densities, with the resulting

negative effect on *Pisonia* trees representing the most obvious threat to the forest ecosystem. The mutualism also appeared to be central to many of the interactions with other arthropod taxa (Chapters 3, 4 & 5). Ant suppression revealed the influence of the mutualists on guilds that they predictably interacted with, such as other soil-surface arthropods (Chapter 3), non-hemipteran canopy herbivores (Chapter 4), and natural enemies of hemipterans (Chapter 5) (Fig. 1). However, it also had broader indirect effects on guilds not obviously associated with the ant, such as predators and parasitoids unrelated to the mutualism (Chapter 5) (Fig. 1). Responses to ant removal varied among guilds and there were specific species that were disproportionately influenced by ant suppression, some of them with important roles in the arthropod food web (Chapter 4 & 5).

These results support the idea that ant-hemipteran mutualisms are keystone interactions that can significantly influence community structure and function (Styrsky & Eubanks 2007). They also agree with a number of studies that have shown that alien removal can have unexpected indirect effects (Bergstrom *et al.* 2009; Caut *et al.* 2009; Courchamp & Caut 2006). In addition, the individual responses suggest that the outcomes of invasive species management are likely to be highly system-specific, depending on the species present and their associations. A basic understanding of the site-specific relationships of the target species to other species in the ecosystem, as well as to abiotic factors, will therefore be of significant practical value to land managers who are planning control programs (Davis 2006). Where resources and time are available, pre-control assessment of ecosystems may be very useful in revealing complex interactions and will enable conservation practitioners to make better predictions on the range of possible management outcomes.

Conclusion

Globally, there has been a steady increase in the size and complexity of management programs being attempted (Clout & Veitch 2002; Donlan *et al.* 2003). Even infestations of species that are considered to be especially challenging, such as social invasive insects (Gillespie & Roderick 2002), are being addressed (Gentz 2009). This growth is encouraging considering that it is a field that can contribute significantly to the conservation of natural systems. This is the main message that is highlighted throughout this project – that it is possible to mitigate the impacts of invasive species and thereby facilitate the recovery of the native ecosystem. Research that further supports these programs will enable practitioners to deal with an increasing range of situations and will contribute to the development of practical solutions (Donlan *et al.* 2003).

References

Adam, P. A., Rocamora, G., Moolna, A., Gaigher, R., Calabrese, L., & Maggs, G. 2012, *Big-headed ant* Pheidole megacephala *eradication plan on Aride, 2012.* Island Conservation Society Report.

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

Bergstrom, D. M., Lucieer, A., Kiefer, K., Wasley, J., Belbin, L., Pedersen, T. K., & Chown, S. L. 2009. Indirect effects of invasive species removal devastate World Heritage Island. *Journal of Applied Ecology* 46, 73-81.

Burwell, C. J., Nakamura, A., McDougall, A., & Neldner, V. J. 2012. Invasive African big-headed ants, *Pheidole megacephala*, on coral cays of the southern Great Barrier Reef: distribution and impacts on other ants. *Journal of Insect Conservation* DOI 10.1007/s10841-012-9463-6.

Callan, S. K. & Majer, J. D. 2009. Impacts of an incursion of African Big-headed ants, *Pheidole megacephala* (Fabricius), in urban bushland in Perth, Western Australia. *Pacific Conservation Biology* 15, 102-115.

Caut, S., Angulo, E., & Courchamp, F. 2009. Avoiding surprise effects on Surprise Island: alien species control in a multitrophic level perspective. *Biological Invasions* 11, 1689-1703.

Clout, M. N. & Veitch, C. R. 2002. Turning the tide of biological invasions: the potential for eradicating invasive species. In: *Turning the tide: the eradication of invasive species.* Proceedings of the international conference on eradication of island invasives, eds.

Veitch, C.R. & Clout, M.N. pp. 1-3. Occasional paper of the IUCN Species Survival Commission no. 27.

Courchamp, F. & Caut, S. 2006. Use of biological invasions and their control to study the dynamics of interacting populations. In *Conceptual ecology and invasion biology: reciprocal approaches to nature*, eds. M. W. Cadotte, S. M. McMahon, & T. Fukami, pp. 243-269. Springer, Dordrecht, Netherlands.

Davis, M. A. 2006. Invasion Biology 1958-2005: The pursuit of science and conservation. In *Conceptual ecology and invasion biology: reciprocal approaches to nature*, eds. M. W. Cadotte, S. M. McMahon, & T. Fukami, pp. 35-64. Springer, Dordrecht, Netherlands.

Dejean, A., Kenne, M., & Moreau, C. S. 2007. Predatory abilities favour the success of the invasive ant *Pheidole megacephala* in an introduced area. *Journal of Applied Entomology* 131, 625-6.

Donlan, C. J., Tershy, B. R., Campbell, K., & Cruz, F. 2003. Research for requiems: the need for more collaborative action in eradication of invasive species. *Conservation Biology* 17, 1850-1851.

Gentz, M. C. 2009. A review of chemical control options for invasive social insects in island ecosystems. *Journal of Applied Entomology* 133, 229-235.

Gillespie, R. G. & Roderick, G. K. 2002. Arthropods on islands: colonization, speciation, and conservation. *Annual Review of Entomology* 47, 595-632.

González-Hernández, H., Johnson, M. W., & Reimer, N. J. 1999. Impact of *Pheidole megacephala* (F.) (Hymenoptera: Formicidae) on the biological control of

Dysmicoccus brevipes (Cockerell) (Homoptera: Pseudococcidae). *Biological Control* 15, 145-152.

Hoffmann, B. D. & O'Connor, S. 2004. Eradication of two exotic ants from Kakadu National Park. *Ecological Management and Restoration* 5, 98-105.

Hoffmann, B. D. & Parr, C. L. 2007. An invasion revisited: the African big-headed ant (*Pheidole megacephala*) in northern Australia. *Biological Invasions* 7, 1171-1181.

Hoffmann, B. D. 2011. Eradication of populations of an invasive ant in northern Australia: successes, failures and lessons for management. *Biodiversity and Conservation* 20, 3267-3278.

Hoffmann, B. D., Abbott, K. L., & Davis, P. 2010. Invasive ant management. In *Ant Ecology*, eds. L. Lach, C. L. Parr, & K. L. Abbott, pp. 287-304. Oxford University Press, New York, USA.

Holway, D. A., Lach, L., Suarez, A. V., Tsutsui, N. D., & Case, T. J. 2002. The causes and consequences of ant invasions. *Annual Review of Ecology and Systematics* 33, 181-233.

Lowe, S., Browne, M., Boudjelas, S., & De Poorter, M. 2000, *100 of the world's worst invasive alien species: a selection from the global invasive species database*, The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN).

O'Dowd, D. J., Green, P. T., & Lake, P. S. 2003. Invasional meltdown on an oceanic island. *Ecology Letters* 6, 812-817.

O'Neill, P., Olds, J., & Elder, R. 1997, *Report on investigations of* Pulvinaria urbicola *infestations of Pisonia* grandis *forests, and masked and brown booby populations in the Coral Sea, 25 Nov-18 Dec 1997*.

Plentovich, S., Eijzenga, J., Eijzenga, H., & Smith, D. 2011. Indirect effects of ant eradication efforts on offshore islets in the Hawaiian Archipelago. *Biological Invasions* 13, 545-557.

Silverman, J. & Brightwell, R. J. 2008. The Argentine Ant: challenges in managing an invasive unicolonial pest. *Annual Review of Entomology* 53, 231-252.

Styrsky, J. D. & Eubanks, M. D. 2007. Ecological consequences of interactions between ants and honeydew-producing insects. *Proceedings of the Royal Society, B-Series* 274, 151-164.

Williams, D. F., Collins, H. L., & Oi, D. H. 2001. The red imported fire ant (Hymenoptera: Formicidae): An historical perspective of treatment programs and the development of chemical baits for control. *American Entomologist* 47, 146-159.

A

B
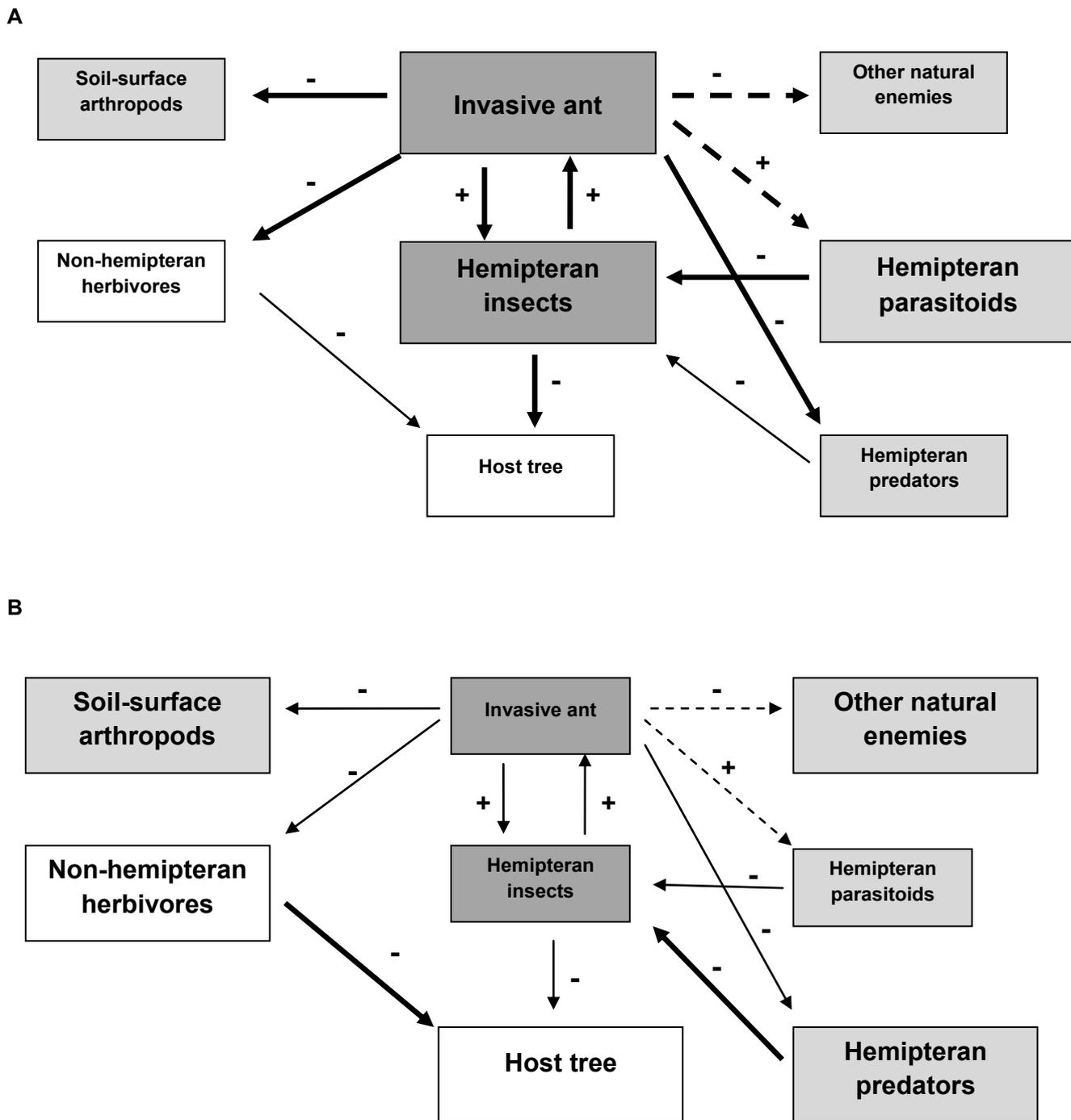
Figure 6.1. Schematic diagram of the arthropod food web related to the ant-hemipteran mutualism on *Pisonia* host trees on Cousine Island a) before ant suppression and b) after ant suppression. Block size is related to population size (or condition in the case of host tree) and arrow width represents interaction strength, with plus and minus signs representing net positive or negative effects. Guilds in white blocks consist mostly of native species, in light grey are both native and non-native species and in dark grey are non-native species.

Appendix A. Checklist of arthropod species recorded on Cousine Island during the surveys in 2008-2011. Only species that could be identified to at least genus level are listed.

Order	Family	Genus	Species
Araneae	Araneidae	*Neoscona*	*subfusca*
Araneae	Salticidae	*Heliophanus*	sp. 1
Araneae	Salticidae	*Heliophanus*	sp. 2
Araneae	Salticidae	*Myrmarachne*	*constricta*
Araneae	Theridiidae	*Theridion*	sp. 1
Araneae	Uloboridae	*Uloborus*	sp. 1
Blattodea	Blaberidae	*Pycnoscelus*	*indicus*
Chilopoda	Scolopendridae	*Otostigmus*	cf *orientalis*
Coleoptera	Coccinelidae	*Rodolia*	*chermesina*
Coleoptera	Coccinellidae	*Chilocorus*	*nigritus*
Coleoptera	Coccinellidae	*Cryptolaemus*	*montrouzeiri*
Coleoptera	Coccinellidae	*Phlyctenolotis*	*scotti*
Coleoptera	Coccinellidae	*Stethorus*	cf *aethiops*
Coleoptera	Coccinellidae	*Sticholotus*	*madagassa*
Hemiptera	Coccidae	*Ceroplastes*	sp. 1
Hemiptera	Coccidae	*Pulvinaria*	*urbicola*
Hemiptera	Diaspididae	*Hemiberlesia*	*lataniae*
Hemiptera	Diaspididae	*Pinnaspis*	*strachani*
Hemiptera	Fulgoridae	*Osaka*	*relata*
Hemiptera	Margarodidae	*Icerya*	*seychellarum*
Hemiptera	Pseudococcidae	*Dysmicoccus*	sp. 1
Hymenoptera	Aphelinidae	*Coccophagus*	sp.1
Hymenoptera	Aphelinidae	*Marietta*	*leopardina*
Hymenoptera	Bethylidae	*Genus A*	sp.1
Hymenoptera	Bethylidae	*Genus B*	sp.1
Hymenoptera	Bethylidae	*Genus C*	sp. 1
Hymenoptera	Braconidae	*Chelonus*	sp.1

Hymenoptera	Ceraphronidae	*Ceraphron*	sp. 1 & 2
Hymenoptera	Chalcididae	*Brachymeria*	sp. 1
Hymenoptera	Chalcididae	*Brachymeria*	sp. 2
Hymenoptera	Chalcididae	*Hockeria*	sp.1
Hymenoptera	Encyrtidae	*Anicetus*	sp.1
Hymenoptera	Encyrtidae	*Aphycus*	sp. 1
Hymenoptera	Encyrtidae	*Cheiloneurus*	probably *cyanonotus*
Hymenoptera	Encyrtidae	*Cheiloneurus*	sp. 2
Hymenoptera	Encyrtidae	*Genus B*	sp. 1
Hymenoptera	Encyrtidae	*Homalolytus*	sp. 1
Hymenoptera	Encyrtidae	*Metaphycus*	sp. 1
Hymenoptera	Eulophidae	*Aprostocetus*	sp. 1
Hymenoptera	Eulophidae	*Pediobius*	sp.1
Hymenoptera	Eulophidae	*Sympiesis*	sp. 1
Hymenoptera	Eupelmidae	*Eupelmus*	sp.1
Hymenoptera	Figitidae	*Ganaspis*	sp 1 & 2
Hymenoptera	Formicidae	*Brachymyrmex*	*cordemoyi*
Hymenoptera	Formicidae	*Camponotus*	*grandidieri*
Hymenoptera	Formicidae	*Camponotus*	*maculatus*
Hymenoptera	Formicidae	*Cardiocondyla*	*emeryi*
Hymenoptera	Formicidae	*Leptogenys*	*maxillosa*
Hymenoptera	Formicidae	*Monomorium*	*floricola*
Hymenoptera	Formicidae	*Monomorium*	*seychellense*
Hymenoptera	Formicidae	*Odontomachus*	*simillimus*
Hymenoptera	Formicidae	*Paratrechina*	*bourbonica*
Hymenoptera	Formicidae	*Paratrechina*	*longicornis*
Hymenoptera	Formicidae	*Pheidole*	*flavens farquharensis*
Hymenoptera	Formicidae	*Pheidole*	*megacephala*
Hymenoptera	Formicidae	*Plagiolepis*	*alluaudi*

Hymenoptera	Formicidae	*Strumigenys*	*emmae*
Hymenoptera	Formicidae	*Tapinoma*	*melanocephalum*
Hymenoptera	Formicidae	*Technomyrmex*	*albipes*
Hymenoptera	Formicidae	*Tetramorium*	*simillimum*
Hymenoptera	Mymaridae	*Gonatocerus*	sp.1
Hymenoptera	Platygastridae	*Gryon*	sp. 1
Hymenoptera	Platygastridae	*Gryon*	sp. 2
Hymenoptera	Platygastridae	*Palpoteleia*	sp. 1
Hymenoptera	Platygastridae	*Synopeas*	sp 1
Hymenoptera	Platygastridae	*Synopeas*	sp. 2
Hymenoptera	Pteromalidae	*Moranila*	sp.1
Hymenoptera	Pteromalidae	*Spalangia*	sp. 1
Hymenoptera	Pteromalidae	*Spalangia*	sp. 2
Hymenoptera	Pteromalidae	*Sycoscapter*	sp.1
Lepidoptera	Heliodinidae	*Epicroesa*	sp. 1
Orthoptera	Gryllidae	*Pteronemobius*	cf *tapobranensis*