Developing species specific management techniques for little fire ant *Wasmannia auropunctata* (Formicidae) in the tropics

Photo by: Melody Euparadorn

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Thesis submitted for the degree of Doctor of Philosophy

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University of Canberra

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Certificate of authorship of thesis

Except where clearly acknowledged in footnotes, quotations, co-author declarations and the bibliography, I certify that I am the sole author of the thesis submitted today entitled:

Developing species specific management strategies for the invasive little fire ant Wasmannia auropunctata (Formicidae) in the tropics

I further certify that to the best of my knowledge the thesis contains no material previously published or written by another person except where due reference is made in the text of the thesis.

The material in the thesis has not been the basis of an award of any other degree or diploma except where due reference is made in the text of the thesis.
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11 August 2022

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14/09/2022

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Abstract

Throughout history, plants, animals, and microorganisms have been intentionally and accidentally transported around the world by humans. Although a small portion of transported organisms become established invasive alien species, those that do frequently impart multi-tiered negative impacts on the ecosystems they are introduced into. Ants are especially easy to transport, are preadapted to successful establishment, and account for over one-third of the terrestrial arthropods on the list of the world's worst 100 invasive species. Impacts and the life histories of many invasive ants have been well documented, however, effective management options for many problematic species is lacking. Current management techniques and methods are generally developed for a few, high profile, target species and are often ineffective against other species. Such is the case with *Wasmannia auropunctata,* an invasive ant species widely distributed throughout the tropics and subtropics.

Wasmannia auropunctata's ability to invade natural ecosystems, agriculture, and urban landscapes leads to milti-tired direct and indirect impacts on people, animals, and plants. Their unique biology, behaviors, and the diversity of habitat preferences has rendered traditional control methods effective under limited circumstances; thus, highlighting the need for species specific management strategies. The aim of this thesis was to consolidate all existing knowledge on *W. auropunctata* and expand on this knowledge base in a way that directly relates to developing effective management strategies for this species. Here, we have investigated chemical sensitivity, dietary effects on foraging, and bait matrix formulations for the densely vegetated habitats typical of the tropical and subtropical regions of the world. This research culminated in a large-scale "proof of concept" demonstration via an eradication effort on the island of Kaua`i, Hawai`i, USA. The management strategies developed in this thesis are suitable for the diverse habitats *W. auropunctata* inhabit while also being readily available to residents and agriculture workers without special permitting. It is also the intent of the authors that the information contained herein should also act a foundation for further research and development of advanced management strategies for area-wide management and eradication projects and site - specific management techniques.

Acknowledgements

For each of us, the search for, pursuit of, and success in a career path take different shapes. One thing is constant, though; no one does it solely on their own. While I have been fortunate enough to have many people in my life who have provided me with the support and opportunity to succeed in life and my career, there are two people, in particular, who have been instrumental throughout this journey. Without them, I likely would have never entered into this career path nor pursued higher education.

First, I would like to thank Dr. David Foote, my first boss and mentor in ecology and entomology. When I met David, I was a young adult, college dropout, with merely a passion for “bugs”. I had no experience to speak of yet David took a chance on me. As it turned out, we both quickly realized that I had an aptitude for entomology and conservation and I really enjoyed the work. The opportunity and support he provided gave me direction in life and inspired me to go back to school and finish my undergraduate degree. Fast forward nearly 20 years later and here I am, submitting my PhD thesis on invasive species management. Without David, there is no telling where I would be today or what I would be doing. I can only hope he knows of the tremendous impact he has had on my life and how grateful I am for his kindness and mentorship.

The other person I would like to highlight is my current boss, mentor, and friend, Dr. Casper Vanderwoude (Cas, aka “Bosso”). Cas’ mentorship has extended far beyond teaching me about applied pest management research. I have learned that my personal success is dependent on success of others. If the public is able to achieve their pest management goals based on my research, then I have personally succeeded as a researcher. If my coworkers and employees are supported enough to excel in their job while maintaining a work-life balance, then I have personally succeeded as leader in the workplace. If our “competitors” can succeed, meet project goals, and maintain their funding needs with our help, then I have personally succeeded as a collaborator and in building important professional relationships. While I have learned a lot and grown as a researcher under Cas’ guidance, it is what I’ve learned about the meaning of success that I value most. Over the past 12 years, Cas has guided me toward personal and professional success. For several years, Cas pressed me to pursue graduate studies, but, I rebuffed the idea of graduate school for financial

reasons, time commitment, and the inability to find a program that didn't require me to move. However, Cas refused to give up and he found an opportunity for me to pursue my PhD through the University of Canberra as a distance education student. I was presented with opportunity to maintain my job and apply my research toward a PhD thesis. This was an unexpected opportunity and one which I couldn't refuse. Even today, his mentorship continues and faith in me as a researcher and leader grows. There are no words to express the gratitude I feel for Cas being a part of my life.

Many other people have contributed to my success. I have been fortunate to have a fantastic supervisory committee with people with various backgrounds and research interests. Arthur Georges played a major role in my being admitted to the University of Canberra's Higher Degree in Research program and has been supportive throughout my candidature. Wayne Robinson guided me in improving my understanding of statistical analyses of experimental research data. Mark Lintermans was talked into joining my supervisory panel late in my candidature but has guided me through the final steps of this process and has held me accountable for required benchmarks and deadlines. Finally, Jasmine Lynch took on a huge role as my primary supervisor and stuck with me despite going through multiple career changes, herself, along the way. Her guidance throughout my candidature has made me a better researcher, writer, and has kept me on track even when professional and life challenges got in the way. I am grateful to each of these people and look forward to maintaining our relationships for many years to come.

This thesis is comprised of individual research projects that fit into a larger framework for developing species specific management tools and plans. Many people helped with each project and some were the culmination of larger collaborative efforts. I'd like to thank all of the people, coauthors, collaborators, and funding agencies who contributed to these projects along the way.

The entire staff (past and present) of the Hawaii Ant Lab

Hawaii Department of Agriculture

Hawaii Invasive Species Council

Pacific Cooperative Studies unit of the University of Hawaii

Kaua’i Invasive Species Committee

US Department of Agriculture – Agriculture Research Services

Without the help, collaboration, and funding support from these groups and agencies, the work contained in this thesis would not have been possible.

Finally, I’d like to thank my family and friends who’s support and faith in me has been unwavering.

- Mahalo Nui Loa

This thesis is dedicated to my grandfather, John P. Montgomery, who was a shining light in my life. When I was a child, I once told him that one day I would earn my PhD to which he replied grumpily “PhD’s are for professional students”. Now I can finally say “Look Grandpa! I’m finally a professional!”

He would be proud.

List of tables and figures

Table of Contents

Preface

This thesis contains 4 peer-reviewed and 1 unpublished manuscripts where I was the primary author and included contributions from co-authors. This constitutes the main body of this thesis which is “framed” by an introduction and a synthesis chapter. The outline of the thesis is as follows:

Introduction

Chapter 1: Montgomery, M. P., C. Vanderwoude, M. Lintermans, A. J. J. Lynch. 2022. The Little Fire Ant (Hymenoptera: Formicidae): A global perspective (Review). Annals of the Entomological Society of America, saac016, https://doi.org/10.1093/aesa/saac016.

Chapter 2: Montgomery, M. P., C. Vanderwoude, A. J. J. Lynch, and W. A. Robinson. 2020. The effects of laboratory rearing diet on recruitment behavior of *Wasmannia auropunctata* (Hymenoptera: Formicidaea). Florida Entomologist 103: 103-111.

Chapter 3: Montgomery, M., J. R. Hagler, C. Vanderwoude, A. J. J. Lynch, and W. A. Robinson. 2019. Laboratory Evaluation of Egg White and Milk External Biomarkers for Wasmannia auropunctata (Hymenoptera: Formicidae). Journal of Insect Science 19: 18.

Chapter 4: Montgomery, M. P., C. Vanderwoude, and A. J. J. Lynch. 2015. Palatability of baits containing (S)-methoprene to *Wasmannia auropunctata* (Hymenoptera: Formicidae). Florida Entomologist 98: 451-455.

Chapter 5: Montgomery, M. P., C, Vanderwoude, T. Keanini, C. Caneshige, A. J. J. Lynch. Eradicating little fire ants (*Wasmannia auropunctata)* from the island of Kaua`I, Hawai`i: process, challenges, and results to date. Unpublished

Chapter 6: Synthesis and Conclusions

The following pages contain the declaration of authorship contribution and extent of contribution for each of the co-authored manuscripts.

Declaration of Co-Authored Publications

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Declaration for Thesis Chapter 1

DECLARATION BY CANDIDATE

In the case of Chapter 1, the nature and extent of my contribution to the work was the following:

Nature of Contribution	Extent of Contributions (%)
Concept, literature search, review, synthesis, writing, editing	90

The following co-authors contributed to the work:

Name	Nature of Contribution	Contributor is also a UC student (Yes/No)
Casper Vanderwoude	Editing	No
Mark Lintermans	editing	No
Jasmyn Lynch	Editing	No

Candidate's Signature

12 / 07 / 2022
Date

DECLARATION BY CO-AUTHORS

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.

(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;

(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;

(4) there are no other authors of the publication according to these criteria;

(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and

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In the case of Chapter 2, the nature and extent of my contribution to the work was the following:

Nature of Contribution	Extent of Contributions (%)
Concept, experimental design, sample collection and processing, statistical analysis, writing, editing	85

The following co-authors contributed to the work:

Name	Nature of Contribution	Contributor is also a UC student (Yes/No)
Casper Vanderwoude	Editing	No
Jasmyn Lynch	Editing	No
Wayne Robinson	Statistical advising, editing	No

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DECLARATION BY CO-AUTHORS

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.

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Declaration for Thesis Chapter 3

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Nature of Contribution	Extent of Contributions (%)
Concept, experimental design, sample collection and processing, statistical analysis, writing, editing	80

The following co-authors contributed to the work:

Name	Nature of Contribution	Contributor is also a UC student (Yes/No)
James Hagler	Sample processing, editing	No
Casper Vanderwoude	Editing	No
Jasmyn Lynch	Editing	No
Wayne Robinson	Statistical advising, editing	No

Candidate's Signature

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DECLARATION BY CO-AUTHORS

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(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.

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	27 July 2022
James R. Hagler	July 26, 2022

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In the case of Chapter 4, the nature and extent of my contribution to the work was the following:

Nature of Contribution	Extent of Contributions (%)
Concept, experimental design, sample collection and processing, statistical analysis, writing, editing	85

The following co-authors contributed to the work:

Name	Nature of Contribution	Contributor is also a UC student (Yes/No)
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Jasmyn Lynch	Editing	No
Wayne Robinson	Statistical advising, editing	No

Candidate's Signature

12 / 07 / 2022
Date

DECLARATION BY CO-AUTHORS

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.

(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;

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	23 July 2022
	27 July 2022

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Declaration for Thesis Chapter 5

DECLARATION BY CANDIDATE

In the case of Chapter 5, the nature and extent of my contribution to the work was the following:

Nature of Contribution	Extent of Contributions (%)
Concept, Project management and implementation, treatments, sample collection and processing, writing, editing	80

The following co-authors contributed to the work:

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Jasmyn Lynch	Editing	No
Tiffani Keanini	Editing, project support	No
Craig Kaneshige	Treatments, sample collection, project support	No

Candidate's Signature

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DECLARATION BY CO-AUTHORS

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.

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Introduction

A Conceptual Framework for Understanding and Managing the Little Fire Ant

Background

Humans have transported exotic species throughout history, either deliberately to gain some benefit from their usage or presence, or accidentally such as with “hitch-hiker” species. The rate at which exotic species are moved beyond their native range and new locations worldwide has increased over time with technological and logistic advances in transportation, increased volume of transported goods, and globalization (Hulme 2009). Although only an estimated 1% of all exotic species introductions establish and become invasive alien species (IAS) (Jeschke et al. 2018), this is a poor metric for the severity and magnitude of impacts caused by IAS. Invasive alien species impart multitiered negative impacts on the ecosystems they invade, are a threat to agriculture and food security, pose human health risks, and result in economic costs through their impacts and in mitigating and managing their spread. These impacts have been estimated at US$ 26 billion annually for North America alone (Crystal-Ornelas et al. 2021).

Lying approximately 3600 km from any continental landmass, the Hawaiian Archipelago is one of the most remote island chains in the world and a hotspot for species radiations (Zimmerman 1970). Adaptive radiations from a few founder species gave rise to the wealth of biodiversity and local endemism in Hawai`i through highly specialized species diversifications (Craddock 2000). Unfortunately, Hawai`i’s unique biodiversity has largely been devistated by IAS (Kraus and Duffy 2010) and Hawai`i’s agricultural industry has been directly and indirectly impacted from the introduction and establishment of quarantine pests such as Tephritid fruit flies (Jang 2007). Over 250 new insect pest species have been recorded in Hawai`i since the year 2000 (Matsunaga et al. 2019). Some of these new pests have been detrimental to Hawaiian native flora such as the Erythrina gall wasp (*Quadrastichus erythrinae* Kim, Hymenoptera: Eulophidae), myoporum thrips (*Klambothrips myopori* Mound & Morris, Thysanoptera: Phaeolthripidae), and ramie moth (*Arcte coerula* [Guenée], Lepidoptera: Noctuidae) while many others are serious agricultural pests. For some of these species, general use insecticides have been effective at managing localized infestations. However, other species, including Erythrina gall wasp, coffee berry borer (*Hypothenemus hampei* [Ferrari], Coleoptera: Curculionidae), coconut rhinoceros beetle (*Oryctes* *rhinoceros* [Linneaus], Coleoptera: Scarabidae), and Queensland longhorn beetle (*Acalolepta aesthetica* [Olliff], Coleoptera: Cerabycidae), possess biological or behavioral traits that render general use insecticides ineffective.

The focal species for my research is the little fire ant, *Wasmannia auropunctata* (Roger) (Hymenoptera: Formicidae). Native to Central America and South America (Wetterer 2013), *W. auropunctata* has spread throughout the Pacific region and parts of Africa, Europe, and Western Asia where it is a pest of natural ecosystems, agriculture, and residential areas. Introduced to Hawai`i sometime in the mid-1990s (Conant and Hirayama 2000), *W. auropunctata* has spread throughout four of the main Hawaiian Islands (Vanderwoude et al. 2015). Since its establishment in Hawai`i, *W. auropunctata* has become a major pest of residential, agriculture, and forest ecosystems. The most notable impact by *W. auropunctata* is its painful sting. However, lower biodisversity and increases of disease vectoring plant pests, such as mealybug and scale insects, are also typical examples of the negative impacts this species imparts on ecosystems it invades. Early eradication efforts showed that traditional ant control methods were ineffective against this species and meant that species-specific control methods and management plans would be needed.

Effective species-specific control methods and management plans can take years to develop and the process is a highly nuanced endeavor. Research of this sort must follow a logical and progressive flow, with each step in the process building upon a previous step to build the evidence basis. This research process begins with a comprehensive literature review of the invasive species’ natural history, impacts, and current knowledge of control methods and their effectiveness. This literature review aids in identifying gaps in knowledge and directing research focus for controlled laboratory trials and field trials (figure 1). Laboratory and field components of the research process are complementary and encompass multiple sub-components which can be further divided into additional subcomponents, and so on (figure 1). Although the finer research points may vary from one species to another, the general process is the same when developing control methods and management plans for all insect pests.

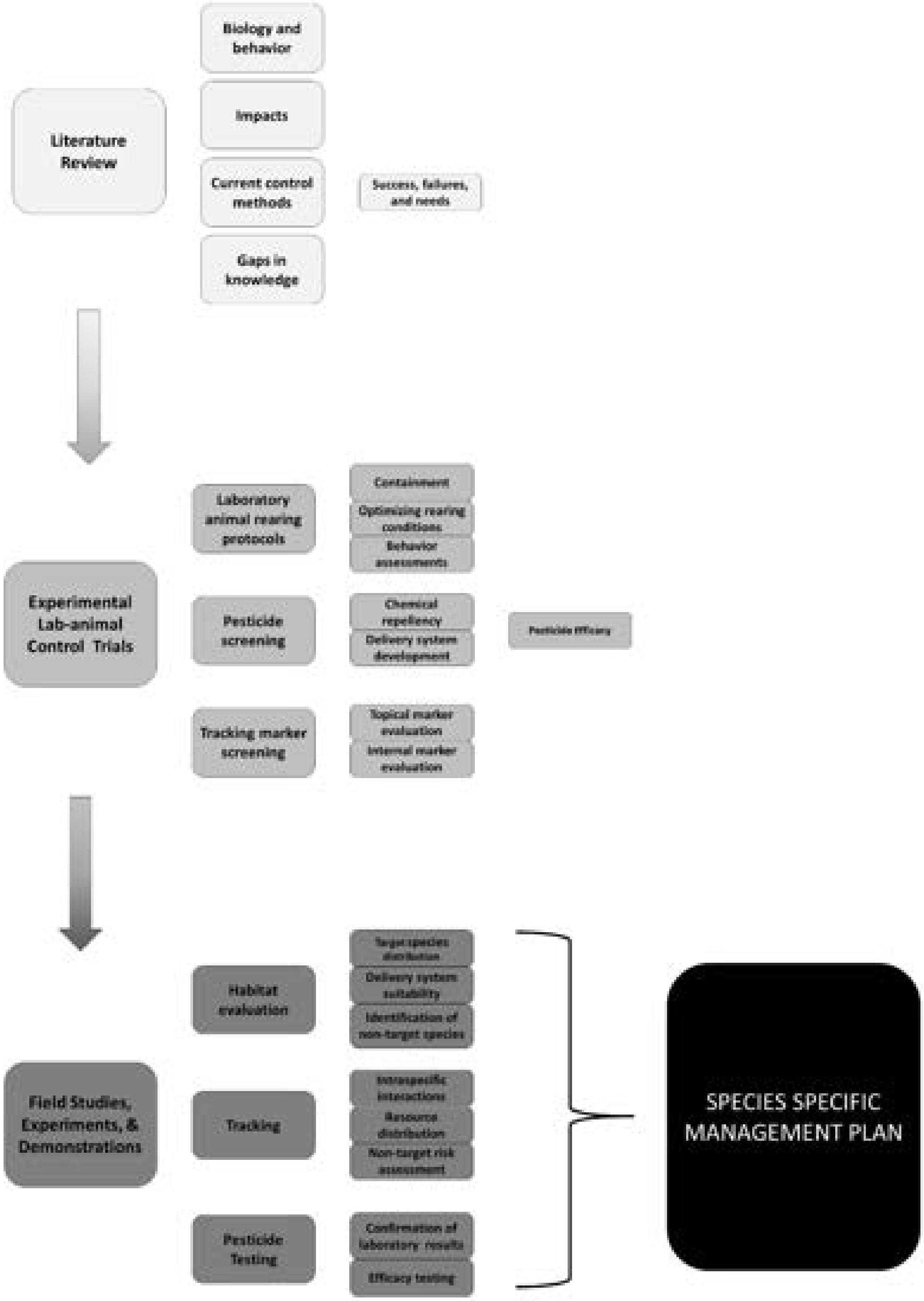

Figure 1: Flow chart showing the direction of research involved in developing species-specific control methods and management plans. Main research categories are divided into subcategories, tertiary subcategories, and so on detailing important specialized research topics. This is the framework adopted by this thesis.

The overarching objective of this thesis is to develop control methods and an effective management plan specifically for *W. auropunctata* in the tropics, with a focus on Hawai`i. While generally relevant to tropical environments, the foundational concepts discussed in each chapter can, and should, be tailored to suit the specific needs of an infested site and the capacity and resources of a land manager. This thesis consists of five published, peer-reviewed manuscripts fitting into the research framework presented in figure 1.

Chapter 1 is a comprehensive review of all publications between 1929 and 2021 where *W. auropunctata* was either the primary focus or one of the primary foci of the study. This review is foundational, not only to show where there are gaps in our knowledge about this species but also to provide context for the rest of the thesis. It synthesizes the global research on the species over the past 92 years into a single document, highlighting areas of consensus and disagreement within the scientific community. This will serve as an important reference for future researchers seeking specific information and further resources on *W. auropunctata* and similar invasive ant species.

Chapter 2 investigates the disparities in foraging behavior between wild and laboratory-raised ants. Ant control relies heavily on the use of insecticidal baits which exploit the natural foraging behavior of ants. However, behavioral differences between wild and laboratory-raised insects are commonplace and often attributed to the dissimilarity of the conditions in which the insects live or have been raised. Nevertheless, laboratory experiments are an important part of the insecticidal bait development process and are used to screen new bait matrices and active ingredients prior to conducting field trials. Differences in foraging behaviors between wild and laboratory-raised ants can confound results from such laboratory experiments. In this chapter, I investigate whether laboratory rearing diet is a driving factor for observed behavioral differences in *W. auropunctata* and whether the diet of laboratory-raised ants can be manipulated in order to illicit a “wild type” foraging response without negatively affecting laboratory colony health.

Chapter 3 examines the potential of topical marking techniques for studies focusing on tracking insect movement and resource flow. Mark – release – recapture (MRR) and mark – capture (MC) studies have been used to investigate insect behavior, population dynamics, dispersion, and food resource allocation for many years (Sunderland et al. 1995, Bowler and Benton 2005, Cordero-Rivera and Stoks 2008, DeGrandi-Hoffmann and Hagler 2000). Tracking individual ants allows us to investigate intra-specific competition and cooperation, foraging dynamics such as how far ants forage from the nest, and to estimate population densities (Buczkowski 2012). Food resources also can be laced with a marker and used to track the flow of resources through a nest, colony, or landscape (Hagler et al. 1992, DeGrandi-Hoffman and Hagler 2000). This kind of information is valuable when determining application rates of insecticidal ant baits or for identifying the number and spacing of bait stations for effective ant control (Song et al. 2017, Hogg et al. 2018). Prior to this research, no marking techniques had been tested for suitability on *W. auropunctata* and this chapter represents the first in a series of ongoing studies dedicated to developing effective marking techniques for this species. Here we test an immuno-marking technique under laboratory conditions, reportedly effective at marking minute parasitoids, for suitability on *W. auropunctata*.

Chapter 4 details the development of a novel ant bait for *W. auropunctata.* Traditionally, commercial insecticidal ant baits have been used to control *W. auropunctata;* however, these baits have proven to be unreliable in tropical habitats. Three factors appear to be the primary contributors to the observed sub-optimal efficacy: 1) the baits are formulated to treat ground-nesting ants rather than ants such as *W. auropunctata* that are also arboreal; 2) the baits become unattractive and rapidly degrade once wetted by dew or rain, a significant consideration in Hawai`i where the rainfall is over 3,000 mm per annum (https://www.usclimatedata.com/climate/hilo/hawaii/united-states/ushi0022, Accessed 16 March 2022); and 3) repellency of some common active ingredients in the baits which reduces their level of uptake by the target ants. Prior to this thesis, a novel gel bait matrix was developed which would adhere to vegetation and was less susceptible to degradation from moisture. The research in this chapter builds upon that earlier work and focuses on the latter point of chemical repellency and the inclusion of adjuvants and phagostimulants to mask the presence of repellent elements, thereby increasing bait acceptance.

Chapter 5 is the final chapter in the main body of the thesis. Here I detail and demonstrate the efficacy of a management plan for the eradication of a historical *W. auropunctata* infestation on the Hawaiian island of Kaua`i. In this chapter, I discuss the challenges faced by practitioners during treatment and monitoring of the site and how the flexibility of an adaptive management approach to invasive species control allows for those challenges to be effectively addressed. An adaptive management method is a structured decision-making approach, which can be active (i.e. hypothesis testing) or passive (incorporating learning), and is founded on the concept that there is no predetermined “correct” method to managing a target pest. It identifies, tests and implements alternative control methods, at an appropriate time, which may be best suited to specific site features or the needs of project stakeholders and land managers.

Although the effective control methods and management plan was developed for *W. auropunctata* through the research presented here, much more can still be done. Research is ongoing to develop a suitable marking technique for this species which will allow better assessment of different bait application methods and pesticide application rates. Future research should also focus on the use of other insecticidal active ingredients (especially reduced risk insecticides) in ant baits, and investigate the potential for identified natural enemies of *W. auropunctata* as biological control agents. While the control methods described in this thesis are effective, the identification and inclusion of additional effective control methods and refinement of existing control methods is essential to improving cost-effectiveness and enabling integrated pest management and adaptive management approaches to pest control. Currently, the control methods and general management plan described in this thesis have become the standard treatment for *W. auropunctata* in Hawai`i and throughout the Pacific. Additionally, the concepts described here are currently being used to develop aerial gel bait application techniques for large-scale landscapes without access for land managers.

Chapter 1

The Little Fire Ant (Hymenoptera: Formicidae): A global perspective (Review)

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Review

Review

The Little Fire Ant (Hymenoptera: Formicidae): A Global Perspective

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Abstract

Wasmannia auropunctata (Roger) is an invasive tramp ant species that has been transported globally since [at least] the early twentieth century. It is often claimed that despite the negative impacts associated with this species and its listing among the world's worst invasive species, very little research attention has been paid to *W. auropuntata*. Although the need for future research exists, there is currently a considerable body of research from around the world and spanning back to the 1920's on this species. Here we synthesize over 200 peer reviewed research manuscripts, book chapters, conference presentations, and media reports of new distributions spanning 1929–2022 culminating in a comprehensive literature review on *W. auropunctata*. This review covers all current knowledge on this species and is intended to serve as a quick reference for future research and provide the reference resources for those seeking more in-depth information on specific topics. Topics included in this review include taxonomic identification, current global distribution and pathways, life history, impacts, detection, and control. We discuss where consensus and ambiguity currently lie within the research community, identify contextual considerations for future researchers when interpreting data, and suggest where we believe more research or clarifications are needed.

Key words: little fire ant, distribution, biology, impact, control

Invasive alien species (IAS) are species that have been introduced beyond their native range and negatively impacts those new areas. The negative impacts associated with IAS range from human health risks to reduced species diversity, plant and animal disease transmission, and altered ecosystem processes (Resnik 2018, Liang 2019). Insects are particularly easy to transport inadvertently from one place to another (Meurisse et al. 2019) and eusocial insects are predisposed to be successful invaders wherever they are introduced (Bertelsmeier 2021, Eyer and Vargo 2021) Specifically, invasive Hymenoptera are well known for causing multitiered negative impacts wherever they are introduced.

Ants (Hymenoptera: Formicidae) are among the most species-rich taxa in the animal kingdom with over 12,000 described species (Ward 2007). They are important for healthy ecological functioning (Andersen 1988, Abbott 1989, Folgarait 1998, Del Toro et al. 2012) but some cause negative ecological and human health impacts. The worst invasive ant species are also notable 'tramp' species due to the ease at which they are transported by anthropogenic means and their ability to thrive in areas disturbed by humans (Wilson and Taylor 1967, McGlynn 1999, Loope and Krushelnycky 2007). Other factors attributed to the success of some invasive ants include a combination of biological and behavioral traits such as polygyny, unicoloniality and low intraspecific aggression, high interspecific aggression, reproduction within the nest, and colony founding via budding instead of nuptial flights (Hölldobler and Wilson 1977, Brandao and Paiva 1994, Passera 1994, Jourdan 1997a, Helms and Vinson 2002, Holway et al. 2002).

The Little fire ant, *Wasmannia auropunctata* (Roger) (Hymenoptera: Formicidae) is an invasive tramp ant listed as one of the world's worst 100 IAS (Lowe et al. 2000), and has an alarming rate of spread globally with 43% of known new introductions occurring since the year 2000 (Wetterer 2013, Gruber et al. 2016, Espadaler et al. 2018, GBIF.org 2021, Vanderwoude et al. 2021, Chen et al. 2022). Consequently, research interest in this species has increased over the past 20 yr. Previous literature reviews on *W. auropunctata* to date have either focused on its global or local distribution, with life history, control, and impacts being minor components (Wetterer and Porter 2003, Herrera and Causon 2008, Wetterer 2013, Vanderwoude et al. 2015) or have focused on the latter three topics while omitting distribution information (Bousseyroux et al. 2019). More recently there has been considerable research into life history, control, and impacts and reports of new distribution data that has yet to be synthesized.

Here, we provide the first comprehensive literature review synthesizing all research on this species before June, 2022. The review is timely given the ongoing spread of this serious global pest and the need for research into cost-effective control methods. We review literature spanning the past 90 yr detailing its taxonomy, life history, distribution, range expansion, impacts, detection, and control of *W. auropunctata*. We provide an overview of its significance as an invasive species and identify priority areas for further research to fill knowledge gaps. Literature included in the review met two primary criteria: 1) it was published in a peer-reviewed journal, book section, government report or document, university scientific research, or extension report; and 2) *W. auropunctata* was either the primary focus or one of the primary foci of the study. Exceptions to these criteria were when the information obtained was novel in context and not reported elsewhere, such as new research presented during a conference or media report detailing a first detection for a locality.

Table 1. Past taxonomic synonyms for *Wasmannia auropunctata* previously described as separate species (Longino and Fernández 2007)

Taxonomic synonyms	
Wasmannia atomum	(Santschi 1914)
W. australis	Emery 1894
W. glabra	Santschi 1894
W. laevifrons	Emery 1894
W. obscura	Forel 1912
W. panamana	(Enzmann 1947)
W. pulla	Santschi 1931
W. nigricans	Emery 1906
W. rugosa	(Forel 1886)

Taxonomy and Systematics

Commonly referred to as little fire ant, *W. auropunctata* was first described by Roger in 1863 and originally included as a species of *Tetramorium* (Smith 1929, Nickerson 1983). After the genus *Wasmannia* was described by Forel in 1893, some arguments persisted around whether the species belonged within *Wasmannia* or the previously described genus *Ochetomyrmex* Mayr (Nickerson 1983, Longino and Fernández 2007). However, Longino and Fernández (2007) conducted a taxonomic review of *Wasmannia*, clearly distinguishing it from *Ochetomyrmex*, and provided a revised key for *Wasmannia*, including *W. auropunctata*.

Currently, ten *Wasmannia* species have been described, with *W. auropunctata* being the most common and widely distributed (Longino and Fernández 2007, Cuezzo et al. 2015). Although 21 *Wasmannia* species have been described historically, over half of these species were subsequently determined to be synonyms of other species and nine are currently considered to be synonymous with *W. auropunctata* (Table 1) (Longino and Fernández 2007).

Generalized ant taxonomy diagrams are presented in Fig. 1 for those unfamiliar with ant taxonomy or taxonomic terminology. Detailed morphological descriptions of *W. auropunctata* may be found in Ulloa-Chacon and Cherix (1990), Wetterer and Porter (2003), and Longino and Fernández (2007), but each description includes different morphological characteristics. Summarizing across all three descriptions, *W. auropunctata* may be identified by the following morphological characteristics:

Workers (Fig. 2) are tiny (~1.2–1.5 mm), monomorphic, rust-colored to pale yellow-brown myrmicine ants with two pedicle segments (petiole and post petiole) and long propodeal spines. The petiole node is roughly quadrate, approximately as high as it is wide. The hind margin of the node is slightly shorter than the fore margin when viewed in profile and meets the peduncle at nearly a 90-degree angle. The head and body are heavily sculptured with transverse

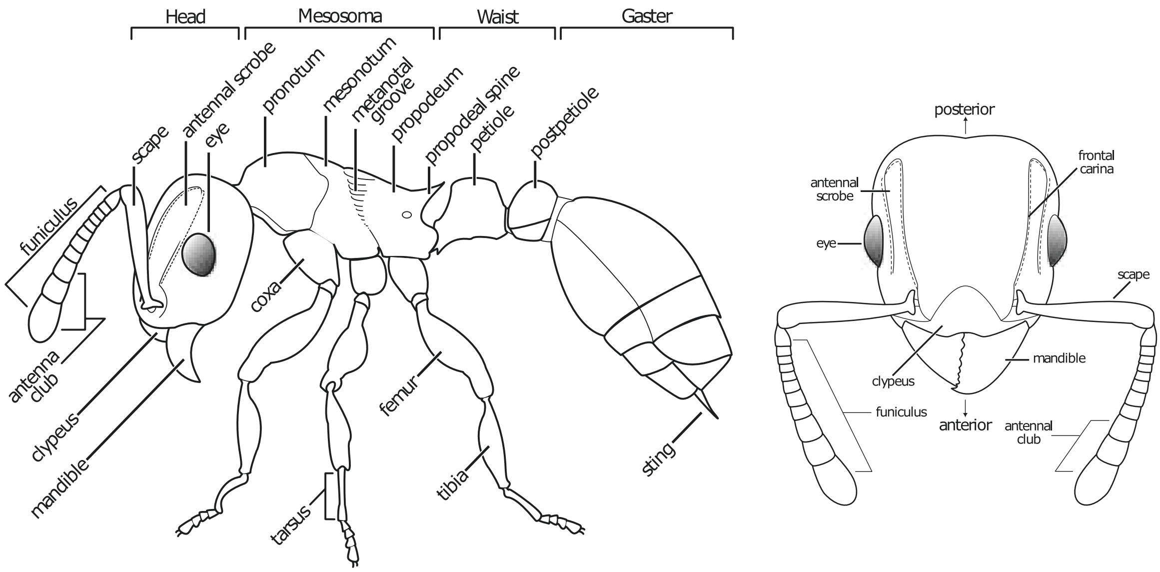

Fig. 1. General ant taxonomy diagrams of the whole body as viewed in profile (left) and the head as viewed from the front (right). Diagrams created by Eli Sarnat and used with permission.

rugules and reticulations, and sparse erect setae. The antenna consists of 11 segments. Funicular segments of the antenna gradually enlarge with the apical 2 segments distinctly larger than the rest and forming a club. Antennal scrobes are present, although shallow, and distinguishable by the presence of two prominent frontal carinae. Although generally considered 'monomorphic', aberrant worker morphs are common in large sample collections.

Reproductives (Figs. 3 and 4) are approximately three to four times larger than the workers (queens: 4.5–5.0 mm, males 4.2–4.5 mm). Two sympatric queen morphs have been documented, large- and small-headed queens. The antenna, sculpturing, and pubescence of the queens are similar to that of the workers. Queen coloration is uniformly dark brown but younger queens may be light brown and darken with age. Propodeal spines are present but shorter than those of workers. The node of the petiole gradually tapers upward but with distinct anterior and posterior angles along the dorsal margin. Wings may or may not be present. When present, the medial and SM1 cells are enclosed and the discoidal cell is absent on the fore wing (Fig. 3c). When not present, the sutures where the wings were detached are clearly visible.

Males (Fig. 4) are dark brown with yellowish antenna, legs, and genitalia. Antenna are long, 13-segmented, and without an apical club. Propodeal spines are absent, but the propodium angles sharply downward at a 90-degree angle. The petiolar node is large and dorsally rounded rather than angular. The parameres of the genital valve are long, curved intero-ventrally, and apically rounded (Fig. 4c.).

Life History

Genetics

Cytogenetics and molecular genetics provide insights on evolutionary and biological processes as well as population dynamics and species identification. To date, the mitochondrial genome has been mapped and macrosatellite markers have been identified for *W. auropunctata* which provided the tools necessary to conduct important foundational research (Fournier et al. 2005b, Souza et al. 2009, de Souza et al. 2011, Duan et al. 2016, Silva et al. 2018). The use of various genetic analyses have allowed researchers to trace the evolutionary history of this species (Chifflet et al. 2016), trace and track historical and current population expansions (Foucaud et al. 2010b, Chifflet et al. 2016, Coulin et al. 2019), distinguish native from exotic populations (Foucaud et al. 2010b), trace the origins of exotic populations (Foucaud et al. 2010b, Coulin et al. 2019), and identify certain biological and behavioral traits linked to invasive potential (Fournier et al. 2005b; Foucaud et al. 2006, 2010b; Mikheyev et al. 2009; Souza et al. 2009; Vonshak et al. 2009; Rey et al. 2011; Tindo et al. 2012). Additionally, genetics can help to identify when and where evolutionary adaptations occurred that has led to *W. auropunctata* being able to invade such a wide range of ecosystems and climates (Rey et al. 2012, Foucaud et al. 2013, Chifflet et al. 2016, Coulin et al. 2019).

The field of genetics is rapidly expanding with new technologies for phylogenetics, species identification, detection, and even pest control. Two technologies with especially promising applications are the use of environmental DNA (eDNA) for detection and RNA interference (RNAi) for control of pest ants. Trace amounts of genetic material are now able to be detected. That, with the advent of metabarcoding, has led to the growing practice of using eDNA to detect species presence from water or substrate samples without direct observation or collection of the target species (Kudoh et al. 2020, Uchida et al. 2020). Proof of concept for using eDNA as an ant detection tool has been reported for *Linepithema humile* Mayr (Yasashimoto et al. 2021). However, eDNA technology requires further development and testing before it can be deemed as a reliable tool for the detection of other species and under different scenarios. Development of RNAi technology for ant control appears to be promising as a future alternative to conventional pesticides. This is the process by which double-stranded RNA (dsRNA) or DNA (dsDNA) is used to stop the normal functioning of messenger RNA (Allen 2021). A construct of dsRNA or dsDNA may be delivered to the target pest via genetically modified crops, sprays, and bait delivery systems (Cagliari et al. 2019) and, when integrated into cells, interferes with gene transcription and effectively activates or silences gene expression. However, efficacy varies depending on target species and delivery system (Allen 2021). Functionality of RNAi on ants has been demonstrated (Allen 2021, List et al. 2022), but considerably more work is needed before the technology can be considered a viable control method (Allen 2021, List et al. 2022). Species-specific target genes must be identified and corresponding dsRNA or dsDNA constructs need to be developed. Degradation of dsRNA and dsDNA due to exposure of digestive enzymes, through trophallaxis, and under field conditions for various delivery systems is currently unknown and needs further investigation (Allen 2021). Finally, standardized testing and evaluation procedures need to be agreed upon

Fig. 2. *Wasmannia auropunctata* worker profile (a) and detail or head (b.). Photographs by: E. M. Sarnet, specimen CASENT 0171093. From www.antweb.org

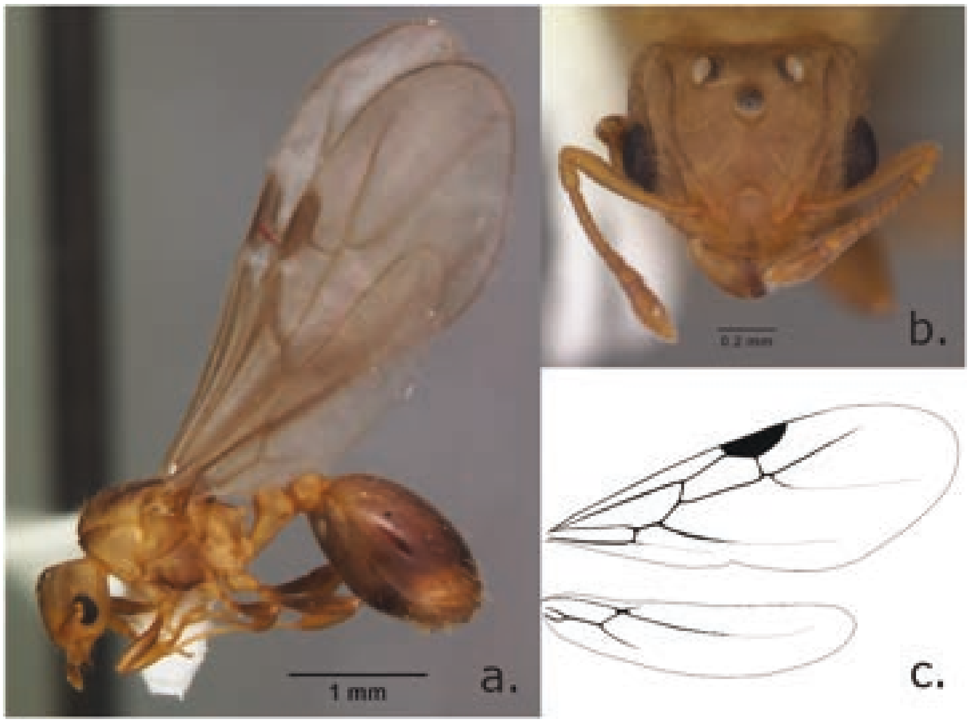

Fig. 3. *Wasmannia auropunctata* queen profile (a), detail of head (b), and wing diagram (c). Photographs by: A. Nobile, specimen CASENT 0102747. From www.antweb.org

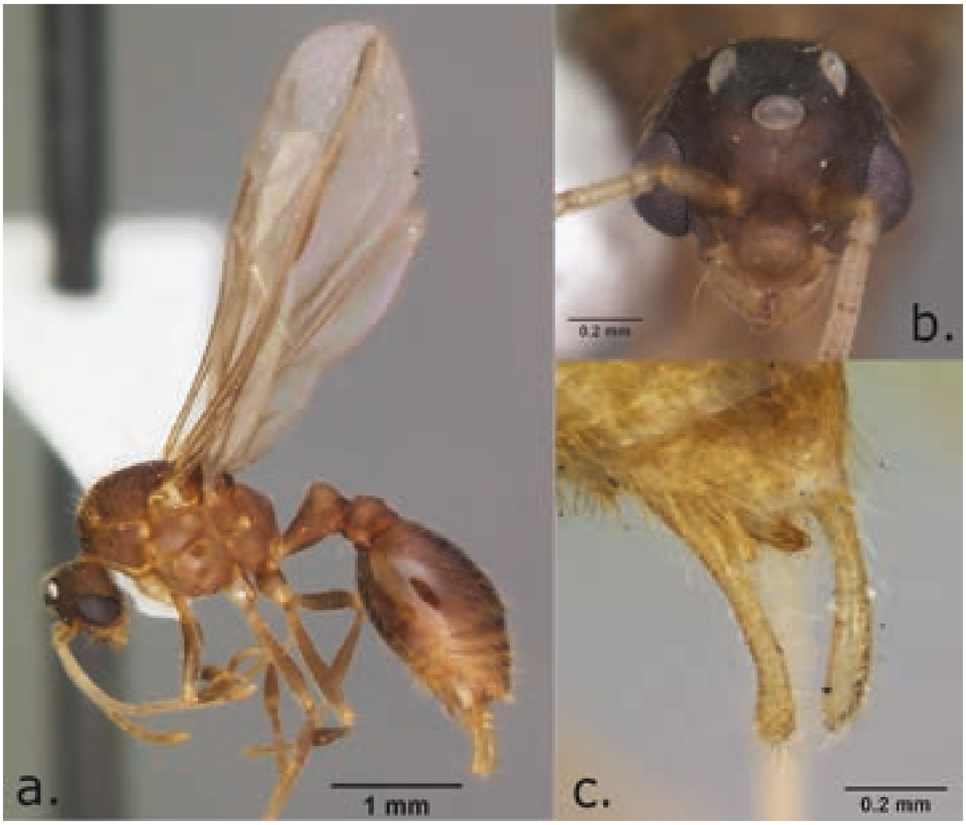

Fig. 4. *Wasmannia auropunctata* male profile (a), detail of head (b), and parameres (c). Profile and head photographs by: A. Nobile, specimen CASENT0102748. Parameres photograph by: A. Nobile, specimen CASENT 0173250. From www.antweb.org.

by the scientific community as it is with conventional pesticides to establish baseline expectancies for field efficacy trials (Allen 2021, List et al. 2022).

Reproduction and Development

Rudimentary aspects of *W. auropunctata* reproduction and development were first described by Ulloa-Chacon and Cherix (1990). Only queens lay eggs and fecundity fluctuates over a queen's lifespan, suggesting that fecundity is influenced by age and colony composition (Ulloa-Chacon and Cherix 1990). While worker brood is produced regularly to maintain nest population, reproductives are produced when high worker:brood ratios occur in the nest or when queen fecundity is low (Ulloa-Chacon and Cherix 1990).

Wasmannia auropunctata reproduction is unique and complex. They are not a typical haplodiploid species as with most social Hymenoptera (Fournier et al. 2005a). In typical haplodiploid reproduction systems, diploid females (queens and workers) are the progeny of sexual reproduction and haploid males are produced through arrhenotokous parthenogenesis, males developing from unfertilized eggs (Normark 2003). However, *W. auropunctata* exhibit a complex reproduction system, where the reproductive mode varies between different populations (Foucaud et al. 2007, 2009, 2010a).

Reproductive castes are produced in several ways. Haploid males are produced either via arrhenotokous parthenogenesis (arrhenotoky), males developing from unfertilized eggs, or via androgenesis, males developing from fertilized eggs through the elimination of the entire maternal genome. The latter process is rare in the animal kingdom and results in male clones that are genetically identical to their fathers. Diploid queens are produced through normal sexual reproduction or via automictic thelytokous parthenogenesis with central fusion (Rey et al. 2011). Automictic thelytokous parthenogenesis occurs from the fusion of two meiotic oocytes. Unusually low recombination rates during the meiotic division process result in clonal diploid queen lineages (Rey et al. 2011).

The occurrence of one reproductive mode or another is linked to *W. auropunctata* ecological dominance (Foucaud et al. 2009). Typical haplodiploid reproduction (sexually produced queens and arrhenotokous males) occurs primarily among nondominant variegate populations whereas clonal reproduction (automictic parthenogenesis and androgenesis) occurs primarily among dominant variegate populations (Foucaud et al. 2009, 2010a). Even though there is a trend linking dominance and reproductive mode, this is not a strict rule. Occasionally dominant sexual population, clonal nondominant populations, and rare occurrences of sexual reproduction within clonal populations have been detected (Foucaud et al. 2006, 2009, 2010b; Tindo et al. 2012). Interestingly, whether males are produced via arrhenotoky or androgenesis is maternally determined (Rey et al. 2013a). Queens from typical haplodiploid nests never produce male clones. They only produce arrhenotokous males whereas clonal queens produce androgen male clones, regardless of insemination or genetic lineage of the fathers (Foucaud et al. 2010a, Rey et al. 2013a). While it is possible for parthenogenic queens to produce arrhenotokous males, this phenomenon has only been documented during a single laboratory experiment (Tindo et al. 2012) and has not been detected among wild populations. It is possible this occurs at such low rates that research to date has failed to detect it.

Speculation around what leads to the expression of one reproductive mode over another is ongoing. An early hypothesis was that endosymbiotic bacteria, such as *Wolbachia,* could be responsible for the shift from typical haplodiploid reproduction to clonal reproduction (Rey et al. 2013b). *Wolbachia* is a widespread arthropod endosymbiont that is maternally inherited and influences sex determination during reproduction, including through the initiation of thelytokous parthenogenesis (Werren et al. 2008). *Wolbachia* is a known endosymbiont of *W. auropunctata*, but *Wolbachia* is less prevalent in clonal populations than in typical haplodiploid populations suggesting that it was not likely responsible for the reproductive difference (Rey et al. 2013b). The current hypothesis surrounding the expression of one reproductive mode over another involves the influence of ecological factors. Some sources suggest *W. auropunctata* are typically found amid floodplains (i.e., creek beds) within primary forests and clonal reproduction may have arisen as an evolutionary response to the repeated disturbance experienced in such habitats (Rey et al. 2012, Chifflet et al. 2018). Nests in floodplains would likely be under pressures such as fragmentation, transportation, and colony founding with every flooding event. Studies on the emergence of clonal reproduction in plants have suggested this reproduction mode may have evolved as an alternative lifecycle loop allowing populations to persist despite the absence of the necessaries sustaining the species normal lifecycle, such as a mate (Honnay and Bossuyt 2005). It is possible that the reproductive plasticity of *W. auropunctata* evolved in a similar way. Repeated flooding events likely increased the frequency of population fragmentation and a need to establish and persist for a short period of time without males. If this is the case, this phenotypic plasticity or adaptation to ecological pressures of floodplain habitats has undoubtedly become a leading factor contributing to their success as an invading species.

The unusual reproductive system of *Wasmannia auropunctata* has been implicated as an important factor contributing to the successful establishment of small founder colonies (Mikheyev et al. 2009). For newly introduced species, the establishment phase is particularly difficult (Foucaud et al. 2009, Mikheyev et al. 2009). In general, founding populations of an introduced species frequently fail to establish as a consequence of the principal cost of sex; the need to find a mate (Smith 1978). For those that do establish, the genetic diversity of the population is low due to few individuals surviving and reproducing. Such a genetic bottleneck leads to inbreeding and loss of heterozygosity over time within sexually reproducing populations and, theoretically, loss of fitness. However, reproductive plasticity in *W. auropunctata* allows for the preservation of heterozygosity when genetic diversity is low and avoids the consequences of inbreeding (Foucaud et al. 2010a, Rey et al. 2013a). It is likely that clonal reproduction via thelytokous parthenogenesis allows for the persistence of genetic adaptations responsible for *W. auropunctata* being able to successfully invade a wide variety of human modified habitats. Indeed, introductions of single female and male genotypes can give rise to area-wide infestations as seen in New Caledonia (Foucaud et al. 2006), Hawai`i (Mikheyev et al. 2009), Cameroon (Mbenoun Masse et al. 2011) and Israel (Vonshak et al. 2009). Rarely, recombination, mutation, and sexually produced queens within clonal populations have been observed which would add small amounts of genetic diversity within otherwise clonal populations (Foucaud et al. 2006, Vonshak et al. 2009, Tindo et al. 2012).

Although insemination does not contribute to *W. auropunctata* genetic diversity and is not strictly required for a queen to lay viable eggs, insemination is necessary to maintain egg development and hatching success (Miyakawa and Mikheyev 2015). Despite the possibility of virgin queens producing viable brood, the rate of successful hatching, pupation, and emergence among uninseminated brood is too low for colony maintenance (Miyakawa and Mikheyev 2015). Sex is therefore an essential part of both reproductive modes.

Nesting and Population Dynamics

Rather than building subterranean nests and mounds like many other ants, *W. auropunctata* prefer to nest opportunistically in warm, moist, and shaded areas (Ulloa-Chacon and Cherix 1990, Wetterer and Porter 2003). They can exploit ecological or man-made features on the ground, in tree canopies, and in other vegetation (Clark et al. 1982, Wetterer and Porter 2003, Le Breton et al. 2005, Mikissa et al. 2013, Álvarez et al. 2018, Santos et al. 2019, Wisniewski et al. 2019). Ideal nesting locations include leaf litter, under rocks and logs, loose bark, clusters of moss, epiphytes, and plant petioles (Wetterer and Porter 2003), but the species also frequently nests in electrical sockets, vehicles, machinery, pallets, metal pipes, furniture, garbage piles, and anywhere that provides shade, moisture, and refuge.

Individual nests are small and often difficult to identify (Clark et al. 1982). Nests are comprised of brood (eggs, larvae, and pupae), sterile workers, multiple queens, and occasionally males. A single nest may be divided into separate aggregations, of which three types have been described; workers + brood + queens, workers + brood, and workers only (Ulloa-Chacon and Cherix 1990). The purpose of separate aggregations and caste segregation has not been identified but could be due to capacity limits at the nest location, protection and defense of brood and queens, or other reasons. Such nesting habits allow *W. auropunctata* to utilize all available nesting sites in an area and support extremely high population densities (Hölldobler and Wilson 1995, Souza et al. 2008).

Typical queen:worker ratios for a *W. auropunctata* nest have been described as ranging between 1:250 and 1:500 with up to 16 queens per nest (Ulloa-Chacon and Cherix 1990). However, one cannot ignore the question of what defines an individual nest within a supercolony and when a 'single' nest may be comprised of separate aggregations. Indeed, many more queens have been observed within proximity to each other amid large infestations (personal observation) but it is uncertain how common this is or what factors influence the number of queens per nest. As with other eusocial insects, older workers forage outside of the nest while younger workers remain within the confines of the nest and care for queens and brood, a process referred to as temporal polytheism (Robinson et al. 1994, Ortiz-Alvarado et al. 2021). Queens are typically relegated to laying eggs but will forage and care for brood when worker populations are low (Ortiz-Alvarado and Rivera-Marchand 2020). This behavior likely contributes to *W. auropunctata* surviving the colony founding period when introduced to new locations and during the budding process.

Two *W. auropunctata* ecological variegates are known throughout their native range: nondominant and dominant (Levings and Franks 1982, Tennant 1994, Foucaud et al. 2009, Orivel et al. 2009). The nondominant variegate typically reproduces sexually and is restricted to natural, primary forests throughout its native range. Although common, nests are diffusely dispersed throughout the forests and these *W. auropunctata* do not display the same level of interspecific aggression as the dominant variegate (Tennant 1994, Salguero Rivera et al. 2011). The dominant variegate typically reproduces clonally and is widely distributed in human modified habitats through its native and introduced ranges (Foucaud et al. 2009, Orivel et al. 2009, Chifflet et al. 2018). The dominant variegate displays high levels of interspecific aggression and other behavioral traits that allow *W. auropunctata* to successfully dominate other ant species. Interestingly, before the 1980s, the nondominant variegate was unknown (Levings and Franks 1982) despite the current hypothesis that the dominant variegate arose from nearby nondominant populations (Foucaud et al. 2007). The behavioral and physiological plasticity of *W. auropunctata* and correlation between human disturbance and dominance has led researchers to label *W. auropunctata* as a 'disturbance specialist' (Majer 1999, Solomon and Mikheyev 2005, Foucaud et al. 2009, Orivel et al. 2009, Chifflet et al. 2018, Achury et al. 2020). Despite this designation, it is still unclear whether certain types and intensity of disturbances create unsuitable habitat (Rojas et al. 2021). For example; although *W. auropunctata* is a well-known agricultural pest, they may not be able to invade annual cropping systems as well as perennial crops and orchards due to annual cropping systems undergoing frequent harvesting, tillage, and replanting (Rojas et al. 2021). Additionally, development of rural areas and increased urbanization dramatically alters landscapes in a way that may reduce suitable habitat for *W. auropunctata* while becoming more suitable to other ant species (Mbenoun Masse et al. 2021). It is likely the effects of disturbance on *W. auropunctata* invasion vary from one location to another and this should be looked into further. Low intraspecific aggression allows workers to freely move between nest aggregates and share food resources, thus forming three dimensional 'supercolonies' (Foucaud et al. 2009). While both the nondominant and dominant variegates build supercolonies, those of the nondominant variegates are smaller and multiple, genetically distinct, supercolonies are present throughout a given landscape (Foucaud et al. 2009). Alternately, the dominant variegate is often unicolonial, building a single expansive supercolony with undefined nest boundaries and extending over hundreds of kilometers (Hölldobler and Wilson 1977, Le Breton et al. 2004, Errard et al. 2005). This unicolonial social organization differs from most ant species (Hölldobler and Wilson 1990) but is common among invasive ants (Holway et al. 2002). Such cooperative networks are conducive to exponential population growth, easily supporting densities of 20,000 workers and 37–52 queens per square meter (200 million workers and 370,000–520,000 queens per ha) (Ulloa-Chacon and Cherix 1990, Souza et al. 2008). This population estimate is over five times that of the estimate for polygynous *Solenopsis invicta* Buren by Macom and Porter (1996) and may be among the highest of all ant species in the world. Hölldobler and Wilson (1990, p. 63) described *W. auropunctata* as '*creating a living blanket of ants that kill and eat nearly all other ants in their path*'.

The underlying cause for the shift towards ecological dominance is not well understood. Foucaud et al. (2009) suggested that human disturbance, rather than unicolonality, is likely responsible for triggering ecological dominance. This hypothesis is supported when the history of invasive populations and the impact of *W. auropunctata* on the local fauna are considered. The alteration of natural ecosystems into agricultural and urban environments results in shifts of biotic and abiotic pressures. Reduced biodiversity in altered ecosystems often creates open niches ready for exploitation. Open niches and the inability of local species to successfully defend against *W. auropunctata* likely allow for the exhibition of dominant behaviors early on in the invasion process and before the formation of large supercolonies. Over time, an invading supercolony is able to expand its boundaries because *W. auropunctata* is able to dominate, outcompete, and displace other species. The degree of impact on local arthropod fauna is not universal since some species are able to successfully defend against *W. auropunctata* (Le Breton et al. 2007a, b; Mbenoun Masse et al. 2019b, 2021; Perfecto and Vandermeer 2020b). Could the presence of highly competitive local arthropod fauna also play a role in regulating ecological dominance of native and exotic *W. auropunctata* populations? It is interesting that the global distribution of other well known invasive ant species overlaps that of *W. aurpounctata* yet their competitive strength against *W. auropunctata* seems to vary from one location to another (Kirschenbaum and Grace 2007a, Mbenoun Masse et al. 2019b).

Future research on the driving factors of ecological dominance, and subsequent ecological impacts should consider the roles different types of human disturbance and multispecies interrelationships have on the degree of ecological dominance (Perfecto and Vandermeer 2020b). Future research should also investigate possible links between *W. auropunctata* genetics and ecological dominance. It is possible that certain haplogroups or haplotypes are more likely to show greater levels of ecological dominance than others.

Social Behaviors

The complex intra- and inter-specific behaviors of *W. auropunctata* provide insight into how this species quickly establishes and eventually dominates in some areas while accounting for a mere fraction of the biodiversity elsewhere. Variation in intra- and interspecific behavior between the dominant and nondominant variegates highlights a behavioral plasticity that is not well understood (Le Breton et al. 2004, 2007a). Since dominant and nondominant variegates exist throughout the native range, it is important to consider behavioral studies in the context of this variation rather than simply as native and nonnative populations. However, most studies focusing on intraspecific aggression were conducted before Foucaud (2009) describing the dominant and nondominant variegates and so intraspecific aggression has typically been reported in the context of native versus nonnative populations rather than according to variegate (Le Breton et al. 2004, Errard et al. 2005). The consensus among these studies is that invasive populations display much lower intraspecific aggression than native populations but it is likely that the native populations studied were of the nondominant variegate since the study sites in question were typically within primary forests rather than disturbed habitats. To date, no studies have examined intraspecific aggression in the context of native and nonnative dominant versus nondominant populations.

Variation in intraspecific aggression and nest-mate recognition is linked to the chemical composition of cuticular hydrocarbons (CHCs) (Errard et al. 2005, Martin and Drijfhout 2009, Vonshak et al. 2009). Errard et al. (2005) reported a correlation between intraspecific aggression and variation of CHC's among native and nonnative *W. auropunctata* populations. Likewise, there is high genetic variability among the nondominant variegate and low genetic variability among the dominant variegate due to their different reproductive modes (Foucaud et al. 2007). This supports the hypothesis that the native population studied by Errard et al. was of the nondominant variate rather than the dominant variegate; however, research is needed to confirm this. Moreover, CHC production and variability are not only governed by genetic factors but are also influenced by environmental factors such as diet, habitat, and season (Vonshak et al. 2009). Studies have shown that CHC production and intraspecific aggression shift when *W. auropunctata* are taken from their natural environment and placed into laboratory culture (Vonshak et al. 2009).

Wasmannia auropunctata has a reputation for being highly agonistic toward other species, with direct aggression being primarily responsible for its success (de la Vega 1994; Kirschenbaum and Grace 2007a, b, 2008; Vonshak et al. 2012). However, although interspecific aggression in *W. auropunctata* is well documented, many factors contribute to its success as an invader. *Wasmannia auropunctata* population densities appear to be an underlying factor influencing interspecific aggression. At low densities or when not numerically dominant, *W. auropunctata* workers are pliant in the presence of other, more dominant species, and act as an insinuator species (Achury et al. 2008, Vonshak et al. 2012, Yitbarek et al. 2017). Its small size may allow *W. auropunctata* to select what type of interaction is most appropriate and beneficial for a given situation (Tennant 1994, Le Breton et al. 2007a, Achury et al. 2008, Vonshak et al. 2012, Yitbarek et al. 2017). Low population densities occur within populations of the nondominate variegate and early in the invasion process, after colony establishment for the dominant variegate. For new introductions, low interspecific aggression may enable *W. auropunctata* to coexist with other species and exploit resources necessary for colony growth (Vonshak et al. 2012, Yitbarek et al. 2017). Once numerically dominant, a behavioral shift occurs and *W. auropunctata* workers become highly agonistic toward other species, excluding them from resources and destroying their nests (Vonshak et al. 2012). The low worker:queen ratio, high fecundity rates, and low intraspecific aggression typical of the dominant variegate allow for quick colony growth and numerical dominance (Clark et al. 1982, Ulloa-Chacon and Cherix 1990, Brandao and Paiva 1994, de la Vega 1994, Way and Bolton 1997, Delsinne 2001, Kirschenbaum and Grace 2008).

The ability of competitor species to fend off *W. auropunctata* attacks and defend resources also plays a role in regulating *W. auropunctata* population dynamics (Le Breton et al. 2007a, Perfecto and Vandermeer 2020a). Controlled behavioral experiments have shown that the presence of *W. auropunctata* elicits reactive responses from some competitor ant species (e.g., immediate recruitment of larger castes able to attack and kill *W. auropunctata*) but not from other species (Kirschenbaum and Grace 2007a, Le Breton et al. 2007a, Kirschenbaum and Grace 2008, Perfecto and Vandermeer 2020b). It's not yet known if successful defense against *W. auropunctata* within its native range is dependent on which ecological variegate is encountered and habitat type (i.e., disturbed or natural). Only the most aggressive competitor ant species seem able to fend off *W. auropunctata* in its native range and these are typically other well-known invasive ant species (Le Breton et al. 2007a, b; Mbenoun Masse et al. 2019b, 2021; Perfecto and Vandermeer 2020b). Interestingly, *Pheidole megacephala* Fabricus has been documented as a displaced species throughout much of *W. auropunctata's* introduced range while also being implicated as a potential cause of a rare invasion contraction event in Cameroon (Mbenoun Masse et al. 2019b, 2021). Additionally, *W. auropunctata* have been documented nesting near and tolerating other species when not competing for food resources (Way and Bolton 1997). This highlights the complexity of interspecific competition and that it is often oversimplified. It is likely that successful competition and resilience against *W. auropunctata* rely on both biotic and abiotic factors (Jourdan et al. 2006, Vandermeer and Perfecto 2020).

Diet

Wasmannia auropunctata is a true generalist, feeding on whatever is available including nectar (floral and extrafloral) (Schemske 1980, Horvitz 1990, Deyrup 2000, Apple 2001), plant parts (Clemente and Whitehead 2020), other invertebrates (Smith 1942, Feinsinger and Swarm 1978, Clark et al. 1982, Way and Bolton 1997), animal feces (Rosumek 2017), and honeydew-producing phytophagous insects (Spencer 1941, Smith 1942, Fabres and Brown 1978, Delabie and Cazorla 1991, Delabie et al. 1994, Naumann 1994, de Souza et al. 1998, Fasi et al. 2013). Few nutrient allocation or dietary studies have been conducted on *W. auropunctata*, but studies on other ant species indicate that dietary preferences and needs may vary seasonally (Stein et al. 1990) and between arboreal and ground-dwelling conspecific ants (Hahn and Wheeler 2002, Bluthgen et al. 2003). Additionally, *W. auropunctata* raised under laboratory conditions can display differences in food-lure preferences when compared to wild conspecifics (Montgomery et al. 2020).

It is common for laboratory-raised insects to behave differently to wild conspecifics and this likely influences observations during laboratory experiments (Herard et al. 1988, Propkopy et al. 1989, Ennis et al. 2015). Since laboratory experiments are valuable components of the research and experimentation process, differences in foraging behaviors should be accounted for when conducting dietary, palatability, and bait efficacy assessments (Montgomery et al. 2020).

Global Distribution

The global distribution and spread of *W. auropunctata* were first outlined in 2003 and has been tracked since (Wetterer and Porter 2003, Wetterer 2013). Currently, *W. auropunctata* is more widespread globally than *S. invicta* (Buren), although the latter receives considerably more attention as a research and legislative priority. *Wasmannia auropunctata* is native to the Neotropics of Central and South America (Wheeler 1929). Its southerly range extends to central Argentina (Chifflet et al. 2016), east of the Andes, and it was recently postulated that *W. auropunctata* may be native as far north as the United States—Mexico border (Mikheyev and Mueller 2007, Wetterer 2013).

Genetic analysis on specimens collected throughout this region has identified two phylogenetic clades (Clad A and Clad B) with numerous haplogroups based on cytochrome c oxidase 1 partial mitochondrial genome analysis (Chifflet et al. 2016). Even though this research has provided empirical evidence for determining places *W. auropunctata* is likely native, there is still uncertainty as to how far the native range extends and where this species has been introduced. For example, *W. auropunctata* is native to northern Argentina, as evidenced by the presence of genetically diverse sexual and clonal populations (Chifflet et al. 2016, 2018). However, the occurrence of only clonal populations of and little genetic variation in central Argentina has been suggested as evidence of range expansion within the past 60 yr and may be indicative of anthropogenic introductions (Chifflet et al. 2016, 2018). Currently, there is a noticeable lack of records from central and western Mexico and no genetic analysis has been done on any *W. auropunctata* collected in Mexico. To tease out the northern limitations of *W. auropuntata's* native range, future research should focus on documenting the ant biodiversity of this area and genetic analysis of *W. auropunctata* populations throughout Mexico should be conducted. Despite the uncertainty surrounding the northern limitations of its native range and the need for additional work, the Mexican populations are presumed part of *W. auropunctata*'s native range in this review due to its contiguous distribution where it is known to occur throughout Central America. We acknowledge that this matter requires further investigation before it is fully reconciled.

Historically, there has been uncertainty surrounding the status of *W. auropunctata* in the Caribbean due to its pervasiveness throughout the region with records dating back to the mid-1800s (Wetterer and Porter 2003, Mikheyev and Mueller 2007, Wetterer 2013). However, molecular genetics and analysis of eco-evolutionary pathways identified the presence of the same clonal lineages being present on multiple islands and in parts of South America (Mikheyev and Mueller 2007, Foucaud et al. 2010b). While this does not preclude the idea of the Caribbean being part of *W. auropunctata*'s native range, it does indicate that numerous introduction events have occurred throughout the region (Mikheyev and Mueller 2007, Foucaud et al. 2010b). The lack of evidence of sexually reproducing populations in the Caribbean is another indication that *W. auropunctata* is not likely native to the region (Mikheyev and Mueller 2007, Foucaud et al. 2010b). It is possible that the Caribbean distribution contains a mix of native and introduced populations (Wetterer 2013) but, no genetic evidence has been presented that suggests this is the case.

The earliest confirmed record of *W. auropunctata* outside of its presumed native range was from Gabon in 1894 by Emery who recognized it as having the potential to become a serious invasive tramp ant (Wetterer 2013). Indeed, since then, *W. auropunctata* has been introduced and established in 23 countries and island groups beyond its presumed native range (Wetterer and Porter 2003, Wetterer 2013, Espadaler et al. 2018, Mayron 2019, Vanderwoude et al. 2021). To date, the nondominant variegate has not been documented outside of *W. auropunctata*'s native range. Since the latest global distribution list (Wetterer 2013) there have been five new detections of established *W. auropunctata* populations in the world. Three new detections were made in Oceania: Wanyaan, Yap, Federated States of Micronesia in 2017 (GBIF.org 2021); Tutuila, American Samoa in 2018 (Gruber et al. 2016); and Suva, Fiji in 2019 (Vanderwoude et al. 2021). A detection in Malaga, Spain in 2018 was confirmed to be the northernmost outdoor population recorded to date (Espadaler et al. 2018). The populations in Israel and Spain represent the only outdoor infestations in Palearctic and Nearctic biogeographical regions. All other infestations in these biogeographical regions were documented in greenhouses and indoor nursery settings (Wetterer and Porter 2003). The most recent detection was confirmed in 2022 from Shantou, Guangdong Province in south-eastern China (Chen et al. 2022). This is the first official record of *W. auropunctata* in the Indomalayan biogeographical region.

The global distribution presented in this review is conservative (Fig. 5), with only confirmed, established populations of *W. auropunctata* as of June of 2021 being included. Not included on the map are *W. auropunctata* occurrences of unconfirmed detections of possible wild populations, which are included on other distribution lists, specifically, detections from California (USA), southern Texas (USA), Lisca Bianca Island (Italy) (Jucker et al. 2008, Wetterer 2013), and Dhaka, Bangladesh (GBIF.org 2022). Unconfirmed detections are either unsubstantiated reports of establishment or instances in which a single specimen was collected or photographed in the wild and presumably positively identified yet no verification, further record, or knowledge exists. It is possible that established *W. auropunctata* populations exist in these locales yet no follow-up sampling was ever conducted to verify their existence. Follow-up surveys and sampling should be a priority for all unconfirmed records of *W. auropunctata* as this information will be invaluable for the understanding of this species presumed native range and potential global distribution. Notably, *W. auropunctata* is regularly intercepted in California by Department of Agriculture quarantine inspectors, but no wild populations have been confirmed in (G. Arakelian, Los Angeles County Entomologist, personal communication) despitean early claim that it was established in Los Angeles County (Keifer 1937) and repeated citing of this claim in numerous distribution lists. Regulatory and responding agencies around the world should be hyper-vigilant, especially in areas where *W. aurounctata* is frequently intercepted but not believed to be established. It is important to acknowledge that *W. auropunctata* are likely established beyond the locations indicated here and have yet to be officially detected due to the ease at which they are distributed and the probability of going unnoticed.

Distribution Pathways

Transportation and dispersion of *W. auropunctata* typically occur in three ways: natural active dispersion, natural passive dispersion, and human-mediated transport. Natural active dispersal occurs primarily via budding (Ulloa-Chacon and Cherix 1990). When a nest becomes

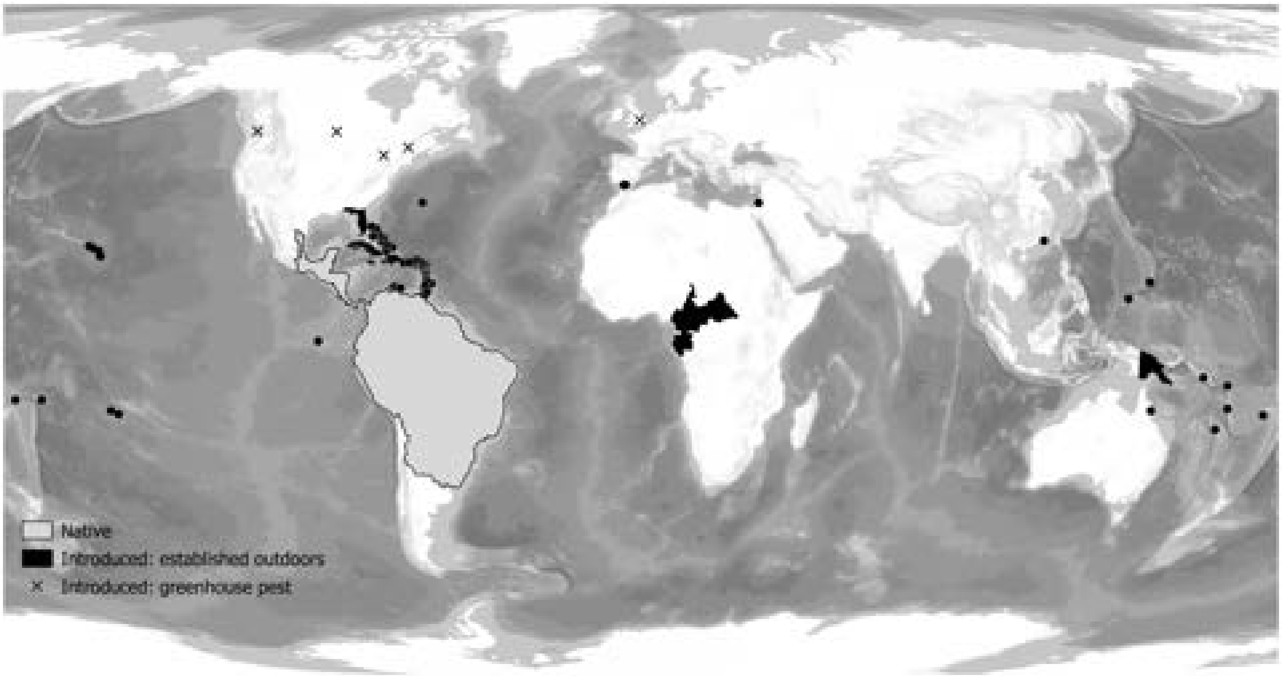

Fig. 5. Global distribution of *W. auropunctata* as of 2022. Gray shaded areas and points indicate the presumed native range. Black shaded areas and points indicate locations where exotic outdoor populations are established. Black x's indicate locations where *W. auropunctata* have been introduced and are documented as indoor greenhouse pests but no outdoor populations have been documented. The current distribution map includes data from J.K. Wetterer's 2013 distribution map and all subsequent records of confirmed established *W. auropunctata* populations detected since 2013.

crowded or the nest is disturbed, a queen will carry a small number of workers to a nearby location and establish a new nest aggregation (Feitosa 2007, Mbenoun Masse et al. 2011). Because of this, outward expansion of an infested area is slow, measured in the tens of meters per year, with dispersal propelled by the ant's direct effort (Walsh et al. 2004). However, the rate of outward expansion may also be influenced by population density and ecological factors (Mikheyev et al. 2008). *Wasmannia auropunctata*'s unicolonial colony structure, low intra-specific aggression, and generalist nesting preferences allow for population densities far beyond that of multicolonial ant species and species with specialized nesting preferences. Therefore, the rate of outward expansion may increase or decrease depending on site features such as nesting site availability and physical barriers between the current infested area and the closest suitable habitat. Invasion contraction events (i.e., reduction of previously invaded area) have been documented, but appear to be rare and warrant further investigation (Lester and Gruber 2016, Mbenoun Masse et al. 2019b).

Modes of natural passive dispersion occur by rafting downstream on waterways and flood water, landslides, and possibly from high winds and storm events (Lubin 1984, Walker 2006, Vanderwoude et al. 2014). Modes of natural passive dispersion enable dispersion along greater distances than through budding and act as pathways for new introductions with transport via moving water being the most frequently observed (Walker 2006, Vanderwoude et al. 2014).

Human-mediated transport is the most common mode of long-distance dispersal (Walsh et al. 2004, Mikheyev et al. 2008, Foucaud et al. 2010b) and both intentional and unintentional introductions have occurred world-wide. *Wasmannia auropunctata* is frequently transported locally, intra- and inter-nationally through human commerce including, but not limited to, the movement of infested nursery stock and planting media, construction materials, vehicle and machinery, stockyard supplies such as wooden pallets, and by the movement of other types of items held at infested sites to uninfested sites (e.g., furniture, portable toilets, salvaged materials, etc.) (Walsh et al. 2004). Phylogenetic analysis allows tracing of the place of origin for invasive populations and their spread (Mikheyev and Mueller 2007, Silva et al. 2018). Distinct genetic similarities have been found between invasive populations of *W. auropunctata* in countries with strong trade ties or sharing established shipping routes (Foucaud et al. 2010b).

Intentional human introduction and movement of *W. auropunctata* has occurred for biocontrol purposes (Bruneau de Miré 1969, Wetterer et al. 1999, Ndoutoume-Ndong and Mikissa 2007, Fasi 2009). Although it is well known that the costs associated with *W. auropunctata* invasion outweigh any potential benefit, it's possible such intentional introductions will continue due to this species being highlighted repeatedly as a natural enemy and potential biocontrol agent for coffee berry borer (Curculionidae: *Hypothenemus hampei* Ferrari) and Asian citrus psyllid (Liviidae: *Diaphorina citri* Kuwayama) (Morris and Perfecto 2016, Kondo et al. 2018, Morris et al. 2018, Jiménez-Carmona et al. 2019, Perfecto and Vandermeer 2020a) (Morris and Perfecto 2022).

Predicting Future Range Expansion

Wasmannia auropunctata has traditionally been considered a tropical and subtropical pest species. The outdoor infestations in Spain and Israel show this is a misleading conception and more attention should be given to the species' current potential range and future range expansion under climate change. 'Clade A' and 'Clade B' each display different range potential (Chifflet et al. 2016) with the former distributed primarily in the tropics and the latter having a much wider range into subtropical and Mediterranean climates (Chifflet et al. 2016).

Species distribution models (SDMs) are often used to predict the potential range expansion of a target species. However, SDMs are far from perfect and ecologists are still trying to determine which models and variables best describe limiting factors, potential range predictions, and eco-evolutionary scenarios (Elith et al. 2010, Rey et al. 2012, Federman et al. 2013, Coulin et al. 2019). To date, only two studies have modeled the potential distribution of *W. auropunctata* (Federman et al. 2013, Coulin et al. 2019). Temperature and precipitation were identified early on as limiting factors for its habitat suitability (Jourdan and Dumas 2004). Since then, minimum and maximum critical thermotolerances of the species have been identified through laboratory experiments and included in SDMs (Rey et al. 2012, Foucaud et al. 2013, Coulin et al. 2019). Some have speculated elevation as a limiting factor due to *W. auropunctata* not occurring above 700 m in New Caledonia (Jourdan et al. 2006), but infestations have been detected in Hawai`i at over 1,200 m (M. Montgomery personal observation). This suggests that elevation is not likely a limiting factor in and of itself, but as it relates to temperature and moisture thresholds. Additionally, although SDMs using climatic factors are undoubtedly useful, the spatial scale at which these models analyze climactic data may not identify localized microclimates and the models do not account for human disturbances that influence local microclimates which may aid in triggering natural adaptations within the species (Federman et al. 2013, Foucaud et al. 2013).

Because *W. auropunctata* is considered a disturbance specialist, human disturbances such as irrigation can transform naturally unsuitable habitats into suitable habitats that are unlikely to be detected by correlative SDMs when using naturally occurring precipitation data (Vonshak 2010, Federman et al. 2013). Federman et al. (2013) demonstrated that using a precipitation correction accounting for irrigation estimates increased the precision and accuracy of model predictions. Models based solely on data collected from the target species native range are also likely to fail to identify all suitable habitat and global distribution potential of highly adaptable species (Elith et al. 2010). Contrary to correlative SDMs that use climatic and known distribution data, mechanistic SDMs use functional attributes, niches, and spatial data to predict potential range expansion. Models combining mechanistic and correlative SDMs that account for human disturbances and incorporate data from the species' known global distribution should be investigated further for their potential to refine predictions of habitat suitability and potential global range expansion.

Impacts

Impacts and benefits associated with *W. auropunctata* are multitiered and may be characterized as direct or indirect. Direct impacts are those caused by *W. auropunctata* while indirect impacts are those caused by other organisms influenced by the presence of *W. auropunctata*. For example, the extirpation of a species due to predation or resource competition by *W. auropunctata* is a direct impact whereas an increase in plant disease due to higher populations of scale insects farmed by *W. auropunctata* would be considered an indirect impact. Humans, animals, and entire ecosystems are known to be affected by *W. auropunctata*. Numerous ecological studies and economic impact assessments have been conducted documenting the multitiered impacts of *W. auropunctata* and have determined that while some impacts may be considered positive (i. e. biological control of coffee berry borer), the vast majority are negative (Bousseyroux et al. 2019). Careful and thorough cost-benefit considerations should be made before the intentional movement or maintenance of *W. auropunctata* infestations.

Mechanisms for Displacement and Impacts on Natural Ecosystems

Displacement of ants and other invertebrates by *W. auropunctata* is well documented (Clark et al. 1982; Lubin 1984; Jourdan 1997a,b Armbrecht and Ulloa-Chacon 2003; Le Breton et al. 2003; Wetterer and Porter 2003; Walker 2006; Grangier et al. 2007; Ndoutoume-Ndong and Mikissa 2007; Kirschenbaum and Grace 2008; Fasi 2009; Vonshak et al. 2010; Gasc et al. 2018; Bousseyroux et al. 2019; Mbenoun Masse et al. 2019b). In fact, Silberglied (1972 p. 13) commented that its impact in the Galapagos was 'the most serious of any introduced animal'. Ecological studies on insect biodiversity between areas and plots with and without *W. auropunctata* (Clark et al. 1982; Lubin 1984; Jourdan 1997b; Roque-Albelo et al. 2000; Le Breton et al. 2003, 2005; Walker 2006; Grangier et al. 2007; Ndoutoume-Ndong and Mikissa 2007; Vonshak et al. 2010; Mbenoun Masse et al. 2017, 2019b; Gasc et al. 2018) along with data on resource interference and competition and interspecific aggression (Grangier et al. 2007, Vonshak et al. 2012, Yitbarek et al. 2017) provide evidence linking this species to the extirpation of other insects within invaded areas). Such studies have built a foundation upon which insect populations and diversity are used as proxies for the impacts of *W. auropunctata* in forests. Additionally, the correlation between biodiversity and *W. auropunctata* presence or absence has led to *W. auropunctata* being used as an indicator species for low insect community diversity (Armbrecht and Ulloa-Chacon 2003, Achury et al. 2008, Berman et al. 2013).

Studies describing *W. auropunctata* impacts on insect communities throughout its native and nonnative ranges appear to make the important assumption that its inherent ability to dominate ecosystems remains constant and that differences in impact magnitude are due to the resilience of competing species. No consideration is given to whether the populations being studied are of the dominant or nondominant variegate described by Foucaud et al (2009). Since the two variegates differ biologically, physiologically, and behaviorally, results from studies that measure impacts between invasive populations and native populations without consideration of ecological variegate may be inherently flawed (Brandao and Silva 2008, Achury et al. 2012, Rojas and Fragoso 2021). Any future study comparing impacts of native versus nonnative populations should take care to ensure that populations being compared are of the same variegate.

Resource and interference competition have been indicated as the mechanisms by which *W. auropunctata* succeed as invaders and displace other insects. Multiple behaviors have been identified contributing to *W. auropunctata*'s competitive strength, including acting as an insinuator species when not numerically dominant (Le Breton et al. 2007a, Yitbarek et al. 2017). *Wasmannia auropunctata* workers frequently take significantly longer to discover and recruit to food resources compared with other ant species (Vonshak et al. 2012, Yitbarek et al. 2017). When first encountering food resources occupied by another species, their passivity toward the other species likely allows *W. auropunctata* to avoid aggressive interactions until enough nestmates can be recruited to successfully defend the resource (Vonshak et al. 2012, Yitbarek et al. 2017). Additionally, native ants often appear incapable of forming appropriate responses are unable to defend nesting and food resources creating niche opportunities that *W. auropunctata* effectively exploit (Le Breton et al. 2005, 2007a).

While the role of resource competition is widely suggested as a mechanism for competitive success, there is some debate as to the magnitude of its contribution. Observations during behavioral laboratory assays suggest that while *W. auropunctata* are aggressive toward some species, they are often unsuccessful defenders of food resources during general interspecific encounters (Kirschenbaum and Grace 2008, Vonshak et al. 2012). However, during one study, although *W. auropunctata* retreated from food resources when confronted by a competitor, they also invaded and completely destroyed the competitor's nests over the course of several days and weeks (Vonshak et al. 2012). Because they are generalist feeders and active 24 h per day, it's possible that the cost of aggressive encounters outweighs the benefit of immediate reward and it is better to wait or find an unoccupied resource. Also, laboratory studies don't often reflect what happens under natural circumstances. For example: in the wild, the dominant variegate is defined, in part, by its propensity to form expansive supercolonies. This allows for massive recruitment of resources. In contrast, laboratory colonies and experiments are highly structured and controlled and use only a single nest at a time. Field studies have suggested that *W. auropunctata* may share food resources when fewer than 500 foragers are present (Achury et al. 2008). If this is the case, it is unlikely that a single nest would provide high enough forager recruitment to a resource to successfully defend it during laboratory experiments and behavioral assays. The dichotomy between laboratory and wild conspecifics and the environmental conditions they are exposed to is an important consideration with interpreting results from such studies.

Although physical aggression, such as biting and stinging, appears to be the primary competitive mechanism by which *W. auropunctata* directly interferes with other species, chemical defenses may also play a role (Howard et al. 1982, Le Breton 2002, Showalter et al. 2010). Ants primarily communicate chemically and the use of pheromones is an important mode of communication for foraging, defense, and regulation of inner-nest functioning (Howard et al. 1982, Martin and Drijfhout 2009, Showalter et al. 2010, Cha et al. 2019). When distressed, *W. auropunctata* secrete an alarm pheromone from the mandibular gland which attracts nestmates and aids in mass recruitment for defensive action (Howard et al. 1982, Showalter et al. 2010). These mandibular gland secretions may also act as a repellant to competitor species (Howard et al. 1982), although this hypothesis has been challenged (Le Breton 2002). It is important to recognize that the two studies investigating repellant properties of the mandibular gland secretions employed very different methodologies and confounding factors may have influenced the results of one or both of the studies. One study tested the effects of extracts from the mandibular gland on the acceptability of mealworms to other ant species (Howard et al. 1982) whereas the other study exposed food lures to *W. auropunctata* in the field and later presented the exposed food lures to other species at different field locations without *W. auropunctata* (Le Breton 2002). Given their behavioral plasticity, it is possible that the mandibular gland excretions are secreted only during interspecific encounters, and thus may not have been present on the food lures during foraging when only *W. auropunctata* was present. Although the mandibular gland excretions are verified alarm pheromones used in mass recruitment and undoubtedly contribute to successful resource defense (Showalter et al. 2010), more research is needed to verify any potential repellant action of *W. auropunctata* mandibular gland secretions.

Wasmannia auropunctata has also been identified as a threat to vertebrates (Jourdan 2001, Walsh et al. 2004, Beavan et al. 2008). Tropical keratopathy (TK) is a condition in which the cornea of the eye becomes clouded over and appears superficially similar to cataracts. Mild cases of TK often appear as small, individual cloudy corneal spots whereas extreme cases appear as clouding over the entire cornea. *Wasmannia auropunctata* has been directly linked to occurrences of widespread TK in domestic animals and with occurrences of TK in humans (Theron 2007, Rosselli and Wetterer 2017, Patael et al. 2019). Although no studies have focused on documenting animal TK occurrences in invaded natural ecosystems, trail cameras in Gabon captured images of a leopard with severe TK (Walsh et al. 2004) and it is likely this is a problem not yet investigated. Agonistic interactions between *W. auropunctata* and several lizard (*Anolis*) species have been documented and it has been speculated that lower herpetofauna diversity may be found throughout infested areas (Jourdan 2001, Jourdan et al. 2001, Wetterer et al. 2007, Perfecto and Vandermeer 2020a). The effects of *W. auropunctata* on ground-nesting seabirds and forest birds has not been formally assessed, but other invasive ant species are known to reduce nesting and hatching success, foraging, and survival rates (Allen et al. 2004, Plentovich et al. 2008, Davis et al. 2009, Kropidlowski 2014). The diversity of suitable habitat for *W. auropunctata* likely increases the chances of ant-bird interactions, and the potential for negative impacts on avifaunal communities. Research is needed to identify possible impacts and the risk *W. auropunctata* poses to various avifaunal communities.

In some areas, *W. auropunctata* may directly and indirectly impact forest plant composition and regeneration through the assistance of or interference with seed production, dispersal, and ant-plant mutualisms (Horvitz 1990, Mikissa et al. 2013, Clemente and Whitehead 2020). *Wasmannia auropucunctata* have been observed harvesting fruits of *Piper sanctifelicis* Trel (Family: Piperaceae), a common South American shrub (Clemente and Whitehead 2020). Although it is not unusual for ants to harvest seeds of plants typically dispersed by vertebrates, it is the first and only record of *W. auropunctata* doing this. Additionally, *W. auropunctata* may aid in fruit set and seed production of some plants through predation on herbivorous insects (Horvitz and Schemske 1984). On the contrary, *W. auropunctata* have been observed interfering with ant-plant mutualisms and indirectly hindering forest regeneration by excluding ant mutualists from their obligate host plants (Mikissa et al. 2013). Other ant species, such as yellow crazy ant (*Anoplolepis gracilipes* [Smith]) have been implicated in 'invasional meltdowns' due to interference with natural ecosystem processes (O'Dowd et al. 2003, Green et al. 2011). For example, on Christmas Island in the Indian Ocean, *A. gracilipes*significantly reduced populations of endemic red land crabs (*Gecarcoidea natalis* Pocock), a keystone species, and is linked to population explosions of scale insect and sooty mold (O'Dowd et al. 2003, Green et al. 2011). These relationships have altered local litter decomposition rates, plant and insect species diversity, and forest structure, and led to secondary invasions within *A. gracilipes* invaded areas (O'Dowd et al. 2003, Green et al. 2011). Although *W. auropunctata* alters ecosystem processes, very little is understood on the ramifications of these relationships on floral communities and whether ecological variegation is a factor influencing the magnitude and direction of the impacts. It is possible that such relationships may also lead to 'invasional meltdowns', especially within insular island communities, but additional research is needed to assess this.

Agricultural Systems

Although ecological impacts of *W. auropunctata* are serious, they are often not enough to trigger concern and human intervention. Agriculture industries are the most affected by *W. auropunctata* according to past economic impact assessments (EIAs) (Lee et al. 2015, Angulo et al. 2021) and their multitiered impacts are perhaps most clearly documented within agriculture systems. Agricultural workers are frequently stung while maintaining and harvesting crops. Reports from Tahiti and Florida indicate that farm workers have been unable or unwilling to harvest crops and to perform other duties in heavily infested citrus orchards and coffee fields (Spencer 1941, Smith 1965, Fabres and Brown 1978, Delabie 1988, Perfecto and Vandermeer 2020b) resulting, in some cases, in the abandonment of family lands (Vanderwoude et al. 2015). A similar trend has been observed in Hawai`i, where farm workers routinely fail to come to work on days when infested fields are scheduled to be picked (M. Montgomery personal observation).

The mutualistic relationship between *W. auropunctata* and honeydew-producing phytophagous insects results in indirect impacts such as population explosions of economically important plant pests (Smith 1965, Fabres and Brown 1978, Delabie 1989, Fasi et al. 2013). In particular, population levels of Homopterans including aphids, scale insects, and mealybugs are positively correlated with *W. auropunctata* population levels and negatively correlated with plant health and crop productivity (Delabie and Cazorla 1991, de Souza et al. 1998, Fasi et al. 2013). These mutualisms are a consequence of the protection that *W. auropunctata* provides the insects against natural enemies and the ants feeding on the sugary exudates (honeydew) of these insects. Many of these plant pests are known vectors of diseases that reduce plant health leading to crop loss and excess honeydew also acts as a medium for sooty mold growth.

Despite the association with economically important plant pests and their direct impact on farm workers, *W. auropunctata* has been intentionally introduced and moved around as a biocontrol agent against significant crop pests in the Solomon Isands and Gabon (Wetterer et al. 1999, Fasi et al. 2013). Recently, research also confirmed *W. auropunctata* is potentially effective at reducing populations of coffee berry borer and Asian citrus psyllid (Kondo et al. 2018, Morris et al. 2018, Jiménez-Carmona et al. 2019, Perfecto and Vandermeer 2020a, Morris and Perfecto 2022). However, researchers of those studies noted that the benefits do not outweigh the costs. Conversely, *W. auropunctata* has been documented interfering with various biocontrol agents (Kondo et al. 2018, Kulikowski 2020, Perfecto and Vandermeer 2020a, Perfecto et al. 2021). Whether intentionally or unintentionally introduced, farmers face a variety of issues due to *W. auropunctata* infestations.

Residential and Urban Areas

Wasmannia auropunctata infestations in urban environments have garnered little attention despite its recognition as a house pest (Smith 1929, Fernald 1947, Delabie 1995, Espadaler et al. 2018, Mbenoun Masse et al. 2019a, Kidon et al. 2022, Mbenoun Masse et al. 2021). The Hawai`i infestation reported by Conan and Hirayama in 2000 represents the first record of *W. auropunctata* as a major residential pest in any western society (Conant and Hirayama 2000). Their small size, brownish-orange color, and cryptic nesting habits allow this species to go unnoticed for many years and it is common for Hawaiian residents to become impacted by *W. auropunctata* only after the infestation has spread throughout the entire property and the ants have begun encroaching into homes and other structures (M. Montgomery personal observation). This is not unique to Hawai`i. The recent detection in Malaga, Spain, was due to a resident reporting an infestation of stinging ants in their home to a pest control company (Espadaler et al. 2018). Follow-up surveys by authorities revealed a 5.8 ha infestation spanning 50 private residences with some ants nesting in electrical receptacles (Espadaler et al. 2018). It was estimated that *W. auropunctata* was introduced over five years before detection but residents reported first noticing them only about two to three years prior (Espadaler et al. 2018). Since the original detection in 2018, two other infestations within the Malaga province have been detected at 13 and 17 km away from the original infestation (Espadaler et al. 2020). At this time, these new detections have not yet been completely delimited and their size is unknown (Espadaler et al. 2020).

Information gathered through public outreach communications in Hawai`i (M. Montgomery, unpublished data) has revealed that residents are frequently stung in their beds, while using the bathroom, or sitting on certain types of furniture (e.g., sofas, recliners). Despite this, individual sentiments toward *W. auropunctata* vary wildly. Some residents appear determined to manage *W. auropunctata* infestations and reduce the impacts themselves while others appear despondent and assume their situation is without a solution (M. Montgomery personal observation). For example, *W. auropunctata* was introduced to the Solomon Islands in the 1960s or early 1970s and continues to spread throughout the archipelago (Fasi et al. 2016). Public attitudes toward *W. auropunctata* appear to shift with communities having dealt with infestations for longer periods being more tolerant of the ant than communities with more recent invasions (Fasi et al. 2016). Once apathy toward *W. auropunctata* begins to take hold in a community, management practices likely decline. Similar attitudes are common in Hawai`i. In many Hawaiian communities, some residents are only persuaded to take management action against *W. auropunctata* when social pressure increases and they fear being stigmatized in their community (Niemiec et al. 2018, 2019).

Economic Impacts

There is no doubt that *W. auropunctata* negatively impacts ecosystem functions and quality of life but the reports are generally qualitative, difficult to quantify into monetary units, and rarely used to guide legislation aimed at biosecurity and prevention. Economic impact assessments (EIAs) and cost-benefit analyses (CBAs) attempt to place monetary values on damages incurred across various economic sectors and costs associated with postarrival management and prevention efforts. These cost factors are used in bioeconomic models to project how economic costs associated with a target species compound change over time under different management strategies. Unfortunately, quantification and reporting of costs associated with *W. auropunctata* are rare. Because EIAs rely on reported costs that can be quantified monetarily and largely ignore qualitative metrics, EIAs tend to grossly underestimate impacts (Angulo et al. 2021), especially in subsistence economies.

Three studies detailing the economic impacts of *W. auropunctata* have been compiled. Two focus on economic impacts in Hawai`i (Motoki et al. 2013, Lee et al. 2015). The other is a global assessment of the economic impact of invasive ants, including *W. auropunctata* (Angulo et al. 2021). In the global assessment, *Wasmannia auropunctata* was identified as one of the most economically impactful species in the world, second only to *S. invicta* with cumulative global costs since 1930 estimated at US$19.91 billion, predominantly incurring over the past ten years (Angulo et al. 2021). The greatest damage and highest costs of management are in the agriculture and public welfare sectors (Lee et al. 2015, Angulo et al. 2021). In Hawai`i, *W. auropunctata* was projected to cost the state US$6.1 billion over 35 yr if the current management efforts are sustained or US$12.9 billion if the management status quo is not maintained (Motoki et al. 2013, Lee et al. 2015). Conversely, economic costs can be greatly reduced with increased management, rapid response efforts to newly detected infestations, and enhanced prevention measures. Despite higher management cost than the status quo and reduced effort scenarios, overall costs (damages + direct costs of management and prevention efforts) of enhanced action plans were projected to reach only $US51 million over 35 yr (Motoki et al. 2013, Lee et al. 2015).

Most of these assessments focus on costs and damages without considering potential economic benefits of the target species. In Hawai`i, the invasion of *W. auropunctata* has spurred business development focusing on ant management in landscapes (M. Montgomery personal observation) and sales of ant baits generate revenues for local businesses. Additionally, the coffee berry borer is a major pest of coffee in Hawai`i with few effective management options. The recent identification of *W. auropunctata* as a potentially effective biological control (Kondo et al. 2018, Morris et al. 2018, Jiménez-Carmona et al. 2019) may provide a valuable nonchemical pest control option for Hawaiian coffee farmers. The reduction in costs associated with coffee berry borer control could be considered as a cost-saving economic benefit when viewed in isolation. However, *W. auropunctata* is also a major pest in coffee and creates costs associated with control efforts.

Detection and Control

Detection

Traditionally, nontoxic food lures have been the primary tool used for detecting *W. auropunctata.* Foods high in lipids and/or protein, such as peanut butter, hotdogs, or tuna fish, are typically used as lures during ecological studies to determine *W. auropunctata* presence with recruitment to the food lures frequently used as a surrogate for population size or density. Food lures are also used to delimit infestations and monitor the effectiveness of control programs. Few studies have investigated the attractiveness of pure lipids, proteins, and carbohydrates to *W. auropunctata* (Williams and Whelan 1992, Montgomery et al. 2020), but the consensus is that lipid-rich foods are the most attractive food lures (Williams and Whelan 1992, Meier 1994, Armbrecht and Ulloa-Chacon 2003, Montgomery et al. 2020). This is important because the use of a suboptimal or less attractive, food lure is likely to underestimate distribution, density, and confound results. Additionally, it is uncertain whether *W. auropunctata* would defend ideal food resources more than suboptimal resources which could influence the results of competition behavior studies.

Given their generalist diet, it is possible that food-lure preference may vary from one site to another or temporally within the same location depending on the season, available resources, nutrient deficiencies, and colony needs. For example, in Hawai`i, *W. auropunctata* recruitment rates to lipid-based food lures have been documented to be 6- —and 11-fold greater than carbohydrate and protein food lures, respectively among wild ants whereas laboratory colonies showed a preference for carbohydrate food lures (Vanderwoude et al. 2015). It is unknown whether fluctuations in dietary needs of wild populations and available resources influence their attraction to different food lures. To minimize possible confounding effects of food-lure selection, more information is needed elucidating food preference variability and the relationship between food preference and interspecific competition.

Despite detection via food lures being the norm, accuracy of area-wide surveys using food-lure detection protocols varies greatly depending on spacing between lures, foraging distance, and operator experience. Additionally, detection of arboreal colonies is difficult with traditional lure-based surveys and require special consideration (Cox et al. 2020). Early detection and control projects often must seek a compromise between feasibility and accuracy depending on personnel, time, and funding restrictions. Such compromises often manifest as wider spacing between lures and fewer survey efforts, and have resulted in false negative results and premature declarations of eradication (Montgomery unpublished data).

Food-lure detection methods are inexpensive and easy to use but these benefits come at a cost. Aside from variations in accuracy and precision, they are not species specific to *W. auropunctata* and ants collected during such surveys must be competently identified. This can be time consuming and further delay time-sensitive control actions. Species-specific detection methods are being developed that hold promise over conventional food-lure detection methods. The use of detector dogs is common for agriculture quarantine inspections and biosecurity programs around the world. Their use in invasive species monitoring programs has also grown in recent years (Lin et al. 2011, Wylie et al. 2016, Baker et al. 2017, Poland and Rassati 2019). Similarly, the development and marketing of lateral flow immunoassay rapid tests has revolutionized detection surveys targeting *S. invicta* by circumventing the need for time-consuming professional or laboratory diagnostics and allowing quick identification in the field (Valles et al. 2017, 2020). Should a comparable rapid test be developed for *W. auropunctata,* it would undoubtedly be an invaluable resource for biosecurity, rapid response and control efforts. The use of alarm pheromones for *W. auropunctata* management has shown to be ineffective, although they may hold potential as a species-specific detection tool in the near future and the development of pheromone lures is ongoing (Troyer et al. 2009, Derstine et al. 2012). Remote sensing has been used to identify *S. invicta* mounds by aerial searches (Vogt et al. 2008). Finally, advances in genetic research will likely lead to improved EDNA technology suitable for testing terrestrial substrates and may be useful for detecting nascent ant colonies. Although the future holds much promise for improved detection, currently, all but the conventional food lure-based and sniffer dog detection methods are still theoretical or unavailable for *W. auropunctata.*

Chemical Control

Ant control is more nuanced than control of most other insect pests. Historically, persistent and nonpersistent general use of contact insecticides, mound treatments, insecticidal ant baits (hereafter referred to as baits), and physical barriers have been used to control pest ants in various habitats (Osburn 1949, Delabie 1989, Williams 1994, Brooks and Nickerson 2008, Cabral et al. 2011). As the term implies, the general use of contact insecticides (hereafter referred to as contact insecticides) kill a wide variety of insects on contact through direct spray or when the insect comes into contact with a treated surface. Horizontal transfer, the acquisition of insecticide through physical contact with a treated individual, of active ingredients has been used to control some species of ants in residential and conservation areas (Soeprono and Rust 2004, Choe and Rust 2008, Klotz et al. 2009, Buczkowski 2019, Buczkowski and Wossler 2019, Zhang et al. 2022, Cabral et al. 2011), but few active ingredients are effective through horizontal transfer. Horizontal transfer, also, has not been tested as a possible control method for *W. auropunctata* and should be investigated further. Other contact insecticides may be used as a soil drench for potted plants or individual nests. While use pattern may be useful to treat infested nursery stock and potted plants at home, it is impractical to attempt control of *W. auropunctata* through drenching individual nests given its nesting behavior. Most contact insecticides are ineffective at producing lasting results for area-wide management because their effects are limited to killing foraging workers only while the rest of the colony within the nest remains unaffected. Baits hold significant advantages over other general use insecticide products in that they are typically lower in toxicity, have fewer nontarget impacts, and minimize insecticide use (Williams 1983, Klotz et al. 2003, Tollerup et al. 2004). Comprised of an attractant, carrier, and small amount of active ingredient, baits use the ant's natural foraging behavior to seek out, collect, and share the bait throughout the colony which affects reproductive, worker, and brood castes alike. To be effective, the active ingredient constituent in baits must be nonrepellant, lethal at very low doses and after dilution by trophallaxis, and also maintain delayed mortality effects (Levy et al. 1973, Williams 1983, Rust et al. 2000, Braness 2002, Tollerup et al. 2004).

Baits developed for 'fire ants' target lipid-responding ants and have been developed primarily for control of *S. invicta.* Although *W. auropunctata* is also a species that is attracted to lipids, there are substantial differences between the two species and the types of habitats they invade which render some baits more effective than others in various situations. For example, *S. invicta* build mounds in soil and prefer open, dry habitats whereas *W. auropunctata* nest opportunistically on the ground and in trees, preferring shady, moist habitats (Perfecto and Vandermeer 2020b). Chemical sensitivity differences between these two species is also apparent which influences efficacy of 'fire ant' baits against *W. auropunctata* (Hara et al. 2014, Montgomery et al. 2015). Site features, chemical sensitivities, and environmental degradation of baits and their active ingredients are the three main issues needing careful consideration when selecting a bait to control *W. auropunctata*. Management and eradication failures against this species have, in part, been attributed to a failure in addressing one or more of these issues during the eradication planning and implementation processes (Bossin and Padovani 2010).

Commercial baits used against *W. auropunctata* are available as granules, pastes and gels and may be used in bait stations, or broadcast treatments. Outdoor control using bait stations has been reported as inconsistent and less effective than broadcast bait applications (Ulloa-Chacón and Cherix 1994, Souza et al. 2008). *Wasmannia auropunctata* do not build mounds, therefore, baits are typically broadcast rather than applied to a mound or individual nests. Although broadcast applications are regarded as more effective than bait stations, there may be situations where bait stations are preferred. To date, no studies have attempted to determine foraging distances and effective bait station densities to control *W. auropunctata*. Such studies often rely on data from mark-capture and mark-release-recapture studies, and marking techniques for this species are under investigation (Montgomery et al. 2019).

Granular baits applied to the ground have been found to have little effect on arboreal ants in most situations. In Hawai`i, *W. auropunctata* nesting in tree canopies, palms, and tall foliage do not always forage on the ground and so some do not encounter the insecticidal bait (Souza et al. 2008, Taniguchi 2008, Montgomery et al. 2015). However, in Cairns, Australia, arboreal *W. auropunctata* appear to descend and forage on the ground at times depending on the availability of food resources in the canopy or foliage in which they reside (G. Morton personal communication). Additionally, the distance to the ground and weather are also factors likely to influence this. Therefore, the efficacy of bait applications on the ground may differ from one site to another. In areas where arboreal *W. auropunctata* do not forage on the ground, bait applications must be three-dimensional and include treatment of tree canopies and vegetation (Souza et al. 2008, Taniguchi 2008, Vanderwoude and Nadeau 2009, Vanderwoude et al. 2010) using a gel or paste bait. Gel bait formulations have the advantage over granules of adhering to vegetation and vertical surfaces, ensuring arboreal ants' access to the bait (Vanderwoude and Nadeau 2009, Vanderwoude et al. 2010).

Another issue is the weathering effects of sunlight, heat, and moisture on bait palatability and degradation. The corn grit carrier of granular baits quickly absorbs moisture from dew and rain making the bait soggy, reducing the oil content, and palatability becomes unpredictable (Banks et al. 1972, Hara et al. 2014, Oi et al. 2022). Oxidation of the soy oil food attractant resulting from heat, light, and humidity exposure also renders baits unpalatable (Markin and Hill 1971, Hara et al. 2014). This can occur from improper or prolonged storage of opened bait containers or after application in the field (M. Montgomery personal observation). Gel and paste baits are also susceptible to weathering. Although dew and light rain may help keep the bait moist and palatable, heavy rains wash the bait away and dry conditions result in desiccation. Additionally, some active ingredients, such as hydramethylnon, undergo rapid aqueous photolysis with a half-life of 42 min when exposed to light and water (Mallipudi 1986). In wet climates, such as windward Hawai`i where average annual rainfall may exceed 3,200 mm (Giambelluca et al. 2013, accessed 12 Oct 2021), there is a short window of opportunity for *W. auropunctata* to find, recruit to, feed on, and share the bait. Ensuring maximum attractiveness is maintained as long as possible and increasing chances for foragers to encounter the bait following broadcast applications is essential for achieving the highest treatment efficacy.

Finally, *W. auropunctata* appear to be more sensitive to certain active ingredients used in 'fire ant' baits. Insect growth regulators (IGRs), such as s-methoprene and pyriproxyfen, appear to be repellant to *W. auropunctata* (Hara et al. 2014, Montgomery et al. 2015). The inclusion of additional phagostimulants or adjuvants has been shown to effectively mask repellent substances, although this is likely only an option for homemade baits as commercial baits are ready-to-use products (Montgomery et al. 2015).

When site features, local climate, and chemical sensitivity are factored into the planning process for control operations, *W. auropunctata* can be effectively controlled using baits (Williams and Whelan 1992, Abedrabbo 1994, Ulloa-Chacón and Cherix 1994, Jourdan and Chazeau 2004, Causton et al. 2005, Vanderwoude et al. 2010, Cabral et al. 2012). Toxic (lethal) baits and IGR baits are equally effective for long-term control and management, but short-term results are drastically different. The use of toxic baits results in the rapid death of ants that ingest a lethal dose of the active ingredient, whereas IGRs are generally considered nonlethal and affect egg production, pupation, and development of reproductive and brood castes (Jourdan and Chazeau 2004; Cabral et al. 2012, 2017). Toxic bait usage results in rapid population knockdown immediately after application followed by a recovery period. Results from IGR bait applications do not show noticeable results immediately after application and instead a gradual population decline is observed over time and with repeated applications. When rapid population knockdown is desired, such as with preharvest treatments to protect harvesters, toxic baits are preferred. However, the nontoxic nature of IGR baits carry lower potential for ecological and nontarget impacts while not compromising long-term efficacy.

Nonchemical Control

When chemical control is either not effective or not desirable, such as for postharvest treatment of horticultural products including produce, cut flowers, and some nursery stock, nonchemical disinfestation methods are needed. Irradiation and hot water treatments have been tested against *W. auropunctata* and both are potentially effective phytosanitary procedures (Hara et al. 2011, Calcaterra et al. 2012). However, neither method is 100% effective and thus may not meet phytosanitary requirements (Hara et al. 2011, Calcaterra et al. 2012).

Biological Control

The parasitoid wasp *Orasema minutissima* is a common parasitoid of *W. auropunctata* throughout the Caribbean. Rather than seeking out *W. auropunctata*, *O. minutissima* oviposit in plant tissue, and emergent planidia (first instar larvae) are brought into the nest via phoretic attachment to a foraging worker or prey (Heraty 1994, Soto et al. 2010). Once inside the nest, they parasitize brood and produce cuticular hydrocarbons that mimic *W. auropunctata* brood leading to their acceptance within the nest. Although widespread and common throughout the Caribbean, parts of Central America, and northern South America, it is uncertain to what degree *O. minutissima* may aid in population control of *W. auropunctata* (Heraty 1994, Burks et al. 2018). The recent introduction and detection of *O. minutissima* on Hawai`i Island (Heraty et al. 2021), where *W. auropuncata* is widespread and a major pest, provides an opportunity to examine *O. minutssima* impacts on *W. auropunctata* over time.

Discussion

Wasmannia auropunctata is a truly fascinating species that has proven adaptable in the most surprising ways. From the unique reproductive systems (Fournier et al. 2005a) to their ability to invade a wide range of habitats and thermotolerance adaptability (Foucaud et al. 2013), *W. auropunctata*provides us with a glimpse of evolution in action. For example, clonality may have arisen to ensure successful establishment under the pressures of constant natural disturbances and the success of *W. auropunctata* in human modified habitats is a consequence of this adaptation. The specific type of clonal reproduction exhibited by *W. auropunctata* allows for the preservation of genotypes responsible for adaptations to specific ecological pressures, such as the expanded thermotolerance seen in Clad B (Chifflet et al. 2016, 2018; Coulin et al. 2019). What is not clear is whether other genetic adaptations are being preserved that influence the success of the different clades, haplogroups, or haplotypes.

The thermotolerance adaptations seen in 'Clade B' has allowed *W. auropunctata* to successfully invade Mediterranean climates previously believed to be unsuitable for the species (Rey et al. 2012; Chifflet et al. 2016, 2018; Coulin et al. 2019). Although rare, sexual reproduction has been documented in invasive clonal populations (Foucaud et al. 2006, Vonshak et al. 2009, Tindo et al. 2012). Could postinvasion sexual reproduction events lead to additional genetic adaptations? While this may be unlikely with the limited genetic diversity of invasive clonal populations, repeated introductions of different genotypes could lead to admixing and possibly the emergence of new adaptive phenotypes that may enhance its invasive potential. Alternately, genetic diversification might act as a regulating factor for invasive populations over time. The Caribbean invasion is believed to be the result of numerous introductions from Central and South America with evidence of genetic recombination and mutation events (Foucaud et al. 2010b). Given that *W. auropunctata* has been present in the region for over 100 yr, studying the Caribbean invasion could provide insight on the emergence of genetic adaptations of historical invasive *W. auropunctata* populations over time.

Wasmannia auropunctata is a serious pest ant throughout the world and is widely distributed, mostly in tropical and subtropical climates. Its ability to establish with low propagule pressure (Mikheyev et al. 2008) and to spread long-distances via human-mediated transport (Walsh et al. 2004, Mikheyev et al. 2008, Foucaud et al. 2010b) will undoubtedly allow this species to continue to spread at an alarming rate in the absence of strong biosecurity and prevention efforts. Once established, *W. auropunctata* is adept at avoiding conflict with competitor species while its population is low (Vonshak et al. 2012, Yitbarek et al. 2017). Infestations frequently go unnoticed for several years (Conant et al. 2007) and by the time a new infestation is detected, colony fragments have likely been inadvertently transported elsewhere. This is evident in Hawai`i, where new infestations on Hawai`i, Maui, Oahu, and Kaua`i islands continue to be detected despite over two decades of state-wide surveys, public outreach, and eradication attempts (Conant and Hirayama 2000, Null and Gundersen 2007, Vanderwoude et al. 2010, Vanderwoude et al. 2015).

Despite several studies investigating the relationship between *W. auropunctata* genetics, reproduction mode, and behavior (Foucaud et al. 2006, 2007, 2009, 2010a,b; Tindo et al. 2012; Rey et al. 2013b) our understanding is still limited and based on data collected from a select few locations. The biology and physiology associated with the ecological variegates and two clades highlight a plasticity rarely seen in nature. Could genetic adaptations be responsible for this plasticity be linked to specific haplogroups or haplotypes? It is possible that competitive, and thus invasion, potential may vary between clades, haplogroups, or haplotypes. It has been pointed out that exotic populations are unlikely to be traced back to their ancestral native population (Foucaud et al. 2010b). However, this may change with expanded sampling, phylogenetic analysis, and the pooling of genetic datasets. Understanding the driving forces, especially the relationships between ecological factors and genetic adaptations, behind biological and physiological shifts in *W. auropunctata* may elucidate how studying different populations could result in the occurrence of conflicting behavioral observations.

Understanding the driving forces behind biological and physiological shifts in *W. auropunctata* may also lead to the development of pesticide-free management tools and methods. For instance, if environmental factors can induce a physiological shift from nondominance to dominance, could the opposite also be true? Could RNAi target genes responsible for the physiological shift between nondominance/dominance? Additionally, rare occurrences of invasion contractions have been documented and it's unclear what led to the contractions (Lester and Gruber 2016; Mbenoun Masse et al. 2019b, 2021). Long term studies looking at expansion and contraction patterns may identify the circumstances necessary for a contraction to occur. If those circumstances were identified, would we be able to artificially induce an invasion contraction?

Interestingly, despite being a known invasive tramp ant and pest since the late 1800's, *W. auropunctata* garnered little attention from researchers and invasive species specialists until the early 2000s. In fact, the number of research publications focusing on *W. auropunctata* between 2000 and 2010 was almost double that published during the previous 80 yr. The importance of *W. auropunctata* as an invasive tramp ant was likely overshadowed by other pest ant species of importance such as *S. invicta* and *L. humile* and interest in it as a research subject was slow to develop. It is also possible that despite knowing the potential impacts of this species, researchers and invasive species specialists have underestimated its distribution potential and adaptability, thus assuming it to be a problem limited to the tropics and not relevant to cooler climates. Regardless, the delay in research focus has slowed the compilation of knowledge and understanding necessary to develop effective prevention and control methods. In this review, we have identified numerous knowledge gaps spanning distribution and status, life history, impacts, biosecurity, chemical ecology, and management. As our knowledge base on this species grows and with technological advancements, new questions arise. We encourage a greater research focus on this species in line with its global importance as an invasive species. The case of *W. auropunctata* not only highlights the importance of species-specific understanding for management and control, but also for effective biosecurity and prevention of accidental new species introductions.

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Data Availability

Data collected during and used for this project may be available upon reasonable request.

Author Contributions

Writing of the original draft was done by Michelle Montgomery and all other authors contributed to revising and editing all subsequent drafts. The final draft has been approved by all authors.

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Chapter 2

The effects of laboratory rearing diet on recruitment behavior of *Wasmannia auropunctata* (Hymenoptera: Formicidaea)

The effects of laboratory rearing diet on recruitment behavior of *Wasmannia auropunctata* (Hymenoptera: Formicidaea)

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Abstract

Wasmannia auropunctata (Roger) (Hymenoptera: Formicidae) is among the world's worst invasive species, and there is an increasing need for effective control methods for this species. Existing chemical treatments and baits used in managing other invasive ant species may not be as effective for managing *W. auropunctata.* Development of effective ant control treatments and baits depends on laboratory experiments to test the potential efficacy of a large number of products and control methods prior to implementation of large-scale field studies. However, anecdotal evidence suggests that laboratory-raised *W. auropunctata* may respond differently than their wild counterparts to bait types, and if this is the case, laboratory trials may not accurately predict results under field conditions. Here we report on experimental research investigating whether ant colonies raised in laboratories, and those in the field, show different patterns of recruitment to non-toxic baits. Laboratory and wild colony recruitment to non-toxic Hawaii Ant Lab gel bait, pureed tuna, and 50% gelled sucrose solution was measured via multi-choice and no-choice field recruitment studies. Secondly, we discuss experiments testing whether the bait preference of laboratory-raised *W. auropuncata* varies with their base diet. We tested 4 base diets: (1) lipid rich, (2) protein rich, (3) carbohydrate rich, and (4) a "complete" diet with lipid, protein, and carbohydrates offered as a buffet. Overall, we found that laboratory colonies differed from wild *W. auropunctata* in their foraging behaviors in no-choice and multi choice experiments, particularly in their levels of recruitment to the Hawaii Ant Lab gel bait. This contrast indicates that experimental trials may give misleading indications of potential outcomes of field trials. Further research is needed on optimal laboratory diets for laboratory-reared ant colonies. However, our results suggest that behavioral differences may be mitigated if colonies are maintained on a nutritionally limited diet while conducting laboratory experiments.

Key Words: little fire ant; bait response; bioassay; Hawaii

Resumen

Wasmannia auropunctata (Roger) (Hymenoptera: Formicidae) se encuentra entre las peores especies invasoras del mundo y existe una creciente necesidad de métodos eficaces para controlar esta especie. Los tratamientos químicos que existen y los cebos utilizados para el manejo de otras especies de hormigas invasoras pueden ser no tan efectivos para el manejo de *W. auropunctata*. El desarrollo de cebos y tratamientos eficaces para el control de hormigas depende de experimentos de laboratorio para probar la eficacia potencial de una gran cantidad de productos y métodos de control antes de la implementación de estudios de campo a gran escala. Sin embargo, la evidencia anecdótica sugiere que las *W. auropunctata* criadas en el laboratorio puede responder de manera diferente a sus contrapartes silvestres a las class de cebo, y si este es el caso, los ensayos de laboratorio no pueden predecir con precisión los resultados en condiciones de campo. Aquí informamos sobre investigaciones experimentales que investigan si las colonias de hormigas criadas en laboratorios, y aquellas de campo, muestran diferentes patrones de reclutamiento de cebos no tóxicos. El reclutamiento de colonias de esta especie criadas en el laboratorio y silvestres al cebo no tóxico de gel Hawaii Ant Lab, el atún en puré y la solución de sacarosa gelificada al 50% fueron medidas por medio de estudios de selección múltiple y de no elección en el campo. En segundo lugar, discutimos los experimentos que prueban si la preferencia de cebo de *W. auropuncata* criado en laboratorio varía con su dieta de base. Probamos dietas de 4 bases: (1) ricas en lípidos, (2) ricas en proteínas, (3) ricas en carbohidratos, y (4) una dieta "completa" con lípidos, proteínas y carbohidratos que se ofrecen como un buffet. En general, encontramos que las colonias de laboratorio de *W. auropunctata* difirieron de las colonias silvestres en su comportamiento de forrajeo en experimentos de no elección y de elección múltiple, particularmente en sus niveles de reclutamiento para el cebo de gel Hawaii Ant Lab. Este contraste indica que los ensayos experimentales pueden dar indicaciones engañosas de los resultados potenciales de los ensayos de campo. Se necesita más investigación sobre dietas de laboratorio óptimas para colonias de hormigas criadas en laboratorio. Sin embargo, nuestros resultados sugieren que las diferencias de comportamiento pueden mitigarse si las colonias se mantienen con una dieta nutricionalmente limitada mientras se realizan experimentos de laboratorio.

Palabras Clave: pequeña hormiga de fuego; respuesta al cebo; bioensayo; Hawaii

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Ants (Hymenoptera: Formicidae) are important terrestrial invertebrates in terms of biomass and filling niches essential for healthy ecological functioning (Andersen 1988; Abbott 1989; Holldobler & Wilson 1990; Porter & Savignano 1990; Folgarait 1998). The vast majority of the more than 12,000 described species (Ward 2007) are innocuous, but some are renowned for their destructive impacts on habitats to which they are introduced and regarded as pests (Zimmerman 1970; Beardsley 1980; Howarth 1985; Cole et al. 1992; Reimer 1994; Daly & Magnacca 2003; Krushelnycky & Gillespie 2008; Bleil et al. 2011; Fasi et al. 2013).

Control of pest ants is difficult because feeding preferences, biology, and behaviors vary between species (Silverman & Brightwell 2008; Gentz 2009). Research on nesting habits, nutrient requirements, food preferences, and chemical sensitivity has resulted in species-specific control methods for some ant species, and formulation of baits for control of a variety of species within a feeding group, such as sugar-loving, lipid-loving, or protein-loving ants (Braness 2002). *Wasmannia auropunctata* (Roger) (Hymenoptera: Formicidae) is among the world's worst invasive species, and there is an increasing need for effective control methods for this species. The International Union of the Conservation of Nature's Invasive Species Specialist Group (Lowe et al. 2000) has listed this species as having negative impacts on agriculture, quality of residential life, and native ecosystems throughout their introduced range (B. M. Drees et al., unpublished; Davis & Van Schagen 1993; Abedrabbo 1994; Haines et al. 1994; Feener 2005; Cooper et al. 2008; Arakaki et al. 2009). This ant species has been reported to reduce biodiversity, farm phytophagous insects that vector plant disease (Smith 1929; Spencer 1941; Fabres & Brown 1978; Fowler et al. 1990; Delabie & Cazorla 1991; Delabie et al. 1994; Jourdan 1997; de Souza et al. 1998; Wetterer et al. 1999; Armbrecht & Ulloa-Chacon 2003; Le Breton et al. 2003, 2005; Walker 2006; Fasi 2009; Vonshak et al. 2009; Berman et al. 2013; Fasi et al. 2013; Vanderwoude et al. 2016), and are linked to the occurrence of tropical keratopathy (clouding of the cornea resembling cataracts) in wild and domestic vertebrates (Roze et al. 2004; Theron 2005; Rosselli & Wetterer 2017). In addition to ecological impacts, *W. auropunctata* is considered a major nuisance pest due to its painful stings in residential and agricultural environments (Spencer 1941; Fabres & Brown 1978; Fasi et al. 2016).

This species has gained little recognition as a pest in the continental USA. Although it has been established in Florida for almost a century, economic and ecological impacts appear to be minimal (Smith 1929; Spencer 1941). This is in contrast to the Pacific region, including Hawaii (Fabres & Brown 1978; Clark et al. 1982; Lubin 1984; De La Vega 1994; Lowe et al. 2000; Jourdan 2001; Holway et al. 2002; Armbrecht & Ulloa-Chacon 2003; Le Breton et al. 2003; Wetterer & Porter 2003; Vanderwoude et al. 2016) where impacts can be severe and widespread. Like *Solenopsis invicta* Buren (Hymenoptera: Formicidae), *W. auropunctata* respond primarily to lipid and protein baits, and it is generally assumed that commercial "fire ant" baits formulated for *S. invicta* also will be suitable for *W. auropunctata*. Despite this assumption, Montgomery et al. (2015) reported that this may not be the case given their observation that *W. auropunctata* is repelled by the insect growth regulator (S)-methoprene, an active ingredient used in some fire ant baits. Chemical sensitivity, biology, behavioral, and ecological differences between *S. invicta* and *W. auropunctata* are all factors influencing the efficacy of currently available baits against the latter species (Montgomery et al. 2015), thus necessitating species-specific bait development and laboratory trials.

As invasive ants continue to spread and new pest species emerge, the need for species-specific research grows. Laboratory experiments are essential to assess biology, behaviors, and screen efficacy of various pesticide formulations prior to implementing management plans for new pests. Generally, laboratory reared insects are frequently used as test subjects for pesticide efficacy trials against ants and other insects (Banks et al. 1983; Braness 2002), including post-harvest treatment studies (Follett & Armstrong 2004), biological control screening (Castillo et al. 2014), dietary and feeding studies (Bhatkar & Whitcomb 1970; Marchioro & Foerster 2012), as well as biological and behavioral observation studies (Adams & Traniello 1981; Howard et al. 1982; Abril et al. 2008; Kirschenbaum & Grace 2008; Rey et al. 2013). Laboratory colonies often are maintained for extended periods of time in controlled environments, often spanning many generations with little resemblance to the insects' natural habitat. This removal and disconnect from natural conditions raises questions regarding the biological and behavioral equivalence of laboratory-reared insects, their suitability as test subjects, quality of test results, and whether or not those results truly predict what should be expected under field conditions. Moreover, insects reared on artificial diets generally differ from their wild counterparts in feeding and foraging behavior (Herard et al. 1988; Propkopy et al. 1989; Ennis et al. 2015), predator evading capabilities (Hendrichs et al. 2007), and response to pheromone cues (Propkopy et al. 1989; Clark et al. 2011), all of which are evolutionary traits essential for species survival. For example, laboratory studies using *S. invicta* found that nutritional voids in diet caused foraging workers to feed on and bring back more food items that contained the limiting nutrient than others (Sorenson et al. 1985; Behmer 2009; Cook et al. 2010).

In addition, laboratory rearing diets are not analogous to natural diets (Marchioro & Foerster 2012; Ennis et al. 2015), and often are formulated to ensure an adequate supply of essential nutrients is available at all times. In contrast, wild populations are limited by irregular supply of at least some of these nutrients that, in turn, may alter feeding preferences when these nutrients become available. Dussutour and Simpson (2008) reported that ant foraging behavior is influenced by nutritional demand signals from their larvae. Cassill and Tschinkel (1999) and Portha et al. (2002) found that foraging workers adjust which resources are collected and shared to meet carbohydrate, protein, and lipid nutritional needs of the colony. In addition, nutrient allocation is regulated by nurse ants and foraging workers to promote and maintain optimal colony growth (Dussutour & Simpson 2008). Several studies have shown that insect rearing diets can influence directly the outcome of laboratory experiments and produce results not indicative of the behavior of that species under field conditions (Huettel 1975; Propkopy et al. 1989; Marchioro & Forester 2012; Ennis et al. 2015). This is especially important when evaluating insecticidal bait matrices and the attractants they may contain.

Nonetheless, bait development requires laboratory experiments to assess the potential of new formulations, and evaluate the efficacy of currently available baits for new pest ant species. The advantages of artificial laboratory diets are numerous and include yr-round availability, and the ability for researchers to manipulate and control nutrient content (Ennis et al. 2015). Laboratory diets for *W. auropunctata*, as with many ant species, requires a mixture of carbohydrates, proteins, and lipids, along with some insect matter as a source of chitin to ensure prolonged colony health and survival (Williams et al. 1987; Porter et al. 2015). Several laboratory diets have been developed and reported as effective for rearing *S. invicta*, a species often used in laboratory studies. All such diets require nutrient supplementation with insect matter such as mealworms, or whole crickets, for continued brood production and colony growth (Bhatkar & Whitcomb 1970; Williams et al. 1987; Keller 1989; Porter et al. 2015). The disadvantage of nutritionally defined laboratory diets is that they are consistent and homogenous, which does not reflect the constantly changing nutritional needs of the colony or the temporal and spatial variability of the supply of these nutrients. Therefore, the objective of our study, reported herein, was to investigate whether rearing diet influenced *W. auropunctata* foraging behaviors on various non-toxic baits in the laboratory when compared with wild colonies.

Materials and Methods

2015 PILOT STUDY

Laboratory and field trials evaluating attractiveness and palatability of non-toxic protein, carbohydrate, and lipid rich baits were conducted between Feb and Jun of 2015. Both trials consisted of no-choice and multi-choice recruitment to assess bait preference. Laboratory experiments were conducted in a greenhouse with a glass roof and screen mesh walls (Hawaii Department of Agriculture, Hilo, Hawaii, USA (19.706494°N, 155.074455°W), where ambient temperature and relative humidity were not controlled, but was comparable to that of exposed outdoor environments. Laboratory colonies of *W. auropunctata* were used in these studies, and had been in continuous culture for approximately 4 mo prior to use. Ants from this colony were sourced originally by extraction from infested banana leaf litter collected from the University of Hawaii Experimental Farm in Pana'ewa, Hawaii Island (19.651408°N, 155.049938°W). To maintain a natural worker to queen ratio of 250 to 500 (Ulloa-Chacon & Cherix 1990), an average of 1,120 workers, 3 queens, and brood were transferred to 35 × 20 cm plastic Sterilite® containers (Sterilite Corporation, Townsend, Massachusetts, USA) with artificial nests made of a 16 × 150 mm glass test tube covered in black paper with approximately 10 mL of water and cotton wool inserted for moisture.

Prior to each experiment, colonies underwent a 4 wk acclimation period during which they were fed a standard diet that was a modified version of Keller's non-desiccating rearing diet (Keller 1989), where crickets were substituted for mealworms (referred to as Keller Cubes). This diet consisted of an oligidic (non-chemically defined) mixture of sugar, protein, lipid, mineral, and vitamins blended together for an "all-in-one" diet. The ingredients in this diet have been accepted among researchers studying laboratory ant colonies as a standard rearing medium. This standard diet was compared with 3 experimental dietary treatments (in no-choice tests) that consisted of (1) a high lipid diet (Great Value™ Creamy Peanut Butter, Walmart Apollo LLC, Bentonville, Arkansas, USA); (2) a high carbohydrate diet (unrefined honey); and (3) a "complete" diet comprised of unrefined honey, Great Value™ Creamy Peanut Butter, 1 quarter of an Up & Up™ brand Jumbo cotton ball (Target Brands Inc., Minneapolis, Minnesota, USA) soaked, but not dripping, with vegetable oil (Great Value™, Walmart Apollo LLC, Bentonville, Arkansas, USA) presented buffet fashion.

Brood production and colony growth of ants is known to cease without insect supplementation (Vogt 2003; Kay et al. 2010; Gavilanez-Slone & Porter 2014; Porter et al. 2015), whereas low carbohydrate diets are known to cause high worker mortality and reduced colony activity (Kay et al. 2006; Cook et al. 2010; Gavilanez-Slone & Porter 2014). Therefore, all colonies received whole dead crickets and 25% sugar water ad libitum in addition to their experimental dietary treatment. Colonies assigned to the standard diet did not receive whole dead crickets because crickets were already an ingredient in the Keller Cubes. Dietary treatments were randomly assigned to experimental colonies within each replicate. Experiments were completed at 28 d and replicated 6 times. Nutritional analyses for each dietary treatment are listed in Table 1. Laboratory experiments were separated temporally by 4 wk for ants to re-habituate to normal rearing conditions and ensure all colonies used in the second experiment were equally healthy. During this time any declining colonies were replaced.

Choice experiments used the same methods as mentioned earlier for no-choice experiments, and consisted of measuring recruitment to 3 non-toxic test baits: (a) Hawaii Ant Lab Gel Bait Matrix (Montgomery et al. 2015) consisting of 40% vegetable oil, 56% water, 0.8% xanthan gum, and 3.2% NOW® Argentine Beef Liver (NOW®, Bloomingdale, Illinois, USA); (b) gelled 50% sucrose solution; and (c) Star Kist® Chunk Light Tuna (Star Kist Co., Pittsburgh, Pennsylvania, USA) in water.

Food was withheld for 24 h prior to exposure to treatments offered in a buffet style by applying approximately 1 g of each diet on 9 × 3 cm plastic-laminated cards placed in the foraging area of each experimental colony. Cards were divided into 3 marked areas of 3 × 3 cm and bait placements randomized on the cards. Recruitment to each bait was measured once per wk for 5 wk by recording and examining high-resolution digital photographs of each card taken 60 min after exposure. Photographs were examined in the laboratory and ants on bait cards (within each of the 3 marked areas) were counted and recorded. By the end of the multi-choice experiment, 1 colony had died completely and several others had greatly declined.

Because *W. auropunctata* is typically controlled in the field through broadcast application of lipid based baits, the no-choice experiment focused only on recruitment to the Hawaii Ant Lab Gel Bait matrix. Experimental colonies were fed the same dietary treatments as in the multi-choice experiment, treatments again were randomly assigned within each replicate, and the experiment was replicated 6 times. Experimental colonies were maintained on their respective diets for 7 wk. Small amounts of Hawaii Ant Lab Gel Bait approximately 1 cm in diameter were applied to 4.5 × 4.5 cm square laminated cards and placed in the foraging area of each experimental colony. Recruitment was measured by recording high-resolution digital photographs of each card taken 60 min after exposure. Photographs were examined in the laboratory, and ants on bait cards were counted and recorded. As observed in the multi-choice experiment, by the end of the no-choice experiment many of the colonies had declined and were visibly unhealthy.

Table 1. Nutritional breakdown as the percent of lipid, carbohydrate, and protein for each dietary treatment for the 2015 pilot study's laboratory component. Sources where nutritional information was obtained for each diet and the ingredients for the Keller Cube diet are provided.

Diet	Percent lipid	Percent protein	Percent carbohydrate	Information source
Keller Cubes	4	7	2	USDA National Nutritional Database, Libby, Niell & Libby® Corned Beef product label, Food Insects Newsletter
Peanut Butter + crickets*	56	35	27	Great Value™ Creamy Peanut Butter product label, Food Insects Newsletter l
Honey + crickets*	6	13	87	USDA National Nutritional Database, Food Insects Newsletter
Buffet + crickets*	100	35	100	USDA National Nutritional Database, Food Insects Newsletter

*Total values for the some dietary treatments may total over 100% due to each element of the diet being offered separately and not being combined into an "all-in-one" diet, as is the case with the Keller Cubes diet.

Field trials were conducted concurrent with laboratory experiments at the University of Hawaii Hilo Experimental Farm in Pana'ewa, Hawaii Island, where the laboratory colonies were initially sourced. The same non-toxic baits tested in the laboratory experiments were placed in the field where forager recruitment to bait was measured at 60 min after exposure. Field experiments also consisted of multi-choice and no-choice experiments; however, the no-choice experiment compared all 3 non-toxic baits.

The field multi-choice experiments consisted of 15 replicates spaced 5 m apart throughout the study site. Replicates were comprised of three 4.5 × 4.5 cm square laminated bait cards arranged in a small 13.5 cm triangle, and bait placement was randomized. The field no-choice experiments were configured in a randomized block design with 10 replicates, where each bait treatment was represented only once per block and spaced 5 m apart to establish independence. Bait stations consisted of a single 4.5 × 4.5 cm laminated card, and treatments were randomly assigned within each block.

At the culmination of the experiment, 2 main issues were identified as detrimental to the experiment and results: (1) the nutritional composition of the dietary treatments overlapped, confounding analyses, and (2) colony health declined to the point that some colonies stopped foraging and 1 colony died during the experiment. These results were deemed to be unreliable and the 2015 experiments were treated as a pilot study and not used in subsequent analyses.

2016 STUDIES

In 2016, no-choice and multi-choice laboratory and field experiments were repeated with the laboratory component being conducted entirely under controlled conditions at the University of Hawaii College of Tropical Agriculture and Human Resources Waiakea Experiment Station (19.643402°N, 155.079969°W). Based on the findings by Galvinez-Slone and Porter (2014) a simple diet of 25% sucrose solution and dead crickets was more suitable for long-term rearing of fire ant colonies than the oligidic diet (Keller 1989) previously used as the standard treatment during the 2015 pilot study. Therefore, the dietary treatments consisted of (1) standard carbohydrate based diet (25% sucrose solution); (2) lipid based diet (small cotton wick saturated in Great Value™ vegetable oil); (3) protein based diet (pureed Star Kist™ Chunk Light Tuna in water); and (4) "complete diet buffet" (i.e., a mixture of diets 1 to 3).

All colonies received whole dead crickets ad libitum in addition to the treatment in order to maintain brood production and maintain colony health. Nutritional analyses for each 2016 dietary treatment are listed in Table 2. Pureed tuna was replaced twice per wk due to rapid dehydration and desiccation. For multi-choice and no-choice experiments, ants were starved for 48 h prior to non-toxic bait exposure. During the multi-choice trial, ant recruitment was recorded at pre-treatment (0 d after treatment) and post-treatment (31 d after treatment). For the no-choice experiment, recruitment data were collected each wk from 0 d (pre-treatment) to 49 d after treatment. Ant recruitment rates to each non-toxic bait followed the same procedures as in the 2015 pilot study. Overall mortality at the end of each experiment was assessed by counting all dead and live ants and comparing between dietary treatments.

Statistical Analysis

Laboratory Multi-Choice Study. To determine if there are interactions between dietary treatment and ant recruitment rate to non-toxic baits, the difference between pre-treatment recruitment rates (0 d after treatment) and the final recruitment rates from each multi-choice experiment was analyzed. Data from each experiment were analyzed separately via Analysis of Covariance (ANCOVA, $P < 0.05$) (R© Statistical software v 3.2.3, R Development Core Team 2012) in order to accommodate negative values that could not be accommodated using a Poisson distributed generalized linear mixed effects model. An ANCOVA was more appropriate than a linear mixed model ANOVA ($P < 0.05$) due to our interest in overall effect of dietary treatment to bait response, as opposed to the change in response to bait over time after treatment (Dimitrov & Rumrill 2003; Knapp & Schafer 2009). Because the model compared the difference between pre- and post-treatment recruitment rates, a covariant was needed to account for variation in baseline recruitment rates between colonies. Therefore, mean pre-treatment recruitment to all 3 baits for each colony was calculated and included in the model as a covariant. Multiple pairwise comparisons were tested via Tukey's post hoc analysis, and the resulting covariant-adjusted means were used to determine whether laboratory ants showed a clear preference for a non-toxic bait when given a choice within each dietary treatment, and between all dietary treatments (Ramsey & Schafer 2002). Figures are presented using non-transformed means.

Laboratory No-Choice Study. Data were analyzed via a generalized linear mixed model using the log link function of Poisson distributed data where colony was a random variable nested in d after treatment and pairwise comparisons obtained via Tukey's post hoc analysis ($P < 0.05$). An observation-level random effect was included in the model to address over dispersion issues (Harrison 2014). Results were exponentially back transformed and interpreted as multiplicative outcomes (Ramsey & Schafer 2002). Overall mortality rate between dietary treatments was analyzed using a 1-way ANOVA ($P < 0.05$).

Field Multi-Choice and No-Choice Studies. To determine which non-toxic bait wild ants prefer, data from no-choice and multi-choice field experiments were analyzed the same way as the no-choice laboratory study. Results of the field experiments were used for qualitative comparison when interpreting the results from the overall bait preference of laboratory colonies in order to determine whether or not laboratory and wild colonies have similar preference for and recruitment rate to non-toxic baits.

Table 2. Nutritional breakdown as the percent of lipid, carbohydrate, and protein for each dietary treatment for the 2016 laboratory experiments are displayed in the table. Sources where nutritional information was obtained for each diet are provided.

Diet	Percent lipid	Percent protein	Percent carbohydrate	Information source
25% sucrose solution + crickets	0	13	30	USDA National Nutritional Database, Food Insects Newsletter
Pureed tuna + crickets	1	23	5	Star Kist™ Chunk Light Tuna in water product label, Food Insects Newsletter
Vegetable oil wick + crickets*	100	13	5	USDA National Nutritional Database, Food Insects Newsletter
Buffet + crickets*	100	32	30	USDA National Nutritional Database, Food Insects Newsletter

*Total values for the some dietary treatments may total over 100% due to each element of the diet being offered separately and not being combined into an "all-in-one" diet.

Results

2016 LABORATORY EXPERIMENTS

Results from the multi-choice experiment identified a significant interaction between dietary treatment and bait ($F_{6,32}$ = 4.01; *P* = 0.003). All colonies recruited to 50% gelled sucrose more than the other baits after 49 d of exposure to their respective dietary treatment (Fig. 1); however, these differences were not significant within the protein based dietary treatment (Sucrose-Hawaii Ant Lab t_{47} = 2.149; *P* = 0.091; Sucrose-Tuna t_{47} = 0.298; *P* = 0.952). Ants maintained on the complete buffet diet recruited to 50% gelled sucrose significantly more than the Hawaii Ant Lab gel bait with a mean difference in recruitment rate of 53.8 ants (t_{47} = 3.212; *P* = 0.007), but recruitment between 50% gelled sucrose and tuna was not significantly different (t_{47} = 1.158; *P* = 0.484). Recruitment to the Hawaii Ant Lab gel bait was also not significantly different compared with tuna (t_{47} = 2.053; *P* = 0.111). Recruitment patterns for ants maintained on the lipid based diet mirrored that of the complete buffet dietary treatment. Recruitment rate to 50% gelled sucrose was significantly greater than to the Hawaii Ant Lab gel bait with a mean difference in recruitment rate of 55.0 ants (t_{47} = 3.283; *P* = 0.005), and no difference in recruitment rate was detected between 50% gelled sucrose and tuna (t_{47} = 1.015; *P* = 0.571) or between the Hawaii Ant Lab gel bait and tuna (t_{47} = 2.268; *P* = 0.070). Recruitment rates to all 3 non-toxic baits were significantly different among ants maintained on the standard carbohydrate based dietary treatment. Ants recruited to 50% gelled sucrose more than both the Hawaii Ant Lab gel bait (mean difference in recruitment = 139.6 ants; t_{47} = 8.33; *P* < 0.0001) and tuna (mean difference in recruitment = 75.4 ants; t_{47} = 4.50; *P* = 0.0001). Greater recruitment rates also were recorded for tuna compared with the Hawaii Ant Lab gel bait (mean difference in recruitment = 64.2 ants; t_{47} =3.832; *P* = 0.001). Among ants maintained on the protein based dietary treatment, no significant differences in recruitment rate were detected between any of the baits (Sucrose-Hawaii Ant LabL t_{47} = 2.149; *P* = 0.091; Sucrose-Tuna t_{47} = 0.298; *P* = 0.952; Tuna-Hawaii Ant Lab t_{47} = 1.851; *P* = 0.165).

Results from the laboratory no-choice experiment indicated diet also influenced recruitment rate to the Hawaii Ant Lab gel bait when no other non-toxic bait was available. Colonies maintained on the standard carbohydrate based diet had an 83% higher median recruitment rate to the Hawaii Ant Lab gel bait compared with colonies maintained on the buffet diet (z = 3.12; *P* = 0.01) and 84% higher median recruitment rate than colonies maintained on the lipid based diet (z = 3.15; *P* = 0.009). Colonies maintained on the protein based diet had a 138% higher median recruitment rate compared with colonies maintained on the buffet diet (z = 4.48; *P* < 0.001) and 140% higher median recruitment rate compared with colonies maintained on the lipid based diet (z = 4.51; *P* < 0.001). Colonies maintained on lipid based and the buffet diets did not differ in recruitment rate to the Hawaii Ant Lab gel bait (z = 0.029; *P* = 0.999). Recruitment rate between colonies maintained on protein and carbohydrate based diets differed only slightly and were not significantly different (z = 1.359; *P* = 0.525). Results from analysis of colony mortality data indicated final mortality rates did not differ significantly regardless of dietary treatment ($F_{3, 16}$ 0.136; *P* = 0.937).

2016 FIELD EXPERIMENTS

Results from the field multi-choice experiment indicated significantly higher recruitment rates to the Hawaii Ant Lab gel bait over all other baits tested (Fig. 2) with an 11-fold greater (z = 9.07; *P* < 0.001) median recruitment rate to the Hawaii Ant Lab gel bait compared with tuna and 6-fold greater recruitment when compared with gelled

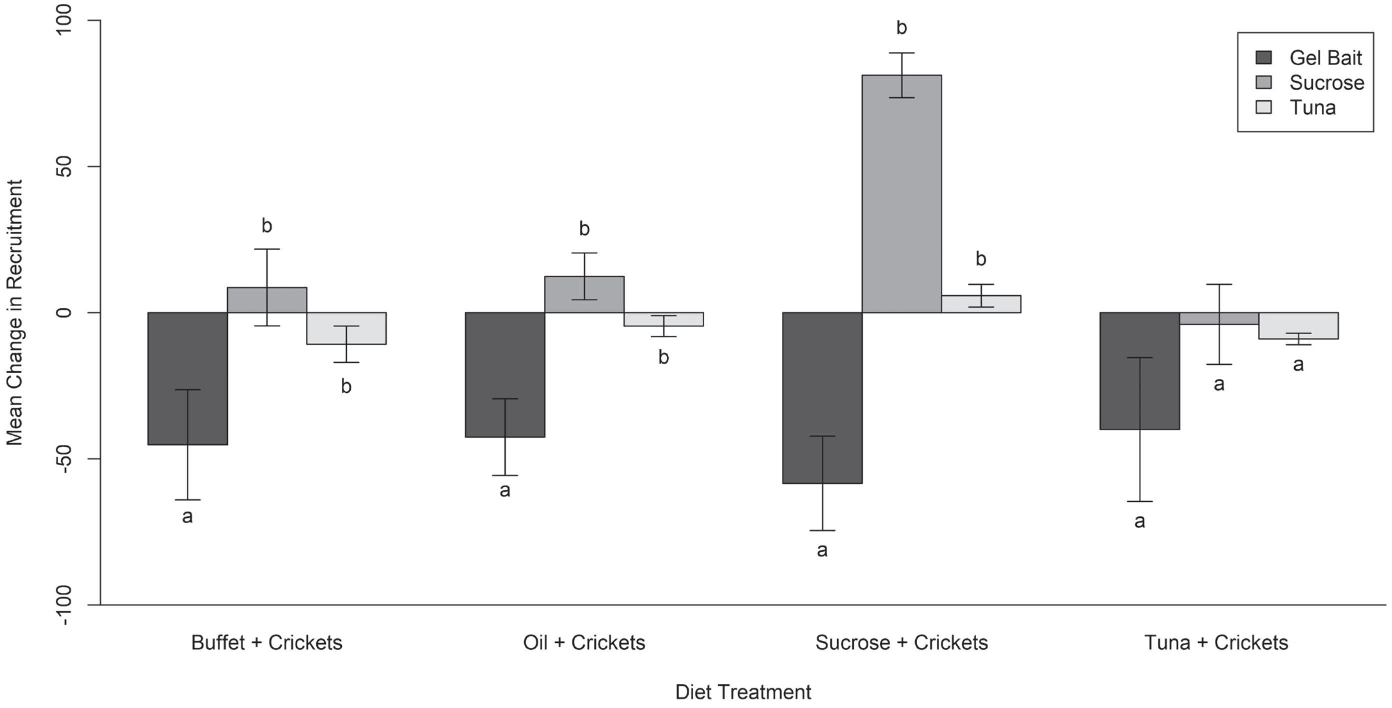

Fig. 1. Mean ± SE difference in recruitment rates of laboratory raised *Wasmannia auropunctata* to non-toxic baits: Hawaii Ant Lab gel bait, 50% gelled sucrose solution, and tuna between pre- and post-treatment measurements of the multi-choice laboratory experiment (n = 5 colonies). Bars in each group with different letters above have statistically different means (*P* < 0.05). Colonies were exposed to their respective dietary treatment (buffet plus crickets: n=5, vegetable oil wick plus crickets: n=5, 25% sucrose solution plus crickets: n=5, and pureed tuna plus crickets: n=5) for 49 d. Means represented in this chart are based on raw data for visualization and are not the reported marginal means.

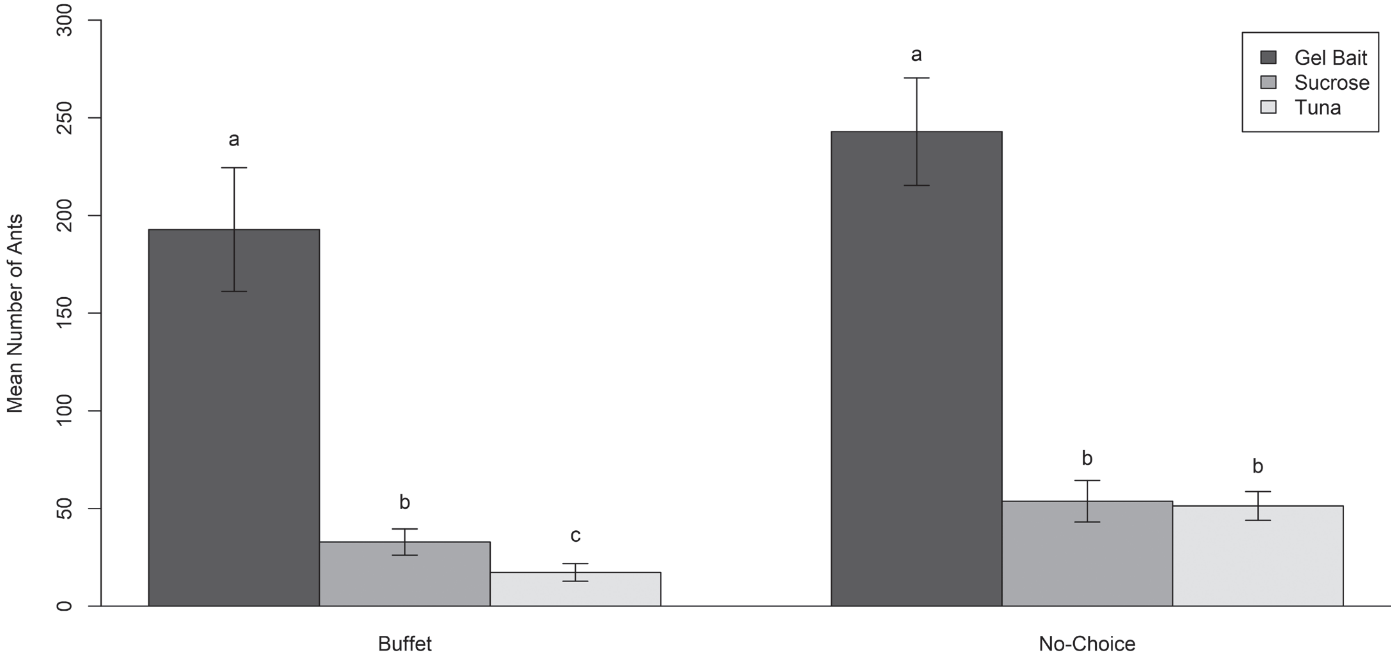

Fig. 2. Recruitment rates (mean number of ants ± SE) of wild *Wasmannia auropunctata* to the Hawaii Ant Lab gel bait, 50% gelled sucrose solution, and tuna for multi-choice (n = 6 per treatment) and no-choice (n = 6 per treatment) field experiments. Bars within clusters with different letters above have statistically different means ($P < 0.05$). Means represented in this chart are based on raw data for visualization and not proportional results from the Poisson distributed generalized linear mixed model as reported.

sucrose. Median recruitment rates to 50% gelled sucrose was 2-fold greater than to tuna (z = 2.41; $P = 0.042$).

Results from the no-choice experiment mirrored the multi-choice experiment. Recruitment rates to the Hawaii Ant Lab gel bait were significantly greater than to the other 2 baits (Fig. 2). Median recruitment rate to the Hawaii Ant Lab gel bait was nearly 5-fold (4.8 times) greater than both tuna (z = 6.39; $P < 0.001$) and 50% gelled sucrose solution (z = 6.64; $P < 0.001$). No significant difference in recruitment rate was detected between tuna and 50% gelled sucrose solution (z = 0.276; $P = 0.959$).

Discussion

Our results from the laboratory experiments suggest diet may affect recruitment rates to resources containing different nutritional profiles; however, the differences in recruitment rates did not appear to be driven entirely by nutritional voids, as described in previous studies conducted on *S. invicta* (Sorenson et. al. 1985; Cassill & Tschinkel 1999; Behmer 2009). When only a single bait option (Hawaii Ant Lab gel bait) was provided to laboratory colonies during the no-choice experiment, a marked difference in recruitment rate was observed. We also found that colonies maintained on diets limited in lipids, such as carbohydrate based (25% sucrose) and protein based diets (tuna), recruited to the Hawaii Ant Lab gel bait significantly more than colonies maintained on diets that included vegetable oil soaked cotton ball (lipid based and complete buffet diets). This supports the hypothesis that nutritional voids influence foraging behavior and bait preferences in laboratory colonies. Conversely, results from the multi-choice experiment indicated a strong preference for 50% gelled sucrose regardless of whether or not carbohydrates were included in their respective dietary treatment.

Results from our field studies indicate *W. auropunctata* are attracted to lipids above other nutrients (Williams & Whelan 1992; Ndueze et al. 2013; Montgomery et al. 2015). This is consistent with historical observations and past studies (Williams & Whelan 1992) and re-affirms the use of lipid-rich lures and baits for survey, detection, and control of this species.

Our studies further suggest a disconnect between the manner in which laboratory *W. auropunctata* and wild *W. auropunctata* behave toward food baits. These results have profound implications in regard to results collected from laboratory bait preference, bait efficacy, and dietary studies. Laboratory reared insects do not behave similarly to wild populations; therefore, the results from laboratory experiments are not necessarily relevant in the context of projecting possible outcomes of large field studies and efforts focused on laboratory experiments may, in fact, be counterproductive.

We were able to elicit a reliable recruitment rate to the Hawaii Ant Lab gel bait from ants maintained on lipid-limited diets during the no-choice laboratory experiment. This suggests reasonably reliable results may be obtained from bait palatability and efficacy experiments on *W. auropunctata* laboratory colonies provided they are maintained on a diet limited in lipids and that all baits tested are formulated with similar nutrient composition. For example, reasonably reliable results can be expected when testing preference between peanut butter, Hawaii Ant Lab gel bait, and various proprietary fire ant baits, because all baits being evaluated are formulated with high lipid content as the primary food attractant. Results from such experiments are more likely to reflect results of future field trials. Experiments comparing baits formulated with carbohydrates as the primary food attractant should not be compared against baits formulated with lipids as the primary food component. Additionally, laboratory data should be paired with field experiments for result validation whenever possible.

Although providing all essential nutrients to laboratory colonies in a buffet, not as an “all-in-one” diet such as the Keller Blocks, is beneficial for colony maintenance and growth (Gavilanez-Slone & Porter 2014), it appears to confound the results of feeding experiments with *W. auropunctata* as test subjects. Since wild *W. auropunctata* preferentially recruit to baits high in lipids, our results suggest it is important to limit the amount of lipids in the rearing diet prior to conducting a laboratory experiment.

Past studies have shown other ant species raised on high protein and low sugar diets can have high mortality rates in laboratory colonies (Dussutour & Simpson 2008; Cook et al. 2010). Our observations, combined with the findings of past research, suggest high protein with low sugar diets also are not appropriate for maintaining laboratory colonies, regardless of the results from the studies reported here. Despite no significant difference in mortality rate between dietary treatments and no significant difference in recruitment rates compared with colonies maintained on carbohydrate rich diets, colonies maintained on a protein-rich diet without carbohydrates generally appeared to be less active, and exhibited slightly higher mortality and lower brood production than treatments where a carbohydrate resource was included. It is our recommendation that laboratory colonies of *W. auropunctata* be maintained on a diet consisting primarily of carbohydrates and crickets with occasional protein supplements in order to maintain healthy colonies while maintaining the integrity of data collected from laboratory experiments and reliability of results. Additional lipids may be supplemented occasionally but should be withheld entirely for 1 to 2 wk prior to conducting a laboratory experiment in order to elicit a reliable recruitment response to lipid based baits.

In conclusion, laboratory experiments are a critical component of managing invasive pest ants and limiting their economic and environmental impacts. Evaluation of suitable rearing diets is an essential aspect of determining the best means of maintaining experimental colonies while also maintaining the integrity of data from experimental research. In addition, the need for species-specific bait development and laboratory trials is an important consideration in developing the best, most effective approach to managing invasive species. Though we were able to identify an interaction between rearing diet and recruitment to non-toxic baits from our statistical model, there appear to be other factors influencing bait preference which we were unable to identify during this study. Studies of various other ant species have indicated foraging preferences change seasonally. This could be due to external factors (e.g., temperature, humidity, type of available resources) or factors within individual colonies (e.g., amount of brood, queen fecundity), or any combination of these factors. Given that invasive *W. auropunctata* are polydomous, it is also possible that the mere separation of a bulk rearing colony into multiple individual experimental colonies could influence colony behavior. Further research is needed to test other factors which could potentially influence laboratory colony behaviors, such as foraging preferences.

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Chapter 3

Laboratory Evaluation of Egg White and Milk External Biomarkers for *Wasmannia auropunctata* (Hymenoptera: Formicidae)

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Research

Laboratory Evaluation of Egg White and Milk External Biomarkers for *Wasmannia auropunctata* (Hymenoptera: Formicidae)

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Abstract

Acquisition and retention of two protein markers were tested on little fire ants, *Wasmannia auropunctata* Roger. Pure (100%) cow's milk and a dilution (10%) of chicken egg whites were applied to *W. auropunctata* directly by contact spray plus residue or indirectly via residual contact only with protein-marked plant debris. Protein-marked ants were held in plastic shoe-box-sized containers, collected at 0, 24, and 48 h after exposure to their respective marks, and then examined for the presence of the marks by a chicken egg albumin and milk casein-specific enzyme-linked immunosorbent assay. Cross-contamination rates were assessed by allowing ants marked with egg whites to interact with an equal number marked milk for 24 and 48 h, and then collected either individually or in bulk. Results indicated that the egg white biomarker was retained longer than milk and that more ants were successfully marked when the direct spray application method was employed. Cross-contamination rates were highest among bulk-collected ants and lowest among ants collected individually after 24 h. However, the rates of cross-contamination among individually collected ants increased and were similar to that of bulk-collected ants after 48 h. On the basis of our results, external protein marking may not be suitable if mass trapping is required or if the study extends beyond 24 h due to high cross-contamination rates among specimens collected in bulk and reduced marker detection rates.

Key words: little fire ant, mark-capture, ELISA, immunomarking

The little fire ant, *Wasmannia auropunctata* Roger (Hymenoptera: Formicidae), is a major pest on the island of Hawaii and is spreading throughout the Hawaiian archipelago and the pacific region (Wetterer and Porter 2003, Vanderwoude et al. 2016, Mayron 2019). It is a very small, nondescript, and rust-colored ant approximately 1.5 mm in length. The destructive nature and mechanism by which *W. auropunctata* succeed over, and at the expense of other species, is well documented (Holway et al. 2002, Wetterer and Porter 2003, Le Breton et al. 2004). Unfortunately, despite being one of the most invasive species in the world (Lowe et al. 2000), little is known about this ant's population dynamics, foraging range, or distribution of food resources.

Wasmannia auropunctata workers forage at least as far as 6 m from their nest (Fernald 1947). However, actual distance is dependent on many factors, including humidity and terrain. No maximum foraging distance has been reported in the literature. Population densities also vary with habitat climate and food availability. The little fire ants build three-dimensional 'super-colonies' that consist of a network of small individual nest aggregations located on the ground and throughout tree canopies, between which workers move freely. To date, the only estimate of population densities was calculated by manually sorting shallow-core thatch and soil samples and counting all adult ants, brood, and larvae (Souza et al. 2008). Although this may be an accurate way to estimate populations of epigeic species, *W. auropunctata* also nest in trees and vegetation (de Souza et al. 1998), so this estimate is likely to be overly conservative.

Attempts to eradicate *W. auropunctata* have been met with varying success (Causton et al. 2005, Vanderwoude et al. 2010). A lack of knowledge of their biological and behavioral traits and the influence of environmental factors on management plans are two leading factors in poor control efficacy (Souza et al. 2008, Taniguchi 2008). Mark-release-recapture (MRR) and mark-capture (MC) techniques offer opportunities to better understand *W. auropunctata* population dynamics, nutrient flow within a colony, and spatial distribution of resources throughout an infested area. Knowledge of parameters, such as foraging distance and rates of trophallaxis, is vital when developing species-specific monitoring and control programs.

MRR and MC research have been integral to elucidating the behavior, dispersion, and population ecology of insects (Sunderland et al. 1995, Bowler and Benton 2005, Cordero-Rivera and Stoks 2008). Numerous marking procedures have been used with insects (e.g., physical tags, paints, inks, dyes, fluorescent dusts, trace elements, genetic markers, and proteins), but very few are useful for marking insects as tiny and delicate as *W. auropunctata* (Su et al. 1991, Evans 1997, Hagler and Jackson 2001). As social insects, behaviors such as grooming and sharing of food resources between individuals require special consideration. Care is needed to ensure that mark retention is uniform among marked individuals; there is minimal risk of cross-contamination between nestmates, and the ant's behavior is not negatively affected (Hayes 1991, Kay et al. 2010, Dickens and Brant 2014). Previous research has shown that mark retention and toxicity vary between markers applied externally and internally and among different species. Fluorescent dusts easily wash away in the rain (Rhodes et al. 1997) and are rapidly removed via grooming in social insects (Evans 1997). Internal dye markers are rapidly excreted by some termite species (Su et al. 1991). In short, finding a suitable marking technique for social insects offers another level of complexity to studying their dispersal behavior.

The methods described above have been previously tested using several ant species (Talbot 1943, Stradling 1970, Young 1980, Wojcik et al. 2000, Vega and Rust 2003); however, no studies have investigated marking techniques for *W. auropunctata*. Given their small size, the use of conventional marking techniques (e.g., topical paints, dyes, tags, etc.) is impractical, because they are likely to alter normal ant behavior (Steiner 1965). Also, preliminary observations showed that fluorescent dusts are not persistent on *W. auropunctata* (e.g., <24 h; M. Montgomery, pers. obs.).

The use of vertebrate immunoglobulin G (IgG) protein biomarkers, detectable by protein-specific enzyme-linked immunosorbent assays (ELISA) (Hagler et al. 1992) have proven useful for marking minute parasitoids (Hagler and Jackson 1998, Irvin et al. 2018) and ants (Buczkowski and Bennett 2007, Song et al. 2017, Hogg et al. 2018). However, the costs associated with IgG protein markers are prohibitive. A more cost-effective protein immunomarking technique using chicken egg whites or whole cow's milk as biomarkers was developed by Jones et al. (2006) and this second-generation marking technique has been subsequently used to mark a wide variety of insects for MC research (Hagler 2019). Protein-specific ELISA can detect protein biomarkers at minute quantities (Hagler 2019). Nevertheless, it is unknown whether biomarkers are transferred in detectable amounts between marked and unmarked individuals through ant social behaviors and specimen collection methods.

This study assesses the efficacy and suitability of using chicken egg whites (hereafter referred to as egg whites) and whole cow's milk (hereafter referred to as milk), applied directly and indirectly, as topical (external) markers for *W. auropunctata* MRR and MC research. Cross-contamination due to social interaction and collection methods (individual or bulk collections) was also examined.

Materials and Methods

The study was conducted within an enclosed rearing facility at the University of Hawaii Experimental Farm near Hilo, HI, operated by the College of Tropical Agriculture and Human Resources (CTAHR; 19°38′36.25″N, 155°84′47.89″W). *Wasmannia auropunctata* workers used in this study were obtained from laboratory colonies maintained at 26.8°C and 71% relative humidity and fed a diet of dead crickets (*Acheta domesticus*), 25% sucrose solution, and water. Experimental containers consisted of clean 35.6 × 20.3 × 12.4-cm (l × w × h) Sterilite plastic tubs (Sterilite Corporation, Townsend, MA, USA) with walls coated in Insect-a-Slip Fluon (BioQuipiProducts, Rancho Dominguez, CA, USA) to prevent from escape. Ants were sourced from stock laboratory colonies and transferred into the experimental containers using a clean, soft-bristled paintbrush before marker application. The study consisted of two components: 1) a marker retention assessment and 2) a marker cross-contamination assessment.

Marker Retention Assessment

Protein Marker Treatments

The two biomarkers tested consisted of cow's milk (Lucern Foods Inc., Boise, ID) and ready-to-use egg whites (Lucern Foods Inc.). The cow's milk application consisted of pure (100%) milk, and the egg white treatment consisted of 10% egg whites homogenized with water (Hagler et al. 2014). The study also contained a water only (negative control) treatment. Each biomarker was administered to cohorts of ~200 ants placed in the experimental containers described above. Two marker application methods, direct contact spray application plus residue and indirect residual contact (self-mark) application, were also examined. A water-only treatment was included to serve as negative control samples.

Acquisition of the Marks by Direct Topical Application Plus Residue

For the direct contact spray application plus residue (hereafter referred to as direct application plus residue), the ants were topically sprayed with ~1.42 ml of biomarker using a Equate hand-spray bottle (Walmart, Bentonville, AR). After the application, the ants were allowed to dry for ~0.5 h at which time a subsample of ants from each treatment was collected and labeled as the 0 h after exposure (HAE) retention treatment. All remaining ants were held in the containers in which they were treated for the duration of the experiment. Additional subsamples were collected at 24 and 48 HAE. Each ant was transferred into a 1.5-ml snap-cap micro centrifuge tube (Biologix Research Company LLC, City, ST) using a clean toothpick, then immediately frozen for later analysis. This experiment was replicated three times.

Acquisition of the Marks by Indirect Residual Contact

For the indirect residual contact mark (hereafter referred to as indirect application), the ants were placed into an experimental container that contained leaf litter that had been treated with milk or egg whites. The leaf litter was composed of *Melaleuca quinquenervia* Blake, *Eucalyptus* sp., and *Metrosideros polymorpha* Gaudich. The leaves were washed with soap and water, air dried and then treated with the respective biomarker by topically spraying the leaf litter with ~13.80 ml of the biomarker until saturated. The protein-marked leaf litter was then placed on the bottom of clean experimental containers and allowed to dry. Once dry, unmarked ants (~200 per container) were transferred from the laboratory colonies into the experimental containers. Ants from these containers were collected into individual tubes as described above at 24 and 48 HAE to the protein-marked leaf litter. This experiment was replicated three times.

Marker Cross-Contamination Assessment

A cross-contamination test was conducted by allowing ants marked with milk to interact with ants marked with egg whites. An equal number of ants treated with each biomarker via a direct application, as described above, were then transferred into a clean experimental container. Cohorts of ants were collected after 24 and 48 h of interacting.

The ants were collected by two different methods. Specifically, ants were collected individually, as described above, or in bulk by sweeping up multiple ants with a clean soft bristled size 3 paintbrush (Crayola, Easton, PA) into a single micro-centrifuge tube. All samples were immediately frozen for later analysis for the presence of the protein marks by ELISA.

Sample Processing

Prior to analysis, each ant sample was removed from the freezer and ants from bulk collected samples were separated into individual clean 1.5-ml microcentrifuge tubes. All ant samples were soaked in 500 µl of Tris-buffered saline for 1 h at 27°C on an orbital shaker set at 100 rpm to remove surface proteins. A 100-µl aliquot of each sample was used for the ELISA. In total, 1,592 ants were assayed for the presence of both chicken egg albumin protein found in egg whites and the bovine casein protein found in milk by the indirect ELISAs described by Jones et al. (2006).

Data Analysis

Each protein-specific ELISA plate contained at least eight negative control ant samples. Positive ELISA reactions for the presence of the egg albumin and bovine casein marks were defined as those specimens that yielded an ELISA optical density (OD) reading exceeding the critical threshold value of the mean value plus 3 SD of the negative control samples (Hagler 1997). Sample sizes for each replicate varied; therefore, data from all replicates were pooled. Descriptive statistics were calculated for all ants from the marker detectability and retention study, whereas cross-contamination rates were calculated as the percent cross-contamination among successfully marked ants only.

Results

Marker Detectability and Retention

Egg whites were more effective as a biomarker than the milk. Additionally, more of the markers were retained by the ants when applied directly than indirectly. After 24 h, the mean OD values for egg whites (0.643) and milk (0.331) applied directly were higher than indirectly applied egg whites (0.273) and milk (0.061). At 0, 24, and 48 h after direct application, egg whites were detected on 96, 98, and 98% of the individuals sampled compared with 91, 87, and 14% for milk (Fig. 1A). When applied indirectly, egg whites were detected on 76 and 12% of the ants after 24 and 48 h, respectively, compared with 18 and 2% for milk (Fig. 1B).

Cross-Contamination

The highest rate of cross-contamination was observed with 29.8% of bulk collected ants at 24 HAE (Fig. 2). The lowest rates of cross-contamination were observed with 1.9% individual ant collections, also at 24 HAE. Cross-contamination rates at 48 h were similar between the two collection methods with 17.3 and 13% cross-contamination observed among bulk and individually collected ants, respectively.

Discussion

The small size and social behavior of *W. auropunctata* limit the options for MRR and MC research. Protein immunomarking techniques have been proven reliable for tagging minute parasitoids (Hagler and Jackson 1998, Hagler et al. 2002, Irvin et al. 2018) and a wide range of social insects, including termites (Buczkowski and Bennett 2007, Baker et al. 2010), bees (DeGrandi-Hoffman and Hagler 2000, Hagler et al. 2011, Boyle et al. 2018), and ants (Buczkowski and Bennett 2007, Song et al. 2017, Hogg et al. 2018). Our study showed that direct application of egg whites was effective for topically marking *W. auropunctata* for up to 48 h. Conversely, the detectability of the milk biomarker rapidly decreased over the same period.

Acquisition and retention of egg whites and milk from the treated leaf tissue were considerably lower in this study than previously reported (Jones et al. 2006, Hagler et al. 2014). Also, the mean OD values observed for egg whites and milk at 24 HAE applied indirectly were considerably lower than the mean OD values observed in the direct application plus residue treatment for egg whites and milk 24 HAE. This suggests that the amount of marker acquired via residual transfer from the treated leaf litter was low and may have been easily removed by *W. auropunctata* through social interactions and self-grooming.

The high rate of cross-contamination among ants collected in bulk suggests that, although collecting ants *en masse* in the field is more convenient and time-efficient, it is likely to result in nearly one-third of the ants with detectable biomarkers being false-positives. The greatest potential for cross-contamination was observed mostly in relation to collection method; however, the increase in

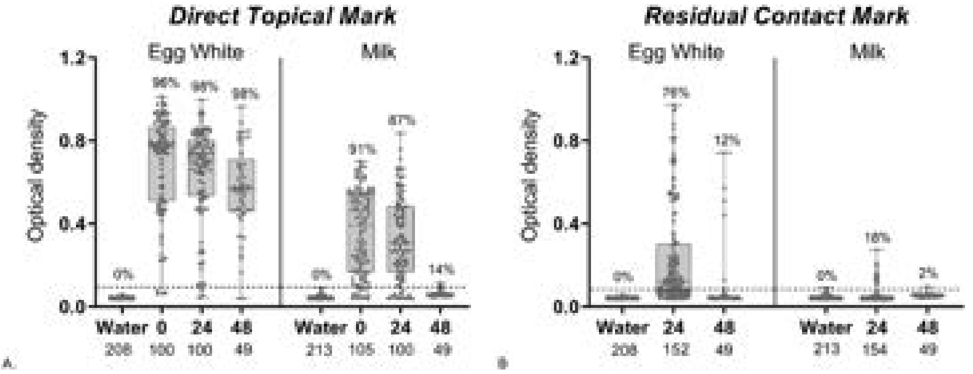

Fig. 1. Box and whisker plots showing the percent positive egg white and milk biomarker reactions for ants marked directly at 0, 24, and 48 HAE (A) or indirectly at 24 and 48 HAE (B). Dots represent individual sample OD values, and the dotted line represent the critical threshold value for a positive ELISA reaction based on the mean negative control (water only) OD value plus three standard deviations. Numbers below each *x*-axis label is the sample size for each mark treatment.

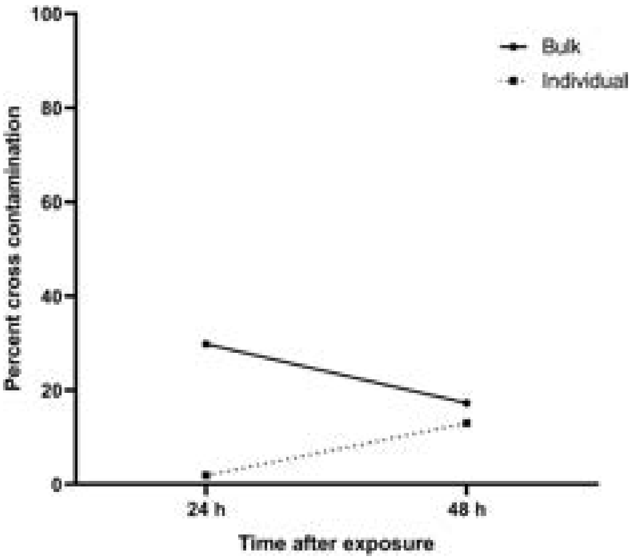

Fig. 2. Line graph showing cross-contamination rates for bulk and individually collected ants at 24 and 48 h after marker exposure.

cross-contamination rates among individually collected ants between 24 and 48 HAE suggests that biomarkers are also passed between individual ants through typical interactions.

In conclusion, selecting an appropriate technique for an ant MRR or MC study can be challenging as many biotic (social behavior, size, etc.) and abiotic factors (collection method, rainfall, temperature, etc.) can affect marker detection and retention. Our study confirms that a 10% egg white solution is retained longer than pure milk (Jones et al. 2006, Slosky et al. 2012, Lessio et al. 2014). However, for *W. auropunctata*, indirect marking by passive exposure to protein-marked leaf debris may not be reliable. Therefore, we recommend applying the marker as a direct spray to foraging trails, aggregations, and exposed nests during field studies. If mass trapping is required to collect many specimens or if the study must extend beyond 24 h, external marking, in general, may not be appropriate due low detection rates beyond 24 h and high cross-contamination rates resulting from bulk specimen collections. Although external marking may not be appropriate for *W. auropunctata* field studies, internal self-marking, whereby individuals acquire the marker by feeding on a food source laced with the marker has been used successfully for ants elsewhere (Buczkowski 2012, Hogg et al. 2018) and other insects (Rhodes et al. 1997, Hagler and Jackson 2001, Hagler and Miller 2002, Hagler et al. 2002) and may be a better marking option for *W. auropunctata*.

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Chapter 4

Palatability of baits containing (S)-methoprene to *Wasmannia auropunctata* (Hymenoptera: Formicidae)

Palatability of baits containing (S)-methoprene to *Wasmannia auropunctata* (Hymenoptera: Formicidae)

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Abstract

Wasmannia auropunctata Roger (Hymenoptera: Formicidae), little fire ant, is recognized as a serious pest ant species that affects agriculture, homes, gardens, and natural ecosystems in Hawaii, USA, and elsewhere. Anecdotal evidence suggests that insecticidal baits containing (S)-methoprene are not effective against this species. We examined whether *W. auropunctata* is repelled by bait formulations containing this compound and whether the addition of torula yeast (*Candida utilis* Lodder; Saccharomycetales: Saccharomycetaceae) increased palatability of these baits. *Wasmannia auropunctata* was found to be repelled by (S)-methoprene concentrations as low as 0.25% regardless of formulation. The addition of torula yeast (3% by weight) significantly increased worker recruitment to baits with and without (S)-methoprene. Our results indicate bait formulations using (S)-methoprene are likely to offer poor efficacy against *Wasmannia auropunctata* without the addition of a feeding stimulant such as torula yeast due to repellency of the active ingredient

Key Words: little fire ant; Tango™; protein adjuvant; recruitment

Resumen

Wasmannia auropunctata Roger (Hymenoptera: Formicidae), hormiga pequeña de fuego, se reconoce como una especie plaga de hormiga plaga que afecta seria a la agricultura, los hogares, los jardines y los ecosistemas naturales en Hawai y en otras partes. La evidencia anecdótica sugiere que los cebos insecticidas que contienen metopreno-(S) no son eficaces contra esta especie. Examinamos si *W. auropunctata* es repelido por formulaciones de cebo que contienen este compuesto y si la adición de levadura de torula (*Candida utilis* Lodder; Saccharomycetales: Saccharomycetaceae) aumenta la palatabilidad de estos cebos. Se encontró que *Wasmannia auropunctata* es repelido por concentraciones tan bajas como 0.25% de metopreno-(S) independientemente de la formulación. La adición de la levadura de torula (3% en peso) aumentó significativamente el reclutamiento trabajadores para cebos con y sin metopreno-(S). Nuestros resultados indican que las formulaciones de cebo usando metopreno-(S) son propensos a ofrecer una pobre eficacia contra *Wasmannia auropunctata,* si no se le adiciona de un estimulante de la alimentación tales como la levadura de torula debido a la repelencia del ingrediente activo.

Palabras Clave: hormiga de fuego pequeña; Tango ™; adyuvantes de proteínas; reclutamiento

Wasmannia auropunctata Roger (Hymenoptera: Formicidae) is among 5 of the most damaging invasive ant species in the world and is widespread throughout the tropics and subtropics, especially throughout the Pacific (Lowe et al. 2000; Holway et al. 2002; Wetterer & Porter 2003). The workers are small, measuring only 1.5 mm in length. However, the sting from this tiny pest causes severe burning sensations, itchy welts, and other symptoms often lasting a week or longer (Spencer 1941; Fabres & Brown Jr 1978; Wetterer & Porter 2003; Taniguchi 2008). The ants infest a wide range of habitats from urban structures to agriculture and forest ecosystems. Unlike many other pest ant species, *W. auropunctata* does not build noticeable nest mounds but creates shallow, sprawling, 3-dimensional "supercolonies" within the leaf litter, vegetation, and tree canopies (Spencer 1941; de Souza et al. 1998; Wetterer & Porter 2003; Le Breton et al. 2004; Vanderwoude & Nadeau 2009). Conservative estimates suggest population densities can be as high as 20,000 workers per square meter in Hawaii (Souza et al. 2008). Worker-to-queen ratios of 385–549: 1 have been recorded (Ulloa-Chacon & Cherix 1990), suggesting that 37–52 queens may be present per square meter. These extraordinary population densities partly explain the difficulties associated with controlling this species.

Humans are the primary mode of dispersal of *W. auropunctata*, a "tramp ant" species, through transportation to new locations in infested potted plants, plant material, produce, or soil for agricultural, industrial, or domestic purposes. Due to the small size and cryptic nature of these ants, home and property owners are often unaware of infestations until ant populations have grown and spread throughout an entire property. Although primarily an outdoor pest, *W. auropunctata* will infest structures and houses when no suitable outdoor habitat is available or if infested plants and materials are brought indoors. Private property owners often feel overwhelmed when managing *W. auropunctata* using current control methods (M. Montgomery, pers. obs.)

Control of invasive ants often involves the use of persistent insecticide sprays, barriers, mound treatments, insecticidal baits (Williams 1994), or a combination of these methods. Baits offer significant advantages over broadcast applications of persistent insecticides (Wil-

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liams et al. 2001), including lower overall insecticide use and reduced non-target impacts (Williams 1983). Baits utilize ant social behaviors of foraging, recruitment, and stomodeal trophallaxis, the mouth-to-mouth transfer of food or excretions, to direct toxicants to nest mates, and most importantly, the queen or queens of a colony. Exploiting the natural behaviors of ants is an efficient management strategy that potentially lowers pesticide and labor costs (Williams 1983; Klotz et al. 2003; Tollerup et al. 2004).

An effective bait formulation is comprised of an attractant (the bait matrix), a toxicant (the active ingredient), and a carrier to facilitate application. Candidate toxicants undergo rigorous testing and must demonstrate specific properties including delayed mortality, non-repellency at high concentrations, and efficacy when diluted by trophallaxis (Williams 1983; Rust et al. 2000; Braness 2002; Tollerup et al. 2004). However, few active ingredients exhibit all of the necessary traits (Levy et al. 1973; Williams 1983).

The 2nd component of an effective ant bait, the attractant, is often species specific, or specific to a sub-group of ants that share feeding preferences. For example, many granular baits marketed for “fire ant control” are formulated using a defatted corn grit carrier impregnated with soya oil as the attractant, and 1 or more active ingredients (Kidd et al. 1985; Williams et al. 2001). Product labels may indicate effective control for a range of ant species, although much of the initial testing may have been conducted using 1 or only a few model ant species.

Assessment of the biological and behavioral characteristics of the target pest is as important in bait development as the physical properties of the formulation. For example, red imported fire ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), prefer to carry granular baits back to the nest rather than consume liquid baits in situ (Kidd et al. 1985). Kidd et al. (1985) hypothesized that *S. invicta* is likely to store bait granules within the nest. Due to their relatively large size, it may be more efficient for *S. invicta* foragers to bring granules back to the nest where they can feed at leisure instead of spending time feeding while being exposed to predators. Underground storage may prevent photodegradation of the active ingredients in the bait and prolong their effectiveness. In contrast, *W. auropunctata* is substantially smaller than *S. invicta*, and mostly feeds on resources in situ, rarely attempting to bring food items back to the nest (M. Montgomery, pers. obs.).

Control of *W. auropunctata* in agricultural production systems in Hawaii is confounded by 3 factors: frequent rain that renders baits unpalatable, an inability to control colonies that nest in vegetation, and an apparent repellency of proprietary baits registered for use on food crops. Firstly, granular baits quickly degrade and become unpalatable when exposed to light and water (Markin & Hill 1971), leaving a narrow window of opportunity for *W. auropunctata* to find the bait, feed, and return to the colony before the bait is rendered ineffective. In wet climates such as the windward coasts of Hawaii, annual rainfall often exceeds 3,200 mm (http://www.prh.noaa.gov/hnl/climate/phto_clim.php), and the window of opportunity may be as short as 42 min (Mallipudi et al. 1986). Any bait applied for control of *W. auropunctata* must therefore be highly attractive to stimulate rapid recruitment and uptake before outdoor exposure renders the bait ineffective or unpalatable.

The 2nd complicating factor is that *W. auropunctata* nests in the upper and mid-story vegetation as well as the ground layer. Typically, ant control using baits involves broadcasting granular baits along the ground, potentially leaving arboreal colonies unaffected (Vanderwoude 2007; Souza et al. 2008; Vanderwoude & Nadeau 2009; Vanderwoude et al. 2010). Souza et al. (2008) observed rapid recolonization within 9 wk of cessation of ground treatments indicating that treatment of arboreal colonies is essential for effective long-term control.

The 3rd issue confounding *W. auropunctata* control is that this species appears to be repelled by certain proprietary “fire ant” baits (Cabral et al. 2012; M. Montgomery, pers. obs.), especially those containing the insect growth regulators pyriproxyfen or (S)-methoprene. Proprietary baits containing these active ingredients are the only formulations permitted for use on many food crops in Hawaii and the continental United States and are therefore vital to agricultural producers. An additional bait formulation therefore is needed for effective control that is not repellent to little fire ants.

Wasmannia auropunctata is a serious pest in Hawaii and presents unique control challenges. This study investigated the utility of a novel gel formulation containing the active ingredient (S)-methoprene (Tango™, Wellmark International, West Schaumburg, Illinois, USA; EPA reg 2724-420), the apparent repellency to *W. auropunctata* of this compound, and potential additives to enhance bait palatability.

Materials and Methods

We investigated the reported repellency of baits containing (S)-methoprene to *W. auropunctata* by observing changes in recruitment over time, as a surrogate measure for bait attractiveness and palatability, to a standard bait matrix mixed with various concentrations of (S)-methoprene. We also tested the effect of adding torula yeast (*C. utilis* Lodder; Saccharomycetales: Saccharomycetaceae) on overall bait recruitment. Both experiments were conducted in a forested area heavily infested with *W. auropunctata* near Hilo on the Island of Hawaii (19°40'N, 155°6'W) during the summer of 2012.

DOSE RESPONSE TO (S)-METHOPRENE

A standard Hawaii Ant Lab (HAL) bait matrix was used for all treatments. The HAL matrix comprised the following ingredients: 1) refined and dewaxed corn oil, (Superb™, Stratas Foods LLC, Memphis, Tennessee, USA) at 350 g/kg; 2) Ziboxan “RD” Rapid Dispersal Xanthan Gum (Deosen Biochemical Ltd, Shandong, China) at 8g/kg; and 3) water at 642 g/kg.

Tango™ (Wellmark International, EPA reg 2724-420; 4.9% [S]-methoprene) was added to this matrix to produce 4 gel formulations with concentrations as follows: 1) control, 0.0%; 2) low dose, 0.25%; 3) medium dose, 0.5%; and 4) high dose, 2.5% (S)-methoprene active ingredient (a.i.) (Table 1). A 5th treatment consisting of a proprietary granular bait that is an effective control product for *S. invicta* and registered for use in and around edible crops in Hawaii, Extinguish® Professional™ (Wellmark International, EPA reg 2724-475) containing 0.5% (S)-methoprene, was included as a standard for comparison.

A randomized block experimental design was chosen for the 1st experiment. Treatments within each block were placed 5 m apart to establish independence and replicated 8 times. Bait stations each consisted of a 4.5 × 4.5 cm laminated card with treatments randomly assigned to bait stations within each block. Recruitment to the bait was measured via high-resolution digital photographs of each plot taken

Table 1. Treatments and concentrations of the active ingredient (S-methoprene used in experiment 1.

Treatment	% (S)-methoprene	Product
1	0	Experimental bait matrix
2	0.25	Experimental bait matrix
3	0.50	Experimental bait matrix
4	2.50	Experimental bait matrix
5	0.50	Extinguish™ Pro™

every 30 min over the course of 3 h. Photographs were examined in the laboratory and ants on bait cards were counted and recorded.

STATISTICAL ANALYSES

All data were analyzed using Minitab statistical software (Minitab version 17, Minitab Ltd., Pennsylvania, USA). Data from treatments 1 through 4 were first analyzed using non-linear regression analysis to identify any correlation trends between ant recruitment and (S)-methoprene concentration. Next, the data from all 5 treatments were analyzed via 2-way ANOVA and Tukey post-hoc test for multiple comparisons between means.

EFFECT OF TORULA YEAST ADJUVANT

The effect of adding torula yeast to the gel formulations was tested by comparing recruitment to HAL gel baits containing combinations of (S)-methoprene 0.25% a.i. and torula yeast (3% by weight of finished bait) in a factorial design. This experiment was laid out as a randomized block design as in experiment 1, differing only in the number of treatments and replications (Table 2). Recruitment to the bait was measured via high-resolution digital photographs of each plot every 30 min over the course of 2 h. Photographs were examined in the laboratory and ants on bait cards were counted and recorded. Data were analyzed using a 2-way ANOVA and Tukey post-hoc test in Minitab statistical software (Minitab version 17, Minitab Ltd.).

Results

DOSE RESPONSE TO (S)-METHOPRENE

The dose response curve for experiment 1 showed a negative exponential correlation between the proportion of (S)-methoprene in the bait and number of ants recorded at bait (Fig. 1). The trend was corroborated by results from the 2-way ANOVA test and Tukey post-hoc test. A significant correlation between treatment and recruitment rate was detected ($F = 129.78$, $P < 0.001$). Recruitment to treatments 1 (0% [S]-methoprene), 2 (0.25% [S]-methoprene), and 4 (2.50% [S]-methoprene) were statistically significant with the greatest recruitment observed at treatments without (S)-methoprene and lowest recruitment observed at treatments with 2.50% (S)-methoprene. No difference in recruitment was detected between treatments 3 (0.5% [S]-methoprene) and 5 (Extinguish® Professional™ standard, 0.5% [S]-methoprene) (Fig. 2). Ant recruitment rates for all treatments remained relatively constant over the course of the 3 h measurement period with no significant difference between recruitment and exposure time detected. During the entire experiment, the high-dose treatment attracted 79% fewer ants than the medium-dose treatment and was the least attractive treatment (means ranging from 18.88 to 40.63 ants per observation).

Table 2. Treatments and concentrations of the active ingredient and the adjuvant used in experiment 2.

Treatment	% (S)-methoprene	% protein adjuvant
Control	0	0
Protein	0	3
(S)-methoprene	0.25	0
Protein + (S)-methoprene	0.25	3

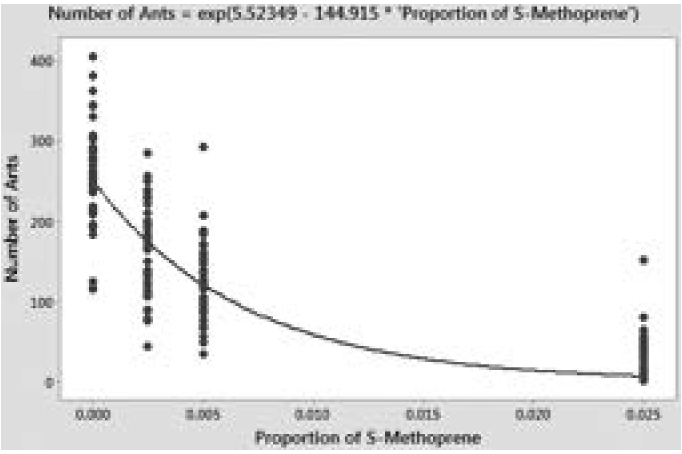

Fig. 1. Non-linear regression curve showing the relationship between ant recruitment and proportion of (S)-methoprene in the Hawaii Ant Lab's novel gel bait.

EFFECT OF TORULA YEAST

There were significant differences in recruitment between treatments with and without (S)-methoprene as well as with and without the protein adjuvant (Fig. 3). Overall, ant recruitment to baits formulated using the product label rate of 0.25% (a.i.) and without protein adjuvant was significantly lower than to baits containing 0% (S)-methoprene ($F = 9.1037$, $P < 0.01$). However, the addition of torula yeast substantially improved recruitment to baits. Mean recruitment to baits with the protein adjuvant was significantly higher than to baits formulated without adjuvant ($F = 22.1801$, $P < 0.001$). Recruitment to bait with (S)-methoprene plus torula yeast was comparable to that of the control with no difference detected during data analysis (Fig. 4).

Discussion

Control measures for *W. auropunctata* have met with limited success throughout the tropics due to a variety of environmental, biological, and behavioral factors. *Wasmannia auropunctata* are repelled by (S)-methoprene baits at concentrations that do not repel some other ant species, as is evident by significantly lower recruitment rates. This sensitivity to baits with even relatively low concentrations of (S)-methoprene has not been reported previously. Our study revealed that the addition of (S)-methoprene significantly reduced recruitment to baits, which may explain reduced efficacy of certain proprietary ant baits against *W.*

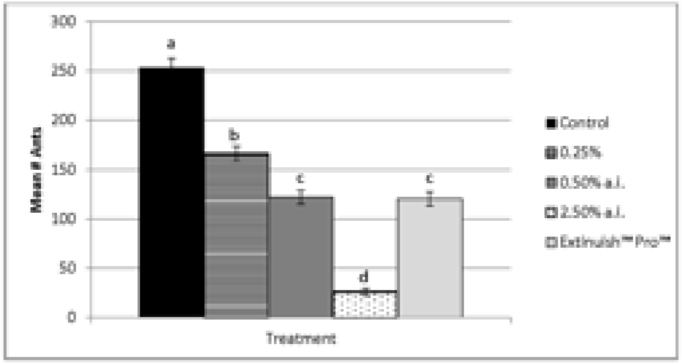

Fig. 2. Mean recruitment of ants to baits containing different concentrations of (S)-methoprene. Treatments with different letters are significantly different ($P < 0.05$).

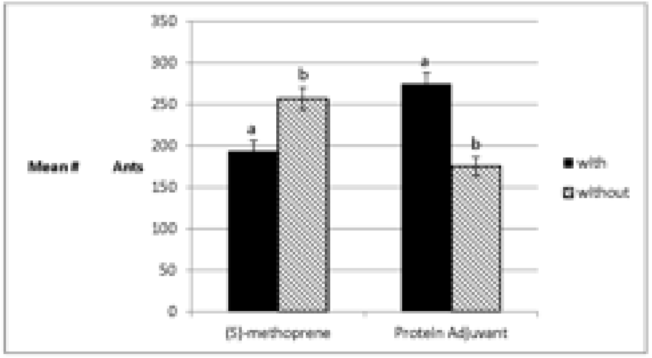

Fig. 3. Differences between all baits with and without (S)-methoprene as well as all baits with and without the adjuvant. Treatments with different letters are significantly different (*P* < 0.05).

auropunctata in Hawaii. Furthermore, we tested a masking agent that effectively increased recruitment to baits containing (S)-methoprene. The proprietary granular bait Extinguish® Professional™ is an effective control product for the red imported fire ant (*S. invicta*) and contains 0.5% (S)-methoprene as the active ingredient. In contrast, the results from our study indicates this rate is moderately repellent to *W. auropunctata* and may explain the reported poor efficacy of Extinguish® Professional™ against *W. auropunctata* in Hawaii. Indeed, the recruitment rate to the standard treatment, Extinguish® Professional™, and that to the medium-dose gel bait treatment containing 0.5% methoprene were comparable, thus corroborating this observation and suggesting that it is the concentration of (S)-methoprene and not the bait formulation that is the repellent factor. Additionally, the labeled concentration for commercially available (S)-methoprene, i.e., Tango™, is 0.25% and even this rate suppressed *W. auropunctata* feeding activity.

Bait efficacy relies on bait consumption and sharing among nestmates via trophallaxis. Therefore efficacy improves with increased recruitment to baits, as it leads to greater consumption by the target species. This is especially so for ants because bait sharing through trophallaxis dilutes the concentration of the active ingredient through the colony. In the case of insect growth regulators, accumulation of the active ingredient in the queen(s) and larval stages is essential to obtain optimum physiological effects. Any bait must therefore be highly attractive and palatable to ensure maximum consumption and effectiveness.

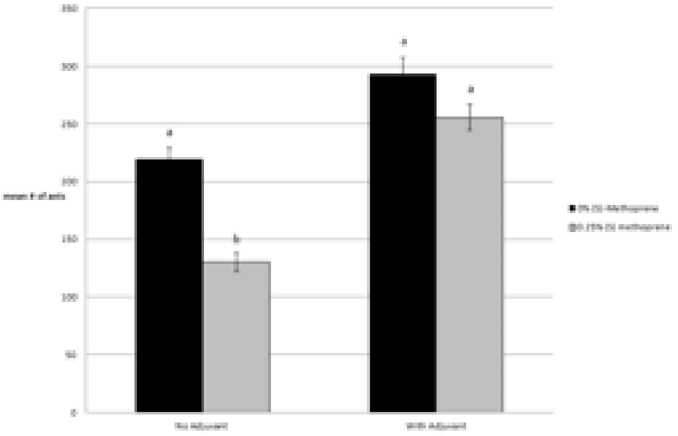

Fig. 4. Effects of addition of 0.25% (S)-methoprene and increase in attractiveness of (S)-methoprene baits by the addition of an adjuvant as a masking agent. Treatments with different letters are significantly different (P*p* < 0.05).

Although *W. auropunctata* did recruit to bait containing the label-recommended dose of 0.25% (S)-methoprene, our results demonstrated overall recruitment was significantly improved with the addition of torula yeast. Our study also demonstrates that the HAL gel matrix (with the addition of torula yeast and (S)-methoprene) is an effective alternative to other baits. The higher recruitment rate at baits containing the protein adjuvant for an extended period of time suggests greater consumption of bait and, in turn, (S)-methoprene, compared with proprietary baits containing the same active ingredient.

The 2 other impediments to control of *W. auropunctata* in Hawaii are the arboreal nesting habit of some colonies and the high rainfall experienced on the windward coasts of the Hawaiian archipelago. Previous efforts to control this species in Hawaii have been hampered by these factors (Souza et al. 2008). However, gel baits incorporating (S)-methoprene can be applied to vegetation where its gelatinous consistency allows it to adhere to leaves and branches. It also appears to be moderately resistant to removal by rainfall, which along with its palatability extends the window of opportunity of recruitment to the bait (M. Montgomery, pers. obs.). Both attributes are essential for an effective control product for *W. auropunctata* in tropical locations.

We provide here evidence supporting observations that classical ant control measures are not suitable for effective control of *W. auropunctata*. When managing pests through baiting, the baits being used must display the specific characteristics of delayed mortality, efficacy at extremely low concentrations, and non-repellency. Products currently available in Hawaii do not meet these requirements for controlling *W. auropunctata*. Our study has developed a bait showing potential as an alternative to the currently available products. Further research will focus on efficacy of this bait and its potential as a viable control method amid a range of land use types rather than mere palatability as was our focus here. Additionally, the bait matrix described in our study may have the potential to be used with a variety of active ingredients previously thought to be repellent, thus unsuitable, to *W. auropunctata*. Further research in this area is also needed.

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Chapter 5

Eradicating little fire ants (*Wasmannia auropunctata*) from the island of Kaua`i, Hawai`i: process, challenges, and results to date

Eradicating little fire ants (*Wasmannia auropunctata*) from the island of Kaua`i, Hawai`i: process, challenges, and results to date

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Abstract

First detected on the Island of Kauai in 1999, the little fire ant (*Wasmannia auropunctata*) has persisted despite early attempts at eradication by state agencies. A multi-agency collaboration was formed in 2011 to develop and implement a two-phase eradication plan. The infestation was delimited to 4.02 ha of rural residences and steep coastline habitat. Treatments regimens were divided between easily accessible infested areas (Phase I) and steep areas requiring rope safety systems (Phase II). The eradication plan included ground and arboreal treatment technologies and long-term post-treatment monitoring. Treatments consisted of broadcasting baits containing s-methoprene (0.25% a.i.), indocaxarb (0.18% a.i), or hydramethylnon (0.73% a.i.) eight times over the course of 12 months. Treatments effectively reduced the population to below detectable levels throughout much of the treatment area, however isolated remnant colonies were detected as recently as September 2019. Remnant colonies were associated with specific site features known to be difficult to treat, such as tall palm trees and large mulch piles. On one occasion, in 2017, two little fire ant detections were confirmed outside of the original treatment area, where no little fire ants had been detected before; highlighting the ability for little fire ants to remain undetected for many years. Current results stress the importance of long-term commitment to post-treatment monitoring and expanded surveys. We recommend development and incorporation of novel detection methods which could increase accuracy and improve efficiency of monitoring and detection surveys.

Keywords: *Wasmannia auropunctata,* gel bait, tropical invasion, invasive alien species, Formicidae

Introduction

An estimated 869 invasive alien species (IAS) with negative impacts have been identified by the International Union for Conservation and Nature's Invasive Species Specialist Group (2015). The negative impacts of IAS are often severe and directly affect ecosystems, the economy, and people. Economic cost-benefit analyses measure the potential impact of a particular species on communities and various economic sectors, placing dollar values on the estimated costs and revenues associated with controlling the species, and assess benefits gained from preventative action and control (Motoki et al. 2013). These analyses are generally conservative yet have significant implications. In the United States, a single ant species (*Solenopsis invicta* Buren) has been estimated to cause multi-billions annually in economic costs (Drees and Lard 2006). International trade is a leading factor contributing to worldwide spread of IAS and, in the Pacific, invasive ants are some of the most widely distributed (Gruber et al. 2017).

Invasive ants are especially successful invaders because they possess the ability to adapt and invade a wide range of habitats. Special biological and behavioral traits, such as polygyny, unicoloniality, high interspecific aggression, symbiotic relationships with honeydew producing insects, and use of human mediated long-distance dispersal, contribute to invasive ants' success as invaders (Holldobler and Wilson 1990; O'Dowd et al. 2003; Passera 1994; Wetterer and Porter 2003).

As many as 147 ant species worldwide are established outside their home range (McGlynn 1999). A subset of these are well known as IAS (McGlynn 1999) with well-documented negative impacts. Five of these ant species feature on the list of the 100 most damaging invasive plants, animals, invertebrates and fungi (Lowe et al. 2000): *Anaplolepis gracilipes* Smith, *Linepithema humile* Mayr, *Pheidole megacephala* Fabr*, Solenopsis invicta* and *Wasmannia auropuncata* Roger. These five species are the most common targets of ant eradication programs worldwide, however the outcomes of ant eradication efforts are still poorly documented and frequently unsuccessful (Hoffmann et al. 2016).

Hawai`i has no known native ant species, yet over 60 species have been introduced and established to date. Many of Hawai`i's introduced ant species are considered invasive "tramp" species due to their propensity to be distributed long distances via human-mediated transport and a unique set of biological characteristics such as polygyny and unicoloniality (Loope and Krushelnycky 2007; Passera 1994). Tramp ants are also known for their negative impacts on residential, agricultural and natural ecosystems

(Krushelnycky 2015; Loope and Krushelnycky 2007) and are frequently the targets of eradication and management programs (Hoffman et al. 2011; Lach and Barker 2013).

The little fire ant (*Wasmannia auropunctata)* is the most impactful invasive tramp ant established in Hawai`i and the target of numerous eradication efforts throughout the archipelago (Conant and Hirayama 2000; Kirschenbaum and Grace 2007; Motoki et al. 2013; Souza et al. 2008; Starr et al. 2008; Vanderwoude et al. 2016). Little fire ants (LFA) sting people, blind animals (Ndoutoume-Ndong and Mikissa 2007; Rosselli and Wetterer 2017), farm Homopteran plant pests that vector plant disease (Delabie et al. 1994; Wetterer and Porter 2003), and reduce biodiversity by effectively extirpating other arthropods (Achury et al. 2008; Kirschenbaum and Grace 2007; Vasconcelos et al. 2008). As is common among invasive tramp ants, LFA primarily spread by people unwittingly moving infested materials (e.g. nursery stock, agricultural materials and commodities, aggregate, vehicles, machinery, etc…) to uninfested locations. Economic costs of LFA infestations in Hawai`i are projected to be $6.1 billion over the next 35 years provided that management efforts are maintained at the current “status quo” level (Lee et al. 2015). Because of this, the state of Hawai`i has a vested interest in eradicating LFA infestations when and where feasible.

Little Fire Ants in Hawai`i

Originally detected in 1999 on Hawai`i island (Conant and Hirayama 2000), LFA have persisted and spread to four of the main Hawai`ian Islands despite early attempts at containment. The size and number of infestations identified in 1999 suggested that LFA had been introduced several years prior. However, it is uncertain exactly when and how LFA had been introduced. Results from rapid response surveys and a publicity campaign indicated that the number of LFA-infested private properties and commercial nurseries on Hawai`i island was such that eradication was not feasible with the resources available at that time. Additionally, in 1999, an isolated infestation was identified at a private property on the north shore of Kaua`i island. The origin of that infestation was likely from a shipment of infested palms from a nursery on the island of Hawai`i (Conant and Hirayama 2000; Null and Gundersen 2007).

Because the 1999 island-wide surveys did not detect any other LFA infestations on Kaua`i (Null and Gundersen 2007), there appeared to be an opportunity for an island-wide eradication. In October 1999, the Hawai’i Department of Agriculture (HDOA) along with the Hawai`i Department of Health Vector Control Branch launched the first LFA eradication effort for the state of Hawai`i focused on the Kaua`i infestation. The infestation was treated using ready-to-use granular insecticidal ant baits, however no documentation could be found regarding methods used to treat the original infestation; such as specific products used, application rates, frequency, and number of applications. The eradication was considered to be successful with LFA declared “eradicated” in HDOA’s Annual Report for FY 2000 (Hawai`i Department of Agriculture 2001). Unfortunately, follow-up surveys of the area in 2003 by the Kaua`i Invasive Species Committee (KISC) revealed that the infestation had rebounded and spread to two adjacent privately-owned properties. A monitoring and containment strategy was then initiated until effective eradication methods and procedures could be developed for this species (Null and Gundersen 2007).

Since the original eradication attempt in 1999, considerable research has been conducted and dedicated to understanding LFA biology and behaviors and improving management strategies and bait efficacy (Montgomery et al. 2015; Montgomery et al. 2020). This new knowledge was used to help formulate appropriate species-specific management strategies. A lipid-based gel bait and new eradication procedure was developed by the Hawai`i Ant Lab (HAL) (hereafter referred to as the HAL gel bait) and this showed great promise against LFA infestations (Vanderwoude and Nadeau 2009; Vanderwoude et al. 2010). In 2011, the HAL partnered with the KISC and the HDOA to develop and implement an LFA eradication plan for the second time on Kaua`i. Here, we detail the process and current status of the second LFA eradication attempt.

Methods and Materials

Site description

The infestation was located in Kalihiwai, on the North Shore of Kaua`i (22°13’17.90” N, 159°25’26.88” W) (figure 5.1) and spanned three private properties. Landscape features included open mown lawn, palm arboretum, dense tropical landscaping and non-native coastal forest, sheer cliffs, and rocky outcrops which were surrounded by ocean at high tide (figure 5.2). Approximately one-third of the infested area consisted of steep terrain requiring the use of specialty equipment and training to navigate. The steep cliffs were wholly infested and when the eradication began there was no viable means of accessing this area nor were there treatment methods developed for such site features. As a result, the eradication plan was divided into two phases (figure 5.3). Phase I consisted of treating all areas accessible without the use of specialty equipment between September 2012 and July 2013. Phase II consisted of treating the remainder of the infested area between September 2014 and June 2015 and once specialty equipment, training and appropriate treatment methods became available. A 20 m buffer surrounding the remaining infestation during Phase II resulted in overlap of the Phase I and Phase II treatment areas.

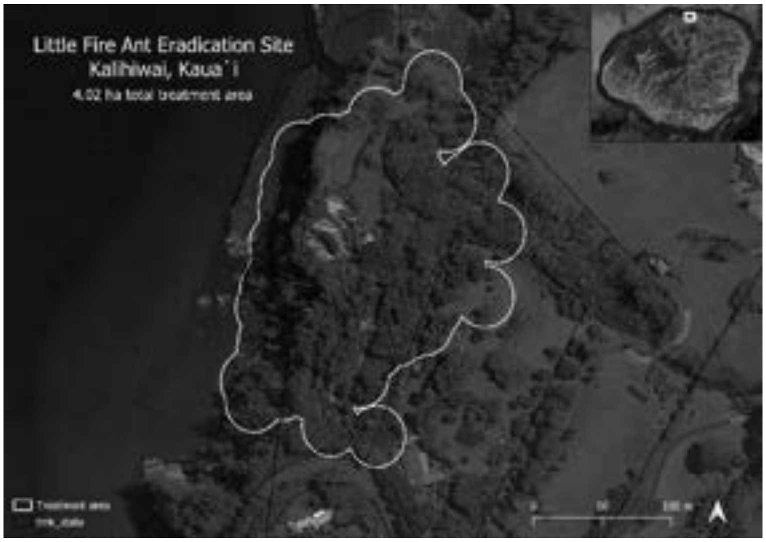

Figure 5.1: The infested area was located in Kalihiwai on the North Shore of Kaua`i island, Hawai`i, USA. The infestation plus 20 m buffer resulted in a 4.02 ha treatment area spanning three private properties.

Figure 5.2: Landscape features within the infested area included grassy areas (a), palm arboretum (b), dense tropical landscaping (c), steeply slopped terrain of non-native costal forest (d), cliffs (e), and a small rock island with patchy vegetation (f)

Figure 5.3: Map of the Kalihiwai, Kauai little fire ant eradication site. The project was divided into a Phase I and Phase II treatment efforts due to steep terrain requiring rope safety equipment for access. The 20 m buffer surrounding the remaining infestation during Phase II resulted in overlap of the Phase I and Phase II treatment areas.

Survey methods

Little fire ant surveys consisted of placing 5-dram plastic vials (20.6 x 52mm, BioQuip® Products, California, USA) laced with peanut butter (hereafter referred to as sample vials) which were collected after 60 min exposure time. The vials then were capped and labelled, with GPS coordinates recorded for each sample site using a GARMIN Foretrex 401 GPS (Garmin International, Missouri, USA). Ants captured in the vials were identified under a dissecting microscope and attributed with the geospatial data. All LFA captured were counted and numbers recorded in the survey database.

Surveys were conducted throughout the eradication effort (2011 – present) for infestation delimitation prior to treatment, population monitoring, and detection of nascent remnant colonies after cessation of treatment regimens. For delimiting surveys, the sample vials were deployed at approximately 10 m intervals along transects radiating outward from known infested areas. The outer boundary of the infestation was determined when sample results revealed zero LFA detections for a minimum of 50 m. Midpoint monitoring surveys were conducted throughout the immediate treatment area (i.e. Phase I vs. Phase II treatment area) and immediately prior to the 5th treatment during both Phase I and Phase II treatment regimens. Sample vials were spaced in a grid-like pattern throughout the treatment areas and at densities of 120 and 190 sample vials/ha for Phase I and Phase II midpoint surveys respectively. The purpose of the midpoint monitoring survey was to assess treatment efficacy and identify areas requiring further attention. This allowed for adjustments to the treatment procedure to be made in a timely manner if and when needed. Immediately prior to each Phase I treatment (September 2012 – July 2013), sample vials were deployed in eight permanent monitoring plots placed randomly throughout the treatment area (table 5.1). The monitoring plots were used to track population dynamics in various habitat types during the Phase I treatment regimen. No monitoring plots were established during Phase II. Post treatment monitoring throughout the Phase I treatment area only occurred between January 2014 and June 2015 with area-wide (Phase I and II treatment areas) post treatment monitoring beginning in January 2016 and continuing to date.

Category	Description	Sample placement	No. plots	Total no. samples
Low vegetation	Spaces dominated by cut grass and low-lying landscaping (<2 m tall)	Ground samples only	4	32
Tall vegetated	Spaces dominated by trees and tall landscaping plants (>2 m tall)	Paired ground and tree samples	4	64
Untreated	Untreated spaces directly adjacent to treated spaces	Ground samples only	2	32
Vegetable garden	A small vegetable garden approximately 3 x 5 m	Ground sample only	1	1

Table 5.1: Descriptions of the 2012-2013 population monitoring plots and sample placements.

Area-wide post-treatment monitoring began after all treatment phases were completed. The number of area-wide post-treatment surveys per year varied due to property access, crew availability, and weather (table 5.2). Vial density was increased in order detect any nascent colonies remaining. High sample vial densities for post-treatment surveys increase the likelihood of detecting nascent remnant colonies present after cessation of the treatment phases. Annual mean sample vial densities from 2016-2019 ranged between 1027 (min) and 3916 (max) per ha. Expanded surveys, extending beyond the treatment area, occurred at least once per year between 2016 and 2021. Additional sample vials were placed in the crowns of all palm trees higher than 3 m. Sample tubes were placed in the tree crowns via a weighed line to which the tubes were attached. These were left in position for 24 hours before collection rather than 60 min due to the time in which it took to deploy arboreal samples. The purpose of the canopy survey was to determine if any arboreal colonies survived.

Total area surveyed was calculated by buffering survey points to 3 m and then calculating the area of the resulting polygon.

Year	No. of surveys	Total no. of samples	Total area surveyed (ha)	Mean survey density (no. sample vials/ha)
2016	3	5,595	4.8	1,158
2017	4	15,925	9.8	1,628
2018	2	24,194	6.2	3,916
2019	2	3,933	3.8	1,027
2020	NA	NA	NA	NA
2021	1	4,110	4.7	874

Table 5.2: Summary of area-wide post-treatment surveys for the years 2016-present. No surveys were conducted in the year 2020 due to the COVID-19 pandemic and associated restrictions.

Treatment Strategy

Approximately 3.2 ha were treated during Phase I and approximately 1.4 ha were treated during Phase II treatment regimens (figure 5.3). The overlap in Phase I and II treatment areas ensured sufficient treatment coverage.

Treatments were focused on the use of insecticidal baits applied to the ground and all vegetation throughout the treatment area. Baits were applied at six-week intervals for 12 months during Phase I and Phase II treatment regimens. The HAL gel bait containing 0.25% s-methoprene (Tango™, EPA reg. 2724-420**,** Wellmark International, Illinois, USA**),** an insect growth regulator (IGR), was applied to the ground and all vegetation for the first half of treatments and the HAL gel bait with 0.18% indoxacarb (Provaunt®, EPA reg. 100-1487, Syngenta Crop Protection LLC., North Carolina, USA), an oxadiazine insecticide, was applied to vegetation only for the final half of treatments during each phase of the treatment regimen. Broadcast applications of Probait® (0.73 hydramethylnon, EPA reg. 73342-1-2724, Wellmark International, Illinois, USA), a ready-to-use granular bait, were made one week following each HAL gel bait application during Phase I; however, Phase II treatments consisted solely of the HAL gel bait with s-methoprene and indoxacarb as described above.

Spot treatments were made as needed throughout the eradication effort and consisted of insecticidal bait applications or use of residual insecticides, such as Talstar® P (7.9% bifenthrin, EPA reg. 279-3206**,** FMC Corporation, Pennsylvania, USA) or Talstar® PL (0.2% bifenthrin, 279-3168, FMC Corporation, Pennsylvania, USA). Other ready-to-use granular bait products used during spot treatments were Amdro Fire Ant Bait (0.73% hydramethylnon, EPA reg. 73342-1, AMBRANDS, Georgia, USA) and Siesta Fire Ant Bait (0.063% metaflumizone, EPA reg. 7969-232, BASF Corporation, North Carolina USA). Total annual amounts of all pesticides used throughout the project are listed in table 5.3.

Product	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**
Amdro	-	-	-	2.27 kg	1.81 kg	-	-	50 g	-
Probait	18.85 kg	27.90 kg	4.31kg	2.95 kg	4.20 kg	0.91 kg	-	-	0.91 kg
Siesta	-	-	-	-	-	1.81 kg	-	-	-
HAL Gel Bait (Provaunt)	-	238.71 L	37.85 L	136.27 L	-	-	-	-	-
HAL Gel Bait (Tango)	181.06 L	63.06 L	31.95 L	26.50 L	-	-	-	1.32 L	1.89 L
Talstar P (tank mix with water)	13.25 L	-	1,210.36 L	473.18 L	-	-	45.2 L	-	-
Talstar PL (granule)	-	-	49.90 kg	56.70 kg	34.02 kg	-	-	-	-

Table 5.3: Total amounts of baits and residual barrier treatments applied between 2012 and 2020

Data Analysis

Descriptive statistics, such as mean number of LFA per sample vial and number of sample vials with positive detections was used as a surrogate for LFA population and to track treatment efficacy over time. Estimates for infested area were calculated by plotting GIS data for positive detections in QGIS version 3.12.3-Bucureșt mapping software, buffering each point to 6 m, and calculating the total area of the buffered points.

Results

The number of LFA positive detections and the mean number of ants per sample vial within the Phase I monitoring plots rapidly decreased upon commencement of the treatment regimen (figure 5.4). Results from the Phase I midpoint monitoring survey indicated the LFA infestation had greatly reduced in size with only 10.5% of deployed sample vials detecting LFA (figure 5.5). Based on the number of sample vials containing LFA, the estimated Phase I infested area had been reduced from 3.22 to 0.39 ha. By January 2014, 4.6% of deployed sample vials were containing LFA, the majority of which were along the boundary dividing the Phase I and II treatment areas. Discounting the positive detections along the Phase I and II boundary, the Phase I infested area was estimated in 2014 to be 0.05 ha. A slower decline was observed during the Phase II treatment regimen. Results from the Phase II midpoint monitoring survey indicated that the infested area had been reduced from 1.35 ha to 0.76 ha with 37.9% of the sample tubes collected containing LFA (figure 5.5). By January 2016, no LFA were detected in either Phase I or Phase II treatment areas.

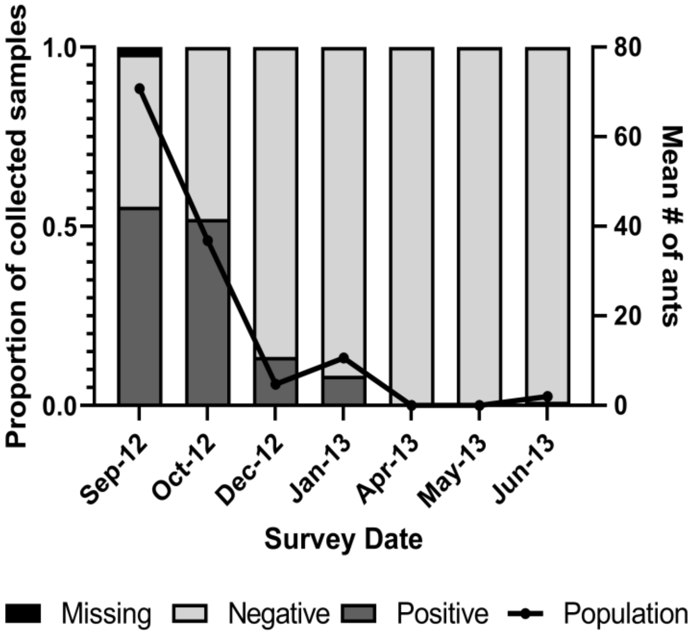

Figure 5.4: Results from the 2012-2013 Phase I monitoring plots showed a decline in both the number of samples traps containing LFA (bars) and the overall LFA population (line). Population was determined as the mean number of LFA per LFA positive sample collected.

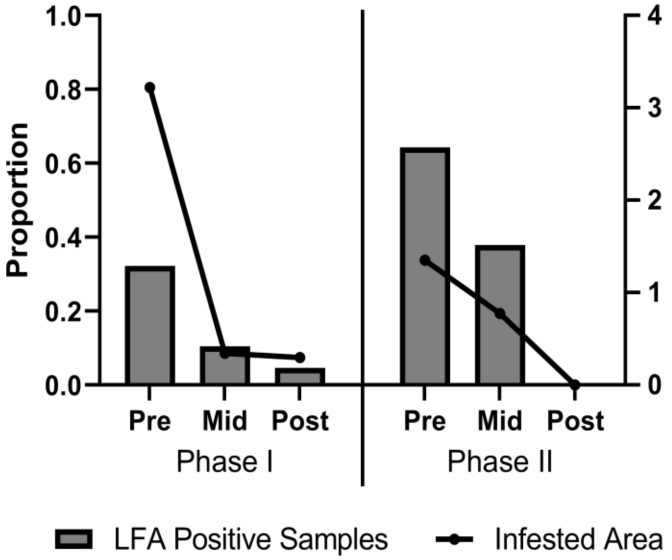

Figure 5.5: Results from Phase I and II pretreatment, midpoint, and post-treatment monitoring surveys. The number of samples detecting LFA (bars) and the infested area (lines) decreased at different rates over time.

A total of 53,757 sample tubes were deployed and collected during post treatment monitoring surveys between 2016 and 2021. Isolated LFA detections (hotspots) were detected on several occasions within the Phase I treatment area; the last being in September 2019. No hotspots have been detected within the Phase II treatment area since area-wide post-treatment monitoring began.

Discussion

Ant eradications are notoriously difficult and many factors can influence the success or failure of an eradication project. The development of the HAL gel bait has provided practitioners with the ability to effectively treat tall vegetation as well as the ground, and has increased the chances of successful eradication and management programs. However, the ability to detect incipient remnant colonies is as important as finding suitable and effective treatment methods. Our post treatment monitoring procedure included closer spacing of sample tubes, palm tree crown surveys, and expanded surveys beyond the treatment area in order to increase the chances of detecting lone, remnant colonies within the landscape. Little fire ant hotspots continued to be detected occasionally until September 2019.

It is not uncommon for incipient colonies to go undetected for multiple years using the ground survey methods described here. We have identified two main factors that likely contributed to the persistence of LFA hotspots within the treatment area and detections outside of the treatment area, despite repeated thorough surveys:

1) under-treated areas or features such as tall trees, dense vegetation, large mulch piles, steep terrain, or a combination of these act as “reservoirs” and “safe havens” for LFA to persist; and

2) movement of infested yard waste containing colony fragments to mulch piles beyond the treatment area allowed ants to be transported after treatments had ceased.

Although the application equipment allowed for baits to be applied up to 9 m horizontally and vertically, and mapping of treatment tracks indicated sufficient coverage, treatments were likely insufficient for palm trees ≥ 15 m or for penetration of dense mulch piles. Additionally, although we surveyed all palm trees throughout the treatment area multiple times, sample tube placement is critical for accurate results. Non-ideal sample tube placement may have resulted in occasional false negative survey results.

Although no LFA have been detected within the Phase II treatment area since area-wide post-treatment monitoring began, post-treatment monitoring should be maintained long term. There is no way to measure the true accuracy of our, or any, survey methods. Not detecting ants during a survey does not necessarily indicate absence, but that the size of a population is below detectable levels. It is possible that remnant LFA colonies remain within small areas that were completely inaccessible despite the use of specialty rappelling equipment.

Treatment options and methodology for LFA control have improved substantially over the past decade. However, the accuracy of monitoring programs continues to be a weak point in the process. The use of baited tubes or other lure-based monitoring methods can be labor intensive and project managers often find themselves in the position of sacrificing accuracy due to funding and personnel constraints. More effective detection methods, that don’t sacrifice accuracy, are needed to seek out and destroy incipient remnant colonies within as soon as possible after cessation of treatments. Detector dogs have been used for post-treatment monitoring during LFA eradications in Australia and this method is able to search larger areas and in a shorter timeframe than currently possible using classical lure-based survey methods (Baker et al. 2017; Wylie et al. 2016). While detector dog programs are promising tools for the future of LFA eradications, they are costly, take many years to develop, and are not infallible (Lin et al. 2011). Other detection methods, such as environmental DNA (eDNA), have been used also with varying degrees of success for a variety of species (Kudoh et al. 2020; Uchida et al. 2020; Valentin et al. 2020). As technologies improve, new detection methods will undoubtedly improve eradication success.

Historically, it has been recommended that post treatment monitoring continue for two years after the last positive detection (Drees et al. 2002) before invasive ants are formally declared to be successfully eradicated. Nevertheless, in the case of LFA, two years is likely insufficient. Little fire ants have been detected at eradication sites in Hawai’i and elsewhere despite two years or more of zero detections during post-treatment monitoring (personal observation). We propose that three years of intensive post-treatment monitoring without LFA detections may be a more appropriate minimum timeframe and we have coined this concept the “three year rule” for LFA eradications. It should be noted that the “three year rule” is somewhat arbitrary and that this value may change over time as more ant eradication efforts are documented. No matter the method, long-term commitment to post-treatment monitoring is vital to the success of any eradication program.

Acknowledgements

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Chapter 6:
Synthesis and Conclusions

Many factors contribute to successful pest management programs but important foundational information is often lacking. Understanding a species' basic biology, behaviors, and behavioral variations is essential to developing proper control and management techniques. *W. auropunctata* is a significant pest ant species that has spread throughout the tropics and subtropics. However, current control methods rely on commercial ant baits developed for other ant species and are only moderately effective against *W. auropunctata*. Species-specific control methods are needed for its effective, long-term management.

The primary objective of the research outlined in this thesis was to develop species-specific ant bait and control techniques for *W. auropunctata* in residential, agricultural, and natural ecosystems. This was achieved by following the progressive research framework detailed in the thesis Introduction (Chapter 1, figure 1). The research presented in this thesis fits with and addresses specific aspects of the research framework through a series of peer-reviewed manuscripts. The academic literature review provides an in-depth examination of what is currently known about the target species, *W. auropunctata*, and identified gaps in research. However, identifying gaps in our knowledge base is not solely an academic venture. Frequently, important knowledge gaps are first identified through challenges faced by field practitioners (i.e. government and non-governmental organization workers, research technicians, pest control operators, residents, etc…). Practitioner observations provided important additional context and a lens through which the existing literature was evaluated throughout the literature review. This is most evident in the section on controlling *W. auropunctata*. Given that species-specific ant control is the focus of this thesis, including practitioner observations was imperative to identifying which knowledge gaps were a priority to fill to improve on-ground management effectiveness in controlling this species and also to guide the progression of applied research through the framework.

The applied research in this thesis was divided into three phases: 1) developing techniques for laboratory bioassays and field trials, 2) pesticide and bait matrix development, and 3) a large-scale demonstration of effective control method implementation under field conditions.

Developing techniques for laboratory bioassays and field trials

Laboratory experiments are an important component of the experimental research process when developing insecticidal ant baits because of the ease of controlling many factors and of observing the experimental outcomes. However, laboratory-raised insects may behave differently from their wild conspecifics and results from experiments and trials using them are therefore often viewed as unreliable (Herard et al. 1988, Propkopy et al. 1989, Hendrichs et al. 2007, Clark et al. 2011, Ennis et al. 2015). This is also true for laboratory-raised ants (Huettel 1975, Propkopy et al. 1989, Marchioro and Foerster 2012, Ennis et al. 2015). Ant foraging behaviors and food preferences shift based on colony needs and the quantity and quality of available food resources (Sorenson et al. 1985; Behmer 2009; Cook et al. 2010). Naturally, such behavioral shifts may vary temporally due to seasonal episodes of colony building and diapause and as available food resources change in abundance and type. The ever-changing colony state and availability of wild food resources likely creates nutritional excesses and deficiencies which have been speculated as driving factors for food resource selection when foraging (Cassill and Tschinkel 1999, Portha et al. 2002, Dussutour and Simpson 2006). In contrast, laboratory-raised ant colonies are maintained in climate-controlled facilities and fed a complete and balanced diet in order to maintain healthy, productive colonies over long periods of time. In other words, laboratory colonies are forced into a constant state of colony building and never experience nutritional voids due to a constantly provided complete diet. Early *in situ* observations during this research study suggested a disparity in foraging preferences between wild and laboratory raised *W. auropunctata* as observed with other insect species.

Insecticidal ant baits are the standard control method for pest ants and development of a species-specific ant bait for *W. auropunctata* was a primary focus of this thesis. Efficacy of ant baits relies on natural foraging behaviors such as bait acceptance, recruitment to the bait, and sharing of the bait to nestmates. Therefore, differences in foraging behaviors between laboratory-raised and wild conspecifics are likely to result in unreliable results from laboratory experiments during the bait development process.

In Chapter 2 I investigated the effects of various rearing diets on the foraging preference of laboratory-raised *W. auropunctata.* Those results were viewed in juxtaposition with results from concurrent field palatability trials measuring foraging preferences of wild *W. auropunctata.* The results from both experiments confirmed that there is, indeed, a disparity in foraging preferences between laboratory-raised and wild *W. auropunctata*. During the 49 day laboratory experiment, I documented a significant increase in preference for carbohydrates over lipids when the ants were fed various laboratory-rearing diets. This was in stark contrast to the foraging preference of wild *W. auropunctata*, which recruited to lipids significantly more than carbohydrates. The observed differences in foraging behavior during this study should be taken into account during further laboratory experiments since they are likely to confound results from feeding experiments such as bait palatability and efficacy trials.

Although foraging preference shifted away from lipids among laboratory colonies during this study, regardless of dietary treatment, the effects of rearing diet may be mitigated. My research found that it is possible to elicit foraging behaviors reminiscent of the wild-type behavior by omitting all lipids from the diet for at least one week and starving the ants of all food for 48 – 72 hours prior to the start of a feeding experiment. However, notwithstanding the possibility of manipulating foraging behavior through diet, laboratory experiments should be generally be considered part of a screening process and results should not be interpreted as definitive unless verified by pertinent field trials with the target species.

Field trails are also an important part of the experimental research process when developing new pest control methods and testing existing control methods on new pest species. They are used to verify the results of laboratory experiments and to demonstrate proof of concept under natural environmental conditions. Currently, there have been few field trials that evaluated the efficacy of insecticide products against *W. auropunctata* and there is uncertainty as to what spatial buffer size is needed between sample locations of neighboring treatment plots to eliminate confounding effects from a neighboring treatment. Additionally, no studies to date have investigated differences in efficacy between different application methods, such as broadcast spread of ant baits versus point location bait stations, for area-wide control of *W. auropunctata*. Foraging distances, intra-specific interactions, and resource flow through an ant colony and throughout an area are all important variables to consider when conducting control method efficacy trials. However, no studies have investigated these factors beyond intra-specific aggression (or lack thereof) and its role in the formation of *W. auropunctata* supercolonies.

Mark – release – recapture (MRR) and mark – capture (MC) studies (referred to as marking studies) have been used to investigate insect behavior, population dynamics, dispersion, and food resource allocation for many years (Sunderland et al. 1995, Bowler and Benton 2005, Cordero-Rivera and Stoks 2008, DeGrandi-Hoffmann and Hagler 2000). Marking studies have been conducted on ants to show how food resources and insecticidal baits are shared throughout a colony, predator-prey interrelationships, population estimates, spatial structure of supercolonies, and to determine distance between bait stations for area-wide control of pest ants (Young 1980, Vega and Rust 2003, Buczkowski and Bennett

2007, Tschinkel 2010, Buczkowski 2012, Buczkowski et al. 2014, Song et al. 2015, Hogg et al. 2018). However, marking studies have not been conducted as yet using *W. auropunctata* as the test subject. Foundational information on which markers work, which do not work, and why is needed to provide the necessary tools for marking studies and research on *W. auropunctata.* Chapter 3 begins the process of identifying an appropriate marker for *W. auropunctata.*

Traditionally, insects are marked with physical tags, fluorescent dusts, pollen, paint, ink, dye, trace elements, animal proteins (immunomarkers), and genetic markers (Hagler et al. 1992, Hagler and Jackson 2001). However, not all markers are suitable for all insects and the type of marker depends on several factors including the size of the insect, the durability of the marker, toxicity, and cost (Hagler and Jackson 2001). *Wasmannia auropunctata* are tiny, less than 2 mm in length, and are a pest primarily in wet, tropical climates. Additionally, as with other social insects, *W. auropunctata* display social behaviors such as grooming and sharing of food resources. These factors are likely to affect marker retention and detectability.

As a first step into investigating suitable markers for *W. auropunctata*, my study focused on topical immuno-marking using whole cow's milk (milk) and 10% chicken egg whites in water (egg whites). These markers are inexpensive, detectable at extremely low quantities, and had been previously successfully tested as external markers on minute parasitoids using direct application and an indirect, self-marking technique whereby parasitoids walked on a treated surface (Irvin et al. 2012). Both application methods effectively marked the parasitoids; therefore, milk and egg whites were viewed as a promising marker candidate for *W. auropunctata.*

The results from my study nevertheless found that the indirect, self-marking technique was an unreliable marking method for *W. auropunctata*, regardless of the type of marker. Also, detectability of milk declined rapidly after exposure even when applied directly. Because of this, milk was deemed an unsuitable marker for *W. auropunctata.* Conversely, chicken egg whites maintained a high rate of detectability when applied directly throughout the 48 hour post-exposure timeframe of the study. It is also possible that topical egg white markers are retained and detectable for much longer than was tested in my study, which would support egg whites as a suitable marker for *W. auropunctata.*

I also demonstrated a high risk of unintentional transfer of the external markers to un-marked ants from nestmate interactions. Therefore, external marking, in general, may not be suitable for *W. auropuctata* and internal marking techniques may be more promising for investigation.

This research is ongoing and internal marking techniques for *W. auropunctata* are in the process of being investigated, although no further data have been published to date. Other studies have shown that minute parasitoids may be effectively marked by feeding on honey laced with a protein marker (Hagler and Jackson 1998). Preliminary data suggest that this may also be a possible technique for *W. auropunctata* (M. Montgomery unpublished data)*;* however, this research is in the early stages and much more work is needed before this technology can be deemed a reliable marking method for this species.

Unfortunately, therefore, we are still without the necessary tools to answer questions related to ant and food resource dispersal throughout a field area. Until a suitable marking procedure can be developed and such questions can be answered, I suggest that field efficacy trials utilize large buffer zones (i.e. ≥ 25 m) between experimental treatment monitoring locations and that area-wide control programs use broadcast insecticidal ant baits rather than using localized bait stations.

Pesticide and bait matrix development

The most effective and efficient way to control pest ants is through the use of insecticidal ant baits. Ant baits are formulated to exploit natural foraging behaviors and eusociality by lacing attractive foods with a small amount of insecticide (active ingredient). Insecticides must meet specific criteria before they are deemed suitable as an active ingredient (a.i.) in an ant bait. Candidate a.i. must be non-repellent, exhibit delayed mortality even when high concentrations are ingested, and maintain efficacy at extremely low concentrations and when diluted by trophallaxis (Williams 1983, Rust et al. 2000, Braness 2002, Tollerup et al. 2004). Unfortunately, few insecticides meet these criteria (Levy et al. 1973, Williams 1983). What's more, differences in food preferences and chemical sensitivities between ant species further complicate efforts to develop a "universal" ant bait. In general, pest ants are categorized into three generic sub-groups (sugar-loving, lipid-loving, and protein-loving), depending on what types of foods cause them to mass recruit, for the purposes of developing and selecting an appropriate ant bait. *Wasmannia auropunctata* belong to the lipid-loving group along with other "fire ant" species.

Historically, it has been assumed that commercial ant baits developed for "fire ants" would be effective against *W. auropunctata*. However, studies have shown this to not be the case, with several factors suspected of limiting their efficacy in tropical ecosystems (Souza et al. 2008, Taniguchi 2008, Vanderwoude and Nadeau 2009). First, commercial "fire ant" baits are formulated as granules using defatted corn grit soaked in soy oil laced with an insecticide (Kidd et al. 1985, Williams et al. 2001).

While granules are easy to use and attractive to *W. auropunctata*, they can be only applied to the ground. However, ground applications have been shown to be ineffective for long-term management since arboreal colonies never encounter the bait and are left unaffected (Souza et al. 2008, Taniguchi 2008). Second, granular ant baits are sensitive to moisture. They become soggy and unpalatable once wetted and rapidly decay. Additionally, some common a.i. have a 45 minute half life once exposed to water and air which greatly reduces the timeframe for which the bait would be effective. The third issue is that some commercial "fire ant" ant baits are not attractive to *W. auropuntata* which suggests that this species may be repelled by some a.i. that other "fire ant" species are not (Hara et al. 2014, Montgomery et al. 2015). This is particularly noticeable with ant baits containing insect growth regulators (IGR) (Hara et al. 2014). The failure of early *W. auropunctata* eradication and control efforts in Hawai`i have been largely attributed to these shortcomings.

Issues relating to arboreal application and weathering have been addressed through the development of a novel home-made gel bait matrix (Vanderwoude and Nadeau 2009, Vanderwoude et al. 2010) with proof of concept demonstrated using the gel bait with indoxacarb as the active ingredient (Vanderwoude et al. 2010). The gel bait matrix easily sticks to vertical surfaces and is easily applied up to nine meters into tree canopies using heavy-duty squirt bottles or backpack sprayers. Because the bait is a gel and not a dry granule, it is not negatively affected by dew or mist. The original intent of developing a home-made gel bait matrix was to offer practitioners flexibility in selecting an a.i.suitable for the particular site being treated (C. Vanderwoude, personal communication). While indoxacarb is considered a reduced risk insecticide (United States Environmental Protection Agency 2000), site use restrictions limit where this insecticide may be used. Alternately, (s)-methoprene, an insect growth regulator (IGR), is one of the few active ingredients (a.i.) without site use restrictions other than "do not apply directly to water" and is exempt from residue tolerances (40 C.F.R § 180). Insect growth regulators are considered generally "non-toxic" since they do not kill target pests directly; rather, they inhibit reproductive and developmental functions. Because of this, ant baits with (s)-methoprene may be used at sites prohibited by other commercial ant baits such as in agriculture production and natural areas. However, issues remained surrounding chemical sensitivity and repellency of *W. auropunctata* to (s)-methoprene and it was the objective of Chapter 4 to investigate this aspect as part of improving bait efficacy.

Results from this study supported earlier observations by Hara et. al (2014) that *W. auropunctata* are repelled by (s)-methoprene. The dose response curve showed a clear decline in *W. auropunctata* recruitment to the ant baits as the concentration of (s)-methoprene increased. Also, (s)-methoprene was found to be repellent even at the very low concentrations found in commercial “fire ant” baits, suggesting that this was a plausible explanation for the poor performance of these ant baits in the field. Once this chemical sensitivity was determined, I was able to modify the gel bait matrix by including a protein adjuvant to mask the presence of (s) – methoprene and thereby develop an attractive IGR ant bait for controlling *W. auropunctata*.

The modified gel bait resolves the three main issues surrounding commercial “fire ant” baits (arboreal treatment, weathering, and repellency). However, the torula yeast used as the protein adjuvant that masks the (s)-methoprene component can be expensive and difficult to acquire for practitioners mixing up their home bait formulations. Since this study was first published, other protein adjuvants (i.e. peanut butter and powdered beef liver) have become the standard due to greater cost effectiveness and the ease at which they can be obtained M. Montgomery personal observation). It should be noted that this is a home-made gel bait recipe, available for all practitioners to use, and is not a shelf stable formulation or commercial product. The gel bait should be used in entirety within 24 hours of mixing, especially when peanut butter is used as the protein adjuvant. Research is ongoing to further improve the gel bait matrix and various preservatives and stabilizers are currently being evaluated for use in order to extend the shelf life of the gel bait without compromising its attractiveness.

Demonstration of effective control under field conditions

One of the main challenges faced by those tasked with controlling or eradicating *W. auropunctata* is the lack of suitable baits and treatment methods. Throughout this research project, a highly attractive ant bait was developed that could be applied in field situations to trees, foliage, and the ground. The final objective of this research project was to create and implement a management plan for *W. auropunctata* using this gel bait for real world situations. In this case, the management plan was used for the purpose of eradicating a persistent *W. auropunctata* infestation on the island of Kaua`i, Hawai`i (Chapter 5).

Specific control methods, such as suitable ant bait and application methods, are essential for effective management of pest ants; however, other elements are needed in order to devise a comprehensive management plan focused on long-term results. The management plan for the *W. auropunctata* eradication project described in Chapter 5 consisted of three essential elements: 1) pre-treatment delimitation, 2) treatment, and 3) post treatment monitoring. Each element is equally important because the failure of one will result in the failure of an entire control effort.

Land usage and the physical features of an infested site are important considerations when deciding what ant baits are the most appropriate to use. Since IGRs do not directly kill ants, rapid population knockdowns do not occur with this type of bait. Instead, an infestation will remain and the ant population will gradually decline over several months due to reduced egg production and the inhibition of development from larval to adult stages. In short, queens are unable to repopulate the colony at the same rate as worker death and colonies slowly decline to the point of collapse. The high numbers of *W. auropunctata* queens increases the risk that some queens will ultimately not be fed the ant bait during any single treatment. This makes repeated treatments necessary to ensure all queens within a control area are fed and affected by the application of IGR ant bait. Additionally, (s)-methoprene does not cause permanent sterility and may be metabolized over time. This further necessitates repeated applications over time to achieve long-term ant control using IGR-based ant baits. In cases where *W. auropunctata* are invading houses and residents are getting stung, residents are often in need of rapid results and do not have the luxury of waiting for gradual population declines over the course of several months.

In contrast to IGR-based ant baits, toxic ant baits result in rapid population knockdown. However, ants and other eusocial insects are able to rapidly recognize when a resource is being detrimental to colony survival. As nestmates become sick and die, ants will begin to avoid the resource that is killing them and they will consequently cease foraging on lethal ant baits. This phenomenon is called bait shyness. Surviving queens can also adjust egg production to suit colony repopulation needs and the rapid knockdown is followed by a period of increased population growth and recovery. Because of the likelihood of bait shyness and survival of some queens following a single treatment with toxic ant baits, repeated applications are also needed when using this type of ant bait against *W. auropunctata.*

The treatment phase of the management plan was centered on the hypothesis that bait shyness was less likely with non-toxic IGR ant baits and may result in greater uptake and distribution of the the gel bait over time compared to lethal ant baits. Therefore, the IGR gel bait may be more effective with higher rather than lower population densities. Additionally, high ant populations result in more IGR bait being brought to a nest and shared than if the population was initially reduced from use of toxic baits.

The eradication was implemented in two phases, each with slightly different treatment strategies due to differences in terrain and land use. The immediate needs of residents and landscapers in the Phase I treatment area required the use of granular baits from the beginning for rapid population knockdown and relief from being stung by the ants on their properties. Gel bait was used to treat all vegetation, tree canopies, and palm crowns. Granular baits were not suitable for the steep terrain of the Phase II treatment area; therefore, only gel bait was applied during Phase II.

The treatment strategies described in Chapter 5 proved effective in reducing *W. auropunctata* populations to below detectable levels and no *W. auropunctata* have been detected since January 2016. However, isolated *W. auropunctata* “hotspots” (remnant colonies) were occasionally detected and treated throughout the Phase I treatment area until September 2019, four years after cessation of the Phase I treatment regimen. Hotspots typically coincided with especially tall palms and large debris piles, but hotspots were also detected beyond the original treatment area on two occasions. This highlights a need for better detection methods as well as prolonged monitoring to detect such resurgences or reinvasions.

The ability to reliably detect a target species is as important as the treatment methods employed. If a target pest remains undetected (false negatives), it will not be treated, even if effective treatment options are available (Britton et al 2011). Non-toxic, lure-based surveys are currently the status quo for *W. auropunctata* detection. Lures consist of highly attractive foods, such as peanut butter, mayonnaise, or hotdogs, and are placed in a grid-like fashion and at a specified distance interval. Since *W. auropunctata* build supercolonies with high population densities, the lure detection method works well within the heart of an infestation. However, at the perimeter of an infestation, when an infestation has been fragmented, after treatment, or when searching for newly introduced incipient colonies, the reliability of lure-based surveys is more variable, depending on lure spacing and placement, foraging distance, and the surveyors’ experience levels. At present, the important foraging dynamics (i.e. foraging distance at various population densities) are unknown and the marking tools needed to investigate this have yet to be refined for *W. auropunctata.*

The methods described in Ch 5 effectively reduced the *W. auropunctata* population to below detectable levels; however, the current lure-based detection method has critical limitations and is insufficient for rapid declaration of eradication. Biosecurity projects, such as those tasked with early detection and control of pest ants, are frequently under-funded and under-staffed; thus, compromises between accuracy and feasibility are common when lure-based survey methods are employed. Small, remnant colonies are difficult to detect and may go undetected for years, but premature declarations of eradication heighten the risk further expansions of *W. auropunctata* infestations and movement of infested materials off-site. Survey repetition and randomization of lure placement between survey efforts is often used in lieu of increasing lure densities throughout the survey area. Traditionally, invasive species eradication projects use a "two-year rule", whereby the target species is not detected for two consecutive years before eradication is declared. In the case of *W. auropunctata,* two years has been found to be an insufficient duration for confirming eradication (Vanderwoude et al. 2015) and this study confirmed that a minimum of three years is more appropriate when lure-based detection methods are employed.

While repetition of surveys over time allows for lower lure densities and fewer resources per survey effort, extending an eradication project's overall timeframe increases overall costs of the project, especially if hotspot infestations continue to be detected. There is no guarantee that funding will continue for an indeterminable amount of time when rapid results are expected by funding agencies. Because of this, better detection methods are needed to expedite the eradication process. Development of novel detection methods and refinement of existing detection methods is the next logical step in improving management strategies for *W. auropunctata.* The use of detector dogs for ants has increased over the past decade (Lin et al. 2011, Baker et al. 2017) and is generally considered more effective than lure-based detection methods. Unfortunately, detector dog programs can be expensive, take years to develop, and there is no standardized method for use or performance evaluation of individual detector dogs. Further refinement and development of standard operating and evaluation procedures of detector dog programs will improve the detection component of control and eradication programs and lead to the global expansion of detector dog programs for species like *W. auropunctata.* Novel detection methods such as pheromone traps, infra-red thermal detection, and environmental DNA are still under development and currently unavailable for this species. However, technological advancements and future research may result in novel detection methods becoming available sooner than expected. This would greatly improve *W. auropunctata* control and eradication successes and within a shorter timeframe than is realistic with current detection methods.

The research presented in this thesis contributes to a greater understanding of *W. auropunctata*, the issues and considerations surrounding effective control of this species, and outlines a framework in which to conduct similar research for further understanding and refinement of species-specific control methods for this and other invasive pest species. More generally, it demonstrates the importance and usefulness of an integrated pest management framework and adaptive management approach to build and test the evidence basis for applied pest control. Such an approach depends on structured, experimental research that builds on past studies to improve techniques and trial innovative tools and protocols. Such intensive applied research is essential for addressing real world challenges but is often bypassed or under-resourced, yet there is a great deal of research left to do.

This thesis has progressed our understanding for controlling W. auropunctata and provided a foundation for future research to build upon. *Wasmannia auropunctata* will continue to spread globally and its biological and ecological plasticity enable this species to invade a wide variety of habitats, each requiring different considerations and approaches to *W. auropunctata* management. Throughout this thesis, problems with the status quo ant control approach were identified, solutions to those problems were developed and described in detail, and further research needs were highlighted. The demonstration phase of this thesis (Chapter 5) highlights the importance of undertaking both laboratory and field research to explore a breadth of factors influencing pesticide efficacy and optimal field usage, and the complementary need to properly assess a site and use site information to develop an effective adaptive management plan specific for both the site and target species. While the strategies described for the eradication effort here are specific for Hawai`i and likely suitable for tropical ecosystems, they may not be suitable for all situations. Practitioners and researchers should take care to understand the behavior of *W. auropunctata* within their local environment, identify site features needing special attention, and use this information to create a management plan best suited to their specific situation. Only this kind of collaborative enhancement of our theoretical and applied knowledge base will lead to the best possible outcome for *W. auropunctata* control and eradication efforts.

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