

EFFORTS TO SLOW THE SPREAD OF INVASIVE SPECIES IN HAWAI‘I: A
FOCUS ON INTEGRATED PEST MANAGEMENT STRATEGIES FOR THE
COCONUT RHINOCEROS BEETLE, *ORYCTES RHINOCEROS* AND THE HALA
SCALE, *THYSANOCOCCUS PANDANI*

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THE DEGREE OF DOCTOR OF PHILOSOPHY IN
ENTOMOLOGY
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I would like to dedicate this dissertation to my parents and in loving memory of Dr. Marty Paulsson, Cherie Kinneman, and Mary Russo.

LAND ACKNOWLEDGEMENT STATEMENT

A land acknowledgement is a step towards our conscious recognition of the place we are in and its Indigenous roots.

Aloha. My name is Mason Russo and I am an immigrant on this ‘āina who now calls Mānoa home. I would like to begin by acknowledging that the ‘āina on which we gather, Mānoa, is part of the larger territory recognized by Indigenous Hawaiians as their ancestral grandmother, Papahānaumoku.

I recognize that her majesty Queen Lili‘uokalani yielded the Hawaiian Kingdom and these territories under duress and protest to the United States to avoid the bloodshed of her people. I recognize that each moment I am in Hawai‘i she nourishes and gifts me the opportunity to breathe her air, eat from her soils, drink from her waters, bathe in her sun, swim in her oceans, be kissed by her rains, and be embraced by her winds. I further recognize that generations of Indigenous Hawaiians and their knowledge systems shaped Hawai‘i in sustainable ways that allow me to enjoy these gifts today.

For this I am grateful and as an immigrant, I seek to support the varied strategies that the Indigenous peoples of Hawai‘i are using to protect their land and their communities.

Mahalo.

ABSTRACT

The Hawaiian islands have been subject to a series of invasive plant, insect, vertebrate, and pathogen introductions. This started with the first Polynesian arrivals, increased with European contact, and substantially increased as globalization ensued. These introductions have resulted in the extinction of many endemic species and have altered many ecosystems from dryland forests to high elevation areas. This dissertation will focus on two specific invasive insects, the coconut rhinoceros beetle, *Oryctes rhinoceros* Linnaeus and the hala scale, *Thysanococcus pandani* Stickney. These insects impact native species and have caused ecological, cultural, and economic damage.

Oryctes rhinoceros is an invasive insect that feeds primarily on coconut palms. It was previously controlled with the release of specimens infected with the virus, *Oryctes rhinoceros* nudivirus (OrNV, *Alphanudivirus oryrhinocerotis*). However, beetles resilient to OrNV were found in Guam, where they became widespread. Since then, this biotype of *O. rhinoceros* was found on the Hawaiian island of O‘ahu in 2013. This triggered an extensive eradication program using tools such as mass trapping, detector dogs, green waste sanitation, and insecticidal treatments. Populations have since spread across O‘ahu, and in 2023 highly localized *O. rhinoceros* populations were found on Maui, Kaua‘i, and Hawai‘i island. The insecticidal studies evaluated the efficacy of systemic insecticides in laboratory and field settings as a potential component of the long-term integrated pest management program for *O. rhinoceros*. The laboratory assays tested the efficacy of imidacloprid, dinotefuran, and abamectin at 25 and 250 parts per million (ppm) on adult *O. rhinoceros* beetles. Two concurrent field trials were conducted to evaluate the preventative and curative effects of injecting imidacloprid, dinotefuran, abamectin, and acephate into coconut palms trunks. Efficacy of treatments was

determined by taking monthly visual measurements of *O. rhinoceros* feeding damage. Palm fronds were removed and tested on laboratory-starved beetles to see if feeding exposure caused negative impacts. The results of the laboratory trials indicated that all the chemicals were effective at both 25 and 250 ppm and that pesticide residue in palm fronds caused negative impacts as late as seven months after treatment. The field trial results indicated that imidacloprid injections reduced the total amount of new feeding damage, with chemical residue remaining seven months post treatment in palms. I conducted an areawide injection trial at this site that had inconclusive results.

I conducted further trials to evaluate commercial and locally occurring entomopathogenic fungi that could be implemented to mitigate *O. rhinoceros* populations. I also conducted trials with locally collected entomopathogenic nematodes that did not yield significant mortality. Our initial laboratory trials in 2018 and 2019 narrowed down five locally collected fungal strains with greater than 60% mortality rates on first instar larvae. In 2021 these strains were tested in conjunction with the fungal insecticide BotaniGard ES, with decreased efficacy in some strains, but greater than 60% mortality with BotaniGard ES at the high label rate. In field trials there was greater than 60% mortality of first instar larvae using BotaniGard ES as a mulch amendment. This treatment is unfortunately difficult to implement in the field due to the shelf life of the product.

The studies pertaining to *T. pandani* covered a variety of knowledge gaps surrounding this rarely studied insect. In Hawai‘i it was first detected in Hāna, Maui in 1995, and its primary host plant is the indigenous hala tree, *Pandanus tectorius*, which is found in coastal and riparian areas throughout the Hawaiian islands. It has been found on Moloka‘i, O‘ahu, Lāna‘i, and Hawai‘i island, but *T. pandani* is only widespread on the islands of Maui and Moloka‘i. The

insect is a sedentary feeder on the leaves and fruit, and heavy infestations result in discoloration, frond, and possible tree death. Experimental field trials were set up to evaluate the efficacy of organic and chemical insecticides as potential treatments to mitigate the damage of *T. pandani* infestations. This was done on the Island of Maui where *T. pandani* is widespread. These trees were inoculated with *T. pandani* and evaluated throughout the trials for changes in plant height, canopy width, scale mortality, and visual infestation ratings. The results of two repeated field trials indicated that flupyradifurone and buprofezin were effective treatments in reducing the hala scale infestation and improving visual aspects related to plant health. In 2021 and 2023, statewide surveys were conducted to evaluate the distribution of *T. pandani*, and a dispersal study was set up on Maui in January 2023 to determine the ability of *T. pandani* to spread from infected to healthy hala trees over time. The visual surveys involved evaluating hala trees throughout plant nurseries, landscaping plantings, urban and residential areas, and forests. The dispersal study evaluated factors such as wind, spatial separation, population levels, and infestation ratings. The visual survey results indicated *T. pandani* is widespread on Moloka'i and Maui, with established populations on O'ahu. The infestations on Lāna'i and Hawai'i island were successfully eradicated. The dispersal study indicated that *T. pandani* was able to infest the entire field plot in the first 10 months.

Available literature indicates that *T. pandani* infestations prevent seedling regrowth, suggesting that the hala forests on Maui and Moloka'i may not regenerate. I carried out a statewide population structure study of coastal *P. tectorius* forests to test if presence of the hala scale was correlated with lower levels of recruitment. In 93 transects across four islands, this study documented population structure, hala scale presence, and biotic and abiotic factors that may also affect regeneration, including elevation, canopy openness, and understory invasive

species cover. Heavy infestations of *T. pandani* infestation were present in all transects across forests on Maui and Moloka'i, while Kaua'i and Hawai'i island did not have any infestations. Canopy cover varied across sites and relatively low levels of invasive plant cover were found in the understory. There was significantly lower sapling recruitment ratio (saplings (1-2m tall)/adult tree) within transects with the hala scale than those without. However, this result was driven by a single outlier. Immediate regeneration of *P. tectorius* may be inhibited by non-native plants and ungulate feeding. We conducted biological control surveys in Thailand, Indonesia, and Singapore to search for insects that could control *T. pandani*. The first trip found generalist coccinellidae predators and a botanical collection where it was found had many *Pandanus* spp. from Madagascar. In a survey to Madagascar, I found three undescribed scale species on various pandanus species, but no immediate evidence of *T. pandani* or biological control.

The results of these studies helped fill knowledge gaps pertaining to integrated pest management of *O. rhinoceros* and *T. pandani*. I was able to conduct a variety of ecological and applied studies for *T. pandani*. These pests can severely alter the aesthetic landscape in urban areas of Hawai'i that are characterized by palm and *Pandanus* plantings. They also have the ability to negatively impact natural areas with endemic *Pritchardia* spp. palms and wide ranging *Pandanus* forests found on coastal areas along the Hawaiian islands. It is possible that the spread of these pests within islands was facilitated by widespread urban planting of these species, thus creating a pathway for widespread dispersal of these pests throughout each island. In general, the accidental transport of these pests results in a range expansion within an island, or to a new area in the state. Public outreach and improved biosecurity protocols can mitigate the impacts of these pests. As these insects are not yet widespread across every Hawaiian island, further research and mitigative measures are necessary to slow the momentum behind the invasion of these pests.

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CHAPTER 1 GENERAL INTRODUCTION AND DISSERTATION STRUCTURE

General introduction background

As the world faces an imminent biodiversity crisis, there have been a multitude of responses to save species from extinction and evaluate the potential impacts associated with this decline (Cardinale et al. 2012, Mendenhall et al. 2012, Gabara et al. 2021). These responses range from policy initiatives with bold promises to mitigate the impacts of climate change, rewilding, reforestation, and the promise of a clean energy revolution. (Pitt and Congreve 2017, Perino et al. 2019, Corlett 2020, Kemppinen et al. 2020, Carver et al. 2021, di Sacco et al. 2021). There has been inspiration for what individuals can do on a smaller scale to reverse the direction of these events. In many areas experiencing drastic ecological decline, there has been an encouragement to plant native ornamental plants, which possibly could have local ecological benefits (Suryaningrum et al. 2021). Planting native plants can have beneficial effects on suburban and urban areas such as increased shade coverage, connecting patches of disturbed land, runoff and flood improvement, and other positive effects that resulted in an increase in programs to encourage residents in urban areas to plant native (Krasny and Tidball 2012, Dawes et al. 2018, Eisenman et al. 2021). Some of these areas are ecologically devoid of native species, and increased planting may facilitate the return of some of the previous ecosystem functions. There also are ecological benefits to strategic planting of native organisms in agricultural areas as well. These include carbon absorption, increased biodiversity, nutrient cycling, and watershed improvement (Cunningham et al. 2015). The implementation of minor changes across a

landscape scale can have far reaching effects on biodiversity loss and possibly even climate change. However, a systemic change faces many societal hurdles that would involve an extensive amount of public outreach. The result could lead to the resumption of some of the ecosystem function to areas that are entirely characterized by anthropogenic activity.

The Hawaiian Archipelago is the most remote island chain in the world and home to a unique variety of endemic species, that are especially vulnerable to these threats, as they face invasive species introductions, habitat loss, and record rates of extinctions (Eldredge and Evenhuis 2003, Doherty et al. 2016). The isolation of these islands has resulted in the evolution of diverse species found nowhere else on the planet, but the anthropogenic introduction of new organisms has resulted in widespread extinction across many taxa. The first Polynesian voyagers brought over 40 species with them such as canoe plants and pigs to sustain their way of life (Cox 1999). As of today, there are an estimated 900 established species of plants introduced to Hawai'i, of which 100 are significant invasive species. The rate of introduction has been exacerbated in recent years through the rapid pace of globalization and continued reliance on imports throughout the State of Hawai'i (Meyerson and Mooney 2007, Hulme 2009). This has created a multitude of pathways for invasive species to arrive and establish on various islands.

The Hawaiian landscape is substantially fragmented, with many endemic species persisting in remote refugia (Sakai et al. 2002). The primary cause of habitat fragmentation is development and the associated habitat destruction, and invasive species readily establish within and threaten the remaining reduced areas. There are excellent conservation efforts in place that have engaged the public to take interest in preserving native habitat and combating invasive species (Niemic et al. 2019). There have been initiatives to make tourism more sustainable and

to educate the public on the ecotourism conservation programs (Sutcliffe and Barnes 2018, Yee 2018).

Effective response programs are in place for the early detection of invasive species and to initiate eradication programs (Daehler et al. 2004, Kraus and Duffy 2010). Each Hawaiian island has invasive species committees: O‘ahu Invasive Species Committee (OISC), Kaua‘i Invasive Species Committee (KISC), Maui Invasive Species Committee (MISC), Moloka‘i Maui Invasive Species Committee (MoMISC), and Big Island Invasive Species Committee (BISC). These coordinate with the Hawai‘i Invasive Species Council (HISC). HISC includes representation of the Hawai‘i Departments of Land and Natural Resources, Agriculture, Health, Transportation, Business, Economic Development and Tourism, and the University of Hawai‘i (“Hawai‘i Invasive Species Council” 2022). These committees have identified numerous target pests that are capable of causing significant economic and ecological damage. Some of these pests are widespread on specific islands, but not yet established on other islands. For example, the coqui frog is widespread on Hawai‘i island likely because initial eradication efforts were hindered by individual homeowner preferences (Kalnicky et al. 2014). Now there are populations under eradication attempts in areas of Maui and O‘ahu. There can be a lack of resources or policy initiatives to enforce the stringent inspection of inter-island transport of pests, resulting in repeated eradication programs in localized areas with difficult parameters to quantify if there is success, or a low-threshold population of the given pest.

The negative impacts of invasive species are well documented in Hawai‘i, as they have negatively impacted economic output, ecological areas, and cultural practices in the state. My dissertation will tie together the various concepts illustrated above, invasive species, invasive species responses, and conservation, ultimately providing recommendations for managing

specific invasive species, while filling knowledge gaps pertaining to these organisms and potential management tactics. The focus of my dissertation will center on invasive insect pest species associated with coconut palms, *Cocos nucifera* (Arecaceae), and hala trees or screwpine, *Pandanus tectorius* (Pandanaceae). These are the coconut rhinoceros beetle, *Oryctes rhinoceros* Linnaeus (Scarabaeidae) and hala scale, *Thysanococcus pandani* Stickney (Halimococcidae). *Thysanococcus pandani* is now widespread on Moloka'i and Maui and contained within a few areas on O'ahu, yet it is not declared as a target pest by OISC. *Oryctes rhinoceros* is part of the CRB-G biotype that is resilient to classical biological control using an insect pathogenic virus (Marshall et al. 2017). *Oryctes rhinoceros* was confined to O'ahu for almost 10 years, until 2023 when it was found on the islands of Maui, Hawai'i island, and Kaua'i. There are ecological, economical, and cultural concerns if control measures cannot eradicate these new introductions. If both species were to become widespread statewide, there would likely be damaging impacts to the economy and ecology of the Hawaiian islands, along with a potential reduction for the continuation of some Native Hawaiian cultural practices attached to impacted plants.

Dissertation Aims and Structure

My dissertation research in Chapters 2 and 3 evaluated additional methods for the management program for the *O. rhinoceros* CRB-G biotype, studying the implementation and effectiveness of chemical and biological control measures. There is a knowledge gap for the efficacy of certain insecticides on *O. rhinoceros* adults and no research regarding widespread field application. Chapter 3 covers the laboratory trials and large-scale field trials related to this. Based upon the results, I can recommend reapplication intervals and an effective pesticide rotation. Studies testing frond feeding insecticidal residue helped determine pesticide longevity

in Chapter 3. Previous research has indicated that local entomopathogenic fungal (EPF) strains exist on O‘ahu with biological control potential for *O. rhinoceros* larvae. In Chapter 2, I evaluated a commercial EPF product that is labeled for use in Hawai‘i and can potentially be incorporated into the management program. The initial goal of Chapter 2 was to collaborate with a company to produce locally collected and tested EPF strains, but this had faced difficulties from regulation, resources, and funding.

Chapters 4 and 5 filled a variety of knowledge gaps pertaining to *T. pandani*. Chapter 5 includes the revised statewide distribution. This survey required extensive public outreach and visiting a variety of urban and rural areas, resorts, schools, botanical gardens, nurseries, airports, and the remaining forested areas of *P. tectorius*. I confirmed the success of *T. pandani* eradication attempts in areas that are now clear of populations. For the island of O‘ahu, public outreach was conducted that helped eliminate one of four hot spots for *T. pandani*. The other three infested areas could be utilized as a source for specimens for future biological control research and testing. A field plot study provided insight into the dispersal capabilities of *T. pandani* in Chapter 5. Multiple rounds of insecticidal trials are presented in Chapter 4, which determined appropriate management tactics for high value trees in areas with widespread infestations. I identified one *Pandanus* sp. variety that is resistant to damage in the Hawaiian islands and multiple species in botanical collections in Asia. The hala forest population survey in Chapter 5 evaluated multiple ecological parameters and scale infestation data to quantify the recruitment ratios for hala forests with and without *T. pandani* infestations. Our natural enemy surveys in Thailand, Singapore, and Indonesia found generalist coccinellidae species feeding on *T. pandani* and are detailed in Chapter 4. This Chapter includes biological control exploration to Madagascar, where multiple undescribed scale species were found on *Pandanus* spp. Chapter 6

concludes the dissertation and includes a reflection on the current challenges we face today with managing invasive species in the Hawaiian islands. This dissertation covers just two of the many damaging invasive insect species in the Hawaiian islands and highlights the challenges that we face in managing these populations due to resource limitations such as the need for updated biological control facilities and funding from the State of Hawai‘i to adequately address individual species of concern. If this situation were to improve, there would be increased potential to conserve species endemic to Hawai‘i, mitigate public health issues with invasive species, and preserve our natural areas for future generations.

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CHAPTER 2: LABORATORY AND FIELD TESTING OF NEW INTEGRATED PEST MANAGEMENT STRATEGIES FOR LONG-TERM CONTROL OF THE COCONUT RHINOCEROS BEETLE, *ORYCTES RHINOCEROS*, USING ENTOMOPATHOGENIC FUNGI AND NEMATODES

Introduction

The isolation of the Hawaiian archipelago has resulted in numerous adaptive radiations and a diversity of endemic species that are particularly susceptible to invasive introductions, owing to their evolution in isolation of other species in terms of coevolved defense mechanisms (Hembry 2021). An estimated 72% of recent recorded extinctions in the United States are species that were endemic to Hawai'i (Cox 1999, Messing and Wright 2006). These high extinction rates are due to the many anthropogenic disturbances to the islands such as development, agricultural expansion, and the introduction of exotic species. Globalization has provided pathways for many invasive species to reach new ecologically compatible habitats, resulting in a three-fold increase in the need for invasive species global eradication programs in the last 50 years (Suckling et al. 2019). Certain species can outcompete endemic organisms, typically resulting in a cascade of negative and sometimes irreversible changes to the original ecosystem (Crowl et al. 2008, Duenas et al 2021).

The coconut rhinoceros beetle (CRB), *Oryctes rhinoceros* (Coleoptera: Scarabaeidae: Dynastinae), was first detected in 2013 on the Hawaiian island of O'ahu. This pest was previously controlled across its invasive range with classical biological control by an entomopathogenic virus, *Oryctes rhinoceros* nudivirus (OrNV, Family: Nudiviridae; *Alphanudivirus oryrhinocerotis*) (Bedford 1980, Caltagirone 1981, Zelazny and Alfiler 1991).

This was considered a success in classic biological control for almost 50 years until a virus resistant haplotype (CRB-G) was detected in Guam in 2007 (Marshall et al. 2016, Marshall et al. 2017). Before nudivirus introductions, there was an estimated 50% palm mortality on larger islands of Palau, and 100% mortality on smaller islands, indicating that the CRB-G haplotype likely will inflict similar damage. The CRB-G haplotype has been found in places such as the Solomon Islands, Papua New Guinea, and Hawai‘i (Marshall et al. 2017). In general, *O. rhinoceros* are highly adaptive to new environments, and they are highly adaptable and able to resist environmental stressors (Han et al. 2024).

This CRB-G haplotype of *O. rhinoceros* is damaging due to its resilience against traditional control methods such as OrNV and it likely can have negative ecological and economic impacts if it were to spread to new areas (Reil et al. 2018). The island of O‘ahu has extensive development with ornamental palm trees that are susceptible to *O. rhinoceros* throughout residential and urban areas (Vargas et al. 2007). The ornamental plantings in communities provides ample habitat for populations of *O. rhinoceros* adults to feed on palms and oviposit in nearby areas, as these zones are characterized by coconut palm planting and accessible green waste. There are legitimate concerns that a widespread population of *O. rhinoceros* could endanger the native Loulu palms, *Pritchardia* spp. On Guam, there was severe mortality associated with unrestricted populations of *O. rhinoceros* destroying endemic palms (Marler et al. 2020). There are already signs that this is happening in remote areas of O‘ahu, as the *Pritchardia* spp. trees are susceptible to minor *O. rhinoceros* damage according to personal communications from a field conservation scientist (Kevin Armstrong, OARNP, pers. comm. October 2023).

The O‘ahu *O. rhinoceros* management program includes green waste management, palm tree surveys, chemical treatment, detector dogs, oviposition site surveys, and public outreach (Coconut Rhinoceros Beetle Response 2024). In the decade since its detection on O‘ahu, the geographic distribution of *O. rhinoceros* has been limited, and localized eradications have been achieved. However, in the last few years there has been an expansion of *O. rhinoceros* populations into agricultural and rural areas on the western and northern shores of the island. These populations are unlikely to be eradicated, and the integration of a biological control agent into the integrated pest management program is necessary to help manage populations. In 2023, *O. rhinoceros* populations were found on the islands of Maui, Hawai‘i island, and Kaua‘i for the first time, indicating that there are pathways facilitating the inter-island movement of this pest. This was likely through the import of green waste or ornamental plants, but cannot be confirmed.

As we face the spread of this pest in the Hawaiian islands, there are opportunities to test novel approaches for this pest, which could potentially be implemented across many Pacific Islands. Previous laboratory studies on O‘ahu evaluated the efficacy of locally collected entomopathogenic fungi (EPF) and entomopathogenic nematodes (EPN) as potential biological control agents of *O. rhinoceros* (Russo 2019, Manandhar et al. 2020). EPF can be applied to manage invasive insect species with applications in conservation, classical, and augmentative biological control programs, utilizing strains of *Beauveria* and *Metarhizium* species (Shah and Pell 2003). Entomopathogens are commonly utilized for insect control and are viewed as an environmentally safer option than insecticides. Their success with insect control has led to the research and development of over 170 mycoinsecticide products in a market that is growing annually (Maina et al. 2018). There are EPN and EPF strains that are unique to the Hawaiian islands that may have greater efficacy against pests that have yet to be exposed to these strains

(Myers et al. 2015, Bisel 2016). The new association approach to biological control has been proven to be effective, as invasive species are from areas where these entomopathogens are not present and they would be at an evolutionary disadvantage when exposed (Hokkanen and Pimentel 1989). It is possible that fungal conidia in Hawai‘i can penetrate the cuticle of CRB-G larvae or adults more readily, or that EPN’s have enhanced searching behavior that the larvae cannot circumvent. These new associations could mean that we already have locally available entomopathogens that can be potentially used for augmentative biological control.

Currently, there are no facilities on O‘ahu for EPF or EPN mass production, and regulatory processes can hamper the possibility of having these produced in different states and shipped to O‘ahu. In the studies reported in this chapter, I aimed to evaluate if locally collected EPF and EPN strains and a readily available commercial EPF product, BotaniGard ES (*Beauveria bassiana*, Bioworks, Victor NY) , would be efficacious against early instar *O. rhinoceros* larvae in both laboratory and field experiments. I hypothesized that the locally collected strains could yield similar or greater rates of mortality to the commercial products. On O‘ahu, *O. rhinoceros* larvae are most likely to be in mulch piles, compost, or soil products that may not have any natural entomopathogens or have some at titers too low to inflict mortality. It is likely that larvae in field populations may not be in areas where endemic or naturalized entomopathogens are present, as these are managed areas such as agricultural operations or residential landscaping. I wanted to determine if the local entomopathogens could be effective against early larval instars in laboratory and small-scale field trials.

I faced obstacles manufacturing our locally collected strains with a company not in Hawai‘i, as I had initially expected to have a collaboration with industry that mass produced local EPF strains. Without logistical and resource limitations, I would have liked to include mass

production of local EPF strains as both a spray and resting spores on a granular substrate, to compare to the spray commercial product. I also would have wanted to include other granular *Metarhizium* products that are not yet labeled in Hawai‘i. I was also limited by the importation of commercial EPN strains that would have added to the repertoire of treatments for larval control, as these may be more effective than the locally collected ones. The following trials in this Chapter evaluated ways to manage the CRB-G haplotype on O‘ahu with a focus on entomopathogen as a soil amendment, using the available resources to conduct this research.

Materials and Methods

EPF laboratory trial

Oryctes rhinoceros adults were collected in the field by CRB Response personnel and then bred in captivity at the University of Hawai‘i’s Arthropod Containment Laboratory (UH-ACL). These adults were kept in a colony, where all life stages were present for experimentation. Laboratory-reared larvae were transported to the Hawai‘i Department of Agriculture Plant Quarantine (HDOA-PQ) facility for the entomopathogenic fungi trials. This reduced any potential contamination of the laboratory colony with experimental fungal strains. The trials had four replicates of four first instar larvae each, per treatment (N=16), with each larva placed in an individual two ounce cup filled with a substrate. Each replicate corresponded with a different shelf in the incubator. The substrate was mulch (from campus landscaping) that was passed through a small particle screen and then autoclaved. Tap water was added to achieve desirable moisture levels, where the material was not visibly dry but also not oversaturated. BotaniGard^(R) ES was evaluated to determine the efficacy of a commercial *B. bassiana* pathovar, compared to a locally collected *Metarhizium anisopliae* (Clavicipitaceae) strains previously evaluated (Russo

2019) that were plated on Potato Dextrose Agar (PDA) plates. These strains were screened from multiple areas, with the Lyon Arboretum (LA) and Koko Crater Arboretum (KO) strains yielding the highest mortality rates among first instar *O. rhinoceros* larvae.

During trials at HDOA-PQ, I utilized BotaniGard ES and applied treatments according to the rate for the plant dipping section on the label. The treatments were applied at a rate of 2.1×10^{10} spores per ml and 5.28×10^9 spores per ml for high and low concentrations, respectively and in line with the suggested label rates. The locally collected *Metarhizium* strains were scraped off colonies in PDA plates, and the spore concentration was quantified using a hemocytometer, with acceptable dilutions having spore concentrations greater than 1×10^7 per ml in a 0.1% Tween 80 suspension solution. EPF strains have maximum efficacy at different concentrations, hence the different treatments (Saif et al. 2024, Cherry et al. 2005). One milliliter of water or 0.1% Tween 80 treatment was directly applied to each larva. An untreated control treatment was included, which had soil with 1 ml of water applied. All the larval cups were placed in an incubator at 25°C for the 14 day trial. There was no photoperiod interval in the incubator, as it did not have internal lighting.

EPF field trial

Oryctes rhinoceros specimens were collected from infested mulch piles at Kunia Agricultural Park in March 2022. Forty-eight first instar larvae were collected and utilized for the Kunia field trial. In the first trial I had to utilize field-caught *O. rhinoceros* larvae due to eradication regulations, but this changed by the second trial where the infestation on O‘ahu was no longer deemed eradicable. The second trial utilized 48 UH-ACL reared first instar larvae that were evaluated at a golf course in a *O. rhinoceros* established area. Each treatment had sixteen first instar *O. rhinoceros* larvae (N=16), each housed in individual mason jars (individual 0.907

kg wide mouth Ball[®]). This prevents cannibalism between larvae, which can occur in a confined environment. The treatments consisted of a tap water control, and BotaniGard ES high and low treatments. I did not have the facilities to set up production of local *Metarhizium* spp. strains for this trial, therefore I only tested the commercial product. The mortality of these local strains had decreased in efficacy from previous trials. The commercial BotaniGard ES doses were based on the label rates for soil applications in orchards for broad spectrum insect control. The maximum label rate was followed for the high treatment (1.89l/464m²), and the low treatment was four times less than this amount, and the minimum label requirement for a hand sprayer (0.47l/464m²).

Mulch from each field site was then sprayed with water, BotaniGard ES low, and BotaniGard ES high solutions on trays until saturated. Each mason jar was labeled and filled with its respective treatment, and then one first instar larva was placed inside. Larvae would burrow to the bottom of each jar, allowing exposure to the treatment. Each jar had holes drilled in the lid with plastic trays above and below each box of 12 jars to allow airflow but prevent drowning of treatments in the event of rain. The boxes of mason jars were then buried approximately one meter into the mulch pile to avoid overheating and simulate the approximate depth of larval developmental sites. Mortality observations were conducted up to three times at 7, 14, and 21 days after treatment. Each observation was conducted by gently pouring the contents of the mason jar onto a plastic tray in search of larvae. In some cases, only head capsules of larvae that died during the study were found.

EPN laboratory trial

Oryctes rhinoceros specimens were tested using the same protocol as stated above in the EPF laboratory trials. However, these trials were conducted within the UH-ACL as there was

low risk of contamination to the colony. Entomopathogenic nematode (EPN) strains *Steinernema feltia* MG 14 and *Heterorhabditus indica* OM 160 were collected on O‘ahu. These strains were maintained on mealworm, *Tenebrio molitor*, and wax moth, *Galleria mellonella*, larvae that were inoculated by Dr. Roxana Meyers, USDA-ARS. The host cadavers were placed on white traps and kept in a dark cabinet at room temperature. Upon emergence of entomopathogenic nematodes, a high and low concentration of these strains were quantified using a counting tray and applied to *O. rhinoceros* larvae.

Single first instar *O. rhinoceros* larvae were placed into individual cups and stored in wooden trays in the UH-ACL. The EPN strains were directly applied to the larvae, and the cup was subsequently filled with mulch substrate. The two nematode treatments were tested at rates determined after white trap emergence. The white trap emergence ratios varied by sample and the following rates were chosen to avoid potentially harming infective juveniles (IJs). If there was success with either strain, follow up trials would have been conducted to determine the efficacies of IJs across a range of concentrations. The concentrations that emerged from white traps for *H. indica* were 544 and 772 IJs at the low and high concentrations for each treatment. *Steinernema feltiae* treatments were applied at rates of 312 and 1112 IJ. These rates were determined from white trap emergence using a counting tray, and the same for both trials. I did not try to modify these rates, as I was concerned that I would cause IJ mortality in the handling process, which would result in an inaccurate assessment of the application rate.

Mortality assessments for each larva were made at three and seven days post treatment, using forceps to sift carefully through the cup, and any dead larvae were placed on white traps. Due to the deteriorated condition of larval cadavers at three days post treatment, I was unsuccessful with white trap isolation of cadavers.

Statistical analysis

The statistical analysis was conducted running a Kruskal Wallis test (JMP Pro 17, SAS 2001-2024). The analysis was conducted to assess the difference between treatments in relation to larval mortality. The response variable in each of the EPF and EPN laboratory trials was the percent mortality of larvae in individual cups, while the response variable in the EPF field trial was the mortality of each beetle in the individual mason jars. If a statistical significance was detected, a Tukey Kramer HSD mean separation test was conducted to determine which treatments were statistically different. The graphs were generated using JMP Software.

Results

EPF laboratory trial

In the commercial product evaluation, the high label rate of BotaniGard ES caused significantly higher mortality compared to the other treatments at day 3 (Figure 1a, $\chi^2 = 10.38$, $P = 0.016$, $DF=3$), and day 8 after treatment (Figure 1b, $\chi^2 = 10.43$, $P = 0.015$, $DF=3$). The second trial evaluated locally collected *Metarhizium* spp. strains as well as the same rates tested in the first BotaniGard ES trial. This trial indicated that there was greater mortality with the high rate of BotaniGard ES compared to the other treatments as early as 3 days after treatment (Figure 2a, $\chi^2 = 19.69$, $P = 0.020$, $DF=3$). The results after seven days showed that BotaniGard ES at a high concentration was more effective than the locally collected strains (Figure 2b, $\chi^2 = 17.75$, $P = 0.038$, $DF = 3$).

EPF field trial

Due to facility restrictions, the only available strain for field testing was the commercially available BotaniGard ES. In the field trials, the high rate of BotaniGard ES caused increased mortality compared to other treatments. In the Kunia field trial with wild-caught specimens, this difference was evident at 14 and 21 days after treatment (Figure 3a, $\chi^2=7.31$, $P= 0.026$, and Figure 3b, $\chi^2=8.06$, $P= 0.018$, $DF=3$). At 21 days after treatment, there was nearly 70% mortality at the higher label rate treatment of BotaniGard ES. The second trial at Ted Makalena Golf Course utilized laboratory reared specimens with greater than 70% mortality one week after treatment (Figure 4a, $\chi^2 =5.379$, $P= 0.068$, $DF=3$). There was no statistical significance at two weeks after treatment due to high control mortality (Figure 4b).

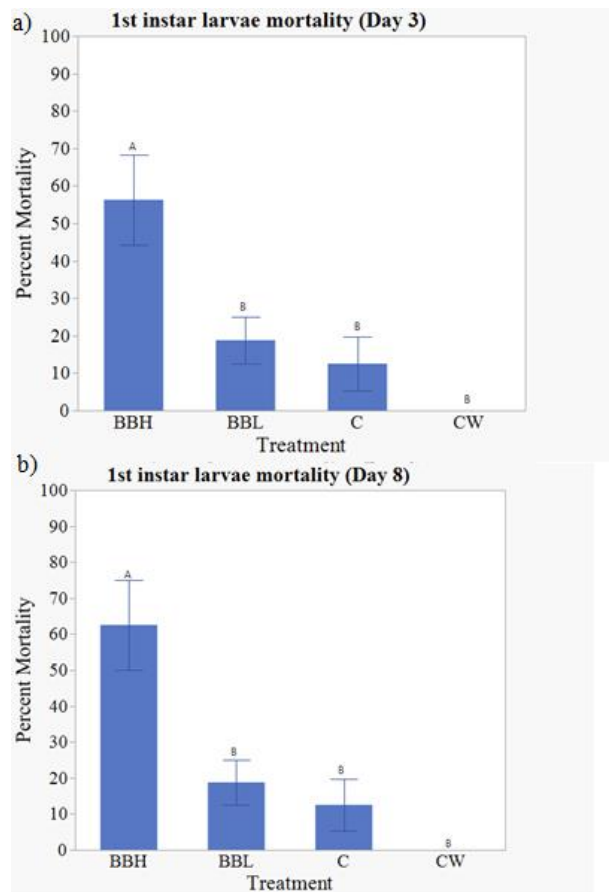


Figure 1: Mortality of 1st instar *Oryctes rhinoceros* larvae following laboratory applications of BotaniGard^(R) ES three (a) and eight (b) days after treatment, mean mortality per treatment (\pm SEM, n=4); columns with same letters were not statistically different, Tukey HSD, $P > 0.050$. BBH and BBL indicate low and high doses of BotaniGard ES, while C indicates untreated control (dry mulch), and CW untreated control plus water.

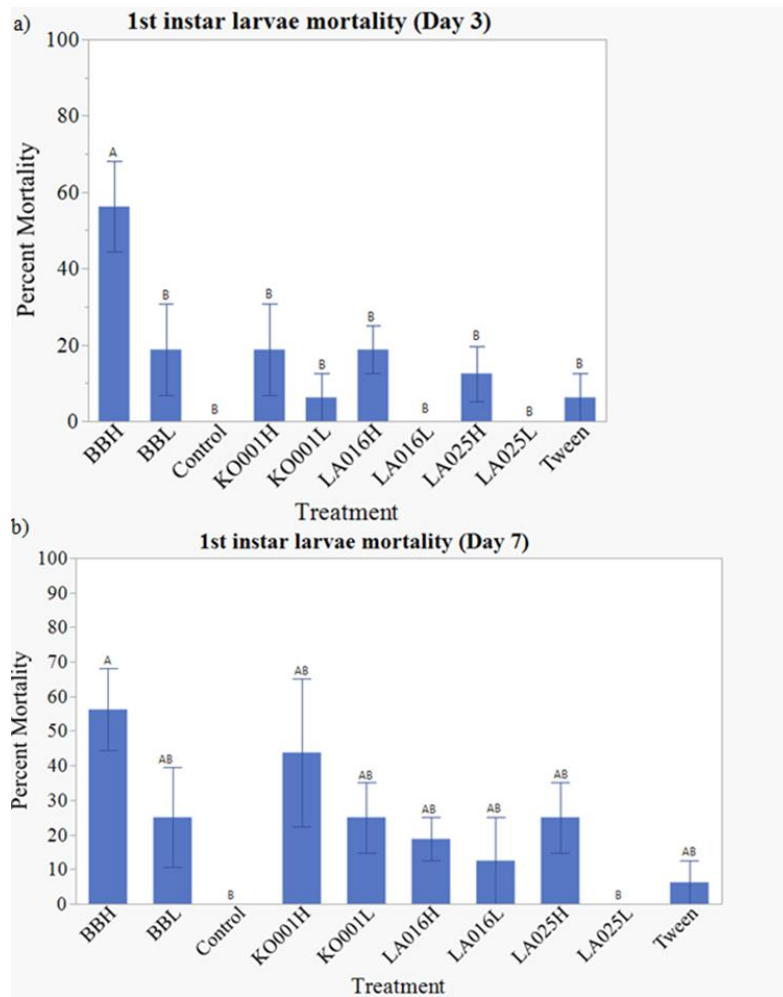


Figure 2: Mortality of 1st instar *Oryctes rhinoceros* larvae following laboratory applications of BotaniGard^(R) ES three (a) and seven (b) days after treatment, mean mortality per treatment (\pm SEM, n=4); columns with same letters were not statistically different, Tukey HSD, $P > 0.050$. BBH and BBL indicate low and high doses of BotaniGard ES, Control (dry mulch), KO

indicates Koko Crater Botanical Garden, while LA indicates Lyon Arboretum EPF strains.

Tween indicates the suspension solution without the spores.

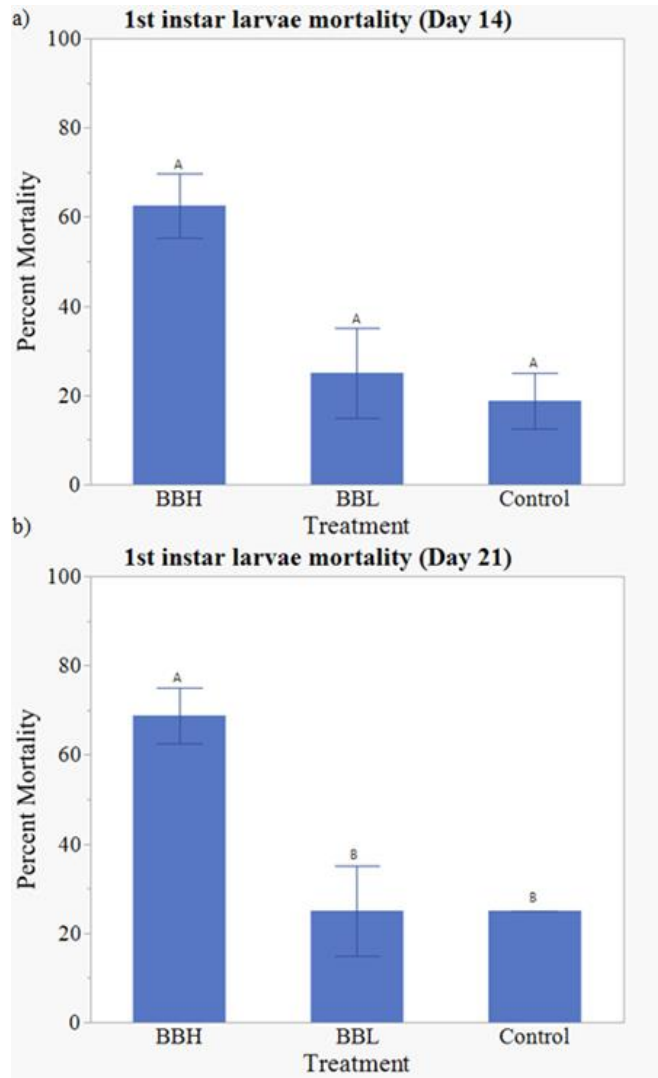


Figure 3: Field trial evaluating BotaniGard ES treated material for first instar *Oryctes rhinoceros* larval mortality showing mean mortality (\pm SEM, n=4) 14 (a) and 21(b) days after treatment; columns with same letters were not statistically different, Tukey HSD, $P > 0.050$. BBH and BBL indicate low and high doses of BotaniGard ES, while Control indicates a water spray.

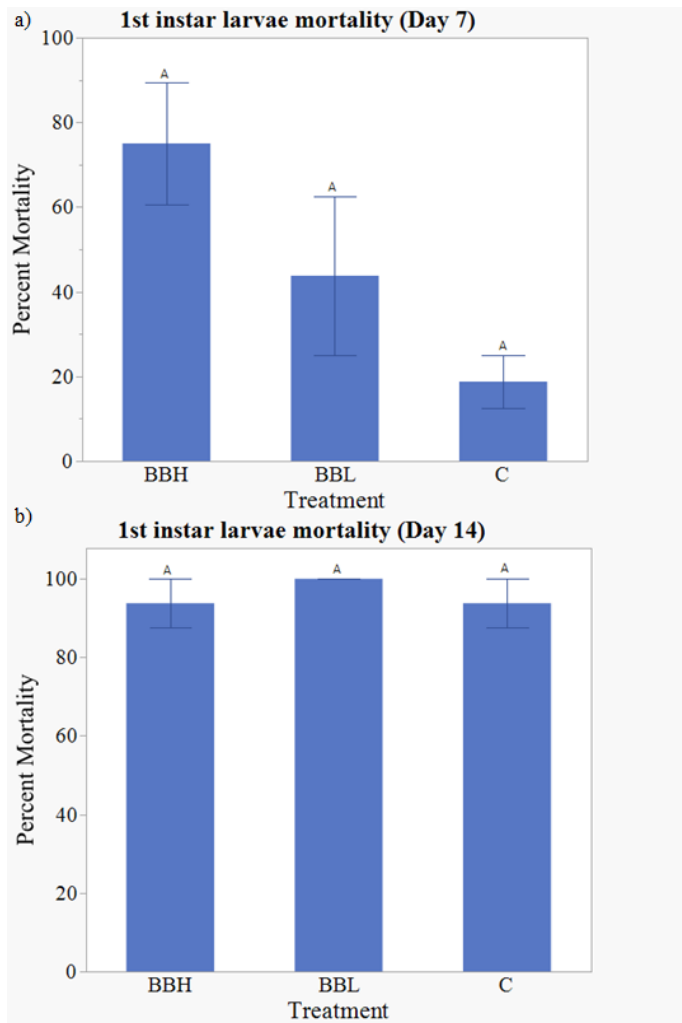


Figure 4: Field trial evaluating BotaniGard ES treated material for 1st instar *Oryctes rhinoceros* larval mortality showing mean mortality (\pm SEM, n=4) 7 (a) and 14(b) days after treatment; columns with same letters were not statistically different, Tukey HSD, $P > 0.050$. BBH and BBL indicate low and high doses of BotaniGard ES, while C indicates untreated control.

EPN laboratory trial

The entomopathogenic nematode trials did not yield any statistically significant differences between treatments. In the first trial, the low concentration of *S. feltiae* resulted in

greater than 60% mortality of 1st instar larvae (Figure 5). A follow up trial evaluating this strain failed to yield greater than 20% mortality of first instar larvae.

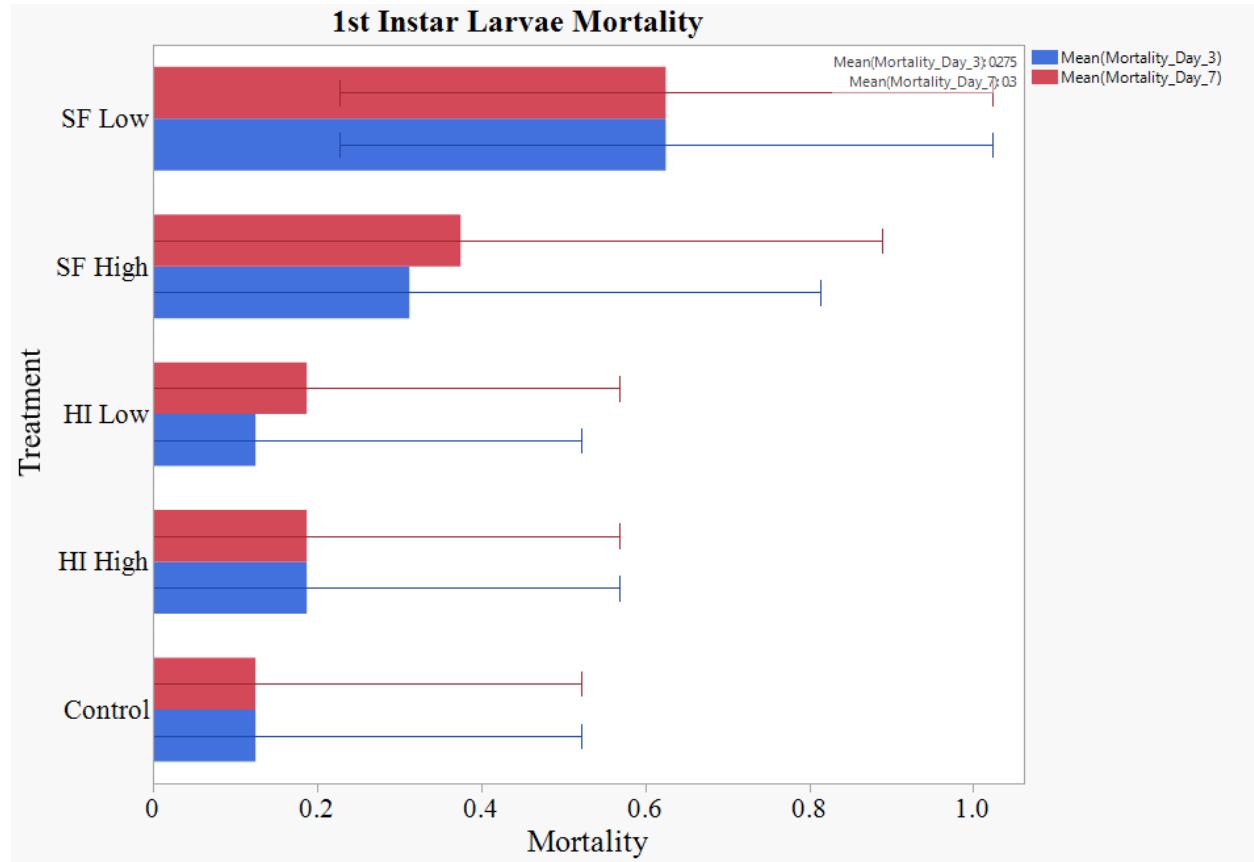


Figure 5: Results of entomopathogenic nematode isolate screening on *Oryctes rhinoceros* larvae after treatment showing mean (\pm 95% CI's, n=4) mortality per trial. SF represents *Steinernema feltiae*, while HI represents *Heterorhabditis indica*.

Discussion

The advent of the nudivirus resistant CRB-G biotype has led to increased management challenges to control populations of *O. rhinoceros* in its expanded range (Marshall et al. 2017). The *O. rhinoceros* population on Guam has destroyed 70 percent of the coconut palms on the

island and threatens its remaining endemic palms (Marshall et al. 2017). The severe impacts of unregulated *O. rhinoceros* populations is a cause for concern, as globalization likely will facilitate a pathway for *O. rhinoceros* to reach the continental United States, or other parts of the world, where there are palms of ecological and economic importance. There would likely be severe damage to date palm production in California and to the numerous native palms in Florida with uncontrolled infestations. Within the Hawaiian islands, the uncontrolled spread of *O. rhinoceros* could result in the extinction of endemic palms.

A recent review of the changing dynamic of global *O. rhinoceros* infestations indicated that entomopathogens have the potential to be an effective tool to manage populations as part of an integrated pest management program (Paudel et al. 2021). In Malaysia, there have been many successful attempts applying powder, granular, and kernel cake formulations of *Metarhizium* spp. for *O. rhinoceros* larval control (Moslim et al. 2007, 2009, Moslim and Kamarudin 2014). Entomopathogenic fungi can be used to reduce larval numbers in vermicomposting sites or as a larval control method beneath cover crops in agricultural areas (Gopal et al. 2006, Moslim et al. 2007). Some limiting factors of successful entomopathogen applications are temperature range, humidity, rainfall, UV exposure, and shelf life. These cases of entomopathogenic management of *O. rhinoceros* are examples where locally collected fungal strains effectively mitigated population growth of the beetle. On O‘ahu, *O. rhinoceros* frequently oviposits in mulch piles, which may lack natural entomopathogens in quantities that could be effective for population reduction. These piles are frequently disturbed and initially are too dry for entomopathogenic fungal growth. A granular treatment utilizing resting spores could potentially be implemented to manage population growth, if a product were labeled for use in Hawai‘i.

Although granular applications of EPF may be an effective way to mitigate populations of *O. rhinoceros*, entomopathogenic fungi do not have the effectiveness that comes with the prey searching behavior of entomopathogenic nematodes. Entomopathogenic nematodes may be effective in integrated pest management programs under specific conditions and can be utilized to reduce pest populations to below economic injury level thresholds (Grewal et al. 2012, Stevens and Lewis 2017). There have been reports of locally collected entomopathogenic nematodes causing mortality on first instar larvae in laboratory trials on *O. rhinoceros* (Sujithra et al. 2022). There was significant field efficacy in a trial testing locally collected *Rhabditis blumi* on *Oryctes agamemnon* (Scarabaeidae), as the larvae of these beetles are found in damp soil near the roots of date palm trees and can be effectively targeted without desiccation of the entomopathogenic nematodes (Tariq 2020). Unfortunately, the larvae of *O. rhinoceros* are found in a variety of breeding sites that may be difficult to target with a spray treatment of entomopathogens.

Despite using different strains the results from the present trials were similar to those of previous studies, in which locally collected entomopathogenic nematode strains were not effective for first instar *O. rhinoceros* control (Manandhar et al. 2020, Russo 2019). The first trial I conducted had a small sample size and high variance, in which *S. feltiae* yielded 60% mortality at the low rate with no statistical significance compared to the control. A follow up trial was conducted that had less than 20% mortality with treatments of *S. feltiae*. There was higher control mortality in the first trial, which may explain the difference in mortality between these trials. If there was any efficacy with the strains tested in our trials on first instar larvae, second and third instar larvae would have been evaluated next. Unfortunately, *O. rhinoceros* larvae can

develop in areas of green waste that may lack natural entomopathogens or are found in the crown detritus of palm trees.

Furthermore, the mulch in which *O. rhinoceros* is typically found on O‘ahu could be considered conventional mulch, which is much more hostile to nematode populations than organic mulch due to its nutritional components (Blanco-Perez et al. 2022). If virulent entomopathogenic nematode strains were to be identified, studies should be conducted with different mulch types to see which would yield the highest mortality. There is existing literature that highlights ways to test different mulch types in conjunction with nematode strains for integrated pest management studies (Duncan et al. 2007, Lacey et al. 2006, Sweeney et al. 1998). If I were to find effective strains of EPN’s in Hawai‘i, I could then test ways to increase their efficacy in different substrates for long term management of *O. rhinoceros* populations. This could prove pivotal for agricultural operations or homeowners to decrease localized populations.

The laboratory and field trials indicated that there is potential to integrate a commercially available entomopathogenic fungal product into the O‘ahu management program for *O. rhinoceros*, but there still should be work on maximizing field application of locally collected strains. The laboratory trial results testing the commercial strain and local strains indicated that mortality occurs within a few days with BotaniGard ES, while the local *Metarhizium* spp. strains take up to 10 days to kill the host insect. The mortality with the BotaniGard ES *B. bassiana* product was evident in field trials.. In these trials, the first instar larvae exposed to mulch treated with the high concentration of BotaniGard ES were lethargic and some of these first instar larvae were found to have died at the next observation point, within seven days. In a pilot study not reported here, the high rate of BotaniGard ES had no effect on *O. rhinoceros* third instar larvae, or an individual field caught adult. This might suggest that treatment of potential breeding

material could impact early larval growth, but likely is not effective on later stages. If there was success on later instars, their cadavers would be able to spread entomopathogens to susceptible larvae, potentially reducing the need for repeated treatments in an augmentative biological control program.

There is no available infrastructure to mass produce the locally collected *Metarhizium* spp. strains on O‘ahu for field testing. If there were facilities to grow *Metarhizium* spp. on rice, sorghum, or another substrate, this may have been more effective on later instars, as resting spores can remain present in potential *O. rhinoceros* breeding sites. These populations are typically found in large mulch piles that could be supplemented with a substrate-based application such as resting spores on rice or sorghum. The results of the field trials indicate that a readily available commercial product could have potential to be implemented into the management program on O‘ahu. However, the practicality of integrating this into a management program is low as the spray cannot penetrate deep mulch piles, the fungal spores are susceptible to environmental conditions, and the spores are only efficacious on early instars. However, this approach could be utilized in agricultural areas such as pineapple fields that may be threatened by the growing *O. rhinoceros* population. Bedford (1980) lists pineapple as a secondary host, but there has not yet been any indication of damage to O‘ahu’s pineapple crop. BotaniGard ES could also be applied to areas with shallow mulch piles. Further studies could explore the rate of depletion of fungal spores in mulch piles and the rate of penetration of topical sprays on larval mortality.

Future studies should evaluate commercial *Metarhizium* spp. products that can be labeled for use in Hawai‘i. The implementation of autodissemination entomopathogenic fungi traps have been successful for *O. rhinoceros* control in Malaysia, and a similar trap could be utilized to

spread spores to areas with developing larvae (Moslim et al. 2011). The EPN trials utilized readily available strains that unfortunately were not effective. It is possible that there are, with yet undiscovered potentially effective, new associations with strains of entomopathogens that can mitigate population growth of *O. rhinoceros*. A way to test this would be to utilize *O. rhinoceros* larvae in the soil screening process, as entomopathogens that immediately cause mortality on these larvae are likely to be able to yield laboratory and field mortality. Screening on wax moths may be an effective way to isolate more strains, but these strains may not be evolved to inflict mortality on coleopteran species. Another tactic could utilize existing entomopathogenic strains and alter them with genetic engineering. Specifically with entomopathogenic fungi, it is possible to produce strains with greater ecological fitness and virulence (Zhao et al. 2016).

With the possible establishment of *O. rhinoceros* in Mexico according to iNaturalist observations, there will be a growing interest in strategies to eradicate or mitigate the damage from populations of this pest (Jackson et al. 2022). Entomopathogenic fungi could serve as a critical tool to manage this pest, and the utilization of readily available commercial products labeled for this pest could help manage populations to a threshold with minimal ecological and economic impacts. This could be integrated with a future biological control agent, such as another nudivirus that can be utilized for population control. Populations of *O. rhinoceros* with consistent nudivirus exposure have been shown to maintain a high level of host immune response in specific localities (Etebari et al. 2021). The population of *O. rhinoceros* in the Hawaiian islands has been geographically separate from virus exposed populations for over ten years. It is possible that this resistance has dropped and that the reintroduction of the virus could be able to manage populations. Further research is integral, as the future of many palm species across the

Pacific may rely on the ingenuity to develop methods to bring *O. rhinoceros* populations back into levels that can coexist with their island ecosystems.

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CHAPTER 3: LABORATORY AND LARGE-SCALE FIELD TRIALS EVALUATING THE EFFICACY OF SYSTEMIC INSECTICIDES AS A TOOL TO MITIGATE COCONUT PALM DAMAGE FROM *ORYCTES RHINOCEROS* ADULTS

Introduction

As previously noted, the Hawaiian islands have experienced many waves of extinction impacting endemic species of all taxa, due to the introduction of invasive species or anthropogenic activities (Regnier et al. 2015, Gagne 1988). The pace of extinction has increased with globalization, but its history dates as far back as the arrival of the first Polynesian voyagers, followed by the first wave of European colonization (Boyer 2008, Olson and James 1982). There are pockets of endemism remaining in the Hawaiian islands, and they are at risk from various factors such as development, climate change, and invasive pest introductions. Invasive species have the capacity to alter island ecosystems and threaten the survival of endemic species (Russel et al. 2017, Reaser et al. 2007). Especially in isolated ecosystems, invasive species can thrive and extirpate native species not adapted to these threats.

The coconut rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae: Dynastinae), is native to parts of SE Asia and is a highly damaging pest of coconut palms that has only been eradicated once throughout its long invasion history (Bedford 1980). The adults feed directly on the crown of palms, causing characteristic V-cuts in the leaves, and bore holes where they penetrate the developing apical meristem, while the larvae are typically found in nearby mulch or organic material. *Oryctes rhinoceros* was previously managed by the introduction of classical biological control by an entomopathogenic virus, *Oryctes rhinoceros nudirus* (OrNV, *Alphanudivirus oryrhinocerotis*). In its native range there likely could be

multiple factors suppressing populations such as parasitic wasps, generalist predators, and OrNV. Populations of *O. rhinoceros* spread across the Pacific during the second World War and were causing significant damage to palm species (Bedford 1980). The introduction of OrNV was considered one of the first instances of successful classical biological control through the utilization of a virus and this was hailed as a success for 50 years. This changed when OrNV resistant beetles were found on Guam known as the CRB-G haplotype. Palms on islands with this haplotype incur severe damage and high population growth of *O. rhinoceros* (Marshall et al. 2017). The OrNV resistant CRB-G haplotype highlights the challenges associated with biological control of an invasive insect through the introduction of a virus, as susceptibility can change over time. The reliance on this haplotype has resulted in a delay in readily available solutions to manage populations. On Palau, *O. rhinoceros* was considered to be responsible for the destruction of half of their palm trees and entire loss of palms on smaller islands (Gressitt 1953). On Guam, CRB-G is the evident cause for high palm mortality rates throughout the island. This new biotype has now spread to new parts of the Pacific such as the Solomon Islands and Hawai'i (Marshall et al. 2017). The impact of these population expansions will likely be quantifiable in the next decade, but the probable scenario is the destruction of endemic and introduced palm species. There may be natural enemies in these areas that may mitigate a damaging population explosion, but so far there have not been any reports of this.

The detection of *O. rhinoceros* on the island of O'ahu in 2013 triggered an extensive multiagency eradication program that has since utilized mass trapping for surveillance, detector dogs, insecticidal treatments, green waste sanitation, palm and breeding site surveys, and public outreach (Rysin et al. 2018, Adams 2019). The impacts of an uncontrolled population could negatively impact native Hawaiian Lulu palms, *Pritchardia* spp., along with the extensive

plantings of coconut palms throughout O‘ahu’s rural, residential, and tourism areas. In 2023, beetles were detected on the islands of Kaua‘i, Hawai‘i island, and Maui. There are multiple populations of *O. rhinoceros* on Kaua‘i, repeated detections in Waikoloa, and limited information as to the extent of the introductions to Maui. The pathway of entry is not certain, but likely was from infested potting material or ornamental plants.

Widespread urban planting of urban trees can facilitate the spread of invasive species, and the extensive planting of coconut palms possibly created a pathway for *O. rhinoceros* to spread throughout O‘ahu (Branco et al. 2019). Trees in residential areas could be utilized as a management strategy for containing or eradicating populations, as invasive species eradication is historically easier to accomplish in man-made areas (Pluess et al. 2012). These managed ecosystems can be utilized to target adult feeding sites, larval development areas, and to mitigate the spread of *O. rhinoceros* to agricultural or rural areas. Systemic insecticides have been shown to be effective in Hawai‘i for mitigating the impacts of invasive pests, while decreasing the negative effects of widespread spraying (Bhandari and Cheng 2016, Bhandari and Cheng 2017, Docola et al. 2009, Norris 1967, Russo et al. 2023). Neonicotinoid insecticides affect the nervous system of insects, with imidacloprid and dinotefuran as some of the leading treatments for crop protection (Byrne et al. 2012, Casida 2018). Some of the benefits of neonicotinoid application are the reduced use of broad application pesticides, resulting in a treatment that has a lower environmental impact, can increase crop yield, has targeted and systemic action, with target plant versatility and cost-effectiveness. Another effective pesticide used in landscape ecosystems is acephate, which is an organophosphate that targets the acetylcholinesterase enzyme (AChE), or is a AChE inhibitor (Raj and Krishan 2023). Abamectin also has been utilized in the urban landscape and is a part of the avermectin family that impacts nerves and can

be applied systemically (Jansson and Dybas 1998). These compounds can be applied through the use of pressurized trunk injection systems to minimize the negative health and environmental risks associated with frequent spraying of insecticides as the product is physically restricted to the application site.

With the benefits of low-risk systemic insecticides for managing invasive species, this study sought to evaluate the efficacy of various insecticides on *O. rhinoceros* adults in laboratory trials, expanding on previous trials (Kellar 2018). Effective insecticides were then evaluated in large-scale field trials to determine if systemic insecticide treatments could be curative, indicating that v-cut damaged palms recover over time, or preventative for *O. rhinoceros* infestation and feeding damage. Fronds removed from these trees were evaluated in laboratory feeding trials to determine the efficacy of pesticide residue over time. Trap catch data for adult *O. rhinoceros* were also collected to try to determine the efficacy of imidacloprid areawide treatments on O'ahu. Our objective was to determine if readily available systemic insecticides with trunk injection application methods could be utilized to reduce populations of *O. rhinoceros*.

Material and methods

Laboratory chemical feeding trials

Imidacloprid, dinotefuran, and abamectin were tested at 25 and 250 parts per million (ppm) concentrations in beetle gel. The gel was prepared by combining Vanilla Whey (Cytosport® Brand), deionized water, sugar, and agar (Fisher BioReagents™). Imidacloprid (IMA-jet, Arborjet, Woburn, MA), dinotefuran (Dino-cide, Mauget, Arcadia, CA), and

abamectin (Aba-cide, Mauget) were the specific chemicals utilized for each treatment. The solutions were poured into ice cube trays and placed into a walk-in-refrigerator to solidify at 2°C following the protocol in Kellar 2018. The chemical trials were conducted at the University of Hawai'i Arthropod Containment Laboratory (UH-ACL) between June 2019 and November 2019. *Oryctes rhinoceros* adults caught by panel traps from around O'ahu were propagated in the UH-ACL. This facility maintains a laboratory reared *O. rhinoceros* colony that is regularly bred with field caught specimens to ensure there is limited genetic variation from the field population. Eggs of field caught beetles were reared for this study, and the emerging adults were marked with individual numbers for Hawai'i Department of Agriculture collection purposes. Adult feeding on a banana diet was monitored prior to the trial to ensure that the beetles would feed on the treatments. The *O. rhinoceros* adults used in this trial emerged from the pupal stage between July and September 2019. Due to uneven sex ratios, there were not enough beetles to have an equally split ratio of male to female beetles. The adults were placed individually in 950-ml wide mouth Ball® mason jars with holes drilled in the lid, and were not fed for three days in an incubator set at 30°C. After three days, the jars were removed from the incubator, and any dead adults were replaced. The replacement beetles were also not fed for three days.

There were seven treatments using the refrigerated gel: no additive (negative control), plus imidacloprid (positive control), dinotefuran, and abamectin each formulated at either 25 ppm or 250 ppm a.i.(active ingredient). Imidacloprid was used as a positive control because it and acephate were effective in previous trials (Kellar 2018). Each treatment had four replicates of five beetles. These replicates were placed in different locations within the incubator in the UH-ACL. The beetles were observed after the gel was placed into the jars at one hour, two, three, four, 24, 48, 72, 96, and 168 hours (7 days). The beetles were checked for signs of

paralysis or mortality at each of these intervals, which was categorized collectively as negative effects. The paralysis observation should indicate that the beetle would not be viable to survive in the field, as there can be minor effects to treatments that can be considered as a partial paralysis. Paralysis was assessed by two observers using clearly defined parameters. These include signs of paralysis such as twitching, minor immobility, and severe immobility. A beetle drop from a height of 7.7 cm was done to assess any movement or reaction, assisting in the paralysis observations. There were not any instances in which the observers disagreed if a specimen was paralyzed or was agile.

Field trials

Two concurrent field studies were set up to assess the impact of laboratory tested systemic insecticides on coconut palms at Ted Makalena Golf Course in Waipahu in December 2019 and concluded in November 2021. This area had low to severe damage associated with *O. rhinoceros* feeding on some portions of the golf course, which is on a peninsula. The rural and residential areas adjacent to it have very few palm trees, and the few trees nearby did not exhibit any signs of *O. rhinoceros* damage during a December 2019 evaluation. It is likely that the population of *O. rhinoceros* on the golf course can be viewed as somewhat isolated from the rest of the population on O‘ahu during the time frame the trial took place.

All 120 coconut palms on the golf course were tagged and visually evaluated by three surveyors for existing *O. rhinoceros* adult damage. Each surveyor looked for bore holes, or characteristic V-cuts associated with adult feeding, and damage was evaluated for the top inner four fronds along with the entire palm tree fronds. The results of these independent observations were averaged into a 0-5 damage score to assign the 70 trees with *O. rhinoceros* damage into respective treatments. No damage was indicated by a 0, 1 indicated 1-29% of the fronds

exhibited some form of damage, 2 indicated 30-49%, 3 indicated 50-74%, 4 reflected palms with greater than 75% of frond damage, while a 5 rating indicated the palm was dead due to *O. rhinoceros* feeding. Overall palm damage ratings were grouped by similar damage levels and then assigned to the five treatments.

These studies aimed to determine if pesticide application by chemical injection may prevent further beetle damage, and allow the palm to recover. Each study utilized 35 palms, one with and one without visible *O. rhinoceros* feeding damage (Figure 6). The undamaged palms potentially could be harboring adult beetles, but during the initial observation window there was no indication of feeding. Palms in each grouping were assigned to one of five treatments (n = 7 per treatment): negative control, acephate (ACE-jet, Arborjet), imidacloprid (IMA-jet, Arborjet), dinotefuran (Dino-cide, Mauget), and abamectin (Abacide, Mauget) at the label rates. Palm trunks were injected using the QUIK-jet AIR Tree Injection System (Arborjet, Woburn, MA). The diameter at breast height or DBH of each individual tree was measured to calculate a specific dose for each palm according to the label rates of application. In the months after application, two observers recorded monthly assessments of the visual frond damage observing the central four fronds and the total count of damaged fronds. The independent observations were averaged.

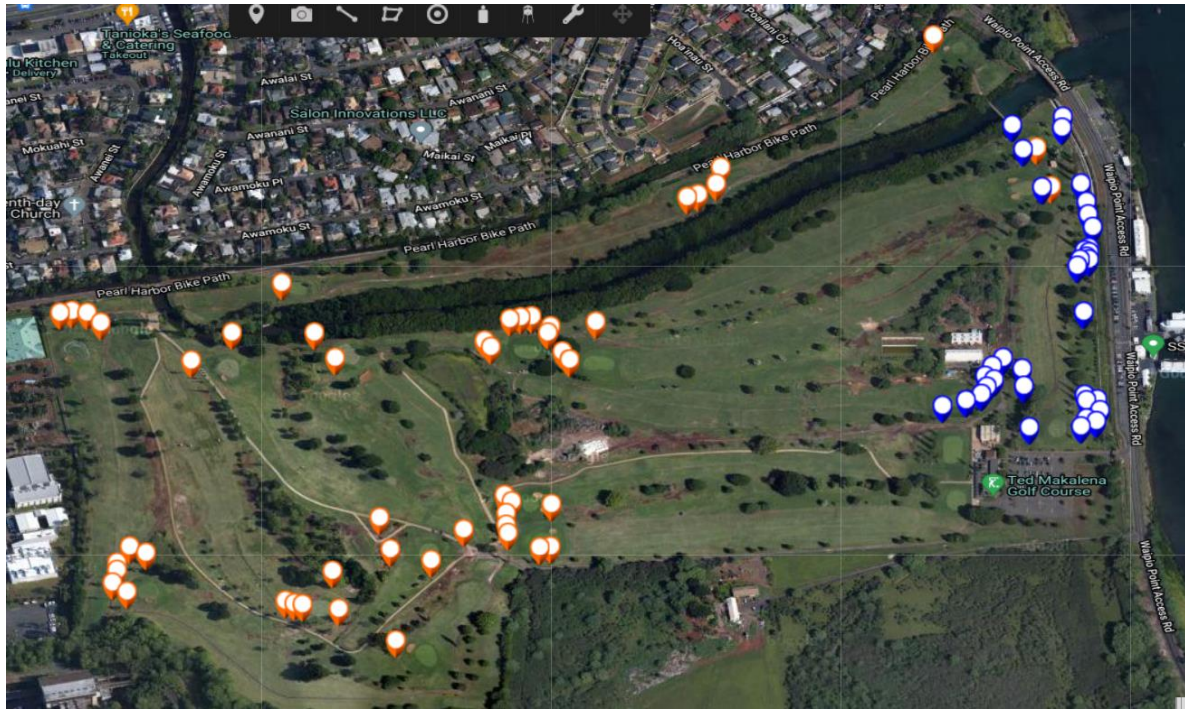


Figure 6: Geographic location of treatments in curative and preventative trials at Ted Makalena Golf Course. Previously damaged palms by *O. rhinoceros* at the beginning of the trial are marked in orange, while the preventative trial has blue markers.

The first injections took place in December 2019, with chemicals reapplied in June 2020. Prior to reapplication of injections in 2020, another visual damage survey was conducted, with the intention to increase the sample size for the curative study. Fifteen additional palms with a similar visual infestation rating were randomly assigned to the five treatments, increasing the replicates for the curative trial from seven to ten palms per treatment. The data collection for this concluded in November 2021. The curative and preventative trials ended as Ted Makalena Golf Course wanted to inject every palm to mitigate the growing damage from their *O. rhinoceros* infestations. I utilized this opportunity to start what was called an areawide field trial in December 2021, where every single coconut palm was treated with the label rate of imidacloprid. This study grouped the palms into clusters based on location and followed the same visual data

collection parameters as the curative and preventative trials (Figure 7). The reasoning for the clusters was to see if there would be individual areas that would improve in their aesthetic appearance during the length of the trial. The observations concluded in August 2022, after eight months of observing the treated palms for any recovery in feeding damage.

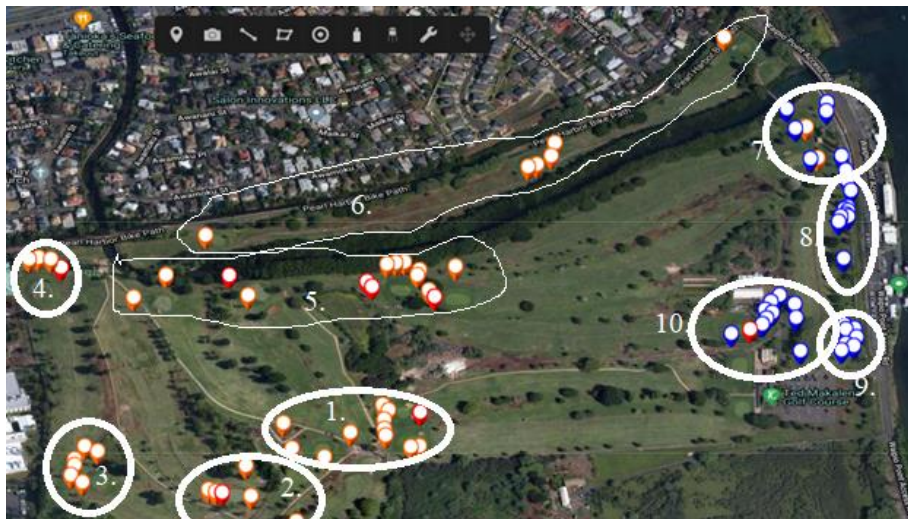


Figure 7: Clusters of similar damage ratios utilized for areawide imidacloprid treatment study. The blue dots indicate the preventative trial cluster, the orange indicates the curative trial, while red dots indicate trees killed by *O. rhinoceros*. I chose 10 distinct geographic clusters to see if there would be improvements in visual palm aesthetic related to feeding damage over time.

Laboratory frond trial

The objective of this experiment was to evaluate if field derived palm material contained enough systemic insecticide to cause negative effects on *O. rhinoceros* adults. This could indicate if changes in visual ratings could be attributed to the efficacy of specific treatments. Palm fronds were removed from treated trees over five meters tall with a bucket truck at Ted Makalena Golf Course. The bucket truck removed one center frond from two palms of each chemical treatment used in the curative and preventative trials described above. These were

chopped into approximately 10-cm long pieces and fed to adult beetles in laboratory assays in mason jars. These trials utilized four replicates of four field-caught adult beetles. These replicates were placed in different locations within the incubator in the UH-ACL. Upon transport to the UH-ACL, these beetles were then placed in individual mason jars. The trial was evaluating a combined response of paralysis and/or mortality across treatments over a 14 day window. The CRB response caught beetles that were not fed for three days prior to the addition of palm material and stored in an incubator set at 30°C, where the trial took place. Extra beetles were kept in the same conditions to account for any mortality prior to the start of the trial.

During observations, the extent of feeding was quantified using a feeding scale of 1-3, with a 1 indicating sawdust or minor interaction with the frond, 2 indicating increased feeding, while a 3 rating was assigned when more than half of the material was consumed (Figure 8). The adults were monitored for 14 days for effects of paralysis and mortality using the same parameters as in the gel feeding trial. Due to the varied ages of the wild caught beetles, not all beetles fed immediately after the three-day starvation period. Evaluations of mortality were collected over a two week interval. This was longer than the gel insecticidal trials due to potential variability in wild caught *O. rhinoceros* beetle age, which can impact the frequency at which they feed.



Figure 8: Frond feeding scale. The extent of frass or disturbed palm material related to *O. rhinoceros* feeding is split into a feeding scale ranging from 1-3, with increasing ratings for increased feeding on the scale. Far left rank is 3, middle is 2, far right is 1.

Trap catch data collection

I was able to receive reports from data collected from the coconut rhinoceros beetle response program, which has a coordinated partnership with the Hawai‘i Department of Agriculture, the University of Hawai‘i, the United States Department of Agriculture and other groups. They inspect more than 3,000 panel traps every two to three weeks and record the number of beetles collected, along with the location where they were found on O‘ahu. This data was especially useful in the eradication portion of the program, as they could track new populations and set up buffer zones around new detections. I received results for the total adults caught in traps in specific areas, with some of the data indicating population drops that happened after widespread palm injections of systemic insecticides. This data was analyzed by the CRB response team and provided to us. I did not receive the number of traps in each location, but the

results are representative of the trapping effort in each area and the number of adults caught over time. Trap densities typically increase in areas with a new detection, or higher populations of *O. rhinoceros*.

Statistical analysis

The statistical analysis was conducted running a Kruskal Wallis test (JMP Pro 17, SAS 2001-2024). The analysis to assess the difference between treatments in relation to combined negative impacts of paralysis and mortality. If statistical significance was detected, a Tukey HSD mean separation test was conducted to determine which treatments were statistically different.

For the preventative, curative, and areawide palm field trials, each month (independent variable) was evaluated using the same analysis, determining if the visual percent of damaged leaves (dependent variable) changed with each treatment over time. This analysis examined the overall health of the palm, including the total damage to all leaves and the crown damage of the top four fronds, using a one-way ANOVA with least significant difference separation. We also used ordinal regression to analyze the impacts on the scores of overall palm damage and top four frond damage (JMP Pro 17, SAS 2001-2024). We used odds ratios to assess the relationship between treatments and the likelihood of observing different scores of tree damage, with the statistical significance determined using 95% CI for the odds ratios.

For the frond exposure trials, a generalized linear model was used with the logit link function and normal distribution using JMP.

Results

The laboratory insecticide gel feeding trials collected data at hourly and daily observation intervals, with every adult in the experiment typically feeding by 48 hours after exposure to the gel. This trial was repeated twice, and at 48 hours imidacloprid and dinotefuran caused 100% negative impacts of combined paralysis and mortality at both 25 and 250 ppm doses, while abamectin was fully effective at causing negative effects at 250 ppm (Trial 1: $\chi^2 = 22.83$, $P < 0.001$, $DF=6$), trial 2: $\chi^2 = 21.99$, $P = 0.001$, $DF=6$). Abamectin at 25 ppm still caused greater than 80% negative effects of combined paralysis and mortality among adult *O. rhinoceros*.

The damaged palm trees in the curative trial were clustered to one side of the golf course, indicating that a breeding site was likely located in that area or that there was an aggregation of beetles in this zone. The undamaged trees in the preventative trial were clustered along the ocean side of the golf course (Figure 8). The monthly visual survey results for these concurrent studies were indicative of the population's preferred feeding locations and aggregations at this site. The trees in the curative portion of the study steadily increased in damage for overall palm damage ratings throughout the duration of the trial. Damage trends of entire palm canopies suggested that imidacloprid treated trees may have had less damage than other treatments from months 17-23, but there was no statistical difference between treatments for any given month (Figure 9a). The odds ratios from the logistic regression analysis indicated that imidacloprid significantly decreased the overall palm canopy damage compared to other treatments (Table 1). For example, the odds ratio of imidacloprid versus the control was 3.79 (with 95% confidence intervals between 2.35 and 6.12, $p < 0.0001$) indicating that imidacloprid significantly increased the odds of reducing insect damage to trees compared to the control and similar effects were present for each treatment.

Imidacloprid-treated palms had statistically less damage to the center crown of the palm, with statistically significant differences between treatments during months 19-23 of the study (Figure 9b, $P < 0.05$). The odds ratios from the logistic indicated that imidacloprid significantly decreased the top four fronds damage compared to other treatments (Table 2). This effect indicates that imidacloprid significantly increased the odds of reducing insect damage to palm crown new growth in the top four fronds compared to the control and similar effects were present for each treatment.

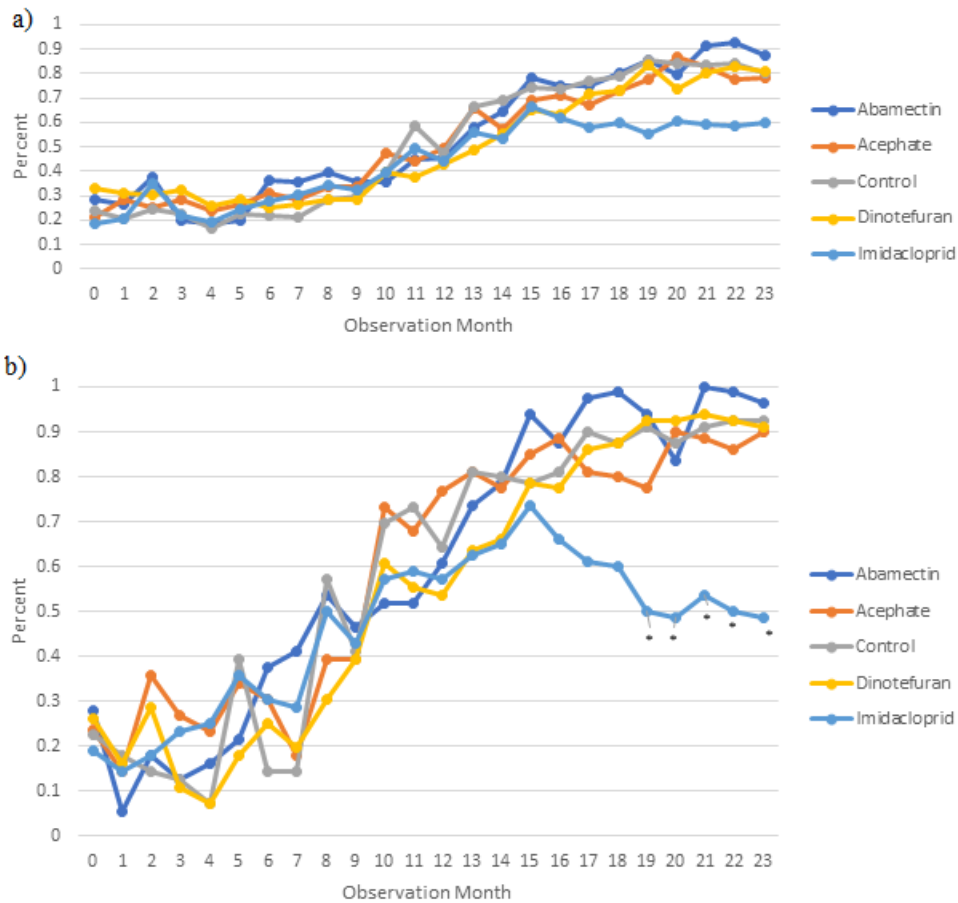


Figure 9: Field trial evaluating whether insecticidal treatments cause curative or visual restoration to *Oryctes rhinoceros* damaged coconut palms through percent quantification of feeding damage. a) Percent of coconut palm entire canopy damage over 23 months b) Percent of

top four new palm fronds damage over 23 months. The observations took place from December 2019 to November 2021. * represents $P < 0.05$ within the specified month after treatment (Least Significant Difference Test).

Table 1: Odds ratios (estimates form ordinal logistic regression analyses) for overall palm rating compared to various insecticides for the curative trial.

Level 1	Level 2	Odds Ratio	P value	95% Confidence Interval (Wald)	
				Lower	Upper
Acephate	Abamectin	2.239	<0.001	1.392	3.601
Control	Abamectin	1.730	0.025	1.073	2.780
Dinotefuran	Abamectin	1.876	0.010	1.161	3.0932
Imidacloprid	Abamectin	6.563	<0.001	4.029	10.693
Control	Acephate	0.773	0.282	0.483	1.237
Dinotefuran	Acephate	0.838	0.463	0.523	1.343
Imidacloprid	Acephate	2.931	<0.001	1.833	4.687
Abamectin	Acephate	0.447	<0.001	0.2777	0.718
Dinotefuran	Control	1.084	0.739	0.673	1.745
Imidacloprid	Control	3.794	<0.001	2.353	6.116
Abamectin	Control	0.578	0.025	0.358	0.932
Acephate	Control	1.294	0.282	0.809	2.072
Imidacloprid	Dinotefuran	3.498	<0.001	2.169	5.643
Abamectin	Dinotefuran	0.533	0.010	0.330	0.862
Acephate	Dinotefuran	1.194	0.463	0.744	1.913
Control	Dinotefuran	0.922	0.739	0.573	1.485
Abamectin	Imidacloprid	0.152	<0.001	0.094	0.248
Acephate	Imidacloprid	0.341	<0.001	0.213	0.546
Control	Imidacloprid	0.264	<0.001	0.164	0.425
Dinotefuran	Imidacloprid	0.286	<0.001	0.177	0.461

Table 2: Odds ratios (estimates form ordinal logistic regression analyses) for top four frond damage rating compared to various insecticides for the curative trial.

Level 1	Level 2	Odds Ratio	P value	95% Confidence Interval	
				Lower	Upper
Acephate	Abamectin	3.328	<0.001	1.976	6.604
Control	Abamectin	2.503	<0.001	1.483	4.224
Dinotefuran	Abamectin	2.124	<0.001	1.256	3.589
Imidacloprid	Abamectin	13.727	<0.001	8.0279	23.471
Control	Acephate	0.752	0.269	0.454	1.246
Dinotefuran	Acephate	0.638	0.085	0.383	1.063
Imidacloprid	Acephate	4.125	<0.001	2.518	6.755
Abamectin	Acephate	0.300	<0.001	0.178	0.506
Dinotefuran	Control	0.848	0.053	0.506	1.422
Imidacloprid	Control	5.484	<0.001	3.304	9.100
Abamectin	Control	0.399	<0.001	0.237	0.674
Acephate	Control	1.329	0.269	0.802	2.202
Imidacloprid	Dinotefuran	6.464	<0.001	3.863	10.817
Abamectin	Dinotefuran	0.471	<0.001	0.279	0.796
Acephate	Dinotefuran	1.567	0.085	0.940	2.612
Control	Dinotefuran	1.179	0.533	0.703	1.976
Abamectin	Imidacloprid	0.073	<0.001	0.043	0.125
Acephate	Imidacloprid	0.242	<0.001	0.148	0.397
Control	Imidacloprid	0.182	<0.001	0.110	0.303
Dinotefuran	Imidacloprid	0.155	<0.001	0.092	0.259

In the preventative trial, there were low damage ratios for all the treatments despite the geographic proximity to the curative trial. The overall visual damage surveys had circumstantial evidence that there was higher damage to control trees than the chemically injected trees (Figure 10). However, the percent of leaves damaged were lower than the curative trial, and there was no statistical difference between treatments for any given month. This was the case as well with the center crown damage throughout the 23 months of the trial (Figure 10b). The odds ratio from the ordinal regression analysis indicated that imidacloprid was more effective than both the control and dinotefuran and had increased likelihood in reducing the damage to the overall palm canopy (Table 3). The odds ratio for the top four frond damage indicated that imidacloprid had an increased likelihood of preventing damage compared to acephate, dinotefuran, and the control (Table 4). For imidacloprid versus the control, the odds ratio was 91.8 (95% CI between 20.3 and 414.48, $P < 0.0001$) indicating that this treatment was extremely effective at minimizing damage to new palm fronds compared to the control.

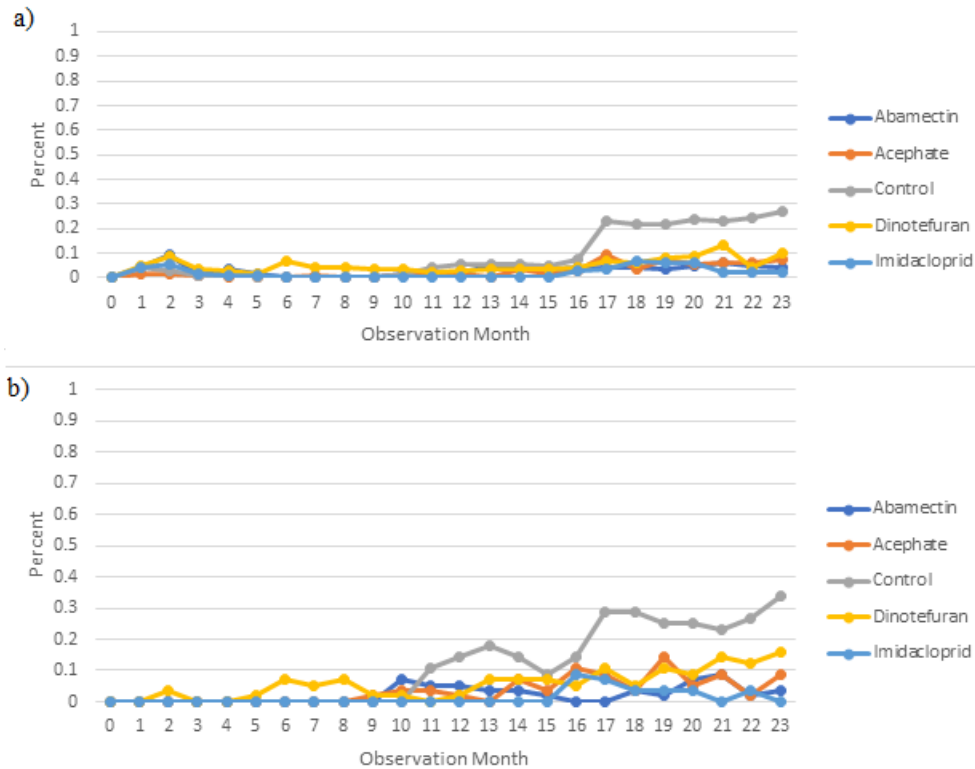


Figure 10: Field trial evaluating whether insecticidal treatments prevent damage to coconut palm from *Oryctes rhinoceros* adults. a) Percent of coconut palm entire fronds damaged by *O. rhinoceros* in preventative trial. b) Percent of top four new palm fronds damaged by *O. rhinoceros* in preventative trial. Observations took place from December 2019 to November 2021. * represents $P < 0.05$ within the specified month after treatment (Least Significant Difference Test).

Table 3: Odds ratios (estimates form ordinal logistic regression analyses) for overall palm rating compared to various insecticides for the preventative trial.

Level 1	Level 2	Odds Ratio	P value	95% Confidence Interval	
				(Wald) Lower	Upper
Acephate	Abamectin	0.958	0.934	0.346	2.655
Control	Abamectin	0.387	0.068	0.140	1.073
Dinotefuran	Abamectin	0.290	0.018	0.104	0.808
Imidacloprid	Abamectin	2.528	0.086	0.875	7.301
Control	Acephate	0.505	0.081	0.146	1.118
Dinotefuran	Acephate	0.303	0.022	0.109	0.842
Imidacloprid	Acephate	2.639	0.073	0.914	7.621
Abamectin	Acephate	1.044	0.934	0.377	2.893
Dinotefuran	Control	0.749	0.568	0.276	2.027
Imidacloprid	Control	6.530	<0.001	2.224	19.147
Abamectin	Control	2.581	0.068	0.932	7.149
Acephate	Control	2.473	0.081	0.895	6.836
Imidacloprid	Dinotefuran	8.718	<0.001	2.939	25.863
Abamectin	Dinotefuran	3.449	0.018	1.237	9.611
Acephate	Dinotefuran	3.304	0.022	1.188	9.188
Control	Dinotefuran	1.336	0.569	0.493	3.617
Abamectin	Imidacloprid	0.396	0.087	0.137	1.143
Acephate	Imidacloprid	0.379	0.073	0.131	1.094
Control	Imidacloprid	0.153	<0.001	0.052	0.450
Dinotefuran	Imidacloprid	0.115	<0.001	0.0386	0.340

Table 4: Odds ratios (estimates form ordinal logistic regression analyses) for top four frond damage rating compared to various insecticides for the preventative trial.

Level 1	Level 2	Odds Ratio	P value	95% Confidence Interval (Wald)	
				Lower	Upper
Acephate	Abamectin	0.432	1.188	0.124	1.505
Control	Abamectin	0.038	<0.001	0.010	0.139
Dinotefuran	Abamectin	0.121	<0.001	0.035	0.421
Imidacloprid	Abamectin	3.463	0.084	0.845	14.188
Control	Acephate	0.087	<0.001	0.027	0.287
Dinotefuran	Acephate	0.2798	0.031	0.088	0.890
Imidacloprid	Acephate	8.008	0.004	1.975	32.472
Abamectin	Acephate	2.312	0.188	0.665	8.045
Dinotefuran	Control	3.201	0.033	1.096	9.352
Imidacloprid	Control	91.806	<0.001	20.336	414.467
Abamectin	Control	26.509	<0.001	7.22	97.338
Acephate	Control	11.463	<0.001	3.484	37.719
Imidacloprid	Dinotefuran	28.679	<0.001	6.826	120.501
Abamectin	Dinotefuran	8.281	<0.001	2.376	28.861
Acephate	Dinotefuran	3.581	0.031	1.124	11.413
Control	Dinotefuran	0.312	0.033	0.107	0.913
Abamectin	Imidacloprid	0.289	0.084	0.07	1.183
Acephate	Imidacloprid	0.125	0.004	0.031	0.506
Control	Imidacloprid	0.011	<0.001	0.008	0.049
Dinotefuran	Imidacloprid	0.035	<0.001	0.008	0.147

A bucket truck was utilized to remove center palm fronds from treated trees over five meters to evaluate the effects of exposure through boring or feeding on field-caught adults. The results from the generalized linear model indicated that there was a significant main effect of treatment and month (Figure 11, $P < 0.0001$). The best fit model did not include the non-significant interaction of month and treatment. This suggests that the effect of treatment did not significantly vary across these months. Over time, treatment efficacy decreased with each treatment and their efficacy for causing negative impacts to *O. rhinoceros* adults. Every treatment was more effective than the control (Figure 12, $P < 0.001$). The residue analysis for these samples did not yield conclusive results when they were sent in for analysis, or these would have been incorporated into the model.

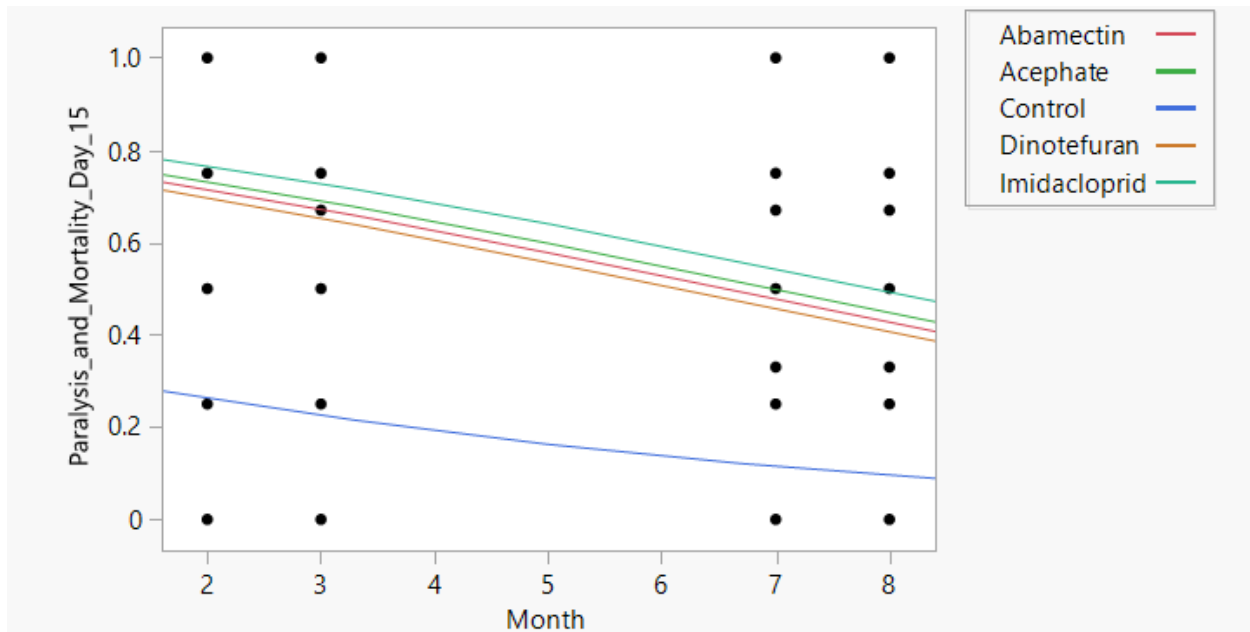


Figure 11: Results of a generalized linear mixed model evaluating the effect of five treatments at two, three, seven, and eight months after treatment. The plot displays the mean response for each insecticidal treatment, with statistical significance in the month ($P < 0.0001$) and treatment ($P < 0.0001$). There was no statistical significance in the interaction of month and treatment.

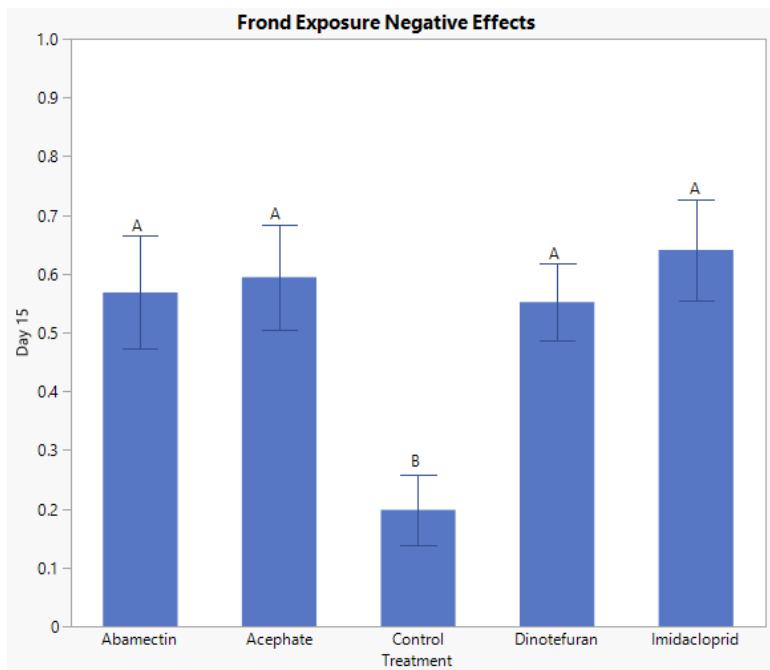


Figure 12: Negative effects of *O. rhinoceros* adults fifteen days after treatment in response to treatment and month after treatment was applied. Bar plot showing proportions for each treatment and the control group, showing mean mortality (\pm SEM, n=4). Bars with different letters were statistically different, $P < 0.050$.

The coconut rhinoceros beetle response team provided us with trap catch data from Ted Makalena Golf Course that explains the steady increase in damage throughout the curative trials (Figure 13). At the start of the trial in December 2019, there were less than 100 beetles found throughout the entire year, while by the end of the trial in December 2021 there were around 800 beetles caught in traps on that individual golf course alone. At the conclusion of this trial, the entire peninsula in Waipahu had their palms treated with imidacloprid injections in December 2021. Trap catch data indicated that there was a steep drop in catch rates following this areawide treatment (Figure 14). In another *O. rhinoceros* infested area of O‘ahu, this trend was evident as well following widespread injections of palms with imidacloprid (Figure 15).

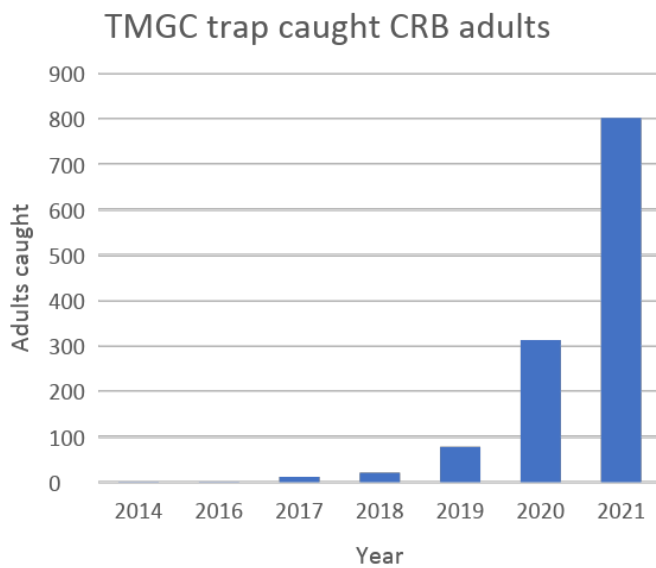


Figure 13: Yearly *Oryctes rhinoceros* trap catch data for Ted Makalena Golf Course through Dec 31, 2021 by the coconut rhinoceros beetle response team.

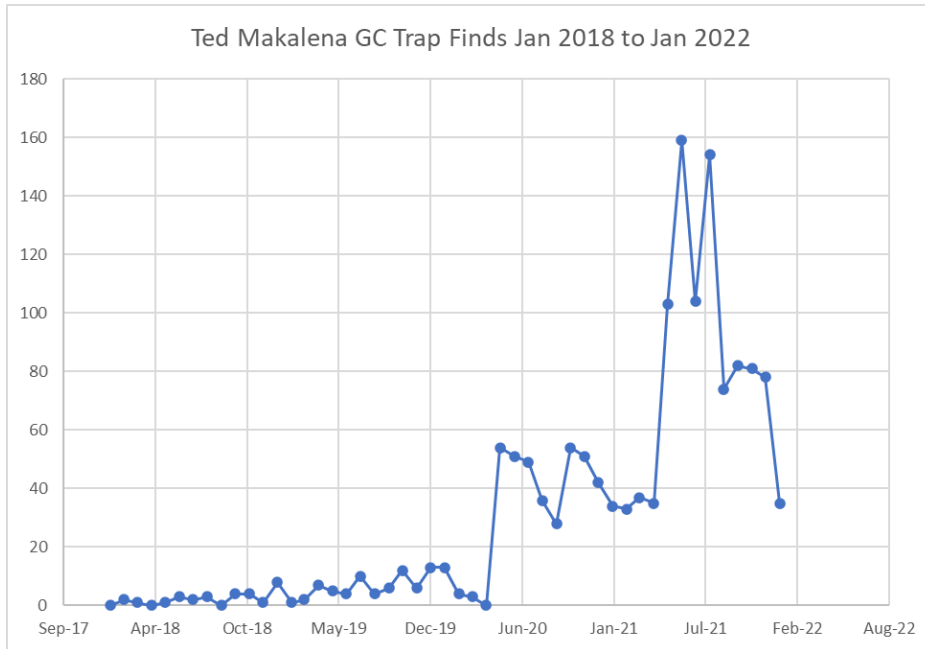


Figure 14: Ted Makalena Golf Course *Oryctes rhinoceros* adult trap catches from January 2018 to January 2022 indicating a rise in trap catches from 2020 to 2022. This is followed by a decline in 2022, coinciding with insecticidal treatments that were conducted in December 2019, December 2020, and December 2021. This indicates a steady increase in CRB response trap catches over time, with two decreases coinciding with areawide injections of palm trees with imidacloprid.

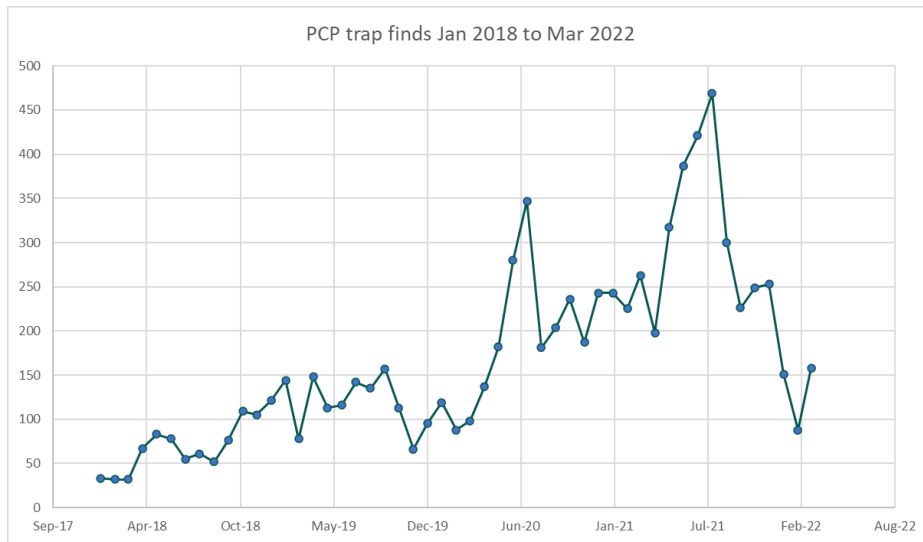


Figure 15: Pearl City Peninsula *Oryctes rhinoceros* adult trap catches from January 2018 to March 2022. This indicates a steady increase in CRB response trap catches over time, with two decreases coinciding with areawide injections of palm trees with imidacloprid. This indicates a steady increase in CRB response trap catches over time, with two decreases coinciding with areawide injections of palm trees with imidacloprid.

At the conclusion of the preventative and curative studies, the areawide treatment was utilized to test the short-term efficacy of treatments on palm recovery. The results indicated that there were minimal differences in damage within the ten specific geographic clusters, indicating that the population was persistent and new damage continued to occur. Areas of the golf course with less damage in the initial observations had an increase in feeding, while the heavily damaged areas of the golf course maintained similar damage percentages. The timeline for this data collection was too short to determine long-term treatment impacts. Although areas of O‘ahu experience decreased *O. rhinoceros* trap catch following areawide injections, this decrease in population may not be immediately reflected in visual damage on palms trees. There may be a

lag effect in palm recovery associated with wild population mortality and decreased *O.rhinoceros* trap catch.

Discussion

The emergence of the *O. rhinoceros* haplotype demonstrates the impacts that larger population sizes can inflict on island ecosystems, while highlighting the timeframe it can take to develop integrated pest management strategies. Without rapid advances in integrated pest management tactics, areas will experience a decline in palms and other species over time. There is a concern that this haplotype could establish itself in areas currently managed by OrNV causing a reverse invasion in its established and native range (Reil et al. 2018). Some of these areas rely on coconut production and the economic, ecological, and cultural consequences can be severe. *Oryctes rhinoceros* is expected to expand its range to new geographic areas with climate change, and the establishment of this biotype back in its native range would pose challenges for managing its populations (Xu et al. 2022). The impacts of populations not restricted by virus biological control of *O. rhinoceros* would have devastating impacts on palms across many regions of the world, impacting ecological and agricultural areas, along with cultural practices. In some areas with potential natural parasitoids and the original CRB biotype, there may be minimal impacts of OrNV resistant *O. rhinoceros* adults, as the populations are held in check by various ecological factors. Mechanical control using methods such as sanitation of areas where larvae and adults develop have been effective, and resulted in the only successful eradication on Niuatoputapu or Keppel Island (Bedform 1980).

Damaging invasive insect pests to North America such as the Asian longhorned beetle, *Anoplophora glabripennis*, and the emerald ash borer, *Agrilus planipennis*, have been controlled using systemic insecticides applied via tree injection (Doccola and Wild 2012). This targeted approach eliminates chemical leaching, drift, photolysis, and microbial degradation typical of surface applications, while protecting the tree against destructive insect pests. Managing or preventing insect pests on landscape trees through chemical injection has numerous benefits, but there are some associated damaging aspects such as damage at the injection site that can lead to secondary pest or pathogen problems. Healthy trees are sometimes able to recover at the injection site over time, but some side effects such as cracking, oozing, or decaying tissue are inevitable with these treatments (Doccola et al. 2011). Systemic insecticide applications on landscape trees via trunk injection are useful to delay the destruction of landscape trees susceptible to invasive insect species, but are only a temporary solution. Insecticide resistance and damage from repeated treatments can reduce the likelihood of these trees surviving over time, unless other tactics are implemented into a wider pest management strategy. The implementation of a chemical rotation and utilizing different modes of action can help reduce the chance of resistance.

The studies I conducted determined that various systemic insecticides could be applied successfully via trunk injection to coconut palms to mitigate their damage and potentially reduce populations of *O. rhinoceros*. Although adults typically oviposit in organic matter or dead standing palms, there has been evidence of larval stages in the organic debris in living palm trees on O‘ahu. This is the case in Guam as well, where all the life stages of *O. rhinoceros* were found inside living palms (Moore et al. 2015). Widescale insecticide injections could also inhibit larval growth in arboreal breeding sites as the organic debris in palms may contain enough pesticide

residue to prevent successful completion of their life cycle. These treatments could be added to an integrated pest management program to either eradicate, or more likely, manage populations of *O. rhinoceros* in other invaded areas around the world. The efficacy of abamectin and dinotefuran in laboratory trials and their ease of field application could be considered in a pesticide rotation with imidacloprid. Imidacloprid was a more effective treatment in the field than other compounds tested, and is currently being implemented in a rotation with acephate by the CRB Response team. This rotation utilizes different modes of action and potentially can avoid the build up of insecticide resistance. The areawide treatments of imidacloprid on O‘ahu coincided with trap catch reduction in treated areas, suggesting a possible population drop related to treatments. This occurred in Pearl City and Iroquois Point. These areas are mostly urban with maintained palms that are trimmed prior to flowering, avoiding the potential for imidacloprid contamination in coconuts and any potentially negative impacts to pollinators. The frond material is typically removed from these sites after tree trimming.

The frond exposure trials determined that all the treatments applied via trunk injection were able to effectively disperse throughout the palm tree and be present at a concentration high enough to cause paralysis or mortality to adult beetles. These beetles were typically boring through the material, rather than ingesting it as it was not the center of the palm. Yet, this exposure to the frond residue was enough to cause negative impacts on the beetles, highlighting the length of field efficacy for these products. It is possible that *O. rhinoceros* adults that feed on this material may recover from low doses of pesticide exposure. In landscape areas such as a golf course, it is unlikely they will survive as mechanical control measures such as mowing likely will kill any lethargic beetles that fall out of the palm, as mowing occurs 2-3 times per week. In some of the laboratory trials the *O. rhinoceros* adults would bore extensively into the frond

before the treatment effect inhibited feeding, indicating that a reduction in damage may not occur for some time after treatment. This delay could be due to decreased pesticide concentration in the frond over time. Control specimens that exhibited negative impacts possibly were exposed to chemical residue in the field, as these trials coincided with the areawide treatments of imidacloprid in areas on O‘ahu. The vane trap caught beetles utilized in this trial, may have had some field pesticide exposure. Public outreach with local golfers at the field site was beneficial, as they alerted us when dead beetles were found at the base of palms and which tree tag number had the beetle. Their anecdotal findings indicated that all four treatments had this effect throughout the study, despite the low sample size.

This Chapter highlights the difficulty with controlling *Oryctes rhinoceros* populations, once they are widespread, as insecticide treatments are not the entirety of the solution for population reduction. Although there are no populations in the continental United States, there have been interceptions in Mexico. There is a possibility that *O. rhinoceros* is now established there according to iNaturalist observations of caught beetles and v-cuts on subsequent palms (Jackson et al. 2022). Within the Hawaiian islands, the expansion of *O. rhinoceros* populations in 2023 to Kaua‘i and Hawai‘i island are another cause for concern. The implementation of the results of this research could be utilized to slow down population growth. The findings from this research indicate that widespread imidacloprid trunk injection could be utilized to manage *O. rhinoceros* if it were to be detected in a new area in conjunction with a variety of other IPM methods mentioned previously. This could result in a localized eradication. The application of these results likely will decrease the rate of palm mortality in some areas in Hawai‘i, but over time it is likely that there still will be palm mortality without proper funding to address the dynamics of a growing population. In July 2024 Governor Josh Green cut \$10 million out of

House Bill 2619, which promised \$20 million to improve biosecurity in Hawai‘i. This funding could have been utilized to mitigate the spread of *O. rhinoceros*, while researching novel ways to manage populations indefinitely. As we approach an uncertain future on how to manage fragile ecological areas, agricultural zones, and landscape plants in Hawai‘i against growing *O. rhinoceros* populations, it will be essential to employ a variety of tactics from chemical control, to novel biological control solutions.

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CHAPTER 4: CHEMICAL CONTROL AND PRELIMINARY BIOLOGICAL CONTROL
EXPLORATION OF THE HALA SCALE, *THYSANOCOCCUS PANDANI* STICKNEY

Introduction

The impacts of anthropogenic activities and globalization on the Hawaiian islands have been implicated in severe decline and extinction of Hawaiian endemic flora and fauna through the introduction of a myriad of invasive species (Warner 1968, Freed et al. 2008, Cox 1999, Wood et al. 2017). The first introduced plants and animals were brought by Polynesian voyagers who navigated the vast ocean and successfully colonized the Hawaiian islands. Today, these introduced plants are referred to as ‘canoe plants’. Subsequent waves of species introductions were brought by Europeans, and each new human contact was typically associated with new species introductions.

The canoe plants and animals were the first human introductions of fauna and flora to the islands, which are present across ecosystems in various areas of each island (Boyer 2008). These have become integrated with existing endemic ecosystems such as indigenous coastal hala forests. The leaves of hala tree, *Pandanus tectorius* (Pandanaceae), are utilized by native Hawaiians for weaving and medicinal practices. Coastal forests of hala used to be found throughout the Hawaiian islands, and today’s remaining forests are considered an effective ecological buffer against invasive plant species, as their leaf litter mitigates the growth of potentially invasive plants (Atkinson 1970). There were indigenous hala species that existed prior to Polynesian contact, but upon arrival, the Polynesians introduced new hala varieties to the Hawaiian islands, as these were among the canoe plants introduced to new Hawaiian settlements (Gallaher 2014). Unfortunately, many areas previously characterized by coastal hala forests have

been removed for development, and some of the remaining forests are threatened by invasive ungulate species.

The hala scale, *Thysanococcus pandani* Stickney (Halimococcidae), was first recorded in Indonesia and Singapore on hala plants (Stickney 1934). It is considered to be the cause of the destruction of *Pandanus* spp. on the Cook Islands of Antiu, Mangaia, and Rarotonga (Beauvais and Chouchan 2006, Speith 2012). However, a google maps search indicates that the Cook island of Mangaia still has pandanus as of a 2016 street view image. *Thysanococcus pandani* has been found in Thailand (Mohsen Ramadan, Hawai'i Department of Agriculture, pers. comm.).

Negative aesthetic and physical impacts of infestations range from foliage distortion, discoloration and yellowing, to crown drop and early plant death, while it impacts the quality of Hawaiian weaving practices (Matsunaga 2021). *Thysanococcus pandani* was first detected in Hawai'i in Hāna, Maui in 1995 and subsequently spread throughout the entire island. A recent survey of the Hawaiian islands indicates that *T. pandani* is now widespread on Moloka'i, and established populations on O'ahu have been detected (Russo and Cheng, 2021). Hala is a very common landscape tree throughout the state, and an expanded range of *T. pandani* could have negative impacts on both urban and rural ornamental plantings (Wong 2008). Widespread distribution of this insect pest throughout the Hawaiian islands could not only cause potential negative ecological impacts in coastal hala forests but also interfere with the continuing of Native Hawaiian weaving practices, specifically ulana lauhala, for future generations (Keawe et al. 2014).

Hala scale adults are around 0.5mm in size and from the naked eye appear as a black dot within the vein lines of a pandanus leaf, with or without a white waxy fringe (Figure 16). Both adult males and females are present, with no evidence of parthenogenesis. Females have three

instars, while males have five instar stages (Stickney 1934). Both stages are flattened ventrally and are dorsally convex, with crawlers emerging from the hard structure that the females form on the leaves. As these are found in tropical areas, there is the potential for multiple generations a year, though they have low fecundity. In Hawai‘i, infestation usually begins with a few adults that are visible with the naked eye on the underside of the leaf. This causes minor discoloration, and is noticeable at a close distance. When there are high densities within the canopy, yellowing is visible as can be aggregations of white waxy fringe that form around *T. pandani* adults. The fringe is usually on the underside of the leaf, while the top typically does not have this substance present.



Figure 16: Images of *T. pandani* at no magnification (left) and 10x magnification (right).

Eradication and management approaches are necessary to manage the infestations of invasive species such as *T. pandani* throughout the Hawaiian islands. For example, treatment with systemic insecticide application is recommended to control the lobate lac scale, *Paratachardina pseudolobata*, another invasive insect pest on ornamental landscape plants in Hawai'i (Bhandari and Cheng 2017). This study aims to fill gaps in population suppression strategies for managing populations of *T. pandani* on ornamental hala trees, as there is little literature regarding this pest. The objectives were to determine the efficacy of various contact and systemic insecticides against the hala scale in repeated field experiments.

Materials and methods

Chemical control

An open-field potted plant experiment was conducted at the Kahului Cooperative Extension Services Garden located at the University of Hawai'i Maui College. This experiment aimed to determine the efficacy of single rates of flupyradifurone, azadirachtin, cyclaniliprole, cyclaniliprole +flonicamid, botanical oil blend, buprofezin, 50% Rosemary Oil concentrate, 50% Rosemary Oil organic, imidacloprid, a high and low rate of afidopyropen (Table 5), and water control on two-year-old *P. tectorius* plants infested with *T. pandani*. Juvenile hala trees in 7.57-liter pots were organized in four rows approximately one meter apart. For the second trial, these were replanted into 11.35-liter pots at one m apart. In both trials fertilizer (Nutricote Total 13-11-11) and soil media (1 bag PRO-MIX combined with ¼ bag perlite) were used, with ten minutes of automatic drip irrigation scheduled twice a day at 7 am and 3 pm HST. Infested hala leaves were used to inoculate these plants over a five-month interval to reach consistent infestation levels across the treatments. Each chemical treatment was applied as a foliar application of 739

ml per plant using a handheld Delta Plant Care Pressure Sprayer (Delta Industries, PA). The experiment was arranged in a randomized complete block design with six replicates per treatment for the first trial and five replicates per treatment for the second trial. The first trial was conducted from June to November 2020, and the second trial was from December 2020 to May 2021. Six assessments of scale mortality were made during the entire experiment at 0-, 7-, 14-, 28-, 56-, and 112-days post-treatment. The second trial followed the same observation schedule, except that it concluded 109 days after application.

At each time of observation, *T. pandani* infestation levels were rated based on a 1-5 visual scale per plant, where 1=no scales present, 1.5=one scale present, 2=two scales minimum, 2.5=increased scale coverage with minimal overall infestation, 3=increased scale coverage on underside and top of new leaves compared to previous rating, 3.5=moderate scale coverage with increased coverage of new and old growth, 4=scale coverage on both sides of the leaves, 4.5=widespread scale coverage on both sides of many of the leaves, and 5=plant is dead due to a severe infestation. The physical appearance of each tree typically reflected the infestation level, as yellowing would increase with a higher density of *T. pandani* populations, indicating decreased plant health. Therefore, the scale infestation was used as a marker of plant health throughout the trials. Upon conclusion of the first trial, the plants were separated based on this plant health infestation rating. These were separated into new replicates of similar infestation levels and then arranged in a randomized complete block design, with five hala trees per treatment.

Thysanococcus pandani were sampled for mortality assessments by using clear scotch tape to dislodge dead specimens from healthy leaves. Alive *T. pandani* specimens remain on the leaf, so this method was effective to determine mortality rates associated with each treatment.

This was evaluated as a visually estimated percent removal, determined by the number of scales that were dislodged from the leaf. For example, if the tape removed half of the scales within the designated area, a 50% mortality rating would be assigned. For each plant the 8th leaf from the spear (center) was selected as a sample. Then three sections of the 8th leaf were sampled with a transparent tape (1 cm × 1 cm area/section), one on the bottom and two on the top of the leaf. The 8th leaf was chosen, as it had new growth of *T.pandani* populations, but would not result in defoliation of new growth during each observation window. These pieces of tape were individually peeled off carefully to avoid unintentionally dislodging scales that may still be alive. Abbott's formula was used to account for control mortality in of these treatments at the end of each trial, as

$$AM = [(X-Y)/X]100$$

(AM = actual mortality, X = the percent living in the check and Y = the percent living in the treated plot) (Abbott 1925). The evaluation of plant growth parameters such as plant height and canopy width were measured before the first chemical application and at the termination of the experiment. In addition, phytotoxicity was evaluated based on the ERWC standard 0-9 scale, where 0 = plant is healthy and 9 = plant is killed due to chemical phytotoxicity.

Statistical analyses were conducted for these trials running a non-parametric Kruskal-Wallis test using R to assess the difference between treatments in relation to mortality, infestation ratings, plant growth, or plant canopy (R Core Team 2021). If statistical significance was detected, a pairwise comparison was conducted using Dunn's method. The graphs and tables were generated using Microsoft Excel 2016.

Biological control exploration

In March 2024, me and Dr. Cheng traveled to Thailand, Singapore, and Indonesia to evaluate coastal pandanus forests for the presence of *T. pandani* and for any potential predators in the native range. We utilized personal communications and Google street view to pick sites of interest that include coastal clusters in sites outside Bangkok and in Phuket, Thailand. We went to locations in Java and Bali, Indonesia, and locations on Sentosa and Lazarus Islands in Singapore. Unfortunately, Dr. Cheng was not able to acquire any collection permits prior to this trip. The findings from this trip indicated that *T. pandani* may have originated in Madagascar, Taiwan, Sri Lanka, or the western pacific as botanical collections had trees from these localities with high levels of *T. pandani* populations. Madagascar has over 70 endemic *Pandanus* spp. and in August 2024, I traveled there. The Madagascar trip was set up to evaluate localities in Ankarana National Park, Analamazaotra National Park, and Masoala National Park, as these areas have *Pandanus* spp. present. Other localities from the gardens were Taiwan and Sri Lanka, but we could not sample there due to funding and time limitations.

We quantified the distribution of *T. pandani* and the percent coverage on *P. tectorius* plants. We did not calculate a percent, but were observing whether densities were high enough for *T. pandani* to also be considered invasive in a different locality. In areas with minimal scale coverage we will collect some material and leave it with local collaborators, if we do not see any visible predators controlling *T. pandani*. If they note that parasites emerged, we will aim to identify these to the lowest possible taxonomic level. If anything promising is collected that is not a generalist and has the potential to reduce populations in Hawai'i, then we will apply for export permits.

Results

Chemical control

Among the 12 treatments tested in two trials, no phytotoxicity was observed on *P. tectorius* by any chemical treatment. There was no treatment effect observed in the final plant growth measurements in terms of canopy width and plant height in either trial. In both trials, there was no significant treatment effect on *T. pandani* mortality on days 7, 14, 28, and 56 (Tables 6-9). However, in Trial 1 there was treatment effect on mortality of *T. pandani* observed at 112 days post treatment indicating that buprofezin was the most effective treatment (Table 7, $H=30.841$, $DF=11$, $P=0.001168$).

Flupyradifurone and buprofezin were potentially effective treatments against *T. pandani* as both treatments had the highest percent of scales dislodged with tape. In terms of reducing infestation ratings of *T. pandani*, flupyradifurone had lower ratings at the 56-day observation point, and at 112 days post treatment buprofezin was the most effective treatment for infestation reduction with the lowest infestation rating (Table 6, $H=31.886$, $DF=11$, $P=0.0008$). Only Buprofezin was statistically different from the control treatment in trial 1.

In the second trial there was no statistical significant effect of the treatments at 56 days post treatment for tape removal or infestation ratings (Tables 8 and 9). At 109 days, buprofezin, Rosemary Oil Organic, and flupyradifurone had the highest tape removal rate (Table 9, $H=20.23$, $DF=11$, $P=0.0423$). The infestation rating was lowest with buprofezin at the conclusion of the trial (Table 8, $F=3.167$, $df=11$, $P=0.0027$). Applying Abbott's formula to the final mortality data for trials 1 and 2 indicated that flupyradifurone and buprofezin were the most effective

treatments in both trials as they had the highest mortality (Table 10). The potential residual effects from the treatments in trial 1 complicated these results.

Table 5: Active ingredients, application rate, application frequency, and product for each treatment applied to *P. tectorius* trees.

Active ingredients	Application Rate (ml/L)	Application Frequency (weekly)	Producer, (Product)
17.09% Flupyradifurone, 82.91% other ingredients	1.09 ml	2	Bayer Environmental Science, Research Triangle Park, NC; (Altus)
3% Azadirachtin, 97% other ingredients	1.25 ml	1	BioSafe Systems LLC, Coldwater, MI; (AzaGuard)
4.55% Cyclaniliprole, 95.45% other ingredients	2.19 ml	3	Ishihara Sangyo Kaisha LTD, Edobori, Osaka, Japan; (Experimental Product)
4.55% Cyclaniliprole, 95.45% other ingredients (50% Flonicamid and 50% other ingredients)	1.29 ml	1	Ishihara Sangyo Kaisha LTD, Edobori, Osaka, Japan; (Experimental Product)
Botanical Oil Blend (10-20% Corn Oil, 3-5% White Mineral Oil (petroleum), 80-90% other components below reportable levels)	10.00 ml	1	Kemin Crop Technologies, Des Moines IA; (Experimental Product)
70% Buprofezin, 30% other ingredients	1.09 ml	2	SePro, Whitakers, NC; (Talus)
50% Rosemary Oil (95-98% Monoterpenes and monoterpene derivatives, 2-5% sesquiterpenes), 50% Inert Ingredients (Glycerol, monooleate, Sorbitol, Lauric acid, Myristic acid, Palmitic acid, Stearic acid, Oleic Acid)	10.00 ml	1	Kemin Crop Technologies, Des Moines IA; (TetraCURB Organic)
50% Rosemary Oil (95-98% Monoterpenes and monoterpene derivatives, 2-5% sesquiterpenes), 3% Clove Oil, 1.95% Peppermint oil(Organic), 45.05% Inert Ingredients (Glycerol monooleate, Ethyl lactate, Ascorbyl palmitate)	10.00 ml	1	Kemin Crop Technologies, Des Moines IA; (TetraCURB Concentrate)
9.78% Afidopyropen, 90.22% other ingredients	0.55 ml	2	BASF, Florham Park, NJ; (Ventigra)
9.78% Afidopyropen, 90.22% other ingredients	0.38 ml	2	BASF, Florham Park, NJ; (Ventigra)

21.4% Imidacloprid, 78.6% other ingredients	0.12 ml	1	Bayer Environmental Science, Research Triangle Park, NC; (Merit)
Water		1	

Table 6: Trial 1. Mean infestation of hala scale based on a 1-5 scale (see methods) affected by various chemical treatments at 112 days post-treatment (DPT). Means in columns with the same letters were not significantly different, Kruskal Wallis test, $P < 0.050$.

Treatment	Day 112
Flupyradifurone	2.00±0.13ab
Azadirachtin	3.58±0.24a
Cyclaniliprole	3.58±0.30a
Cyclaniliprole+Flonicamid	3.42±0.44ab
Botanical Oil Blend	2.58±0.27ab
Buprofezin	1.42±0.24b
Rosemary Oil Concentrate	2.75±0.31ab
Rosemary Oil Organic	3.42±0.35ab
Afidopyropen (Low)	3.00±0.47ab
Afidopyropen (High)	2.50±0.41ab

Imidacloprid	3.75±0.25a
Water	3.33±0.40ab

Table 7: Mean percent of hala scale dislodged from an infested hala leaf using a clear tape assessed at 0, 7, 14, 28, 56, and 112 days post-treatment (DPT). Means in columns with the same letters were not statistically different, Kruskal Wallis test, P <0.50.

Treatment	Day 112
Flupyradifurone	94.17±3.38ab
Azadirachtin	46.00±9.77ab
Cyclaniliprole	72.17±13.16ab
Cyclaniliprole+Flonicamid	35.00±9.30b
Botanical Oil Blend	50.72±11.07ab
Buprofezin	99.44±0.55a
Rosemary Oil Concentrate	62.72±14.39ab
Rosemary Oil Organic	36.67±12.79b
Afidopyropen (Low)	50.06±13.07ab
Afidopyropen (High)	77.06±13.62ab
Imidacloprid	45.83±11.43ab

Water	34.72±13.37b
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Table 8: Trial 2. Infestation of hala scale based on a 1-5 scale (1 = no scales present; 5 = kill plants) affected by various chemical treatments at 109-day post-treatment (DPT). Means and standard error in columns with the same letters were not statistically different, Kruskal Wallis test, P <0.50.

Treatment	Day 109
Flupyradifurone	3.00±0.44 ab
Azadirachtin	3.60± 0.29 ab
Cyclaniliprole	4.10± 0.29 b
Cyclaniliprole+Flonicamid	3.70±0.25 ab
Botanical Oil Blend	3.00±0.29 ab
Buprofezin	2.60±0.29 a
Rosemary Oil Concentrate	3.60±0.29 ab
Rosemary Oil Organic	3.40±0.32 ab
Afidopyropen (Low)	3.20±0.43ab
Afidopyropen (High)	3.30±0.50 ab

Imidacloprid	3.60±0.29 ab
Water	3.70±0.32 ab

Table 9: Percent of hala scale dislodged from an infested hala leaf using a clear tape, as a surrogate measure of insect mortality, assessed at 109 days post-treatment with insecticides (DPT). Means and standard error in columns with the same letters were not statistically different, Kruskal Wallis test, P <0.50.

Treatment	Day 109
Flupyradifurone	85.67±5.82a
Azadirachtin	79.00±7.35a
Cyclaniliprole	61.73±9.77a
Cyclaniliprole+Flonicamid	76.87±6.50a
Botanical Oil Blend	38.67±14.00a
Buprofezin	90.33±7.75a
Rosemary Oil Concentrate	72.53±11.42a
Rosemary Oil Organic	85.67±6.50a
Afidopyropen (Low)	65.67±6.58a
Afidopyropen (High)	70.33±11.58a

Imidacloprid	77.53±7.69a
Water	53.67±7.43a

Table 10: Abbott's formula applied to the mortality results of each trial at 109 days (trial 1) and 112 days (trial 2) after treatment. This is an estimate of mortality of *T. pandani* using Abbott's formula.

Treatment	Rate(s)	Mode of Action	Trial 1	Trial 2
<i>Flupyradifurone</i>	/100 gal	Systemic	90.8%	69.1%
<i>Azadirachtin</i>	14 fl oz	Contact	16.9%	54.7%
<i>Cyclaniliprole</i>	16 fl oz	Contact	56.9%	17.4%
<i>Cyclaniliprole</i> +	28 fl oz	Contact	0%	50.1%
<i>Botanical Oil Blend</i>	16.5 fl oz	Contact	24.6%	-32%
<i>Buprofezin</i>	128 fl oz	Insect Growth Regulator	98.5%	79.1%
<i>Rosemary Oil</i>	14 fl oz	Contact	43.1%	40.7%
<i>Rosemary Oil</i>	128 fl oz	Contact	3.1%	69.1%
<i>Afidopyropen</i>	128 fl oz	Chordotonal organ TRPV channel modulator	64.4%	25.9%
<i>Afidopyropen</i>	7 fl oz	Chordotonal organ TRPV channel modulator	23.1%	36%
<i>Imidacloprid</i>	4.8 fl oz	Systemic	16.9%	51.5%

Biological control exploration

We conducted two trips to search for the native range of *T. pandani* and any natural enemies that potentially could be used as biological control agents. The first trip was a brief visit to existing countries where *T. pandani* has been confirmed to be present. We did not find *T. pandani* in our four day survey of Thailand as we searched the outer Pattaya area and across the extensive *Pandanus* sp. located across Phuket and in the Phi Phi Islands (Figure 17). In Indonesia, Dr. Mohsen Ramadan confirmed that *T. pandani* was in Yogyakarta. In Bogor, there was a pandanus collection that had *T. pandani* present (Figure 18). *Pandanus polycephalus*, *P. parkinson*, *P. pygmaeus*, *P. affinis*, and *P. odorifer* had confirmed *T. pandani* populations with Coleopteran Cybocephalidae sp. and Coccinellidae sp. (Coleoptera) as active predators. We did not find any Cecidomyiidae predators that were observed on a previous trip (Mohsen Ramadan, personal communication). *Pandanus sulawesi*, *P. furcatis*, and *P. livingstonianus* were present in the collection and did not have any *T. pandani*.

In our four days in Singapore, we visited a variety of sites from natural areas, botanical gardens, and nearby islands (Figure 19). We found similar species of Coleopteran natural enemies that we found in Indonesia. The infestations of *T. pandani* were clustered around the Singapore Botanical Garden, Gardens by the Bay, Santosa Island, and Lazarus Island. The infested varieties were *P. utilis* and *P. tectorius*. The densities of *T. pandani* were similar to levels seen in Hawai'i, suggesting that these could be introduced populations.

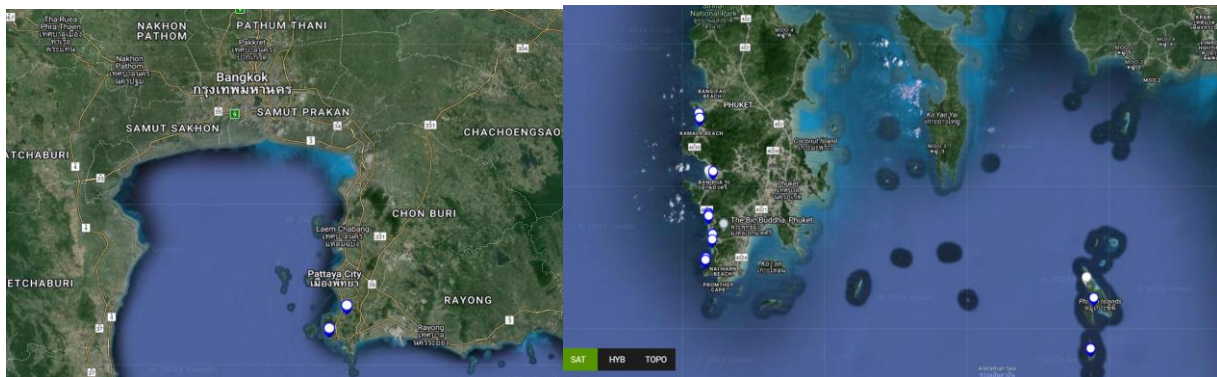


Figure 17: *Pandanus* spp. locations surveyed in Thailand. Blue markers indicate no *T. pandani* present, gray markers indicate trees that were too tall to survey or sites we did not get to.



Figure 18: *Pandanus* spp. surveyed in Indonesia. Orange marker indicates positive detection of *P. tectorius*, while blue does not.

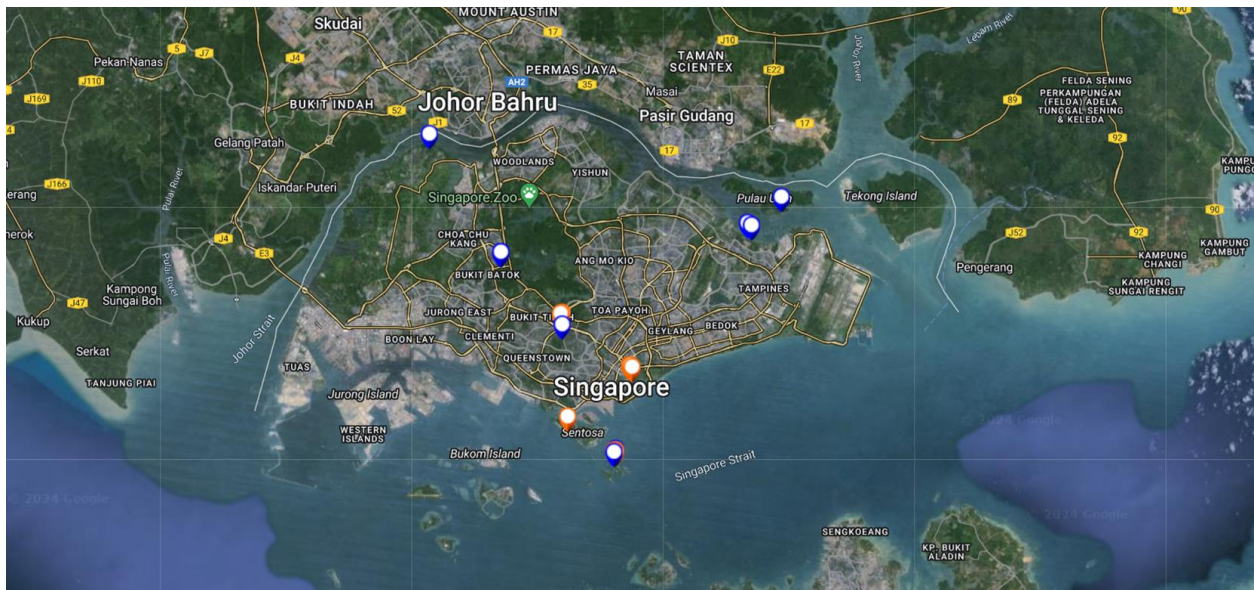




Figure 19: Top: *Pandanus* spp. surveyed in Singapore. Orange marker indicates positive detection of *P. tectorius*, while blue indicates no detection. Bottom: Coleopteran biological natural enemies found feeding on *T. pandani* in Singapore. Five times magnification zoom with a hand lens.

In Madagascar I sought to determine which sites had the highest likelihood of finding *T. pandani* with a limited time of three weeks during the winter. In Ankarana National Park, the park ranger indicated that there are around 80 species of *Pandanus* in the country. I picked two parks in areas that have tropical climates and another site that has a higher species count of *pandanus* and close proximity to Antananarivo. These sites were all I could fit in with the limited time, budget, and taking into account safety considerations. I anticipated that Masoala National Park would be the most likely site to find *T. pandani*. During this trip I found 15 different species of *Pandanus*, and three underscribed scale insects that are specific to *Pandanus* (Figure 20). The first two insects I found in Ankarana National Park, and in Masoala National Park I found the first and third species. The last species shown in Figure 21, was similar to *T. pandani* and DNA sequence data are needed to determine how closely related these taxa are. The scale insects that I did see were on very few trees around each park, and limited to just a few leaves on those trees.

This is different from the infestations of *T. pandani* that I see in Hawai‘i, where every leaf is typically infested. There was one specimen of the first species I found with an exit hole, but I did not observe any active predators on the very few *Pandanus* spp. that had scale insects.

Interestingly, in both national parks, two different rangers indicated that *Phelsuma* spp. (Gekkonidae) geckos predate these scale insects and have been seen licking the leaves. I did not observe this behavior during our stay, as some of the species are nocturnal and I was not permitted into some areas after dark. There are at least two *Phelsuma* spp. geckos introduced to Hawai‘i, the giant day gecko, *Phelsuma grandis*, and the gold dust day gecko, *Phelsuma laticauda*.

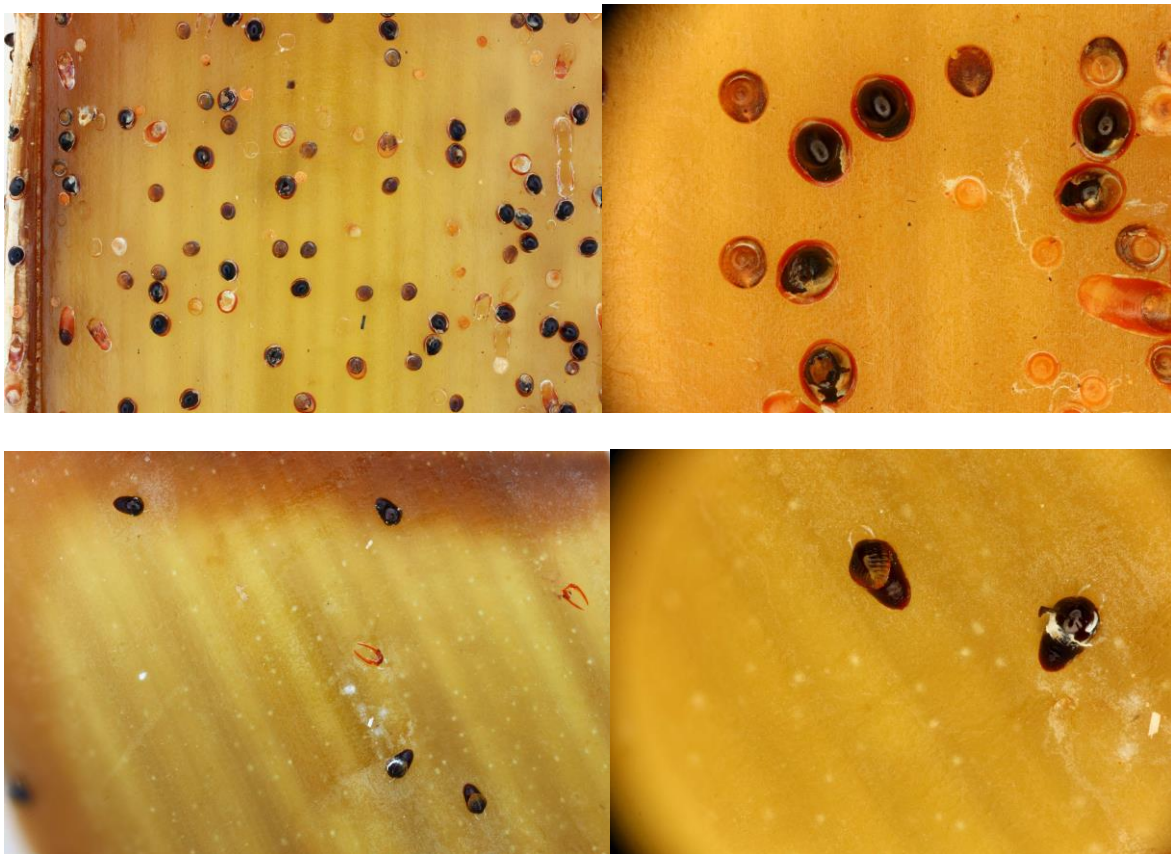




Figure 20: Images of each undescribed scale species found on Pandanus trees in Madagascar at 4x (left) and 10x (right) magnification.

Discussion

In Hawai'i, invasive insects and pathogens have taken advantage of landscape plantings to move across areas that may have not been suitable for establishment previously, eventually reaching remote native ecosystems. One potential example was with the planting of *Erythrina* sp. windbreaks, taxonomically similar to the endemic dryland endemic forest tree wiliwili, *Erythrina sandwicensis*. The detection of the invasive *Erythrina* gall wasp, *Quadrastichus erythrinae*, in 2005 was followed by the death of many of these ornamental trees, along with trees in the native dryland forests (Heu et al. 2008). Insecticides were used as a mitigation tactic until a biological control agent was found that was able to save what remained of this endemic species (Doccola et al. 2009, Kaufman et al. 2020). This could be similar to what is happening with *T. pandani*, as there have been extensive planting of *P. tectorius* trees in areas where it previously was not present. This might result in a similar chain of events, facilitating the pathway for an invasive insect pest to spread across a landscape, eventually reaching remote areas where this indigenous

plant resides. This chapter explores the use of insecticides as a mitigative measure, and expands upon the search for broader control mechanisms by searching for natural enemies.

Thysanococcus pandani is currently widespread on the islands of Moloka‘i and Maui, with a limited population on O‘ahu. Hala trees are widely planted as ornamental plants on these islands, which possibly facilitated the spread of *T. pandani* from residential to rural areas. Pandanus trees were present in areas that they possibly would not be in without anthropogenic assistance. This might facilitate the spread of *T. pandani* across the residential landscape, and it probably reached very remote areas likely through wind dispersal. The exact life history of the hala scale is not fully understood, but in armored scale insects such as the lobate lac scale, *Paratachardina pseudolobata*, crawlers emerge from reproductive adults and infest new plant material (Howard et al. 2014). In general, scale insects are notoriously difficult to control in the ornamental landscapes, and targeted insecticide application has limited efficacy (Quesada et al. 2018). This can be due to the difficulty with eliminating entire populations, insecticide resistance, defenses to treatment methods, or other environmental factors

In the insecticidal trials on Maui, the final observation data with Abbott’s formula corrections indicated that flupyradifurone and buprofezin yielded the highest mortality rates per treatment, with buprofezin having higher mortality than flupyradifurone in both trials. Buprofezin acts as an insect growth regulator and can be applied for scale insect mitigation (Grafton-Cardwell et al. 2006). However, these products were not significantly different from the control, and further testing is necessary to evaluate this. For the widespread infestations of *T. pandani* on Moloka‘i and Maui, this research provides potential management strategies that can be applied to mitigate the negative impacts of a heavy *T. pandani* infestation. These products would first need to be labeled for use on *T. pandani*. After further testing and label changes,

these treatments could be applied to target ornamental trees, as a landscape wide approach would not be practical. A pesticide rotation could be implemented with flupyradifurone and buprofezin to maintain the health of hala plants against the negative impacts of the damage caused by *T. pandani*, while avoiding potential insecticidal resistance issues. A previous unpublished study indicated that imidacloprid application via soil drench or trunk injection can be utilized for *T. pandani* control and would be readily available for the general public to use to target multiple pests on *P. tectorius* trees.

Although insecticidal treatments can be applied to areas with widespread infestations, there are further steps that can be taken to reduce the chance of this damaging insect spreading via anthropogenic means. Mitigation methods could be enacted to prevent the transport of *Pandanus* spp. from Maui and Moloka'i to other islands that do not have infestations. There is ongoing public outreach on O'ahu to increase awareness about this pest and to achieve eradication in the few established areas. This has already resulted in one localized eradication and positive media releases related to the efforts to mitigate the damage of this pest. There has been an increase in outreach that focuses on the lau hala weaving community. This could reduce the inadvertent spread to other areas through sharing hala leaves. In 2024, I attended the first annual hala festival on Hawai'i island and was able to share information about *T. pandani* to cultural practitioners, visitors, and local residents. This festival was an opportune chance to teach early identification methods to a vast audience through personal conversations, fliers, and a talk story session. I also participated in speaking to a group of Lauhala weavers on O'ahu, and have been invited to speak again in late 2024.

Any accidental introduction of *T. pandani* to a new geographic location near a port or plant nursery in Hawai'i could eventually result in its spread across another Hawaiian island.

Hala trees are found throughout the state from the ports of entry, through residential areas, to the remaining hala forests. I hypothesize that widespread planting of hala has created a natural pathway for *T. pandani* to spread throughout any Hawaiian island if it escapes detection. It is likely that early infestations will be difficult to spot, but increased public outreach may combat this deficit. Future studies should evaluate further products and different insecticide application techniques such as soil drench or chemical injection to treat *T. pandani* infestations. There are upcoming trials on Maui in Fall 2024 that will be utilizing both soil drench and spray applications for a variety of insecticides that have not been tested. This includes soil drench application of imidacloprid, dinotefuran, acephate, and azadirachtin, along with 11 spray treatments recommended by the IR-4 Project Hawaii. The 2024 biological control exploration trips were a start, but there is much more to be done to determine where the native range is for *T. pandani*.

The brief biological control survey to Thailand, Indonesia, and Singapore identified generalist natural enemies that didn't reduce populations of the scales there to lower levels than what I see on *T. pandani* in Hawai'i. The populations that were found were located in botanical gardens and disturbed areas. These may be locations where the scale was introduced, rather than the true native range. There are Coccinellidae on O'ahu that have been recorded predating *T. pandani*. Perhaps increased densities of these could suppress populations of *T. pandani* until a specialist predator can be found, if laboratory testing indicates effective population control. The *T. pandani* infested *Pandanus* varieties within the botanical gardens may provide a better framework for determining the pathway of entry and areas that should have natural populations of *T. pandani*. Within the *Pandanus* spp. varieties that were infested in Indonesia and Singapore, *P. polycephalus* is from the Western Pacific, *P. pygmaeus* is from Madagascar, *P. utilis* is from

Madagascar, and *P. odorifer* is from Sri Lanka, Taiwan, and other areas around the South China Sea (Gallaher 2013). *Pandanus affinis* has been recorded in Madagascar, New Guinea, and the South Pacific. A follow up trip to these areas would narrow down the search for the natural range of *T. pandani*. As varieties in Singapore and Indonesia were both from Madagascar, I hypothesized that East Africa could be part of the native range of *T. pandani*. There are 74 *Pandanus* spp. endemic to Madagascar (Stone 1970), increasing the likelihood that scale insects may be present that have a host preference for Pandanaceae. The utilization of botanical collections for biological control exploration has previously been successful in narrowing down the native range of pests of an unknown origin (Messing et al. 2009). The findings from these gardens of *Pandanus* spp. originating from Madagascar suggests it is a possible location for the native range of *T. pandani*. It is also possible that *T. pandani* is from locations in east Africa, Sri Lanka, or Taiwan.

This led to a collaboration with the University of Antananarivo in Madagascar, where I acquired permits to visit multiple national parks and search for the presence of *T. pandani* and any natural enemies that may be present. I traveled there in August 2024, and was able to visit Ankarana National Park, Analamazaotra National Park, and Masoala National Park. These localities were highlighted in the Stone 1970 *Pandanus* survey as places with easy to find *Pandanus* spp. trees. There was only time for a three week trip, and I was able to cover as many areas as I could. This trip yielded a few interesting results that hopefully will lead to further exploration. The first was the sign of an exit hole, but no parasites. This may be due to the visit taking place in winter, but the temperature was around 27 degrees Celsius that day. The next was the presence of multiple species of scale insects between the vein lines on *Pandanus* spp., but in quantities that suggest the species are under control. This could be due to the nutritional

composition of the plants, biological control, or seasonal dynamics. The first and third undescribed species infest leaves in a similar manner to *T. pandani*, with an aggregation of insects along the inside of the leaves at higher densities. The difference was that these populations did not cover the entirety of the plant, and from a conservative estimate were only on 2% of the trees I inspected. This suggests some sort of control measure, whether it is physiological or biological. DNA analysis hopefully will reveal genetic similarities between these species and *T. pandani* if our collaboration can continue. It is well documented that *Phelsuma* spp. and other reptiles have a commensal association with *Pandanus* spp. and utilize it to hide from predators, increase offspring survival, and for predation on insects in Madagascar (Lehtinen 2002). In 2025, I will be testing whether the *Phelsuma* geckos that are currently in Hawai'i feed on populations of *T. pandani*. This may be able to be implemented into a control program if there is evidence that *T. pandani* population reduction can occur as a result of gecko feeding. At the conclusion of this chapter and my time working on *T. pandani*, it looks like chemical and mechanical control are the best measures that can be implemented currently, but that future research into resident or new natural enemies should remain a priority.

Chapter 4 References

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CHAPTER 5: AN EVALUATION OF THE REGENERATIVE CAPABILITIES OF HALA
FORESTS IN THE HAWAIIAN ISLANDS WITH UPDATES ON THE CURRENT
GEOGRAPHIC DISTRIBUTION AND DISPERSAL CAPABILITIES OF THE HALA
SCALE, *THYSANOCOCCUS PANDANI* STICKNEY

Introduction

Isolation and island size are key drivers of endemism, with these aspects of island biogeography influencing the high number of endemic radiations in the Hawaiian islands (Chen and He 2009, Price 2004). However, these species are experiencing very high levels of endangerment of the endemic flora and fauna, with half of the native flora considered at risk species (Sakai et al. 2002, Weisenberger and Keir 2014). This pattern of endangerment has become common for Hawaiian ecosystems. These areas of endemism have been severely impacted by a variety of factors, such as the introduction of invasive species, which has resulted in ecological shifts where much of the remaining endemism is only found at higher elevations (Koide et al. 2017, Hibit and Daehler 2023 preprint). The impacts of these invasive species tend to be more severe on islands due to compounding factors of land use, climate change, and the disruption of ecological processes (Russell et al. 2017).

Invasive insects can negatively impact native plants and their associated ecosystems. There are many native Hawaiian forest species that have been impacted by the black twig borer, *Xylosandrus compactus*, such as koa, *Acacia koa*; māmakī, *Pipterus* spp.; the endangered mehamehame, *Flueggea noewaraea*, and ‘Ōhi‘a lehua, *Metrosideros polymorpha* (Roy et al. 2023). *Metrosideros polymorpha*, one of the most widespread trees in Hawai‘i, is also under threat from devastating fungal pathogens *Ceratocystis* spp., which causes Rapid ‘Ōhi‘a Death

(Fortini et al. 2019). These forests may face the same rapid decline that Wiliwili, *Erythrina sandwicensis*, encountered in dryland forests, from an invasive gall forming wasp, *Quadrastichus erythrinae*, before biological control agents could be released (Kaufman et al. 2020). The invasive seed-beetle *Specularis impressithorax* was also found to hinder the recovery of these forests (Medeiros et al. 2008). Many endemic Loulou palms (*Pritchardia* spp.) and related species are at risk, as the polyphagous coconut rhinoceros beetle, *Oryctes rhinoceros*, has been detected on the islands of Maui, Hawai'i island, and Kaua'i and is widespread across O'ahu. The impacts of non-native species on Hawaiian ecosystems have already disrupted larger ecological processes, ecosystem function, and threaten the ability for these endemic systems to endure for future generations (Barton et al. 2021).

One of the species possibly at risk from invasive species is the hala tree, *Pandanus tectorius*. The *Pandanus tectorius* complex refers to an extensive radiation of Pandanaceae screwpines across South and Southeast Asia, Australia, and Pacific Islands from Micronesia to Melanesia to Polynesia (Gallaher et al. 2015, Lim 2012). These iconic trees were successfully able to reach and colonize new areas through floatation. This dispersal took place across a wide area from the Western Pacific to the Indian Ocean, and the South-Central Pacific to the Hawaiian islands, indicating the large distances over which dispersal may occur, in which floating seeds can remain viable (Gallaher et al. 2016). As indicated before, the Hawaiian islands are the most remote island archipelago in the world, characterized by high endemism and more recently by high rates of invasive species introductions and extinction (Hembry 2021, Freed et al. 2008, Cox 1999, Leibherr and Polhemus 1997). *Pandanus tectorius* dispersal among the Hawaiian islands is influenced by ocean currents and there is random variation between Hawaiian localities due to

high migration rates with inland populations being less genetically diverse than coastal forests (Gallaher 2017).

Hala trees are found throughout coastal and riparian areas of the Hawaiian islands, and provide valuable ecosystem services, prevent the spread of invasive species, and are integral for Native Hawaiian cultural practitioners (Atkinson 1970, Keawe et al. 2014, McGuire 2022). Hala tree roots and seeds are used for traditional medicine and food. Native Hawaiian weavers utilize dried hala leaves to make everything from floor mats to leis and sails (Keawe et al 2014). Unfortunately, the trees and associated traditions may be threatened by the introduction of the invasive hala scale, *Thysanococcus pandani* Stickney (Halimococcidae), which was first detected in Hāna, Maui in 1995 and subsequently spread across the island (Matsunaga 2021). Since then, it has been detected on multiple Hawaiian islands, with extensive populations on Maui and Moloka‘i and smaller established populations on O‘ahu. This scale species is not parthenogenic (Stickney 1934). Introduction of *T. pandani* on the Cook Islands of Antiu, Mangaia, and Rarotonga resulted in population decline of *Pandanus* spp. (Beauvais and Chouchan 2006, Speith 2012). There are insecticides labeled to treat select individual hala trees in Hawai‘i, but this is not a feasible strategy to protect larger natural areas (Russo et al. 2023).

Infestations of *T. pandani* cause a variety of negative effects from yellowing, early crown drop, branch dieback, and potentially early hala tree death. Matsunaga (2021) suggested that infestations of *T. pandani* could prevent the regeneration of hala forests by causing seedling mortality. If this holds true, the extensive hala forests in Hāna, Maui and in remote areas from Kalaupapa to Hālawa, Moloka‘i are potentially at risk of disappearing. *Pandanus tectorius* trees in Fiji take 10-20 years of growth to reach their flowering stage that can last for 40 years (Ash 1987). Infestations of *T. pandani* reached Hāna in 1995, and Moloka‘i around 2007 (Lola

Spencer, MoPePP staff, April 2023, pers. comm.). Since these introductions, populations of *T. pandani* have become widespread across these islands, providing some information on their dispersal capabilities. There have been at least 15 years of hala forest growth in the presence of *T. pandani* as it spread to remote locations, making it an opportune time to assess if seedlings emerging in forests subject to the hala scale can grow to flowering adults.

Despite hala's significant ecological and cultural importance, to date there are no demographic studies on this species in the Hawaiian islands. Population structure, based on size-classes, is often a good indication of population status and regeneration potential (Rabatnov 1985), and has been used in many studies to assess the effects of threats on plant populations (Szczecinska et al. 2016, Iriando 1996). The population structure can be quantified to assess parameters such as size class, survival, growth and fecundity (Chu and Adler 2014). In this study I conducted a comprehensive hala population survey to evaluate whether *T. pandani* infestations may be associated with reduced seedling and sapling recruitment. Since other factors can also lead to lowered recruitment, including canopy cover, understory invasive species cover, and non-native ungulates (Ticktin et al. 2023, Denslow et al. 2006), I assessed some of these variables including invasive plant species understory cover and canopy coverage. An understanding of the impacts of hala scale can help inform if early mitigating actions are required to conserve hala forests. I also sought to evaluate the current range of *T. pandani* across the Hawaiian islands and to determine its dispersal capability with a small scale field experiment. The combination of these three studies will help determine how fast *T. pandani* can spread, its current geographic distribution in the Hawaiian islands, and if there are long-term ecological concerns with infestations of this pest.

Materials and methods

Statewide survey for T. pandani

In 2021 and 2023 I conducted extensive surveys of hala trees throughout urban, residential, forested areas and rural landscapes on O‘ahu, Kaua‘i, Moloka‘i, Lāna‘i, and Hawai‘i island. These surveys included plant nurseries, and hala trees at airports, universities, hotels, resorts, and ornamental hala trees near potential pathways of entry. Areas of cultural significance, botanical gardens, popular hiking trails and places frequented with tourism were included. In some cases, I visited private residences to inspect hala trees and conduct public outreach about *T. pandani*.

The surveys were relatively straightforward, as healthy trees typically have a green, or variegated appearance, and heavily infested ones will have distinct symptoms (Figure 21). From a distance, a heavily infested hala tree will have discoloration such as yellowing leaves. A closer inspection of the underside of the leaves will reveal a pattern of black dots, with a white waxy fringe. The waxy fringe may be absent from the top of the leaf, but adult *T. pandani* can be present. Trees that exhibited symptoms were observed by hand or with binoculars, while other accessible non-symptomatic trees were surveyed randomly to detect early signs of infestations. The waxy fringe surrounding the hala scale can be wiped off, leaving the entrenched scale insect on the leaf. This method is a good way to screen through residue left by Psuedococcidae and other insects. If a plant has recently become colonized by a few adult *T. pandani*, a close up visual inspection may be necessary to confirm this. It is difficult to detect an infestation in the early stages. After the survey was complete for each tree, a geolocation pin was dropped using

BaseMap™ App. Upon completion of the survey, these pins were downloaded as a KML file and uploaded to Google Earth.



Figure 21: A healthy hala forest (left) compared with a forest with an established *T. pandani* infestation (right).

Dispersal study

Hala seedlings without *T. pandani* were collected from O‘ahu in August 2022 and transported to Maui in January 2023 to the Kahului Cooperative Extension Service Garden located at Maui College. Irrigation lines were set up that watered the potted trees twice a day. The available trial space utilized a grid that is 21.3 by 11.3m. In this rectangular design, one hala tree was placed in the center and inoculated with *T. pandani* specimens. This was done by taking a dozen heavily infested leaves and placing them around the new growth.

The design of the grid was oriented around the existing irrigation lines, which were placed 2 meters apart across the east west axis and longer side of the rectangular field plot. Trees

were spaced 3 meters apart on each of these irrigation lines (Figure 22). Reduced density over distance in the plot was added to evaluate if *T. pandani* would not be able to disperse to the corners of the field plot within the timeframe of the trial, or if they would be able to utilize the adjacent plants as a pathway to spread.

An abundance rating system was used to categorize numbers of *T. pandani* on individual early growth saplings. Adult scales were counted on both sides of the leaf, as other life stages were not visible with a 30x-21mm hand lens. The rating scale was: 1= no *T. pandani* present, 1.5= one *T. pandani*, 2= 2-10 *T. pandani*, 2.5=11-50 *T. pandani*, 3=51-100 *T. pandani*, 3.5=101-150 *T. pandani*, 4= 151-250 *T. pandani*, 4.5=251-350 *T. pandani*, and 5=351 or more *T. pandani* present. I used a rating system as I anticipated faster population growth, and populations can easily exceed 1,000 per plant. This was the best way to quantify growth to higher densities accurately, as some adults develop in frond areas where they are not visible. A visual inspection was conducted monthly to evaluate the spread of *T. pandani* in the experimental plot.

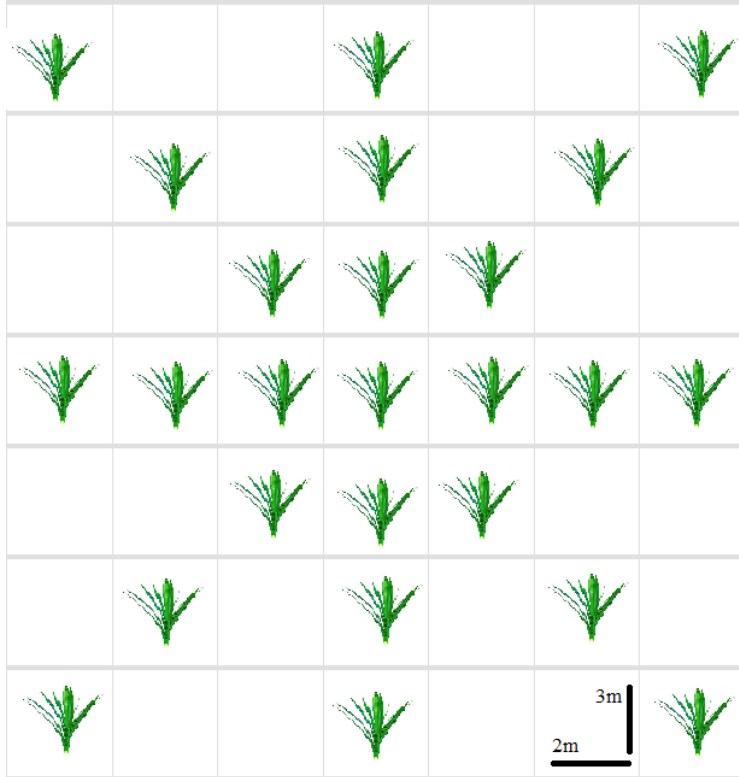


Figure 22: Field plot for *T. pandani* dispersal trial design on Maui. The center tree in the plot was artificially inoculated with *T. pandani* and monthly observations were conducted to determine the rate of spread over time and distance.

Forest Population Structure

I carried out hala population structure surveys between March and November 2023. I surveyed a total of 93 transects in 24 sites representing most of the accessible hala forest areas in the state. The survey sites on Maui were at two coastal forests in Waiānapapa, Hāna airport, and in the National Tropical Botanical Garden (NTBG). On Moloka‘i the survey site accessible by foot was Hālawā Valley, and the remaining sites were accessed via helicopter including Kikipua Peninsula, Hākāano Peninsula, Kaho‘olaiki Bay, and Waiehu Peninsula. Hawai‘i island survey sites were coastal forests in Puna, Hilo, and along the Hāmākua coast. Kaua‘i survey sites were

coastal forests near Hanalei, Hā‘ena, and Anahola. These survey sites were on the northeast coastlines of each Hawaiian island, which is the environmental range suitable for coastal hala forest growth. Areas not surveyed included hala forests on terrain too steep or dangerous to survey, kapu (forbidden) areas with cultural sensitivity, one site at Kalaupapa National Historic Park on Moloka‘i due to permit issues, and one peninsula off Waimanu Valley on Hawai‘i island that was not accessible. In each site I established rectangular transects, 20 m by 6 m in size, with the directions of origin chosen using a random number generator of predetermined compass angles. If any random direction led to insufficient forest or to inaccessible terrain, the next number in the list was chosen for that transect. The transect width was limited to 6 m due to constraints of the terrain.

Within each transect, I recorded information on hala population structure, canopy cover, hala scale presence or absence, elevation, and invasive plant cover in the understory. The *P. tectorius* trees were classified as small saplings at 0-1 m height, larger saplings at 1-2 m height, pre-reproductive trees at 2-3 m height, and adult reproductive size (either male or female) at greater than 3m in height. I made the cut-off for adults at 3m because there were no trees less than 3m in height that had visible seeds or flowering. I used visual estimates of invasive plant understory cover using a rating of 1 indicating 0-20%, 2 for 21-40%, 3 for 41-60%, 4, for 61-80%, and 5 for 81% and above. This was measured at 5, 10 and 15m intervals along the transect. The transect was split into these three centralized points. At each of these increments, a visual assessment was made of the invasive understory cover for the surrounding radius of 5 m. Then the canopy coverage was measured using a spherical densiometer (Forestry Suppliers Inc.) at the

same centralized points the transect. Basemap™ software was used to map out the exact survey sites.

Statistical analysis

I calculated recruitment by dividing the number of saplings of each size class by the total number of adult mature trees. I then divided that by two, to obtain the number of recruits per adult female, with a 50:50 female to male ratio. I excluded one transect because it had no mature trees, preventing the calculation of recruitment ratio, resulting in 92 transects in the total data set. I used generalized linear mixed models to evaluate the potential influence of hala scale and plant cover on hala recruitment ratios. For each size class, I fit models with the recruitment ratio as the response and one or more of the following predictors: hala scale presence/absence, percent canopy cover, invasive understory cover class, and interactions between hala scale and canopy cover and between hala scale and invasive understory cover. Because there were multiple transects per site, I also included the site as a random factor in each model. Models were fit with the zero-inflated gamma distribution and log link function using the glmmTMB package in R (R Core Development Team). I used the dredge function in the MuMIn package to evaluate all possible combinations of predictor variables for each response, resulting in 13 models in the candidate set for each of the three recruitment ratios. I considered all models with AICc values within 2 of the best-fitting models as plausible models explaining hala recruitment ratios. Because the best-fitting model for the larger sapling recruitment ratio was heavily influenced by a single outlier point (see Results), I repeated the model fitting and selection procedure with the outlier excluded for this response.

Results

Statewide Survey for T. pandani

Kaua'i: The 2021 Kaua'i survey evaluated Kaua'i Nursery and Landscaping, Hideaway beach trail and parking lot, Wyndham Bali Hai Villas, Hanalei Valley Lookout, Kolopua Apartments, An'aina Hou Community Park, Kaluakai Beach, Larsen's Beach, Anahola Beach, Kumu Camp, Kapa'a Beach, Līhu'e Airport, Marriott Kauai, Kaua'i High School, Mauka'uwahi Cave Trail, Pō'ipu residential area, Waimea Canyon State Park, and various other locations with ornamental or forested hala (Figure 23). Meetings were set up to evaluate the hala plants at the Allerton Garden, National Tropical Botanical Gardens McBryde Garden, and Na 'āina Kai Botanical Gardens and Sculpture Park. Another visit evaluated the extensive hala forest along the Kalalau trail of the Nā Pali Coast. The Nā Pali Coast attracts many off-island visitors that could be a pathway for *T. pandani* to invade its hala forests. There was no detection of *T. pandani* during these trips. In 2023 these locations were revisited, and other gaps from the previous survey such as the Limahuli Garden were visited. There was no detection of *T. pandani* on either of these surveys.

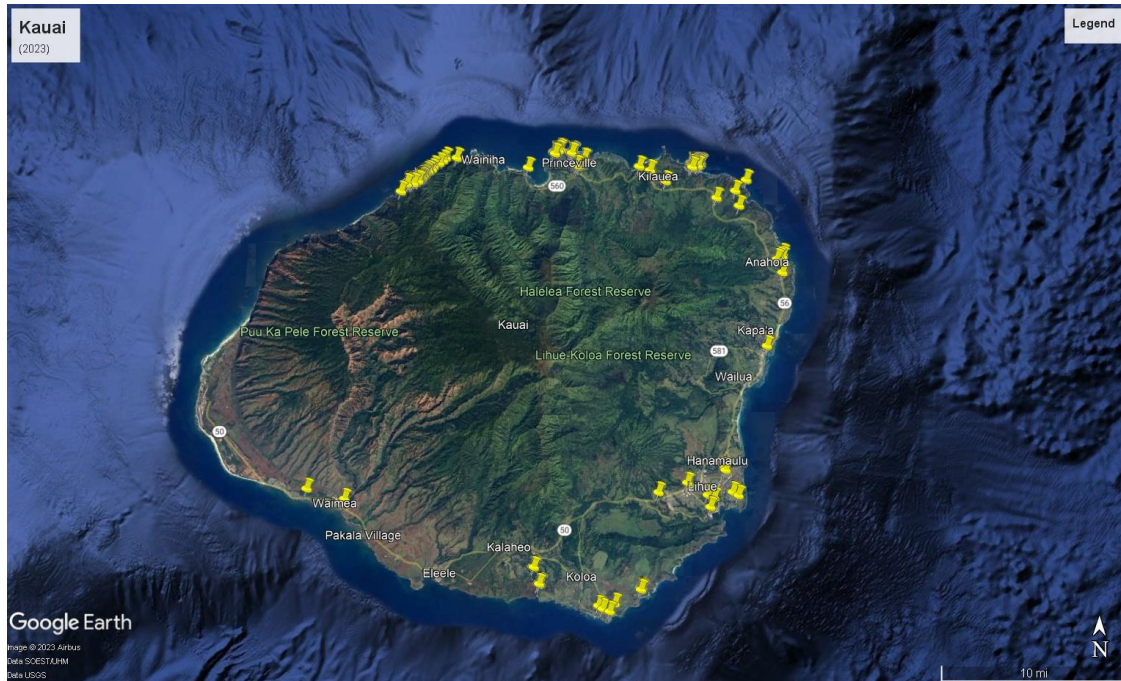


Figure 23: Kaua‘i surveys conducted during 2021 and 2023. Yellow pins indicate no infestation of *T. pandani* at available survey sites.

O‘ahu: The O‘ahu survey (Figure 24) re-confirmed the known hala scale populations on the Chaminade University Campus, University of Hawai‘i at Mānoa campus and at various resorts in Waikīkī (Matsunaga 2021). In 2022, there were successful efforts to eradicate the University of Hawai‘i at Mānoa population. Around 15 infected trees were cut down, and incinerated. Healthy trees on the University of Hawai‘i at Mānoa campus received preventative imidacloprid treatments during this timeframe. In 2024, a population was detected at Punahou high school by its staff and was brought to our attention.



Figure 24: O‘ahu surveys conducted during 2021 and 2023. Yellow pins indicate no infestation of *T. pandani* at available survey sites, while a red pin indicates infested plant clusters.

Maui: There was no survey conducted for the island of Maui as the insect was already considered widespread throughout the island. This was confirmed during visits to Maui for other studies.

Moloka‘i: With an established population known on Moloka‘i, the 2021 survey aimed to evaluate the extent of the population (Figure 25). The survey sites were the Molokai airport, Hālawā Valley, Pu‘u O hoku Ranch Store, Pūko'o Beach, Kanewai Schools Molokai Regional Resource center, Ke Nani Kai Vacation rentals, Manae Goods and Grindz, and various sites along the road to Hālawā valley. The result of this survey confirmed the established populations and indicated that the population of *T. pandani* is widespread across the island. In 2023, I visited the coastal hala forests located in the northeast areas of Molokai via helicopter and confirmed that the hala scale is widespread across the area from Kikipua and Hākāno peninsula, to

Kaho'olaiki Bay to Waiehu peninsula. The hala forests near Kalaupapa National Historic Park had yellow discoloration visible from the helicopter, but I was not permitted to land there due to restrictions.



Figure 25: Moloka'i surveys during 2021 and 2023. Yellow pins indicate no infestation of *T. pandani* at available survey sites, while red pins indicate infested plant clusters.

Lāna'i: This survey was conducted alongside Pūlama Lāna'i employees in 2021 and in 2023 they conducted a follow up survey on their own (Figure 26). The sites included Koele Resort, City Nursery, Club Lāna'i, Laser Lāna'i Archery and Shooting Range, City Nursery Sod Farm, Airport Nursery, Kaunoa drive and Four Seasons Lāna'iat Mānele Bay. I was unable to access the hala trees present at Kapano gulch due to a rockslide that blocked the road. However, the previous hala scale population near Mānele Bay was successfully eradicated. Their eradication tactic was to spray bifenthrin (Talstar®), cut down the tree, bury the plant debris, and then soak

the soil with dinotefuran (Safari[®]). As of 2023 there has been no further detection of *T. pandani* in the surveyed locations.

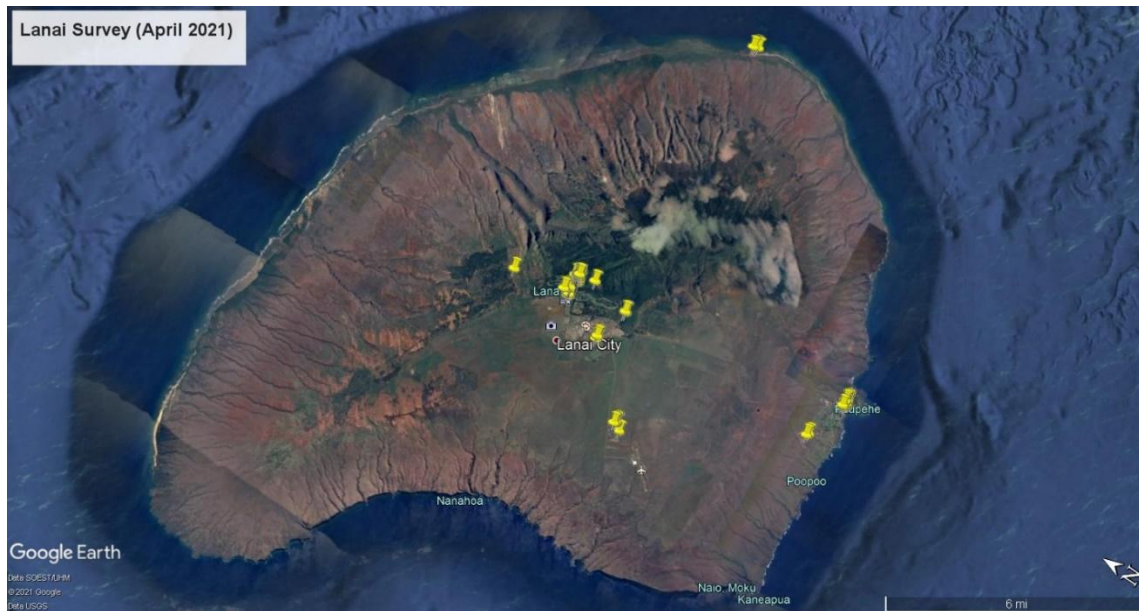


Figure 26: Lāna‘i survey conducted during April 2021. Yellow pins indicate no infestation of *T. pandani* at available survey sites, while red pins indicate infested plant clusters.

Hawai‘i island: Multiple surveys of Hawai‘i island were conducted in 2021 and 2023 (Figure 27). Survey sites included Kona Airport, Puma Nursery, Pu‘uhonua o Hōnaunau, Hōnaunau school, Amy B. H. Greenwell Ethnobotanical Garden, Keauhou Resort Condominiums, Hilo Walmart, Mountain Meadows Nursery, Queen Lili‘uokalani gardens, Waipi‘o Valley Trail, Hilo airport, Waiākea Peninsula, Carlsmith Beach Park, Lāupahoehoe Beach Park, and the University of Hawai‘i at Hilo. Conversations with plant nurseries indicated they do not import hala but grow it from locally collected seeds. There was difficulty accessing the site on the Hamakua Coast in 2021 previously infested with *T. pandani*. In 2023 this location was accessed, along with a comprehensive survey of the Kona coastline resorts, Waimanu valley, and Puna hala forests. The

eradication strategy at the Hamakua site was to cut down and bury the infested trees and treat the trees nearby. This site and all other sites were confirmed as negative in 2023 for *T. pandani*.

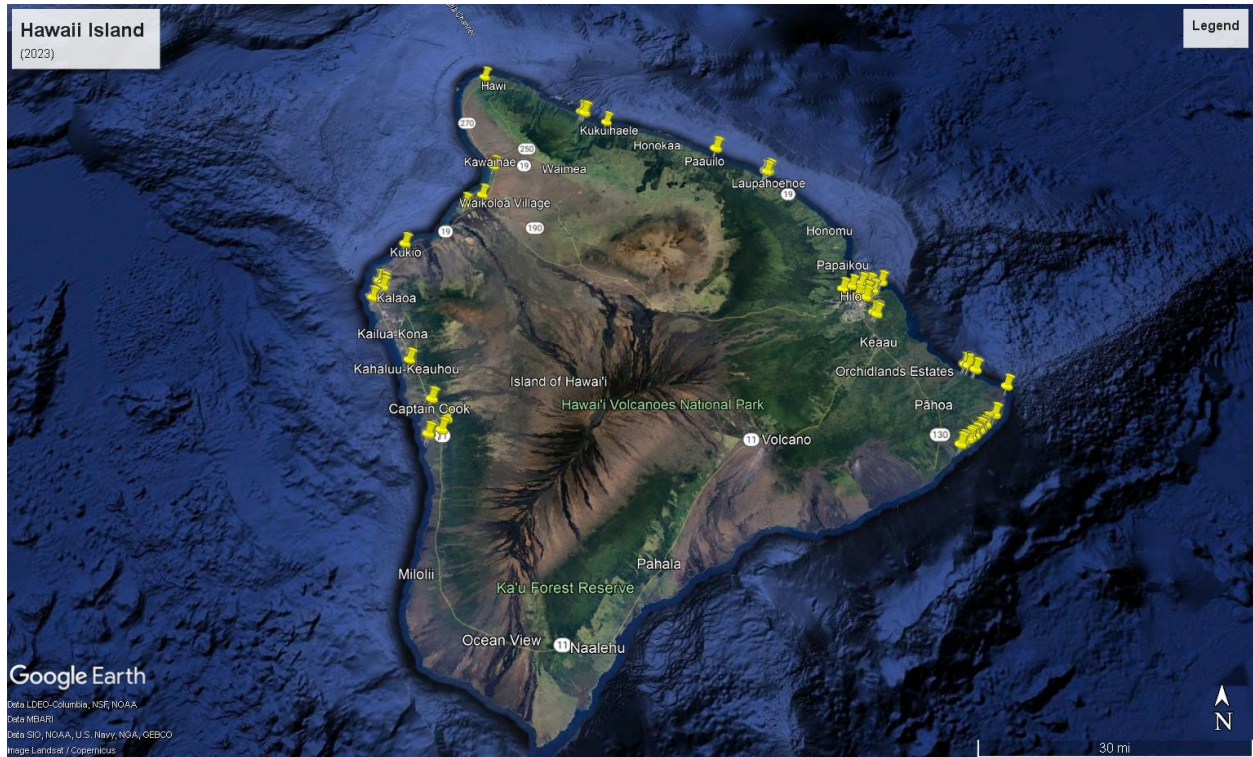


Figure 27: Hawai'i island survey conducted during April 2021. Yellow pins indicate no infestation of *T. pandani* at available survey sites.

Kaho'olawe and Ni'ihau

I did not survey Ni'ihau. Kaho'olawe had the last hala tree die in early 2021.

Dispersal study

After inoculation, there were no visible signs of *T. pandani* until three months into the trial on the center tree, upon which a few scales were present. Seven months into the trial, two trees downwind of the inoculated center had scale detections (Figure 28). The population of *T. pandani* was below ten adults per infested plant at this point. Eight months into the trial, the

population spread to an additional seven trees, while at month 9, there were only 4 trees remaining without *T. pandani* present. At month 10 *T. pandani* was confirmed on every single tree in the plot (Figure 29). Abundances remained between 1-10 total scales for many of the trees. The center tree had an infestation level that increased from less than 10 scales at month 8, to over 200 scales at month 9, with over 250 scales (rating of 4.5) present 10 months into the study.

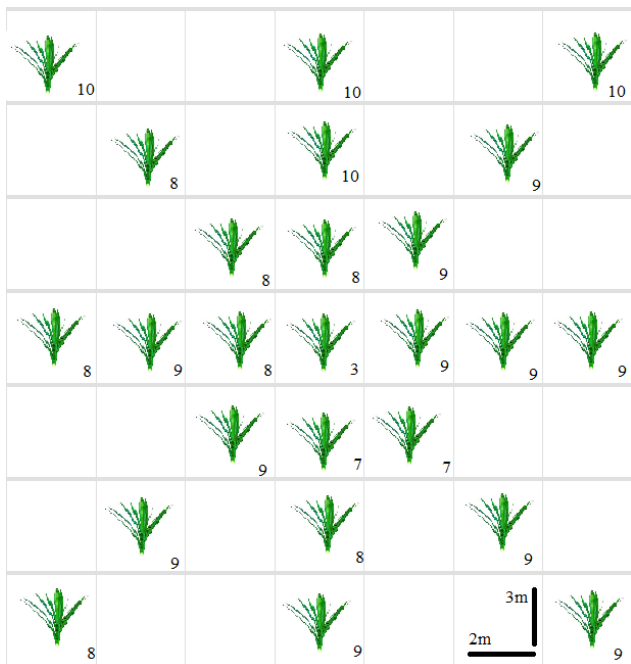


Figure 28: Aerial illustration of field plot with the first observed adult of *T. pandani* on each hala tree. The center tree took three months from initial artificial inoculation to have visible signs of *T. pandani*. Each grid contains the number for the month in which *T. pandani* was detected.

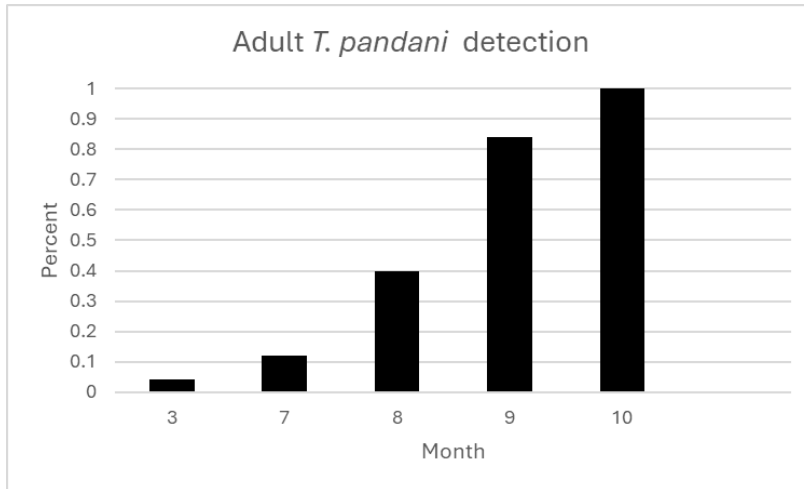


Figure 29: The spread of *T. pandani* across hala trees within the field plot quantified as the percentage of plants over time.

Forest Population Structure

Across four of the major Hawaiian islands I recorded 6599 total hala trees in our 93 transects (Table 11). This included 4922 small saplings, 385 larger saplings, 349 pre-reproductive trees, and 943 adults (reproductive size trees). Transects varied from hala monostands to mixed forests, with differing levels of invasive understory cover under 25%. Transects on Maui had less than 70% canopy coverage, compared to greater than 80% coverage on Moloka'i, Hawai'i Island, and Kaua'i (Figure 30). Figure 33b shows that islands with hala scale had decreased canopy, compared to islands that did not. Moloka'i, Maui, and Hawai'i island had coastal hala forests at elevations of 30m or less, while Kaua'i had forests higher than 60m.

Table 11: Total survey sites and transects by island for the forest population survey.

	Hawai'i island	Kaua'i	Maui	Moloka'i
Sites	9	6	4	5
Transects	23	20	19	31

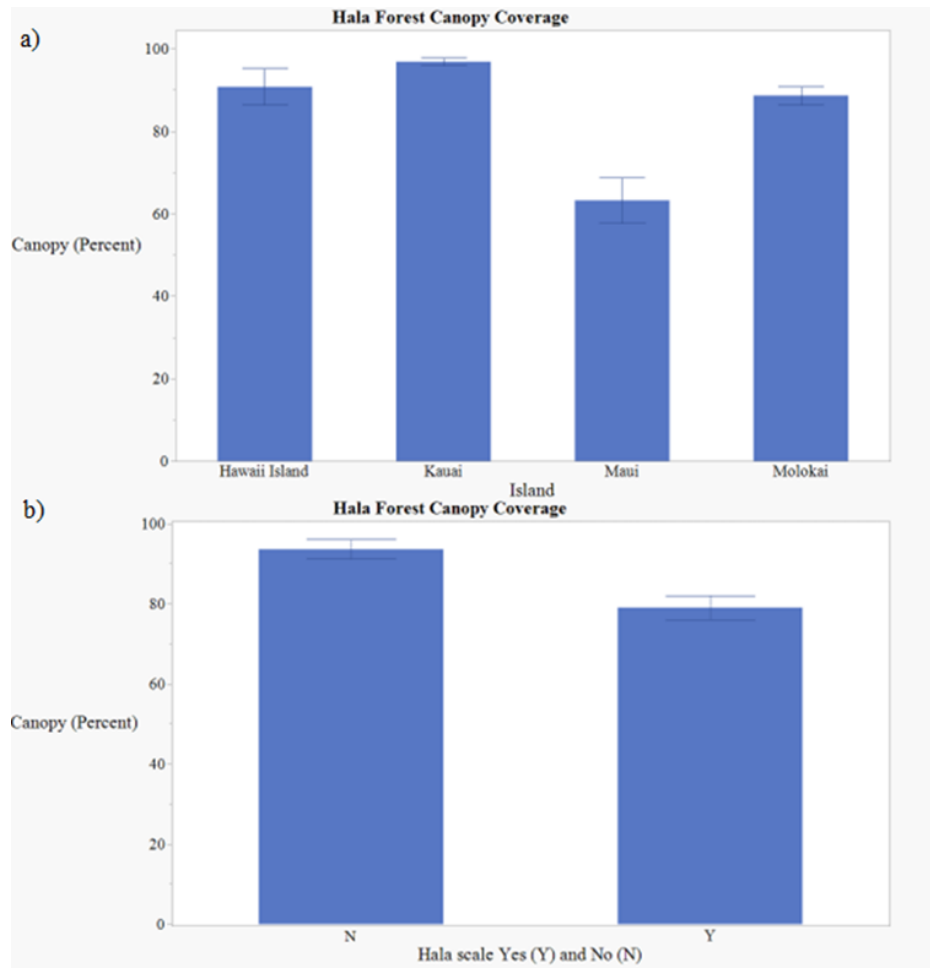


Figure 30: The percent canopy coverage by island (a), and by hala scale presence or absence (b).

This was measured over 12 data points per transect using a spherical densiometer across all forest sites. Error bars are one standard error from the mean.

Six models had reasonably similar fits for the smaller sapling recruitment ratio response, with AICc values within 2 of the best-fitting model (Table 12). These included the null model, and for all of the other models, each of the predictors had coefficients whose confidence intervals spanned zero. This suggests that none of the predictors, including hala scale presence, explained

much variation in the smaller sapling recruitment ratio. For the pre-reproductive recruitment ratio response, the best fitting model included only percent canopy cover as a predictor (Table 13). However, the confidence intervals for this coefficient spanned zero, and the model confidence set for this response also included the null model. This again suggests that none of the predictors explained much variation in the pre-reproductive recruitment ratio.

Table 12: Confidence set of best fitting models fit to the smaller sapling recruitment ratio.

Model _a	Model ID	Log-Likelihood	AICc	ΔAICc	Weight	df
HS	1	-134.69	280.08	0	0.27	5
US	2	-135.34	281.38	1.30	0.14	5
Null	3	-136.48	281.42	1.34	0.14	4
HS, US	4	-134.44	281.88	1.80	0.11	6
CC, HS, US	5	-409.45	831.88	1.51	0.09	6
HS, US, HS*U	6	-409.51	831.99	1.62	0.08	6

a cc indicates canopy coverage, HS indicates Hala scale present/absent, and US indicates invasive understory.

Table 13: Confidence set of best fitting models fit to the Pre-Reproductive recruitment ratio.

Model _a	Model ID	Log-Likelihood	AICc	Δ _i	Weight	df
CC	1	-125.52	261.74	0.0	0.27	5
Null	2	-127.17	262.81	1.1	0.16	4

a cc indicates canopy coverage, HS indicates Hala scale present/absent, and US indicates invasive understory.

In contrast, the candidate set for the larger sapling recruitment ratio response included only a single model, with all other models possessing delta AICc values >2 (Table 14). Furthermore,

the best-fitting model had statistically significant coefficients for the interactions of hala scale presence with both canopy cover and invasive understory cover (Table 15). This suggests that in the presence of hala scale, larger sapling recruitment is higher at lower percent canopy cover and at higher invasive understory cover (Figure 31). However, examination of these results revealed that these patterns appeared to be driven by a single outlier data point, involving one transect with zero percent canopy cover. When models were rerun without this point, four models had reasonably similar fit, including the null model (Table 16). Furthermore, none of the predictors in the other models in the confidence set had coefficients whose confidence intervals did not span zero. This suggests that when this outlier data point is excluded, none of the predictors explained much variation in the larger sapling recruitment ratio, similar to the other two recruitment ratios.

Table 14: Top four best-fitting models fit to the larger sapling recruitment ratio.

Model _a	Model ID	Log-Likelihood	AICc	Δ AICc	Weight	df
cc, HS, US, cc*HS, US*HS	1	-153.13	326.44	0	0.77	9
cc, HS, cc*HS	2	-157.80	330.92	4.48	0.08	7
cc, HS, US, cc*HS	3	-156.89	331.50	5.07	0.06	8
cc	4	-161.30	333.29	6.85	0.03	5

a cc indicates canopy coverage, HS indicates Hala scale present/absent, and US indicates invasive understory. Interaction is indicated by *.

Table 15: a. Best-fitting model explaining the larger sapling recruitment ratio, using the full dataset including outlier point. b. Coefficients and CI for statistically significant interactions.

Predictor	Chi-square	DF	Pr(>chisq)
Hala Scale (Y, N)	1.44	1	0.2306
Canopy Coverage	1.91	1	0.1671
Invasive Understory	2.1	1	0.1471
Hala Scale (Y, N)*Canopy Coverage	13.93	1	0.0002
Hala Scale (Y, N)*Invasive Understory	10.32	1	0.0013

a.

Predictor	Coefficient	SE	asyp UCL	asyp LCL
Hala Scale (Y)*Canopy Coverage	0.022	0.011	-0.00141	0.0454
Hala Scale (N)*Canopy Coverage	-0.038	0.012	-0.0592	-0.0169
Hala Scale (Y)*Invasive Understory	0.260	0.075	0.1135	0.4068
Hala Scale (N)*Invasive Understory	0.011	0.020	-0.0278	0.0506

b.

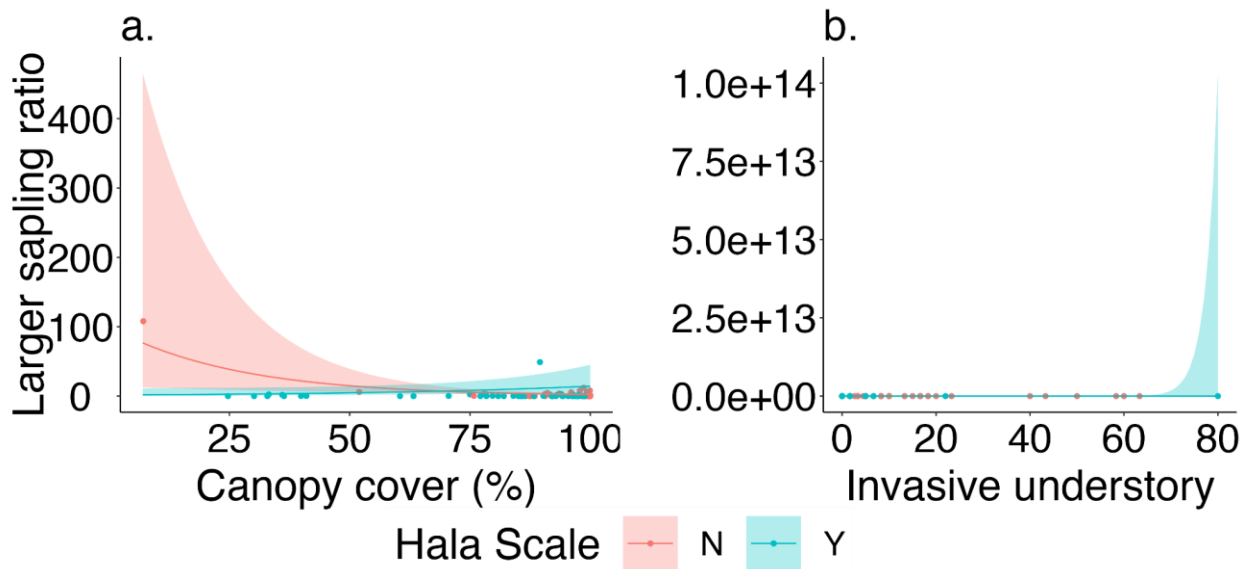


Figure 31: a. The relationship between hala canopy coverage, larger sapling ratio, and the presence or absence of hala scale on survey sites. b. The relationship between invasive plant understory, larger sapling ratio, and the presence or absence of hala scale. These include a single site with zero canopy and no scale presence.

Table 16: Confidence set of best fitting models for the larger sapling ratio excluding an outlier point. 91 transects are included.

Model _a	Model ID	Log-Likelihood	AICc	ΔAICc	Weight	df
HS	1	-134.69	280.08	0	0.27	5
US	2	-135.34	281.38	1.30	0.14	5
Null	3	-136.48	281.42	1.34	0.14	4
HS, US	4	-134.44	281.88	1.80	0.11	6

a cc indicates canopy coverage, HS indicates Hala scale present/absent, and US indicates invasive understory.

Discussion

The flora of the Hawaiian islands has been highly impacted by introduced plants, mammals and insects (Barton et al. 2021). This dissertation has shown many examples where Hawaiian ecosystems are negatively altered by the introduction of invasive species. I wanted to conduct an early analysis to determine if the introduction of *T. pandani* poses a threat to the long-term status of hala forests in the Hawaiian islands. Our forest population study was the first to evaluate whether *T. pandani* presence may be associated with decreased *P. tectorius* recruitment. Although there are still uninfested hala forests throughout the islands, the early detection of damage from invasive species is essential to set up long term management plans. The hala forests I surveyed on Moloka‘i are estimated to be only 150 years old, as the areas were previously disturbed by Hawaiian settlements (Ane Bakutis, MoPEPP, personal communications April 2023). The previous disturbance highlights the success for *P. tectorius* to re-establish in areas following disruptions. As there was no statistical difference in recruitment of the smallest saplings, this may indicate that *T. pandani* does not affect fruiting or the potential to germinate, and thus has no fitness impact on plants of this size. As hala trees mature and the infestations

develop, the presence of thousands of adults of *T. pandani* on leaves across the canopy can potentially decrease photosynthesis, canopy size, tree vigor, and serve as a reservoir for wind transport of further *T. pandani* populations. In some cases, invasive scale insects can cause significant damage without the presence of biological control agents (Hanson and Miller 1984). It is possible that the pre-reproductive stages were not impacted as they were already established prior to the introduction of *T. pandani*. I noted that *T. pandani* infestations did not cause the dieback of adult (reproductively mature) trees, therefore our regeneration ratio of recruits: adults is an appropriate measure for identifying the effects of *T. pandani* on regeneration. The larger saplings have developed from smaller saplings with exposure to high levels of *T. pandani* populations, potentially impacting their fitness. As it was noted in the results, the statistical significance was influenced by one outlier. The effect vanished with the removal of this outlier, but this outlier was the only point that reflected the growth of *P. tectorius* in an area with zero canopy on islands without *T. pandani*. Regeneration in canopy gaps can be an early indicator for the success of forest regeneration (Denslow et al. 2006). There is a chance that there would have been more data points with a similar distribution if I had more transects in areas with these factors.

With invasive insects in other systems, such as the invasive emerald ash borer, *Agrilus planipennis*, the establishment of permanent populations led to the decreased viability of regenerative stems, and the authors suggest that regeneration of tree populations will likely not occur with existing seedling population levels (Aubin et al. 2015). Although a wood-boring insect has different impacts on a tree than scale insects, the introduction of an invasive species can have the capacity to alter entire ecosystems and disrupt the flow of ecosystem services (Kenis et al. 2009). The impact of *T. pandani* on hala monostand coastal forests could result in

an eventual mixed forest structure, allowing non-native species to occupy areas previously with hala trees. Hala stands on islands with *T. pandani* had numerically less pre-reproductive trees. Perhaps a future survey could evaluate factors that may be limiting this size class.

There are many factors that impact forest regeneration such as invasive ungulates, non-native trees, and topography. Ungulates in Hākāno and Kikipua Peninsulas on Moloka‘i reduced regeneration in general, as they were characterized by limited regrowth and unmanaged goat and deer populations. Management efforts are currently underway to address this issue (Ane Bakutis, MoPEPP, Personal communication September 2023). Transects in the hala forests on Maui, Hawai‘i island, and Kaua‘i showed that populations are being encroached upon by ironwood, *Casuarina equisetifolia*. This species has inhibited the growth of native forests on other Pacific Islands (Hata et al. 2009). Ironwood was chosen as a reforestation tree in the Hawaiian islands due to its ability to grow in poor soils through nitrogen fixation. At many of our sites, hala forest appeared to be constrained by the expansion of *C. equisetifolia*. *Pandanus tectorius* forests and *C. equisetifolia* both grow in the same coastal areas. Pandanus forests likely provide better ecological services as *C. equisetifolia* changes the forest water balance, by changing soil conditions (Hata et al. 2015). Unfortunately, due to the status as a reforestation tree, there are no management efforts in place to mitigate the expansion of *C. equisetifolia*.

Studies on the efficacy of insecticides have been conducted, showing that it is possible to protect individual landscape hala trees from *T. pandani* infestations, but this approach cannot practically be applied on an entire forest (Russo et al. 2023). The dispersal study on Maui determined that within a short time frame, *T. pandani* can easily spread throughout a patch of healthy hala saplings. It is unlikely that crawlers reached the trial in the wind from outside areas. There are no visible *P. tectorius* trees within sight of the field plot, but there is one tree that is

800m away. It would have been preferable to have a much larger field plot, as this was a limitation to the study. Nonetheless, this trial filled a knowledge gap, as there is little literature available about this insect.

From an urban landscape perspective, hala trees are easy to manage. This makes them frequently utilized as landscape elements and potentially provides a pathway for *T. pandani* to spread across an island from urban to rural areas. The dispersal mechanism for the populations in the remote forests of Moloka'i is unknown. The probable scenario was that populations arrived on landscape plants from Maui, and the scale insect subsequently spread widely on the island via wind dispersal. Southerly Kona winds also could have facilitated the spread of *T. pandani* to Moloka'i from Maui. According to the landowners, the eradicated populations of *T. pandani* on Lāna'i and Hawai'i island arrived via landscape plants (Personal communications Pūlama Lāna'i May 2021 and Kamran Fujimoto March 2023). Unfortunately, the most effective eradication tactics in these areas are to cut down, treat, and bury or incinerate the infected trees to ensure complete removal of insect populations. Preventative measures with the interisland movement of potentially infested material can help mitigate or stop the arrival of *T. pandani* for years to come. The results of the surveys showed that populations of *T. pandani* are not yet widespread across all major Hawaiian islands and that efforts to contain infestations detected by private individuals were successful.

As we experience a resurgence in Hawaiian cultural practices, a unique opportunity to shift attention to conserving ecosystems associated with these customs presents itself. Until more studies are done on the impact of *T. pandani*, mitigative measures should be enacted with the transportation of *P. tectorius* material between Hawaiian islands to prevent spread. As Hawaiian traditions continue to be passed down, the presence of native plant based weaving materials is

integral to a number of Hawaiian traditional practices. The following quote highlights the necessity of weaving to cultural practitioners: “We cannot weave without lau niu (coconut palm leaves) or lau hala (pandanus leaves) or makaloa (sedge), or the aerial roots of the ie’ie (freycinetia). We cannot practice our art without those plants. We are related to these plants. Our legends tell us that when certain of our plants disappear, we too, will disappear” (MacDowell et al. 2014).

As Chapter 4 mentions, biological control exploration is being conducted by our group to determine if there are any insects in the other *T. pandani* positive locations that can be eventually utilized to reduce populations to lower levels of infestation. Stickney (1934) utilized specimens from a Botanical Garden in Bogor for *T. pandani* description, and recent surveys have found *T. pandani* in Singapore as well. A 2024 survey to these areas assumed that these are part of an introduced range, as the density of *T. pandani* was similar to the Hawaiian islands and were only found in managed areas. It is possible that this level of infestation is present in a native range and that *P. tectorius* trees are tolerant to this feeding. Future surveys will hopefully be conducted to determine if trees in the botanical collections are from the native range of *T. pandani*, as the collection had specimens from Madagascar, Sri Lanka, Taiwan, and other Pacific islands. It is likely that one of these may be the native range and may host potential classical biological control agents. A 2024 trip to Madagascar found three species of scale on *Pandanus* spp., one of which had similar morphology and behavioral characteristics associated with *T. pandani*, along with the presence of exit holes of a possible parasitoid on one of these species. The local guides there suggested that *Phelsuma* spp. geckos have been seen feeding on these scales and are associated with *Pandanus* species across the country. Hala forests in the Hawaiian islands can be protected in the short term by tending populations to remove non-native plant species to ensure

gaps for regeneration, and also exclude ungulates. Monitoring these forests over many years is recommended, as possible differences in regeneration may become more apparent over time.

Chapter 5 References

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CHAPTER 6: GENERAL CONCLUSION, NOTES ON BIOSECURITY, AND DISSERTATION FINDINGS

General Conclusion and the Current Status of Biosecurity in Hawai‘i

Although Hawai‘i is one of the most remote archipelagos in the world, they are no longer the thriving endemic ecosystems that the Polynesians encountered upon their arrival. By the arrival of the distinguished entomologist Robert CL Perkins in the late 1800’s, he had noted that the changes to the ecosystems due to alien organisms were already underway (New 2009). The journals of RCL Perkins documented areas across the Hawaiian islands under enormous anthropogenic stressors, and some of these areas today are devoid of their original plants and insects. His bird and arthropod collections provide an excellent snapshot of an era in Hawai‘i that is lost, and his journals serve as a reminder for us to try to save what is left. Many chapters in this dissertation have noted the ecological decline due to the introduction of a myriad of invasive species. These plants, animals, and pathogens have been detrimental to the native ecosystems and caused irreversible shifts to areas once characterized by endemic species. Many of these shifts are ongoing, and the results will only be quantifiable for generations to come.

The chapters in this dissertation encompass a variety of studies or experiments that set out to mitigate the impacts of *T. pandani* and *O. rhinoceros* on Hawai‘i’s urban and forest ecosystems. The exploration of different integrated pest management tactics are not always successful, but important in detailing the scientific process for figuring out which methods will be able to be utilized for immediate implementation and which require further exploration. The research pertaining to *O. rhinoceros* will help manage populations but will not eradicate populations from O‘ahu. The combination of these methods with other integrated pest

management strategies may be enough to eradicate populations on other islands, but the initial pathways of entry need to be contained to ensure continued eradication. The current regulations for green waste transport are improving, but there are gaps that can still facilitate the pathway of larvae or eggs to new islands or the mainland. For example, sod grass facilities in Kunia, O‘ahu, utilize mulch that is likely contaminated with *O. rhinoceros* for their operations, but the grass shipments to other islands may not be regulated or fumigated for potential eggs or 1st instar larvae in the material. This is one of many small flaws that *O. rhinoceros* could utilize to escape detection and be moved unintentionally. There needs to be consistent exclusion protocols in place to ensure that there are not repeated introductions to other islands, for eradication to be considered possible. This is assuming that the populations on other islands are at a threshold that is still possible to eradicate. This is not the case on Kaua‘i, but Hawai‘i island is still eradicable as of Fall 2024.

An efficient biosecurity protocol can increase interceptions of target pests. Some analyses of pest interceptions indicate that new detections are most likely to occur at airports, with the remainder taking place at land crossings and marine ports (Rainwater 1963, McCullough et al. 2006). A study indicated that most insect interceptions are found in the baggage of airline travelers, which could be a cause for concern given the reliance on tourism throughout the state of Hawai‘i (Leibold et al. 2006). Insects are least likely to be detected in refrigerated air and boat cargo and Hawai‘i’s reliance on large scale importation of fresh produce, packaged food and supplies creates an ideal pathway for insects to enter undetected (Work et al. 2005, HB547 2019). There are mitigation methods that can prevent the spread of invasive insects through these likely points of entry such as light trapping on cargo ships, the development of port trapping systems, and enhanced port biosecurity (Gressitt and Nakata 1958, Oteng-Amoako 2012). There

also could be the implementation of ecologically inhospitable areas near ports that can mitigate the spread of species that are hitchhiking to Hawai‘i. These areas would be devoid of plantings that can serve as a pathway for high risk invasive species to spread. An emphasis on pre-border inspection can help decrease the introduction of pest species of concern to Hawai‘i.

An effective biosecurity plan will utilize pre-border, border, and post-border detection systems to mitigate invasive species introductions. Hawai‘i’s interagency biosecurity plan was set up to mitigate the impacts of invasive species on tourism, watersheds, horticulture, native species, agriculture, and impacts on human health. Despite the wide reach of invasive species across many areas of economic importance, many programs are underfunded (State of Hawai‘i Department of Agriculture 2017). The top five most costly current invasive species in 2017 for Hawai‘i were feral ungulates, coffee berry borer, *Hypothenemus hampei*, Miconia, *Miconia calvescens*, small predators and rodents, and the little fire ant, *W. auropunctata* (State of Hawai‘i Department of Agriculture 2017). These are the costliest, but similar funding is likely necessary to manage other pests that are expanding their post-border distribution. Pre-border detection is preferable as costs are less than managing eradication programs. The brown tree snake, *Boiga irregularis* is predicted to cause \$2.14 billion in economic damage to the Hawaiian islands per year if it were to become established, while the red imported fire ant, *Solenopsis invicta*, would be estimated to cause \$211 million a year in management costs. Surely the economic impacts of these pests would instigate rigorous monitoring for their arrivals? This monitoring could be utilized to monitor many other species within or not yet in Hawai‘i. The establishment of *S. invicta* in Europe for the first time was confirmed in 2023, highlighting that pathways exist for this pest to travel across the world and evade detection (Menchetti et al 2023). Biosecurity protocols for *S. invicta* could also reduce interisland spread of *W. auropunctata* and other

potential pests of concern. There are already severe agricultural impacts of *W. auropunctata* on Hawai‘i island, as growers are having trouble retaining employees due to extensive stinging during papaya harvest (personal communication, Shipman family 2023). The immediate impacts on harvest efficacy highlight the need to protect agricultural areas not impacted by this pest through enhanced biosecurity.

Even with improved biosecurity, there should be an emphasis on public outreach to educate individual landowners or businesses regarding the economic and ecological impacts of the widespread distribution of invasive pests. Nursery growers, landscaping businesses, and box stores with compost may be hesitant to report the presence of *W. auropunctata* or *O. rhinoceros* larvae out of the fear of a loss in profits. The results of a few bad actors can be the failure of eradication programs, as the pathways of dispersal reach a point that cannot be contained, with the later economic cost being much larger than the underfunded initial eradication budgets set out to manage these populations. This is likely to be the eventual scenario for many pests in the Hawaiian Islands. Unfortunately, there is not enough funding to eradicate pests of concern, conserve entire ecosystems, or protect every endangered species (State of Hawaii Department of Agriculture 2017). There are many initiatives to motivate the public to plant native species and pursue conservation goals through community service.

The Plant Pono initiative is a great example of a multiagency group that educates homeowners and businesses on the safety of planting certain species. It is a partnership between the Hawai‘i Invasive Species Council (HISC), Coordinating Group on Alien Pest Species (CGAPS), the Hawai‘i Biological Information Network, and the Landscape Industry Council of Hawai‘i (LICH) (<https://plantpono.org/>). This resource utilizes a Hawai‘i Pacific Weed Risk Assessment criterion to inform landowners about the benefits or risks of planting certain species

on their property. Plant risk assessments are used globally to determine the risks of a plant or pest species becoming economically or ecologically damaging, considering introductions in previous areas (EFSA Panel on Plant Health 2018). These are very accurate and can be helpful to mitigate the introduction of many species of concern. The scoring systems in these assessments can be utilized to predict the risks of introducing specific plant species to an area as an ornamental planting.

The urban and residential areas of the Hawaiian islands fit a relatively similar ecological profile, with choice landscape plants being utilized across the landscape to provide various benefits to the homeowner or area. There is a familiar pattern of fruit trees, landscape trees and palms, ornamental shrubs, and native plants that can be seen in residential areas. The predictability and diversity of plants throughout the landscape could create pathways that invasive species can utilize to facilitate their movement across an island. There are many pathways of entry for a pest in Hawai'i, but the spread of the pest could be mitigated upon entry if there is not enough habitat to survive. The ecologically predictable areas around harbors, residential areas, agricultural zones, and rural ecosystems provide a path for insects and pathogens to spread and reach areas where containment is no longer feasible. The establishment of a biologically hostile area around ports could prevent this but is not feasible in Hawai'i. This leaves an urban landscape that is exposed to invasion and that also potentially facilitates a pathway to areas of ecological vulnerability.

There are many examples where agricultural plants, or landscape plants in Hawai'i facilitate the spread of invasive insects or pathogens. Naupaka, *Scaevola taccada* is frequently planted at resorts and even inland areas as an ornamental plant. This species is susceptible to cucumber mosaic virus, which also negatively impacts agricultural plants such as banana,

lettuce, cucumber, and tomatoes (Dragich et al. 2014). There are at least 15 endemic species of *Hibiscus* (Malvaceae) in the Hawaiian islands, and it is likely that these varieties are susceptible to infestations of the lobate lac scale, *Paratarchardina pseudolobata*, a polyphagous scale insect that is found throughout the Hawaiian islands (Roe 1961, Cheng and Bhandari 2015). There are extensive non-native plantings of *Hibiscus* across the Hawaiian islands that facilitate a pathway for this insect to spread. There are other ornamental plants that are non-native but highlight the ability of invasive species to quickly spread. Plumeria rust, *Coleosporium plumeriae*, was detected on O‘ahu in 1991 and is now found across the Hawaiian islands (Ogata and Gardener 1992). Ornamental banyan trees such as *Ficus microcarpa* and *F. benjamina* are frequently found throughout the urban landscape and in forests. They are currently under threat from *Josephellia* spp. leaf and stem gall wasps, *P. pseudolobata*, and a psyllid pest, *Macrohomotoma gladiate* (Matsunaga 2022, Russo et al. 2023). These pests have prompted recommendations to plant different *Ficus* spp. However, they may serve as biological control to mitigate the growth of these trees in Hawai‘i’s forests as they reduce canopy and fitness (Dr. Zhiqiang Cheng unpublished data). The avocado lace bug, *Pseudacysta perseae* was found on O‘ahu in 2019 and has reduced fruit yield on some avocado varieties in some areas on O‘ahu (Matsunaga 2022). Papaya is found throughout the residential to agricultural landscape and is host to a series of pests such as papaya ringspot virus, white peach scale, *Pseudaulacaspis pentagona*, several mite species, and the papaya mealybug, *Paracoccus marginatus* (Constantinides 2005). Bananas also are planted in similar areas and a report in the 1990’s highlighted how simultaneous detections in residential and agricultural areas were found indicating the presence of banana bunchy top disease (Conant 1992).

The Hawai'i Data book has indicated that 47% of land in the state is designated as agricultural, 48% is for conservation, while 5% is urban (Land Use Division). This highlights the percentage of human managed ecosystems and conservation areas under threat from invasive species. There is not enough funding to adequately manage the threats from invasive species across these diverse areas. The design of the urban and agricultural landscapes has helped to facilitate the spread of invasive species to Hawaii's forests and remote areas. Chapter 5 discussed the devastation of Hawaii's dryland Wiliwili trees, *Erythrina sandwicensis* forests from the *Erythrina* gall wasp, *Quadrastichus erythrinae*, that was brought under control from the release of a classical biological control agent. Hawaii's urban forests, botanical gardens, and agricultural windbreaks utilized a non-native *E. variagata* (Kaufman et al. 2020). Prior to this, there were only 5-10% of the original dryland forests remaining, which are home to endangered species (Bell et al. 2013). Mehamehame trees, *Flueggea neowawraea*, are nearly extinct due to the introduction of the black twig borer, *Xylosandrus compactus* Eichoff (Hara and Beardsley 1979). The polyphagous nature and feeding behavior of *X. compactus* likely contributed to its ability to spread to remote areas.

Chapter 5 also mentions the impacts of two *Ceratocystis* spp. fungal pathogens, the causal agents of Rapid 'Ōhi'a Death (ROD) and their association with the widespread decline of the ecologically important endemic 'Ōhi'a lehua trees, *Metrosideros polymorpha* (Myrtaceae) (Fortini et al. 2019). 'Ōhi'a forests provide habitat for more than half of Hawaii's endangered plants, suggesting that the loss of these forests would have cascading effects on a variety of endemic species. 'Ōhi'a are planted as ornamental trees in the urban landscape and in botanical gardens. ROD is becoming widespread across the forests of the Hawai'i island and its spread can be facilitated through spore contaminated material or equipment. The spread of this pathogen is

directly facilitated by four invasive *Xyleborous* spp. ambrosia beetles (Roy et al. 2023). These invasive insects have been found to attack both healthy and damaged trees. The presence of *M. polymorpha* in urban areas and botanical gardens could serve as a reservoir for ROD. Planting Pono with *M. polymorpha* should only be considered in areas that would not facilitate the spread of pathogens to nearby forests. There is also concern that a species of Neotropical rust fungus, *Puccini psidii* could impact other trees in the Myrtaceae family (Loope and La Rosa 2010). There are more than 90 *Eucalyptus* spp. in the Hawaiian islands that are used for timber, ornamental landscaping, and in botanical gardens, and eight species in the Myrtaceae family that are native to Hawaii. There already have been five of the eight native species and 12 of the non-native species that have been found with *P. psidii* infections. The planting pattern across Hawaii's landscape will likely facilitate this to become widespread, if it is not already.

These examples could be a result of State laws (Act 73 and 276) that required the use of native Hawaiian plants in state funded projects, to supplement the Endangered Species Act and to increase both the plant's populations, and public's knowledge of these plants (Tamimi 1996). Although there was the best of intentions with these plantings, this may have contributed to planting native species in an area that can facilitate the pathway for invasives to spread to a new area. These examples also highlight a pattern where pre-border detection did not take place for a variety of pests or pathogens, followed by a subsequent invasion that utilized the urban landscape and, in some instances, severely altered the ecology of entire ecosystems. Unfortunately, there is an impending expansion of a current invasion that may result in the loss of Hawaii's endemic palms and indigenous pandanus species. *Oryctes rhinoceros* was first detected more than ten years ago on O'ahu and the extensive eradication program significantly decreased its spread for many years. However, partly due to funding limitations it was not

successful in complete eradication and the populations spread to agricultural areas of the island, and subsequently populations began to be detected across the island of O‘ahu. In 2023, detections of *O. rhinoceros* were found on Kaua‘i, Maui, and Hawai‘i island, with no detections on Lāna‘i or Moloka‘i as of 2024. The infestation on Kaua‘i is currently spreading faster than Maui and Hawai‘i island. Kaua‘i has extensive ornamental plantings of palm species that will provide ample areas for populations to develop. This is the same for Maui, while there is a chance that the infestation on Hawai‘i island will remain confined to the Kona area and not reach Hilo. This is due to the geography of the island, but public outreach will be essential to prevent any interisland movement of potentially infested material.

The location in Kona will require extensive public coordination with homeowners to eradicate, while the geographic distribution of the Maui infestation is unclear as of April 2024. These are the two populations that may be eradicable, but it depends on the ability to locate breeding sites and to identify the initial pathway of entry. The status of O‘ahu’s palms shows a severe decline in heavily infested areas. An assessment during November 2024 across the island, revealed that there are many palms on the west side and north shore that are exhibiting damage symptoms like heavily infested areas in Kunia and Pearl City, that likely will be dead standing palms within the year. It is likely that the north and northeast shores will first experience higher palm mortality due to the increased breeding material available and wetter climate of the area, while the west side will have a slower decline as the leeward side receives less rain. Mulch piles of a certain moisture and particle size are optimal for larval development (Manley et al. 2018), but I also observed third instar larvae growing in detritus of a live coconut palm tree on Ted Makalena Golf Course.

In areas of the island with areawide injections of palms with imidacloprid, there still was palm mortality due to excessive feeding. I have heard of people charging between \$50-75 per tree to inject them with imidacloprid (Julie Coughlin, personal communications). I have injected many trees with imidacloprid and estimate that the costs per tree for Imajet™ and plugs are about \$30 per tree depending on the DBH, not factoring in costs of insurance, equipment repair, commercial applicators license and transportation costs. These estimates seem rather low per tree and some residents have indicated they only use one plug per tree, which is something I was rarely able to do. As we enter the long-term management phase, it is important for the public to trust the science of these treatments, but it is difficult to quantify if these individuals are injecting the full dose into each tree. This could lead to eventual insecticidal resistance, especially if a chemical rotation with acephate is not incorporated. Uneven implementation of these insecticidal treatments may mitigate the damage to treated trees but will not reduce populations in an area if there are many trees that did not receive treatment.

The likely trend regarding palm health on O‘ahu will be on a downward trajectory, as there already is evidence of native *Pritchardia* spp. palms in remote areas experiencing heavy damage from *O. rhinoceros* populations. There aren’t any current management methods that can save these palm trees in remote areas, as there is hesitancy to apply insecticides in areas of high endemism. These ethical dilemmas are becoming more common as we try to salvage ecosystem function and the survival of particular species in the face of the multitude of threats mentioned in Chapter 1 such as habitat loss, climate change, biodiversity collapse, and a variety of other anthropogenic stressors. The spread of *O. rhinoceros* to new regions may expedite the already ongoing process of the unprecedented extinction of palm species across the globe (Bellott et al. 2022). Hawai‘i’s many endemic palm species are at risk from the increasing infestations, whose

spread has been potentially expanded by the easily available ornamental palms across the Hawaiian islands. The polyphagous feeding habits will impact pandanus as well, with evidence from Palau indicating that *O. rhinoceros* destroyed pandanus trees after many of the coconut palms were killed (Gressitt 1953). The exclusion of this pest from Moloka‘i is crucial to protect some of the remaining pristine Hawaiian ecosystems. The protection of mountain to sea linkages is crucial for ecosystem health of both terrestrial and aquatic ecosystems, and the loss of endemic species across Hawai‘i’s coastal ecosystems will pave the way for invasive plants to change these historic functions (Wada et al. 2020).

Chapter 5 details studies on the regeneration of coastal *P. tectorius* forests, which face immediate threats from invasive plants and ungulate feeding, with long term reduction uncertain related to *T. pandani* infestations. The addition of *O. rhinoceros* feeding threatens this ecosystem as well. The infested range of *T. pandani* may remain confined to its current geographic distribution, with slight expansions likely on O‘ahu from the Waikīkī, Punahou, or Chaminade infestations. There is an ample pathway for *T. pandani* to spread around the island via ornamental plants, but it may take decades to reach the limited forests around Kahana Bay through this movement. On Kaua‘i, there is enough ornamental *P. tectorius* to facilitate a faster spread to the forests. The fastest mechanism for spreading to rural areas is through transporting materials, as the infestation on Lāna‘i was through an ornamental planting at a resort and the Pa‘auilo infestation on Hawai‘i island was from a tree gifted from someone on Maui. Both were eradicated as mentioned in Chapter 4, but if the infestations had time to spread there would have been ample pathways for these to expand across the limited *P. tectorius* plantings on Lāna‘i and the extensive forests across Hawai‘i island. Some of the gulches on Hawai‘i island near this site would have been too steep to eradicate a new infestation if it were given time to spread. This

highlights another example of ornamental planting that either facilitated the spread of an invasive insect or provided a pathway for it to spread to a remote area. This was likely how the infestation spread from residential areas of Moloka‘i, across the island to the remote valleys between Kalaupapa National Historic District and Hālawā valley. The following sections detail the findings of this dissertation and the comprehensive approaches taken for evaluating novel approaches to study and manage *O. rhinoceros* and *T. Pandani*.

Chemical control to manage the CRB-G biotype in Hawai‘i

Prior to this dissertation, there were only a few trials that evaluated the efficacy of systemic insecticides on *O. rhinoceros* in laboratory conditions. These determined that acephate and imidacloprid were efficient at causing laboratory mortality. I expanded these trials to include systemic insecticides such as dinotefuran and abamectin. I determined that abamectin, dinotefuran, and imidacloprid caused significant mortality at 25 and 250 ppm. I studied these treatments, as I was already familiar with Arborjet injection systems, and these insecticides are able to be applied via trunk injection. Our goal was to identify treatments that could be applied efficiently and with minimal training to eradicate localized populations of *O. rhinoceros* on O‘ahu.

During the beginning of these field trials, the population of *O. rhinoceros* on O‘ahu was in a quarantine phase, with a limited distribution. As our field trials progressed, there was an exponential increase in population levels at this site. This provided valuable data on the efficacy of each treatment, along with lessons for an integrated pest management program. Imidacloprid

had increased odds of reducing damage to the entire palm canopy and to the damage to the crown compared to other treatments.

Imidacloprid injections were used as a widespread management strategy by the CRB Response team on a larger scale across areas of the island. Their data indicated that vane trap catches decreased in months following treatment in residential areas. This can be utilized with an acephate rotation via trunk injection to avoid resistance. Acephate did not yield statistically significant results but can be used to prevent the buildup of insecticidal resistance. I anticipate that treated palms will still die in areas closer to rural or agricultural zones as these treatments alone cannot mitigate excessive crown feeding. The exposure trials with field collected palm fronds indicated that all four treatments were more effective than the control up to 8 months after treatment. However, chemical residue was not detectable in most of these samples that were shipped for analysis. This is why methods for larval population control were studied in the next section.

Entomopathogens as augmentative biological control to manage the CRB-G biotype

I had the goal to assess the impact of local and commercial entomopathogens for managing larval populations of *O. rhinoceros*. In our entomopathogenic fungal trials I determined that local *Metarhizium* strains and a commercial *Beauveria* product were efficient at causing laboratory mortality for first instar larvae. This is likely due to new associations with the CRB-G biotype – the lack of previous exposure of the beetles to these pathogens. Our efforts to have the strains mass produced as a spray or granular substrate were mitigated by APHIS regulations and a mainland facility with the capacity to produce this commercially. Therefore I

conducted field testing of BotaniGard ES as a spray amendment to mulch. This yielded results that showed increased mortality to first instar larvae. I also conducted trials with locally collected entomopathogenic nematodes that did not have significant results. These were the most comprehensive trials to date evaluating whether these would have been successful, and the goal was to have these tested to a much broader extent and integrated into an augmentative biological control program.

Evaluating IPM strategies to manage *T. pandani*

I conducted the first repeated studies testing the effect of various treatments that can be applied to manage populations of *T. pandani*. Buprofezin and flupyradifurone were effective treatments for managing infestations on select landscape plants. The results of this could be used in areas where *T. pandani* is not yet widespread, but it is not feasible to remove the infested *Pandanus* material using careful protocol.

Thysanococcus pandani Hawaiian Distribution and Dispersal

The statewide survey for populations of *T. pandani* helped fill knowledge gaps on its range and was an effective public outreach mechanism for this insect. I was able to note that the Moloka'i population was widespread to the most remote areas of the islands that are inaccessible to most people. I was able to confirm three successful eradications. Lāna'i and Hawai'i island successfully eradicated populations on their own and carefully monitored for any relapse. The UH infestation was successfully eradicated, and new trees were planted in this area. They

published an article online that details the procedure used for eradication and how to effectively mitigate its spread (<https://www.hawaii.edu/news/2022/09/14/hala-scale-eradicated-at-uh-manoa/>). They were successful in preventing other areas on campus from becoming infested during the removal process. If *T. pandani* were to spread to other areas of Hawai'i island or Kaua'i; it likely would not be eradicable.

As I noted in this dissertation, the dispersal study did not have the replication or field plot size that I had hoped for. This still filled important knowledge gaps about wind dispersal and the distance *T. pandani* can spread over time.

Pandanus forest survival in the presence of widespread *T. pandani* infestations

With extensive coordination and many days of walking I was able to effectively cover a wide range of accessible indigenous *Pandanus tectorius* forests across the Hawaiian islands. I conducted this study to determine the ecological impacts and if a crucial cultural resource would be available for future generations. The outlier driven results indicated there were some issues with the larger sapling regeneration ratio. I have compiled the most comprehensive data set on these forests to date and time will tell if the *T. pandani* infested forests on Maui and Moloka'i will have long-term decreased regeneration or persist with these infestations.

Biological control exploration for *T. pandani*

We had hoped to find specialist predators of *T. pandani* in the brief survey trip to Singapore, Thailand, and Indonesia. Due to late permit applications, limited field time, and some field sites we could not visit, this trip was not successful in the sense of discovering its goal. If I

was in charge of coordinating it, the timeframe would have been much longer, and the field sites would have been more comprehensive. The beneficial outcomes were the discovery of generalist predators and the range of *P. tectorius* trees that can be infested. We also were able to screen infested trees from the botanical gardens and their collection of global *Pandanus* spp. This indicated that a potential introduction to these gardens could have come from the Western Pacific, Madagascar, Sri Lanka, Taiwan, and other areas in the Pacific. Both Indonesia and Singapore had an overlap with pandanus from Madagascar, which has a radiation of over 75 *Pandanus* spp.

I traveled to Madagascar to see if there could be unknown populations of *T. pandani*. Using a map from the 1960's I determined sites that would have Pandanus and potential access. Although I did not find *T. pandani* I did find three undescribed scale species that were previously unknown on Pandanus in the available literature, with one having an exit hole on a single specimen. This was an important discovery that could be explored further, as I only surveyed 14 out of the 79 or more species that are endemic there. These insects were not widely distributed across the Pandanus there and under some type of control. The high endemic diversity of *Pandanus* spp. in Madagascar may have resulted in a commensal evolution between the Pandanus, the undescribed scale insects and the *Phelsumia* spp. geckos (I did not observe feeding on scale insects). The lack of diversity in Hawai'i compared to Madagascar may be promoting the spread of *T. pandani* across our Pandanus forests. Finally, I did observe v-cuts and bore holes on coconut palms in Masoala National Park (S 15.72989, E 49.959655). The translation indicated a big black beetle caused the damage, but I did not collect or see any specimens. It is possible the distribution of *O. rhinoceros* goes farther to Madagascar, and that populations are under control there.

Future study and Recommendations

There is potential for the *O. rhinoceros* and *T. pandani* to spread throughout Polynesia through the current pathways in place with globalization. These specific pests would have detrimental effects on areas that are already facing a series of threats mentioned previously. This dissertation provided up to date research for *T. pandani* on its statewide distribution, dispersal capabilities, ecological impacts, and management strategies for their populations. This will expand on the limited literature about this insect. There also was a comprehensive approach to evaluate the potential for management strategies for *O. rhinoceros*; and during the timeframe of the research was able to identify an effective treatment regimen for decreasing the population of adult beetles through systemic insecticide applications. The practicality of the entomopathogen research results is limited for actual integration into the management or residential programs in Hawai'i. I would recommend the exploration of granular based *Metarhizium* spp. strains for product development and application in ecologically strategic areas. We spent 13 months going through a patent, non-disclosure agreement, and communication process with a company to attempt this, but it was not successful. Perhaps the expansion of the current population will have greater incentives for investment in local facilities as the damage worsens across the state.

This is where the substantial investment in a state-of-the-art biological control facility in Honolulu may be able to reduce the invasions that are overwhelming our biosecurity and ecological areas of the state. There are ranges from 8:1 to 3000:1 benefit to cost ratios associated with the returns on the investment for these facilities (van Wilgen et al. 2020). As many species of concern are of a limited distribution within Hawai'i, this is an excellent opportunity to have a

facility that can be the flagship of the Pacific and provide a management framework for pests that are expanding across many islands within and outside of Hawai‘i. HISC has already made goals to establish biocontrol facilities for Hawai‘i’s future that have more space, progress, and savings, but there is not a specific date or funding source for these to be built currently (HISC 2023). The development of a new nudivirus for *O. rhinoceros* would have profound implications for the Pacific, before it spreads and causes greater ecological and economic impacts than it has already. The forest population study was a complex undertaking and in itself could have been an entire dissertation. I would be interested to see another student explore this over multiple years and to a greater extent than I could. The testing of *Phelsuma* spp. geckos as biological control agents is another interesting route to explore as well, as there are multiple species in Hawai‘i that hypothetically could reduce *T. pandani* populations in established areas. This will be taking place in 2025, as vertebrate permits are acquired. There was parasitism evidence in Madagascar as well, suggesting that there could still be a classical biological control agent out there for *T. pandani*. If someone has the opportunity to travel to Masoala National Park for a classical biological control trip, this may yield interesting results for the biological control of both *T. pandani* and *O. rhinoceros* and for the enjoyment of the coral that is adjacent to these field sites. The Hawaiian islands will continue to face problems from these pests and the multitude of established and unestablished invasive species that are making their way around the world. The revitalization of Hawaii’s biological control facilities would have positive environmental impacts for generations to come and could preserve the unique biodiversity that is remaining across the Hawaiian islands.

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