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

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Abstract

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

Introduction

Invasive ants are especially successful invaders because they possess the ability to adapt and invade a wide range of habitats. Special biological and behavioral traits, such as polygyny, unicoloniality, high interspecific aggression, symbiotic relationships with honeydew producing insects, and use of human mediated long-distance dispersal, contribute to the success of invasive ants as invaders (Holldobler and Wilson 1990; O'Dowd et al. 2003; Passera 1994; Wetterer and Porter 2003). Of the 147 ant species which have been transported beyond their native range, only a small subset are known as IAS (McGlynn 1999) with well-documented negative impacts. Five ant species are the most common targets of ant eradication programs worldwide:

Anoplolepis gracilipes Smith, *Linepithema humile* Mayr, *Pheidole megacephala* Fabr, *Solenopsis invicta* Buren and *Wasmannia auropunctata* Roger. Despite the frequency at which ant eradications take place, the outcomes of ant eradication efforts are still poorly documented and frequently unsuccessful (Hoffmann et al. 2016).

Hawaiʻi has no known native ant species, yet over 60 species have been introduced and established to date. Many of Hawaiʻi's introduced ant species are considered invasive "tramp" species owing to their propensity to be distributed long distances via human-mediated transport and a unique set of biological characteristics such as polygyny and unicoloniality (Loope and Krushelnycky 2007; Passera 1994). Tramp ants are also known for their negative impacts on residential, agricultural and natural ecosystems (Krushelnycky 2015; Loope and Krushelnycky 2007) and are frequently the targets of eradication and management programs (Hoffman et al. 2011; Lach and Barker 2013).

The little fire ant (*Wasmannia auropunctata*) is one of the most impactful invasive tramp ants established in Hawai

i and the target of numerous eradication efforts throughout the archipelago (Conant and Hiramatsu 2000; Kirschenbaum and Grace 2007; Moore et al. 2015).

It is projected to be \$6.1 billion over the next 35 years provided that management efforts are maintained at the current "status quo" level (Lee et al. 2015). Because of this, the state of Hawaiʻi has a vested interest in eradicating LFA infestations when and where feasible.

Little Fire Ants in Hawaiʻi

Originally detected in 1999 on Kaua

i, the LFA (*Wasmannia auropunctata*) has persisted and spread throughout the island (Conant and Hiramatsu 2000). The LFA is genetically distinct and identical to those in Florida, suggesting Florida as the likely source of the Hawaiʻi population (Foucaud et al. 2010).

Results from response surveys and publicity relating to these detections indicated that the number of LFA-infested private properties and commercial nurseries on Kaua

i was high. The origin of that infestation was likely from a shipment of infested palms from a nursery on the island of Hawaiʻi (Conant and Hiramatsu 2000; Null and Gundersen 2007).

An island-wide survey in 1999 did not detect any other LFA infestations on Kaua

i (Null and Gundersen 2007). In 2003, the LFA was detected on Oahu and Maui, and eradication efforts were initiated.

The Department of Agriculture (HDOA) along with the Hawaii Department of Health Vector Control Branch launched the first LFA eradication effort for the state of Hawaii focused on the Kauaʻi infestation. The infestation was treated using ready-to-use granular insecticidal ant baits (Amdro®, hydramethylnon 0.739% a.i.), however no documentation could be found regarding methods used to treat the original infestation such as application rates, frequency, and number of applications. At the time, the eradication was considered successful with LFA declared "eradicated" in HDOA's Annual Report for FY 2000 (Hawaii Department of Agriculture 2001). Unfortunately, follow-up surveys of the area in 2003 by the Kauai Invasive Species Committee (KISC) revealed that the infestation had rebounded and spread to two adjacent privately-owned properties. A monitoring and containment strategy was initiated until effective eradication methods and procedures could be developed for this species (Null and Gundersen 2007).

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

Methods And Materials

Site description

The infestation was located in Kalihiwai, on the North Shore of Kauaʻi (22°13'17.90" N, 159°25'26.88" W) (figure 1) and spanned three private properties. Landscape features included open mown lawn, palm arboretum, dense tropical landscaping and non-native coastal forest, sheer cliffs, and rocky outcrops which were surrounded by ocean at high tide (figure 2). Approximately one-third of the infested area consisted of steep terrain requiring the use of specialty equipment and training to navigate. The steep cliffs were wholly infested and when the eradication began there was no viable means of accessing this area nor were there treatment methods developed for such site features. As a result, the eradication plan was divided into two phases (figure 3). Phase I consisted of treating all areas accessible without the use of specialty equipment between September 2012 and July 2013. Phase II consisted of treating the remainder of the infested area between September 2014 and June 2015 and once specialty equipment, training and appropriate treatment methods became available. A 20 m buffer surrounding the remaining infestation during Phase II resulted in overlap of the Phase I and Phase II treatment areas.

Survey methods

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

Surveys were conducted throughout the eradication effort (2011 – present) for infestation delimitation prior to treatment, population monitoring, and detection of nascent remnant colonies after cessation of treatment regimens. For delimiting surveys, the sample vials were deployed at approximately 10 m intervals along transects radiating outward from known infested areas. The outer boundary of the infestation was determined when sample results revealed zero LFA detections for a minimum of 50 m. Midpoint monitoring surveys were conducted throughout the immediate treatment area (i.e. Phase I vs. Phase II treatment area) and immediately prior to the 5th treatment during both Phase I and Phase II treatment regimens. Sample vials were spaced in a 10 x 10 m grid-like pattern throughout the treatment areas for Phase I and Phase II midpoint surveys. The purpose of the midpoint monitoring survey was to assess treatment efficacy and identify areas requiring further attention. This allowed for adjustments to the treatment procedure to be made in a timely manner if and when needed. Immediately prior to each Phase I treatment (September 2012 – July 2013), sample vials were deployed in eight permanent monitoring plots placed randomly throughout the treatment area (table 1). The monitoring plots were used to track population dynamics in various habitat types during the Phase I treatment regimen. No monitoring plots were established during Phase II. Post treatment monitoring throughout the Phase I treatment area only occurred between January 2014 and June 2015 with area-wide (Phase I and II treatment areas) post treatment monitoring beginning in January 2016 and continuing to date.

Category	Description	Sample Placement	# Plots	Total # Samples
Low vegetation	Spaces dominated by cut grass and low-lying landscaping (<2 m tall)	Ground samples only	4	32
Tall vegetated	Spaces dominated by trees and tall landscaping plants (>2 m tall)	Paired ground and tree samples	4	64
Untreated	Untreated spaces directly adjacent to treated spaces.	Ground samples only	2	32
Vegetable garden	A small vegetable garden approximately 3 x 5 m	Ground sample only	1	1

Table 1: Descriptions of the 2012-2013 population monitoring plots and sample placements.

Area-wide post-treatment monitoring began after all treatment phases were completed. The number of area-wide post-treatment surveys per year varied due to property access, crew availability, and weather (table 2). Vial density was increased in order detect any nascent colonies remaining and the sample grid spacing was reduced to an average of 2.5 x 2.5 m. High sample vial densities for post-treatment surveys increase the likelihood of detecting nascent remnant colonies present after cessation of the treatment phases. Expanded surveys, extending beyond the treatment area, occurred at least once per year between 2016 and 2021. Additional sample vials were placed in the crowns of all palm trees higher than 3 m. Sample tubes were placed in the tree crowns via a weighed line. These were left in position for 24 h before collection rather than 60 min due to the time in which it took to deploy arboreal samples. The purpose of the canopy survey was to determine if any arboreal colonies survived.

Year	No. of surveys	Total no. of samples	Total area surveyed (ha)	Inter-vial distance (m)
2016	3	5,595	4.8	3
2017	4	15,925	9.8	2.5
2018	2	24,194	6.2	1.6
2019	2	3,933	3.8	3
2020	NA	NA	NA	NA
2021	1	4,110	4.7	3.4

Table 2: Summary of area-wide post-treatment surveys for the years 2016-present. No surveys were conducted in the year 2020 due to the COVID-19 pandemic and associated restrictions.

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

Treatment Strategy

Approximately 3.2 ha were treated during Phase I and approximately 1.4 ha were treated during Phase II treatment regimens (figure 3). The overlap in Phase I and II treatment areas ensured sufficient treatment coverage.

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

Spot treatments were made as needed throughout the eradication effort and consisted of insecticidal bait applications or use of residual insecticides, such as Talstar® P (7.9% bifenthrin, EPA reg. 279-3206, FMC Corporation, Pennsylvania, USA) or Talstar® PL (0.2% bifenthrin, 279-3168, FMC Corporation, Pennsylvania, USA). Other ready-to-use granular bait products used during spot treatments were Amdro Fire Ant Bait (0.73% hydramethylNon, EPA reg. 73342-1, AMBRANDS, Georgia, USA) and Siesta Fire Ant Bait (0.063% metaflumizone, EPA reg. 7969-232, BASF Corporation, North Carolina USA). Total annual amounts of all pesticides used throughout the project are listed in table 3.

Product	2012	2013	2014	2015	2016	2017	2018	2019	2020
Amdro	-	-	-	2.27 kg	1.81 kg	-	-	50 g	-
Pro bait	18.85 kg	27.90 kg	4.31 kg	2.95 kg	4.20 kg	0.91 kg	-	-	0.91 kg
HAL Gel Bait with Provaunt	-	238.71 L	37.85 L	136.27 L	-	-	-	-	-
Siesta	-	-	-	-	-	1.81 kg	-	-	-
HAL Gel Bait with Tango	181.06 L	63.06 L	31.95 L	26.50 L	-	-	-	1.32 L	1.89 L
Talstar P (tank mix with water)	13.25 L	-	1,210.36 L	473.18 L	-	-	45.42 L	-	-
Talstr PL (granule)	-	-	49.90 kg	56.70 kg	34.02 kg	-	-	-	-

Table 3: Total amounts of baits and residual barrier treatments applied annually between 2012 and 2020

Data Analysis

Descriptive statistics, such as mean number of LFA per sample vial and number of sample vials with positive detections was used as a surrogate for LFA population and to track treatment efficacy over time. Estimates for infested area were calculated by plotting GIS data for positive detections in QGIS version 3.22.0- Białowieża mapping software, buffering each point to 6 m, and calculating the total area of the buffered points.

Results

The number of LFA positive detections and the mean number of ants per sample vial within the Phase I monitoring plots rapidly decreased upon commencement of the treatment regimen (figure 4). Results from the Phase I midpoint monitoring survey indicated the LFA infestation had greatly reduced in size with only 10.5% of deployed sample vials detecting LFA (figure 5). Based on the number of sample vials containing LFA, the estimated Phase I infested area had been reduced from 3.22 to 0.39 ha. By January 2014, 4.6% of deployed sample vials were containing LFA, the majority of which were along the boundary dividing the Phase I and II treatment areas. Discounting the positive detections along the Phase I and II boundary, the Phase I infested area was

estimated in 2014 to be 0.05 ha. A slower decline was observed during the Phase II treatment regimen. Results from the Phase II midpoint monitoring survey indicated that the infested area had been reduced from 1.35 ha to 0.76 ha with 37.9% of the sample tubes collected containing LFA (figure 5). By January 2016, no LFA were detected in either Phase I or Phase II treatment areas.

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

Discussion

Ant eradications are notoriously difficult and many factors can influence the success or failure of an eradication project. The development of the HAL gel bait has provided practitioners with the ability to effectively treat tall vegetation as well as the ground, and has increased the chances of successful eradication and management programs. Since site features and vegetation varied between the Phase I and II treatment areas, it is difficult to tell with certainty whether the different treatment methods or site features and vegetation had a greater impact on the results of monitoring efforts for each phase. However, the general trends observed at the midpoint and post treatment monitoring surveys showed rapid population knockdown during Phase I and a gradual population decline over time during Phase II (figure 5). The difference was likely due to the incorporation of toxic granular baits throughout the Phase I treatment regimen which did not occur during Phase II. At the same time, the early reduction in population due to the use of toxic granular baits concurrently with the HAL gel bait containing s-methoprene likely reduced the amount of IGR shared throughout the super colony; thus, the residual effects of the IGR may not have been as pronounced during Phase I as in Phase II. It is also possible that the vegetation composition throughout the Phase I treatment area was such that more areas of refuge were available for hot spots to persist compared to the Phase II treatment area.

Treatment methodology has greatly improved since the original eradication attempt in 2000 due to dedicated research on species specific treatment methods for LFA. However, the ability to detect incipient remnant colonies is as important as finding suitable and effective treatment methods. Our post treatment monitoring procedure included closer spacing of sample tubes, palm tree crown surveys, and expanded surveys beyond the treatment area in order to increase the chances of detecting lone, remnant colonies within the landscape. Little fire ant hotspots continued to be detected occasionally until September 2019.

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

- 1) under-treated areas or features such as tall trees, dense vegetation, large mulch piles, steep terrain, or a combination of these act as reservoirs and safe havens for LFA to persist; and
- 2) movement of infested yard waste containing colony fragments to mulch piles beyond the treatment area allowed ants to be transported after treatments had ceased.

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

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

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

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

Declarations

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Conflicts of interest/Competing interests

The authors of this manuscript do not have any conflict of or competing interests.

Data and material availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request. **Code availability**

Not Applicable

Authors' contributions

All authors: Michelle P. Montgomery, Casper Vanderwoude, Tiffani Keanini, Craig Kaneshige, and Jasmyn Lynch contributed to the conceptualization, planning, implementation, data collection and/or data analysis for this project. Writing of the original draft was done by Michelle Montgomery and all authors contributed to revising and editing all subsequent drafts. The final draft has been approved by all authors.

Ethics approval

Not Applicable

Consent to participate

Not Applicable

Consent for publication

Not Applicable

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Figures

Figure 1: Map of the little fire ant eradication site. The infested area was located in Kalihiwai on the North Shore of Kaua'i island, Hawaii, USA (inset). The infestation plus 20 m buffer resulted in a 4.02 ha treatment area spanning three private properties. Map created in QGIS 3.22.0 Białowieża.

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Figure 2

Photos of landscape features within the infested area: (a) grassy areas; (b) palm arboretum; (c) dense tropical landscaping; (d) steeply sloped terrain of non-native coastal forest; (e) cliffs; (f) and a small rock island with patchy vegetation.

Figure 3: Map of the Kalihiwai, Kauai little fire ant eradication site showing the Phase I and Phase II treatment areas. The project was divided into Phase I and Phase II treatment efforts due to steep terrain requiring rope safety equipment for access. The 20 m buffer surrounding the remaining infestation during Phase II resulted in overlap of the Phase I and Phase II treatment areas. Map

Figure 3

Map of the Kalihiwai, Kauai little fire ant eradication site showing the Phase I and Phase II treatment areas. The project was divided into Phase I and Phase II treatment efforts due to steep terrain requiring rope safety equipment for access. The 20 m buffer surrounding the remaining infestation during Phase II resulted in overlap of the Phase I and Phase II treatment areas. Map created in QGIS 3.22.0 Białowieża.

Figure 4

Results from the 2012-2013 Phase I monitoring plots. There was a decline in both the number of sample traps containing LFA (bars) and the overall LFA population (line). Population was determined as the mean number of LFA per LFA positive sample collected. Chart created in GraphPad Prism 8.4.3.

Figure 5: Results from Phase I and II pretreatment, midpoint, and post-treatment monitoring surveys. The number of samples detecting LFA (bars) and the infested area (lines) decreased at different rates over time. Chart created in GraphPad Prism 8.4.3.

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