MANAGEMENT STRATEGIES OF NEW INVASIVE INSECT PESTS OF LANDSCAPE PLANTS IN HAWAIʻI

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ABSTRACT

Landscape plants provide beneficial effects such as increasing air quality, promoting mental health, and improving aesthetics. The need to have effective control methods for pests is important to maintain these benefits. This study investigated manamgent tactics for *Oryctes rhinoceros*, *Josephiella* spp., and *Paratachardina pseudolobata*, pests that attack important landscape plants in Hawai‘i including coconut palm, Chinese banyan and weeping banyan. Various systemic insecticides were tested on *O. rhinoceros* in the laboratory. The highest percent of affected beetles were observed in acepahte and imidacloprid treatments. Irrigation did not impose negative impacts on insecticide efficacy and followed similar trends to insecticide treatmetns alone against banyan pests. A comparative study on different systemic application methods on controlling *P. pseudolobata* found that both injection and soil drench were effective at suppressing *P. paratachardina*.

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Trees and other plants are important in urban and suburban landscape settings. They produce shade and oxygen, increase air quality and wildlife, promote mental health, and provide a natural aesthetic. As urbanization increases, it becomes paramount to ensure that natural and landscaping environments stay healthy. There are a number of pests that attack trees in landscaped areas. Given the risks to human, animal, and environmental health, the management of some insect pests, particularly selection of insecticides with reduced risks and development of application methods with low non-target exposure becomes very important.

Many insecticides are applied through a foliar spray method, but because of drift or the need for additional equipment on tall trees and, it can be difficult to cover the entire tree if the canopy is large. Soil drenching and trunk injection can be systemically applied at ground level, allowing the plant to draw the insecticide to the canopy through its vascular system. Both trunk injection and soil drench have shown high success on controlling insects (Bhandari and Cheng, 2016; Bhandari and Cheng, 2018; Doccola et al., 2009; Howard and Steinberg, 2005; Miranda et al. 2016).

Insecticides applied through trunk injection can be done with lower active ingredients as the insecticide is applied directly into the tree and so can be a safer and more environmentally friendly way to administer pesticides. This can be especially beneficial in urban settings where the risk of off target effects is high (Docolla and Wild 2012). Application of systemic insecticides via trunk injection is an effective and low-risk approach to managing pests of trees in urban landscapes. However, these application methods need to be tested for each targeted pest because the level of exposure of the pest to the pesticide may be different than a foliar spray.

In Hawai‘i, landscaping trees are of particular value. One of the reasons why Hawai‘i is a tourist destination for people worldwide is because of the scenery. Even in urban settings, a diverse range of flora can be found. In January of 2018 alone, approximately 800,000 visitors traveled to Hawai‘i, spending $1.89 billion (Hawaii Tourism Authority, 2018). With high traffic and diverse flora, invasive insect pests can arrive and establish and negatively affect endemic and other important flora and fauna. An integrated pest management (IPM) system is most often the ideal strategy to manage invasive pests. In Hawai‘i, if invasive pests establish, it can be difficult to eradicate, partially because biological control can take years to pass through bureaucracy unless already found on the

islands (Messing, 2005). Because of this, chemical control can be an effective method initially as insecticides that are registered for appropriate plants and pests can be used early.

Landscaping plants such as banyans and coconut palms are important trees in both Hawai‘i and other tropical regions of the world. Relatively new insect pests to Hawai‘i, the Chinese banyan stem and leaf gall wasps, lobate lac scale, and the coconut rhinoceros beetle all can detrimentally affect certain landscaping plants, eventually causing death to trees; and there are limited management options for these pests. This project tests the application of insecticides by trunk injection and the influence of irrigation, a control tactic, on three insect pests in prominent landscape trees found in Hawai‘i.

1. Coconut rhinoceros beetle

Biology:

The life cycle of the coconut rhinoceros beetle, *O. rhinoceros*, consists of an egg stage, three larval instars, a pupa stage and adulthood. Adults oviposit into decaying green waste or dead standing palms in which the larvae develop (Hinckley, 1973; Bedford, 1976). Larvae have also been found in palms with debris in the crowns in Guam, but it has been suggested that this is because predators such as rats and birds are rare and so do not pose an impact at these sites (Moore et al. 2015). Larval sex ratios is about 1:1 (Gressitt 1953; Hinckley 1973) while adults caught in different traps seem to vary (1.5:1 male:female according to Hinckley 1973). Adults lay 4 to 5 eggs a day and could oviposit a new clutch every three weeks (Hinckley 1973). In one clutch, females can lay 62-65 eggs (Hinckley 1973; Beford 1976). Females are able to lay viable eggs several months after an initial mating, but can also mate multiple times (Hinckley 1973). After pupation, adults remain in their earthen cocoon while their exoskeleton darkens and hardens before they fly to nearby food sources to feed on palms and other agriculturally important monocots.

Eggs are a cream-white color and are initially ovoid, but become spherical as the larva develops before hatching. Larvae are similar in color to the eggs, but have a burgundy head capsule with spiracles running the length of the larva’s body on both flanks. Larvae typically lie on their sides and curl their abdomen beneath their heads to form a C-shape. As larvae prepare to pupate, the pre-pupae form becomes shriveled and is slightly smaller than the third instar. Pupae are copper and show evidence of future horn and wings. Adults are dark brown or black and show sexual dimorphism. Males generally have a larger horn for their size and a rounder abdomen with little hair. The posterior end of the female’s abdomen is hairier than the males and is also more pointed.

The duration of *O. rhinoceros* stages varies considerably from one author to another, but the general consensus is that the beetle spends about half of its life, 2 to 9 months, as an adult (Leefmans 1920; Cherian & Antananarayan 1939; Gressitt 1953; Goonewardene 1958; Kurian & Pilai 1964; Hürpin & Fresneau 1967; Catley 1969; Bedford 1976; Hinckley 1973). All authors recently mentioned that eggs take 1-1.5 weeks to hatch. First instar larvae transitioned to second instar after 10 days (Hürpin & Fresneau 1967) to 21 days (Hinckley 1973). Second instars for 12 (Hürpin & Fresneau 1967; Catley, 1969) to 21 days (Hinckley 1973; Gressitt 1953) before becoming third instars. Third instar larvae show a wide variation of duration depending on the author ranging from 39 days (Gressitt 1953) to 165 days (Hürpin & Fresneau 1967). In optimal conditions, the egg to egg generation time can be as little as 20 weeks (DeNitto et al. 2015). Development can slow if nutrition is poor or climate is not ideal (Catley 1969). Larvae show preference to dead plant media between 27-29° C at high relative humidity and are attracted to ammonia and acetone (Bedford 1980). *O. rhinoceros* can experience a population boon after hurricanes such as Typhoon Dolphin in Guam as the debris allows for new breeding sites (Moore 2016).

Damage from *O.* rhinoceros is mainly from the adult stage as larvae consume decaying matter. Adults burrow into the meristem of the palm to feed on the phloem. Newer fronds are affected by the burrowing, which cause distinct bore holes in the midrib and v-shaped cuts in the leaflets to indicate that a beetle has fed from a specific plant. Adults can stay in a tree on average of six days (Hinckley, 1973). Beetles that burrow into the meristem can remain until the entrance is moved by palm growth to allow easy exit for adults (Young, 1975). Each feeding event can cause damage to multiple fronds (Hinckley, 1966) and different beetles generally will not use premade holes to feed (Hinckley 1973). Damage to the palms can attract secondary pests such as weevils (Bedford, 1980).

Geography:

O. rhinoceros is native to South Asia through the South Pacific (Bedford 1980; Gressitt 1953; Hinckley 1967). It has since migrated to locales such as Myanmar, Samoa, Guam (Bedford 1980), and more recently, Hawai‘i. The migration events are suspected to be from military and trade transport (Gressitt 1953). The main form of long-distance movement of *O. rhinoceros* is through hitchhiking. The beetle can be in a variety of media or wood and transported through cargo. In June 2014, a live adult *O. rhinoceros* was found in a shipment of palm furniture to Jalisco, Mexico from Indonesia (Quiroz et al., 2017)

On Guam, *O. rhinoceros* was first detected in the third quarter of 2007 (Moore 2016). Mass trapping and sanitation efforts to eradicate the beetle failed which allowed *O. rhinoceros* to spread throughout the island by 2010. The nudivirus used to control *O. rhinoceros* throughout the pacific was resisted by *O. rhinoceros* populations on Guam, indicating a new biotype of the beetle. The CRB-G biotype has since invaded Hawaii, Solomon Islands, and Papua New Guinea (Moore 2016). The first wild *O. rhinoceros* breeding population detected in Hawaii was in December, 2013 on Oahu and was of the CRB-G biotype.

Control:

Althought *O. rhinoceros* has natural predators; the effects on the population are difficult to assess (Bedford 1976). Insects that feed on *O. rhinoceros* include the assassin bug *Platymeris laevicollis* and two types of ants, *Pheidole megacephalai* and *odontomachus haematoda*; however, the assassin bug only showed effectiveness in insectaries (Beford 1976) and the two ants did not actively seek out *O. rhinoceros* larvae, instead only attacking exposed eggs and larvae (Hinckley 1976). Reptiles showed to not feed on any stage of *O. rhinoceros*, while chickens consumed larvae and eggs when close to the surface of the breeding site (Hinckley 1976).

Several mammals have shown evidence of feeding such as the flying phalanger *Petaurus breviceps papuanus* (Bedford 1976), the roof rat *Rattus rattus*, and the house mouse *Mus musculus* (Hinckley 1976). Although pigs have been shown to eat the larvae of *O. rhinoceros*, many prefer other food sources available (Hinckley 1976). Through personal observation, elytra of *O. rhinoceros* were found in mongoose (*Herpestes javanicus*) fecal matter. Although there are many natural enemies of *O. rhinoceros*, many of these enemies cannot establish or do not significantly affect the population in locations that *O. rhinoceros* has invaded (Bedford, 1980).

The fungus *Metarhizium anisopliae* has shown success against *O. rhinoceros*, but there has been debate on if *O. rhinoceros* is only affected by isolates of other members of its species (Bedford, 1980). The *Oryctes rhinoceros nudivirus* has shown to infect *O. rhinoceros*, but in the presence of copious amounts of organic matter, a low infection rate will allow a majority of the population of beetles to mature before becoming infected (Ramle et al. 2005) A new biotype of *O. rhinoceros* has also been found to be resistant altogether to the virus (Marshall et al. 2017). A potential problem when using *Metarhizium* spp. exclusively is that breeding sites can potentially be inaccessible allowing uninfected *O. rhinoceros* to continue to survive (Moore 2016).

Cultural/mechanical control of *O. rhinoceros* includes hooking adults from feeding holes with wires, destroying breeding sites, and trapping (Bedford, 1980). Trapping and hooking are not

the preferred method as trapping is more useful as surveillance rather than eradication and hooking can cause damage to the palm (Bedford 1980).

Some insecticides have been tested as a form of chemical control such as cypermethrin showing significant effects (Moore 2013). Other insecticides have shown success on various life stages of scarab beetles such as neem, which can inhibit growth and doubles as an organic fertilizer, on larvae (Mohan & Padmanaban, 2013), as well as imidacloprid, fipronil, and λ-cyhalothrin (Martinez et al., 2014).

Cocos nucifera biology and geography:

Cocos nucifera L. is a monoecious monocot with a fibrous root system. Different varieties of *C. nucifera* can grow to 31 m tall (Broschat and Crane, 2014). Leaves can grow to 5.5 m long and 1.2 m wide with a pinnate formation (Broschat and Crane, 2014). Both male and female flowers are yellow in color and are primarily pollinated by honeybees (Free et al., 1975). Female flowers are larger than male flowers and located at the basal end of the inflorescence. *Cocos nucifera* have a high tolerance to salt and so can grow along shorelines, though can grow inland as well as long as the soil pH is between 5.0-8.0 and has proper drainage (Broschat and Crane, 2014). Common pests on *C. nucifera* include the coconut rhinoceros beetle, *Oryctes rhinoceros*, two-colored coconut leaf beetle, *Brontispa longissima*, coconut black-head caterpillar, *Opisina arenosella*, and the red palm weevil, *Rhynchophorus ferrugineus* (Samseemoung et al. 2017).

Although it is unclear where *C. nucifera* originated due to its wide distribution, it is suspected to be native to the South Pacific (Broschat and Crane, 2014). Coconut palms are widespread in the tropics and distribution can occur when the fruit drifts across bodies of water. Germination has occurred in fruits that have been floating in sea water for 110 days and could allow for natural expansion (Edmondson, 1941). Ward and Brookfield (1992) suggest *C. nucifera* have been transported predominantly out of the pacific via seafaring vessels to aide their distribution as currents and wind would not be sufficient in transporting the fruit to a new location before no longer being viable (Ward and Brookfield, 1992).

2. Ficus leaf and stem gall wasps

Biology:

Plants often produce galls in response to pests, including bacteria, fungi, and insects (Davies, 1988). The galls are made of enlarged cells around the foreign organism and can be found on any part of a plant. Of the various insects that cause galls, two have been identified on *Ficus microcarpa*,

the Chinese banyan leaf gall wasp, *Josephiella microcarpae* Beardsley and Rasplus, and a similar wasp, the Chinese banyan stem gall wasp, *Josephiella* sp.

Adult Chinese banyan leaf gall wasps are a dark brown color with females being 2.2 mm long and males about half the size (1.1 mm, Beardsley and Rasplus, 2001). Females oviposit in the leaves of *F. microcarpa* causing the galls to form while the larvae develop. Larvae can continue to pupate in galls regardless if the leafs have abscised from the tree. The average life cycle of *J. microcarpae* is three to four months.

A similar wasp was observed for the first time in 2012 in Hawai‘i and was found to produce galls on the stems of *F. microcarpa*. Unlike the Chinese banyan leaf gall wasp, which caused minor damage on leaves of *F. microcarpa*, this new wasp was determined to cause leaf abscission, dieback of newer stems, and potential death of the affected tree. Although not described yet, the new wasp behaves similarly to other wasps in the *Josephiella* genus. The life cycle of the Chinese banyan stem gall wasp was also found to be five to six months.

Geography:

The Chinese banyan leaf gall wasp was first identified in Hawai‘i in 1989, and is found also in California, Florida, and the Canary Islands, although it’s native range is unknown (Beardsely and Rasplus, 2001; Caldwell, 2008). Similarly, the Chinese banyan stem gall wasps native range is also unknown as it is only known to be in Hawai‘i. Currently, the Chinese banyan stem gall wasp has not been observed outside of Hawai‘i and so its native range is not fully known.

Control:

Little research has been conducted on either of the Chinese banyan gall wasps. Emamectin benzoate and imidacloprid have shown success on other gall inducing insects (Doccola et al., 2009). Bhandari and Cheng (2016) showed similar conclusions finding that both insecticides showed success against both the Chinese banyan stem all leaf gall wasps; however, emamectin benzoate showed a larger reduction in both stem and leaf infestation levels.

Ficus microcarpa biology and geography:

The Chinese banyan, *Ficus microcarpa* L.f. is a dicot that is pollinated by a host specific agaonid wasp, *Euprestina verticillata* Waterson. Although *E. verticillata* is the only pollinating wasp of *F. microcarpa*, eight other agaonid wasps have been found in the fruit of *F. microcarpa* which can potentially cause negative reproductive success of both *F. microcarpa* as well as *E. verticillata*

(Cardona et al., 2013). This agrees with Kobbi et al. (1996), which found that higher parasitism of the fruit of *F. microcarpa* is inversely proportionate to the number of pollinating wasps that occur. *Ficus microcarpa* has been prized as a landscaping tree due to its small fruit size as well as due to asynchronous leaf abscission and fruiting cycle, allowing for the canopy to remain full and allowing *E. verticillata* to reproduce throughout the year. Common pests of *F. microcarpa* include the Chinese banyan leaf gall wasp, *Josephiella microcarpae*, Chinese banyan stem gall wasp, *J.* spp., lobate lac scale, *Paratachardina pseudolobata*, and Cuban laurel thrips, *Gynaikothrips ficorum*.

Ficus microcarpa is native to South/South-East Asia including Ceylon, India, China, Ryukyu islands, as well as into the pacific in Australia and New Caledonia (Starr et al., 2003). It is also widely found in a landscape setting throughout tropical and subtropical regions and was introduced to Hawai‘i in 1921 (Ramirez et al., 1988) and can grow in moist and dry environments up to an elevation of 6,000 feet above sea level (Starr et al., 2003). Chinese banyan has spread throughout the tropical regions of the world due to its landscaping capabilities and is commonly found in the Hawaiian Islands.

3. Lobate lac scale

Biology:

The lobate lac scale, *Paratachardina pseudolobata* Kondo and Gullan, was initially believed to be *P. lobata* until 2007 when a taxonomic revision was conducted (Kondo and Gullan, 2007). Adults are approximately 2.2 mm with an X-shaped scale and are a dark, maroon color. No males have been observed and so it believed to be parthenogenic, similar to various other scale insects (Kondo and Gullen, 2007). Because of this, one adult on a plant can lead to heavy infestation (Howard et al. 2010). Adults are immobile; however, larvae emerge through the dorsal opening and disperses via wind or animals to new branches or plants. *Paratachardina pseudolobata* has a wide host range, including over 110 plant species in Hawai‘i and over 300 woody plants in Florida (Bhandari and Cheng, 2018; Howard et al., 2010). Many of the host plants have been found in landscape settings such as hibiscus, *Hibiscus* spp, Chinese banyan, *Ficus microcarpa*, and weeping banyan, *F. benjamina* (Garcia, 2013).

Geography:

Although the native range of *P. pseudolobata* is not fully known, it is likely native to tropical regions of Asia as the other species in the genus are native to Asia (Howard el al. 2010). In the United States, *P. pseudolobata* has been observed in Florida and Hawai‘i to date.

Control:

Similar to the Chinese banyan gall wasps, little research has been conducted on control methods of *P. pseudolobata.* Imidacloprid showed a complete suppression of adult *P. lobata* by 334 days post-treatment, but reduced live adults observed in half by 61 days post-treatment when used in soil drench applications (Howard & Steinberg, 2005). Imidacloprid via trunk injection has also shown high success on controlling lobate lac scale on both weeping banyan and Chinese banyan (Bhandari and Cheng, 2018). Biological control has occurred from parasitoid wasps, but at very low levels (Schroer et al., 2008; Howard et al., 2010).

Ficus benjamina biology and geography:

The weeping banyan, *Ficus benjamina* L. is a dicot pollinated by a host specific agaonid wasp, *Eupristina adempta* Wiebes (Ramirez and Montero, 1988). *Ficus benjamina* has been used as both a landscaping tree in urban and suburban environments as well as used in bonsai. A full grown tree can to 60 feet tall. The canopy has a symmetrical canopy with drooping branches, giving the plant its name (Gilman and Watson, 1993).

Ficus benjamina is native to South and Southeast Asia, including India, China, pacific islands such as Malaysia, and Australia with preference to tropical and sub-tropical regions. As a landscaping plant, *F. benjamina* has been transported throughout the world, but has a low frost tolerance and needs well-drained soil (Gilman and Watson, 1993).

4. Low-risk systemic insecticides and trunk injection method

Members of hymenoptera and coleoptera have shown to be susceptible to imidacloprid (Fossen, 2006). Imidacloprid can enter insect pests through either ingestion or direct contact and disrupts nicotinic acetylcholine receptors in the central nervous system (Fossen, 2006). The half-life of imidacloprid in the soil ranges from 26.5 to 229 days and can be continuously taken up by roots during this time. Acephate is also used as a common insecticide in agriculture due to its high efficacy on controlling insect pests (An et al., 2017). Similar to imidacloprid, acephate disrupts acetylcholinesterase, inhibiting motor and sensory neurons. Emamectin benzoate is a synthetic derivative of avermectin, normally produced by *Streptomyces avermitilis* (Jansson et al., 1997). Emamectin benzoate binds to glutamate and GABA receptors in insect chloride channels resulting in loss of cell function as well as negatively affecting nerve impulses, causing paralysis and eventual death in four days (Jansson et al., 2007). The compound is generally safe in mammals as emamectin benzoate has a difficult time to cross the blood-brain barrier and the lack of glutamate-gated chloride

channels in mammals. Previous studies (Bhandari and Cheng, 2016; Bhandari and Cheng, 2018) showed high efficacy to *Ficus* pests with imidacloprid and emamectin benzoate.

5. Irrigation

Promoting the health of a plant can influence the presence of pests (Asiimwe et al., 2014). One potential strategy to improve the health of a landscape plant is proper irrigation. A lack of water can cause stress to plants, causing an increased chance of pests and pathogens (Asiimwe et al., 2014). In locations with little rainfall, irrigation becomes crucial to maintain plants. If irrigation negatively affects the efficacy of insecticides, other control strategies will need to be developed. Few studies have shown effects of a combination of irrigation and insecticides on controlling insect pests. Asiimwe et al. (2014) found that irrigation does not affect the efficacy of contact insecticides, but no studies have shown if irrigation can affect the efficacy of soil drenching or trunk injection.

Research Objectives

In urban and suburban environments, where landscaping plants receive irrigation, it is important to know if insecticides are affected by the addition of water, especially soil drenching applications. Similarly, it is important to determine if various application methods show similar results on controlling insect pests.

The objective of this project is to investigate the effectiveness of trunk injection of systemic insecticides and irrigation on three pests of landscape trees. Specifically

1. Evaluate lower-risk insecticide treatment strategies on *O. rhinoceros.*
2. Determine if irrigation affects the efficacy of systemic applications, both through soil drenching and trunk injection.
3. Determine if different systemic application methods show similar efficacy.

Chapter 2. Management strategies of coconut rhinoceros beetle, *Oryctes rhinoceros* in Hawai‘i

Introduction

The coconut rhinoceros beetle, *Oryctes rhinoceros* (L.) (Coleoptera: Scarabaeidae), is native to South/Southeast Asia, but the first recorded detection outside of its native range was in Samoa in 1909 (Catley, 1969). The life cycle of *O. rhinoceros* consists of an egg stage, three larval instar stages, pupa, and adulthood. Adults oviposit into decaying green waste or plant based debris, which the larvae consume (Moore et al. 2015). Although larvae do not cause economic damage, their relatively diverse breeding site locations can allow a population to establish without resistance from outside forces (Bedford, 2013). Adults are invasive pests in non-native ranges of *O. rhinoceros* as the adult bores into the meristem of vegetation to feed on phloem. This can cause distinct damage patterns to newer fronds of coconut palms, *Cocos nucifera* (L.), such as bore holes in the midrib and horizontal v-shaped cuts in the leaflets. Infestation of palms can cause a reduction in fruit count and eventual death or avenues for secondary pests to infect palms (Bedford, 2013, Catley, 1969). Adults can be difficult to track as they are nocturnal and can oviposit near palms or outside of adult feeding locations (Bedford, 2013).

O. rhinoceros was first detected in 2013 in Hawai‘i on the island of Oahu and an eradication program was shortly established. In an integrated pest management system, several different methods are used to control a pest, usually cultural/mechanical control, biological control, and chemical control. While effective, mechanical control can be difficult as *O. rhinoceros* has cryptic breeding sites that range from mulch and green waste to cow dung (Bedford, 2013). Because of this, mechanical control requires active hunting and eliminating of potential breeding sites which can take coordination between personnel and land owners. Biological control has shown some success with both *Metarhizium* spp. Bedford, 2013) and the *O. rhinoceros* nudivirus (Marshall et al., 2017) in the pacific. The variety currently in Hawai‘i has shown resistance to the nudivirus (Marshall et al., 2017) and biological control agents not present in Hawai‘i need to be vetted to make sure they do not go after off target species and can take time to ensure safety which can take long periods of time for paperwork alone to go through (Messing, 2005). Chemical control, however, can be applied if registered in the State. On the island of Oahu, *C. nucifera* is predominantly used as an ornamental plant instead of as a food source, applying insecticides is a viable option in an attempt to eradicate *O. rhinoceros*.

Previous studies have shown insecticides such as imidacloprid have detrimental effects on adult scarab beetles (Martinez et al., 2004). Fox et al. (1995) also showed that acephate reduced leafminer herbivory on turkey oak trees. Neem has also shown to be an effective method of controlling *O. rhinoceros* larvae, although adults have not been tested (Mohan & Padmanaban, 2013). The deterrent effects of neem could cause adult *O. rhinoceros* to starve similar to larvae. These suggest that various insecticides can be effective on controlling damage to coleopterans, including in systemic application methods. Because of detrimental effects to similar insects, various insecticides were tested on adult *O. rhinoceros* both in the lab and the field to determine efficacy.

The overall objective of this experiment was to identify effective low-risk systemic insecticides against adult *O. rhinoceros* in the lab and field. It is hypothesized that insecticides such as imidacloprid, acephate, and emamectin benzoate will show success in controlling adult *O. rhinoceros* both in lab trials as well as in a field site. In successful field control, both fewer adult *O. rhinoceros* should be caught in panel traps as well as less feeding damage should be observed in *C. nucifera* crowns.

Materials and Methods

Lab Trials:

Six bioassay lab trials were conducted on adult *O. rhinoceros* between September 2015 through July 2017. The first four trials were conducted at the Hawai‘i Department of Agriculture Plant Quarantine Station (Honolulu, Oahu, HI), while the final two trials were conducted at the University of Hawai‘i at Mānoa (Honolulu, Oahu, HI). All trials included adult *O. rhinoceros* from a colony of both reared and lab caught beetles. All treatments used 10 adults in separate wide-mouth mason jars (0.95 L) with holes drilled (0.32 cm diameter) into the lids for ventilation. Beetles were observed daily and livelihood was recorded for two weeks. Adults were picked as close to a 1:1 female:male ratio as possible in case sex determined efficacy of treatments. Trials were conducted based on availability of adult beetles and so each trial consisted of different treatments.

The food source was removed three days after treatment (DAT) to simulate real life scenarios as the adults would not stay in a tree for two weeks at a time. The final trail also included hourly observations on the first day. Each adult was briefly removed from the mason jar at each observation period to determine health. Three classifications of observations were then recorded (0=alive, 1=paralyzed, 2=dead). Alive beetles showed normal activity. Paralyzed beetles showed ataxia and would fall on their backs when attempting to walk as well as exhibited twitching of antennae and

legs. Dead beetles showed no signs of life. Mortality rates among treatments were corrected for mortality among control adults using Abbott's formula ($\frac{(Treatment\ mortality - Control\ mortality)}{(100 - Control\ mortality)} \times 100\%$).

Sugar cane stalks were used in the first three trials as a food source for the adults while the final three trials used a lab prepared food source. Sugar cane was donated by a farm in Waimanalo and were cut into 5x5 cm segments and sprayed with 91% isopropyl alcohol to prevent fungal growth. Each sugar cane stalks were then dipped into a solution of their respective treatments. A single paper towel folded into a 5 x 5 cm square was placed in the mason jar and was moistened with 20 mL of water to prevent desiccation. A segment of sugar cane was then added to each mason jar with the corresponding treatment before an adult was added to the jar.

The lab produced food was made by following the protocol produced by the CRB colony team, but treatments were added during the creation process for each treatment. Each mason jar was filled with 200 mL of mulch substrate to prevent desiccation. Eight mL of beetle food was then placed on the lid of a condiment container and placed on the substrate.

Trial One:

The first trial was conducted from the first of September to the fourteenth of September in 2015 and tested the insecticides acephate and emamectin benzoate (EB) at both 100 and 1000 parts per million (ppm). The acephate solution was prepared by diluting 0.55 g ACE-jet (acepahte 97.4%) into 550 mL of water and mixed to solution to create the 1000 ppm solution. Fifty mL was then extracted and diluted into 450 mL to create a 100 ppm solution. Similarly, EB was prepared by diluting 12.5 mL TREE-äge (emamectin benzoate 4.0%) into 500 mL of water to produce the 1000 ppm solution. Fifty mL of the solution was then extracted into 450 mL of water to create the 100 ppm solution. Sugar cane segments fed to control beetles were dipped in 500 mL of water.

Trial Two:

The second trial was conducted from the fourth until the nineteenth of January 2016. The second trial tested the efficacy of acephate, azadirachtin, and imidacloprid. Both azadiractin and imidacloprid were tested at 1000 ppm, while acephate was tested at 100 and 10 ppm. Azadirachtin was prepared by mixing 4.0g of AzaSol (6% azadirachtin) into 250 mL of water to produce the 1000 ppm solution. The 1000 ppm solution of imidacloprid was prepared by diluting 5 mL of Ima-jet into 250 mL of water. The two acepahte concentrations were prepared by first producing a 1000 ppm stock solution (0.25g ACE-jet into 250 mL of water) and extracted 25 mL into 225 mL of water to create the 100 ppm solution. The solution was then mixed well and 25 mL was then extracted into

225 mL of water again to create a 10 ppm solution. Sugar cane segments used for controls were dipped in 250 mL of water.

Trial 3:

Trial three was conducted from the eleventh until the twenty-fifth of April, 2016 testing the efficacy of acephate and imidacloprid. Acephate was tested at 100 ppm, while imidacloprid was tested at 10, 100, and 1000 ppm. The acephate solution was prepared by diluting 0.25 g ACE-jet into 250mL of water and mixed to solution to create a 1000ppm stock solution. From the stock solution, 25mL were extracted and diluted in 225mL of water to create the 100ppm solution to be used in the experiment. To make the 1000ppm solution of imidacloprid, 5mL of Ima-jet was added to 250mL of water and mixed into solution. Twenty five mililiters of the solution was extracted and added to 225mL of water and mixed to create the 100ppm solution. Of the 100ppm solution, 25mL was extracted and added to 225mL of water to create the 10ppm solution. Control sugar cane segments were dipped into 25mL of water.

Trial 4:

Trial four was conducted from the sixth until the twentieth of June, 2016 and tested again acephate and imidacloprid. Both acephate and imidacloprid included 10, 100, and 1000ppm concentrations. Beetle food was prepared by combining 5.2g of vanilla whey with 48mL of coconut water. Sugar (6.6g) was then mixed in. 2g of agar was then mixed into 152mL of boiling water to dissolve then added into the solution. The solution was then placed into a walk-in refrigerator to cool and solidify. Acephate beetle food was prepared by adding 0.002g of Ace-jet into the beetle food solution before cooling to produce the 10ppm acephate food source. Higher concentrations of the acephate used 0.02g and 0.2 g for 100ppm and 1000ppm food sources, respectively. To produce the 10ppm imidacloprid food source, 0.04mL of Ima-jet were added into the beetle food solution before cooling. When preparing the 100ppm and 1000ppm dilutions of beetle food, 0.4mL and 4mL of imidacloprid were applied, respectively.

Trial 5*:*

The fifth lab trial was conducted from 24, January, through 6, February 2017 testing both acephate and imidacloprid at 10 and 100ppm as well as Palm Weevil Killer (PWK) mix (clove oil 14%, thyme oil 14%, sodium lauryl sulfate 20%) at maximum and minimum concentrations according to the label (600:1 and 150:1 water: concentrate, respectively). Acephate food was prepared by adding 0.002g of Ace-jet into the beetle food solution before cooling to produce 10ppm acephate food source. The 100ppm acephate food was prepared by adding 0.02g of Ace-jet instead. To produce the 10ppm imidacloprid food source, 0.04mL of Ima-jet was added into the food solution

before cooling, while 0.4mL was used for the 100ppm food. When producing the minimum concentration of the palm weevil killer formula, 0.083mL was added to the food before cooling. Maximum concentrations received 0.32mL of the base before cooling.

Trial 6*:*

The last lab trial was conducted from 20, June through 05, July 2017 testing acephate and imidacloprid at 10 and 100ppm as well as the PWK solution at the max concentration on the label. The food source with acephate was prepared by adding Ace-jet into the beetle food solution before cooling to produce 10ppm acephate food source. The 100ppm acephate food was prepared by adding 0.02g of Ace-jet instead. To produce the 10ppm imidacloprid food source, 0.04mL of Ima-jet was added into the food solution before cooling, while 0.4mL was used for the 100ppm food. When producing the maximum concentration of the PWK food source, 0.32mL of the PWK solution was added to the food solution before cooling.

*Field Trial**:***

Efficacy of four insecticide types and a control on *O. rhinoceros* adults in an ornamental setting was conducted at Iroquois Point, Oahu, Hawai‘i between May 2016 through November 2017. One hundred and twenty-five coconut palms were chosen and split into 25 blocks by proximity and assigned a treatment randomly in each block. The following treatments were used according to label rates and administered through the quick-jet air (Arborjet, Woburn, MA); acephate (ACE-jet, Arborjet), emamectin benzoate (Tree-äge G4, Arborjet), imidacloprid (IMA-jet, Arborjet), and a combination of acephate and imidacloprid. Insecticides were reapplied when needed; however, EB only received the initial treatment.

Each tree was observed monthly and was given two different scores based on the overall tree health and the innermost four frond health. Two observers took independent data which was averaged each month. Scores were given on a 0-5 rating system with a score of 0 for no damage and 5 for tree death. The amount of adult *O. rhinoceros* collected from panel traps was obtained from the CRB eradication program both at the location of the field trial as well as from a grid two miles away to determine the effects of treatments. These data were taken from December 2015 through November 2017.

*Statistical Analysis**:***

Statistics were run using JMP Pro 13. Lab assays were analyzed using contingency tables and using Fisher’s Exact Test (α=0.05) to determine overall differences as well as how each treatment compared to the control group. Field trial results were analyzed using Kruskall-Wallis and Steel-Dwass test (α=0.05) to determine differences in overall and inner frond damage between treatments

and a least squares model to determine treatment effects on trap data by location, time, and location by time.

Results

*Lab Trials***:**

Trial 1*:*

Sixty percent of beetles treated with acephate at 1000 ppm were affected one day after treatment (DAT) which was significantly more affected compared to control adults ($p<0.004$, Figure 2.1). This trend continued throughout the trial where by seven DAT, 90% of adults treated with acephate at 1000 ppm were dead (Table 2.1). Acephate at 100 ppm and emamectin benzoate at 1000 ppm also affected adults one DAT (table 2.1). Forty percent of adults exposed to acephate at 100 ppm were dead by three DAT and another 30% were paralyzed which was significantly more beetles affected compared to the control (0% affected; $p<0.033$

Acephate at 100 ppm and emamectin benzoate at 1000 ppm showed statistical significance at three DAT ($p<0.033$) as well as 13 DAT ($p<0.02$). Acephate at both concentrations also showed at least a 40% affected rate starting one DAT and both concentrations of acepahte and emamectin benzoate at 1000 ppm showed at least 50% affected rate starting three DAT (Table 2.1). Emamectin Benzoate at 100 ppm did not show significant results compared to controls through the trial ($p=1$).

Trial 2*:*

No treatments were significantly different compared to controls until three DAT, in which imidacloprid at 1000 ppm affected 70% of adults ($p=0.0031$; Table 2.2) and continued to affect adults throughout the trial. No other treatments were statistically significant compared to control beetles. No effects were observed in adults treated with azadirachtin through the trial (Figure 2.2).

Trial 3*:*

At least one beetle was paralyzed by each insecticidal treatment one DAT (Table 2.3); however, imidacloprid at 1000 ppm was the only treatment that was statistically significant compared to control adults ($p<0.02$). Three DAT, acephate at 100 ppm killed 50% of adults, while imidacloprid at 1000 ppm killed 30% of adults and were statistically more than the zero control adults affected (Figure 2.3, $p<0.03$). All treatments affected beetles three DAT, although imidacloprid at 10 and 100 ppm were not statistically significant compared to adult until seven DAT ($p<0.0003$)

Acephate at 100 ppm and imidacloprid at 1000 ppm showed statistical significance compared to the control three DAT ($p<0.03$. Figure 2.3). All treatments showed statistical significance

compared to controls starting seven DAT ($p<0.003$) through the end of the trial (all treatments $p<004$ at 14 DAT; table 2.3). Mortality rate was higher in acephate compared to all imidacloprid concentrations, but by seven DAT, imidacloprid was still highly effective (≥90%, table 2.3).

Trial 4*:*

All insecticide treatments affected beetles as early as one DAT (Figure 2.4). Acephate at 10 ppm affected 60% of adults one DAT, but was the lowest of all treatments, where at least 90% of beetles were affected (Table 2.4). At least 60% of adults were dead three DAT in both acephate treatments with 30% mortality observed in adults treated with imidacloprid (Table 2.4). Imidacloprid at all concentrations showed similar affected rates to acephate, but mortality rates ranged from 30-70%. At the end of the trial, at least 90% of all adults were affected in each treatment, while 30% of control adults were dead (Table 2.4). All treatments were statistically significance compared to controls one DAT ($p<0.02$) and continued to show statistical significance throughout the trial.

Trial 5*:*

Beginning one DAT, 70% of adults exposed to acephate at 10 ppm were dead, while 70% of adults exposed to acephate at 100 ppm and 90% of adults with imidacloprid at 100 ppm were affected which were statistically significant compared to the zero control adults affected (Table 2.5, $p<0.05$). This trend continued throughout the trial. Beginning 3 DAT, imidacloprid at 10 ppm was also statistically significance compared to controls with 80% of adults affected compared to 20% mortality in controls ($p<0.05$). Similar mortality rates were observed between Palm Weevil Killer at both concentrations and controls were observed throughout the trial (Figure 2.5).

Trial 6*:*

During the first four hours (Figure 2.6), no feeding was observed and so it was difficult to determine exactly which beetles fed at each hour after treatment. Forty percent of adults treated with imidacloprid at 10 ppm were paralyzed beginning one hour after treatment which continued through the first day of observations, whereas, 10% of the 100 ppm concentration of imidacloprid were paralyzed for the first three hours then increased to 20% at the fourth-hour observation point. No paralysis was observed in any other treatment over the first day.

At least 10% mortality was observed in all treatments other than Palm Weevil Killer at high concentration starting one DAT which was statistically significant compared to the zero control adults affected (Figure 2.7, $p<0.02$). At least 80% of adults were affected in both concentrations of imidacloprid and acephate three DAT (Table 2.6). Despite 60% mortality and a 70% overall affected rate in imidacloprid at 10 ppm, the treatment was not statistically significant from the control adults

(20% mortality) seven dAT ($p=0.70$, figure 2.7) until 14 DAT where a 90% of adults treated with imidacloprid at 10 ppm were affected compared to 30% mortality in controls ($p<0.02$).

Field Trial:

Although all treatment scores primarily consisted of 0-1, acephate resulted in consistent overall frond condition improvement until eight MAT, and remained the least damaged palm treatment among all treatments up to 15 months post initial treatment (Figure 2.8). Starting from 16 months post initial treatment, both imidacloprid and combo (imidacloprid + acephate) treatments began to show good effects. Inner frond damage was the lowest among all treatments when acephate was applied up to seven months after initial treatment. At eight months after treatment, the application of combo became the lowest inner frond damage (Figure 2.9). The similarities between the damage ratings on the overall tree health and the inner four fronds suggest that CRB adults do not prefer one tree over another as far as feeding behavior which allows for adults to become affected by the insecticides. Two adult *O. rhinoceros* were found on the ground where the field trial is located.

Panel traps in the treated region showed a marginally significant difference in beetles caught compared to panel traps in a neighboring area ($p=0.061$) suggesting that the two locations had different catch rates throughout the experiment. Neither time, nor a cross of time and location showed significance. Panel traps in the treated location showed a decreasing trend of adult *O. rhinoceros* caught over time, whereas traps in the non-treated location showed an increase in beetles caught over time (Figure 2.10).

Discussion

Due to the limited number of adult *O. rhinoceros* adults, different trials were used to test different insecticides in the lab. Acephate and imidacloprid were the two most effective insecticides throughout the six trials. Elzen, Maldonado, and Rojas (2000) observed some differences in insecticide efficacy between male and female boll weevils; however, no differences were noticed in any of the lab trials. In five of the six trials, acephate effectively killed and paralyzed adult *O. rhinoceros*, while in all five of the imicloprid trials resulted in high paralysis and eventual mortality. Both insecticides resulted in little to no recovery over the two weeks observed. Paralysis and mortality was observed as early as one day after treatment in both acephate and imidacloprid and imidacloprid paralyzed beetles as early as one hour after treatment (Figure 2.6). This suggests that imidacloprid can work almost immediately at affecting adult *O. rhinoceros*. Because these two insecticides at both concentrations used affected adults quickly, it is feasible that theoretically once a

beetle begins to feed in the wild on treated trees, it might become affected enough to at least slow feeding as it loses some motor controls.

Paralysis and mortality of adults exposed to emamectin benzoate and PWK was observed at higher concentrations, but only towards the end of their trials. Due to the slow effects, both emamectin benzoate and palm tree weevil killer were not considered to be effective insecticides against *O. rhinoceros* adults. This disagreed with Jansson et al. (1997) who suggested the maximum time needed for mortality of emamectin benzoate was four days. As azadirachtin can be used as a deterrent (Mohan & Padmanaban, 2013), the sugar cane pieces dipped in the insecticide were not fed upon, but adults exposed to it did not die suggesting that it could take longer than two weeks for adults to die of starvation. On Oahu, *O. rhinoceros* is also localized around Pearl Harbor. Using feeding deterrents in a field setting might cause adults to fly further from their current sites causing an expansion of range on the island. These two factors eliminated azadirachtin from being used on adults in future trials and experiments.

No differences were observed in overall tree health or inner petiole health in field trials between treatments suggesting that treatments did not affect these scores. This could mean that the amount of insecticides in each tree was not high enough to affect adult *O. rhinoceros* or that the number of adult *O. rhinoceros* is low. A large majority of all trees received overall tree health and inner frond health scores of zero throughout the experiment suggesting a low overall beetle count in the area. Although it is unknown how many adults are in a given area, a large majority of the ratings were one for both overall tree health and inner four petiole health suggesting few beetles are in the area of the experiment (Figure 2.8, 2.9). A reduction in adult *O. rhinoceros* caught in panel traps in the treated location compared to an increase in beetles caught over time in the neighboring location suggesting that treatments showed some negative effects to *O. rhinoceros* in the field (Figure 2.10).

The efficacy trials in the lab showed acephate and imidacloprid as the two most effective among all of the insecticides tested which suggests that they may be effective chemical control to adult *O. rhinoceros* in landscape settings. The field trial did not consistently show significant results, but trap data did show a decrease in overall beetles caught compared to similar levels of beetles caught in the neighboring location suggests that there was some effect of insecticide application (Figure 2.10). This could be due to beetles feeding in control trees would not be exposed to insecticides and so would have no effects, allowing for continued breeding and survival. In the future, more trees could be treated with exclusively acephate, imidacloprid, or a combination of the two. Other factors can also be looked at to try to increase field efficacy to match lab results. Hawai‘i has a relatively strict biocontrol vetting system and so it can take several years before biocontrol

options to surface and so effective chemical and mechanical control are vital in keeping invasive species from expanding. Based on the killing power of these insecticides, theoretically, they could be used in assisting eradication efforts on Oahu. Both acephate and imidacloprid should be used in a landscape or ornamental palm setting and on Oahu, most palms are used in an ornamental setting and so would be viable for application. In locations where coconuts are used as a food source, the palms would not be able to receive either acepahte or imidacloprid and so would not be viable for this control method. Future experiments will focus on determining the efficacy of other insecticides on adults and larval *O. rhinoceros* that can be applied systemically.

Tables

Table 2.1 Percent mortality and paralysis of adult *O. rhinoceros* exposed to each treatment throughout trial one. Corrected percent mortality was obtained by using Abbott's Formula.

	Day After Treatment							
	1		3		7		13	
Treatment	% Mortality	% Paralysis	% Mortality	% Paralysis	% Mortality	% Paralysis	% Mortality	% Paralysis
Control	0	0	0	0	10	0	10	0
Acephate 100 ppm	0	40	40	30	30	10	100	0
Corrected	0		40		22.22		100	
Acephate 1000 ppm	30	30	40	30	90	0	90	0
Corrected	30		40		88.89		89.89	
Emamectin Benzoate 100 ppm	0	0	0	0	10	0	20	0
Corrected	0		0		0		11.11	
Emamectin Benzoate 1000 ppm	0	20	20	30	40	20	60	10
Corrected	0		20		33.33		55.56	

Table 2.2 Percent mortality and paralysis of adult *O. rhinoceros* exposed to each treatment in the second trial. Corrected percent mortality was obtained by using Abbott's Formula.

	Day After Treatment							
	1		3		7		15	
Treatment	% Mortality	% Paralysis	% Mortality	% Paralysis	% Mortality	% Paralysis	% Mortality	% Paralysis
Control	0	0	0	0	0	0	10	0
Acephate 10 ppm	10	10	10	0	10	10	40	0
Corrected	10		10		10		33.33	
Acephate 100 ppm	0	0	0	0	10	0	20	20
Corrected	0		0		10		11.11	
Azadirachtin 1000 ppm	0	0	0	0	0	0	0	0
Corrected	0		0		0		0	
Imidacloprid 1000 ppm	10	30	50	20	70	20	90	0
Corrected	10		50		70		88.89	

Table 2.3 Percent mortality and paralysis of adult *O. rhinoceros* exposed to each treatment throughout trial three. Corrected percent mortality was obtained by using Abbott's Formula.

	Day After Treatment							
	1		3		7		14	
Treatment	% Mortality	% Paralysis	% Mortality	% Paralysis	% Mortality	% Paralysis	% Mortality	% Paralysis
Control	0	0	0	0	0	0	10	0
Acephate 100 ppm	0	10	50	0	60	30	80	20
Corrected	0		50		60		71.43	
Imidacloprid 10 ppm	0	30	0	10	10	90	10	90
Corrected	0		0		10		0	
Imidacloprid 100 ppm	0	20	0	30	0	90	10	90
Corrected	0		0		0		0	
Imidacloprid 1000 ppm	0	60	30	60	40	60	50	50
Corrected	0		30		40		28.57	

Table 2.4 Percent mortality and paralysis of adult *O. rhinoceros* exposed to each treatment in the fourth trial over time. Corrected percent mortality was obtained by using Abbott's Formula.

	Day After Treatment							
	1		3		7		14	
Treatment	% Mortality	% Paralysis	% Mortality	% Paralysis	% Mortality	% Paralysis	% Mortality	% Paralysis
Control	0	0	0	0	0	0	30	0
Acephate 10 ppm	40	20	70	0	70	20	80	10
Corrected	40		70		70		71.43	
Acephate 100 ppm	20	70	60	30	60	40	70	30
Corrected	20		60		60		57.14	
Acephate 1000 ppm	40	60	70	30	90	10	90	10
Corrected	40		70		90		85.71	
Imidacloprid 10 ppm	0	100	30	60	30	70	30	60
Corrected	0		30		30		0	
Imidacloprid 100 ppm	20	70	50	30	50	40	60	30
Corrected	20		50		50		42.86	
Imidacloprid 1000 ppm	10	80	70	30	70	30	70	30
Corrected	10		70		70		57.14	

Table 2.5 Percent mortality and paralysis of adult *O. rhinoceros* exposed to each treatment in the fifth trial over time. Corrected percent mortality was obtained by using Abbott's Formula.

	Day After Treatment							
	1		3		7		14	
Treatment	% Mortality	% Paralysis	% Mortality	% Paralysis	% Mortality	% Paralysis	% Mortality	% Paralysis
Control	0	0	20	0	20	0	50	0
Acephate 10 ppm	70	0	70	20	80	10	90	10
Corrected	70		62.5		75		80	
Acephate 100 ppm	40	30	70	20	80	10	90	10
Corrected	40		62.5		75		80	
Imidacloprid 10 ppm	20	10	40	40	40	20	60	40
Corrected	20		25		25		20	
Imidacloprid 100 ppm	50	40	70	20	70	20	90	10
Corrected	50		62.5		62.5		80	
Palm Weevil Killer Low	0	20	20	20	20	30	50	10
Corrected	0		0		0		0	
Palm Weevil Killer High	0	0	0	0	10	10	10	50
Corrected	0		0		0		0	

Table 2.6 Percent mortality and paralysis of adult O. rhinoceros exposed to each treatment throughout trial six. Corrected percent mortality was obtained by using Abbott's Formula.

	Day After Treatment							
	1		3		7		15	
Treatment	% Mortality	% Paralysis	% Mortality	% Paralysis	% Mortality	% Paralysis	% Mortality	% Paralysis
Control	0	0	0	0	20	0	30	0
Acephate 10 ppm	20	60	80	0	80	0	80	10
Corrected	20		80		75		71.43	
Acephate 100 ppm	40	40	80	10	80	10	90	0
Corrected	40		80		75		85.71	
Imidacloprid 10 ppm	40	20	60	20	60	10	70	20
Corrected	40		60		50		57.14	
Imidacloprid 100 ppm	10	70	50	40	80	0	80	10
Corrected	10		50		75		71.43	
Palm Weevil Killer High	0	10	10	10	10	10	30	40
Corrected	0		10		0		0	

Figures

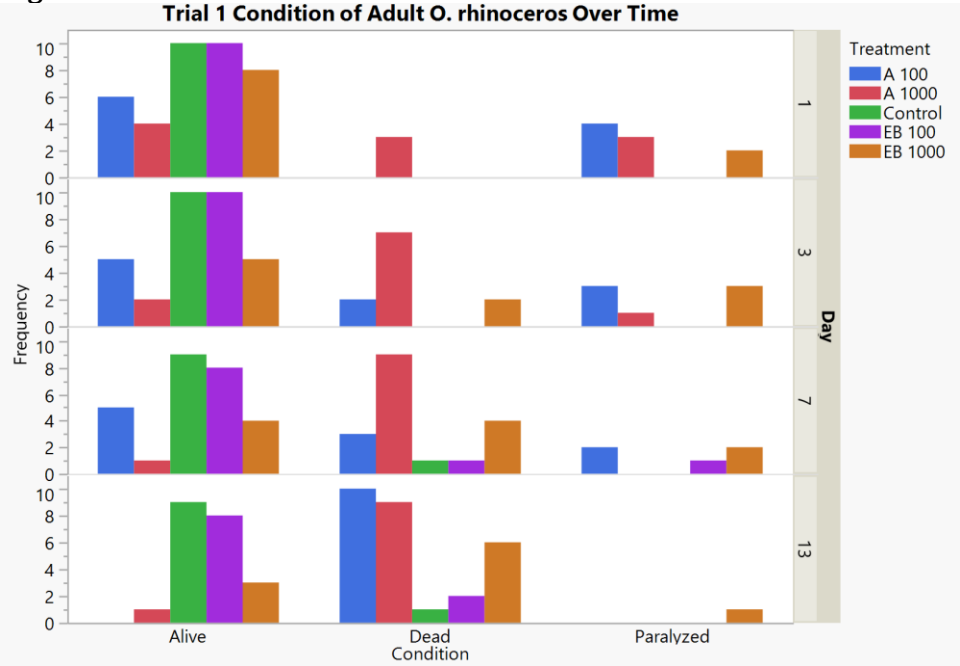

Figure 2.1. Number of adult *O. rhinoceros* for each condition rating over time in trial one. Control: Untreated, A 100: Acephate 100 ppm, A 1000: Acephate 1000 ppm, EB 100: Emamectin Benzoate 100 ppm, EB 1000: Emamectin Benzoate 1000 ppm

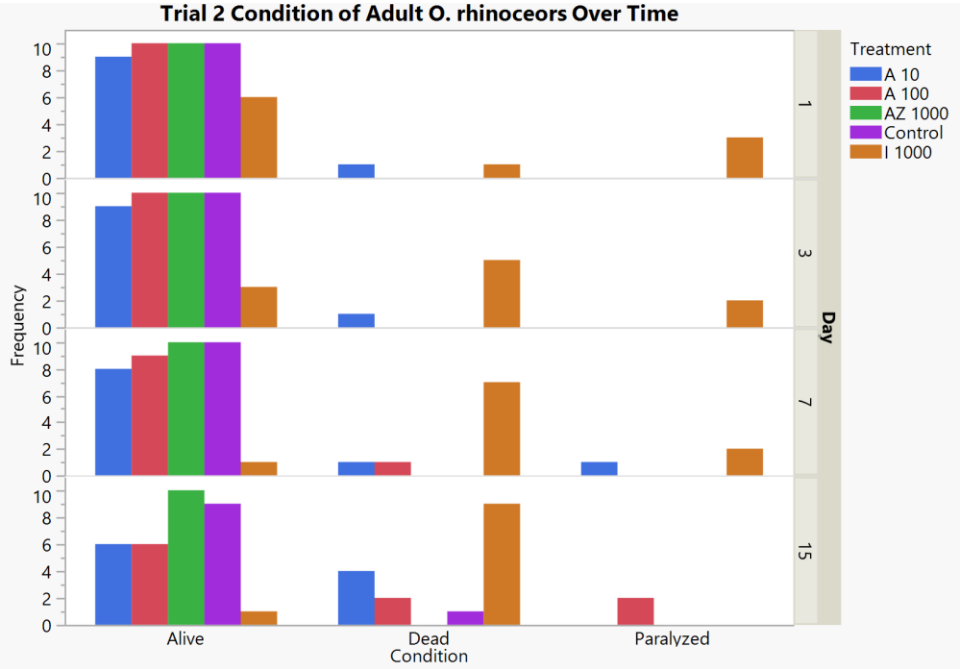

Figure 2.2 Number of adult *O. rhinoceros* for each condition rating over time in trial two. Control= Untreated, A 10= Acephate 10 ppm, A 100= Acephate 100 ppm, AZ 1000= Azadirachtin 1000 ppm, I 1000= Imidacloprid 1000 ppm

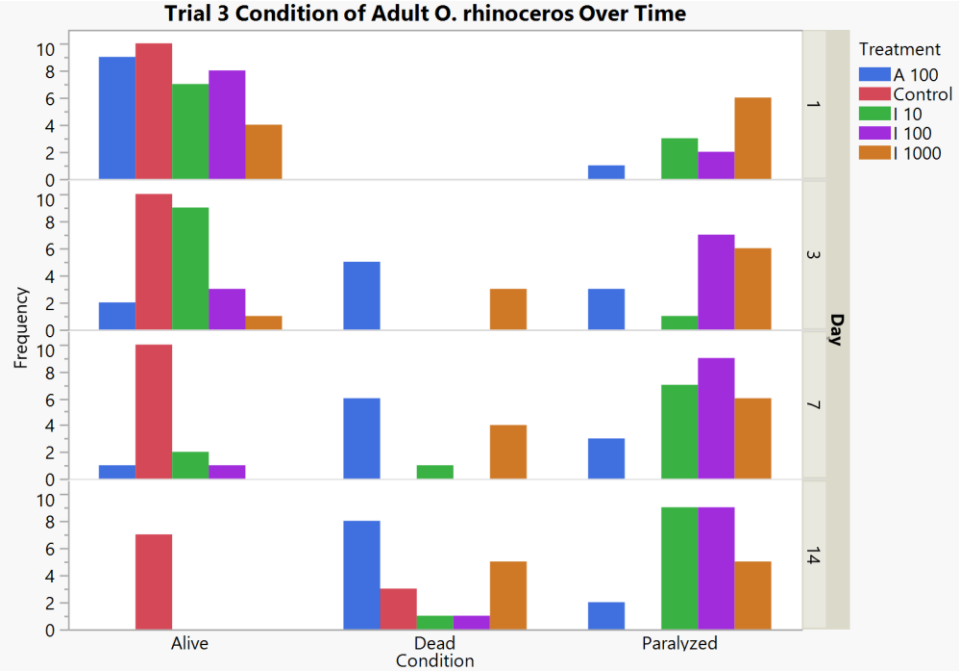

Figure 2.3. Number of adult *O. rhinoceros* for each condition rating over time in trial three. Control= Untreated, A 100= Acephate 100 ppm, I 10= Imidacloprid 10 ppm, I 100= Imidacloprid 100 ppm, I 1000= Imidacloprid 1000 ppm.

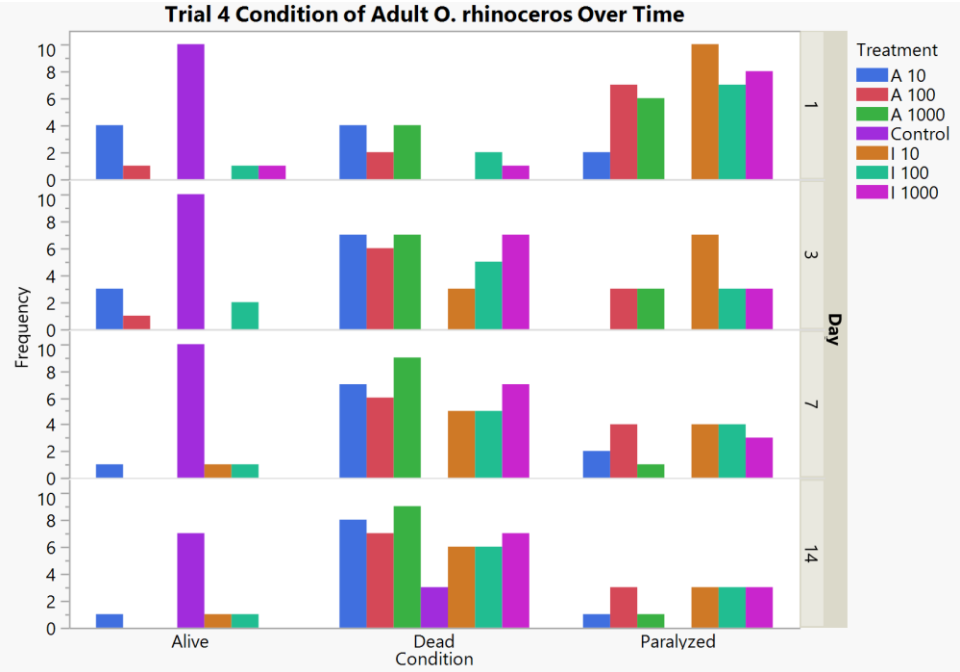

Figure 2.4. Number of *O. rhinoceros* for each condition rating over time in trial four. Control= Untreated, A 10= Acephate 10 ppm, A 100= Acephate 100 ppm, A 1000= Acephate 1000 ppm, I 10= Imidacloprid 10 ppm, I 100= Imidacloprid 100 ppm, I 1000= Imidacloprid 1000 ppm.

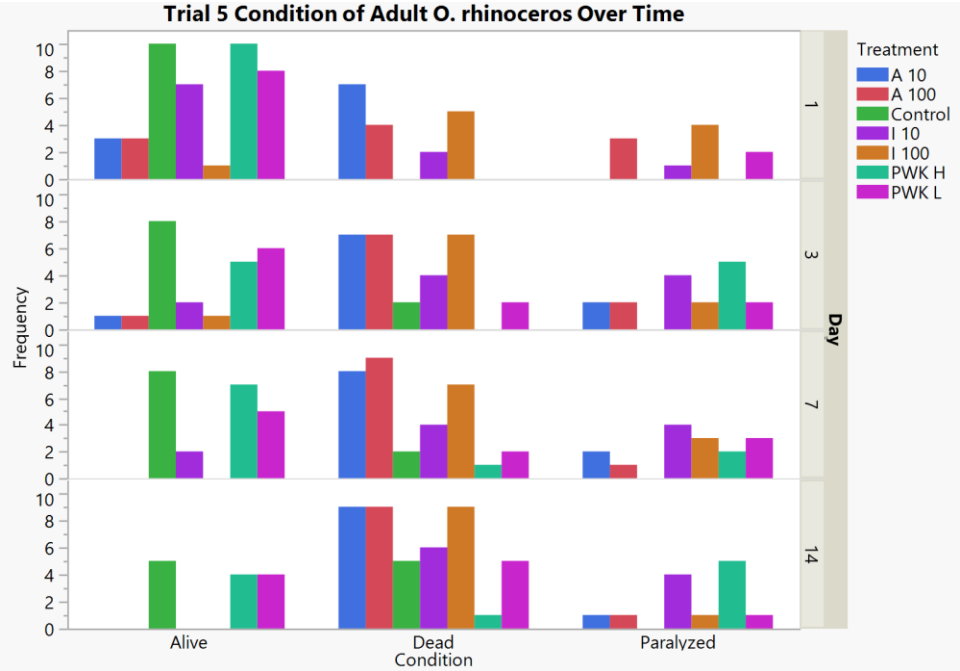

Figure 2.5. Number of adult *O. rhinoceros* for each condition rating over time in trial five. Control= Untreated, A 10= Acephate 10 ppm, A 100= Acephate 100 ppm, I 10= Imidacloprid 10 ppm, I 100= Imidacloprid 100 ppm, PWK L= Palm Weevil Killer solution at the low concentration, PWK H= Palm Weevil Killer solution at the high concentration.

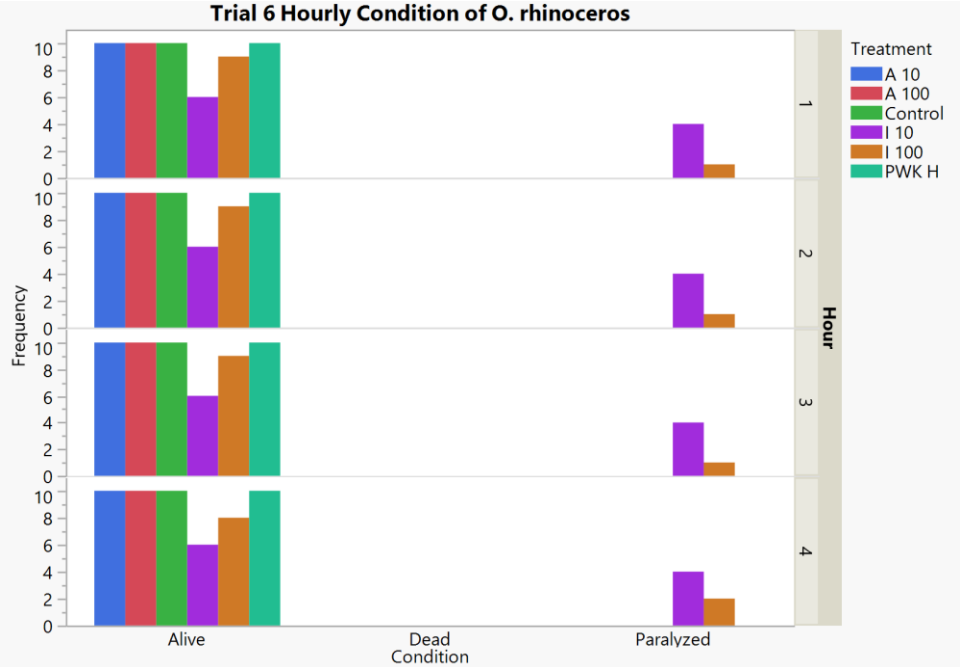

Figure 2.6. Condition of *O. rhinoceros* over the first day of treatment in trial six. A 10= Acepahte 10 ppm, A 100= Acephate 100 ppm, Control= Untreated, I 10= Imidacloprid 10 ppm, I 100= Imidacloprid 100 ppm, PWK H= Palm Weevil Killer solution at the high concentration.

Figure 2.7. Number of adult *O. rhinoceros* for each condition rating over time in trial six. A 10= Acepahte 10 ppm, A 100= Acephate 100 ppm, Control= Untreated, I 10= Imidacloprid 10 ppm, I 100= Imidacloprid 100 ppm, PWK H= Palm Weevil Killer solution at the high concentration.

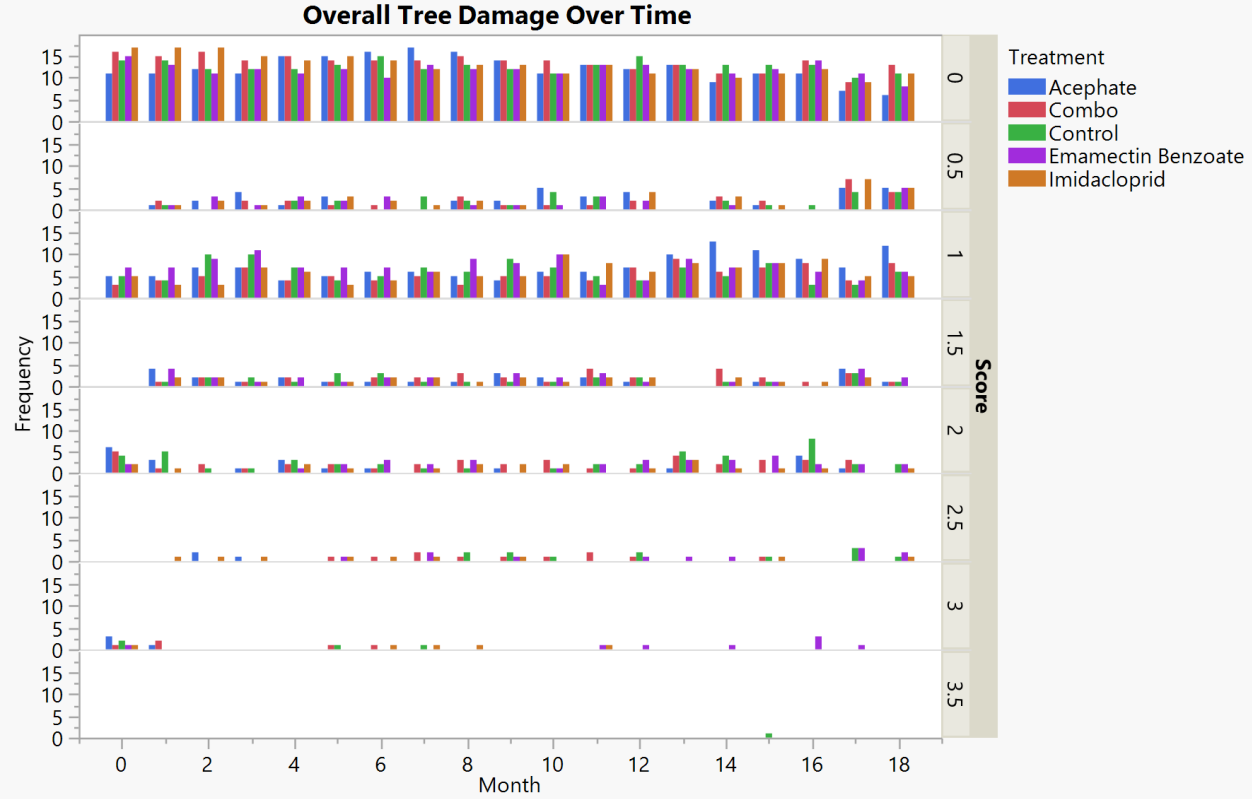

Figure 2.8. Number of *C. nucifera* at each overall palm damage over time by treatment. Scores are on a 0-5 scale with 0 indicating no damage and 5 being a dead tree.

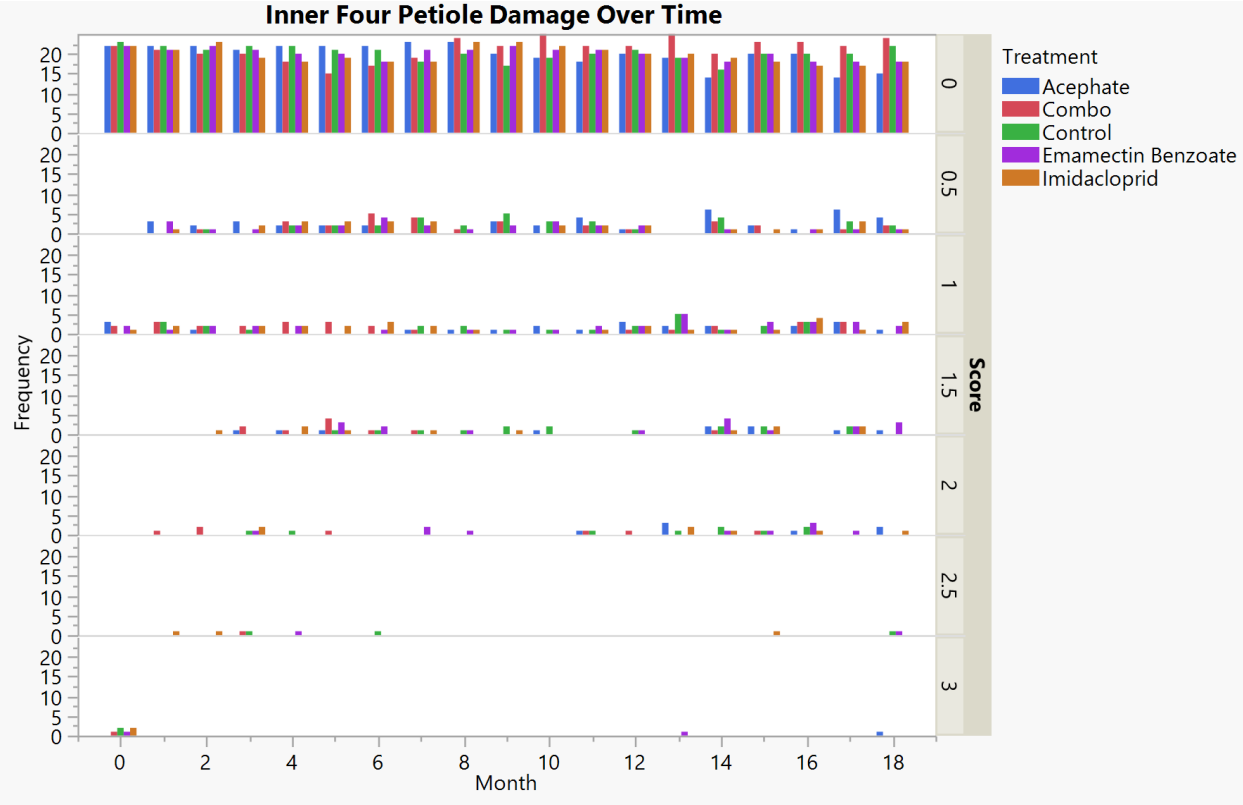

Figure 2.9. Inner four petiole damage over time by treatment type. Scores are on a 0-5 scale with 0 indicating no damage and 5 being a dead tree.

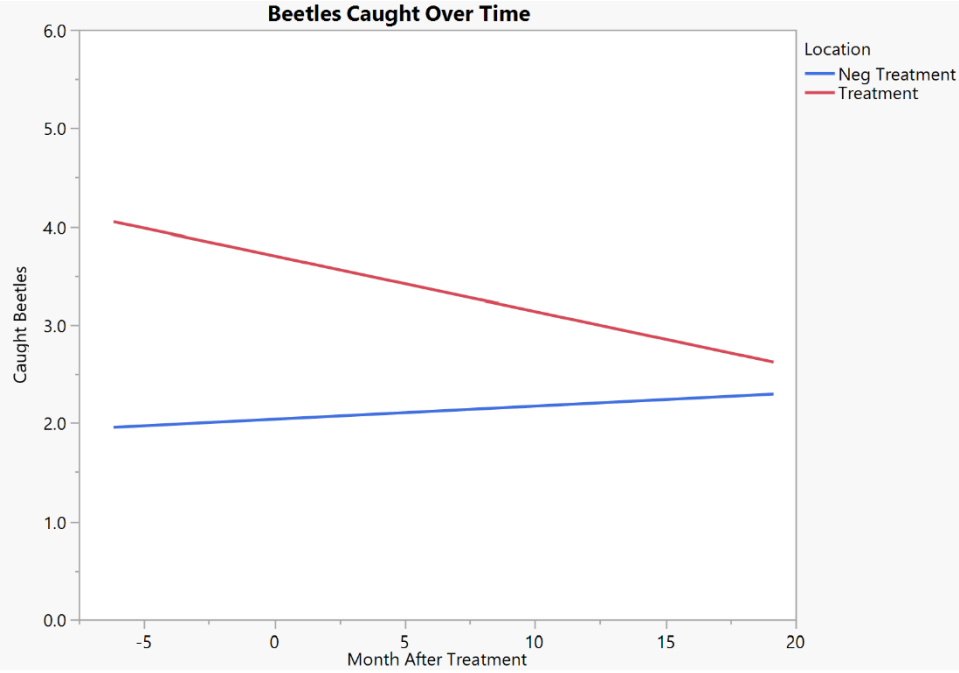

Figure 2.10. Panel Trap caught adult *O. rhinoceros* over time. Treatment indicates panel traps near treated trees, Neg Treatment indicates panel traps off site.

Chapter 3. Management strategies of *Ficus* stem and leaf gall wasps, *Josephiella* spp. on Chinese Banyan *Ficus microcarpa* in Hawai‘i

Introduction

Chinese banyan, *Ficus microparpa* L.f. (Rosales: Moraceae) is native to South/South-East Asia including Ceylon, India, China, Ryukyu islands, as well as into the pacific in Australia and New Caledonia (Starr et al., 2003). It is also widely found in a landscape setting throughout tropical and subtropical regions and was introduced to Hawai‘i in 1921 (Ramirez et al., 1988) and can grow in moist and dry environments (Starr et al., 2003). *Ficus microcarpa* has a small fruit size which allows for various methods of dispersal and fruits can be pollinated throughout the year by *Eupristina verticillata* Waterson (Hymenoptera: Agaonidae) (Starr et al., 2003; Pemberton, 1939).

In 1989, a non-pollinating agaonid fig wasp, *Josephiella microcarpae* was found in Hawai‘i (Starr et al. 2003). Adult *J. microcarpae* oviposit in the leaves of *F. microcarpa* which create galls. Adults are small (2.2mm females, 1.1mm males), dark brown, and have yellow appendages and females show preference to oviposit on younger terminal leaves over mature leaves and can oviposit multiple eggs per leaf (Beardsley and Rasplus, 2001). The pupal stage can develop in the leaf galls both on the tree or on abscised leaves.

A second species of *Josephiella* wasp was discovered in July 2012 in Hawai‘i and was found to be more damaging to *F. microcarpa* by induce galls on young stems (HDOA, 2012). The galls caused defoliation and eventual death of the injected shoots. This new agaonid wasp is currently undescribed and has only been found in Hawai‘i. Previous lab studies showed a longer life cycle of the stem gall wasp (5-6 months) compared to *J. microcarpae* (3-4 months) and that the stem gall wasp will not oviposit in leaves and *J. microcarpae* will not oviposit in the stem (Bhandari and Cheng, 2016).

Currently, no predators or parasitoids are known for either of these wasp species and so biocontrol is not an option for management. Insecticides such as emamectin benzoate have shown some success on gall forming insects (Doccola et al., 2009) and emamectin benzoate has shown success against both gall wasps for up to 14 months after treatment (Bhandari and Cheng, 2016). Emamectin benzoate can also be applied through systemic trunk injections which can lower the risk of off target effects as the chemical is applied directly into the vascular system, unlike soil drenching or foliar spraying, which would also allow for less active ingredients (Norris, 1965; Doccola and

Wild, 2012). Proper irrigation techniques can also reduce stress to plants which allows the plant to fend off detrimental predators, parasitoids, or pathogens more effectively.

Because of previous success with emamectin benzoate, it was used again to test efficacy of systemic insecticides on both of the gall wasps as well as to see how irrigation practices might affect the efficacy. It was hypothesized that the emamectin benzoate should still show efficacy compared to untreated trees and that the addition of monthly watering would also be beneficial to tree health both by itself and in tandem with emamectin benzoate.

Materials and Methods

Experimental Design

Twigs of adult *F. microcarpa* trees on the University of Hawai‘i at Mānoa campus in Honolulu, Hawai‘i were collected to determine initial leaf gall wasp and stem gall wasp presence as well as overall tree health and new shoot emergence. Twenty of the most similar *F. microcarpa* were chosen and placed into five nonrandom blocks to ensure highest similarity of metrics among groups. Trees in each group were randomly assigned a treatment type (injection, injection plus irrigation, irrigation alone, and untreated control). Emamectin benzoate was administered to injected insecticide trees via a TREE I.V. system (Arborjet) according to label rates based on tree diameter at breast height (DBH). Trees that received irrigation were given an additional 10 gallons of water per month in addition to natural rain water. Preexisting irrigation systems were not in place and so *F. microcarpa* trees were not exposed to any other water source.

Data Collection

Monthly observations beginning November 2016 (one month after treatment, MAT) until December 2017 (14 MAT) were conducted by randomly clipping three new terminal shoots from each tree approximately 45 cm long using a tree pruner (ATSS PSPL30NC, American Tree Service Supply, Greenville, RI, USA). Observations included measurements of stem gall counts, average stem infestation level (1-5 scale, 1 showing no infestation and 5 showing heavy infestation), percentage of infested leaves, average leaf infestation level (1-5 scale, 1 showing no infestation, 5 showing multiple galls on infested leaves and greater than 75% of leaves having infestation), as well as visual ratings on overall tree health (1-5 scale, 1=full canopy with no dead shoots/branches, 2=mostly full canopy with little to no dead shoots/branches, 3=moderate canopy coverage with some dead shoots/branches, 4=poor canopy coverage with dead shoots/branches, 5=dead tree) and new shoot emergence (1-5 scale, 1=many, 2=moderate, 3=some, 4=little, 5=none). Galls showing exit

holes were not considered as far as current infestation levels as the wasps would have completed their life cycle, but were used to determine approximately how many gall wasps could occupy a given area.

Statistical Analysis

Statistical analysis was conducted using JMP Pro 13. Number of stem galls and percent leaf infestation were analyzed using an ANOVA and Tukey's test (α=0.05), while contingency tables and a X^2 test was run for both visual rating systems (α=0.05). A bivariate model was also produced with a regression line to determine if overall tree health and new shoot emergence was impacted by treatment type between 0 and 14 months' post application (α=0.05).

Results

Overall tree health remained consistent over time in all treatments and control trees (Figure 3.1). Emamectin benzoate alone was the only treatment to receive an overall tree score of 5 (seven, nine, and ten MAT, Figure 3.1), but occurred in one sample. Trees of all treatments consistently received overall tree health scores of 2 or three. One tree treated with irrigation received a new shoot emergence score of five, one MAT, while no other treatment received a score of five through the trial (Figure 3.2). Similar to overall tree health, new shoot emergence scores were primarily two to three; however, a higher frequency of one scores were observed in new shoot emergence suggesting a large presence of new shoots (Figure 3.2)

Injection alone resulted in the lowest stem gall wasp number at month 14 and also showed the largest reduction in terms of stem gall number compared to initial condition (Figure 3.3).

Emamectin benzoate treatments as well as irrigation were able to maintain a similar percent of infested leaves at a lower level compared to control; however, these results were non-significant (Figure 3.4). Irrigated trees consistently produced fewer leaf galls compared to other treatments, while fewer infested leaves were observed in all treatments compared to controls (Figure 3.4).

Discussion

Similar trends were observed in overall tree health and new shoot emergence among treatment types with a majority of overall tree health and new shoot emergence scores among treatments being three (Figure 3.1, 3.2). This might suggest that either different treatments have little to no effect on overall tree health or that the timetable used for this experiment was not long enough to discern a pattern. Consistent new shoot emergence scores were observed throughout the

experiment in all treatments. Despite no treatment significantly differencing from controls, fewer leaf and stem galls were observed in all treatments compared to controls beginning five MAT suggesting that treatments have some beneficial effects on *Josephiella* spp. Other insects were found during observations, though, such as thrips and scales which may have some impact, but were not considered for this experiment.

Due to feasibility, trees were only watered 10 gallons per month. Consistently fewer leaf galls were present in irrigated trees as well as trees treated with emamectin benzoate compared to controls suggesting that irrigation might have some beneficial effects against *J. microcarpae* (Figure 3.4). Similar effects in irrigated trees were not observed against the stem gall wasp, but could be used as a lower cost management method if *J. microcarpae* is the only wasp attacking *F. microcarpa*. As no current literature standardizes an appropriate volume of water per tree or DBH, trees may have received too much or too little water. Potential remedies to resolve this could be to determine soil moisture content and identify proper irrigation for *F. microcarpa*.

The current results partially agree with previous studies on the *Josephiella* spp. on *F. microcarpa* (Bhandari and Cheng, 2016) that injection with emamectin benzoate was able to moderately suppress both *Ficus* stem and leaf gall wasps. However, new management strategies tested in this study (injection with irrigation and irrigation alone) did not result in significant suppression against both gall wasp species.

Figures

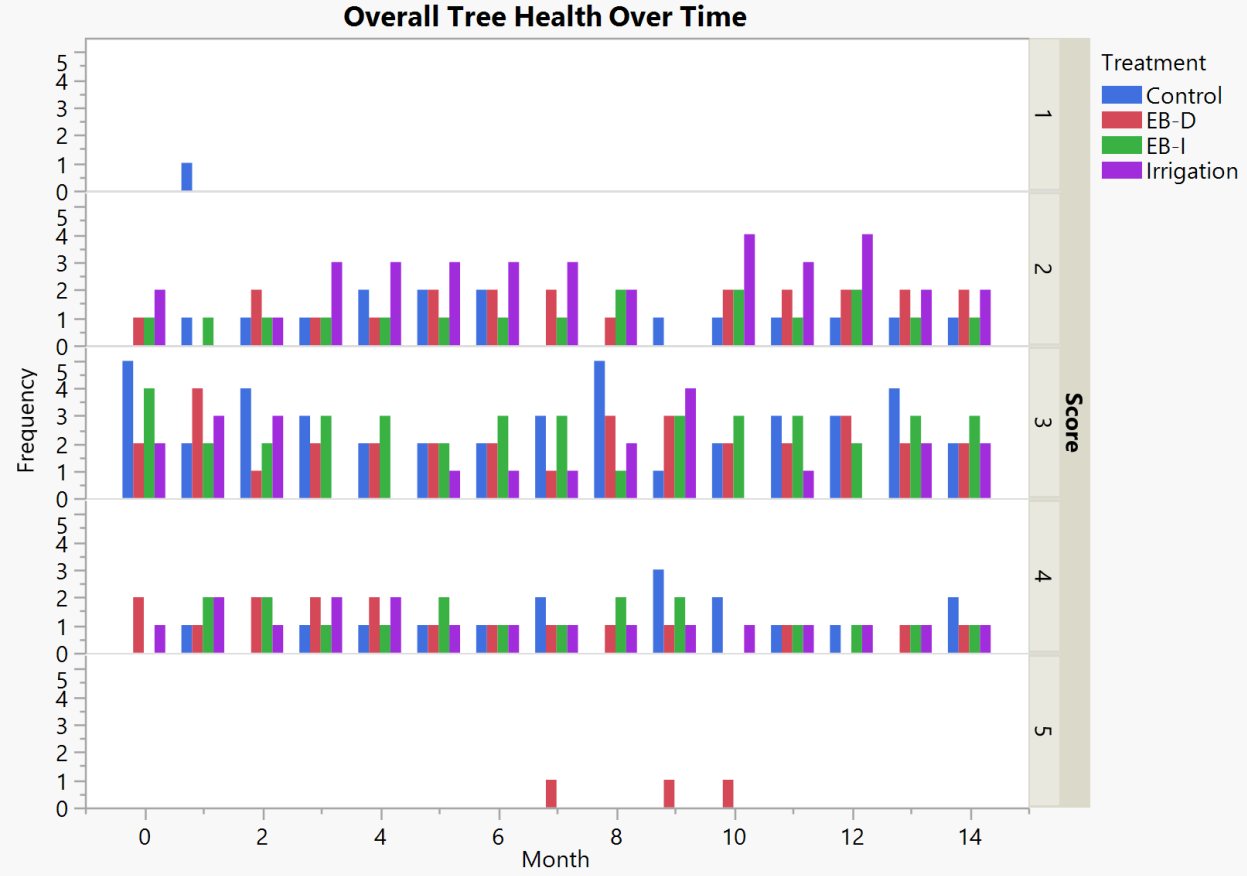

Figure 3.1. Number of *F. microcarpa* at each tree health score over time by treatment. Rating scale 1-5, 1 indicating full canopy with no dead shoots/branches and 5 indicating a dead tree. Control= Untreated, EB-D= Emamectin Benzoate alone, EB-I: Emamectin Benzoate plus Irrigation, Irrigation= Monthly Watering

Figure 3.2. Number of *F. microcarpa* over time by new shoot emergence score for each treatment type. Graph presented using means for ease of understanding. Rating scale 1-5, 1 indicating many new shoots and 5 representing no new shoots. Control= Untreated, EB-D= Emamectin Benzoate alone, EB-I: Emamectin Benzoate plus Irrigation, Irrigation= Monthly Watering

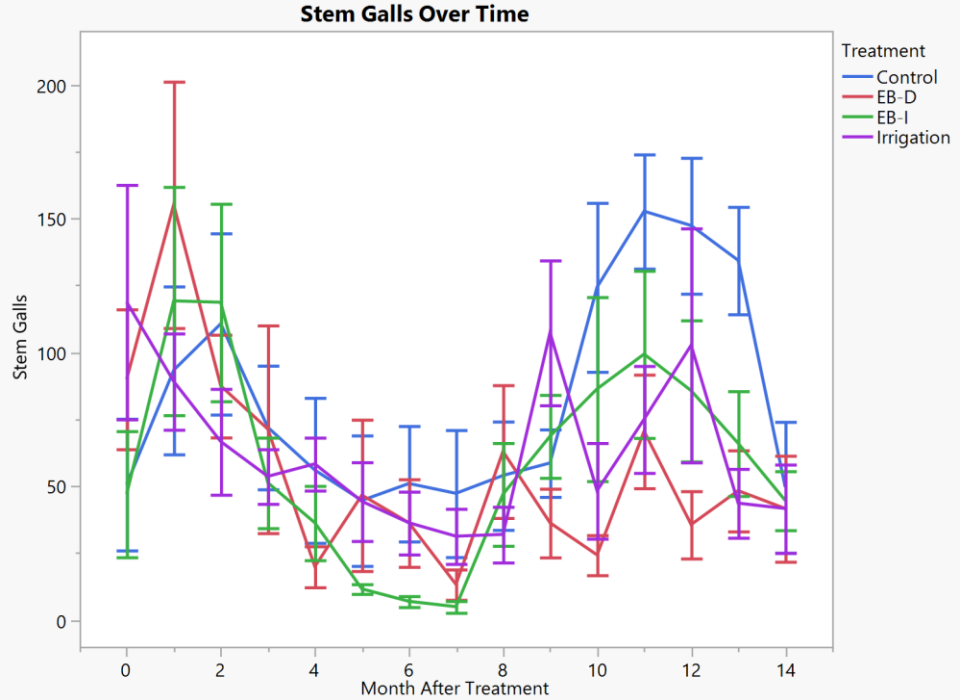

Figure 3.3. Average number of stem galls on each branch over time by treatment type. Control: untreated, EB-D: emamectin benzoate alone, EB-I: emamectin benzoate plus irrigation, Irrigation: monthly watering.

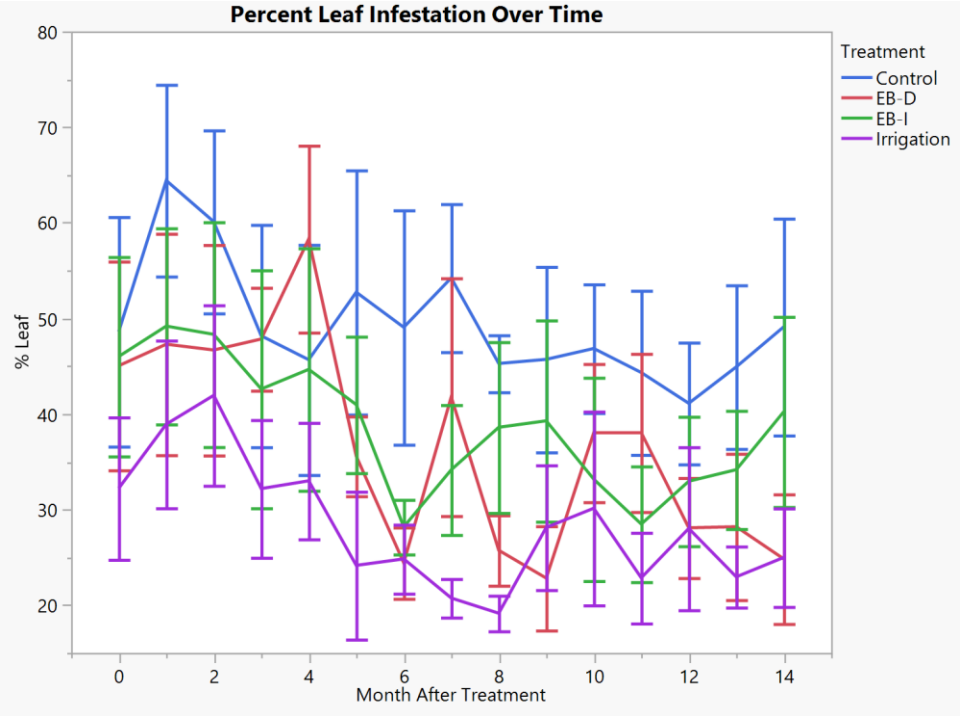

Figure 3.4 Percent of leaves infected with leaf galls over time by treatment. Control: untreated, EB-D: emamectin benzoate alone, EB-I: emamectin benzoate plus irrigation, Irrigation: monthly watering.

Chapter 4. Management strategies of lobate lac scale, *Paratachardina pseudolobata* on weeping banyan *Ficus benjamina* in Hawai‘i

Introduction

The lobate lac scale, *Paratachardina pseudolobata* Kundo and Gullan (Kerriidae: Coccoidae: Hemiptera) is suspected to be native to South Asia, but has been found in Hawai‘i, Florida, Bahamas, Christmas Island, Puerto Rico, and Cuba (Garcia, 2013). Until 2007, *P. pseudolobata* was synonymous with *P. lobate* until molecular analysis and differences in morphological features were identified (Kondo and Gullan, 2007). *Paratachardina pseudolobata* was first observed in Hawai‘i on *Ficus benjamina* in 2012 and has become a severe pest, promoting sooty mold and can cause thinning foliage, dieback of branches or twigs, and eventual death if not controlled (Garcia, 2013). All life stages can be found on woody branches less than 2 cm in diameter, but larvae disperse via wind or animals.

Larvae, or the crawler stage, are bright red and are about 0.4 mm long. Female adults are sedentary and about 2 mm diameter and form an x-shaped shellac and are a dark red color. No males have been observed and so are assumed to be parthenogenic. *Paratacharidina pseudolobata* was been found on over 110 plant species in Hawai‘i and over 300 woody plants in Florida showing that it has a diverse host range and can be a pest in many tropical environments (Bhandari and Cheng, 2018; Howard et al., 2010). Many of the host plants have been found in landscape settings such as hibiscus, *Hibiscus* spp, Chinese banyan, *Ficus microcarpa*, and weeping banyan, *F. benjamina* (Garcia, 2013). Currently, no research has shown effective biological control agents, but imidaclorpid has shown success both via soil drench and injection methods (Howard and Steinberg, 2005, Cheng & Bhandari, 2015). This study attempts to determine if one application method is more effective than another and if irrigation methods can improve these effects further or if irrigation alone can allow sufficient effects to alleviate the presence of *P. pseudolobata*.

As both injection and soil drenching has shown highly effective results on suppressing *P. pseudolobata* on multiple plant species from various studies, it is hypothesized that there will be little difference between the two application methods. It is also hypothesized that the irrigation will improve overall tree health and indirectly help trees to resist lobate lac scale infestation. We also aimed to test if irrigation practice will affect the efficacy of trunk injection and soil drench, or not.

Materials and Methods

Experimental Design

Twigs of adult *F. benjamina* trees on the University of Hawai‘i at Mānoa campus and Ala Wai Golf Course in Honolulu, Hawai‘i were collected to determine initial *P. pseudolobata* presence as well as overall tree health and new shoot emergence. Twenty-four of the most similar *F. benjamina*, six at the University of Hawai‘i at Mānoa and 18 at Ala Wai Golf Course, were chosen and placed into four nonrandom blocks (One block at the University of Hawai‘i at Mānoa and three at Ala Wai Golf Course) to ensure highest similarity of metrics among groups. Trees in each group were randomly assigned a treatment type (injection, injection plus irrigation, soil drench, soil drench plus irrigation, irrigation alone, and untreated control). Imidacloprid was administered to injected insecticide trees via a TREE I.V. system (Arborjet) according to label rates based on tree diameter at breast height (DBH). Soil drenching was applied at the base of each treated tree according to label rates with one gallon of water dilutions. Trees that received irrigation were given an additional 10 gallons of water per month in addition to natural rain water. Preexisting irrigation systems were not in place at either location and so *F. benjamina* trees were not exposed to any other water source.

Data Collection

Monthly observations beginning November 2016 (one month after treatment, MAT) until December 2017 (14 MAT) were conducted by randomly clipping three new terminal shoots from each tree approximately 45 cm long using a tree pruner (ATSS PSPL30NC, American Tree Service Supply, Greenville, RI, USA). Observations included measurements of presence of *P. pseudolobata* (1-5 scale, 1 showing no infestation or no living individuals and 5 showing heavy infestation), overall tree health (1-5 scale, 1=full canopy with no dead shoots/branches, 2=mostly full canopy with little to no dead shoots/branches, 3=moderate canopy coverage with some dead shoots/branches, 4=poor canopy coverage with dead shoots/branches, 5=dead tree) and new shoot emergence (1-5 scale, 1=many, 2=moderate, 3=some, 4=little, 5=none). Juvenile *P. pseudolobata* were all treated as alive, whereas 10 adults were randomly pressed with a nail to determine vitality. If adults excreted hemolymph, they were considered alive, otherwise would be considered dead.

Statistical Analysis

Statistical analysis was conducted using JMP Pro 13. Contingency tables were created and X^2 tests were run to test for significance ($\alpha=0.05$) for lobate lac scale ratings as well as for both visual rating systems. A bivariate model was also produced with a regression line to determine if overall

tree health and new shoot emergence was impacted by treatment type between 0 and 14 months' post application (α=0.05).

Results

Overall tree health scores in injection alone treatments fluctuated the most in the trial with the only treatment having at least one tree in each treatment over the course of the experiment. Beginning five MAT, overall tree health scores of one were observed in injection plus irrigation and soil drench alone treatments (Figure 4.1). Twelve months after treatment, four trees treated received overall tree health scores of two, which was significantly less than controls (p=0.0183, Figure 4.1), whereas no other treatment showed statistical significance compared to controls through the course of the experiment.

New shoot emergence scores were consistent throughout the trial among treatments; however, soil drench plus irrigation slightly improved over time (Figure 4.2). Both soil drench treatments differed from controls five MAT where more new shoots were observed in control trees (p=0.0285, Figure 4.2). At 11 MAT, soil drench plus irrigation again differed from controls, but more new shoots were observed in soil drench plus irrigation trees compared to controls (p=0.0460).

Paratachardina pseudolobata was found on a majority of the trees throughout the experiment, but numerous samples included dead specimen which were not counted in the study as even dead adults remain attached to branches as long as no disturbances occur. Fewer living *P. pseudolobata* were observed in all treatments over time; however, no treatment was statistically significant compared to controls (p>0.05, Figure 4.3). Beginning seven MAT, no live lobate lac scale were observed in injection alone trees and beginning eight MAT, no live lobate lac scale were observed in soil drench alone trees (Figure 4.3).

Discussion

Similar to results found previously by Bhandari and Cheng (2018), there did not appear to be seasonal trends in presence of *P. pseudolobata* in this study. As *P. pseudolobata* can cause defoliation and death in over 110 species in Hawai‘i and over 300 species in Florida, a need for effective control methods is necessary (Bhandari and Cheng, 2018; Howard et al., 2010). Soil drench and injection techniques have both been successful on suppressing *P. pseudolobata* on *Ficus* spp. (Howard and Steinberg, 2005, Cheng & Bhandari, 2015). This study combined the previously mentioned two studies to determine if one method was more effective than another and also if irrigation played a factor by itself or amended the insecticides.

A slight improvement of overall tree health in soil drench plus irrigation treatments was observed; however, overall tree health and new shoot emergence remained consistent over time in all treatments. As these scores were primarily between two and four, the trees in this experiment had a relatively full canopy and new shoots throughout the year. At the Ala Wai Golf course, there were trees that had no lobate lac scale presence (either living or dead); however, some branches had no foliage suggesting some other factor could affect overall tree health. No other pests were observed on *F. benjamina* over the course of this experiment.

A reduction of *P. pseudolobata* was observed in all treatment types over the course of the experiment agreeing with both Howard and Steinberg (2005) and Bhandari and Cheng (2018). Trees that were treated with trunk injection fully suppressed *P. pseudolobata* beginning seven MAT with two instances in injection plus irrigation where *P. pseudolobata* was observed after seven MAT (Figure 4.3). Suppression of lobate lac scale was observed in a majority of injection plus irrigation trees as early as four MAT. Similarly, full suppression was observed in soil drench alone beginning eight MAT, and a majority of trees suppressed *P. pseudolobata* presence as early as five MAT. This suggests that both treatments can be used as effective chemical control on *P. pseudolobata* with injection killing lobate lac scale more quickly. This could be due to the application method where the insecticide is applied into the system directly and so does not need to be absorbed through the roots.

The addition of watering, even after applying imidacloprid via soil drench did not appear to dilute the concentration enough to cause diminished effects. Similarly, injection methods did not seem to be negatively affected by the addition of watering trees. Although irrigation alone did not have as much success on controlling *P. pseudolobata* compared to soil drench and injection, it is still important as it can keep the canopy of *F. benjamina* fuller which may slow the effects of *P. pseudolobata* until other control methods are implemented.

Figures

Figure 4.1. *F. benjamina* tree health over time by frequency of score. Rating scale 1-5, 1 indicating full canopy with no dead shoots/branches and 5 indicating a dead tree. Control= Untreated, Injection-D= Imidacloprid Injection alone, Injection-I=Imidacloprid injection plus Irrigation, Soil Drench-D= Imidacloprid Soil Drench alone, Soil Drench-I= Imidacloprid Soil Drench plus Irrigation, Irrigation= Monthly Watering

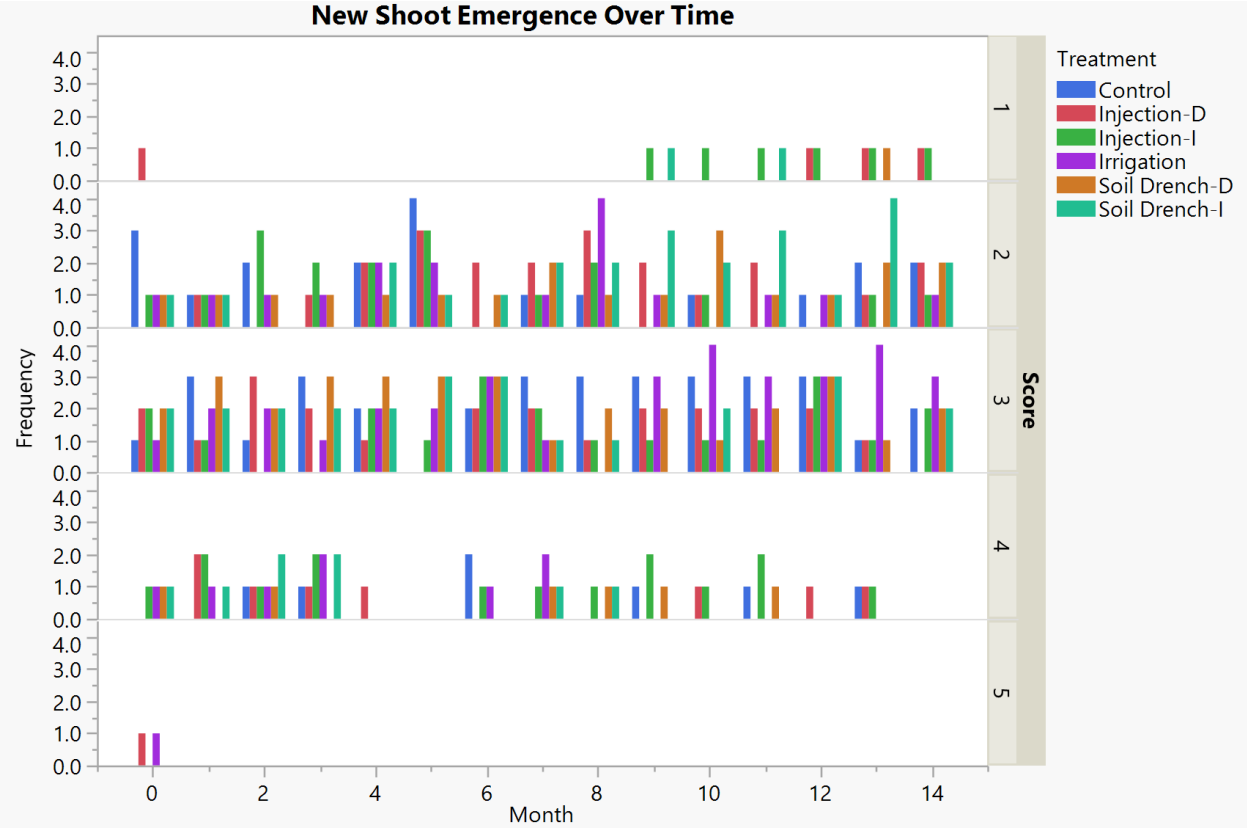

Figure 4.2. New shoot emergence in *F. benjamina* over time for each treatment type. Rating scale 1-5, 1 indicating many new shoots and 5 representing no new shoots. Control= Untreated, Injection-D= Imidacloprid Injection alone, Injection-I=Imidacloprid injection plus Irrigation, Soil Drench-D= Imidacloprid Soil Drench alone, Soil Drench-I= Imidacloprid Soil Drench plus Irrigation, Irrigation= Monthly Watering

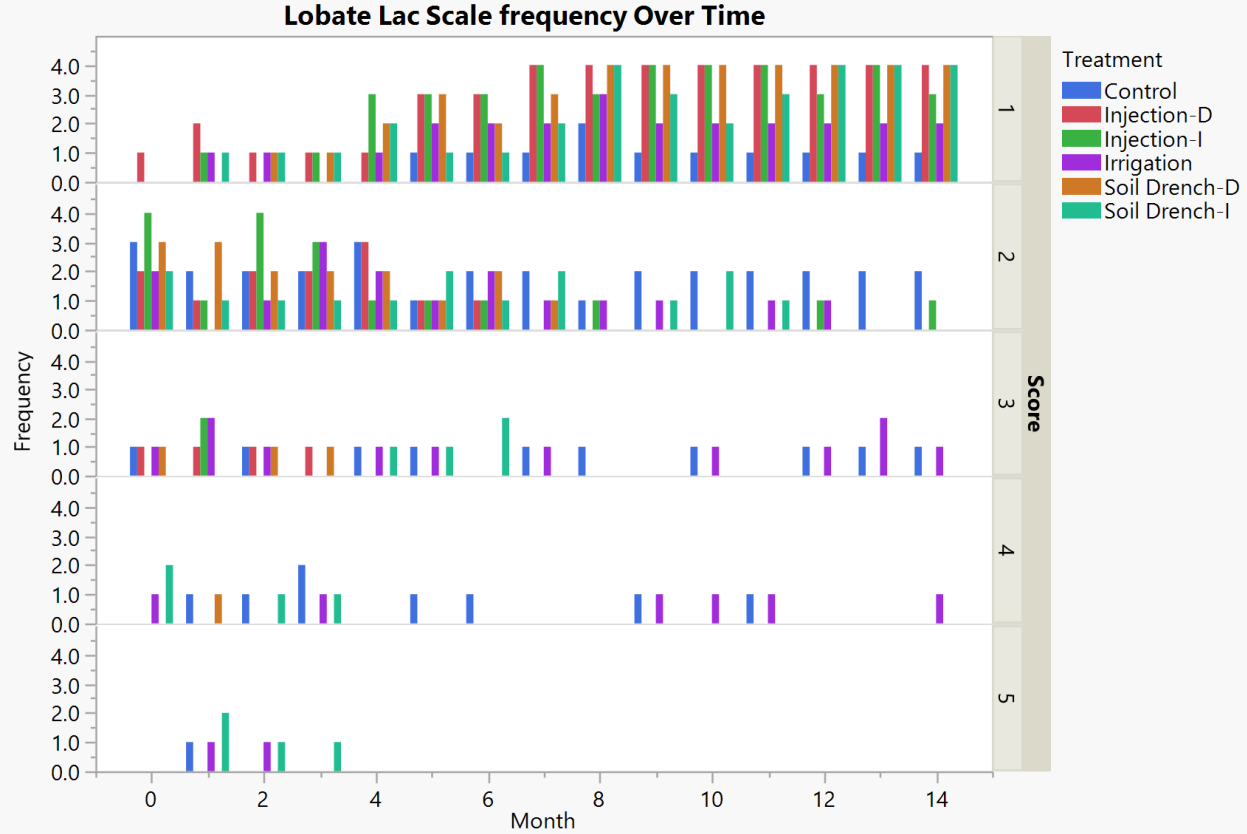

Figure 4.3. Frequency of each score each month of living *P. pseudolobata*. 1 showing no infestation or no living individuals and 5 showing heaving infestation of living *P. pseudolobata*. Control= Untreated, Injection-D= Imidacloprid Injection alone, Injection-I=Imidacloprid injection plus Irrigation, Soil Drench-D= Imidacloprid Soil Drench alone, Soil Drench-I= Imidacloprid Soil Drench plus Irrigation, Irrigation= Monthly Watering

Chapter 5. Overall Conclusions and Future Directions

As Hawai‘i has become increasingly urbanized, the need for environmentally friendly and effective control methods on invasive species attacking urban landscape plants becomes necessary. Employing a proper IPM strategy is an effective method of controlling pests, and in many cases requires the inclusion of insecticides. The challenge for using insecticides in urban settings is delivering the active ingredient in a way that is effective, i.e. kills the pest, and has very low non-target impacts.

Insecticides have shown to be successful in controlling insect pests and can be applied via trunk injection, soil drenching, or foliar sprays. In urban and suburban settings, foliar spraying is not as feasible compared to trunk injection and soil drenching as chemical drift can affect flora and fauna down-wind and full application may not occur depending on the size of flora. Trunk injection is generally considered the safest and most efficient method (Doccola and Wild, 2012).

Trunk injection alone, injection plus irrigation, and soil drench alone using imidacloprid effectively controlled *Paratachardina pseudolobata* beginning seven MAT and as early as four MAT in injection plus irrigation. Despite similar results to soil drenching, trunk injection is more environmentally friendly and so would be the best application method currently available. This is because the insecticide can be applied at a lower active ingredient concentration and be applied directly into the system and so off target risks are reduced.

Irrigation alone showed some benefit towards *Ficus microcarpa* health as overall tree health and new shoot growth did not significantly deteriorate over the course of the project (Figure 3.1, Figure 3.2). Throughout both the *F. microcarpa* and *F. benjamina* experiments, irrigation and insecticides did not decrease tree health or new shoot growth compared to insecticides alone, suggesting that proper irrigation will not affect the efficacy of insecticides, including soil drenching methods. This suggests that irrigation, as a general good cultural practice, can be used in conjunction with insecticides with little to no negative effects.

Common insecticides that can be administered in a low-risk manor, namely trunk injection, such as imidacloprid and acephate showed high efficacy on *O. rhinoceros* adults in the lab setting (Figures 2.1-2.7). High efficacy was observed in lab settings with acephate and imidacloprid at low concentrations (10 ppm). Field trials did not reflect similar results compared to the lab; however, low palm affected rates could have impacted the study; however, fewer adult *O. rhinoceros* were caught in panel traps at the end of the trial compared to the beginning in the treated region (Figures 2.8-2.10). It is unlikely that beetles traveled to neighboring regions as there were ample palms to feed on

and no treatments have shown repellent effects. Future field studies in Hawai‘i can be difficult in controlling adult *O. rhinoceros* using insecticides as there is a low concentration on beetles currently in Oahu. That being said, if a large stand of coconut palms are present, different areas could be treated with different insecticides to determine how each treatment affects adult *O. rhinoceros* as the current setup could allow for refuges in the control palms.

Continuing to search for natural enemies of these pests can be a future direction to fortify an IPM strategy for each pest. Another direction could be to determine if/how quickly various insect pests build resistances to successful insecticides. Future insecticidal experiments on *O. rhinoceros* in a lab setting could first be done with the synthesized beetle food to determine optimal condition efficacy before switching to a sugar cane diet for more realistic results. The sugar cane could also be dipped on one side only so that capillary action could draw pesticides into the remainder of the sugar cane to also emulate real life conditions. Mock field trials could also be conducted in quarantined areas where a known population of beetles were present to determine how many are affected by insecticides as currently it is unknown the number of beetles at Iroquois point and so it is difficult to determine the true efficacy of the insecticides treated.

Proper irrigation could also be conducted by using a mechanism to determine relative moisture content in the soil. Trees of similar DBH were chosen for our experiments, but moisture in various areas could theoretically be different and so maintaining similar moisture levels would allow for a more accurate representation of the effects of irrigation.

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