Department of Defense
Legacy Resource Management Program

PROJECT 18-844

Managing invasive ants as new and emerging threats to endangered insect species on DoD lands in Hawai‘i

Paul D. Krushelnycky
University of Hawai‘i at Mānoa

June 30, 2021

TABLE OF CONTENTS

EXECUTIVE SUMMARY

Natural resource managers on DoD installations are mandated to manage for federally listed threatened and endangered (T&E) species that may be impacted by training on their lands. At least four species of endangered picture-winged *Drosophila* flies and one to two species of endangered yellow-faced *Hylaeus* bees occur on lands used by the Army, Navy and Marine Corps in Hawai‘i. All of these species share a common vulnerability to predation and population depletion by invasive ant species. The present project addressed two main objectives: 1) understanding invasive ant distributions around T&E insect breeding habitats on DoD installations on the islands of O‘ahu and Kaua‘i, and 2) conducting efficacy and non-target risk studies for two different ant control approaches that target two different groups of invasive ants that currently impact T&E insects on DoD lands.

Thirteen ant species were detected in surveys of yellow-faced bee habitats on Marine Corps Base Hawai‘i (MCBH), with *Pheidole megacephala* being present at a majority of both ground and arboreal sampling points. *Ochetellus glaber* was the second most common species, occurring at 11% and 20% of ground and arboreal points, respectively. Neither species appears to completely exclude yellow-faced bees at current densities. The largest management concern at MCBH is the presence of a population of *Anoplolepis gracilipes* along the eastern coastline. This species is likely to exclude yellow-faced bees at current densities.

Nine ant species were detected in surveys of yellow-faced bee habitats on Dillingham Military Reservation (DMR). *Pheidole megacephala* was again the most common species at both ground and arboreal sampling points, and *O. glaber* was the second most common. No yellow-faced bees were observed while conducting the ant surveys, although *Hylaeus anthracinus* has recently been observed approximately 0.8 km to the east of the DMR boundary. Repeated searches for yellow-faced bees at DMR would be advisable, as portions of the coastal strand appear to support suitable habitat for them.

Eight ant species were detected across nine montane mesic forest sites in the Wai‘anae Mountains of O‘ahu that support picture-winged flies or their host plants, and that are managed by the Army Natural Resources Program of O‘ahu at Schofield Barracks Army Base (SBAB). *Solenopsis papuana* was the most common ant detected in ground baiting surveys of the Waianae sites, while ants were uncommon on picture-winged fly host plants across nearly all of the sites. *Solenopsis papuana* is presently the largest ant threat to endangered picture-winged flies at SBAB, given its prevalence and known impact on fly reproduction. Management at breeding sites with high *S. papuana* prevalence should be attempted.

Eight ant species were detected across five sites in the Kōke‘e area of Kaua‘i under lease by the Pacific Missile Range Facility (PMRF), which abut or overlap with designated critical habitat for a listed picture-winged fly species. All ant species were relatively uncommon at these sites. Nearly all ant detections at ground baits occurred in paved, mowed, or otherwise open areas, and few if any ants appeared to be present in closed canopy forest surrounding the modified areas. Similarly, ants were detected on only three of the 93 sampled host plant trees. Nevertheless, several of the sites supported relatively high diversities of invasive ants, and some efforts to control these may be advisable to help forestall spread into surrounding forests.

Amdro Ant Block is a granular bait that is attractive to a variety of invasive ant species predominantly in the subfamily Myrmicinae, including *S. papuana*. A single broadcast application of Amdro Ant Block bait at the label rate in 20 x 20 m field plots was very effective for suppressing *S. papuana* abundances in mesic montane forests for a period of at least six

months, with somewhat weaker effects persisting for up to one year. Broadcast of Amdro Ant Block bait did not appear to have strong negative impacts on non-target invertebrates in these forests at the scale investigated: no significant declines were detected in Amdro-treated plots for any taxonomic group either immediately after application or six months later. Furthermore, cage trials suggest that picture-winged flies themselves are not strongly attracted to Amdro Ant Block bait. Only a single brief feeding episode was observed among 23 flies of three non-listed surrogate species tested, and there was no difference in time to death between flies placed in cages provisioned with Amdro and flies placed in control cages. Collectively, the results suggest that a single broadcast of Amdro Ant Block is effective for long-term suppression of *S. papuana* and should pose little risk to non-target ground-dwelling mesic forest invertebrate communities as well as to picture-winged flies.

Invasive ant species in the subfamilies Formicinae and Dolichoderinae tend to be strongly attracted to sugar water-based baits. Three types of experimental water storing granules (WSG) formulated to deliver sugar water bait were tested in a series of field trials. The three WSG were polyacrylamide crystals, alginate beads, and textured vegetable protein (TVP). Drying trials found that water evaporated from polyacrylamide crystals more slowly than from alginate beads or TVP, suggesting that the period of attractiveness is longer for polyacrylamide. Bait preference trials found little evidence of differential attractiveness among the WSG types for three ant species tested (*Linepithema humile*, *A. gracilipes*, and *Wasmannia auropunctata*). Repellency trials, however, found that different ant species are sensitive to different pesticide active ingredients. Field efficacy trials found that indoxacarb at 0.05% concentration is highly effective in reducing densities of *L. humile*, and thiamethoxam at 0.0005% concentration also exerts good but possibly inconsistent control of *L. humile*. For *A. gracilipes*, dinotefuran formulated at 0.05% or 0.005% yielded good control, while formulations with indoxacarb generally performed more poorly. Despite differences in drying rates among the three WSG types, all three were highly successful in controlling both *L. humile* and *A. gracilipes* when formulated with the right active ingredients and concentrations. There are nevertheless substantial differences in cost and ease of use among the three granule types.

Non-target attraction to WSG baits was assessed through video observation and protein marking methods. Both suggested that pollinating insects are not strongly attracted to the granules, but will readily feed on the granules if encountered near flowers. Several taxa, including some native species, were regularly marked when collected in field plots where WSG were broadcast, indicating that some non-target mortality can be expected. Some indirect exposure to the active ingredients may also occur via pesticide residues. Residues of the two neonicotinoid pesticides tested, dinotefuran and thiamethoxam, were generally relatively low in and around the field efficacy plots. Residues tended to be highest in plant tissues, and were higher when bait formulations used higher concentrations of the AI. Surprisingly, indoxacarb residues were considerably higher than residues of either neonicotinoid in plant tissues, even after accounting for differences in bait formulation concentrations. Because indoxacarb is not generally reported to be a systemic pesticide, the opposite pattern was expected. Pesticide behavior and persistence in the environment is dependent on many complex factors. The residues measured in the present study should therefore be viewed as only approximate guidelines for pesticide behavior when broadcast in WSG baits.

INTRODUCTION

Natural resource managers on DoD installations in Hawai‘i are tasked with addressing the threats and limiting factors for a large number of federally listed threatened and endangered (T&E) species that may be impacted by training on their lands. In recent years, Hawaiian insects have increasingly been considered for addition to the list of T&E species, and this trend is likely to continue. Over the past decade, 22 insect species have been listed in Hawai‘i, and several of these occur on a variety of military lands across the state. At least four species of endangered picture-winged *Drosophila* flies occur on lands used by the Army and Navy in Hawai‘i. Similarly, one to two species of endangered yellow-faced *Hylaeus* bees occur on a Marine Corps base and in Army training areas, and a recently listed *Megalagrion* damselfly occurs on Army land.

While members of this diverse group of insects have various ecological roles and needs, most share a common vulnerability to predation and population depletion by invasive ant species (USFWS 2006, 2016). Information on the distribution of invasive ants in relation to T&E insect breeding habitats on DoD lands, and tools for mitigating their impacts, are therefore of direct relevance for DoD land managers attempting to meet their compliance obligations, under the US Endangered Species Act, of stabilizing these listed insect species. Such information and tools also advance installation objectives to control invasive species under the Invasive Species Executive Order, and contribute to the overall ecological health of military-operated natural areas as dictated under the Sikes Act.

Hawai‘i is thought to have few if any native ant species (Wilson 1996), but today over 60 non-native ant species have become established in the state. Certain invasive ants are commonly recognized to be among the most important threats to the native insect fauna in Hawai‘i and elsewhere, owing to their exceptional predatory and competitive abilities (Zimmerman 1970, Holway et al. 2002, Krushelnycky et al. 2005). Some of these ant species have been present in Hawai‘i for over a century, but others continue to spread into new natural areas and exert new threats (Hartley et al. 2010, Vanderwoude et al. 2016), and new ant species continue to arrive and establish (Krushelnycky et al. 2005, Buczkowski and Krushelnycky 2010). Efforts to remove invasive ants, using pesticide-laden baits, have become a central strategy for conserving native insects and other animals in Hawai‘i, and indeed around the world (Krushelnycky et al. 2004, Plentovich et al. 2011, Hoffmann et al. 2016).

Most such efforts have focused on eradication of incipient invaders, and in these situations a certain level of non-target environmental damage during short-term campaigns can be an acceptable cost. However, management of T&E insect populations may often require the ongoing, periodic suppression of invasive ants that are now beyond the point of eradication, in order to allow rare native populations to recover and stabilize. Effective, efficient, and safe methods for controlling problematic invasive ants on a longer-term basis therefore need to be developed. This includes an assessment of the potential non-target impacts that might be expected from such a management strategy. Furthermore, different target ant species and different management situations will often require different methods to be employed, and these may involve different types of non-target risks.

The present project aimed to fulfill two main objectives: 1) conduct invasive ant distribution mapping around T&E insect breeding habitats across a number of DoD installations on the islands of O‘ahu and Kaua‘i, including sites being considered for re-introduction of listed species to meet stabilization goals; 2) conduct efficacy and non-target risk studies for two

different ant control approaches that target two different groups of invasive ants that currently impact T&E insects on DoD lands.

Many invasive ant species of Hawai‘i belonging to the subfamily Myrmicinae are strongly attracted to an oil-based commercial granular bait, Amdro Ant Block. Several of these species, including *Pheidole megacephala*, *Tetramorium* spp., *Solenopsis abdita*, and especially *Solenopsis papuana*, are widely distributed in mesic to wet montane forests of O‘ahu and Kaua‘i (P. Krushelnycky unpub. data, Plentovich 2010), including habitats supporting listed picture-winged *Drosophila* flies. Experimental methods used to control *S. papuana* to date have distributed Amdro Ant Block within bait stations to minimize effects on non-ant insects. While effective over the short term (Ogura-Yamada and Krushelnycky 2016), this method is very labor intensive in comparison to broadcasting the bait. Amdro Ant Block is labeled for broadcast application in forests in Hawai‘i, but the attendant risks to native insects, including endangered *Drosophila* flies, are unknown.

Invasive ant species belonging to the subfamilies Formicinae and Dolichoderinae, including *Anoplolepis gracilipes*, *Ochetellus glaber*, and *Linepithema humile*, are more strongly attracted to sugar water-based baits. The first two species are common in coastal areas supporting endangered yellow-faced *Hylaeus* bees, while the third is more common in montane mesic forests and shrublands that may support both picture-winged flies and yellow-faced bees. Although sugar-water baits are highly attractive to these target ant species, the application of liquid baits in natural areas is a major challenge, requiring the use of numerous expensive and laborious bait station dispensers. Recently, “hydrogels” (polyacrylamide crystals) and other water-storing granules (WSG) have been used experimentally to transform liquid baits into a granular form that can be easily broadcast to control invasive ants (Boser et al. 2014, Buczkowski et al. 2014a,b, Peck et al. 2016). However, the efficacy of this method has been shown to vary depending on the active ingredient used and the species of ant targeted. In addition, biodegradable granule alternatives to the polyacrylamide crystals may represent a preferable option in natural areas, but their relative effectiveness needs to be investigated. Finally, no commercial pesticides are yet labelled for this type of use pattern, and additional data are needed to seek a Special Local Need label that would allow such use for the recovery of endangered insects.

Both approaches to ant control show promise for suppressing these damaging invasive species and concurrently recovering endangered native insects. For example, recent research has demonstrated that suppressing ants in montane forests can result in a 140% increase in the reproductive success of *Drosophila* flies (Krushelnycky et al. 2017). Similarly, research has shown that excluding ants from *Hylaeus* bee nesting blocks in Hawaiian coastal sites greatly increases their nesting success (Plentovich et al. 2021). In support of these goals, the present report provides information on ant distributions at breeding habitats of Hawaiian T&E insects occurring on DoD installations or associated management lands (Section I), efficacy and non-target risk data associated with the broadcast of Amdro Ant Block granular bait in mesic montane forests (Section II), and efficacy and non-target risk data associated with the broadcast of sugar water-based WSG in coastal and montane shrubland habitats (Section III).

SECTION I. Distributions of invasive ants at T&E breeding habitats on DoD lands

INTRODUCTION

Invasive ants are listed as important threats for listed species of both picture-winged flies (*Drosophila* spp.) and yellow-faced bees (*Hylaeus* spp.) in Hawai‘i ((USFWS 2006, 2016). At least four species of endangered picture-winged *Drosophila* flies occur on lands used or managed by the Army and Navy in Hawai‘i. *Drosophila montgomeryi*, *D. substenoptera*, and *D. obatai* are currently known from a fairly small number of breeding sites in mesic to wet montane forests of O‘ahu that are managed by the Army Natural Resources Program of O‘ahu (ANRPO), along with state partners (Magnacca 2014). Most of these sites occur across the Wai‘anae Mountains, although one known breeding site for *D. substenoptera* is in the Ko‘olau Mountains. The fourth species, *D. musaphilia*, is known from a few mesic to wet forest locations on Kaua‘i, and its designated critical habitat overlaps with some of the Navy's Pacific Missile Range Facility sites situated within Kōke‘e State Park (USFWS 2006, HHFP 2010).

Among the seven listed species of yellow-faced bees, *Hylaeus anthracinus* occurs in coastal strand habitat along much of the northern-facing shores of Marine Corps Base Hawai‘i on O‘ahu (Magnacca 2017). *Hylaeus anthracinus* has also been seen in coastal habitat just east of the boundary of Dillingham Military Reservation (Army) on O‘ahu, which contains similar habitat. In addition, *H. longiceps* has been known to co-occur with *H. anthracinus* at several coastal O‘ahu sites, and so might also be found at either the Marine Corps base or Dillingham Military Reservation. Finally, a third species, *H. kuakea*, is known from a single gulch in the Wai‘anae Mountains of O‘ahu, but has not been sighted since its only collection in 1997 (Magnacca 2007, USFWS 2016), and was not addressed here.

Baseline documentation of the ant species present at the breeding sites of listed insect species is prerequisite information needed in order to develop any subsequent management strategies concerning invasive ants. For this project, ants were surveyed in four areas covering the main known breeding sites of the above species on DoD-associated lands. These were 1) coastal strand breeding habitats of *H. anthracinus* along the northern and eastern shores of Marine Corps Base of Hawai‘i, 2) coastal strand habitat along the length of shoreline at Dillingham Military Reservation, 3) nine breeding sites of *D. montgomeryi*, *D. substenoptera*, and *D. obatai* across the Wai‘anae Mountains of O‘ahu, and 4) the built-up land and surrounding mesic forest habitat at five Pacific Missile Range Facility sites in the Kōke‘e area of Kaua‘i, which overlap with or border critical habitat for *D. musaphilia*.

MATERIALS AND METHODS

Installation site descriptions

Marine Corps Base Hawai‘i (MCBH). MCBH encompasses all of Mōkapu Peninsula in eastern O‘ahu. *Hylaeus anthracinus* populations have been detected along most of the length of north-facing coastal portion of the peninsula, and in a small patch on the eastern side of the peninsula at Fort Hase Beach (Magnacca 2017). Bees occur in these areas in a narrow band of

coastal vegetation dominated by *Scaevola taccada* and *Heliotropium foertherianum*. *Hylaeus anthracinus* is known to breed in hollow twigs of both of these species, and also to rely heavily on them for floral resources (Graham and King 2016, Graham 2018). Additional plant species that likely serve as preferred floral resources include *Sida fallax*, *Sesuvium portulacastrum*, *Lycium sandwicense, and Euphorbia degeneri*. Ant surveys focused on the relatively narrow band of coastal vegetation on the northern shore stretching from Pyramid Rock in the west to the end of North Beach in the east, and coastal vegetation on the eastern shore from the northern end of Fort Hase Beach to the southeastern installation boundary (Fig. 1-1).

Dillingham Military Reservation (DMR). DMR encompasses Dillingham Airfield in the Mokulēʻia area of northwest Oʻahu, and includes both coastal habitat seaward of the airfield and lowland and montane habitat inland of the airfield. Vegetation suitable for breeding of *Hylaeus anthracinus* occurs only along the coastal strand community north of the airfield and Farrington Highway (Hwy 930), and this parcel was therefore the target of ant surveys (Fig. 1-7). Native coastal vegetation at DMR is comprised mainly of *S. taccada*, and ant surveys focused on habitat dominated by this shrub. This habitat also supports scattered individuals of the coastal herbs or shrubs *E. degeneri*, *S. fallax* and *Sesbania tomentosa*, all of which also serve as floral resources for *H. anthracinus* (Hopper 2002, Graham 2018). Large monotypic stands of the Christmasberry tree (*Schinus terebinthefolius*) were avoided, as this was not considered suitable breeding habitat. Although *H. anthracinus* has not been recorded within the boundaries of DMR in recent years, it has been seen 0.8 km east of DMR (Graham and King 2016), and its occupation of similar coastal habitat within DMR is therefore not unlikely.

Schofield Barracks Army Base (SBAB) and affiliated lands. SBAB occupies large parcels of land in the central portion of Oʻahu, including training areas in the Waiʻanae and Koʻolau Mountains. Host plants for three listed species of picture-winged *Drosophila* are scattered throughout SBAB properties and a number of adjacent state-owned lands that are managed in part by ANRPO staff based at SBAB. These lands collectively encompass a wide range of mesic to wet montane forest communities. They typically include a mixture of alien and native flora, in addition to the flies' host species: *Urera glabra* and *U. kaalae* (host for *D. montgomeryi*), *Cheirodendron* spp. and *Polyscias oahuensis* (host for *D. substenoptera*), and *Chrysodracon* spp. (host for *D. obatai*) (Magnacca 2014). Ant surveys targeted patches of forest directly surrounding host plant trees that were either known or potential fly breeding sites in the Waiʻanae Mountains (Fig. 1-12).

Pacific Missile Range Facility (PMRF). The majority of PMRF is located on the western coast of Kauaʻi at Barking Sands, however, there are five small parcels in the Kōkeʻe area leased by PMRF from the state of Hawaiʻi known as the Kōkeʻe Sites (HHFP 2010). These sites, designated sites A through E, are situated in mesic montane forest within Kōkeʻe State Park (Fig. 1-22). The site footprints are mostly occupied by buildings and surrounding pavement, but each also extends into the surrounding forest and includes numerous *Acacia koa* trees, the host plant for *D. musaphilia* (USFWS 2006). The footprints of sites B and D overlap with designated critical habitat (CH) for *D. musaphilia*, while the other three sites are separated from the CH by short distances (Figs. 1-22 – 1-27). Ant surveys covered the entire footprints of each of the five sites, because ant species vectored into or exhibiting preference for the modified portions of the sites may nevertheless spread over time into surrounding forests.

Survey methods

Ants were surveyed with the use of non-toxic baits (lures) placed throughout endangered insect breeding habitats. Surveys using baits do not usually detect the full diversity of ant species present, both because many cryptic species are not easily attracted to baits, and because dominant species often exclude subordinate species from baits (Holway et al. 2002). Nevertheless, baiting is the most efficient and effective method for surveying the distributions, and obtaining a rough estimate of relative abundances, of the most ecologically prominent invasive ants species. Preliminary trials found that among several baits tested (peanut butter, peanut butter blended with corn syrup, spam, spam blended with corn syrup, and tuna blended with corn syrup), a spam and corn syrup blend was most effective for attracting large numbers of ants of a variety of species, including species in the three main ant subfamilies Myrmicinae, Formicinae and Dolichoderinae. The recipe used for all subsequent surveys was 41.7% spam, 41.7% corn syrup, and 16.7% water by weight, blended to a fine slurry. The placement of baits varied among sites, microhabitats, and target ant communities, as follows.

At coastal breeding sites of *Hylaeus anthracinus* (DMR and MCBH), ground-active ant communities were surveyed with bait cards placed on the ground in shaded locations at intervals of approximately 5-10 m throughout coastal shrubland/woodland habitat dominated by *S. taccada* and *H. foertherianum*. Bait cards consisted of one half of a 7.6 x 12.7 cm index card provisioned with bait, and left in place for 45-60 minutes. Arboreal ant communities were surveyed with pieces of baited sponge (approximately 3 x 4 cm) tied to branches of *S. taccada*, *H. foertherianum*, and occasionally other shrub or tree species, at intervals of approximately 20 m throughout the same habitat. Sponges were left in place for 45-90 minutes.

At montane forest breeding sites of *Drosophila montgomeryi*, *D. substenoptera*, and *D. obatai* (SBAB and affiliated lands), ground-active ant communities were surveyed with bait cards, as described above, at intervals of approximately 2-5 m throughout forest patches supporting the fly host plants. Cards were left in place for 60-90 minutes to accommodate the slower recruitment and more patchy distributions of the dominant ants occurring in these habitats. Arboreal ant communities were surveyed with pieces of baited sponge, as described above, tied to host plant trees at the sites, also left in place for 60-90 minutes. Surveys at montane forest breeding sites of *D. musaphilia* (PMRF) were conducted in a similar manner, except that ground baits were spaced at intervals of approximately 5-10 m throughout the site footprints, and as many host trees as possible were baited in the same areas.

Bait cards or sponges were retrieved after the specified intervals, at which time ant species visiting the baits were identified and counted. Unknown species were collected for later identification. A reference collection of ant specimens is housed in the PDK Collection, University of Hawai‘i. Each bait was georeferenced by recording a point with a Garmin eTrex 20x handheld GPS unit. GIS shapefiles and corresponding maps of ant surveys were subsequently created in ArcMap 10.2.2. Survey dates are recorded in shapefile and spreadsheet products.

RESULTS AND DISCUSSION

Marine Corps Base Hawai‘i

A total of 3,184 ground sampling points and 549 arboreal sampling points were completed at MCBH (Figs. 1-1 – 1-6). Thirteen ant species were detected with ground baits, although a single species, *P. megacephala*, occurred at over three-fourths of sampling points (Table 1-1). The remaining 12 species were detected at <11% of sampling points each.

Six ant species were detected with arboreal baits at MCBH (Table 1-2). These formed a subset of the 13 species detected on the ground. *Pheidole megacephala* was again the most common species, but occurred at a smaller percentage of arboreal baits (53.6%) than ground baits (77.8%). *Ochetellus glaber* was present at a higher fraction of arboreal baits (20.0%) than ground baits (10.9%), consistent with its arboreal nesting preference. Almost a quarter of arboreal baits (22.4%) attracted no ants, a considerably higher percentage than occurred for ground baits (6.6%). Two-thirds of arboreal baits (66.6%) were placed on *H. foertherianum* while one-third was placed on *S. taccada*. Prevalence of most ant species was slightly higher on *S. taccada*, resulting in a somewhat higher rate of ant absences on *H. foertherianum* (Table 1-2).

An additional three ant species, *Camponotus variegatus*, *Pseudomyrmex gracilis*, and *Leptogenys falcigera*, were observed and collected away from baits at several locations within the sampled areas.

Table 1-1. Percent occurrence and mean abundance per sampling point of ant species detected with ground baits at MCBH.

Species	**Occurrence (%)**	**Mean abundance**
Pheidole megacephala	77.8	67.8
Ochetellus glaber	10.9	33.8
none	6.6	--
Anoplolepis gracilipes	2.9	26.0
Monomorium floricola	1.9	113.6
Teteramorium simillimum	0.5	12.7
Nylanderia bourbonica	0.3	16.0
Technomyrmex difficilis	0.3	185.6
Brachymyrmex obscurior	0.06	3.0
Paratrechina longicornis	0.06	3.0
Trichomyrmex destructor	0.06	35.0
Plagiolepis alluaudi	0.03	20.0
Solenopsis geminata	0.03	25.0
Tetramorium caldarium	0.03	12.0

Table 1-2. Percent occurrence and mean abundance per sampling point of ant species detected with arboreal baits at MCBH.

Species	Occurrence overall (%)	Occurrence on Helfoe[1] (%)	Occurrence on Scatac[2] (%)	Mean abundance
Pheidole megacephala	53.6	47.0	56.4	61.5
none	22.4	29.5	18.8	--
Ochetellus glaber	20.0	17.5	21.2	41.6
Anoplolepis gracilipes	3.5	6.0	2.2	31.4
Monomorium floricola	0.5	0	0.8	200.0
Paratrechina longicornis	0.2	0	0.3	70.0
Technomyrmex difficilis	0.2	0	0.3	30.0

[1]*Heliotropium foertherianum*
[2]*Scaevola taccada*

Ant communities across most of the native coastal strand habitat at MCBH are dominated by *P. megacephala*, the big-headed ant. This species is considered to be one of the most ecologically damaging invasive ants worldwide (Holway et al. 2002, Wetterer 2007). It has been established in Hawai‘i since at least 1879, and has long been associated with destruction of the native arthropod fauna (Krushelnycky et al. 2005). It is today one of the most widespread species in Hawai‘i, generally occurring at elevations below approximately 1000 m (Reimer 1994, Krushelnycky et al. 2005).

Several sections of vegetation along North Beach had only sparse presence of *P. megacephala*, and these tended to be dominated by *O. glaber* (Figs. 1-2 – 1-4). These areas also sometimes had higher diversity of other ant species, suggesting that more species can coexist with *O. glaber* than *P. megacephala*. However, there was no clear distributional pattern of yellow-faced bee occurrences with respect to these two ants. Of 35 documented locations where *H. anthracinus* occurs at MCBH, 57% are locations dominated by *P. megacephala*, 17% are locations dominated by *O. glaber* and coexisting species, and 26% are locations where both *P. megacephala* and *O. glaber* occur. Neither of these species, therefore, appear to completely exclude yellow-faced bees, at least not when occurring at present densities.

One large area (~850 m long) on the eastern shore is dominated by *Anoplolepis gracilipes*, the yellow crazy ant. This species can be ecologically devastating to both invertebrates and vertebrates (Lach and Hooper-Bùi 2010), and anecdotal observations in Hawai‘i suggest that native *Hylaeus* bees cannot coexist with *A. gracilipes* when it occurs at high densities. No *Hylaeus* have been observed along this stretch of coastal habitat, although it has not been surveyed for bees as extensively as the northern shores.

Management recommendations

The population of *A. gracilipes* on the eastern shore currently presents the largest invasive ant problem at MCBH. The population appears restricted to this portion of the peninsula, but a more complete delineation effort should be undertaken to more clearly define its boundaries, particularly inland. Ideally, this population should be eradicated if it proves to be a discrete entity. However, registered bait products that could be used for this purpose are currently limited in Hawai‘i, making management a challenge. Newer tools may become

available in the future (see Section III). In the meantime, care should be taken to avoid transporting this species to other parts of the peninsula.

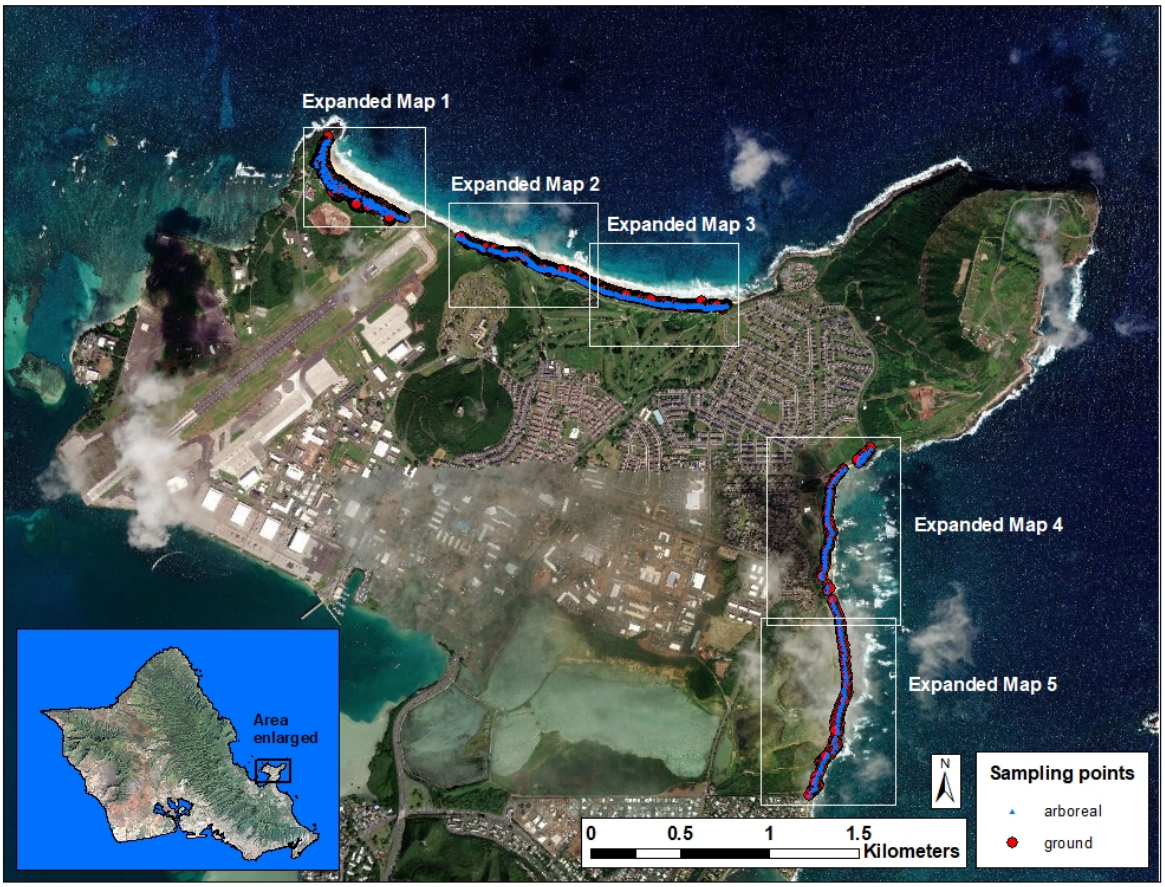

Figure 1-1. Overview map of coastal habitats surveyed for ants at MCBH, including arboreal and ground sampling points. Expanded map areas enlarged in Figures 1-2 through 1-6.

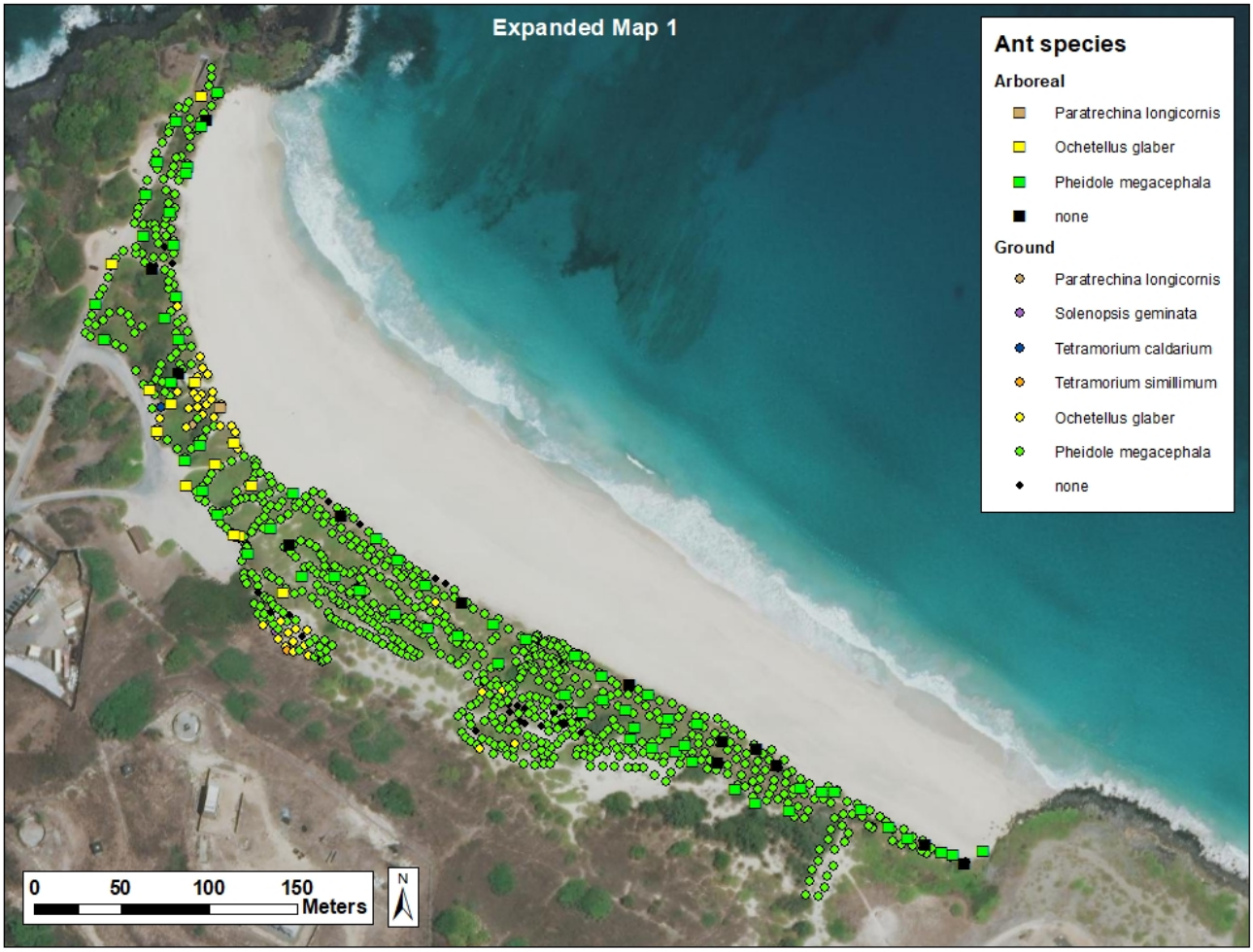

Figure 1-2. Expanded map 1 at MCBH, showing ant species detected at arboreal and ground sampling points.

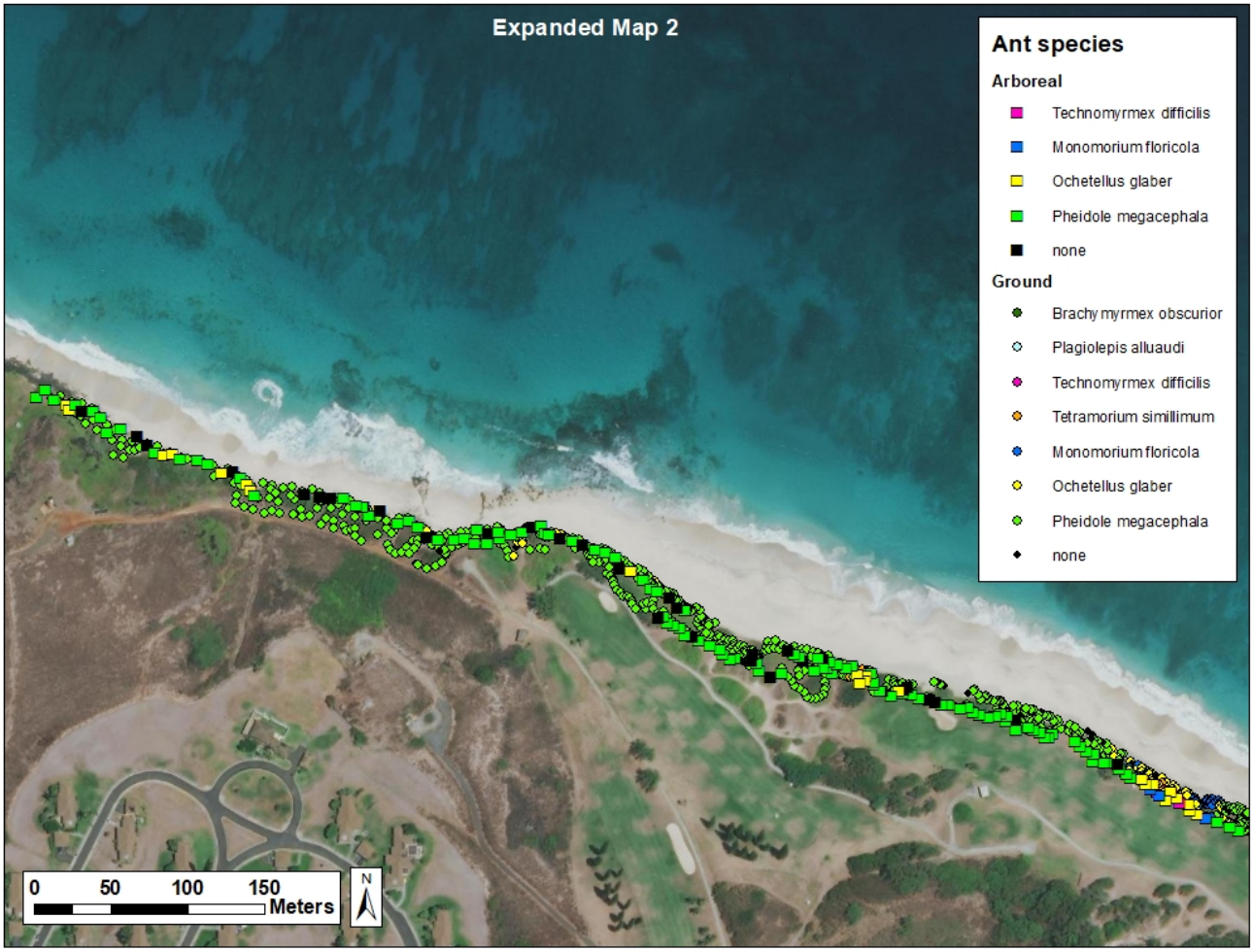

Figure 1-3. Expanded map 2 at MCBH, showing ant species detected at arboreal and ground sampling points.

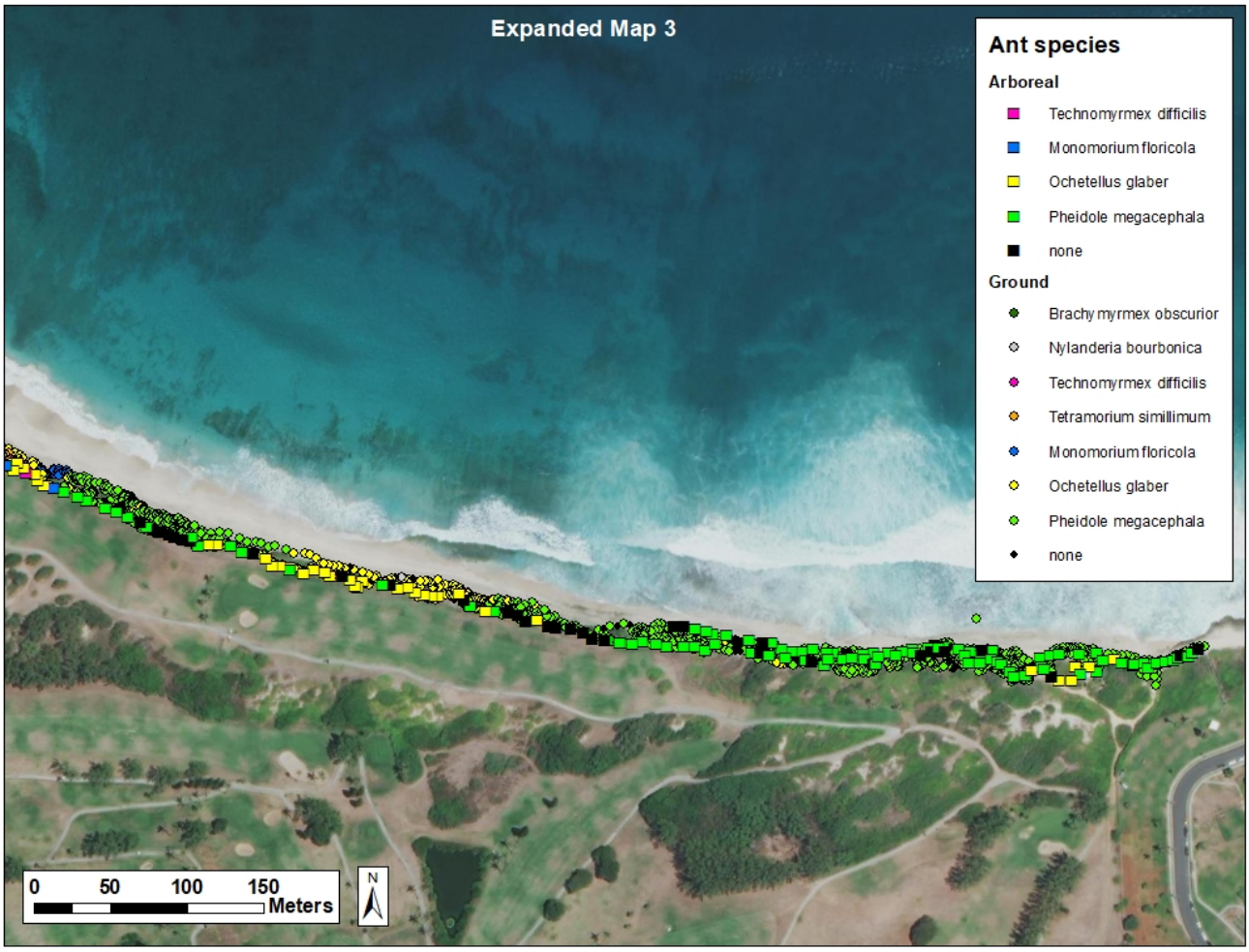

Figure 1-4. Expanded map 3 at MCBH, showing ant species detected at arboreal and ground sampling points.

Figure 1-5. Expanded map 4 at MCBH, showing ant species detected at arboreal and ground sampling points.

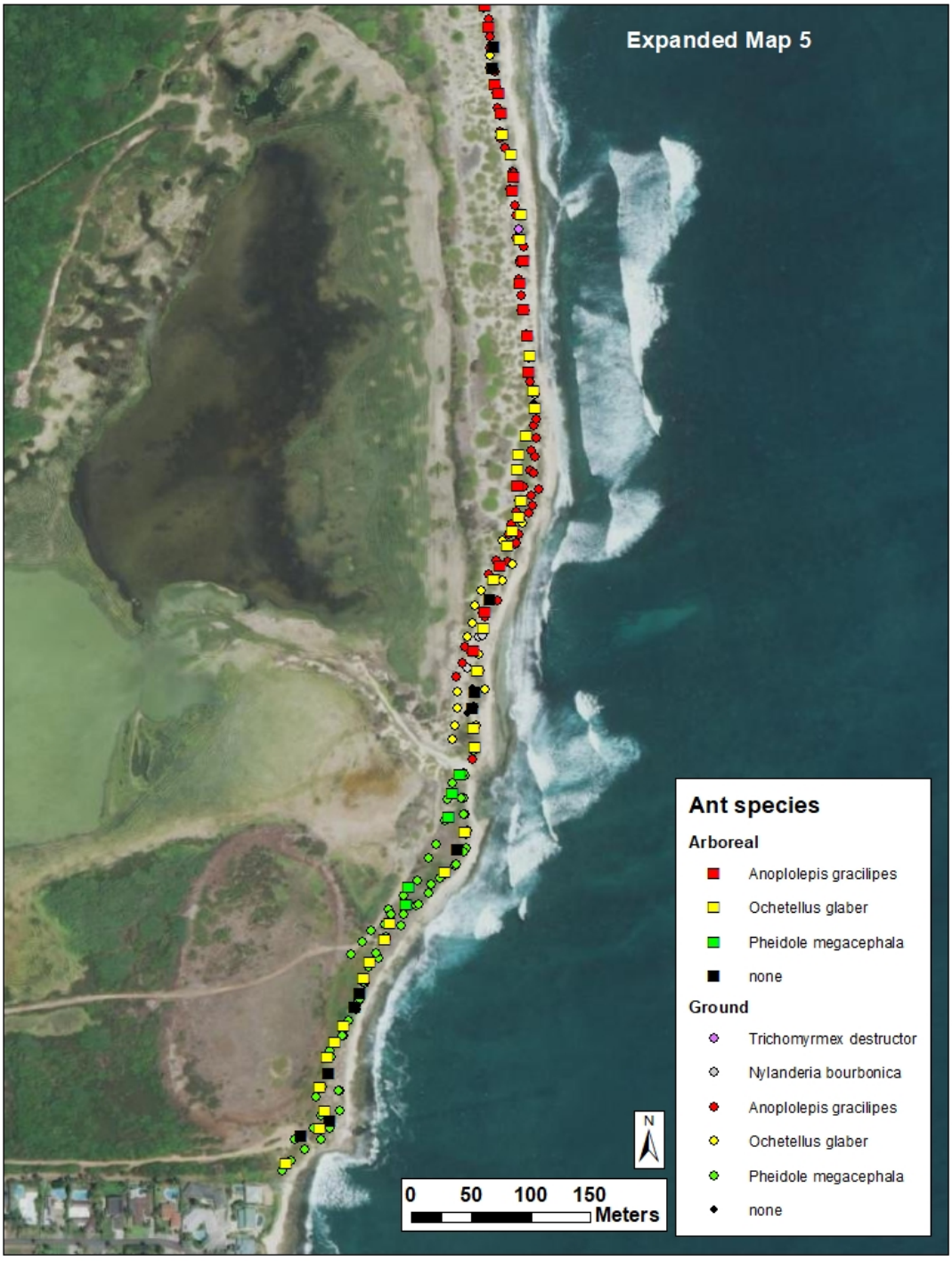

Figure 1-6. Expanded map 5 at MCBH, showing ant species detected at arboreal and ground sampling points.

Dillingham Military Reservation

A total of 1,302 ground sampling points and 147 arboreal sampling points were completed at DMR (Figs. 1-7 – 1-11). Nine ant species were detected with ground baits. Like MCBH, *P. megacephala* dominated the DMR coastal strand habitat, recruiting to nearly 84% of ground sampling baits (Table 1-3). The remaining eight species were detected at 5% or fewer of the sampling points. Most of these other species were concentrated in two locations: a small area near a common parking area (Fig. 1-9), and the disjunct smaller coastal parcel to the east of the main parcel (Fig. 1-11) where *P. megacephala* was much less common. Where present, *P. megacephala* tended to recruit substantially more workers to baits than the other species (Table 1-3).

Only three species were detected with arboreal baits at DMR, primarily *P. megacephala* and *O. glaber* (Table 1-4). The most common ant on shrubs and trees was again *P. megacephala*, and no ants were detected at nearly one-third (32.6%) of sampling points. Most arboreal sampling was conducted on *S. taccada* (82.3% of points), which dominated the native portions of coastal strand vegetation at this site. Only 9.5% of points were on *H. foertherianum*, and *O. glaber* occurred somewhat more frequently (14.3%) on this species than overall (7.5%). The remaining 8.2% of arboreal sampling points were conducted on the endangered shrub *Sesbania tomentosa*, and these were dominated by *P. megacephala* (91.7% occurrence).

The suite of ant species occurring at DMR is similar to that at MCBH but slightly less diverse. Unlike MCBH, however, *A. gracilipes* appears to occur in low densities and is restricted to the small eastern parcel. It therefore does not currently present a major issue.

Management recommendations

No yellow-faced bees were observed while conducting the ant surveys, although *H. anthracinus* has recently been observed approximately 0.8 km to the east of the DMR boundary. Repeated dedicated searches for yellow-faced bees at DMR would be advisable, as portions of the coastal strand appear to support suitable habitat for them.

Table 1-3. Percent occurrence and mean abundance per sampling point of ant species detected with ground baits at DMR.

Species	**Occurrence (%)**	**Mean abundance**
Pheidole megacephala	83.9	60.2
none	9.1	--
Ochetellus glaber	5.2	17.1
Anoplolepis gracilipes	1.5	5.0
Solenopsis geminata	0.8	17.9
Monomorium floricola	0.5	21.2
Tetramorium simillimum	0.4	14.4
Cardiocondyla emeryi	0.2	2.5
Paratrechina longicornis	0.2	7.0
Brachymyrmex obscurior	0.08	1.0

Table 1-4. Percent occurrence and mean abundance per sampling point of ant species detected with arboreal baits at DMR.

Species	Occurrence overall (%)	Occurrence on Helfoe[1] (%)	Occurrence on Scatac[2] (%)	Occurrence on Sestom[3] (%)	Mean abundance
Pheidole megacephala	59.2	42.8	57.8	91.7	57.4
none	32.6	42.8	33.9	8.3	--
Ochetellus glaber	7.5	14.3	7.4	0	33.4
Tapinoma melanocephalum	0.7	0	0	0	40.0

[1]*Heliotropium foertherianum*
[2]*Scaevola taccada*
[3]*Sesbania tomentosa*

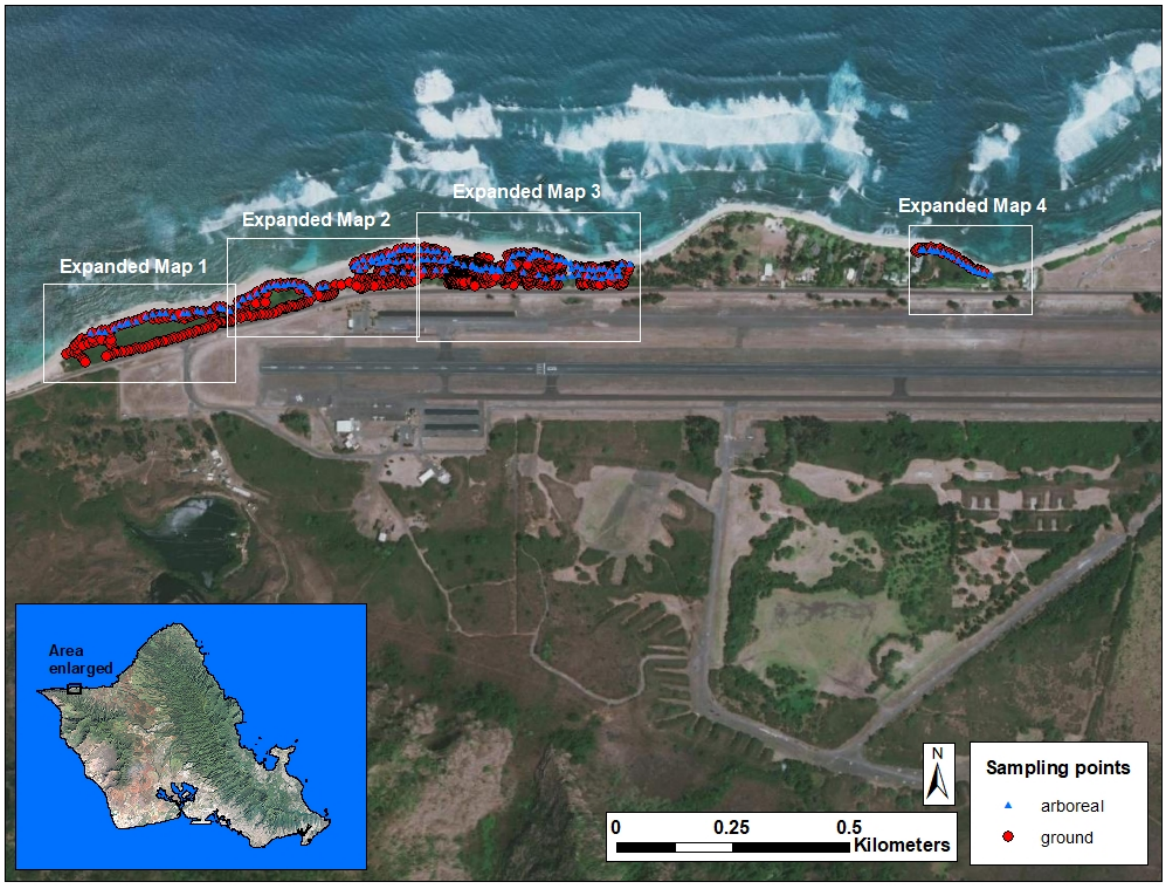

Figure 1-7. Overview map of coastal habitats surveyed for ants at DMR, including arboreal and ground sampling points. Area between expanded maps 3 and 4 is private land. Expanded map areas enlarged in Figures 1-8 through 1-11.

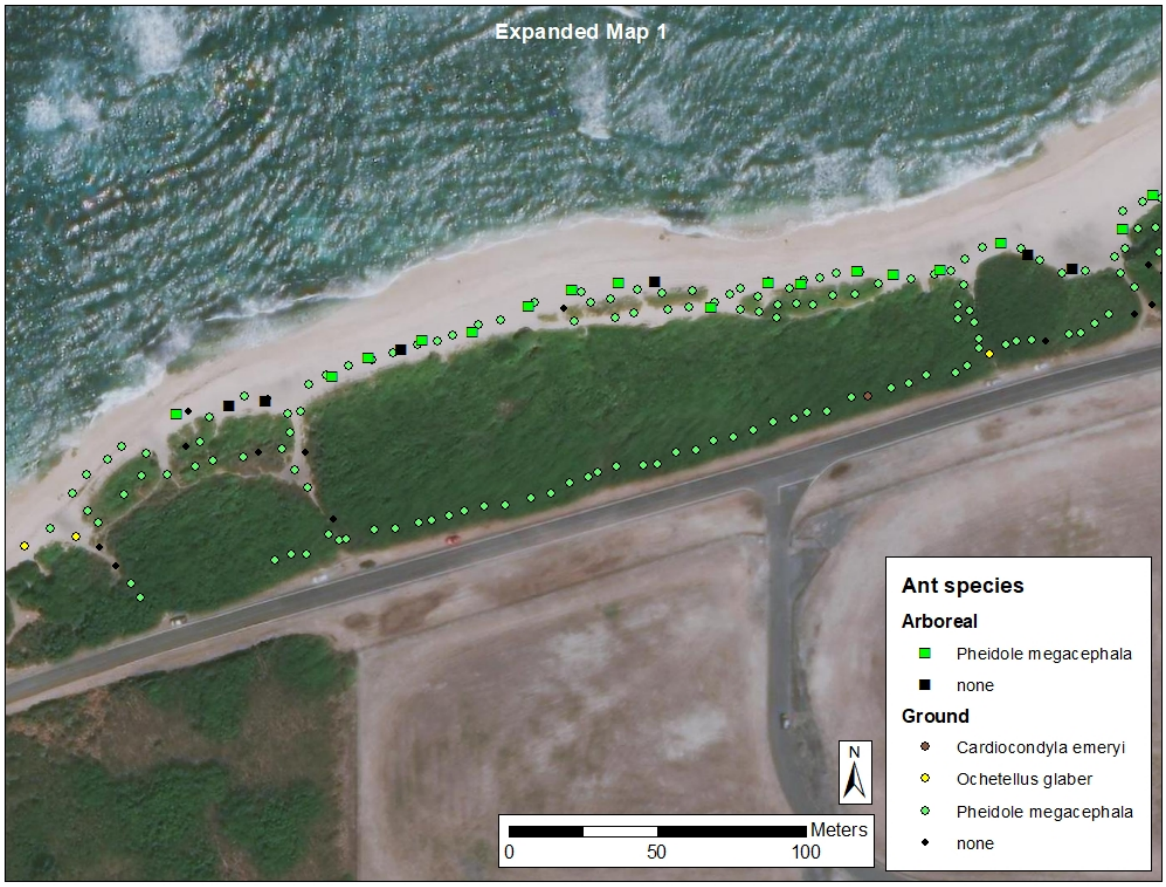

Figure 1-8. Expanded map 1 at DMR, showing ant species detected at arboreal and ground sampling points. Unsampled areas interior of the seaward edge of native coastal strand vegetation were unsuitable bee habitat.

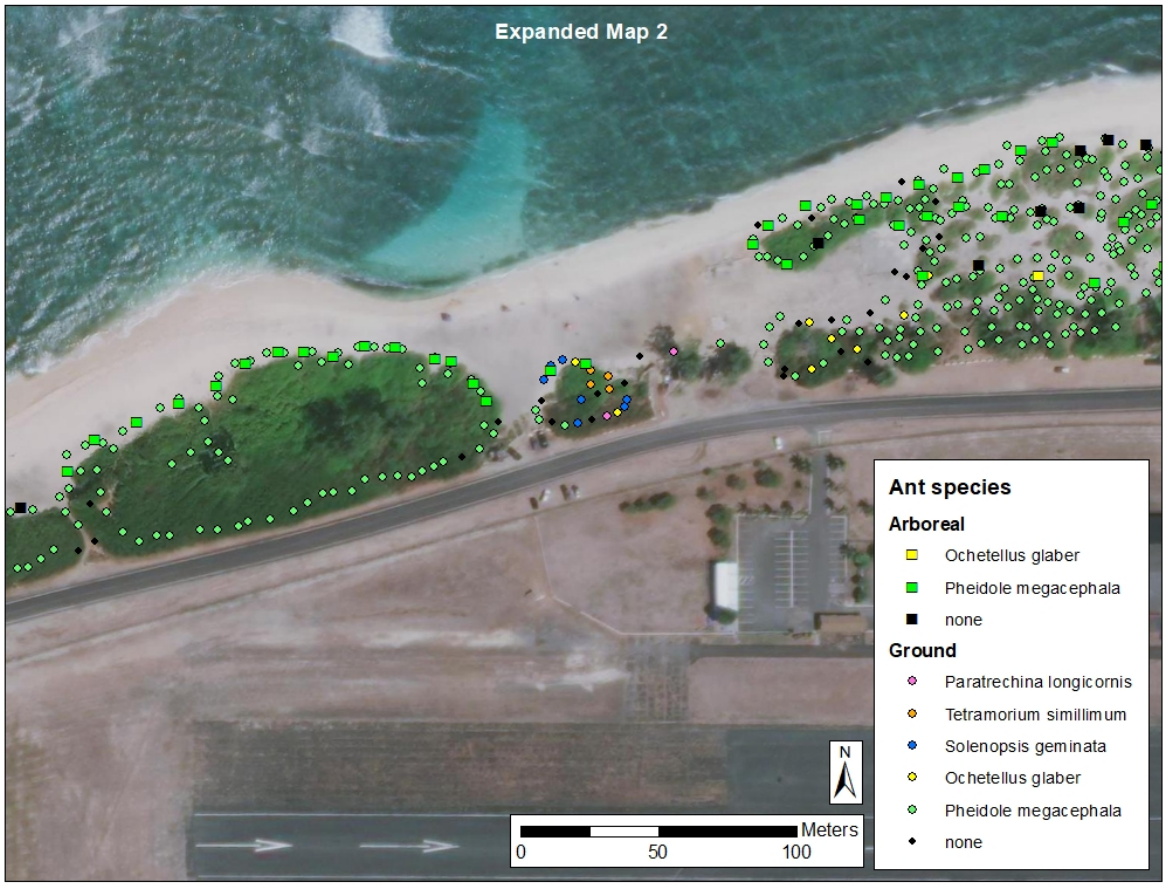

Figure 1-9. Expanded map 2 at DMR, showing ant species detected at arboreal and ground sampling points. Unsampled areas interior of the seaward edge of native coastal strand vegetation were unsuitable bee habitat.

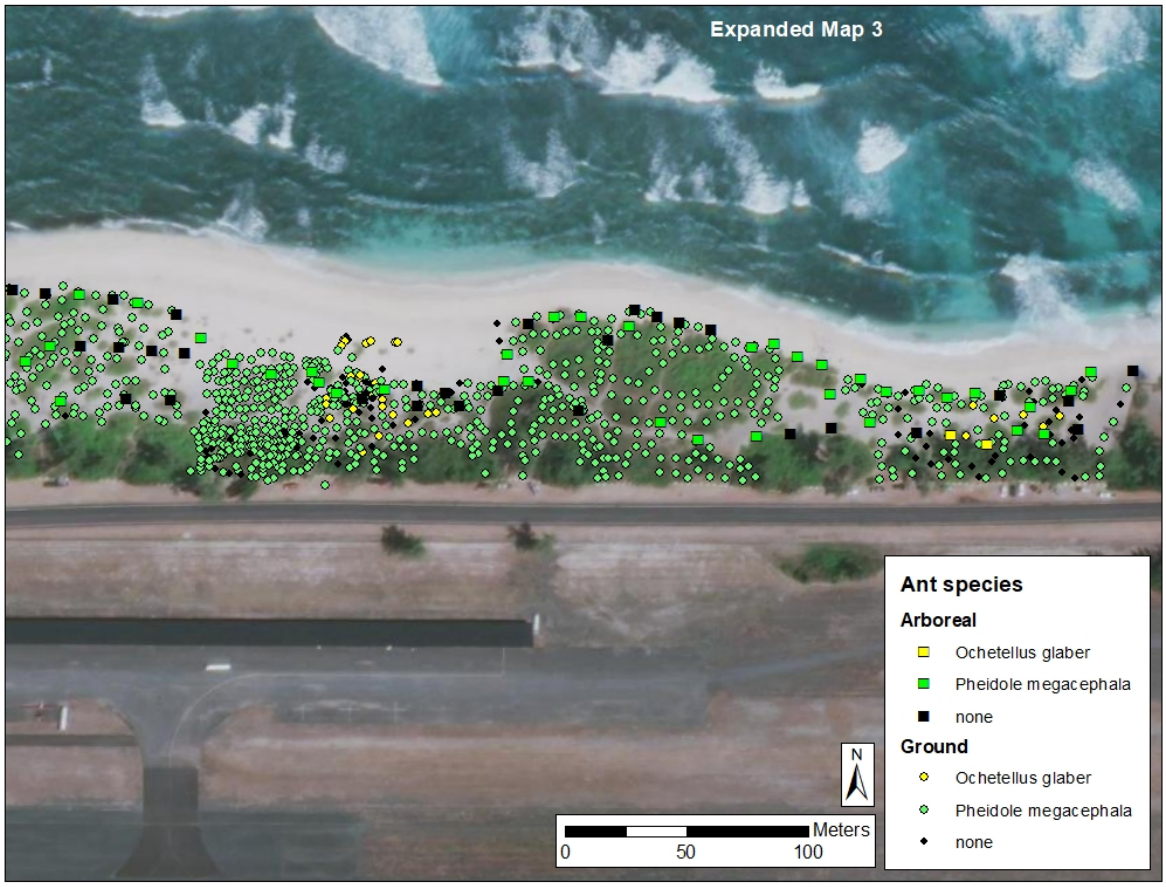

Figure 1-10. Expanded map 3 at DMR, showing ant species detected at arboreal and ground sampling points.

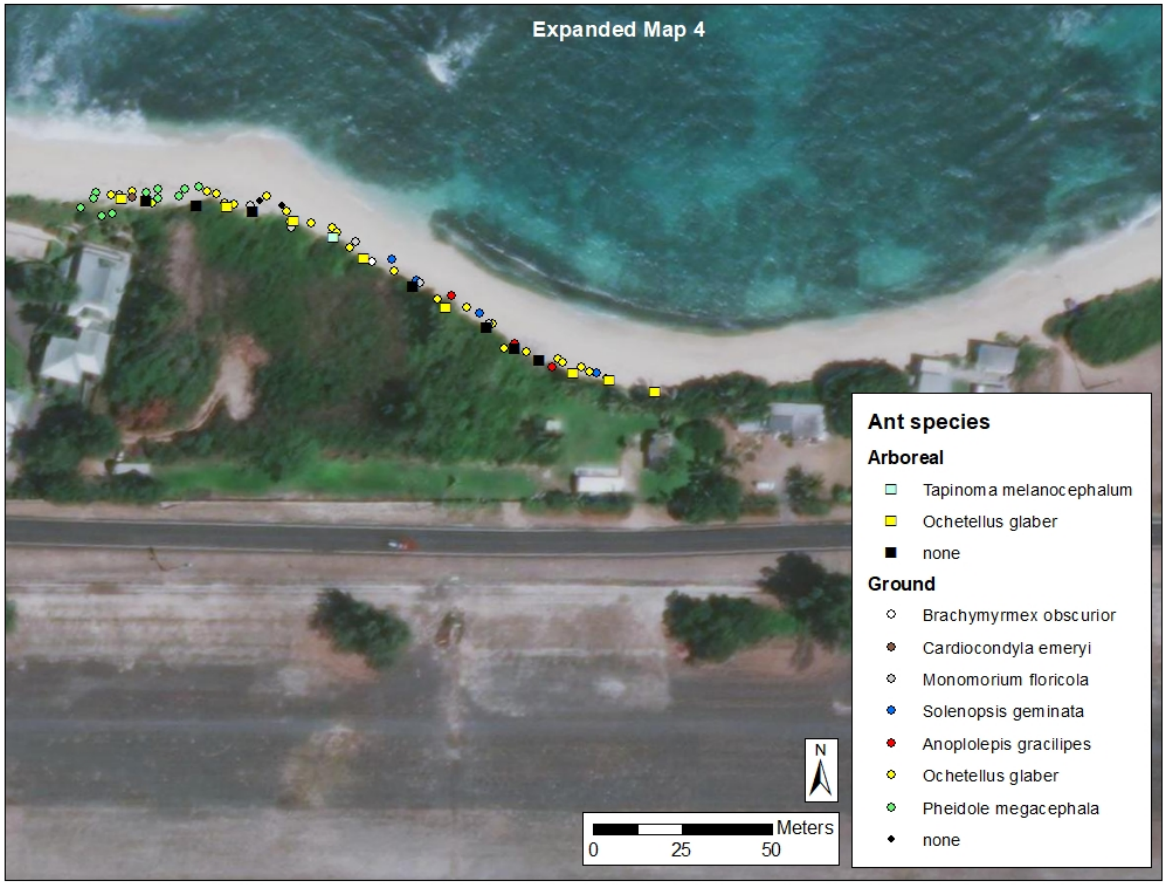

Figure 1-11. Expanded map 4 at DMR, showing ant species detected at arboreal and ground sampling points. Unsampled areas interior of the seaward edge of native coastal strand vegetation were unsuitable bee habitat.

Schofield Barracks Army Base

A total of 1,196 ground sampling points and 161 arboreal sampling points were completed across the nine Waiʻanae Mountain sites under ANRPO management that support picture-winged flies or their host plants (Figs. 1-12 – 1-21). The following results group sites by the fly host plants present at each.

Urera glabra and *U. kaalae*, the host plants for *D. montgomeryi*, occur at six sites, and a total of eight ant species were detected across them (Tables 1-5 and 1-6). *Solenopsis papuana* was the most common ant species detected with ground baits at five of the six sites, occurring at 34.4% to 62.8% of sampling points (Table 1-5). Very few ants were detected at the sixth site, Palikea, with 93.8% of sampling baits attracting no ants. There were no ants detected at 16.7% to 57.4% of sampling points at the remaining five sites. Very few ants were detected with the arboreal baits placed on host plants. Only four ant species were attracted to the baits, while 83.3% to 100% of baits attracted no ants across the six sites (Table 1-6).

Table 1-5. Percent occurrence and mean abundance per sampling point of ant species detected with ground baits at Waiʻanae sites supporting host plants for *D. montgomeryi*. Percent occurrence calculated within each site.

Site	**Species**	**Occurrence (%)**	**Mean abundance**
Waiʻanae Kai	none	57.4	--
	Solenopsis papuana	40.4	23.3
	Plagiolepis alluaudi	1.4	1.5
	Cardiocondyla wroughtonii	0.8	1.0
Puʻu Hāpapa	*Solenopsis papuana*	56.2	56.2
	none	41.6	--
	Cardiocondyla obscurior	1.4	2.0
	Technomyrmex albipes	1.4	31.0
Kaluaʻa north	none	47.7	--
	Solenopsis papuana	34.4	38.9
	Plagiolepis alluaudi	20.0	17.0
	Cardiocondyla obscurior	2.2	1.0
Kaluaʻa central, gulch 1	none	55.0	--
	Solenopsis papuana	42.6	28.6
	Plagiolepis alluaudi	3.0	8.2
	Cardiocondyla obscurior	0.6	4.0
	Solenopsis abdita	0.6	7.0
ʻĒkahanui, Palai gulch	*Solenopsis papuana*	62.8	38.7
	Solenopsis abdita	20.5	12.3
	none	16.7	--
	Nylanderia bourbonica	2.6	22.5
	Plagiolepis alluaudi	1.3	50.0
Palikea	none	93.8	--
	Cardiocondyla kagutsuchi	2.8	3.8
	Solenopsis abdita	2.1	3.3
	Solenopsis papuana	1.4	15.0

Table 1-6. Percent occurrence and mean abundance per sampling point of ant species detected with arboreal baits at Waiʻanae sites supporting host plants for *D. montgomeryi*. Percent occurrence calculated within each site.

Site	Species	Occurrence on Uregla[1] (%)	Occurrence on Urekaa[2] (%)	Mean abundance
Waiʻanae Kai	none	88.9	--	--
	Solenopsis papuana	11.1	--	15.5
Puʻu Hāpapa	none	86.7	100	--
	Technomyrmex albipes	6.7	0	40.5
	Solenopsis papuana	3.3	0	3.0
	Cardiocondyla obscurior	3.3	0	4.0
Kaluaʻa north	none	83.3	--	--
	Plagiolepis alluaudi	16.7	--	10.3
Kaluaʻa central, gulch 1	none	100	100	--
ʻĒkahanui, Palai gulch	none	100	--	--
Palikea	none	100	--	--

[1]*Urera glabra*
[2]*Urera kaalae*

Cheirodendron platyphyllum and *C. trigynum*, the host plants for *D. substenoptera*, occur at two of the surveyed sites. *Cheirodendron* is relatively common at the Kaʻala site, where no ants were detected with either ground or arboreal baits (Tables 1-7 and 1-8). *Cheirodendron* trees are scattered around the Palikea site, which similarly had very few ants detected. Only three ant species were detected at Palikea, at a combined occurrence of only 6.2% of ground baits; 93.8% of ground baits attracted no ants (Table 1-7). No ants were detected on the few *C. platyphyllum* trees baited (Table 1-8).

Table 1-7. Percent occurrence and mean abundance per sampling point of ant species detected with ground baits at Waiʻanae sites supporting host plants for *D. substenoptera*. Percent occurrence calculated within each site.

Site	Species	Occurrence (%)	Mean abundance
Kaʻala	none	100	--
Palikea	none	93.8	--
	Cardiocondyla kagutsuchi	2.8	3.8
	Solenopsis abdita	2.1	3.3
	Solenopsis papuana	1.4	15.0

Table 1-8. Percent occurrence and mean abundance per sampling point of ant species detected with arboreal baits at Wai‘anae sites supporting host plants for *D. substenoptera*. Percent occurrence calculated within each site.

Site	**Species**	**Occurrence on Chepla[1] (%)**	**Occurrence on Chetri[2] (%)**	**Mean abundance**
Ka‘ala	none	100	100	--
Palikea	none	--	100	--

[1]*Cheirodendron platyphyllum*
[2]*Cheirodendron trigynum*

Chrysodracon halapepe, a host plant for *D. obatai*, occurs at three of the surveyed sites, and a total of four ant species were detected across them (Tables 1-9 and 1-10). *Solenopsis papuana* was the most common ant species detected with ground baits at all three sites, occurring at 34.4% to 59.6% of sampling points (Table 1-9). *Plagiolepis alluaudi* was also relatively common at all three sites, occurring at 18.1% to 22.2% of sampling points. *Plagiolepis alluaudi* was also the most common ant attracted to arboreal baits at two of the three sites, occurring at 12.5% to 33.3% of baits (Table 1-10). No ants were detected with arboreal baits at the third site, Kalua‘a north, although there is only one large host tree there.

Table 1-9. Percent occurrence and mean abundance per sampling point of ant species detected with ground baits at Wai‘anae sites supporting host plants for *D. obatai*. Percent occurrence calculated within each site.

Site	**Species**	**Occurrence (%)**	**Mean abundance**
Kalua‘a north	none	47.7	--
	Solenopsis papuana	34.4	38.9
	Plagiolepis alluaudi	20.0	17.0
	Cardiocondyla obscurior	2.2	1.0
Manuwai	*Solenopsis papuana*	59.6	40.0
	none	23.4	--
	Plagiolepis alluaudi	18.1	11.2
	Solenopsis abdita	8.8	11.3
	Cardiocondyla sp.[1]	0.6	1.0
Līhu‘e, Guava gulch	none	42.4	--
	Solenopsis papuana	36.4	23.9
	Plagiolepis alluaudi	22.2	18.7
	Solenopsis abdita	7.1	17.0

[1]Ant escaped, likely *C. obscurior*.

Table 1-10. Percent occurrence and mean abundance per sampling point of ant species detected with arboreal baits at Waiʻanae sites supporting host plants for *D. obatai*. Percent occurrence calculated within each site.

Site	**Species**	**Occurrence on Chrhal[1] (%)**	**Mean abundance**
Kaluaʻa north	none	100	--
Manuwai	none	87.5	--
	Plagiolepis alluaudi	12.5	1.0
Līhuʻe, Guava gulch	none	53.3	--
	Plagiolepis alluaudi	33.3	14.2
	Solenopsis papuana	13.3	31.5

[1]*Chrysodracon halapepe*

As expected, *S. papuana* was the most common ant detected in ground baiting surveys of the Waiʻanae sites. Excluding the two sites at which few or no ants were detected (Palikea and Kaʻala), *S. papuana* occurred at 47.9% of ground sampling points across the remaining seven sites. The next two most common species, *P. alluaudi* and *S. abdita*, occurred at 8.9% and 4.4% of ground sampling points, respectively, across the same seven sites. The five remaining species detected all occurred at less than 0.7% of sampling points. *Solenopsis papuana* also recruited more workers to baits than other species, attracting 37.2 workers on average, compared to 14.7 and 12.6 workers for *P. alluaudi* and *S. abdita*, respectively. *Technomyrmex albipes* and *Nylanderia bourbonica* also recruited relatively large numbers of workers to baits (31.0 and 22.5 per bait, respectively), but these species were only detected at two ground baits each.

Substantial variation in ant presence was observed among the Waiʻanae sites. As mentioned, the two sites supporting host trees for *D. substenoptera*, Palikea and Kaʻala, had few to no ants attracted to ground baits. While cryptic species that are not easily attracted to baits, such as *Hypoponera* spp., may be present at one or both sites, most such species form small colonies and likely pose relatively minor ecological threats. Palikea and Kaʻala are the two highest sites surveyed, and the cooler temperatures and wet microhabitats that exist there are likely responsible for their lower ant diversities and abundances.

Among the remaining sites, Palai gulch in ʻĒkahanui had the highest prevalence of *S. papuana* (62.8% of ground baits) and ants overall (83.3% of ground baits). The second most common ant at Palai gulch was *S. abdita*, another small thief ant that until recently (Sharaf et al. 2020) went under the provisional names *Solenopsis* sp. (Gruner et al. 2003) or *Solenopsis* HI01 (Ogura-Yamada and Krushelnycky 2020). This ant may have similar biology to *S. papuana*, and together the two *Solenopsis* species occurred at over 80% of ground baits at Palai gulch. Ants were generally less prevalent at the remaining six sites, with no ants attracted to ground baits at 41.6% to 57.4% of sampling points. At half of these six sites (Manuwai, Guava gulch in Līhuʻe, Kaluaʻa north), *P. alluaudi* was notably more common, occurring at 18.1% to 22.2% of ground baits.

Ants were uncommon on picture-winged fly host plants across nearly all of the sites. The one exception was Guava gulch in Līhuʻe, where either *P. alluaudi* or *S. papuana* was detected at baits on 46.7% of surveyed *C. halapepe* trees. *Plagiolepis alluaudi* was recently found to be

the most common ant on several species of trees surveyed in the northern Wai‘anae Mountains, with evidence suggesting that its presence was associated with lower numbers of native Lepidoptera larvae (Krushelnycky 2015). At the remaining eight sites, no ants were detected on 83.3% to 100% of baits placed on host plants.

Management recommendations

Solenopsis papuana is presently the largest ant threat to endangered picture-winged flies at the Wai‘anae Mountain sites, given its prevalence and known impact on fly reproduction (Krushelnycky et al. 2017). Research suggests that broadcasting Amdro Ant Block granular bait around fly host plants should be effective for suppressing *S. papuana*, and should pose few non-target risks (Section II). Management at breeding sites with high *S. papuana* prevalence should be attempted. Suppression of *S. papuana* ahead of any planned translocations of flies to new sites should also be considered.

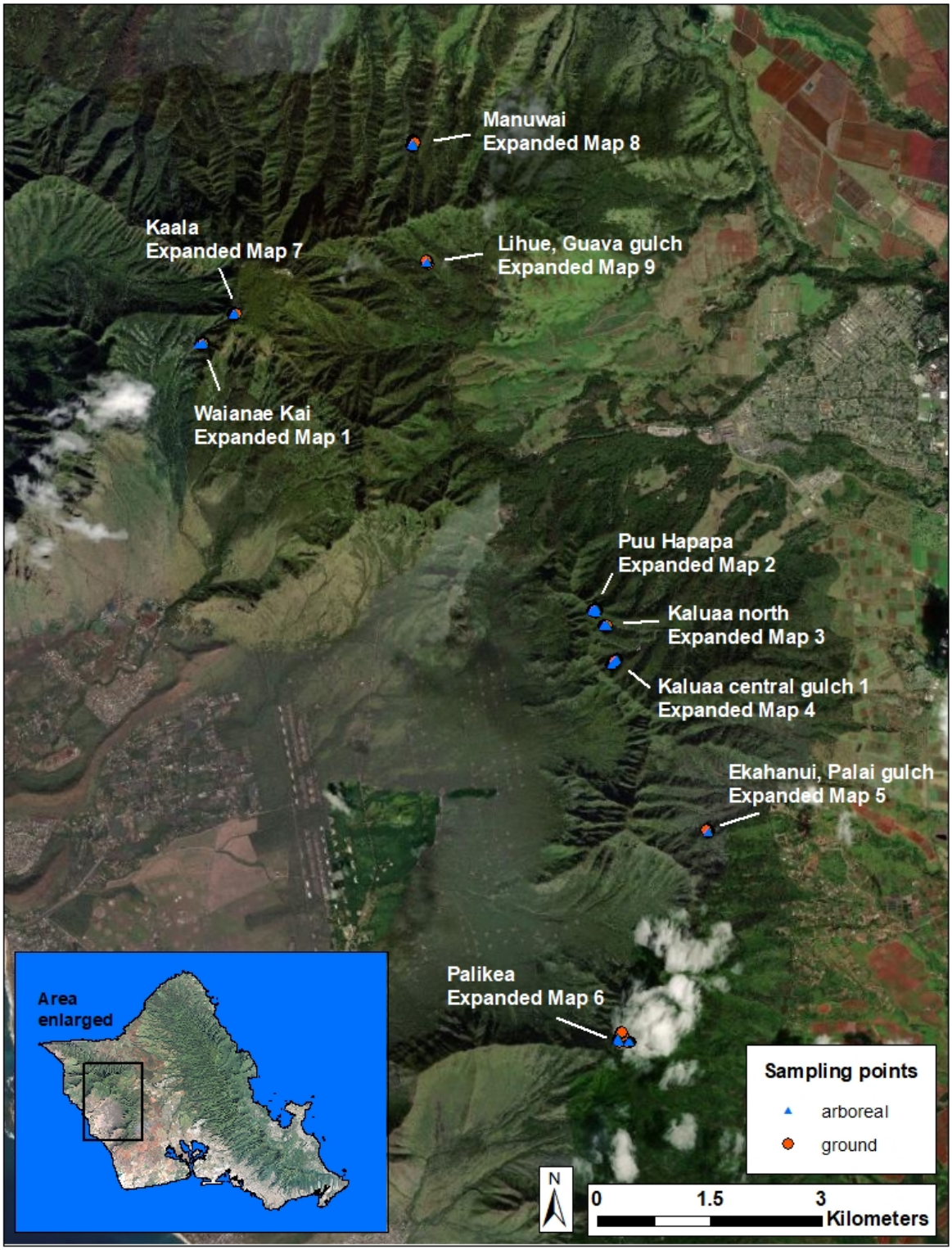

Figure 1-12. Overview map of Wai‘anae Mountain picture-winged fly breeding sites surveyed for ants, including arboreal and ground sampling points. Individual sites are enlarged in Figures 1-13 through 1-21.

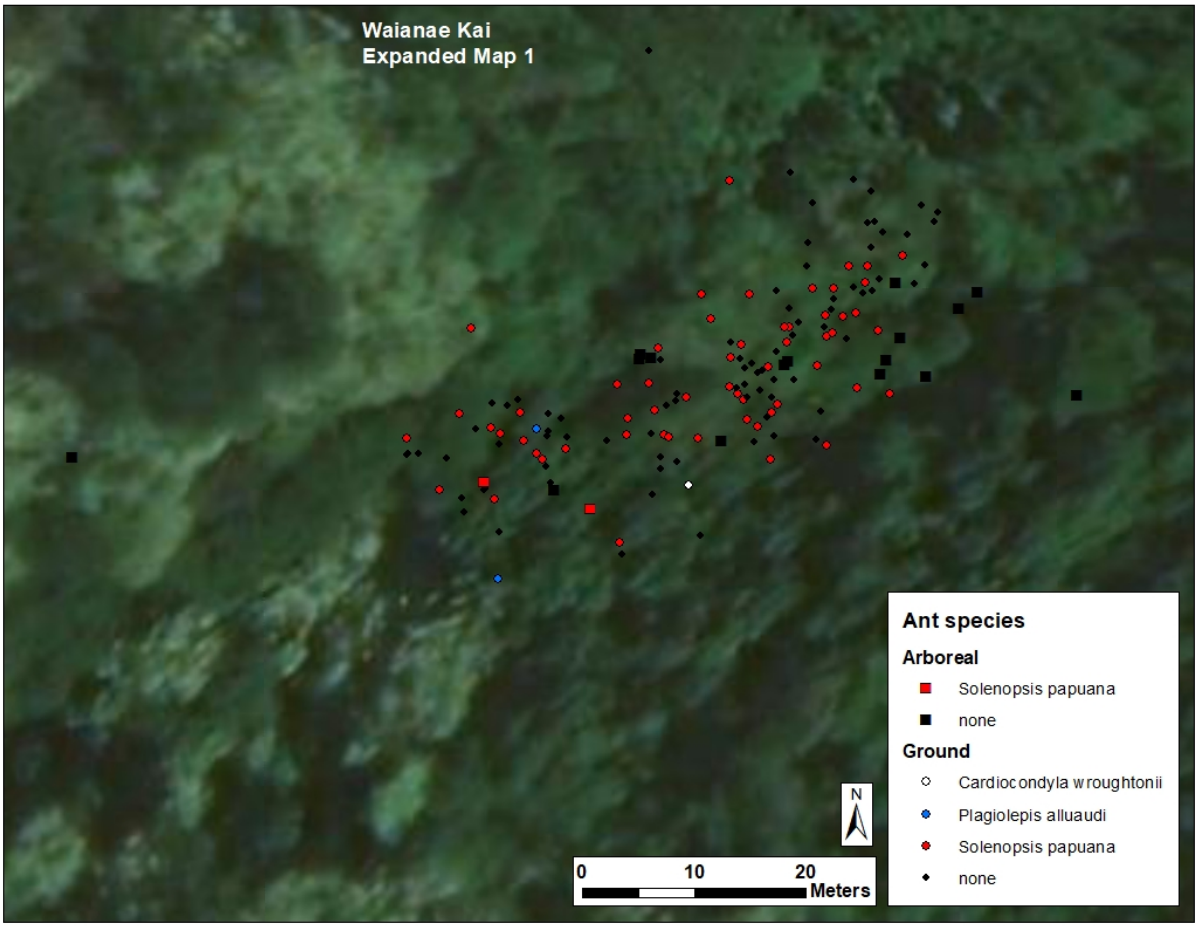

Figure 1-13. Expanded map 1 showing ant species detected at arboreal and ground sampling points at the Wai‘anae Kai site. This site supports host plants for *D. montgomeryi*.

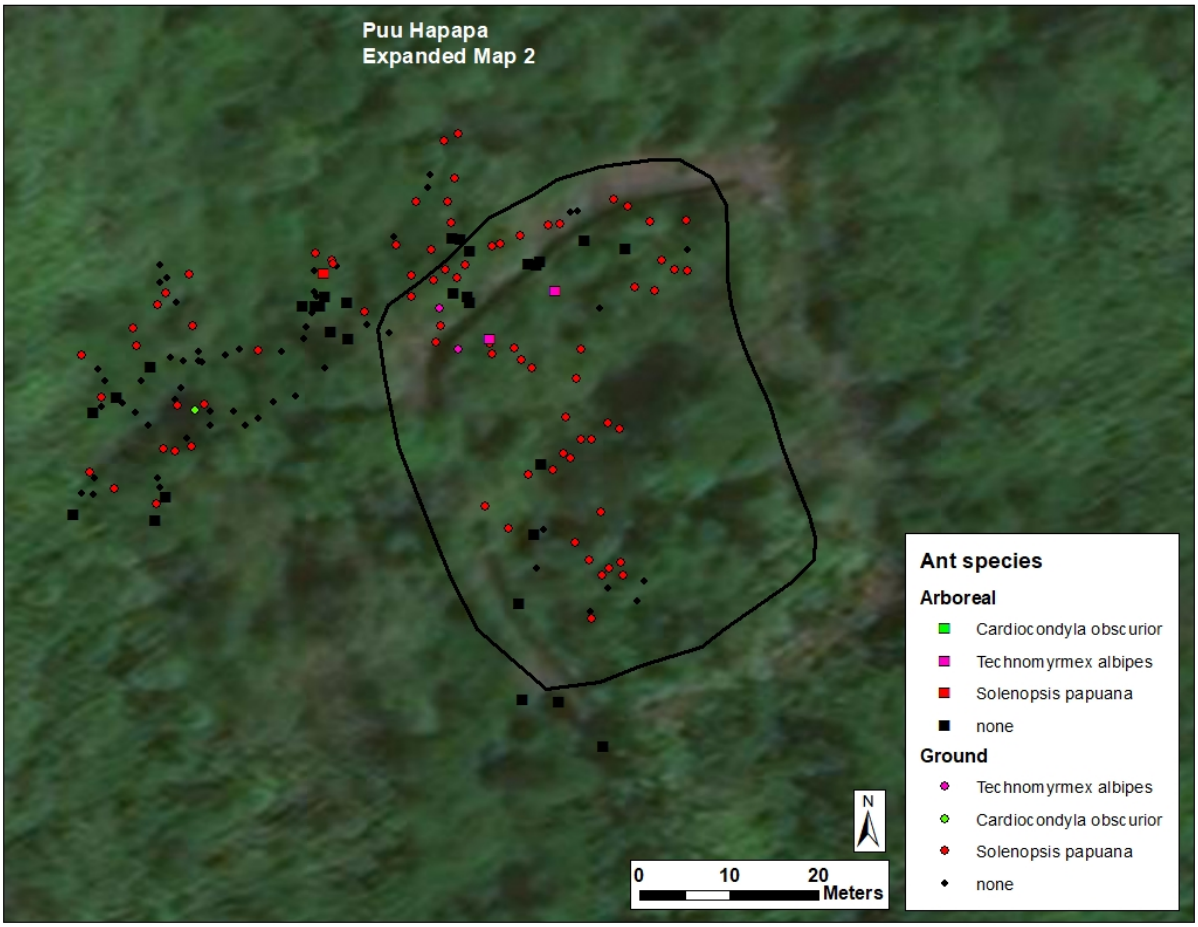

Figure 1-14. Expanded map 2 showing ant species detected at arboreal and ground sampling points at the Pu‘u Hāpapa site. This site supports host plants for *D. montgomeryi*. Note that the survey points are offset slightly from their true locations relative to the snail enclosure that is visible in the background image; the black polygon shows a more accurate representation of the position of the snail enclosure with respect to the survey points.

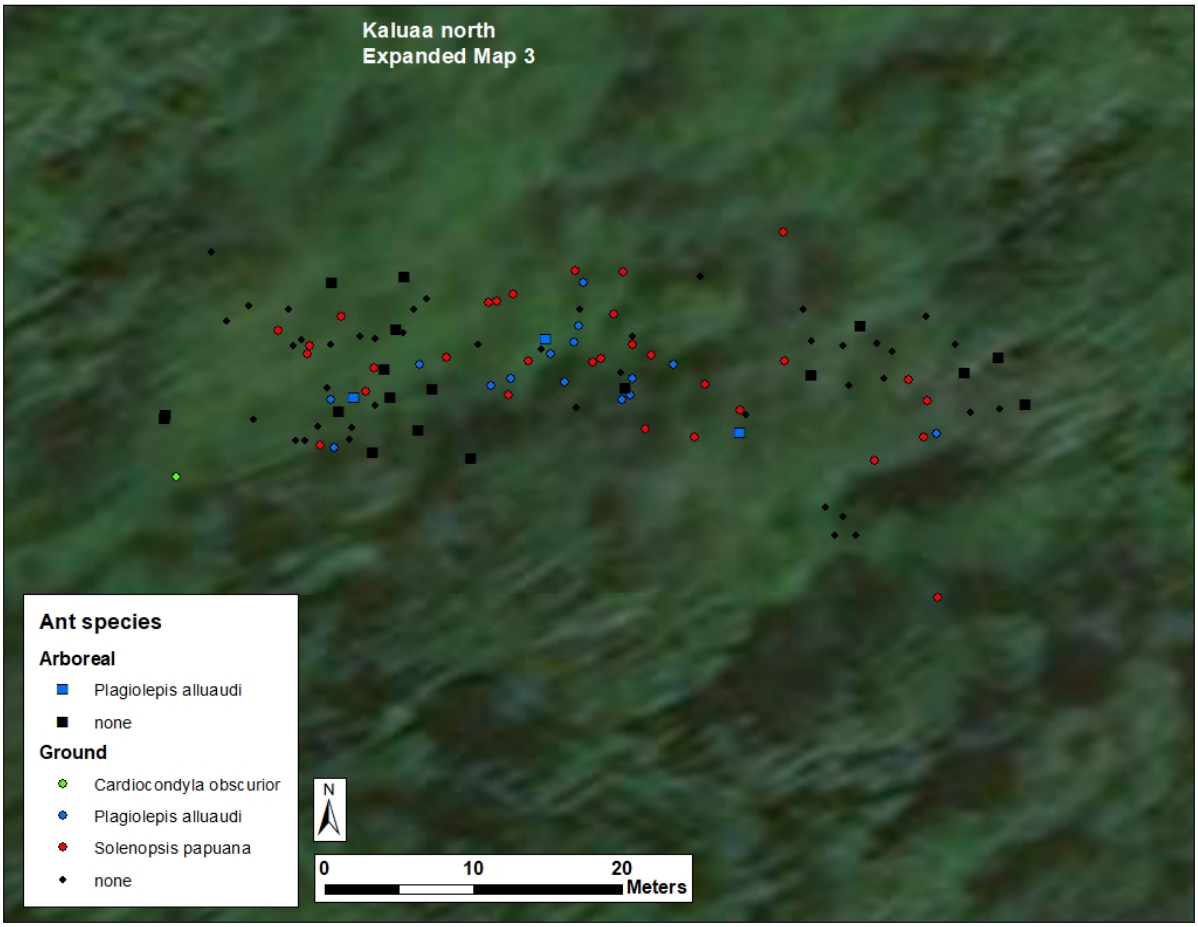

Figure 1-15. Expanded map 3 showing ant species detected at arboreal and ground sampling points at the Kalua‘a north site. This site supports host plants for *D. montgomeryi* and *D. obatai*.

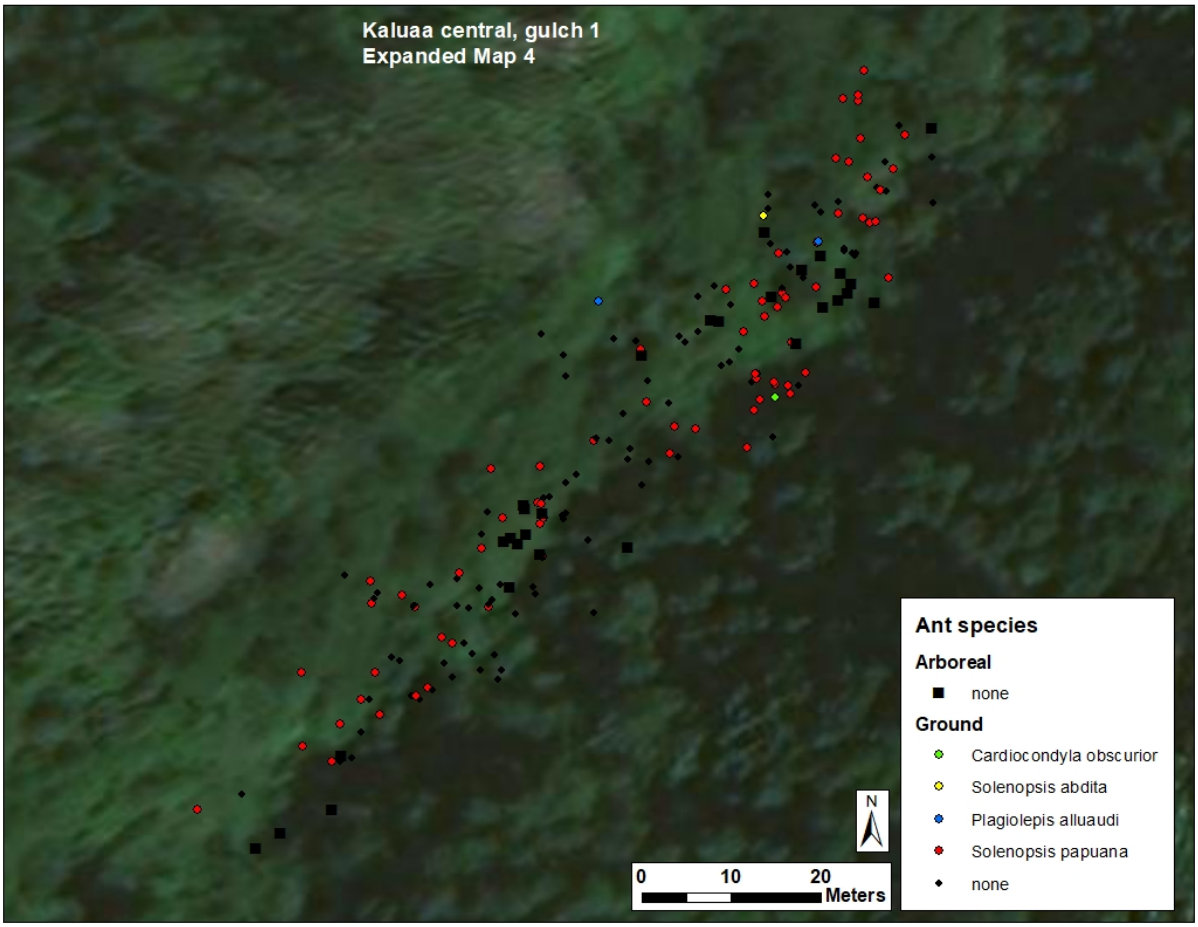

Figure 1-16. Expanded map 4 showing ant species detected at arboreal and ground sampling points at the Kalua‘a central, gulch 1 site. This site supports host plants for *D. montgomeryi*.

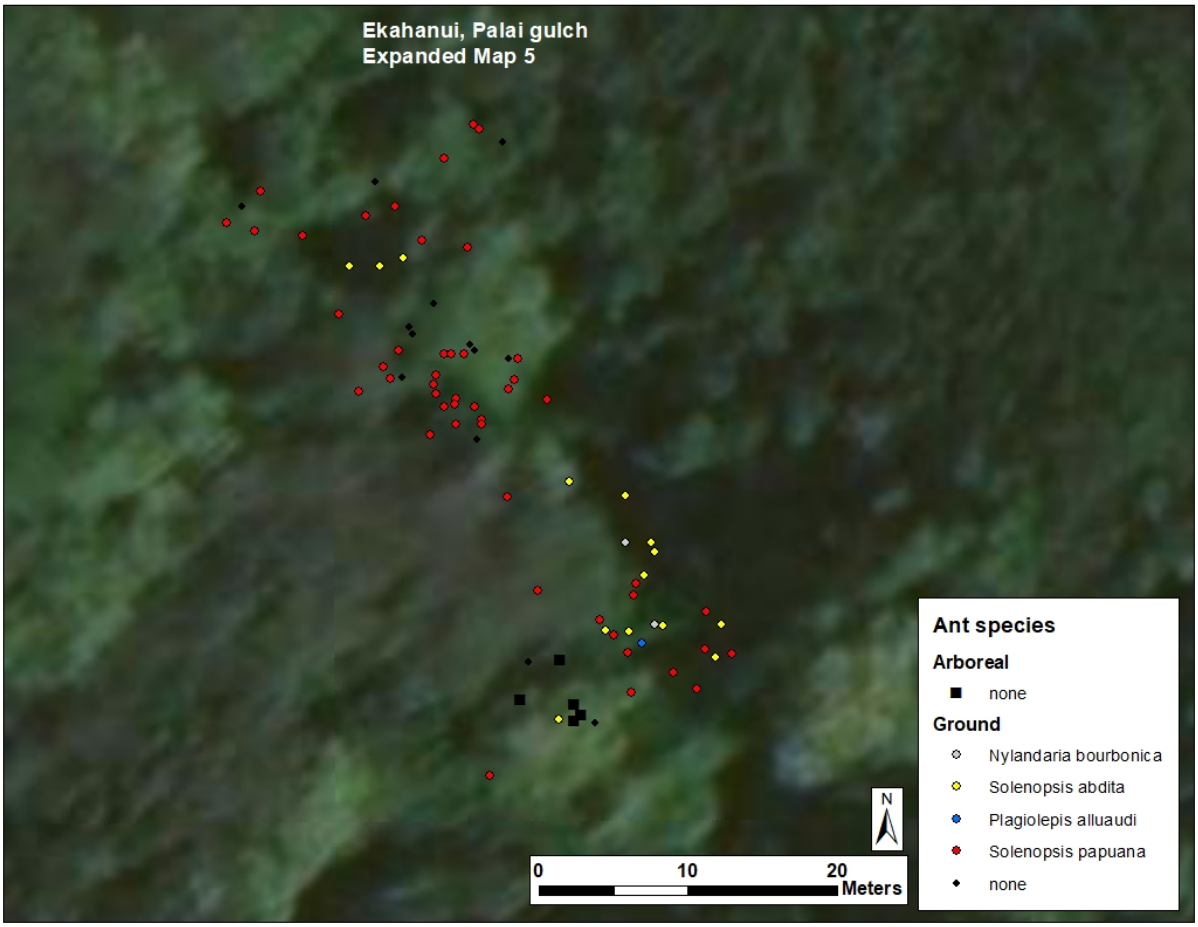

Figure 1-17. Expanded map 5 showing ant species detected at arboreal and ground sampling points at the ʻĒkahanui, Palai gulch site. This site supports host plants for *D. montgomeryi*.

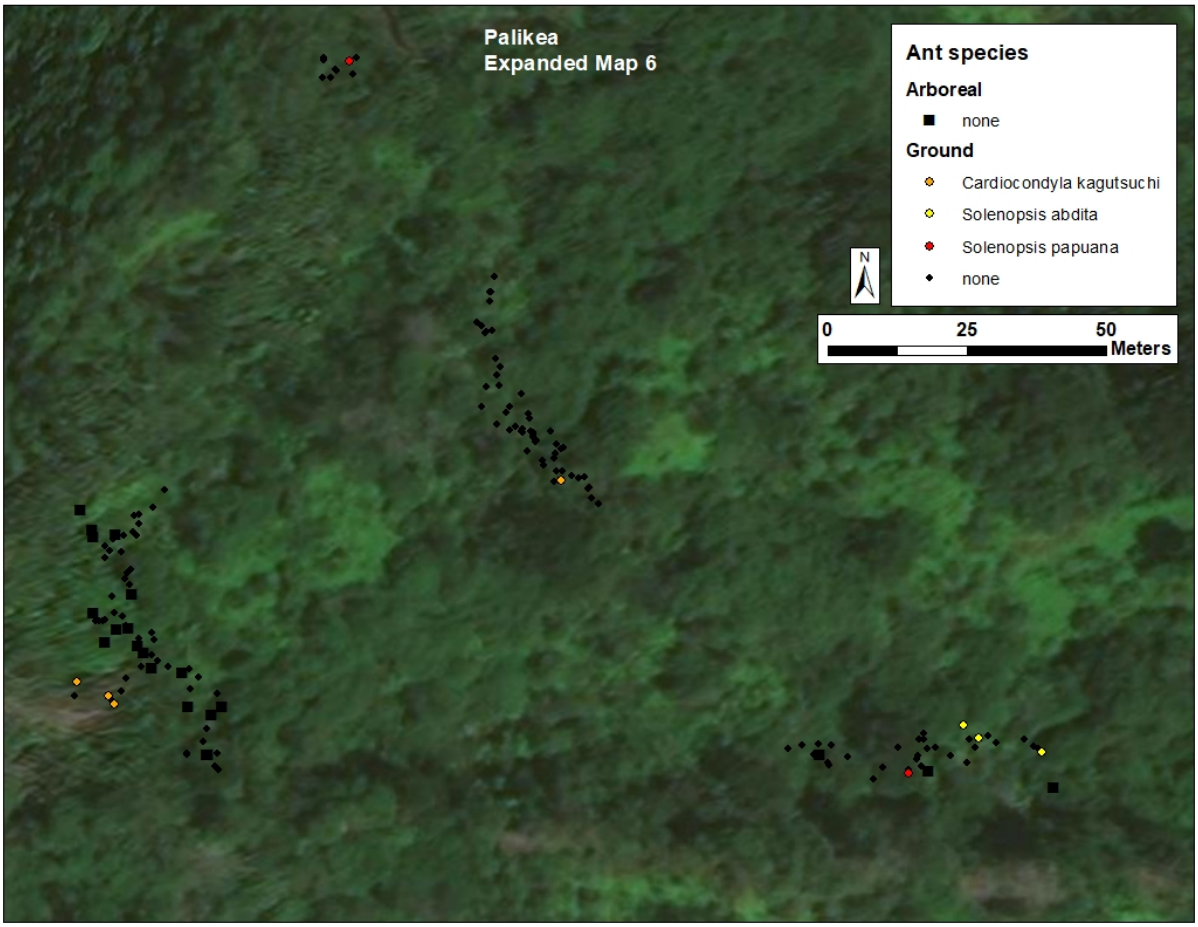

Figure 1-18. Expanded map 6 showing ant species detected at arboreal and ground sampling points at the Palikea site. This site supports host plants for *D. montgomeryi* and *D. substenoptera*.

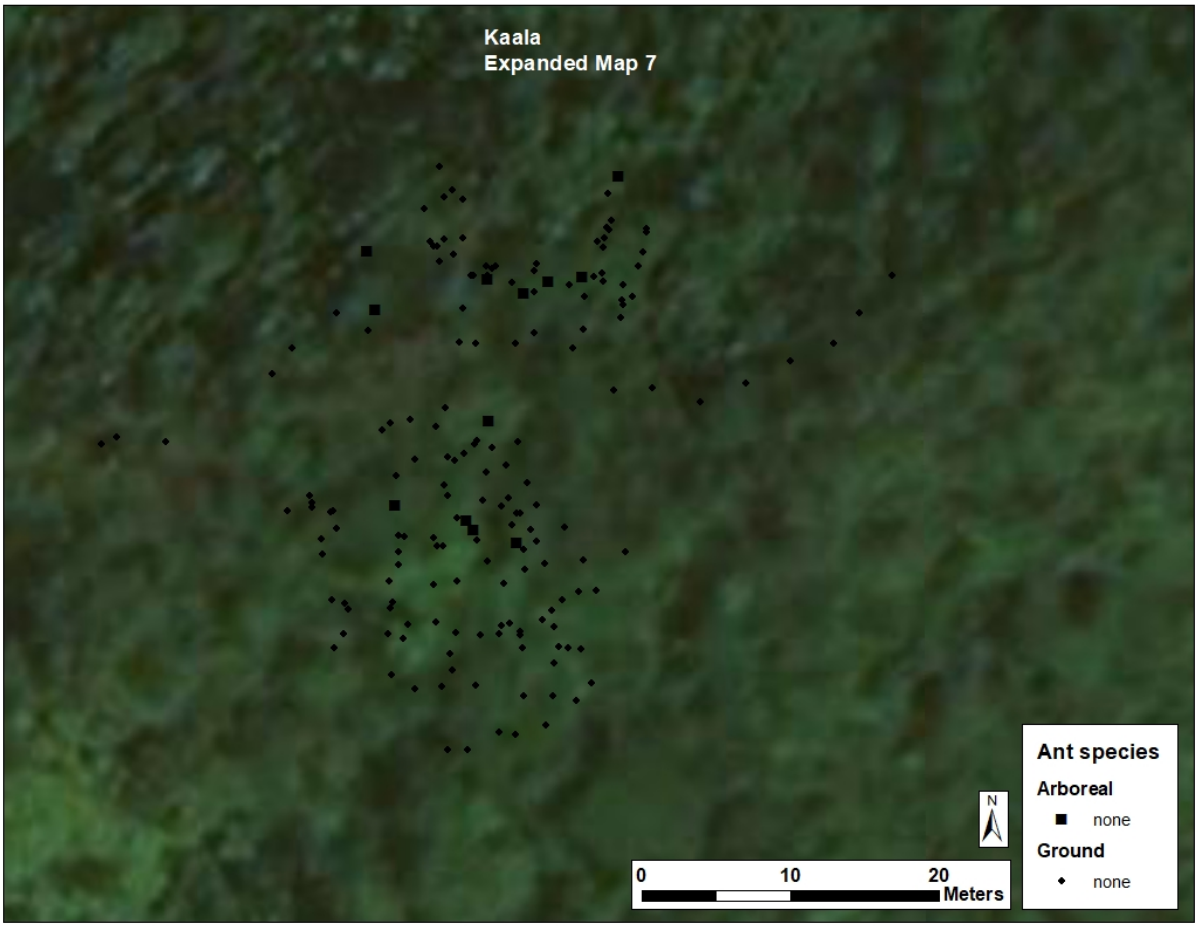

Figure 1-19. Expanded map 7 showing ant species detected at arboreal and ground sampling points at the Ka‘ala site. This site supports host plants for *D. substenoptera*.

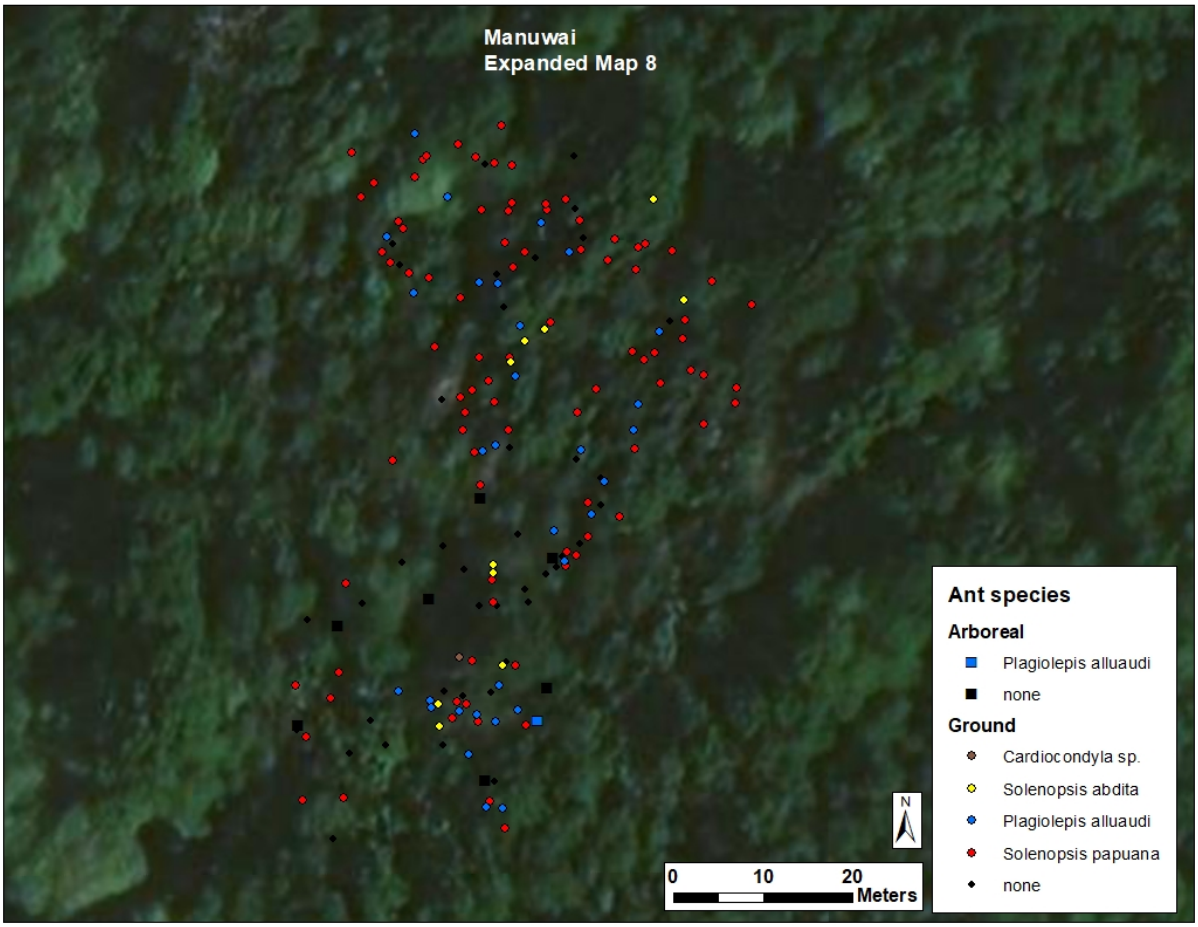

Figure 1-20. Expanded map 8 showing ant species detected at arboreal and ground sampling points at the Manuwai site. This site supports host plants for *D. obatai*.

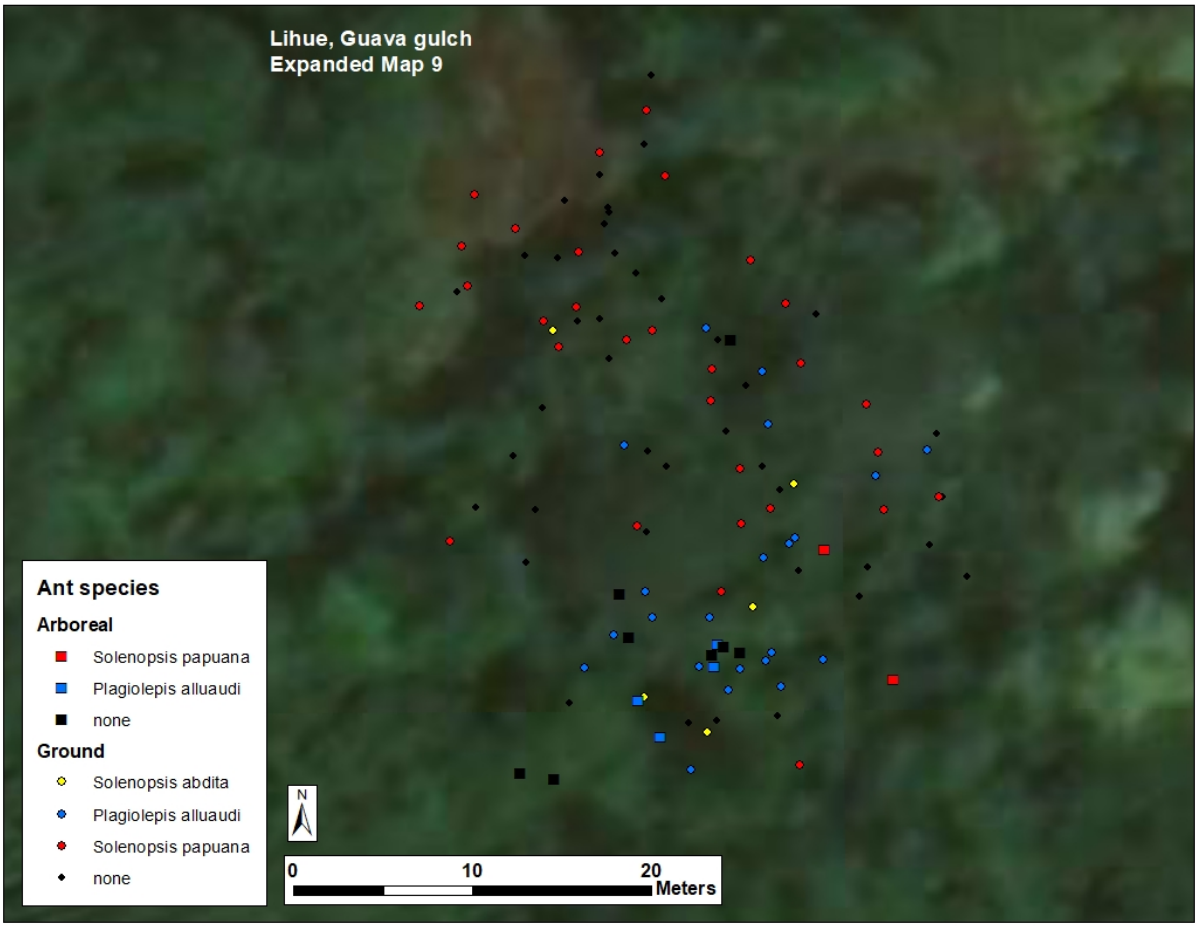

Figure 1-21. Expanded map 9 showing ant species detected at arboreal and ground sampling points at the Līhu‘e, Guava gulch site. This site supports host plants for *D. obatai*.

Pacific Missile Range Facility

A total of 840 ground sampling points and 93 arboreal sampling points were completed across the five PMRF Kōkeʻe sites (Figs. 1-22 – 1-27). Eight ant species were detected with ground baits, although all of them were relatively uncommon: 79% of ground baits attracted no ants (Table 1-11). One of the species collected, *Cardiocondyla kagutsuchi*, is difficult to separate morphologically from a related species, *C. venustula*. Both species are known to occur in the area (P. Krushelnycky unpub. data), so it is possible that some or even all of the ants identified as *C. kagutsuchi* may actually be *C. venustula*.

Nearly all ant detections at ground baits occurred in paved, mowed, or otherwise open areas of the sites (Figs. 1-23 – 1-27). Few if any ants appeared to be present in closed canopy forest surrounding the modified areas. Similarly, ants were detected on only three of the 93 sampled *A. koa* trees, the host plant for *D. musaphilia* (Table 1-12). This included two trees with *Paratrechina longicornis* at site A (Fig. 1-23) and one tree with *O. glaber* at site D (Fig. 1-26).

Table 1-11. Percent occurrence and mean abundance per sampling point of ant species detected with ground baits at PMRF.

Species	**Occurrence (%)**	**Mean abundance**
none	79.0	--
Tetramorium caldarium	11.9	7.0
Paratrechina longicornis	3.1	22.9
Pheidole megacephala	2.7	15.5
Linepithema humile	1.8	54.5
Nylanderia bourbonica	1.5	6.9
Cardiocondyla kagutsuchi[1]	1.0	1.0
Ochetellus glaber	1.0	2.4
Solenopsis abdita	0.7	6.3

[1]Some or all of these may belong to *Cardiocondyla venustula*, which is difficult to separate from *C. kagutsuchi* morphologically.

Table 1-12. Percent occurrence and mean abundance per sampling point of ant species detected with arboreal baits at PMRF.

Species	**Occurrence on Acakoa[1] (%)**	**Mean abundance**
none	96.8	--
Paratrechina longicornis	2.2	15.0
Ochetellus glaber	1.1	1.0

[1]*Acacia koa*

The potential for ants to spread from modified habitats into surrounding forests, and thus to pose a threat to *D. musaphilia* and other native arthropods, likely varies by species. Currently all of the species detected appear unable to penetrate forested habitats in this area, likely because of the colder and wetter conditions prevailing under the closed canopy. However, this barrier may become weaker with a changing climate, and it is possible that some of the species have been introduced to the sites only recently, and thus have had insufficient time to spread into the forest.

Tetramorium caldarium was the most common ant encountered in the survey, occurring at all five sites. This small species generally forms small colonies, and is not typically considered to be highly invasive or ecologically destructive. It is possible, however, that it could exert predatory pressure on picture-winged flies similar to *S. papuana*, if it were to become established and attain higher densities in forested habitats.

Pheidole megacephala, *P. longicornis*, and *L. humile* each pose greater ecological threats, but each was found to be abundant only in localized portions of the study site. An informal survey of the wider Kōkeʻe area in 2001 found *P. megacephala* to be generally restricted to open ridges below approximately 950 m elevation (P. Krushelnycky unpub. data). The population of *P. megacephala* found only at Site E in the present survey, therefore, could represent a discrete, human-vectored introduction. The same 2001 survey found *L. humile*, the Argentine ant, to be widely established around the vicinity of the PMRF Kōkeʻe sites, but mostly restricted to roadsides, trails and other open areas. The continued presence of *L. humile* around the buildings of Site B and roadside at Site A is therefore not surprising. *Paratrechina longicornis*, the longhorn crazy ant, however, was not detected in the 2001 survey, and was only found at Site A in the present survey. It is possible that this species was accidentally introduced to Site A through human activities.

Management recommendations

Sites A and E, and to a lesser extent Site B, supported relatively high diversities of invasive ants, and some efforts to control these may be advisable. In particular, at site E, *P. megacephala* should be relatively easy to eradicate with one to several applications of Amdro Ant Block granular bait (see Section II). Treatment of this site might also eliminate *S. abdita*, which was only detected at a few points near the main building at Site E (and none of the other sites), and which should also be attracted to Amdro bait. Amdro bait is likely also attractive to *T. caldarium*, so treatments with this bait may suppress this species as well.

The remaining problematic species – *L. humile*, *P. longicornis*, and to a lesser extent *O. glaber* and *N. bourbonica* – are generally more strongly attracted to sugar water-based baits. Until new baiting tools like water-storing granules are available for use in Hawaiʻi (Section III), control options are limited. Deployment of bait stations containing sugar water bait formulated with boric acid (e.g. Terro) is one option, but these would likely need to be maintained over longer time periods (weeks to months) and may not be practical. Another option is the granular bait Maxforce Complete, which can be broadcast with relatively little effort. This bait may be less attractive to these species than sugar water baits, but sufficient control at sites A and B may nonetheless be achieved. Maxforce Complete contains two granule types, one of which should also be attractive to the myrmecine species mentioned above (*P. megacephala*, *S. abdita*, *T. caldarium*).

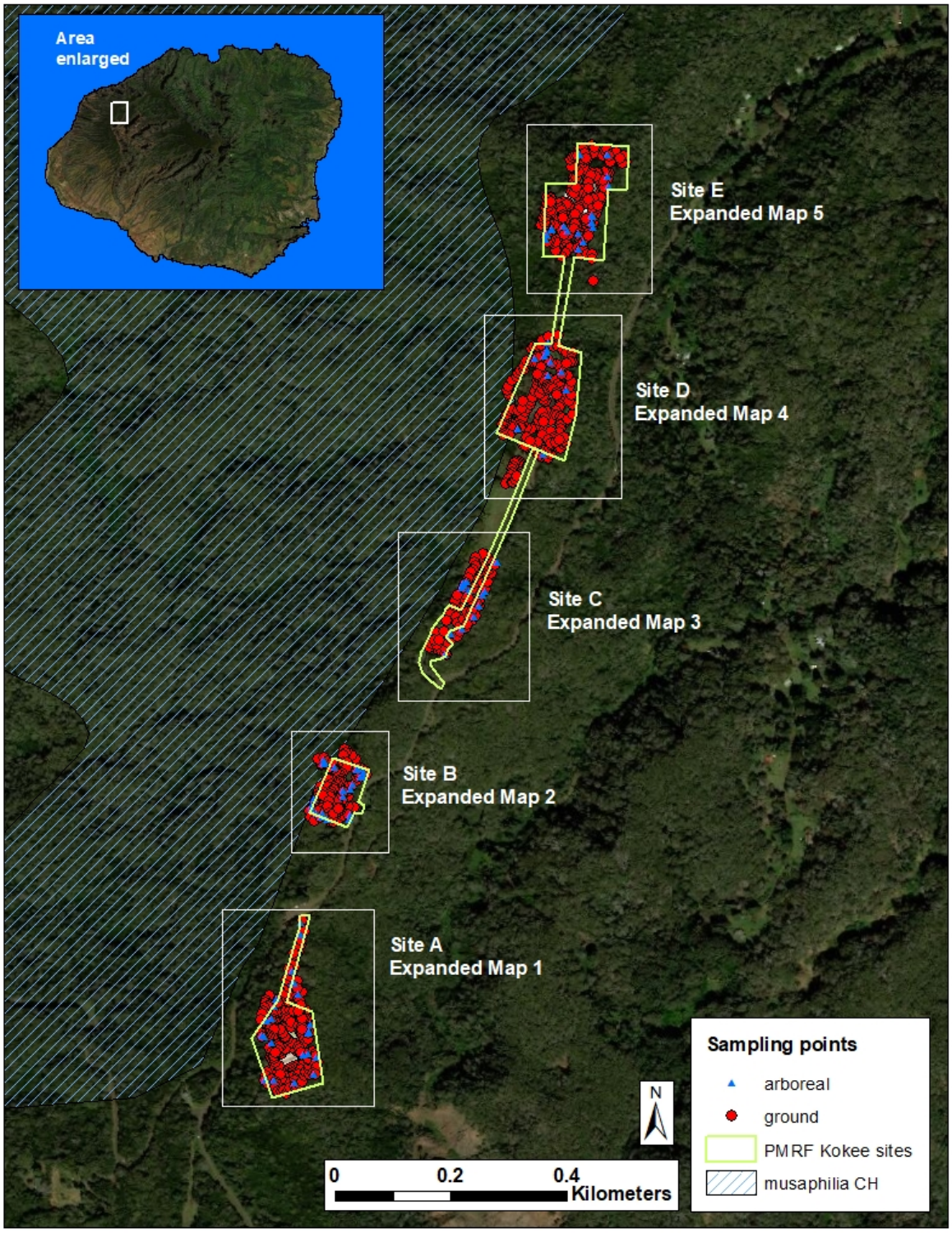

Figure 1-22. Overview map of PMRF Kōkeʻe sites surveyed for ants, including arboreal and ground sampling points. Individual sites are enlarged in Figures 1-23 through 1-27. Approximate site boundaries indicated with light green polygons, and designated critical habitat for *D. musaphilia* shown with blue hatching.

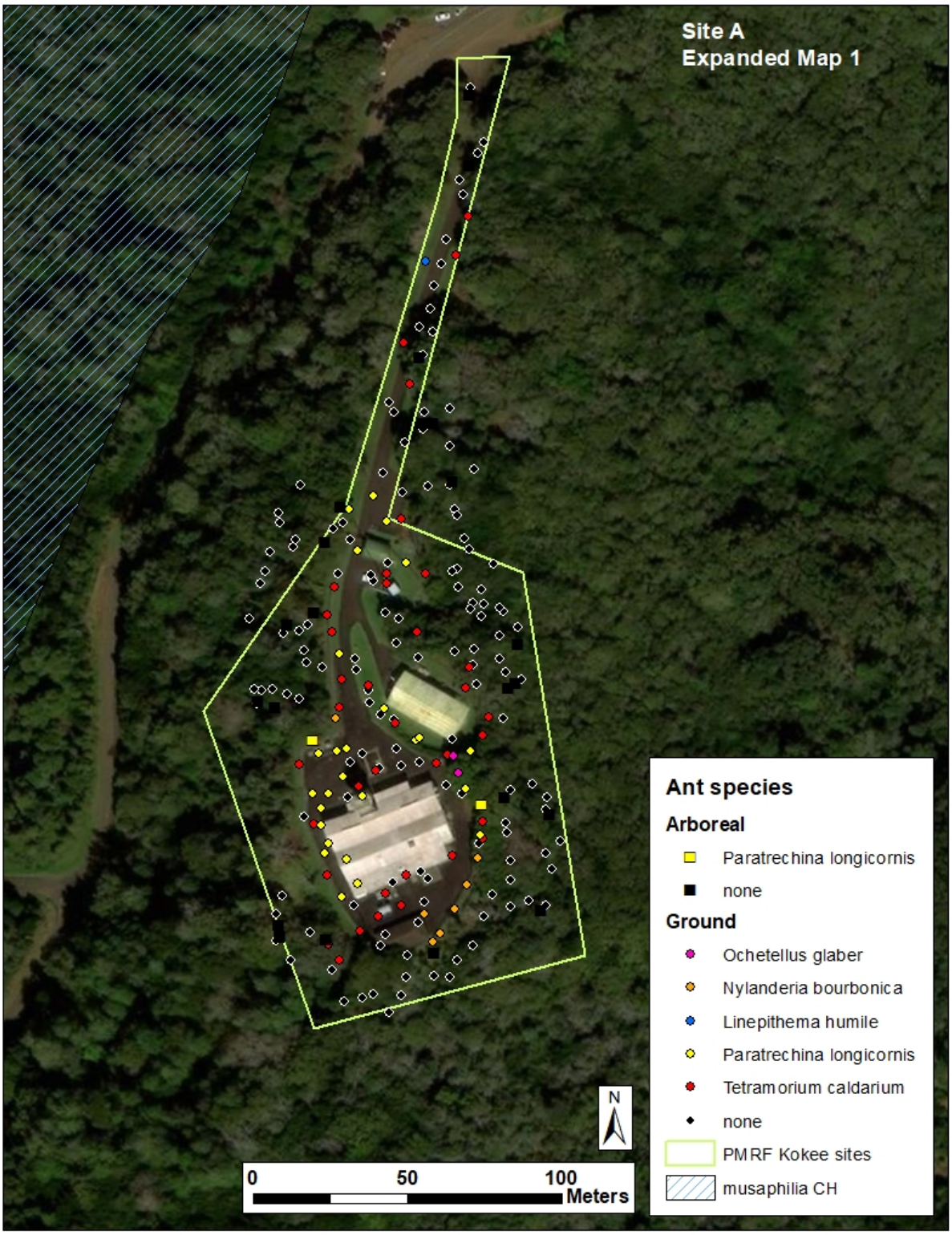

Figure 1-23. Expanded map 1 showing ant species detected at arboreal and ground sampling points at Site A. Ground points with no ants detected are ringed with white to aid visualization.

Figure 1-24. Expanded map 2 showing ant species detected at arboreal and ground sampling points at Site B. Ground points with no ants detected are ringed with white to aid visualization.

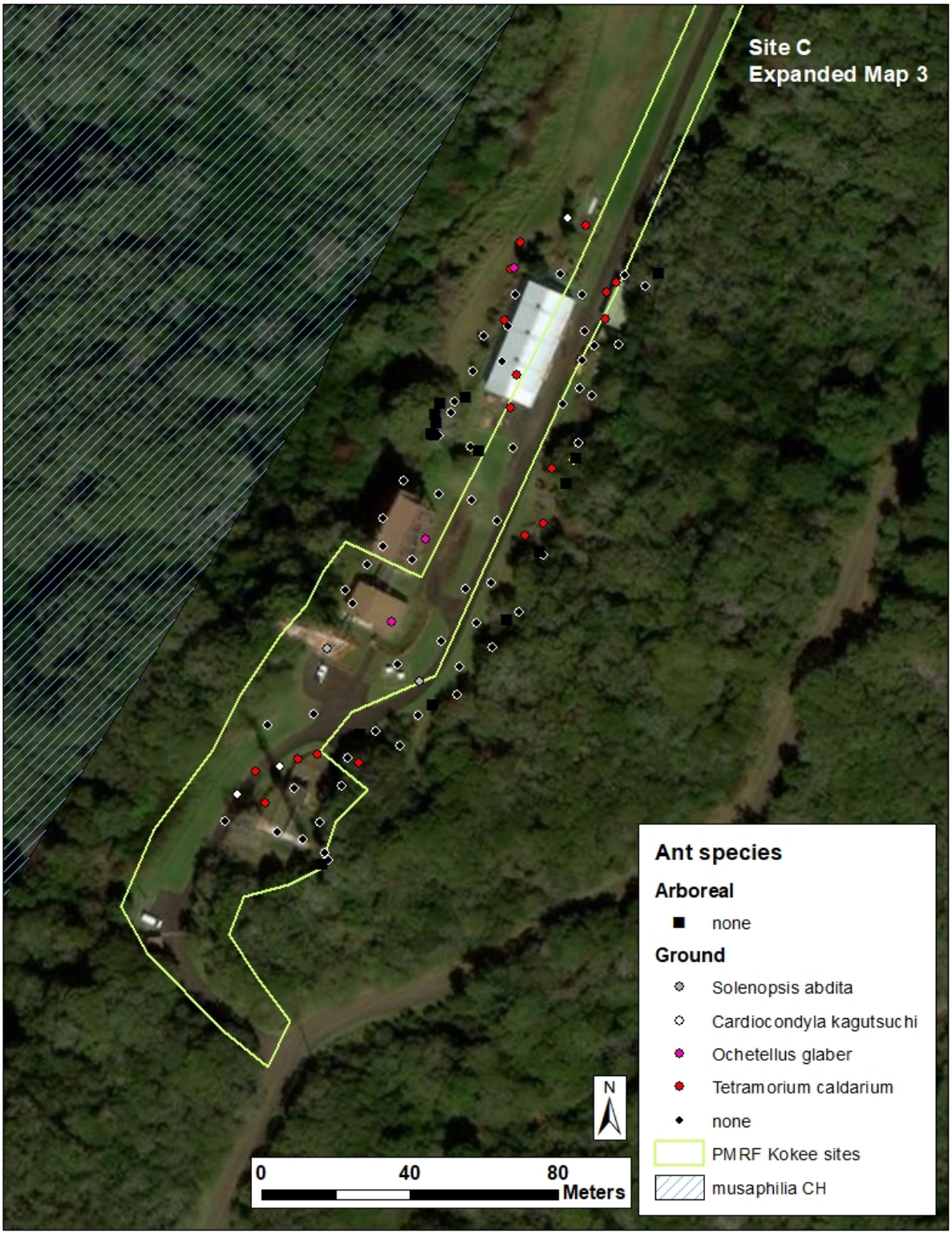

Figure 1-25. Expanded map 3 showing ant species detected at arboreal and ground sampling points at Site C. Ground points with no ants detected are ringed with white to aid visualization.

Figure 1-26. Expanded map 4 showing ant species detected at arboreal and ground sampling points at Site D. Ground points with no ants detected are ringed with white to aid visualization.

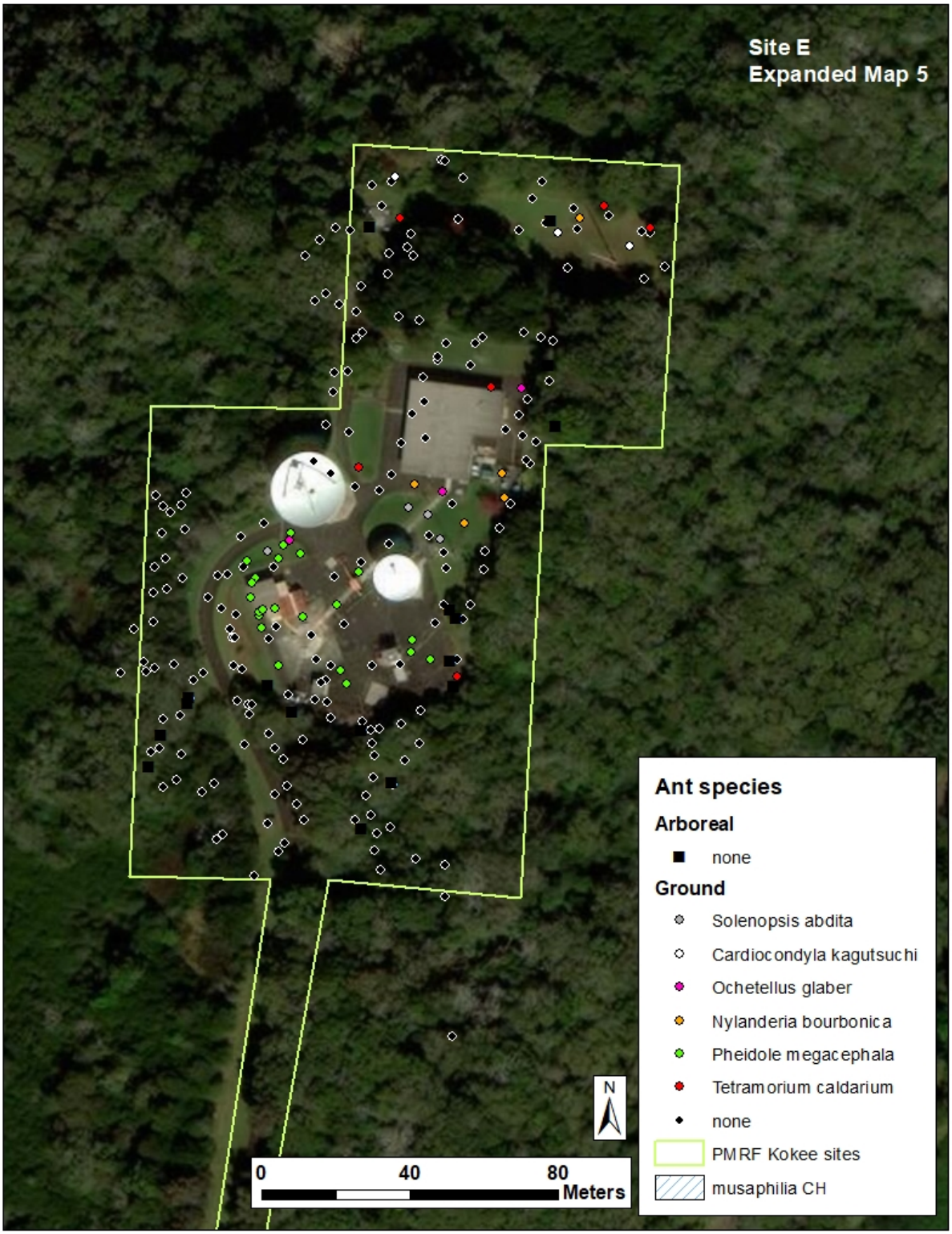

Figure 1-27. Expanded map 5 showing ant species detected at arboreal and ground sampling points at Site E. Ground points with no ants detected are ringed with white to aid visualization.

SECTION II. Efficacy and non-target risks of broadcasting Amdro bait to control ants attracted to oil-based baits

INTRODUCTION

Many invasive ant species of Hawai‘i belonging to the subfamily Myrmicinae are strongly attracted to an oil-based commercial granular bait, Amdro Ant Block. Several of these species, including *Pheidole megacephala*, *Tetramorium* spp., *Solenopsis abdita*, and especially *Solenopsis papuana*, are widely distributed in mesic to wet montane forests of O‘ahu and Kaua‘i (P. Krushelnycky unpub. data, Plentovich 2010), including habitats supporting listed picture-winged *Drosophila* flies (see Section I). The presence of *S. papuana* in the breeding habitats of picture-winged flies can greatly reduce their reproductive success (Krushelnycky et al. 2017). Experimental methods used to control *S. papuana* to date have distributed Amdro Ant Block within bait stations to minimize potential impacts on non-ant insects. While effective, this method is very labor intensive. Because of the short foraging distances of *S. papuana*, bait stations need to be placed at intervals of approximately 2.5 m to be effective, and the bait inside needs to be replaced every four to six weeks because of molding (Ogura-Yamada and Krushelnycky 2016). Broadcasting the bait is far easier, and because of better distribution might be more effective in suppressing ants. Amdro Ant Block is labeled for broadcast application in forests in Hawai‘i, but the efficacy and non-target risks of doing so have not been quantified.

This section reports on a series of studies that investigated 1) the efficacy of broadcasting Amdro Ant Block for suppressing *S. papuana* in mesic montane forests of O‘ahu, 2) the non-target effects of broadcasting Amdro Ant Block on invertebrate communities in such forests, and 3) attraction of picture-winged flies to Amdro Ant Block bait. The results provide information directly relevant to the utility and safety of broadcasting this ant bait as a management tool aimed at recovering listed picture-winged *Drosophila* in Hawai‘i.

MATERIALS AND METHODS

Efficacy and non-target effects of Amdro broadcast

Study Sites

The efficacy of broadcasting Amdro Ant Block bait for suppressing *S. papuana*, and the resultant effects on non-ant ground-dwelling invertebrates of Wai‘anae Mountain mesic forests, were evaluated at three sites that supported moderate to high densities of *S. papuana*. The sites were Kahanahāiki Valley (583-665 m elevation, 1347-1407 mm mean annual rainfall) under management of SBAB, Pahole Natural Area Reserve (475 m elevation, 1339 mm mean annual rainfall), and Kalua‘a (482-585 m elevation, 1160-1198 mm mean annual rainfall) in Honouliuli Forest Reserve. All sites were situated in mesic montane forest supporting a mixture of native and alien vegetation. Reported estimates of mean annual rainfall were obtained from the Rainfall Atlas of Hawai‘i (Giambelluca et al. 2013).

Study Design

Seven pairs of 20 x 20 m plots were established across the three study sites: three pairs at Kahanahāiki and two pairs each at Pahole and Kaluaʻa. One plot in each pair was randomly assigned to either Amdro Ant Block broadcast (Amdro) or unmanipulated control (control). Amdro plots were treated with Amdro Ant Block Home Perimeter Ant Bait (0.88% hydramethylnon, EPA No. 73342-2, AMBRANDS, Atlanta, Georgia) once each at the label rate of 0.37 kg/ha (2 lbs/acre) between 8/26/19 and 8/30/19.

Solenopsis papuana abundances were monitored in the plots using peanut butter-baited monitoring cards. A smear of peanut butter was placed on each of 16 cards (one-half of a 7.6 x 12.7 cm index card) spaced 5 m apart in a grid pattern in the central 15 x 15 m portion of each plot. After 90 minutes, *S. papuana* ants on the top and bottom of the cards were counted and summed. Abundances were monitored one week prior to bait application, two weeks after application, and then every five to 10 weeks thereafter for one year.

Ground-dwelling invertebrates were sampled in each plot using pitfall traps and leaf litter extraction on three occasions: immediately before bait application (starting 8/19/19-8/23/19 and ending 8/26/19-8/30/19), two weeks after bait application (starting 9/9/19-9/13/19 and ending 9/16/19-9/20/19) to assess direct impacts from bait consumption, and six months after bait application (starting 2/24/20-2/28/20 and ending 3/2/20-3/6/20) to assess the longer-term effects on any taxonomic groups that may have been impacted. Both sample types were collected at four fixed points at the corners of a 5 x 5 m square at the center of each plot. Litter samples were obtained by collecting approximately 3-4 L of leaf litter from an area extending 1 m around the sampling point, removing 2 L of this litter in the lab, and placing the resultant sample in a Berlese funnel for approximately 72 hours. Pitfall traps consisted of 10 oz. plastic cups (#TP10D, Solo® Cup Company, Lake Forest, Illinois) buried flush with the ground, partially filled with a 50% propylene glycol and 50% water solution, and shaded with a square plastic cover. Pitfalls were opened for seven days during each sampling event.

Invertebrate Identification

Invertebrate samples were sorted to the following taxonomic levels. Snails were not identified beyond the level of class (Gastropoda). Arthropods were sorted to class or subclass (in the case of Acari, Chilopoda and Diplopoda) or order (remaining groups), then individuals in Amphipoda, Blattodea, Chilopoda, Coleoptera, Dermaptera, Diplopoda, and Hemiptera were identified to species or morphospecies (or to genus in the case of native *Proterhinus* spp., order Coleoptera). Several additional lower taxonomic groups were identified to genus or species, including all Formicidae (ants, order Hymenoptera), *Hyposmocoma* spp. (native case-making caterpillars, order Lepidoptera), and *Laupala* spp. (native crickets, order Orthoptera). If immature individuals could not be identified to species or morphospecies for groups that were sorted beyond order, individuals identified to at least genus or family were allocated to species in proportion to the number of adults of species of the same taxonomic group occurring in the sample. All individuals were then classified as native, non-native (accidentally or purposely introduced), or of unknown origin based on Nishida (2002) and other taxonomic literature.

Only a subset of taxonomic groups were sorted, as described above, for samples collected at six months after bait application. These were Acari, Araneae, Blattodea, Collembola, Hymenoptera, Isopoda, Lepidoptera, Orthoptera, Psocoptera, Thysanoptera, and Gastropoda.

Analysis

Data were pooled across both sample type (pitfall, leaf litter extraction) and sampling points within each plot for each sampling event. To assess impacts from Amdro bait application, changes in abundance from pre-application to two weeks post-application were calculated in Amdro and control plots (n = 7 for each) for major taxonomic groups. Because data for some groups were not normally distributed, abundance changes were compared between Amdro and control plots with non-parametric Wilcoxon tests. Longer-term effects were similarly assessed by comparing abundance changes from pre-application to six months post-application for Amdro and control plots.

Bait attraction among picture-winged flies

Attraction of picture-winged flies to Amdro bait was assessed by placing individual flies of surrogate, non-listed species in small laboratory cages provisioned with the bait, and observing feeding behavior and comparing survival times to those of flies in control cages. Cages were plexiglass boxes with mesh screen tops that were 40 cm L x 30 cm W x 30 cm H (Fig. 2-1). Damp sand was placed in the bottom of each cage to a depth of approximately 2 cm, and a wooden dowel for perching was placed inside. In Amdro cages, a small pile of Amdro Ant Block granular bait was placed on a piece of plastic in the center of the cage immediately prior to fly addition (Fig. 2-1). Control cages were identical except no Amdro bait was added. Wild picture-winged flies of three non-listed species were captured in the field by K. Magnacca of ANRPO for use in the cage trials: *D. ambochila* (9 Amdro cages, 8 control cages), *D. crucigera* (6 Amdro cages, 6 control cages), and *D. punalua* (8 Amdro cages, 8 control cages).

On the evening of capture, flies were placed individually in snap-cap vials that were provisioned with paper towel soaked in 40% sucrose solution. Trials began the following morning, at which point flies were randomly assigned to either Amdro or control treatment, and were placed individually into their respective cages for seven days. No alternative food was provided in the cages, but a small dish with water-soaked paper towel was placed in one corner. Survival of flies was monitored and recorded each morning; flies that were found moribund (e.g. lying on their backs or sides with legs barely moving) were scored as dead. Survival times in cages were compared using log-rank chi-square values from Kaplan-Meier time-to-event survival analysis, where the event was the day on which fly death was first observed. A video camera (Sony HDR-CX405) was used to film the Amdro bait in Amdro cages for nine daytime hours on each of the first two days of each trial (Fig. 2-1), and this video was subsequently viewed to see if flies visited and appeared to feed on the bait granules.

To confirm that the seven-day trial period was sufficiently long to assess feeding exposure to Amdro Ant Block bait in the cage trials, additional wild flies were placed in either small, one dram (3.5 ml) vials half-filled with Amdro bait (Amdro) or empty vials (control) for five hours on the morning after capture (Fig. 2-2). Vial tops were covered with mesh screen after flies were inserted. Flies in Amdro vials were therefore forced to at least touch the bait during the five-hour exposure period. Following the five-hour exposure period, flies were returned to their original snap-cap vials provisioned with 40% sucrose solution. Survival was assessed at the end of the five-hour period, and subsequently every morning for seven days. The same three species used in the cage trials were also used in the vial trials: *D. ambochila* (9 Amdro vials, 10 control vials), *D. crucigera* (7 Amdro vials, 7 control vials), and *D. punalua* (3 Amdro vials, 3 control vials).

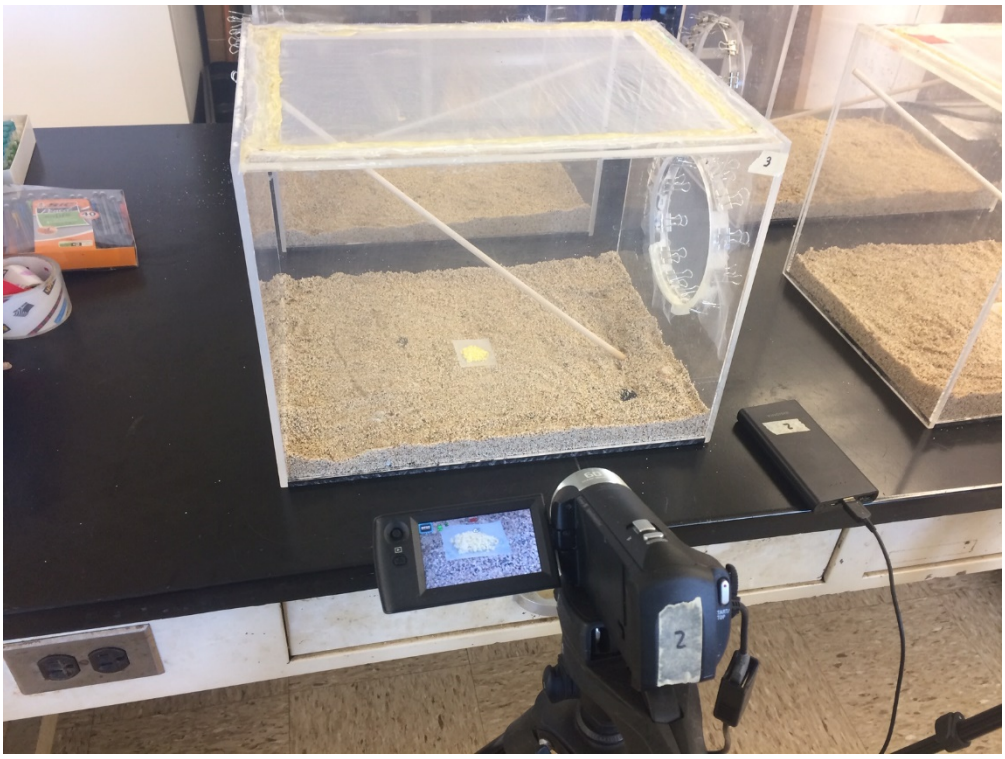

Figure 2-1. Example of cage used to test attraction of picture-winged flies to Amdro Ant Block bait. A small pile of Amdro bait, placed on a piece of plastic, sits in the center of the cage. At bottom of image is a video camera, zoomed in on the Amdro bait, used to film potential fly visitation to bait. Control cages were identical except no Amdro Ant Block bait was provided.

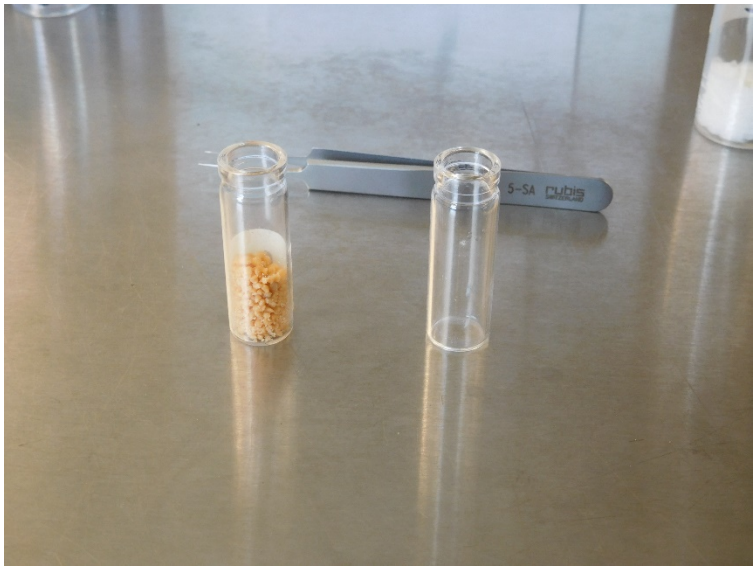

Figure 2-2. Examples of Amdro and control vials used to test effects of Amdro exposure on picture-winged flies.

RESULTS

Efficacy for controlling *S. papuana*

A single broadcast of Amdro Ant Block bait yielded very strong suppression of *S. papuana* two weeks after application, as measured with peanut butter bait cards across the seven pairs of 20 x 20 m field plots (Fig 2-3). This suppression was also evident in each of the seven Amdro plots (Fig. 2-4). Suppression of *S. papuana* persisted for at least six months (26 weeks), after which ant numbers began to increase moderately, but still remained substantially lower than numbers in some of the control plots for one year (Figs. 2-3, 2-4). The convergence in ant numbers between Amdro and control plots towards the end of the study period resulted largely from declines in control ant numbers at some of the sites.

Seven other ant species were detected in low numbers in the study plots with the peanut butter bait card monitoring (*Solenopsis abdita, Nylanderia bourbonica, Ochetellus glaber, Pheidole navigans, Plagiolepis alluaudi, Cardiocondyla obscurior, Anoplolepis gracilipes*). However, suppression of *S. papuana* did not lead to an obvious increase in numbers of these ants in Amdro plots, as numbers of all other ant species combined remained very low during the study (Fig. 2-5).

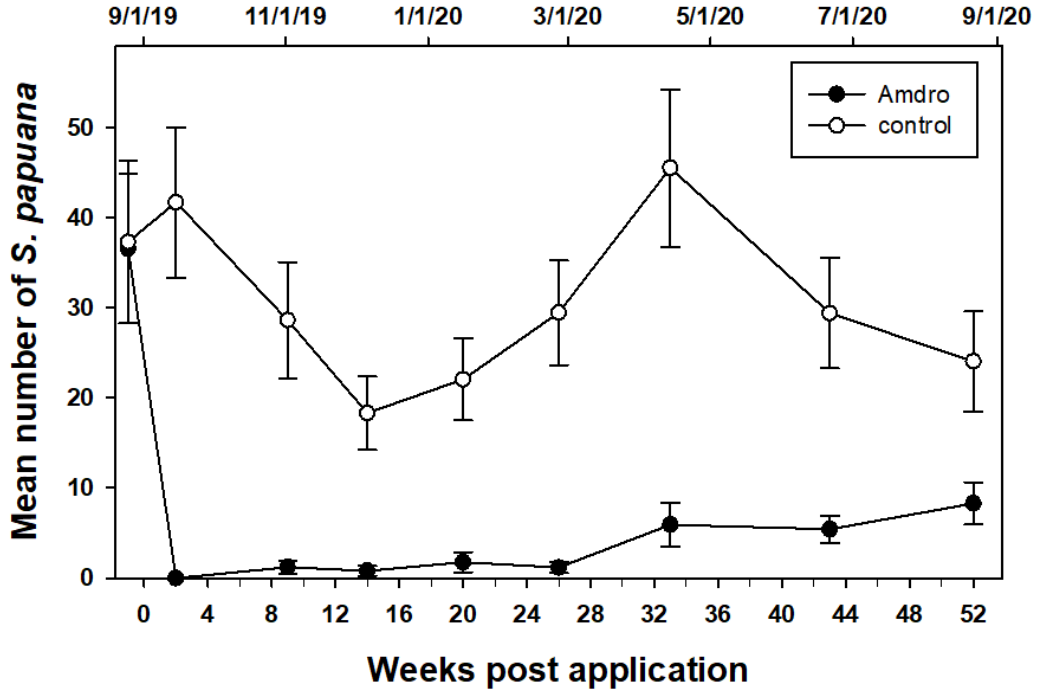

Figure 2-3. Mean numbers of *S. papuana* over the course of the study measured with peanut butter bait cards, averaged over the seven pairs of Amdro and control plots. Amdro broadcast was completed at week 0.

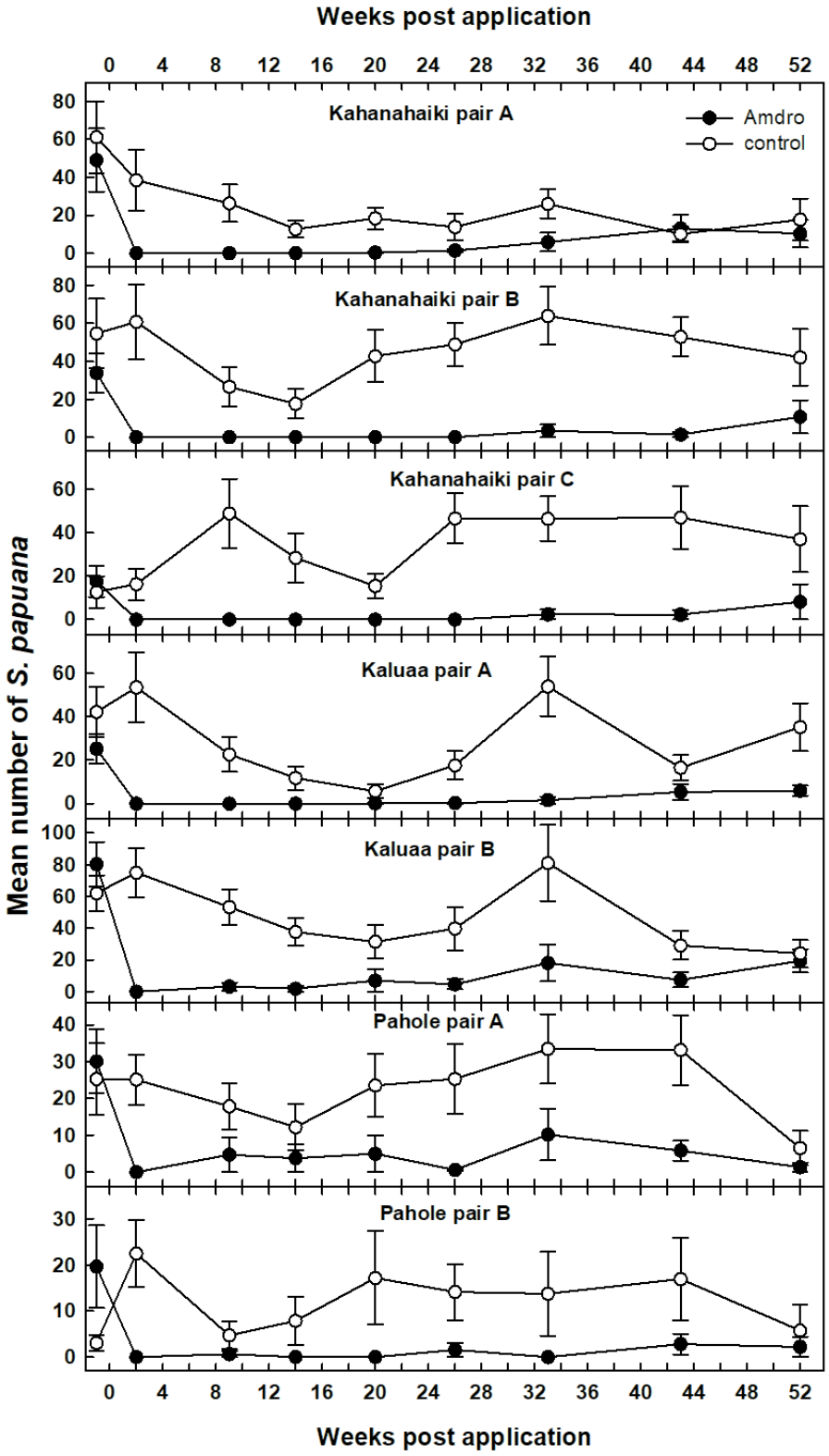

Figure 2-4. Mean numbers of *S. papuana* over the course of the study measured with peanut butter bait cards in each of the seven pairs of Amdro and control plots. Amdro broadcast was completed at week 0.

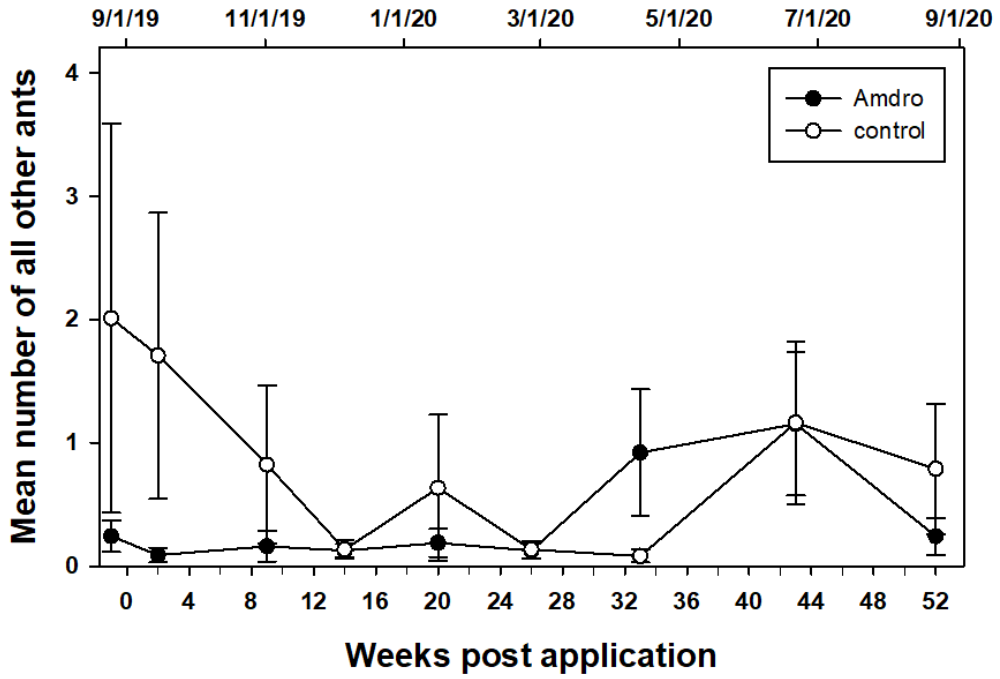

Figure 2-5. Mean numbers of all non-*S. papuana* ants combined over the course of the study as measured with peanut butter bait cards, averaged over the seven pairs of Amdro and control plots. Note the low numbers on the y axis compared to Figures 2-3 and 2-4. Amdro broadcast was completed at week 0.

Effects on non-ant invertebrates

A total of 91,956 invertebrates were captured with the pitfall and leaf litter sampling and sorted for analysis. Invertebrate abundances did not significantly decline in Amdro plots relative to control plots at two weeks after the Amdro Ant Block broadcast application for any taxonomic group examined, or for arthropods or gastropods overall (Table 2-1). The only statistically significant change was an increase of native hemipterans in Amdro plots relative to control plots (Table 2-1). This was largely driven by a marginally significant ($p = 0.062$) increase in *Nesidiorchestes hawaiiensis*, a flightless mirid bug that is common in leaf litter, in Amdro plots relative to control plots (Table 2-1). It is important to note, however, that this single significant result out of 30 statistical comparisons is fewer than would be expected from chance sampling error (1.7 out of 30 at $\alpha = 0.05$), so this result should be viewed with caution.

Statistical power for these comparisons was not high due to the logistical challenges of implementing many replicate field plots for this type of study. However, there was no evidence that the lack of a measureable impact from Amdro broadcast on non-ant invertebrates was driven by weak statistical power. Among the 30 taxonomic comparisons made, half (15) had mean decreases in abundance in Amdro plots relative to control plots, while the other half had mean increases in Amdro plots relative to control plots. Although this does not preclude the possibility that impacts on some sensitive taxonomic groups went undetected, it is consistent with the

interpretation that invertebrate abundance changes from pre-application to two weeks post application largely represented random fluctuations.

Table 2-1. Mean changes in invertebrate abundances (± SE) in Amdro and control plots at two weeks and six months after Amdro Ant Block broadcast application.

Taxon	2 weeks			6 months		
	Amdro	**control**	**p[1]**	**Amdro**	**control**	**p[1]**
Acari	758.6 ± 315.3	715.1 ± 430.3	0.655	984.4 ± 702.7	680..0 ± 651.7	0.142
Amphipoda	-36.6 ± 33.6	-47.6 ± 29.1	0.441			
Araneae	0.8 ± 9.9	32.1 ± 13.0	0.159	-10.0 ± 7.5	-5.1 ± 12.0	0.406
Blattodea	-0.7 ± 0.5	0.0 ± 0.4	0.179	-0.3 ± 0.4	1.4 ± 1.4	0.645
Chilopoda	1.0 ± 0.8	-1.3 ± 0.7	0.072			
Coleoptera						
native	2.6 ± 2.2	4.3 ± 2.2	0.700			
Proterhinus spp.[2]	0.1 ± 0.9	0.4 ± 0.7	0.732			
introduced	3.4 ± 2.8	3.8 ± 3.5	0.898			
total	-12.7 ± 13.6	-9.6 ± 26.2	0.798			
Collembola	100.6 ± 70.4	116.7 ± 25.7	0.406	100.0 ± 78.7	32.4 ± 63.6	0.406
Dermaptera						
native	-3.1 ± 1.8	-10.3 ± 6.0	0.556			
introduced	-11.3 ± 2.5	-6.3 ± 3.6	0.336			
total	-14.4 ± 3.5	-16.6 ± 6.6	0.798			
Diplopoda	-13.3 ± 13.7	6.1 ± 10.9	0.224			
Diptera	2.1 ± 1.7	0.3 ± 2.9	1.000			
Hemiptera						
native	4.7 ± 5.4	-4.1 ± 1.2	*0.043*			
Nesidiorchestes hawaiiensis[3]	3.6 ± 5.0	-3.7 ± 2.6	0.062			
introduced	3.1 ± 2.7	3.4 ± 3.2	0.795			
total	8.3 ± 6.1	-0.4 ± 3.4	0.200			
Hymenoptera						
non-ant total	0.1 ± 0.4	-0.4 ± 0.8	0.395	-0.1 ± 0.6	-0.8 ± 0.5	0.554
Isopoda	223.3 ± 86.4	24.7 ± 93.7	0.180	336.6 ± 144.9	142.0 ± 122.1	0.338
Lepidoptera						
Hyposmocoma spp.[4]	0.4 ± 0.4	0.7 ± 0.6	0.385	-0.4 ± 0.4	-0.4 ± 0.6	0.793
total	-4.8 ± 3.9	5.3 ± 7.3	0.480	-26.8 ± 9.0	-52.3 ± 21.8	0.898
Orthoptera						
Laupala spp.[5]	-1.1 ± 1.0	-0.1 ± 0.5	0.205	-0.8 ± 1.0	1.6 ± 1.9	0.274
Psocoptera	106.4 ± 41.2	60.8 ± 18.7	0.371	-15.0 ± 4.8	-8.1 ± 4.8	0.949
Thysanoptera	22.8 ± 16.1	-48.0 ± 47.0	0.064	-17.1 ± 10.3	-86.3 ± 75.9	0.607
Arthropoda						
native	-22.3 ± 21.3	-22.1 ± 13.0	0.482			
introduced	-96.6 ± 63.0	-67.0 ± 40.6	0.794			
total	1060.1 ± 419.9	804.3 ± 478.4	0.482			
Gastropoda	6.0 ± 3.4	1.3 ± 0.3	0.321	7.0 ± 2.6	7.0 ± 2.1	0.334

[1]Statistical significance of difference between Amdro and control plots determined with Wilcoxon Test.
[2]The most common native Coleoptera collected.
[3]The most common native Hemiptera collected.
[4]The only identifiable group of native immature Lepidoptera.
[5]Native *Laupala* spp. were the only Orthoptera collected.

There were similarly no significant differences in abundance changes between Amdro and control plots at six months post bait application for any taxonomic groups examined (Table 2-1). In addition, non-*S. papuana* ants, as a group, did not increase in abundance in Amdro plots relative to control plots at six months post broadcast, indicating no evidence for competitive release of these other ant species following suppression of *S. papuana* (-0.4 ± 11.3 SE abundance change in Amdro plots versus 14.3 ± 16.8 SE in control plots, p = 0.482). Other ant species collected with pitfall and litter sampling included *S. abdita, N. bourbonica, P. navigans, P. alluaudi, C. obscurior, A. gracilipes, Brachymyrmex* cf. *obscurior, Hypoponera punctatissima, Hypoponera* cf. *johannae, Hypoponera* sp. HI01*, Monomorium floricola, Strumigenys rogeri, Tapinoma melanocephalum, Technomyrmex albipes, and Technomyrmex difficilis.*

Bait attraction among picture-winged flies

The vial trials confirmed that picture-winged flies forcibly exposed to Amdro Ant Block bait usually died within several days. Seventeen of 19 flies placed in Amdro vials (89%) died within 48 hours of exposure, with the remaining two still alive after seven days. In comparison, only three of 20 flies placed in control vials (15%) died within 48 hours, with 13 of the remaining flies still alive after seven days. This pattern was consistent among the three test species (Fig. 2-6), and indicates that the seven-day duration of the cage trials was sufficiently long to assess whether lethal quantities of Amdro were consumed by caged flies.

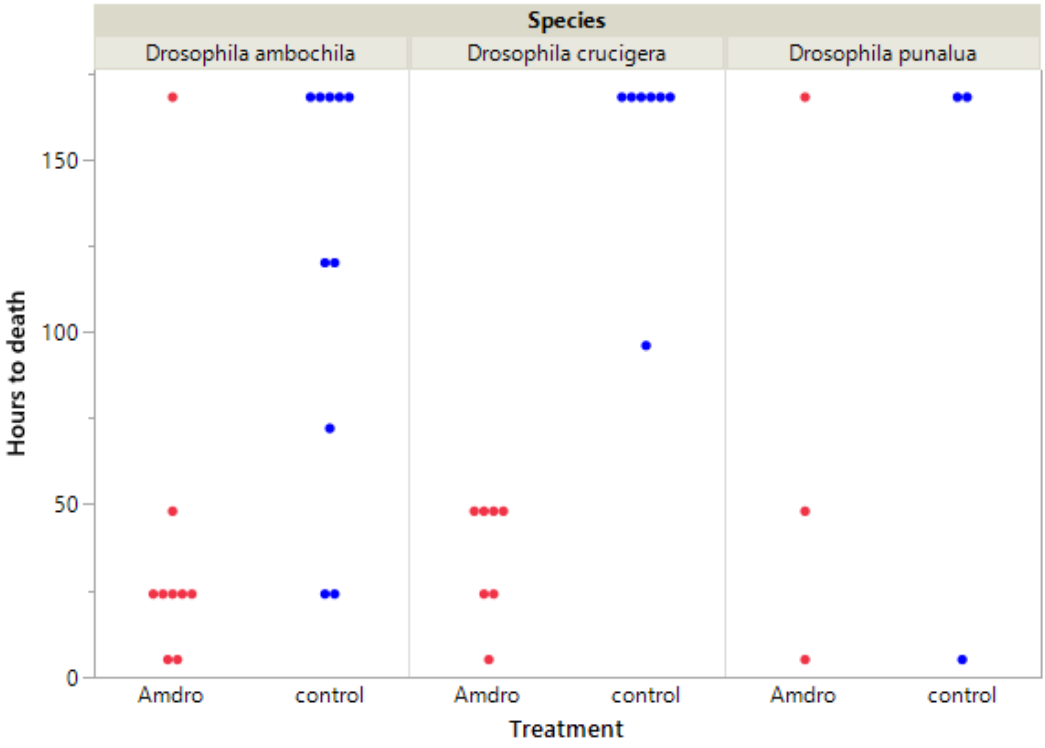

Figure 2-6. Time to death of three picture-winged fly species placed in Amdro or control vials for a five-hour exposure period. Each point represents one replicate fly tested.

Of the 23 picture-winged flies placed in Amdro cages, only three flies were observed to land on or near the Amdro bait pile during the 18 daytime hours that were filmed over the first two days of each trial. Two of these visits lasted approximately one and 10 seconds, respectively, and while one of the two flies may have touched the bait with a leg, the second did not approach the bait itself, and neither fly was observed to feed on the bait during the visits. The third fly, a male *D. punalua*, visited the bait pile on one occasion for approximately 34 seconds, and clearly appeared to sponge the bait with its mouthparts about 12-15 times over the course of five seconds.

There was no significant difference in time to death between flies placed in Amdro versus control cages for each of the three test species or when pooled across species (Table 2-2). Survival curves for Amdro and control groups, pooled across species, are shown in Figure 2-7.

Table 2-2. Mean time to death and results of survival analysis for the cage trials.

Species	Mean days to death ± SE (n)		Log-ranked chi-square	p
	Amdro	Control		
Drosophila ambochila	4.4 ± 0.4 (9)	4.0 ± 0.5 (8)	0.686	0.408
Drosophila crucigera	5.5 ± 0.7 (6)	4.8 ± 0.4 (6)	2.02	0.155
Drosophila punalua	4.5 ± 0.6 (8)	5.6 ± 0.4 (8)	0.761	0.383
combined	4.8 ± 0.3 (23)	4.8 ± 0.3 (22)	0.302	0.583

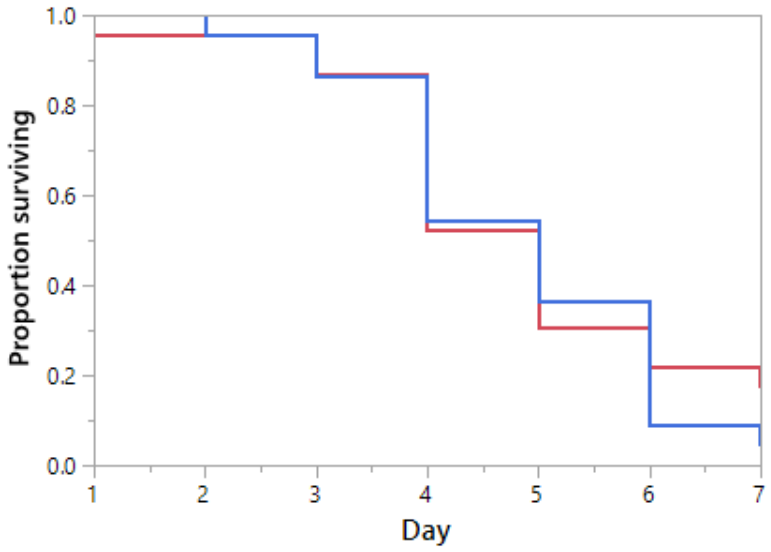

Figure 2-7. Kaplan-Meier survival curves for flies placed in Amdro (red) and control (blue) cages.

DISCUSSION

A single broadcast application of Amdro Ant Block bait at the label rate was very effective for suppressing *S. papuana* abundances in mesic montane forests for a period of at least six months, with somewhat weaker effects persisting for up to one year. Because Amdro granules degrade quickly in wet conditions, and because the active ingredient hydramethylnon also degrades quickly when exposed to sunlight and other environmental conditions (Vander Meer et al. 1982, Mallipudi et al. 1986, NPIC 2002), this long-lasting suppression likely resulted from an inability of *S. papuana* colonies to quickly rebound and/or recolonize rather than from persistence of the bait and pesticide. Broadcast application of Amdro Ant Block was substantially more effective than application with bait stations. Amdro in bait stations needs to be replaced approximately every six weeks to maintain suppression of *S. papuana* (Ogura-Yamada and Krushelnycky 2016), likely because bait coverage is less complete even when bait stations are spaced every 2.5 m, and because bait within stations becomes very moldy after several weeks in the field. The level of actual ant mortality, as opposed to estimated mortality measured with monitoring bait cards, is therefore likely higher with broadcast application compared to bait station application, resulting in longer-lasting suppression.

Although a wide variety of other ant species were detected in the study plots, most occurred at much lower densities than *S. papuana*. For example, *S. papuana* individuals represented 94.8% of all ants sampled through bait cards, pitfalls and leaf litter extraction combined. Other ant species did not increase in abundance in Amdro plots following suppression of *S. papuana*, suggesting that control of *S. papuana* in mesic forests of O‘ahu will not simply result in the release and subsequent dominance of another invasive ant species (e.g. Plentovich et al. 2011). Small thief ants like *S. papuana* may not provide substantial resistance to larger and more actively foraging invasive ant species; instead, behaviorally dominant epigaeic invasive ants often co-exist with hypogaeic species by partitioning behavioral and ecological space (Holway et al. 2002). Hence, the low densities of other dominant invasive ant species at the study sites, both before and after Amdro broadcast, more likely resulted from environmental constraints, as few ant species besides *S. papuana* appear capable of attaining high densities in closed-canopy, mesic to wet montane forests in Hawai‘i (Reimer 1994, Krushelnycky et al. 2005, Ogura-Yamada and Krushelnycky unpublished data).

Broadcast of Amdro Ant Block bait did not appear to have strong negative impacts on non-target invertebrates in mesic montane forests at the scale investigated. No significant declines were detected in Amdro-treated plots for any taxonomic group either immediately after application or six months later. It seems likely that at least some generalist feeders would consume the bait, but modest impacts from doing so may have been masked by rapid immigration into the small plots by vagile species. The plot size tested, however, approximates areas that might typically be treated to suppress *S. papuana* around clusters of picture-winged fly host plants, so this type of rapid equilibration would likely be a normal occurrence following this management action. Non-target impacts may also be reduced by the fact that hydramethylnon has low toxicity to invertebrates through dermal contact, and generally must be ingested to be effective (NPIC 2002). Few non-target impacts on arthropods were similarly observed in a lowland ant control effort using Amdro bait in Hawai‘i (Plentovich et al. 2011).

Finally, cage trials suggest that picture-winged flies themselves are not strongly attracted to Amdro Ant Block bait. During the first two days of the trials, there was only a single brief feeding observation among 23 test flies belonging to three species, even under extreme

conditions in which flies were confined to a small space with the bait and provided no alternative food. An absence of attraction to the Amdro bait was supported by the cage survival analysis, in which there was no difference in time to death between flies placed in cages provisioned with Amdro and flies placed in control cages. Furthermore, bait granules are more dispersed in the environment when broadcast, and presumably emit less odor than the small bait piles that were offered in the cage trials. While it is always possible that some picture-winged flies will encounter granules by chance and feed on them, the available evidence suggests that this will be rare.

Collectively, the results from the present study suggest that a single broadcast of Amdro Ant Block is highly effective for long-term suppression of *S. papuana*, that such a management action should have minimal long-term non-target impacts on ground-dwelling invertebrate communities in mesic montane forests of O‘ahu, and should pose little risk to picture-winged flies.

SECTION III. Efficacy and non-target risks of water-storing granules as a baiting method to control ants attracted to sugar water-based baits

INTRODUCTION

Many ant species are strongly attracted to sweet liquid foods, including many invasive species in the subfamilies Formicinae and Dolichoderinae. However, an absence of practical methods for controlling invasive ants that are primarily attracted to sweet liquid foods has been a persistent problem. These species are often targeted with sugar water-based baits dispensed in bait stations. In natural landscape settings, deployment of numerous bait stations quickly becomes extremely laborious, costly, and in most cases prohibitive. Unfortunately, several of the most destructive established ant species in Hawai‘i belong to this sugar-loving group, including species that invade and impact natural areas. These include the yellow crazy ant (*Anoplolepis gracilipes*) and glaber ant (*Ochetellus glaber*), which are common in coastal areas supporting endangered yellow-faced bees, and the Argentine ant (*Linepithema humile*), which is more common in montane mesic forests and shrublands that may support endangered picture-winged flies and/or yellow-faced bees. In addition, some worrisome new threats, like the tawny crazy ant (*Nylanderia fulva*) currently invading the US mainland, belong to this group.

A recent advance has employed polyacrylamide crystals, or hydrogels, to convert liquid baits into an easily dispersed granular form (Buczkowski et al. 2014a,b; Boser et al. 2017). These hydrogels, used as soil amendments in horticultural and forestry applications, absorb many times their weight in water and then slowly release it as they dry. They also absorb water containing dissolved sugar and pesticides, which ants can imbibe directly from the dispersed granules. This approach is being used experimentally in attempts to eradicate Argentine ants in the California Channel Islands and yellow crazy ants at Johnston Atoll and Australia (Boser et al. 2017; Peck et al. 2017; B. Hoffmann, CSIRO Australia, pers. comm.). Textured vegetable protein (TVP) also has water-absorbing properties, but has the advantage of being biodegradable, and showed promising results in initial testing at Johnston Atoll (Peck et al. 2016, 2017). Another biodegradable water-absorbing medium based on alginate was recently developed at UC Riverside (Tay et al. 2017). These media, which are here referred to collectively as water-storing granules (WSG), represent a highly promising new tool for invasive ant control in Hawai‘i.

However, no commercial pesticides are yet labelled for this use pattern, and a variety of questions need to be addressed to develop this as a usable approach in Hawai‘i. This section reports on a series of studies to investigate some of the questions concerning the use of WSG as a new ant control tool, pertaining both to aspects of their effectiveness and their non-target risks. These included: 1) drying rate of the three WSG types under investigation (polyacrylamide, TVP, and alginate beads), which influences duration of bait attractiveness, 2) bait preference among the three WSG types for three target ant species (yellow crazy ant, Argentine ant, and little fire ant (*Wasmannia auropunctata*)), 3) repellency of three pesticides under investigation (thiamethoxam, dinotefuran, and indoxacarb) when formulated in WSG to the three target ant species, 4) efficacy of the most promising bait and pesticide formulations for controlling Argentine ants and yellow crazy ants in field plots, 5) non-target species attraction to WSG baits, focusing on pollinating insects and ground-foraging birds, and 6) indirect non-target risks via pesticide residues resulting from this application method.

MATERIALS AND METHODS

Target ant species and study sites

Work on this project focused on three highly invasive and problematic ants species: the yellow crazy ant (YCA, *A. gracilipes*), the Argentine ant (AA, *L. humile*), and the little fire ant (LFA, *W. auropunctata*). Studies involving YCA took place at disturbed lowland grassland and shrubland at James Campbell National Wildlife Refuge (JCNWR) and adjacent county property, Oʻahu. Studies involving AA took place in native subalpine shrubland at Haleakalā National Park (HALE), Maui. Studies involving LFA took place at several rural residential properties in the Puna District, Hawaiʻi Island. Non-target attraction studies were conducted at some of the same sites, as well as in native coastal strand communities at Kaiwi State Scenic Shoreline (KSSS), Oʻahu, and Kaʻena Point Natural Area Reserve (KPNAR), Oʻahu.

Preparation of WSG formulations

The three WSG types were used to deliver a 25% sucrose solution as the bait attractant. All unspecified references to sucrose solutions in this report refer to solutions made with table sugar in tap water at a concentration of 25% (w/vol). For repellency and efficacy trials, pesticide active ingredients were mixed into the sucrose solution at the stated concentrations (w/vol) prior to absorption with WSG. WSG were allowed to absorb bait solutions for approximately 24 hours prior to use. The commercial products Safari 20 SG (EPA Reg. No. 86203-11-59639), Optigard Flex Liquid (EPA Reg. No. 100-1306), and Provaunt (EPA Reg. No. 100-1487) were used to supply the active ingredients (AI) dinotefuran, thiamethoxam, and indoxacarb, respectively.

Miracle-Gro® Water Storing Crystals were used for the polyacrylamide WSG, at a rate of 20 g per L of bait. Bob's Red Mill® Textured Vegetable Protein was used for the TVP WSG, at a rate of 350 g per L of bait. Alginate bead WSG were manufactured for the study following the protocol developed by Tay et al. (2017). Alginate beads were mass-produced by allowing a 10 g/L sodium alginate solution (Na-Alg, Sigma-Aldrich, CAS 9005-38-3, in distilled water) to gravity drip from a 100-nozzle shower head into a 5 g/L calcium chloride solution ($CaCl_2$, Sigma-Aldrich, CAS 10043-52-4, in distilled water). Beads were allowed to cross-link in the calcium chloride solution for approximately five minutes, after which they were rinsed with distilled water, producing beads that were >98% water by weight. Finished beads were then conditioned in a bait solution for approximately 24 hours to produce the WSG for the various trials. During the conditioning period, solutes (sugar and AI, if applicable) equilibrated between the water within the beads and the conditioning solution as the beads absorbed more liquid, increasing in weight by approximately 30% in the case of mass-produced beads. The conditioning solution was typically formulated with concentrations of solutes that were twice the target concentrations obtained after equilibration. Equilibration was confirmed by measuring the final sucrose concentration of the excess conditioning solution with a hand-held refractometer (Eclipse model 45-03, Bellingham + Stanley Ltd.). After equilibration, excess conditioning solution was drained prior to use of the alginate WSG.

WSG drying rates

Prior work found that WSG formulated with sucrose become less attractive to ants once approximately 50% of the water in the granules has evaporated (Rust et al. 2015, Tay et al. 2017), which can in turn reduce their efficacy (Buczkowski et al. 2014a). To estimate the rate of water loss of the three WSG types, drying trials were conducted on the roof of Gilmore Hall on the University of Hawai‘i campus in Honolulu (49 m elevation) to approximate lowland natural areas, and at 2070 m elevation at HALE to represent high elevation natural areas. One trial each was conducted in full sun and in full shade on the roof of Gilmore Hall. A single HALE trial was initiated in full sun, but intermittent low clouds occurred during later portions of the trial. For each trial, 10 individual granules of each of the three WSG types formulated with sucrose solution were randomly assigned to an array of 30 petri dishes (6 cm diameter). For polyacrylamide and TVP, an attempt was made to select 10 granules that spanned the majority of the range of granule sizes observed in a sample of granules; alginate beads were much more uniform in size, so 10 granules were selected haphazardly. Dishes were weighed at the start of the trial, and then approximately every hour for 5 hours after the array was placed outdoors. The low elevation, full sun trial was started at 9:41 am on 6/21/18, and the low elevation full shade trial was started at 10:23 am on 6/22/18. The high elevation trial was started at 9:26 am on 8/11/18. Air temperature and relative humidity was measured hourly for the two low elevation trials using a sensor (HOBO UX100-001, Onset Computer Corp.) placed in the shade next to the dish array. Air temperature and relative humidity was logged every three minutes during the high elevation trial, using a sensor mounted within a radiation shield (HOBO U23-002, Onset Computer Corp.) and placed next to the dish array. Final dry weights of granules were calculated by letting them air dry in the lab for at least 1 week after the trial. Percent water loss was subsequently calculated for each granule at each hourly measurement interval.

The time to reach 50% water loss (T50) was estimated for each granule from the slope of the line joining the two successive hourly measurements that spanned this percentage. T50 was then regressed against the initial saturated weight for each granule (natural log transformed) to determine the relationship between granule size and T50 for each WSG type. To estimate typical T50 values for each WSG type under each of the three trial scenarios, the regression relationships were applied to 50 individually weighed granules of each WSG type formulated with sucrose solution. The 50 granules were the first 50 encountered within approximately 15 g batches of formulated WSG, and were therefore haphazardly selected. Median estimated T50 values were compared among WSG types with box plots.

Bait preference among WSG

For each of the three target ant species, a combination of choice trials and no-choice trials were conducted to test the relative attraction to the three WSG formulated with 25% sucrose solution (no AI). In choice trials, 20 replicate stations were established along transects at each site, with stations separated by approximately 5 m or more. At each station, the three WSG were offered side by side on laminated cards (4.5 x 3.5 cm) placed on the ground (Fig. 3-1). Relative positions of the baits to one another at each station were assigned haphazardly. Baits were photographed at 30 min, 60 min, 120 min and 180 min after placement, and numbers of ants at each were subsequently counted in the digital images. In no-choice trials, 60 stations were established along multiple transects at each site, with stations separated by approximately 5 m or

more. Each station received only one of the three WSG types, with each WSG type being randomly assigned to 20 of the 60 stations. Baits were offered on laminated cards (4.5 x 3.5 cm) placed on the ground, were photographed at 30 min, 60 min, 120 min and 180 min after placement. Bait preference tests for YCA were conducted at JCNWR on 5/8/18 and 5/15/18. Bait preference tests for AA were conducted at HALE on 6/28/18 and 6/29/18. Bait preference tests for LFA were conducted at a residence in Nānāwale Estates, Puna, Hawai‘i Island on 6/19/18 and 6/21/18. Numbers of ants were compared among the three WSG types at each time interval using generalized linear models fit with the log link function and negative binomial distribution. Prior to analysis, data were excluded for stations in which one or more of the baits were blown away by wind or removed by rodents or chickens. For two time intervals at the HALE site where nearly all ant counts were zero, data were not analyzed statistically.

Figure 3-1. Example station from choice bait preference trial with AA. Top left card contains polyacrylamide, top right contains TVP, bottom contains alginate beads.

Pesticide repellency trials

Repellency towards different concentrations of the three active ingredients (AI) being tested was assessed with choice trials for each of the three target ant species. For each ant species, trials for the three AI were run sequentially on the same day for a given WSG type. In each trial, three concentrations of the AI (w/vol) formulated in 25% sucrose solution were compared with a control (sucrose solution only), at each of 20 replicate stations. Stations were established along transects at each site, and were separated by approximately 5 m or more. The concentrations of AI tested were 0.25%, 0.05%, 0.005%, and 0% (control) for indoxacarb and

dinotefuran, and were 0.025%, 0.005%, 0.0005%, and 0% (control) for thiamethoxam. At each station, the four baits were offered side by side on laminated cards (4.5 x 3.5 cm) placed on the ground (Fig. 3-2). Relative positions of the baits to one another at each station were assigned randomly. Baits were photographed at 30 and 60 min after placement, and numbers of ants at each were subsequently counted in the digital images. Repellency trials were conducted for YCA on county land adjacent to JCNWR on 7/24/18, 7/26/18 and 7/27/18. Trials were conducted for AA at HALE on 7/31/18, 8/1/18, and 8/2/18. Trials were conducted for LFA at several sites in Puna, Hawai‘i Island, on 8/16/18 and 10/1/18. Because attractiveness of the WSG typically decreases with time as the granules dry (see Bait preference results) irrespective of any repellency to AIs, the repellency analysis used ants counts from only the higher of the two station counts (30 and 60 minutes post placement): the time interval with the higher total across all four cards at each station was used, to account for possible differences in discovery time and recruitment rate to different stations. Numbers of ants were compared among the three concentrations of AI and control for each AI and WSG type using generalized linear models fit with the log link function and negative binomial distribution. Prior to analysis, data were excluded for stations in which one or more of the baits were blown away by wind or removed by rodents or chickens.

Figure 3-2. Example station from trial testing repellency of indoxacarb formulated in alginate beads to AA. Card 9 is the control (sucrose solution only), card 10 is 0.25% indoxacarb, card 11 is 0.005% indoxacarb, and card 12 is 0.05% indoxacarb.

Efficacy testing

Nine WSG formulations were selected for the initial round of efficacy screening for AA, based on the results of the bait preference and pesticide repellency trials. These were 0.0005% thiamethoxam (w/vol) formulated in each of the three WSG types, 0.005% indoxacarb (w/vol) formulated in each of the three WSG types, and 0.05% indoxacarb (w/vol) formulated in each of the three WSG types. Each of the nine formulations was tested in one 25 x 25 m plot at HALE. In each plot, WSG were broadcast by hand at a rate of 55 L of absorbed sucrose bait (with AI) per ha, which is similar to rates found to be effective in prior studies using the WSG approach (Rust et al. 2015; Peck et al. 2016, 2017; Boser et al. 2017). Each plot was treated twice, first on 10/31/18 and again on 11/13/18.

Based on results of the initial round of testing for AA, a second round of testing was conducted to confirm the efficacy of the two best pesticide formulations, and to test the efficacy of a lower bait application rate. The two formulations tested were 0.0005% thiamethoxam and 0.05% indoxacarb, each broadcast at a rate of 55 L/ha and 25 L/ha, using only the polyacrylamide WSG. The four resulting treatments were each replicated three times in 25 x 25 m plots at HALE, with each plot being treated a single time on 10/23/19.

Twelve WSG formulations were selected for the initial round of efficacy screening for YCA, based on the results of the bait preference and pesticide repellency trials. These were 0.005% dinotefuran (w/vol) formulated in each of the three WSG types, 0.05% dinotefuran (w/vol) formulated in each of the three WSG types, 0.005% indoxacarb (w/vol) formulated in each of the three WSG types, and 0.05% indoxacarb (w/vol) formulated in each of the three WSG types. Each of the 12 formulations was tested in one 25 x 25 m plot at JCNWR. In each plot, WSG were broadcast by hand at a rate of 55 L of absorbed sucrose bait (with AI) per ha. The nine plots testing formulations using 0.005% dinotefuran, 0.05% dinotefuran, and 0.005% indoxacarb were treated once on 6/14/19. The three plots testing 0.05% indoxacarb were also treated on 6/14/19, and were treated a second time on 6/29/19 to test whether a second application would yield good levels of control.

Based on results of the initial round of testing for YCA, a second round of testing was conducted to assess the efficacy of lower concentrations of the best AI and the efficacy of a lower bait application rate. The two pesticide formulations tested were 0.005% dinotefuran and 0.0005% dinotefuran, each broadcast at a rate of 55 L/ha and 25 L/ha, using only the polyacrylamide WSG. The four resulting treatments were each replicated three times in 25 x 25 m plots at JCNWR, with each plot being treated a single time on 8/21/20.

Results of each efficacy trial were assessed by comparing numbers of ants attracted to baited monitoring cards in each plot and in an untreated control plot at each site. Monitoring was conducted two days before the application, then on two, four, and six days after each application. On each monitoring date, 12 monitoring cards were baited (with a blend of tuna and corn syrup for AA and a blend of spam and corn syrup for YCA) and placed on the ground within 5 m of the center of each plot, and numbers of ants were counted after 60 to 75 minutes for AA and after 30 to 40 minutes for YCA. Percent reduction in ant numbers relative to pretreatment numbers was calculated for each plot on each monitoring date, pooling the 12 monitoring cards on each date. Mean percent reduction was calculated after each bait application by averaging the percent reductions on each of the three monitoring days (two, four and six days) post treatment for each plot. Trends in percent reduction across plots at each site following each application were analyzed with a two-factor ANOVA, in which the mean percent reduction for each plot (arcsine

square root transformed) was the response, and granule type and AI formulation were the factors in the model. In the first round of efficacy testing for YCA, mean reductions following the second application in the three 0.05% indoxacarb plots were compared to means following the first application for the remaining plots, which were not treated a second time. Significant differences among levels of factors in the model were assessed with Tukey HSD pairwise comparisons (at $\alpha = 0.05$).

Non-target species attraction: video observations of pollinators

Attraction of pollinators and other insects to WSG was assessed by filming small clumps of WSG formulated with sucrose (no AI) that were placed either on the ground or near flowers at several sites. Observations were conducted only on sunny days between approximately 10 am and 3 pm, at sites known to support abundant populations of *Hylaeus* bees (Hymenoptera: Colletidae), other bees, and/or other pollinating insects. For ground observations, approximately one spoonful of WSG was placed on the ground, near the base of vegetation around which pollinating insects were observed to be active. For flower observations, as much WSG as was practical, up to approximately one spoonful, was perched on or near individual flowers or flowering panicles, depending on the plant species. During each observation event, nine video cameras (Sony HDR-CX405) were used to film three replicates of each of the three WSG types, usually for a duration of four to five hours. Replicates were separated by approximately 1 m or more (Fig. 3-3). Videos were subsequently viewed and all non-ant visitors that made contact with the WSG were noted. For flower observations, visits to the adjacent flowers (>2 sec duration) were also noted. Length of each visit, time of day of each visit, and identity of each visitor to the lowest taxonomic level recognizable was tabulated for each replicate observation. Total number of visiting taxa, and total number of individual visits was also calculated for each replicate observation, with the latter defined as the total number of visits that were separated from previous visits by at least one minute.

Ground observations were conducted at JCNWR on 5/15/18 and 6/14/18 (n = 6 replicates per WSG type), at KPNAR on 5/10/18, 5/11/18, and 5/17/18 (n = 9 replicates per WSG type), at KSSS on 5/24/18 and 6/8/18 (n = 6 replicates per WSG type), and at HALE on 5/30/18, 5/31/18, and 6/1/18 (n = 9 replicates per WSG type). Only videos with at least 210 minutes of usable footage were included in analyses, resulting in 29 replicate ground observations for polyacrylamide and TVP granules, and 28 replicate observations for alginate beads. These comprised 120 to 125+ hours of video footage for each WSG type.

Flower observations were conducted at JCNWR on 8/31/18 and 9/19/18 (n = 6 replicates per WSG type), at KSSS on 9/21/18, 10/25/18, and 1/9/19 (n = 9 replicates per WSG type), and at HALE on 6/26/18, 6/27/18, and 6/29/18 (n = 9 replicates per WSG type). Only videos with at least 210 minutes of usable footage were included in analyses, resulting in 22, 21, and 23 replicate flower observations for alginate, polyacrylamide, and TVP granules, respectively (comprising 91 to 100+ hours of video footage for each). Plant species used for flower observations were *Scaevola taccada* and *Heliotropium foertherianum* at JCNWR and KSSS, and *Geranium cuneatum* and *Santalum haleakalae* at HALE (Fig. 3-4). For each observation event, two replicates of each WSG type were assigned to one of the focal plant species at the site, and the third replicate of each WSG type was assigned to the second focal plant species at the site. The total number of replicate flower observations that were analyzed for each plant species were as follows: *S. taccada* (19), *H. foertherianum* (21), *G. cuneatum* (17), and *S. haleakalae* (9).

Fig. 3-3. Examples of non-target species attraction video observation. Top: three of nine cameras at Kaʻena Point Natural Area Reserve filming small clumps of WSG placed on the ground near the base of vegetation that was actively visited by insect pollinators. Bottom: four of nine cameras at James Campbell National Wildlife Refuge filming small clumps of WSG placed on or near flowers of *Heliotropium foertherianum* and *Scaevola taccada*.

Numbers of visitors per observation event, number of taxa per observation event, and duration of visits were compared among WSG types for both ground and flower observations with generalized linear models, using the log link function and negative binomial distribution. For flower observations, number of visits and number of visiting taxa to both flowers and adjacent granules were also compared among plant species (pooling granule types), with generalized linear models using the log link function and negative binomial distribution.

Figure 3-4. Examples of polyacrylamide WSG granules placed next to flowers of *S. taccada* (top left*), H. foertherianum* (top right), *G. cuneatum* (bottom left), and *S. haleakalae* (bottom right), to test for pollinator attraction. Location of granules indicated with red arrows.

Non-target species attraction: video observations of birds

An attempt was made to assess bird attraction to WSG using two video filming methods. In the first method, three replicate clumps of each WSG type (formulated with sucrose and no AI) were placed on the ground at JCNWR in an area where shorebirds were active, and cameras were set up to film each clump at a distance of approximately 10 to 20 m. This method was attempted on 9/25/18 and 10/23/18. In both events, the act of setting up the baits and cameras caused the birds to leave the area, and they did not return for the duration of filming (approximately four hours). In the second method, three 20 x 20 m plots were established at JCNWR near the northeast corner of the refuge, in coastal flats inland of the coastal dune system, an area where shorebirds and other birds were commonly active. On each of three dates, WSG were broadcast by hand in the plots at an application rate of 55 L/ha of absorbed sucrose bait (with no AI). One WSG type was allocated to each plot. WSG were broadcast at 11:30 am on 2/13/19, at 7:45 am on 2/28/19, and at 1:45 pm on 3/4/19. After broadcast, cameras positioned unobtrusively 30-50 m away near the coastal dune vegetation recorded bird activity in the plots.

On the first two dates, two cameras were used for each plot, one set up with a wide angle to capture the entire plot, and the second focused on an area several meters wide that was provisioned with a high density of WSG. On the third date, three cameras were used per plot, with all three focused on different areas within the plot. At the same time, two observers monitored the plots with binoculars in an effort to observe bird feeding behavior. Across the three observation dates, a total of over 9 hours of observation was performed for each plot.

Non-target species bait consumption: protein marking and detection

A bait marking and detection approach was used to test consumption of sucrose bait in broadcast WSG. A common approach uses mammalian IgG as a marking protein, whose presence in the gut of a target insect can be screened after exposure using ELISA (Hagler 1997, DeGrandi-Hoffman and Hagler 2000, Buczkowski and Bennett 2006). However, the large quantity of bait required when broadcasting WSG in test plots necessitated a cheaper marking method. Consultation with James Hagler, an expert in the field, suggested that rabbit serum would be an effective way of delivering IgG much more cheaply than using standard, purified IgG. To confirm this, concentrations of rabbit serum (Sigma Aldrich R4505) ranging from 0.5% to 20% in sucrose solution were fed to individual honey bees (*Apis mellifera*, Hymenoptera: Apidae) and white-footed ants (*Technomyrmex difficilis*, Hymenoptera: Formicidae) in the lab in preliminary trials, and these insects were screened with the ELISA procedure (see below). This determined that insects feeding on sucrose solutions containing rabbit serum at or above 2% concentration were consistently and strongly marked. All subsequent tests used 2% rabbit serum in sucrose solution as bait.

To confirm that pollinating insects would be marked when feeding on sucrose bait absorbed in WSG, honey bees and non-native solitary bees were fed in the lab using polyacrylamide WSG formulated with 2% rabbit serum in sucrose solution (no AI). Wild *A. mellifera* were captured on the UH campus, were individually restrained in harnesses, and fed from a polyacrylamide granule by eliciting the proboscis extension reflex. Only bees that fed for at least 30 seconds were retained for analysis (n = 30). Wild solitary bees (belonging to *Ceratina smaragdula* (Hymenoptera: Apidae), *Hylaeus strenuus* (Hymenoptera: Colletidae) and *Lasioglossum microlepoides* (Hymenoptera: Halictidae)) were captured at various coastal locations and were placed in individual cages in the lab for 48 hrs. Each cage was provisioned with a single artificial flower that contained a central receptacle holding a polyacrylamide granule, and bees were allowed to feed naturally and self-mark in the process. Only bees that survived the 48 hr period were retained for analysis (n = 30).

To test consumption of WSG bait by pollinating and other insects under natural conditions, WSG formulated with 2% rabbit serum in sucrose solution (and no AI) were broadcast in a total of 18 10 x 10 m plots, 6 plots for each of the three WSG types. Three plots each were treated on 5/11/18 and 5/17/18 at KPNAR, both sunny days with ample flying insect activity. The six plots were located in dune habitat dominated by *Scaevola taccada*, *Sesbania tomentosa* and *Euphorbia degeneri*. Three plots each were treated on 5/30/18, 5/31/18, 6/26/18, and 6/27/18 at HALE, also on sunny days with ample flying insect activity. The 12 plots were located in native shrubland habitat dominated by *Leptecophylla tameiameiae*, *Sophora chrysophylla, Santalum haleakalae, Dubautia menziesii, Geranium cuneatum, Dodonea viscosa and Coprosma montana*. On each treatment date, granules were broadcast in the three plots by hand between 9:30 and 10:30 am at a rate of 55 L of absorbed liquid bait per ha, with one plot

allocated to each WSG type. Beginning 60 to 75 minutes after WSG broadcast, flying insects were sampled during four 10 minute periods in each plot, rotating between plots for each successive sampling period. During each 10 minute period, as many flying insects as possible were collected with a sweep net, focusing on bees and other common flower-visiting insects. A total of 441 insects were collected across the 18 plots. To confirm that the protein marker remained active within WSG under field conditions, 25 to 28 foraging ants (*Anoplolepis gracilipes, Ochetellus glaber* and *Paratrechina longicornis*) were also collected within each of the three plots at KPNAR on 5/17/18, focusing on ants observed near broadcast granules.

Because the sweep net sometimes came into contact with WSG lodged in vegetation during sampling, it was possible that bait absorbed by the net could externally mark captured insects that did not feed on WSG, resulting in false positive detections. To test for this type of net contamination, flying insects were captured with the same sweep net at a location approximately 1 km from the test plots after normal sampling was completed on 5/31/18 (n = 36 insects) and 6/27/18 (n = 17 insects) at HALE.

All insects were stored at -20° C until they were screened for the presence of the protein marker with double-antibody sandwich ELISA, using the following procedure. All wells of 96-well microplates were coated with 100 µL of goat anti-rabbit IgG (Sigma Aldrich AP132) diluted 1:500 in distilled water and incubated overnight at 4° C. Primary antibodies were then discarded and 310 µL of 1% non-fat dry milk in distilled water was added to each well to block remaining non-specific binding sites. After incubation for 30 minutes at 26° C, the milk was discarded and plates were rinsed five times with phosphate buffered saline (PBS) Tween 20 (0.05%). Insect samples were individually homogenized in PBS; 200 µL of PBS was used for small insects (e.g. ants), 0.5 ml was used for medium insects (e.g. solitary bees), and 1.0 ml was used for large insects (e.g. honey bees). Each well then received 100 µL aliquots of a sample: 84 wells on each plate received test samples (insects exposed to bait treatments), eight wells received negative controls (insects never exposed to bait treatments), and four wells received positive controls (100 µL of the 2% rabbit serum in sucrose solution bait). Plates were incubated for 2 hrs at 26° C, after which samples were discarded and plates were rinsed five times with PBS Tween 20. Next, 100 µL of goat anti-rabbit IgG alkaline phosphatase conjugate (Sigma Aldrich A3687) diluted 1:5000 with 1% non-fat dry milk in distilled water was added to each well and incubated for 2 hrs at 26° C, after which antibodies were discarded and plates were again rinsed five times with PBS Tween 20. Finally, 100 µL of phosphatase substrate (Sigma Aldrich, CAS 333338-18-4) was added to each well, and after 30 minutes plates were read on a Biotek Epoch Microplate Spectrophotometer set at 405 nm.

Samples were scored as positive for the presence of the protein marker if their optical density reading exceeded the mean negative control reading by three standard deviations (SD) (Hagler 1997, Hagler et al. 2014). Because some of the net contamination samples scored positive according to this threshold despite not being exposed to bait (see Results), a second threshold was used for the samples collected in the field test plots: the mean + 3 SD of the net contamination sample readings. A chi-square test of association was used to compare incidences of marked to unmarked individuals among the three WSG types and among the main orders of insects sampled.

Indirect exposure risk for non-target species: pesticide residues in efficacy field plots

Various samples were collected in the efficacy field trials to assess the levels of pesticide residues that can be expected when broadcasting WSG baits. In the first round of efficacy tests for YCA at JCNWR, plant tissues, floral nectar, and soil samples were collected in the plots. In addition, water samples were collected from three fresh water locations along an artificial waterway connected to man-made ponds and wetlands near the study site (310 to 320 m from the nearest efficacy plot), and from three sea water locations along the shoreline in front of the study site (62 to 98 m from the nearest efficacy plots). Plant tissue samples included leaves and flowers of plant species visited by bees and other pollinators, with plants sampled throughout each plot in rough proportion to their cover and composited for a single sample of approximately 15 g (fresh weight) per plot per date. Nectar was sampled by inserting microcapillary tubes (Drummond Scientific Co.) into flowers to draw up the liquid, sampling as many flowers as possible of a given plant species per plot per date to maximize quantities of nectar for analysis. Due to the small quantities obtained, nectar was subsequently composited from all species sampled and from all three plots testing the same AI concentration (grouping across granule types) on each date to increase total nectar volume. Soil was sampled by collecting five subsamples (approximately 10 cm in diameter and 10 cm deep) with a trowel from the center and each quadrant of each plot, mixing the subsamples together and extracting approximately 40-45 cubic centimeters of the mixed soil. Water samples consisted of approximately 120 ml of water collected from each sampling point on each date. Plant tissue and nectar samples were collected two to eight days prior to bait application, then five, 10, and 30 to 31 days after application. Soil samples were collected eight to nine days prior to application, then 10, 30 to 31, and 90 days after application. Water samples were collected nine days prior to application, then 10 and 31 days after application. Due to cost, plant tissue and soil samples were analyzed only from plots treated using the polyacrylamide granules.

In the second round of efficacy tests for YCA at JCNWR, only plant tissue samples were collected. Plant tissues were sampled, as described above, in one replicate plot of each treatment immediately prior to application, then five, 10, 31 and 90 days after application.

No residue samples were collected in the first round of efficacy tests for AA at HALE. In the second round of tests, plant tissue and soil samples were collected, as described above, in one replicate plot of each treatment. Plant tissue samples were collected eight days prior to application, then five, 10, and 30 days after application. Soil samples were collected eight days prior to application, then 10, 30, and 90 days after application.

After collection, all samples were stored in the dark at -20° C. Plant samples were subsequently homogenized in a blender and freeze-dried, and soil samples were freeze-dried, prior to shipping to Dr. Daniel Snow of the Water Sciences Laboratory at the University of Nebraska for analysis.

Extraction methods for plant tissues and nectar composite followed procedures outlined in Botías et al. (2015), while soil and water used processing from Snow et al (2012). Briefly, 1 g of plant tissue was spiked with a surrogate mix and mixed with 8 milliliters (mL) of acetonitrile and 0.75 mL hexane in a Teflon^{TM} microwave extraction tube. The sample was extracted using microwave-assisted solvent extraction (MASE) at 90 °C for 10 minutes. After cooling, liquid extract was transferred to a centrifuge tube containing SupelQuE PSA/C18/ENVI-Carb (Sigma Aldrich, St. Louis, MO) solid phase dispersion media, and solids were mixed with 2 milliliters acetonitrile to rinse remaining extract into the centrifuge tube. The liquid was again separated by

centrifugation, combined with first portion and then filtered in a RapidVap evaporation tube (Labconco, St. Joseph, MO) with a glass microfiber filter. Nectar composites (0.2 grams) were spiked with a surrogate mix to measure recovery and mixed with water, acetonitrile and hexane at room temperature on a shaker. Target compounds were salted out and interferences removed by shaking with solid phase dispersion media. The liquid extract was separated by centrifugation, evaporated to near dryness, spiked with labelled internal standards, and mixed with 200 µL of purified reagent water to a solvent ratio 20:80 methanol:water prior to transfer to an autosampler vial containing a deactivated glass insert.

Soil samples (2 grams) were weighed into Teflon™ microwave tubes, spiked with a surrogate mix to measure recovery and mixed with acetonitrile. The sample was then extracted using microwave-assisted solvent extraction (MASE) at 90 °C for 10 minutes. The liquid extract was separated by centrifugation, filtered with a glass microfiber filter, and evaporated to near dryness. Once spiked with labelled internal standards, the extracts were mixed with 200 µL of purified reagent water to a solvent ratio of 20:80 methanol and water. Water samples were pre-weighed into 50 gram portions and spiked with a surrogate mix to measure recovery. The sample was then extracted using aspiration onto polymeric HLB 6 cc, 200 mg solid-phase extraction cartridges (Waters Corporation, Manchester, UK). The sample containers were rinsed with 8 mL of methanol to dissolve any analytes that may be sorbed to the glass, and this methanol was used to elute cartridges into 10 mL glass culture tubes. The eluent is then evaporated to near dryness under nitrogen, spiked with labelled internal standards, and mixed with 200 µL of purified reagent water to a solvent ratio of 20:80 methanol and water. Compounds were separated and analyzed on an Aquity™ UPLC interfaced with a Xevo TQS triple quadrupole mass spectrometer using a UniSpray™ source (Waters Corporation, Manchester, UK). Multiple reaction monitoring was used for each compound and five deuterium -labeled internal standards were used for quantitation. Method detection limits were determined from 8-10 replicates of a low-level fortified blank in each matrix (USEPA 1986).

RESULTS

WSG drying rates

Granules of all three WSG types dried fairly quickly, reaching 50% water loss, on average, in under 2 hours in all three scenarios (Fig. 3-5). The mean drying rate among the 10 selected granules of each WSG type was substantially lower for polyacrylamide compared to alginate beads and TVP, and drying rates were lower in the shade at low elevation and at high elevation (Fig. 3-5). Air temperature and relative humidity averaged 31.5° C and 37.7% during the low elevation full sun trial, 30.0° C and 45.2% during the low elevation full shade trial, and 22.1° C and 72.6% during the high elevation full sun trial. However, the air temperature/relative humidity sensor was shaded in all three trials, so did not accurately capture the temperature differences between the low elevation full sun and full shade trials.

For polyacrylamide and TVP granules, there were strong and highly statistically significant positive linear relationships between granule mass (ln-transformed) and T50 in all three scenarios (Fig. 3-6) (polyacrylamide low elevation, full sun: $r^2 = 0.947$, $p < 0.001$; polyacrylamide low elevation, full shade: $r^2 = 0.986$, $p < 0.001$; polyacrylamide high elevation, full sun: $r^2 = 0.961$, $p < 0.001$; TVP low elevation, full sun: $r^2 = 0.844$, $p < 0.001$; TVP low

elevation, full shade: $r^2 = 0.813$, $p < 0.001$; TVP high elevation, full sun: $r^2 = 0.818$, $p < 0.001$). There were also positive linear relationships between granule mass and T50 for alginate beads, but these were generally weaker and less consistent, likely because of the much smaller size range of the highly uniform beads (Fig. 3-6) (alginate low elevation, full sun: $r^2 = 0.184$, $p = 0.215$; alginate low elevation, full shade: $r^2 = 0.864$, $p < 0.001$; alginate high elevation, full sun: $r^2 = 0.496$, $p = 0.023$). In all three scenarios, polyacrylamide had higher T50 values than TVP for all but the smallest granule sizes, with the difference increasing as granules increase in size (Fig. 3-6). This indicates that water evaporates from polyacrylamide granules more slowly than from TVP granules, even after adjusting for granule size. The equivalent comparisons with alginate beads are more difficult because of their smaller size and small size range, but where alginate beads overlap in size with polyacrylamide granules, the data suggest that they retain water similarly (e.g. Fig. 3-6, middle panel).

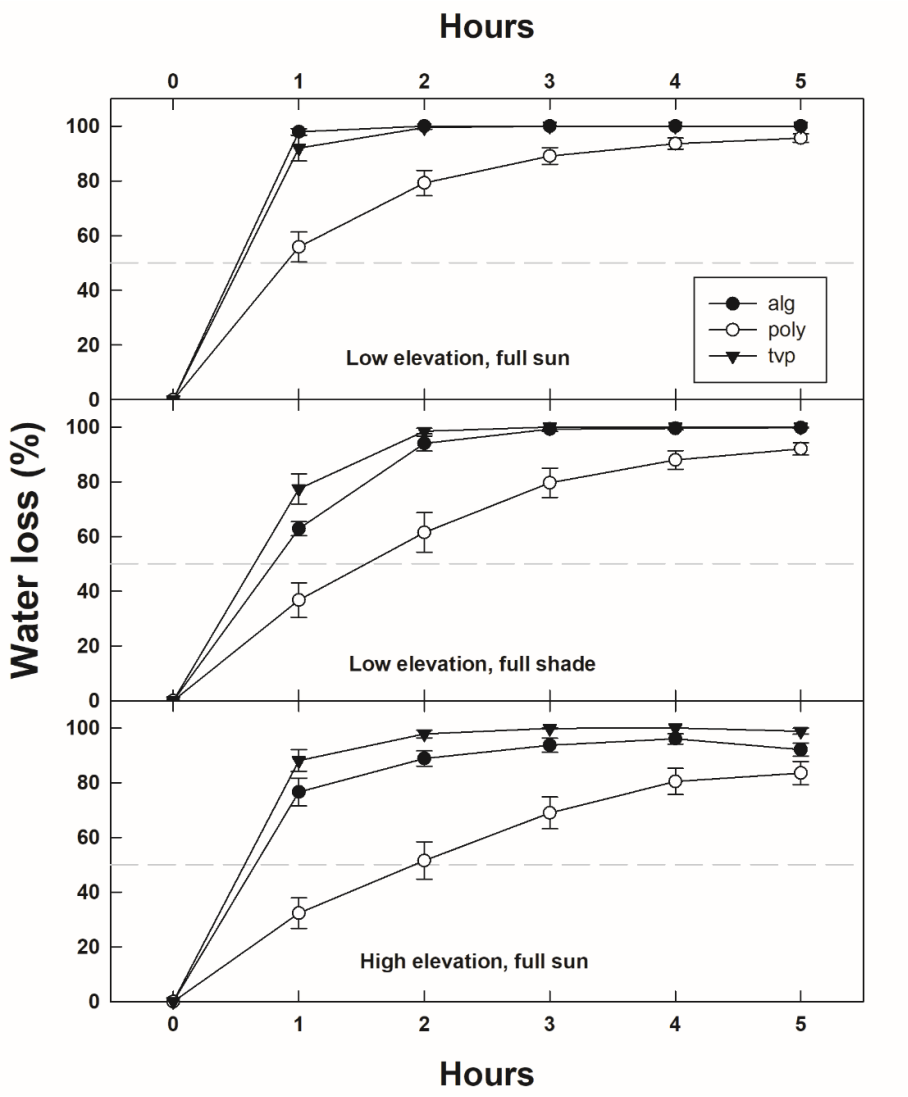

Figure 3-5. Mean percent water loss (± SE) over time for individual granules of each WSG type for three scenarios: low elevation in full sun (top panel), low elevation in full shade (middle panel), and high elevation in full sun (bottom panel). 50% water loss indicated with grey dashed line.

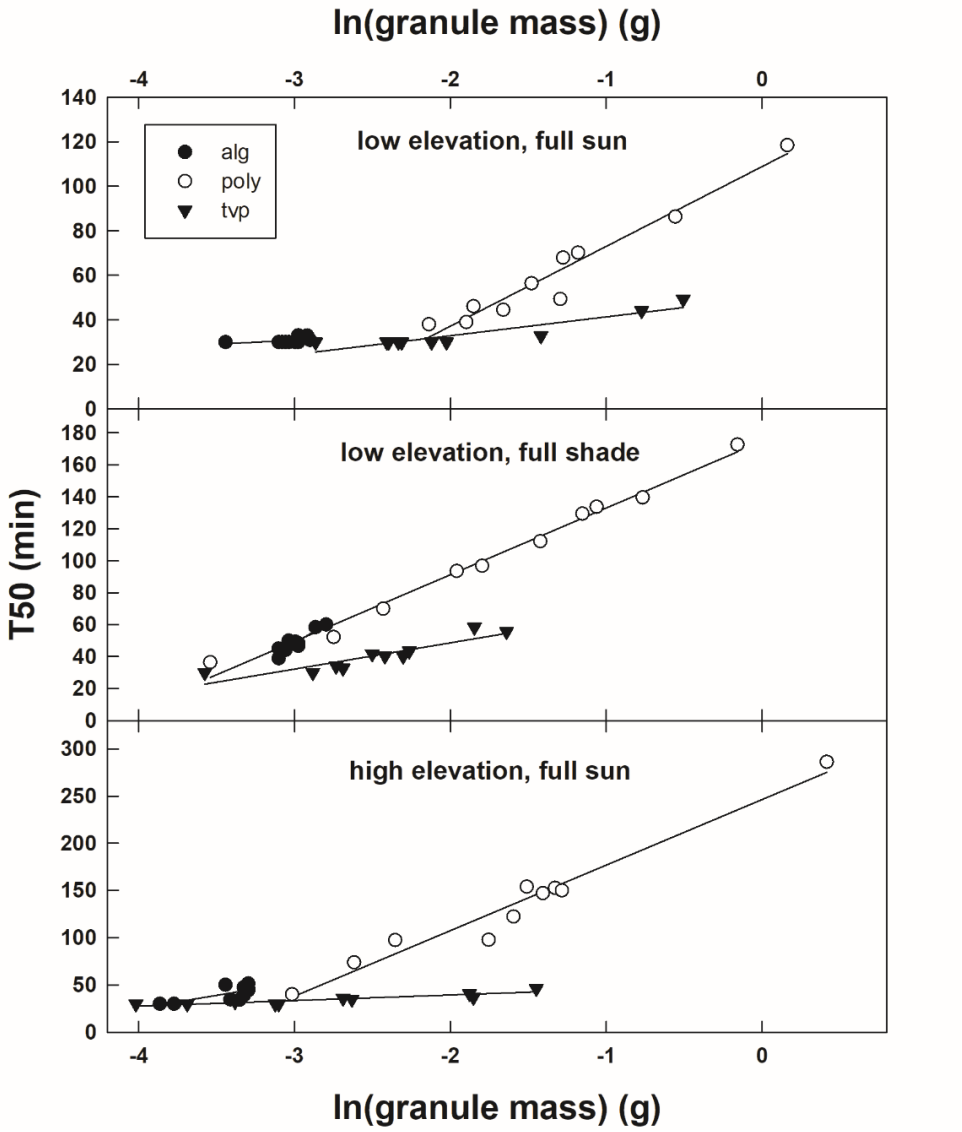

Figure 3-6. Relationships between granule mass (ln transformed) and the time to reach 50% water loss (T50) for the three WSG types under three scenarios. All linear relationships are statistically significant ($\alpha = 0.05$) except for alginate beads at low elevation in full sun (see text).

Estimated typical T50 values differed substantially among the WSG types and environmental scenarios (Fig. 3-7). These estimates, based on the regression relationships in Figure 3-6, were calculated for 50 haphazardly selected granules, and should approximate performance in a typical batch of formulated WSG of each type. The median time for granules to lose 50% of their water, and therefore to decline in attractiveness to ants, was substantially longer for polyacrylamide than for alginate or TVP (Fig. 3-7). The range of values was also much larger for polyacrylamide, indicating that many granules should stay attractive for considerably longer than the median time. In comparison, the ranges of T50 values were much smaller for alginate and TVP (Fig. 3-7).

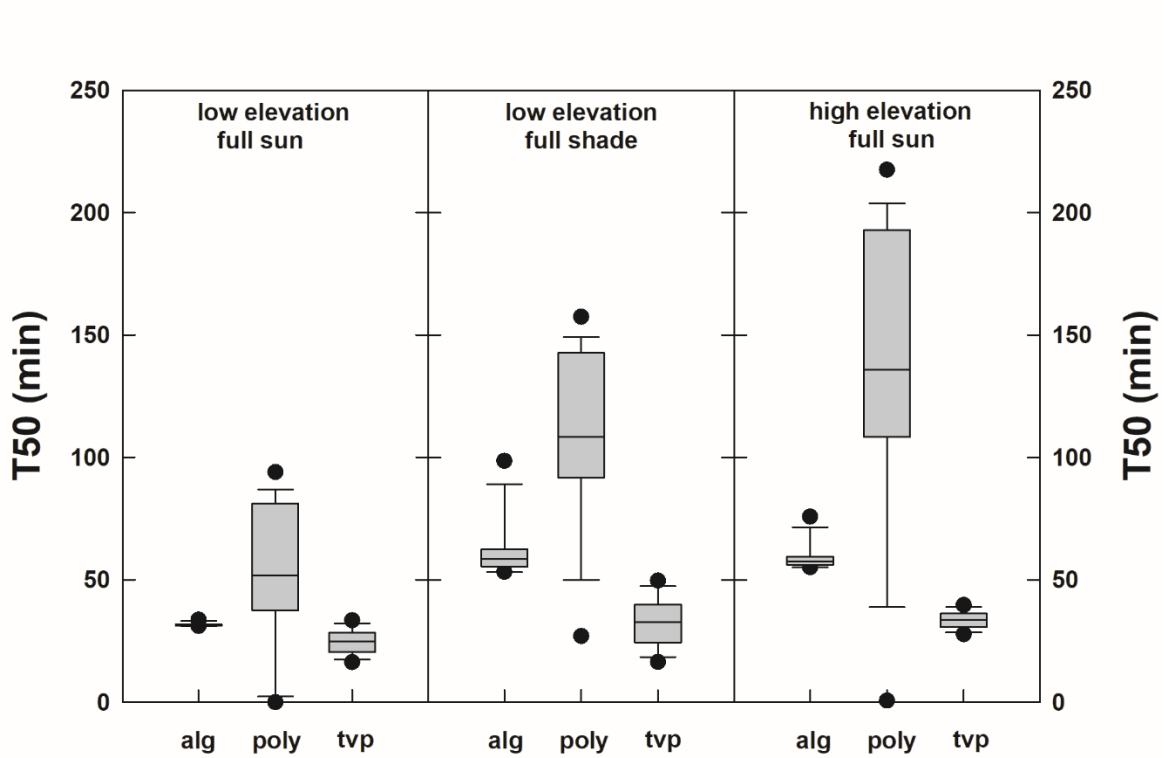

Figure 3-7. Box plots of estimated T50 values for each of 50 typical granules of each WSG type, under three environmental scenarios. Boxes span 25th to 75th percentiles with median indicated with horizontal line; whiskers indicate 10th and 90th percentiles, and outliers up to 5th and 95th percentiles are shown with dots.

Bait preference among WSG

At 30 minutes after bait placement YCA recruited significantly fewer workers to alginate formulated with sucrose compared to both polyacrylamide and TVP in the choice trial, but attraction was not significantly different among the WSG types in the no-choice trial (Fig. 3-8). Attraction of ants to the baits generally declined after 30 minutes post-placement, likely owing to drying of the baits. This decline was especially pronounced for TVP, which attracted significantly fewer YCA after 60 minutes post-placement than the other two WSG in both trials, with this pattern persisting through 180 minutes post bait placement (Fig. 3-8). TVP granules likely dried more quickly than the other WSG, probably both from evaporation and from consumption of sucrose by ants, given that YCA recruited the highest mean number of ants to TVP at 30 minutes. By 180 minutes post placement, attraction was attraction was low for all three WSG.

Attraction of AA at 30 minutes after placement was not significantly different among the three WSG types in both the choice trial and the no-choice trial (Fig. 3-9). As in the YCA trial, numbers of ants attracted to all three WSG subsequently decreased, with this decline again especially strong for TVP, at least in the choice trial. Numbers of ants were low at all three baits by 120 minutes post placement in both trials, and there were almost no ants attracted to the baits by 180 minutes post placement (Fig. 3-9).

In contrast to YCA and AA, LFA recruitment to the WSG baits increased over time (Fig. 3-10). This difference may have resulted from slower recruitment and less rapid consumption of sucrose due to the smaller size of LFA workers, combined with more humid conditions at the site of the trials in Puna, Hawai‘i. Although TVP and alginate beads generally attracted more LFA than polyacrylamide in both choice and no-choice trials, these differences were usually not statistically significant.

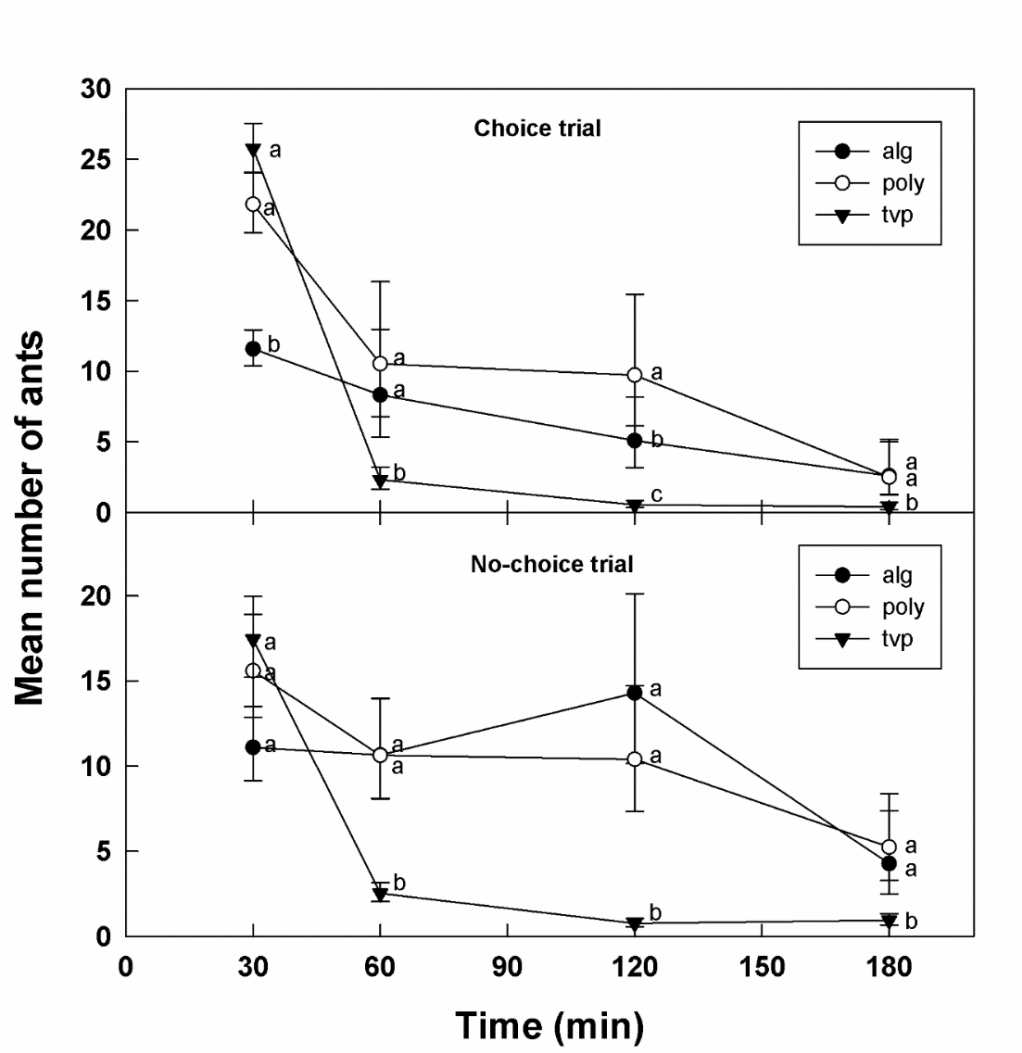

Figure 3-8. Bait preference trials with YCA. Back-transformed mean number of ants (±SE) attracted to the three WSG types over time shown for the choice trial (top panel) and no-choice trial (bottom panel). Means sharing the same letter within each time interval are not significantly different (at $\alpha = 0.05$).

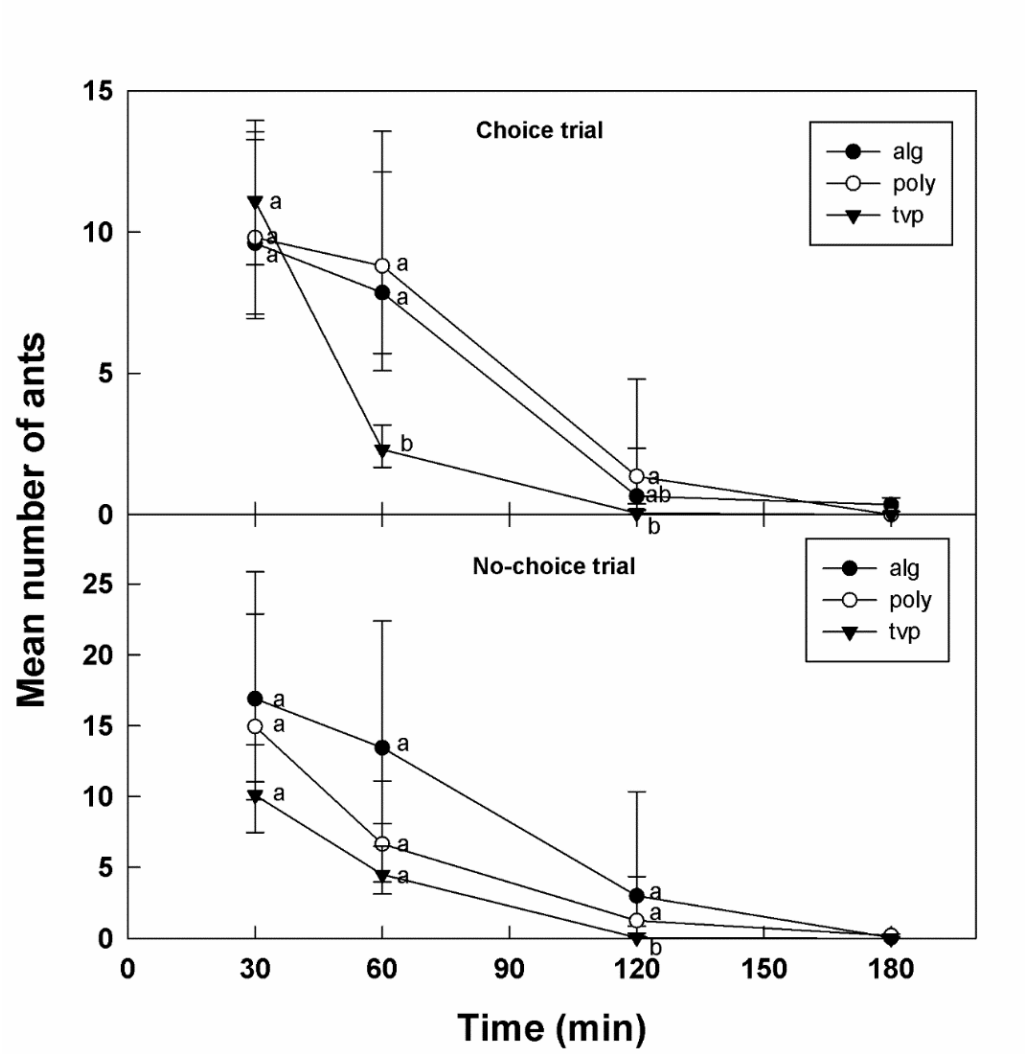

Figure 3-9. Bait preference trials with AA. Back-transformed mean number of ants (±SE) attracted to the three WSG types over time shown for the choice trial (top panel) and no-choice trial (bottom panel). Means sharing the same letter within each time interval are not significantly different (at $\alpha = 0.05$).

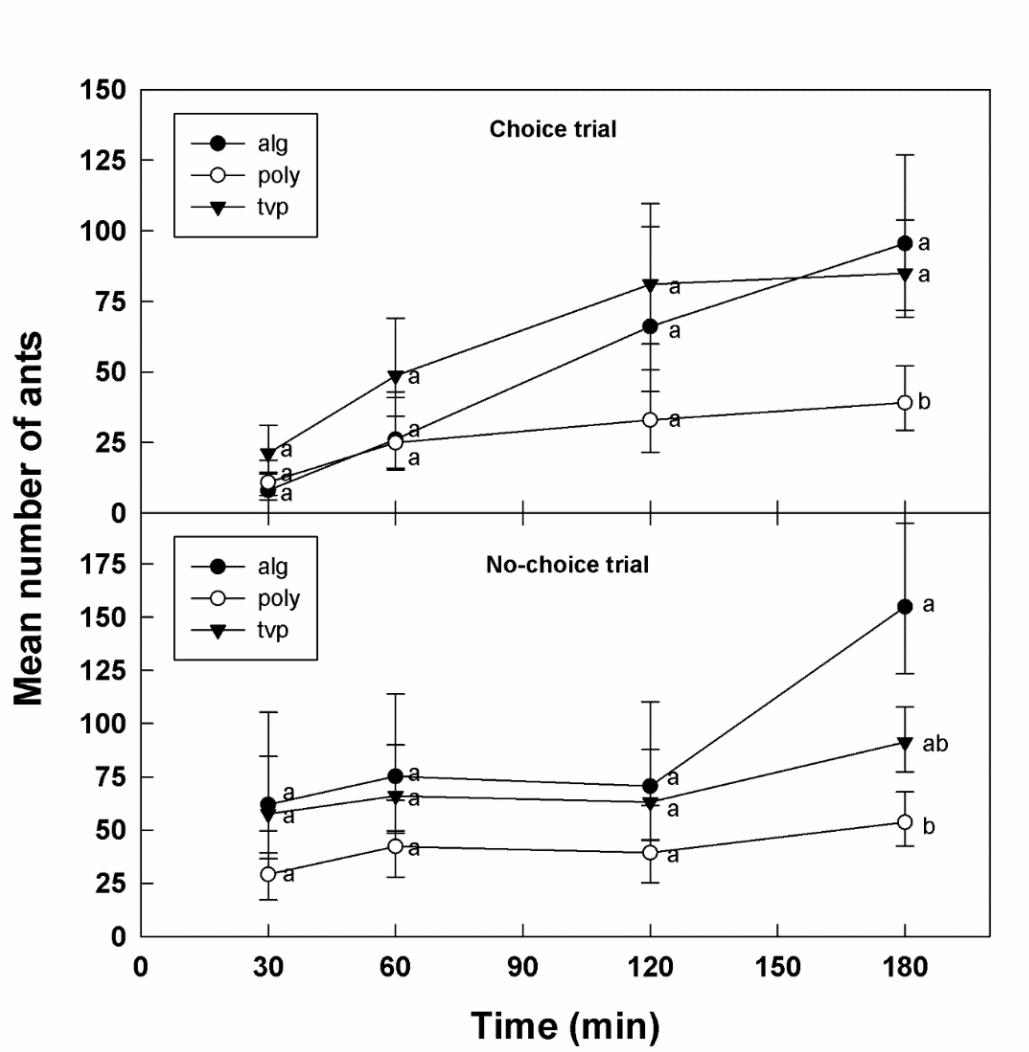

Figure 3-10. Bait preference trials with LFA. Back-transformed mean number of ants (±SE) attracted to the three WSG types over time shown for the choice trial (top panel) and no-choice trial (bottom panel). Means sharing the same letter within each time interval are not significantly different (at $\alpha = 0.05$).

Pesticide repellency trials

YCA exhibited little repellency to both dinotefuran and indoxacarb, with the possible exception of the highest concentrations tested (0.25%) in some formulations (Fig. 3-11). In contrast, YCA exhibited clear repellency to thiamethoxam at concentrations at or above 0.005% (Fig. 3-11). The patterns of repellency were quite consistent across all three WSG types.

AA exhibited little repellency to indoxacarb and thiamethoxam below the highest concentrations tested (0.25% and 0.025%, respectively) (Fig. 3-12). However, there was evidence of repellency to dinotefuran at even the lowest concentration tested (0.005%) with alginate beads, as well as above 0.05% concentration with polyacrylamide. The patterns of repellency were again fairly consistent across the WSG types.

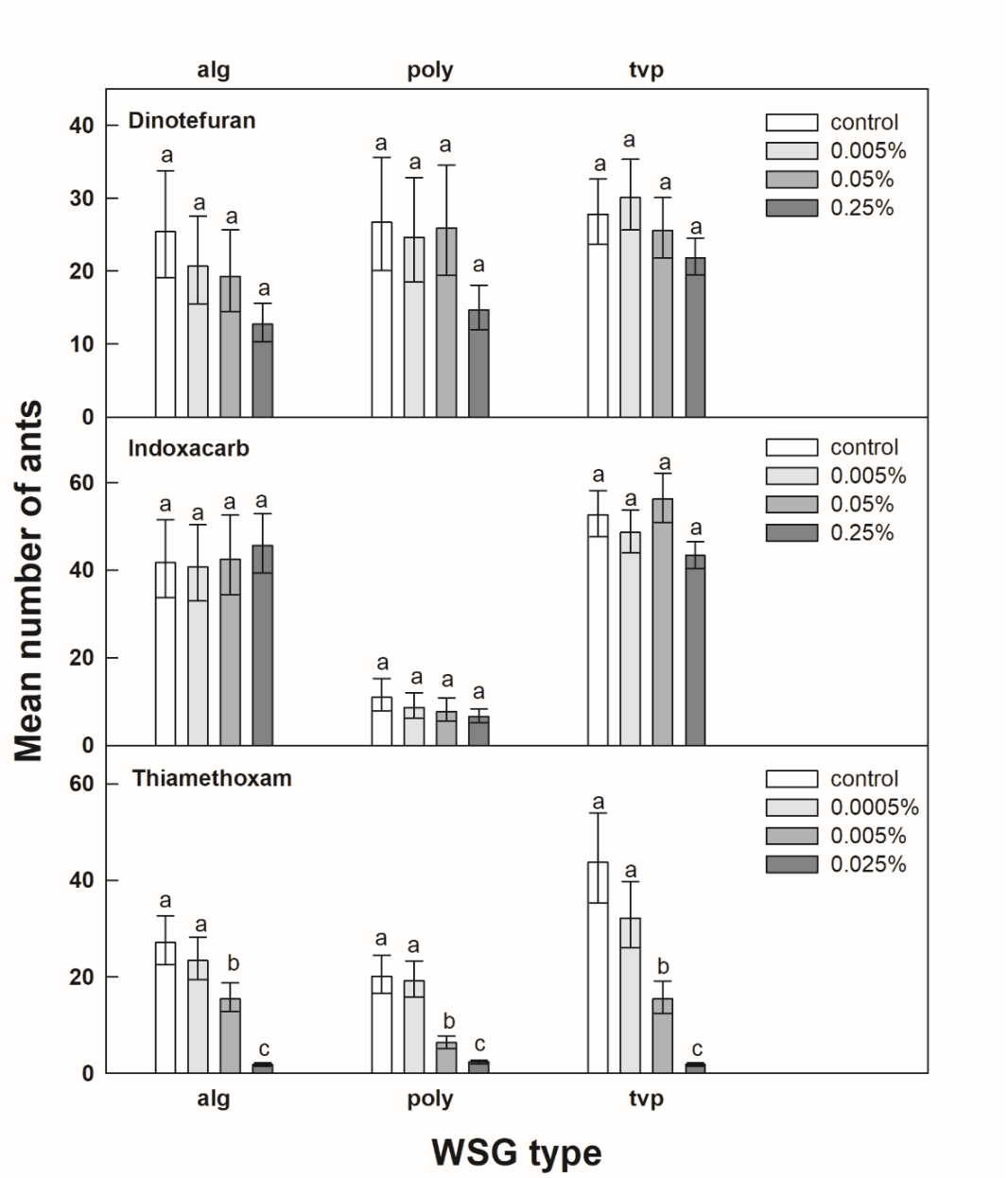

Figure 3-11. Pesticide repellency trials with YCA. Back-transformed mean number of ants (±SE) attracted to different concentrations of each AI are shown in separate panels, and grouped by WSG type. Means sharing the same letter within each WSG grouping are not significantly different (at $\alpha = 0.05$). Several comparisons were marginally significantly different: 0.25% dinotefuran vs. control formulated in alginate beads ($p = 0.081$), and 0.25% vs. 0.05% indoxacarb formulated in TVP ($p = 0.052$).

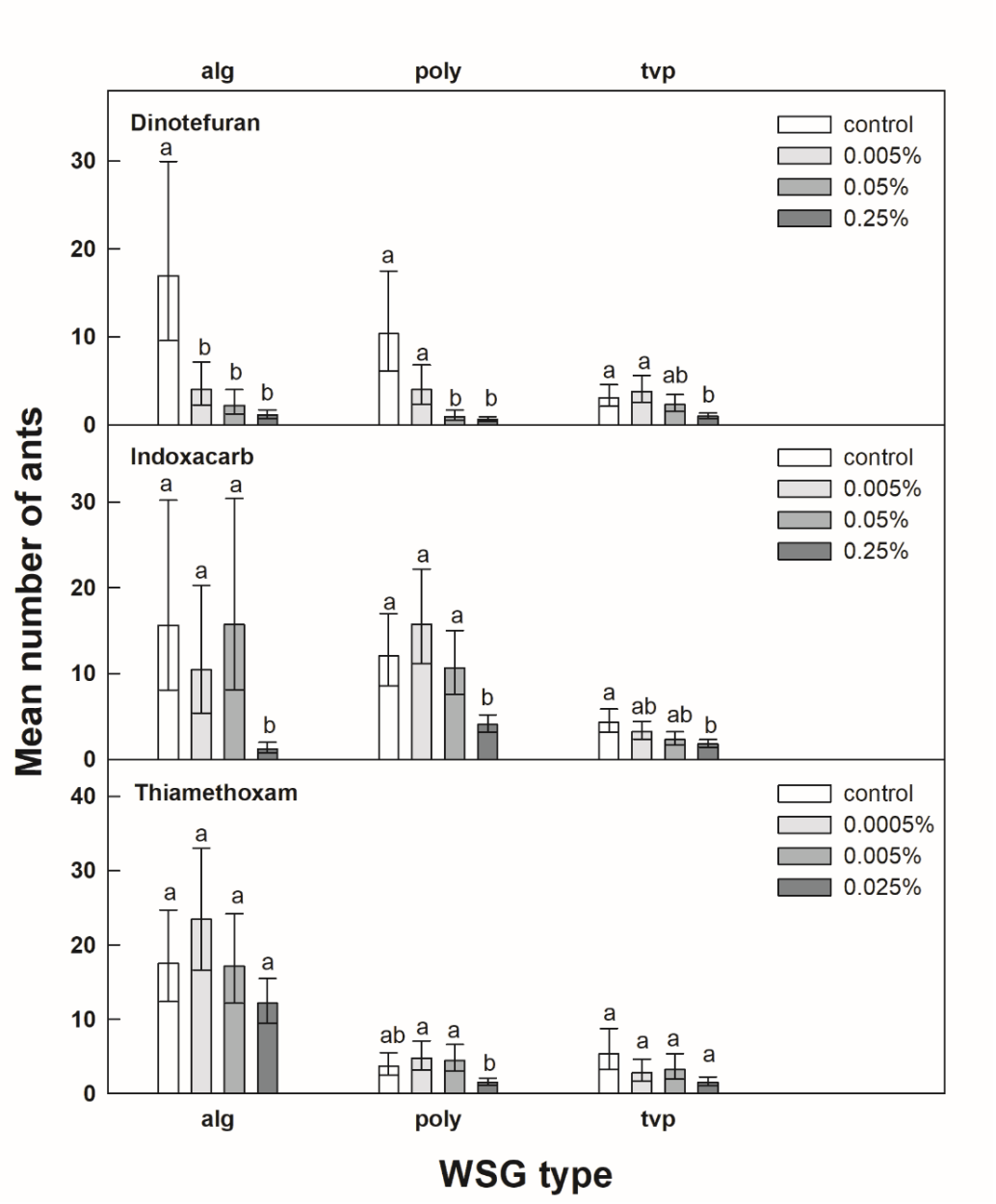

Figure 3-12. Pesticide repellency trials with AA. Back-transformed mean number of ants (±SE) attracted to different concentrations of each AI are shown in separate panels, and grouped by WSG type. Means sharing the same letter within each WSG grouping are not significantly different (at $\alpha = 0.05$). One comparison was marginally significantly different: 0.025% thiamethoxam vs. control formulated in TVP ($p = 0.067$).

For LFA, indoxacarb appears to be non-repellant at concentrations below 0.25% (Fig. 3-13). In contrast, thiamethoxam appears to be repellant to LFA at concentrations at or above 0.005%, and even exhibited signs of repellency at concentrations of 0.0005% (Fig. 3-13). Repellency to dinotefuran was less clear, in part because of low overall recruitment rates in two of the trials (Fig. 3-13). In the trial using polyacrylamide, recruitment was highest to the

formulation with the highest concentration of dinotefuran (0.25%), suggesting that this AI is not repellent to LFA. However, recruitment to intermediate concentrations of dinotefuran in the polyacrylamide trial was very low (Fig. 3-13), which is difficult to explain. The latter may have been a spurious result.

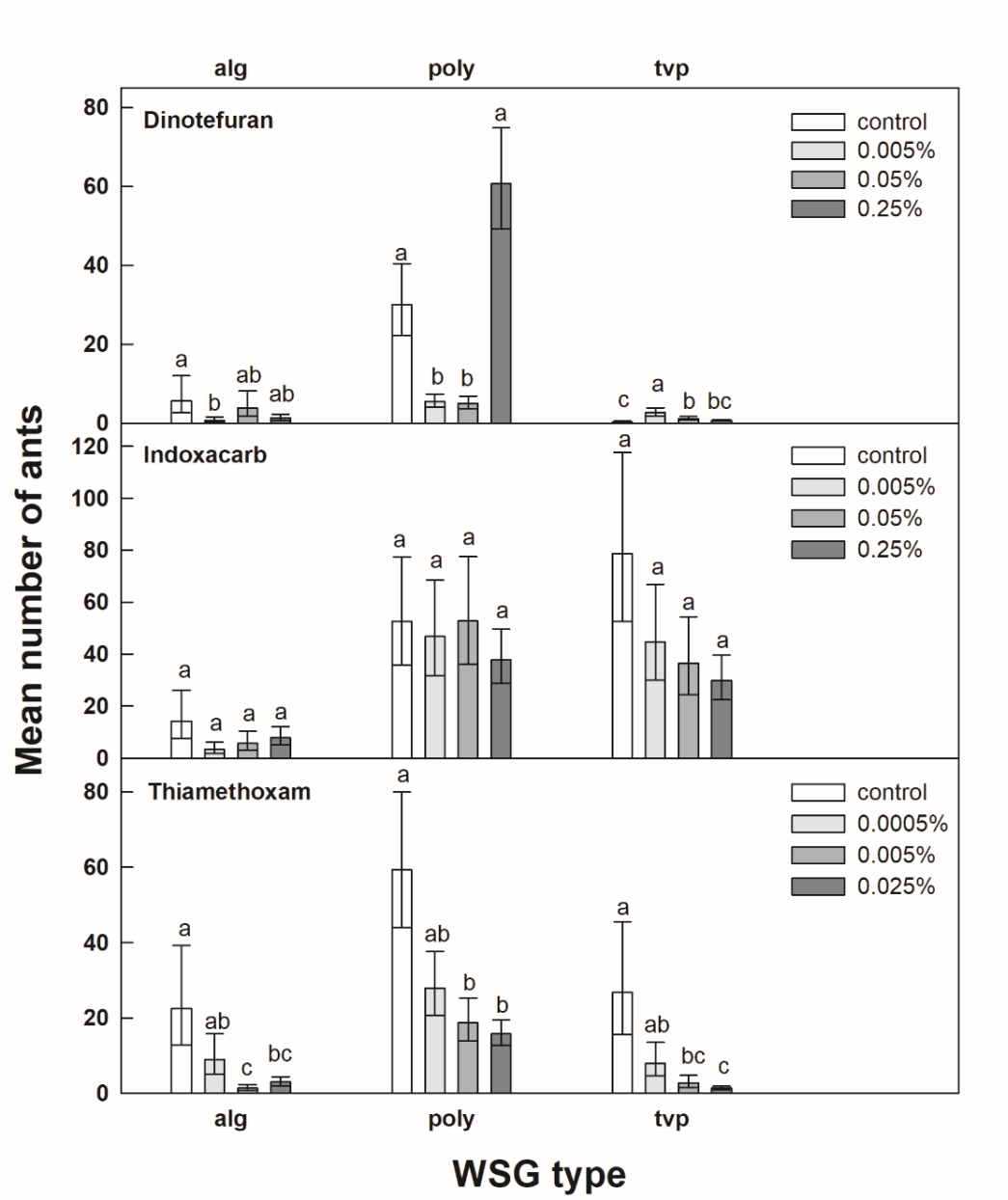

Figure 3-13. Pesticide repellency trials with LFA. Back-transformed mean number of ants (±SE) attracted to different concentrations of each AI are shown in separate panels, and grouped by WSG type. Means sharing the same letter within each WSG grouping are not significantly different (at $\alpha = 0.05$). Several comparisons were marginally significantly different: 0.25% dinotefuran vs. control formulated in polyacrylamide ($p = 0.095$), 0.005% indoxacarb vs. control formulated in alginate beads ($p = 0.098$), 0.25% indoxacarb vs. control formulated in TVP ($p = 0.082$), 0.0005% thiamethoxam vs. control formulated in polyacrylamide ($p = 0.061$), and 0.0005% thiamethoxam vs. control formulated in TVP ($p = 0.080$).

For all three species, differences in overall recruitment rates (i.e., maximum numbers) to different WSG types should not be taken to indicate differences in attraction to those granule types, as each WSG-specific repellency trial was conducted on a different transect and in some cases at different sites. Differences in local densities of ants are therefore likely responsible for these differences in recruitment.

Efficacy testing

Trends in ant numbers over time in the first AA efficacy trial are shown in Figure 3-14. Ant numbers at monitoring cards were reduced by over 98% after the first application of WSG baits in all plots except two of the plots treated with 0.005% indoxacarb (Table 3-1). However, across all plots, differences in mean percent reduction after the first application were not significantly associated with either granule type ($F = 1.734$, $p = 0.287$) or AI formulation ($F = 2.277$, $p = 0.219$). Least squares means for granule types and AI formulations are shown in Table 3-2. Results after the second application were similar, except that ant numbers dropped substantially further in at least one of the plots treated with 0.005% indoxacarb (Fig. 3-14, Table 3-1). Differences in percent reduction were again not significantly associated with either granule type ($F = 1.271$, $p = 0.374$) or AI formulation ($F = 3.989$, $p = 0.115$) after the second application (Table 3-2).

Table 3-1. Mean percent reduction in ant numbers in each of the plots after each bait application in the first AA efficacy trial. Reduction in each plot averaged across the three monitoring dates (two, four and six days) after each application.

AI	**Granule**	**Mean % reduction after 1st application**	**Mean % reduction after 2nd application**
0.005% indoxacarb	alginate	83.9	98.8
0.005% indoxacarb	polyacrylamide	100	96.4
0.005% indoxacarb	tvp	77.4	86.9
0.05% indoxacarb	alginate	98.1	100
0.05% indoxacarb	polyacrylamide	99.9	100
0.05% indoxacarb	tvp	98.7	99.9
0.0005% thiamethoxam	alginate	99.6	100
0.0005% thiamethoxam	polyacrylamide	99.1	95.6
0.0005% thiamethoxam	tvp	98.9	99.6
control	none	13.4	44.4

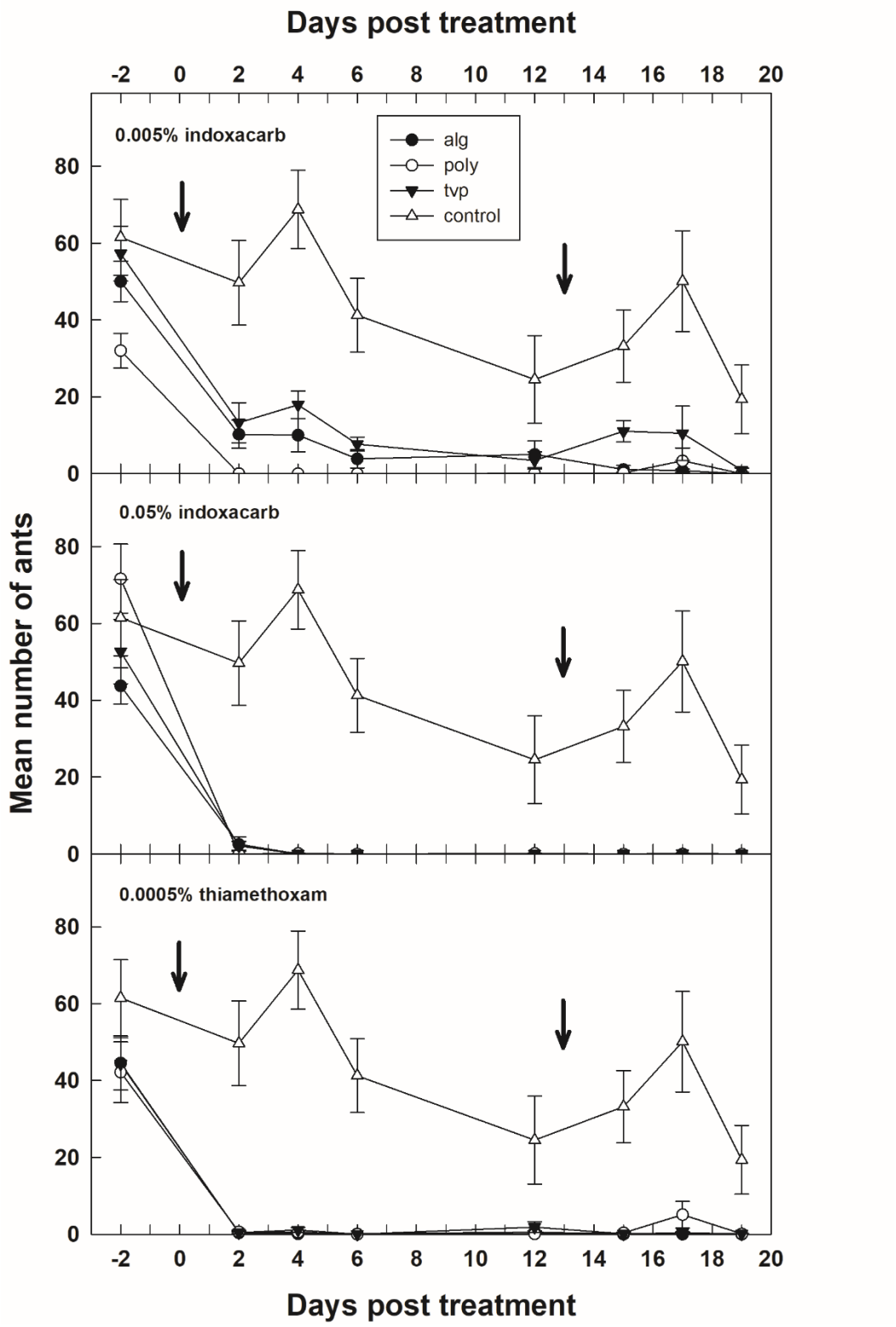

Figure 3-14. First AA efficacy trial. Mean numbers of ants (± SE) in treatment and control plots are shown over time, grouped by AI formulation in separate panels. Timing of the two WSG bait applications shown with arrows.

Table 3-2. Least squares means of percent reduction in AA numbers (back-transformed) following each application for the two factors included in the model, in the first AA efficacy trial.

Factor	Mean % reduction (95% CI), 1st application[1]	Mean % reduction (95% CI), 2nd application[1]
Granule type		
alginate	95.9 (82.5-99.9) a	99.9 (96.5-100.0) a
polyacrylamide	99.8 (93.2-96.4) a	98.2 (92.0-100.0) a
tvp	94.4 (79.8-100.0) a	97.7 (91.1-100.0) a
AI formulation		
0.005% indoxacarb	91.1 (74.5-99.4) a	95.0 (86.5-99.5) a
0.05% indoxacarb	99.2 (90.2-98.2) a	100.0 (97.5-100.0) a
0.0005% thiamethoxam	99.2 (90.5-98.0) a	99.2 (94.2-100.0) a

[1]Means sharing the same letter within each factor and application are not significantly different according to Tukey HSD pairwise comparisons ($\alpha = 0.05$). ANOVA performed on arcsine square root transformed data.

Trends in ant numbers over time for the second AA efficacy trial are shown in Figure 3-15. Ant numbers at monitoring cards were reduced by over 97% on average in all plots treated with 0.05% indoxacarb at either application rate (Table 3-3). In contrast, reductions in ant numbers were substantially lower in plots treated with 0.0005% thiamethoxam at both application rates, but especially at the higher application rate (Table 3-3). Across all plots, differences in mean percent reduction were highly significantly associated with AI formulation ($F = 24.934$, $p < 0.001$), but were not significantly associated with application rate ($F = 1.917$, $p = 0.200$). Least squares means for AI formulations and application rates are shown in Table 3-4, and indicate that reductions in indoxacarb plots were significantly higher than those in the thiamethoxam plots. Across formulations, the lower application rate worked at least as well as the higher application rate (Table 3-4).

Table 3-3. Mean percent reduction in ant numbers for each treatment in the second AA efficacy trial, averaged across the three monitoring dates (two, four and six days) after application and across the three replicate plots for each treatment.

AI	Application rate (L/ha)	Mean % reduction
0.05% indoxacarb	55	97.4
0.05% indoxacarb	25	98.8
control for indoxacarb	n/a	27.4
0.0005% thiamethoxam	55	72.9
0.0005% thiamethoxam	25	86.8
control for thiamethoxam	n/a	-36.2

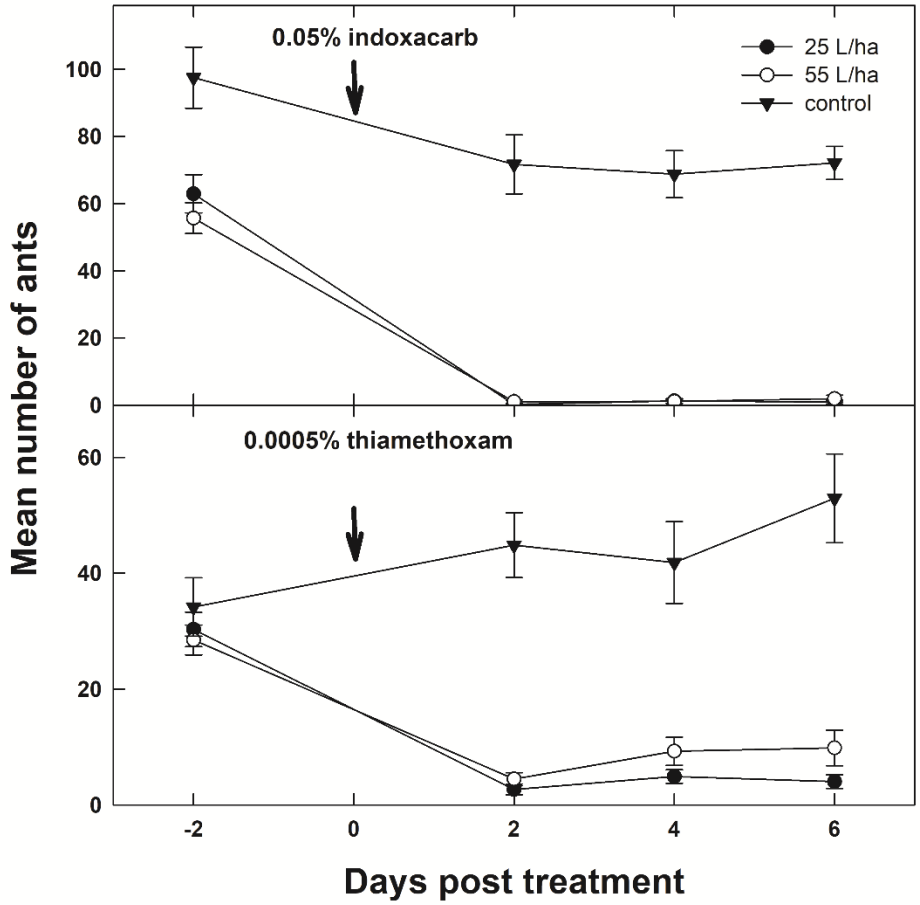

Figure 3-15. Mean numbers of ants (± SE) in treatment and control plots over time in the second AA efficacy trial, grouped by AI formulation in separate panels. Timing of the WSG bait applications shown with arrows.

Table 3-4. Least squares means of percent reduction in ant numbers (back-transformed) in the second AA efficacy trial, for the two factors included in the ANOVA model.

Factor	**Mean % reduction (95% CI)[1]**
AI formulation	
0.05% indoxacarb	98.7 (95.2-99.9) a
0.0005% thiamethoxam	80.8 (71.6-88.6) b
Application rate	
55 L/ha	89.5 (81.9-95.2) a
25 L/ha	94.6 (88.6-98.4) a

[1]Means sharing the same letter within each factor are not significantly different according to Tukey HSD pairwise comparisons ($\alpha = 0.05$). ANOVA performed on arcsine square root transformed data.

Trends in ant numbers over time for the first YCA efficacy trial are shown in Figure 3-16. Ant numbers at monitoring cards were reduced by over 90% after the first application of WSG baits in all plots treated with either concentration of dinotefuran (Table 3-5). In contrast, reductions in ant numbers were substantially lower in plots treated with indoxacarb, especially those treated at the lower concentration of 0.005% (Table 3-5). Ant numbers increased to some degree in most plots at six days after the first application (Fig. 3-16), which may have resulted in part from reinvasion of the plots from the periphery. YCA densities are very high in the study area, and this very active ant may be capable of recolonizing the plots more quickly than AA. Across all plots, differences in mean percent reduction after the first application were not significantly associated with granule type ($F = 1.099$, $p = 0.392$), but were highly significantly associated with AI formulation ($F = 14.314$, $p = 0.004$). Least squares means for granule types and AI formulations are shown in Table 3-6, and indicate that reductions in dinotefuran plots were significantly higher than those in the 0.005% indoxacarb plots, with reductions in 0.05% indoxacarb plots being intermediate.

Table 3-5. Mean percent reduction in ant numbers in each of the plots after each bait application in the first YCA efficacy trial. Reduction in each plot averaged across the three monitoring dates (two, four and six days) after each application. Only 0.05% indoxacarb plots received a second application.

AI	**Granule**	**Mean % reduction after 1st application**	**Mean % reduction after 2nd application**
0.005% indoxacarb	alginate	54.3	
0.005% indoxacarb	polyacrylamide	74.7	
0.005% indoxacarb	tvp	49.0	
0.05% indoxacarb	alginate	73.7	67.6
0.05% indoxacarb	polyacrylamide	88.0	88.6
0.05% indoxacarb	tvp	65.8	83.2
0.005% dinotefuran	alginate	97.0	
0.005% dinotefuran	polyacrylamide	91.7	
0.005% dinotefuran	tvp	94.0	
0.05% dinotefuran	alginate	98.8	
0.05% dinotefuran	polyacrylamide	94.0	
0.05% dinotefuran	tvp	96.2	
control	none	-3.8	2.4

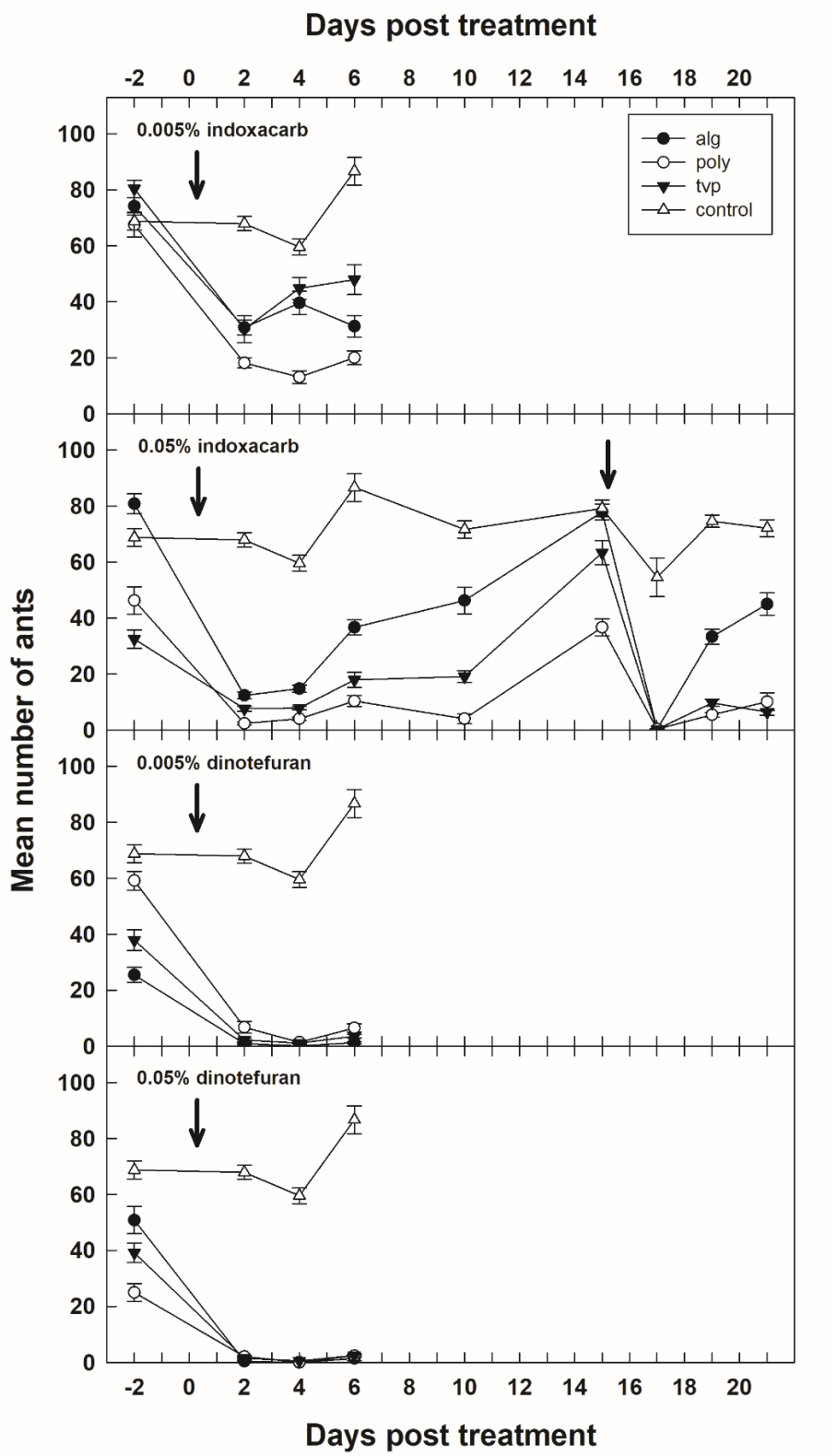

Figure 3-16. First YCA efficacy trial. Mean numbers of ants (± SE) in treatment and control plots are shown over time, grouped by AI formulation in separate panels. Timing of the WSG bait applications shown with arrows.

Table 3-6. Least squares means of percent reduction in YCA numbers (back-transformed) following each application for the two factors included in the model, in the first YCA efficacy trial. Model for second application uses data for first application for all plots treated only once.

Factor	Mean % reduction (95% CI), 1st application[1]	Mean % reduction (95% CI), 2nd application[1]
Granule type		
alginate	85.5 (75.1-93.4) a	84.3 (72.5-93.2) a
polyacrylamide	87.9 (78.1-95.1) a	88.0 (77.2-95.7) a
tvp	79.8 (68.3-89.2) a	83.7 (71.8-92.8) a
AI formulation		
0.005% indoxacarb	59.6 (44.5-73.9) c	59.6 (43.0-75.2) b
0.05% indoxacarb	76.5 (62.6-88.0) bc	80.5 (65.8-91.8) ab
0.005% dinotefuran	94.5 (85.6-99.3) ab	94.5 (84.5-99.5) a
0.05% dinotefuran	96.6 (89.0-99.9) a	96.6 (88.1-100.0) a

[1]Means sharing the same letter within each factor and application are not significantly different according to Tukey HSD pairwise comparisons ($\alpha = 0.05$). ANOVA performed on arcsine square root transformed data.

The level of control achieved with the first application of 0.005% indoxacarb formulations was deemed insufficiently effective to pursue further. However, a second application of the 0.05% indoxacarb formulations was conducted to determine whether two treatments at this concentration could achieve control similar to a single treatment with dinotefuran. The second application achieved strong immediate reductions of foraging ant numbers in each of the three plots, but ant numbers rebounded four to six days after the second application, especially in the plot using alginate beads (Fig. 3-16). Levels of control averaged over the three monitoring dates following the second application were similar to those achieved after the first, with the exception of the TVP plot that had somewhat better control after the second application (Table 3-5). When analyzed across all plots, however, results were very similar to those achieved after the first application: differences in mean percent reduction were not significantly associated with granule type ($F = 0.331$, $p = 0.730$), but were again highly significantly associated with AI formulation ($F = 11.336$, $p = 0.007$). Least squares means for granule types and AI formulations are shown in Table 3-6, and indicate that reductions in dinotefuran plots were significantly higher than those in the 0.005% indoxacarb plots, with reductions in 0.05% indoxacarb plots again being intermediate.

Trends in ant numbers over time for the second YCA efficacy trial are shown in Figure 3-17. This trial tested a lower dinotefuran concentration and a lower bait application rate than the first trial. Percent reduction in ant numbers was progressively lower as the amount of dinotefuran applied in the plots decreased (Table 3-7). Despite this progression, differences in mean percent reduction were significantly associated only with dinotefuran concentration ($F = 12.949$, $p = 0.0058$), and not with application rate ($F = 3.510$, $p = 0.0938$). Least squares means for AI concentrations and application rates are shown in Table 3-8, and indicate that ant reductions in 0.005% dinotefuran plots were significantly higher than those in 0.0005% dinotefuran plots, but that percent reductions were not significantly different between application rates.

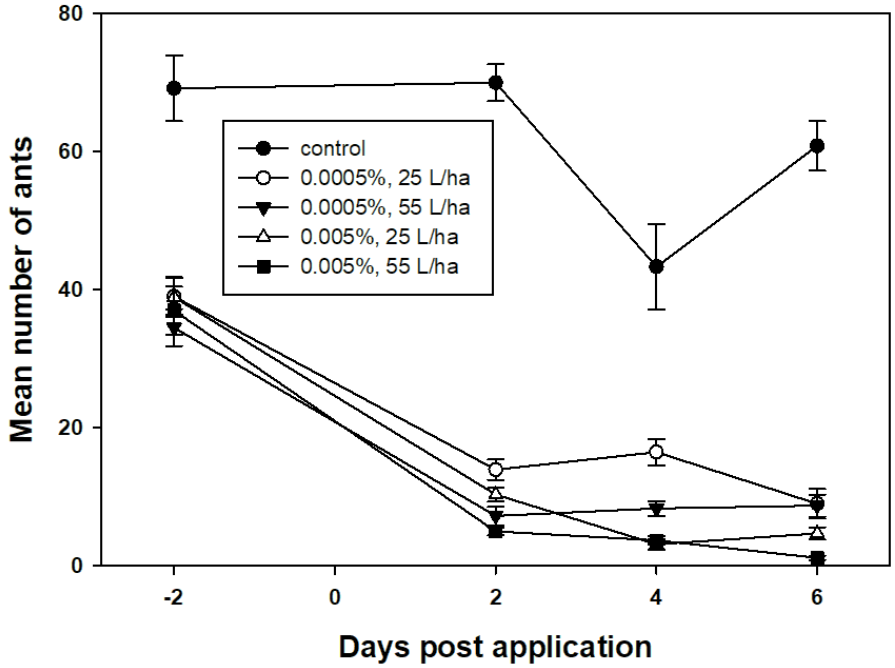

Figure 3-17. Mean numbers of ants (± SE) in dinotefuran treatment and control plots over time in the second YCA efficacy trial.

Table 3-7. Mean percent reduction in ant numbers for each of the treatments in the second YCA efficacy trial, averaged across the three monitoring dates (two, four and six days) after bait application.

AI	Application rate (L/ha)	Mean % reduction
0.005% dinotefuran	55	88.1
0.005% dinotefuran	25	84.5
0.0005% dinotefuran	55	77.4
0.0005% dinotefuran	25	66.3
control	none	16.1

Table 3-8. Least squares means of percent reduction in ant numbers (back-transformed) in the second YCA efficacy trial, for the two factors included in the ANOVA model.

Factor	Mean % reduction (95% CI)[1]
AI concentration	
0.005% dinotefuran	86.8 (80.9-91.8) a
0.0005% dinotefuran	72.3 (64.7-79.2) b
Application rate	
55 L/ha	83.7 (77.3-89.3) a
25 L/ha	76.1 (68.8-82.7) a

[1]Means sharing the same letter within each factor are not significantly different according to Tukey HSD pairwise comparisons ($\alpha = 0.05$). ANOVA performed on arcsine square root transformed data.

Non-target species attraction: video observations of pollinators

Videos of WSG placed on the ground in the vicinity of active pollinators recorded 132 non-ant visitors to the 90 replicate bait piles observed for a collective >384 hours (Table 3-9). The most common visitors were overwhelmingly flies (Diptera) of various types (85), followed by parasitic Hymenoptera (17) and a variety of taxa with seven or fewer visits each. Among common pollinating insects, hover flies (Diptera: Syrphidae) made 5 visits to WSG baits, a single native *Hylaeus volatilis* bee visited alginate beads, and four visits were made by the non-native solitary bee *Ceratina dentipes*.

Table 3-9. Total number of visitors to WSG placed on the ground during video observation events (n = 30 per WSG type).

Taxon	Alginate	Poly.	TVP	Total
Acari Total	5	2	0	7
Araneae Total	0	4	1	5
Chilopoda Total	0	0	1	1
Isopoda Total	0	1	0	1
Collembola Total	1	0	3	4
Diptera Total	9	21	55	85
Sarcophagidae	1	11	30	42
Syrphidae	3	2	0	5
Other/unknown	5	8	25	38
Hymenoptera Total	6	3	14	23
Bees Total (Apidae or Colletidae)	2	0	3	5
Ceratina dentipes	1	0	3	4
Hylaeus volatilis	1	0	0	1
Sphecidae Total	0	0	1	1
Tachysphex apicalis	0	0	1	1
Parasitoids Total	4	3	10	17
Unknown Total	2	2	2	6
Grand Total	23	33	76	132

Most visits to ground baits occurred during the first two hours after placement (Fig. 3-18). TVP baits attracted the most visitors and the greatest diversity of taxa per observation event, although differences among WSG types in these two metrics were statistically significant only between TVP and alginate beads (Fig. 3-19). Duration of visits were not significantly different among any of the WSG types (Fig. 3-19).

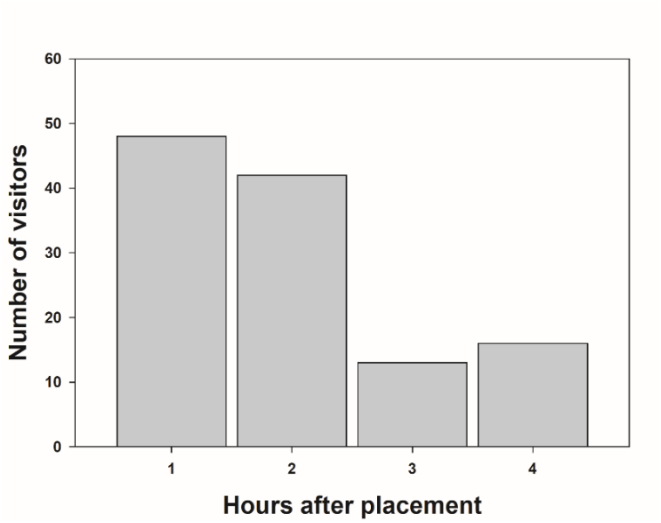

Figure 3-18. Histogram showing frequency of visitation during each hour after bait placement for WSG placed on the ground. Number of visitors shown for each 60 minute period ending in the hour indicated.

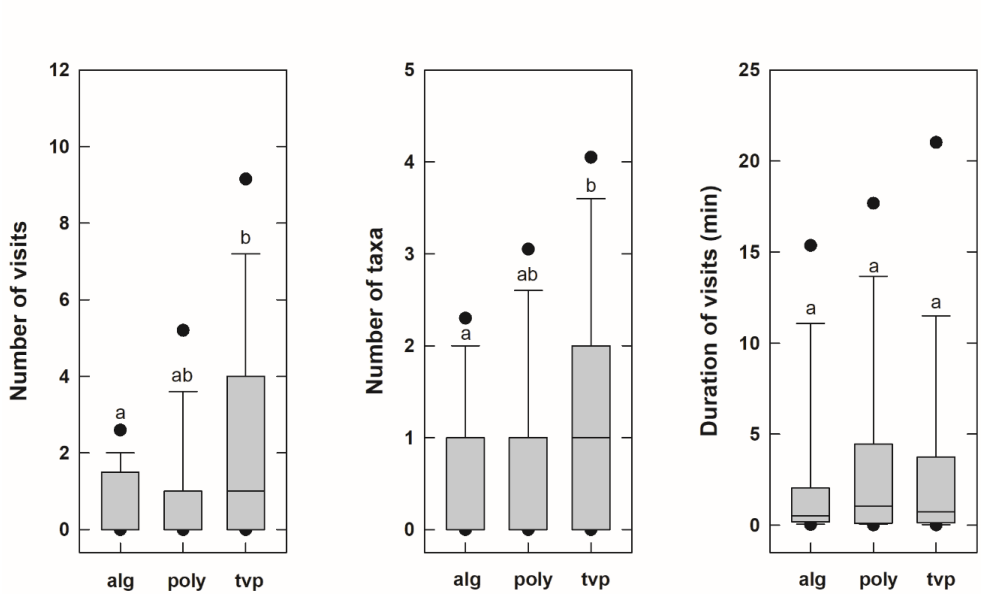

Figure 3-19. Box plots showing the number of non-ant visits (left panel), the number of non-ant taxa (middle panel), and the duration of non-ant visits (right panel) to WSG of each type placed on the ground in the vicinity of active pollinators. Boxes, whiskers and outliers as in Fig. 3-7. In all cases where median line is not visible, the median is 0. WSG types sharing the same letter within each panel are not significantly different (generalized linear models, $\alpha = 0.05$). Number of visits were marginally significantly different between polyacrylamide and TVP ($p = 0.092$).

Videos of WSG placed adjacent to flowers recorded many more non-ant visitors compared to those of granules placed on the ground: 394 visits to the 72 replicate bait piles occurred during the collective >307 hours of observation (Table 3-10). These visits were dominated by Hymenoptera, and to a lesser extent, Lepidoptera. Among Hymenoptera, bees were the most common visitors, predominantly honey bees (*Apis mellifera*), but also native and non-native *Hylaeus* yellow-faced bees. Vespid wasps were also observed visiting granules. Lepidoptera visiting granules were represented by several unidentified species of crambid moths occurring only in the trials conducted at HALE, and are quite possibly native species. Surprisingly few visits were made by syrphid flies.

Table 3-10. Total number of visitors to WSG placed near flowers during video observation events (n = 24 per WSG type).

Taxon	**Alginate**	**Poly.**	**TVP**	**Total**
Araneae Total	0	4	1	5
Diptera Total	8	3	6	17
Syrphidae	5	0	2	7
Other/unknown	3	3	4	10
Hemiptera Total	0	0	2	2
Miridae	0	0	2	2
Hymenoptera Total	113	115	68	296
Bees Total (Apidae or Colletidae)	98	111	61	270
Apis mellifera	73	83	45	201
Hylaeus spp. (native)	3	12	6	21
Hylaeus strenuus (non-native)	22	16	10	48
Vespidae Total	14	4	7	25
Pachodynerus nasidens	1	2	0	3
Polistes aurifer	13	2	7	22
Parasitoids Total	1	0	0	1
Lepidoptera Total	8	32	33	73
Crambidae	8	32	33	73
Orthoptera Total	0	1	0	1
Tettigoniidae Total	0	1	0	1
Elimaea punctifera	0	1	0	1
Grand Total	129	155	110	394

Unlike baits placed on the ground, baits placed near flowers attracted similar numbers of visitors throughout each of the first four hours after placement (Fig. 3-20). Among the four plant species investigated, numbers of visitors to WSG granules was highest on *S. haleakalae* and *H. foertherianum*, intermediate on *G. cuneatum*, and lowest on *S. taccada* (Fig. 3-21). This pattern largely followed rates of visitation to the flowers of these species: floral visitation was high for *S. haleakalae*, *H. foertherianum* and *G. cuneatum*, and much lower for *S. taccada* (Fig. 3-21). The lower visitation rate to WSG granules near *G. cuneatum* flowers, compared to those near *S. haleakalae* flowers, likely resulted from the fact floral morphology required placement of granules further from flowers for *G. cuneatum*, whereas granules could be perched immediately adjacent to *S. haleakalae* flowers (and *H. foertherianum* flowers, Fig. 3-4). The number of taxa visiting WSG granules placed near flowers followed similar patterns among plant species as the

number of visitors, with more taxa visiting granules placed near flowers of *S. haleakalae*, *G. cuneatum* and *H. foertherianum*, and fewer taxa visiting granules placed near flowers of *S. taccada* (Fig. 3-22). This pattern again generally followed the number of taxa visiting the flowers of those plant species (Fig. 3-22).

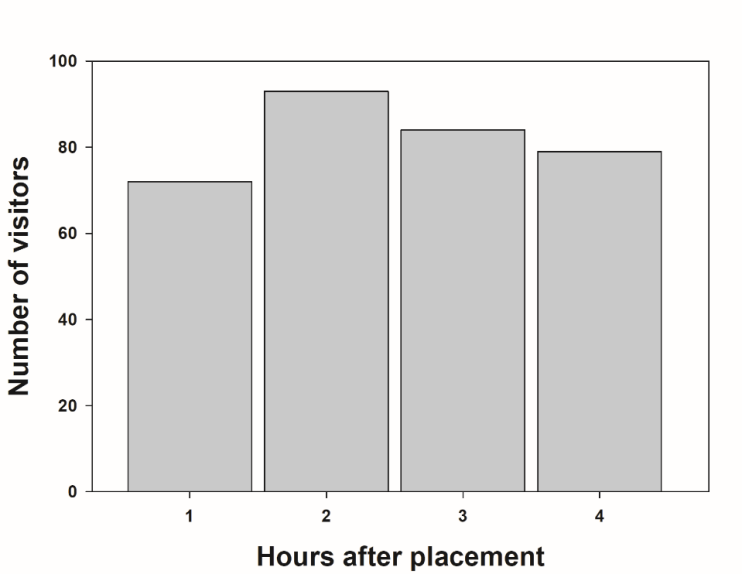

Figure 3-20. Histogram showing frequency of visitation during each hour after bait placement for WSG placed near flowers. Number of visitors shown for each 60 minute period ending in the hour indicated.

When visitation to WSG granules was examined across the four plant species, there were no significant differences in the number of visits per observation event, the number of visiting taxa per event, or the duration of visits among the three WSG types (Fig. 3-23). (To facilitate comparison of the majority of visit durations, 9 visits (out of 385 analyzed) that lasted longer than 15 minutes each were excluded from this analysis. These were all made by small crambid moths or in one case a katydid.) The lack of differences in visitation among WSG types supports the inference that differences in visitation to WSG on different plant species (Figs. 3-21, 3-22) is driven by differences in attraction to the flowers of those species, rather than differences in attraction to the WSG types.

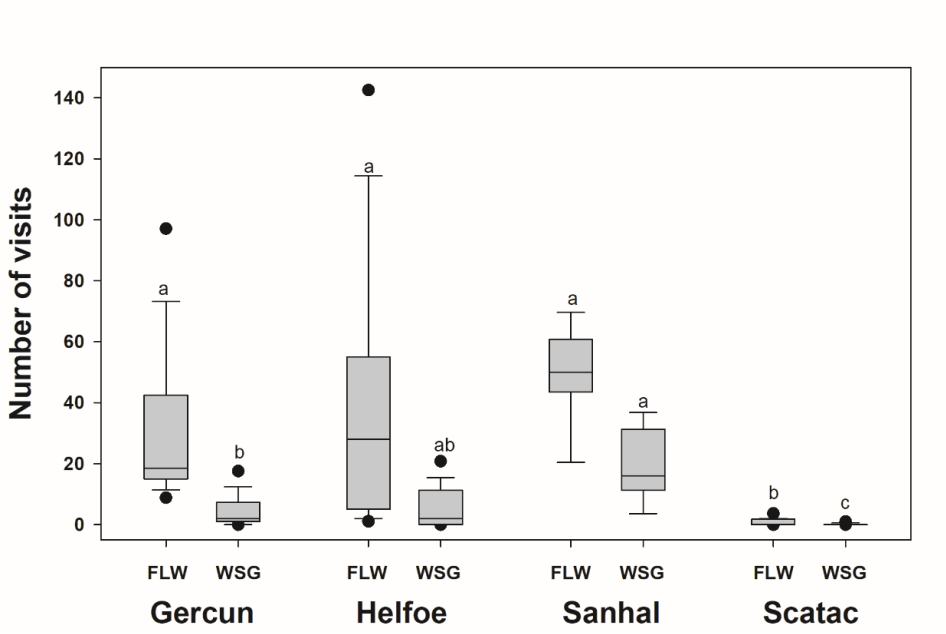

Figure 3-21. Box plots showing the number of non-ant visits to flowers and to WSG granules placed adjacent to flowers for each of four plant species. Boxes, whiskers and outliers as in Fig. 3-7. Boxes sharing the same letter within each substrate type (flower or WSG) are not significantly different (generalized linear models, $\alpha = 0.05$). Number of visits to WSG granules on Sanhal and Helfoe were marginally significantly different ($p = 0.057$).

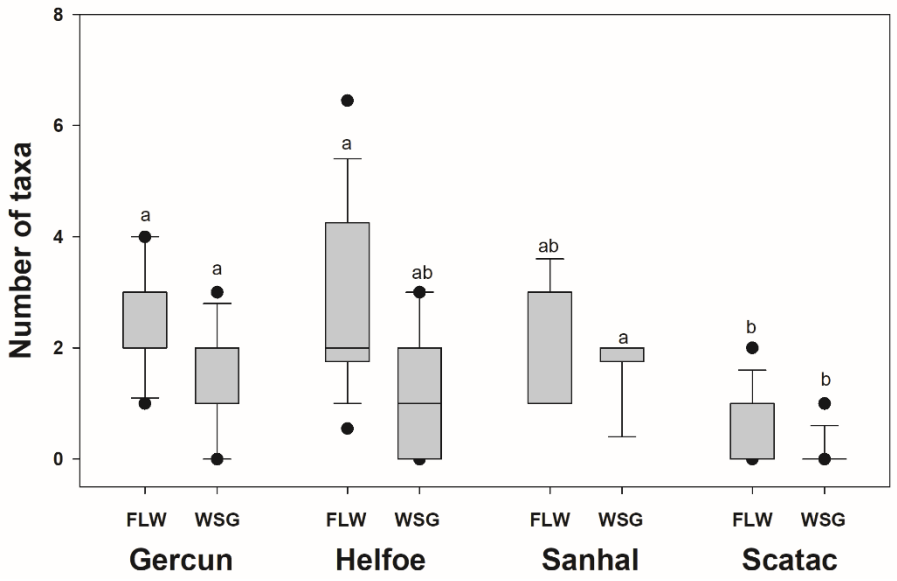

Figure 3-22. Box plots showing the number of non-ant taxa visiting flowers and WSG granules placed adjacent to flowers for each of four plant species. Boxes, whiskers and outliers as in Fig. 3-7. Boxes sharing the same letter within each substrate type (flower or WSG) are not significantly different (generalized linear models, $\alpha = 0.05$). Number of visits to WSG granules on Helfoe and Scatac were marginally significantly different ($p = 0.066$).

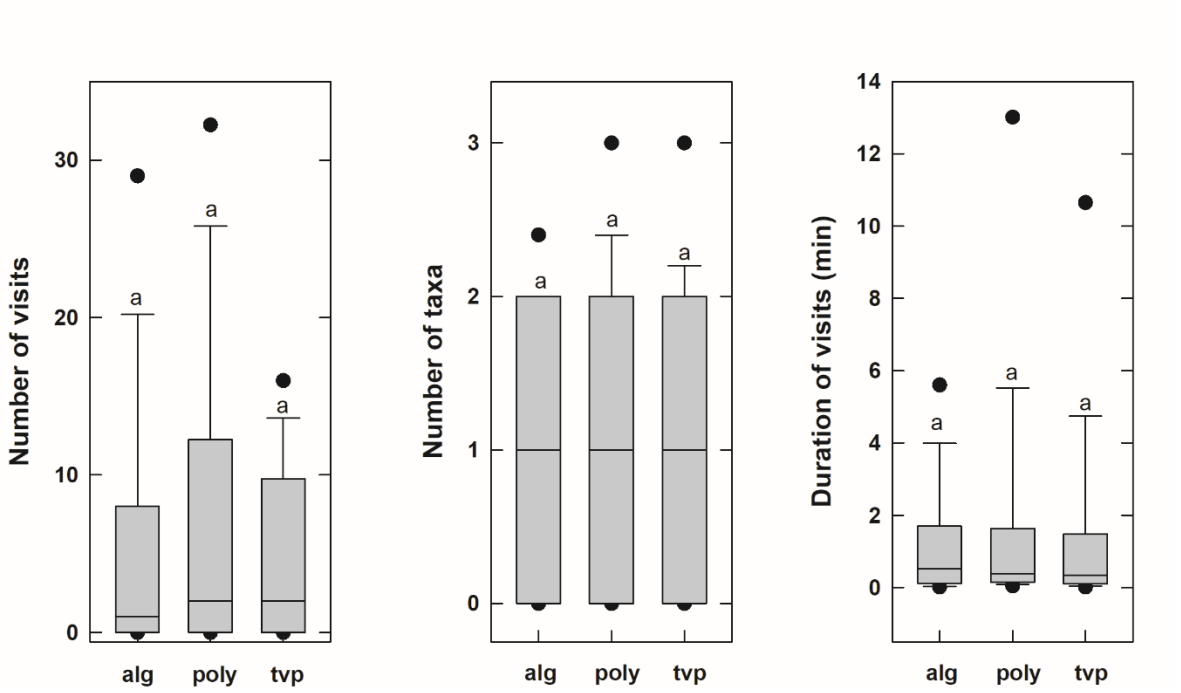

Figure 3-23. Box plots showing the number of non-ant visits (left panel), the number of non-ant taxa (middle panel), and the duration of non-ant visits (right panel) to WSG of each type placed near flowers. Boxes, whiskers and outliers as in Fig. 3-7. WSG types sharing the same letter within each panel are not significantly different (generalized linear models, $\alpha = 0.05$).

Non-target species attraction: video observations of birds

Attempts to observe attraction of birds to WSG baits were largely unsuccessful. In the two attempts to film individual piles of granules, birds fled the area and failed to return during the filming period. During the three events in which WSG were broadcast in 20 x 20 m plots, some birds did return to the area and did in fact enter the plots. These included Ruddy turnstones, Pacific golden plovers, Mynahs, Mourning doves and Common waxbills. However, it was not possible to discern the targets of foraging in either the video footage or when observing from a distance with binoculars. Behavior of the birds did not suggest unusual attraction to the baits, as birds did not appear to linger within the plot boundaries, unusual numbers of birds did not congregate in the plots, and foraging behavior (e.g., rate or nature of ground pecking) did not appear to differ when birds were within plot boundaries as compared to when they exited the plots. It is nevertheless not possible to state whether birds did or did not consume WSG bait while foraging within the plots.

Non-target species bait consumption: protein marking and detection

Among honey bees fed with polyacrylamide granules for at least 30 seconds via the proboscis extension reflex, 100% (30 of 30) were clearly marked relative to the negative control-based threshold (mean + 3SD). Among the solitary bees that self-fed on polyacrylamide in lab cages, 70% (21 of 30) tested positive for the protein marker. The rate of marking varied strongly

among species: 100% (4 of 4) of *H. strenuus* bees were marked, 86% (12 of 14) of *L. microlepoides* bees were marked, and 42% (5 of 12) of *C. smaragdula* bees were marked. The relatively low marking rate among *C. smaragdula* likely resulted from lower tolerance of the lab protocol (the mortality rate among additional, un-analyzed individuals was noticeably higher for this species), which probably also impacted their likelihood of feeding. Notwithstanding, the combined results indicate that both honey bees and solitary bees are clearly marked when they feed on WSG formulated with 2% rabbit serum.

Ants collected in three of the test plots treated with WSG at KPNAR also demonstrated moderate to high rates of marking relative to the negative control-based threshold. In the plot treated with alginate bead WSG, 100% of ants were marked (28 of 28 *A. gracilipes*); in the plot treated with polyacrylamide, 96% of ants were marked (8 of 8 *A. gracilipes*, and 19 of 20 *O. glaber*); in the plot treated with TVP WSG, 54% of ants were marked (10 of 15 *O. glaber*, and 5 of 13 *P. longicornis*). These results indicate that the rabbit serum marker remains highly active in WSG broadcast in field conditions.

Insects that were collected to test for the possibility of external contamination from the sweep net used during sampling had optical density readings ranging from 0.071 to 0.866. The mean reading was 0.117, and the mean + 3SD equaled 0.507. According to the standard negative control-based threshold, 4 of these insects (out of 53) tested positive for the marker. These were one *Hylaeus nivicola* (Hymenoptera: Colletidae) and three individuals of an unidentified *Hyposmocoma* species (Lepidoptera: Cosmopterigidae). This indicates that some insects can become externally marked when captured in the net, without feeding on the bait. A higher marking threshold based on the mean + 3SD of the net contamination readings may therefore be more appropriate when evaluating test samples collected with the sweep net method. Although this higher threshold still results in one individual being scored as positively marked among the net contamination samples, it reduces the incidence of false positive detections among the treatment samples.

When using the higher net contamination-based threshold, 9.3% of the 441 flying insects sampled across the 18 plots treated with WSG baits were marked (Table 3-11), suggesting that they fed on or at least came into contact with the baits. When using the lower negative control-based threshold, the percentage increased slightly to 14.0% (not shown). Using the net contamination-based threshold, the incidence of marked vs. unmarked individuals of all taxa combined was significantly associated with the type of WSG used (Pearson Chi-Square = 14.23, $p = 0.001$), with marking rates higher than expected for alginate beads, lower than expected for polyacrylamide, and similar to expected for TVP. Similarly, the incidence of marked vs. unmarked individuals across all WSG types was significantly associated with taxonomic order (Pearson Chi-Square = 15.11, $p = 0.001$), with marking rates higher than expected for Hymenoptera, lower than expected for Lepidoptera, and similar to expected for Diptera (the single Hemiptera individual was excluded from analysis).

Most taxa were either consistently unmarked or exhibited low rates of marking, while several taxa had higher rates of marking (Table 3-11). Among the latter, the non-native sphecid wasp *Bembecinus* sp. showed the most consistent evidence of feeding on the baits, with 46.9% of the 49 captured individuals being marked. Interestingly, none of the five individuals of the native sphecid wasp *Ectemnius nesiotes* were marked. Marking rates were also high among several species of vespid wasps, including the native *Nesodynerus molokaiensis*, but sample sizes were very low for these taxa so the reported rates should be viewed with caution. Among bees, marking rates were generally low, the highest being for *A. mellifera* (6.7%). However, one of the

three marked honey bees captured had an optical density reading of only 0.856 (compared to 3.499 and 3.691 for the remaining two), which is lower than the highest reading among the net contamination samples (0.866). It is therefore possible that this bee may have been externally contaminated in the net. The same may be true for the single native *Hylaeus* bee that was marked, out of 70 captured, as it had an optical density of only 0.678. Other native insects that exhibited at least some incidence of marking were the tephritid fruit fly *Trupanea cratericola* (21.4% marking rate) and an abundant but unidentified case-making moth in the genus *Hyposmocoma* (5.0% marking rate).

Table 3-11. Percent of individuals that were positively marked (and number of individuals captured) among taxa sampled in the WSG treatment plots. Percents and sample sizes tabulated for each WSG type and for all plots combined. Native taxa denoted with asterisk.

Taxon	Alginate % marked (n)	Poly. % marked (n)	TVP % marked (n)	All WSG % marked (n)
Diptera Total	14.3 (14)	5.9 (17)	15.4 (13)	11.4 (44)
Calliphoridae Total	0 (1)	0 (1)	0 (1)	0 (3)
Eucalliphora latifrons	0 (1)	0 (1)		0 (2)
Gonia longipulvilli			0 (1)	0 (1)
Muscidae Total		0 (1)	0 (1)	0 (2)
Muscidae sp.		0 (1)	0 (1)	0 (2)
Pterophoridae Total	0 (3)	0 (3)	0 (4)	0 (10)
Stenoptilodes littoralis rhynchophora	0 (3)	0 (3)	0 (4)	0 (10)
Sarcophagidae Total	33.3 (3)	0 (2)	0 (1)	16.7 (6)
Blaesoxipha plinthopyga	50.0 (2)	0 (1)	0 (1)	25.0 (4)
Ravinia anandra		0 (1)		0 (1)
Sarcophaga albiceps	0 (1)			0 (1)
Syrphidae Total	0 (3)	0 (3)	0 (2)	0 (8)
Allograpta exotica	0 (2)	0 (1)	0 (1)	0 (4)
Simosyrphus grandicornis	0 (1)	0 (2)	0 (1)	0 (4)
Tephritidae Total	25.0 (4)	14.3 (7)	50.0 (4)	26.7 (15)
Bactrocera dorsalis			100 (1)	100 (1)
**Trupanea cratericola*	25.0 (4)	14.3 (7)	33.3 (3)	21.4 (14)
Hemiptera Total		0 (1)		0 (1)
Lygaeidae Total		0 (1)		0 (1)
**Nysius* sp.nr. *abnormis*		0 (1)		0 (1)
Hymenoptera Total	30.2 (63)	6.3 (79)	9.1 (77)	14.2 (219)
Apidae Total	8.3 (12)	0 (27)	9.4 (32)	5.6 (71)
Apis mellifera	9.1 (11)	0 (16)	11.1 (18)	6.7 (45)
Ceratina dentipes		0 (1)		0 (1)
Ceratina smaragdula	0 (1)	0 (10)	7.1 (14)	4.0 (25)
Colletidae Total	0 (20)	0 (31)	5.3 (19)	1.4 (70)
**Hylaeus nivicola*	0 (17)	0 (22)	6.7 (15)	1.8 (54)
**Hylaeus volatilis*	0 (3)	0 (9)	0 (4)	0 (16)
Halictidae Total	0 (3)	0 (7)	0 (3)	0 (13)
Lasioglossum imbrex	0 (2)	0 (1)		0 (3)
Lasioglossum microlepoides	0 (1)	0 (6)	0 (3)	0 (10)
Ichneumonidae Total		0 (2)	0 (4)	0 (6)
Calliephialtes grapholithae			0 (1)	0 (1)
Diadegma blackburni		0 (2)	0 (3)	0 (5)

Table 3-11, Continued. Percent of individuals that were positively marked (and number of individuals captured) among taxa sampled in the WSG treatment plots. Percents and sample sizes tabulated for each WSG type and for all plots combined. Native taxa denoted with asterisk.

Taxon	Alginate % marked (n)	Poly. % marked (n)	TVP % marked (n)	All WSG % marked (n)
Sphecidae Total	65.4 (26)	40.0 (10)	11.1 (18)	42.6 (54)
Bembecinus sp.	70.8 (24)	50.0 (8)	11.8 (17)	46.9 (49)
**Ectemnius nesiotes*	0 (2)	0 (2)	0 (1)	0 (5)
Vespidae Total	50.0 (2)	50.0 (2)	100 (1)	60.0 (5)
**Nesodynerus molokaiensis*	0 (1)	100 (1)		50.0 (2)
**Pachodynerus nasidens*		0 (1)		0 (1)
Polistes aurifer	100 (1)		100 (1)	100 (2)
Lepidoptera Total	1.8 (54)	0 (57)	6.1 (66)	2.8 (177)
Cosmopterigidae Total	3.8 (26)	0 (31)	9.1 (44)	5.0 (101)
**Hyposmocoma* sp.	3.8 (26)	0 (31)	9.1 (44)	5.0 (101)
Lycaenidae Total	0 (2)	0 (2)	0 (3)	0 (7)
Brephidium exilis			0 (1)	0 (1)
Lampides boeticus	0 (1)	0 (2)	0 (2)	0 (5)
**Udara blackburni*	0 (1)			0 (1)
micro-Lepidoptera Total	0 (26)	0 (24)	0 (19)	0 (69)
micro-lep sp.1	0 (3)	0 (4)	0 (3)	0 (10)
micro-lep sp.2	0 (1)	0 (5)	0 (3)	0 (9)
micro-lep other spp.	0 (22)	0 (15)	0 (13)	0 (50)
Grand Total	16.8 (131)	3.9 (154)	8.3 (156)	9.3 (441)

Indirect exposure risk for non-target species: pesticide residues in efficacy field plots

Dinotefuran and indoxacarb residues in plant tissues and floral nectar sampled in the YCA efficacy plots treated with polyacrylamide granules at JCNWR are reported in Tables 3-12 and 3-13. Dinotefuran residues in plant tissues composited across entire plots were less than 10 ng/g, or ppb. Residue values were lower as concentrations in bait formulations decreased, being undetectable in the plots treated with 0.0005% dinotefuran (Table 3-12). Dinotefuran was also undetectable in floral nectar samples (Table 3-13). Indoxacarb residues in plant tissues in the YCA plots were considerably higher than those of dinotefuran (Table 3-12), but were also undetectable in floral nectar (Table 3-13).

Interestingly, dinotefuran residues in plant tissues increased from 10 days post treatment to 31 days post treatment in the YCA plots (Table 3-12, Fig. 3-24), contrary to the expectation of residue degradation over time. One possible explanation for this pattern is that dinotefuran not consumed by ants remains bound in the polyacrylamide granules until it is flushed out by significant rainfall and subsequently translocated to plant tissues (Fig. 3-24). Alternatively, it may represent slower rates of uptake by woody shrubs, which comprised a substantial fraction of the plant cover in some plots. Indoxacarb residues, in contrast, did not increase in plant tissues between 10 and 46 days post treatment (Table 3-12, Fig. 3-25), perhaps because indoxacarb does not remain bound in the granules as tightly as dinotefuran. Instead, values generally declined over time.

Table 3-12. Pesticide residues in plant tissues sampled in the YCA efficacy plots. Concentrations reported in terms of fresh plant tissue weight.

Treatment	**days post application**	**dinotefuran residue (ng/g)[1]**	**indoxacarb residue (ng/g)[2]**
0.05% dinotefuran 55 L/ha	-2	0.00	0.00
	5	1.50	0.00
	10	0.86	0.00
	31	9.74	0.02
0.005% dinotefuran 55 L/ha (first efficacy trial)	-2	0.00	0.00
	5	0.03	0.00
	10	0.01	0.00
	31	2.42	0.00
0.005% dinotefuran 55 L/ha (second efficacy trial)	0	0.00	0.00
	5	0.16	0.00
	10	0.00	0.00
	31	0.03	0.00
	90	0.01	0.00
0.005% dinotefuran 25 L/ha	0	0.00	0.00
	5	0.00	0.00
	10	0.02	0.00
	31	0.00	0.00
	90	0.00	0.00
0.0005% dinotefuran 55 L/ha	0	0.00	0.00
	5	0.00	0.00
	10	0.00	0.00
	31	0.00	0.00
	90	0.00	0.00
0.0005% dinotefuran 25 L/ha	0	0.00	0.03
	5	0.00	0.00
	10	0.00	0.00
	31	0.00	0.00
	90	0.00	0.00
0.05% indoxacarb 55 L/ha	-2	0.00	0.00
	5	0.00	119.84
	10	0.00	61.49
	31[3]	0.00	12.38
0.005% indoxacarb 55 L/ha	-2	0.00	0.06
	5	0.00	65.91
	10	0.02	0.96
	31	0.00	6.58

[1]Method detection limit = 0.03 ng/g dry weight.
[2]Method detection limit = 0.05 ng/g dry weight.
[3]31 days after the second bait application, but 46 days after the first application.

Table 3-13. Pesticide residues in nectar sampled in the YCA efficacy plots.

Treatment	days post application	dinotefuran residue (ng/g)[1]	indoxacarb residue (ng/g)[2]
0.05% dinotefuran 55 L/ha	-2	0.00	0.00
	5	0.00	0.00
	10	0.00	0.00
	31	0.00	0.00
0.005% dinotefuran 55 L/ha	-2	0.00	0.00
	5	0.00	0.00
	10	0.00	0.00
	31	0.00	0.00
0.05% indoxacarb 55 L/ha	-2	0.00	0.00
	5	0.00	0.00
	10	0.00	0.00
	31[3]	0.00	0.00
0.005% indoxacarb 55 L/ha	-2	0.00	0.00
	5	0.00	0.00
	10	0.00	0.00
	31	0.00	0.00

[1]Method detection limit = 0.05 ng/g.
[2]Method detection limit = 0.05 ng/g.
[3]31 days after the second bait application, but 46 days after the first application.

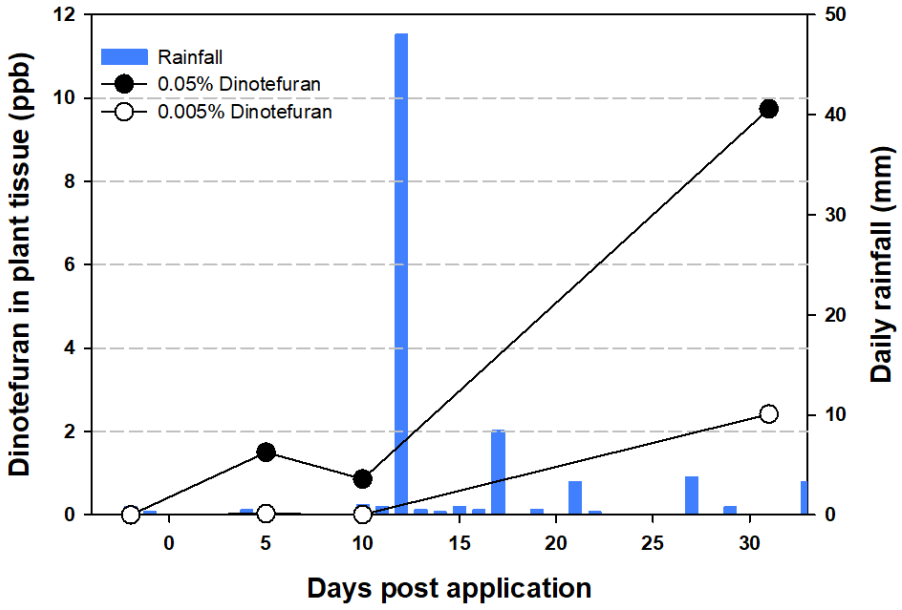

Figure 3-24. Dinotefuran residues in plant tissues relative to time post treatment in the first YCA efficacy trial at JCNWR. Both plots were treated a single time on day 0. Also shown are daily rainfall totals for Kahuku, O‘ahu (rainfall data from National Climatic Data Center).

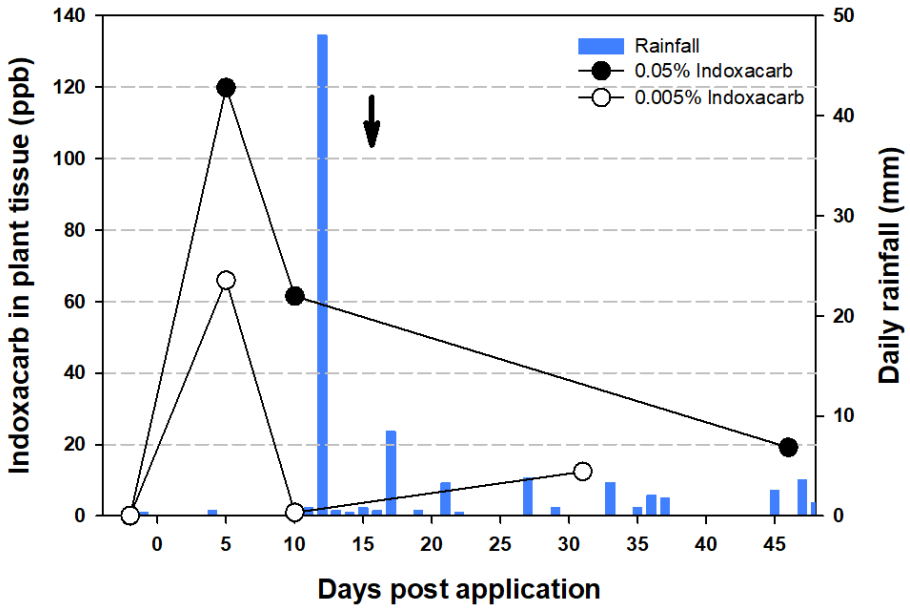

Figure 3-25. Indoxacarb residues in plant tissues relative to time post treatment in the first YCA efficacy trial at JCNWR. Both plots were treated on day 0, and the 0.05% indoxacarb plot was treated a second time on day 15 (arrow). Also shown are daily rainfall totals for Kahuku, O‘ahu (rainfall data from National Climatic Data Center).

Dinotefuran and indoxacarb residues in soil and water samples collected in the YCA efficacy trials are reported in Tables 3-14 and 3-15. Dinotefuran residues in soil were generally lower than in plant tissues, with all measurements less than 2 ppb (Table 3-14). This likely reflects the high solubility of dinotefuran, suggesting that most of the pesticide is translocated in water and little adsorbs to soil. Indoxacarb residues in soil were generally less than or equal to approximately 5 ppb, with the exception of one soil sample that had concentrations over 2 ppm (Table 3-14). It is likely that this sample contained one or more water storing granules with undispersed pesticide.

Dinotefuran concentrations of up to 2 ppt and 15 ppt were measured in freshwater and sea water samples, respectively, collected near the YCA efficacy plots at JCNWR (Table 3-15). Curiously, one measurement of 6 ppt was made in a sea water sample collected prior to bait application. It is possible, but seemingly unlikely, that this could have resulted from very small scale pesticide repellency testing conducted near the site approximately 10 months prior. Alternatively, it could reflect general environmental contamination from commercial pesticide use unrelated to the study. For example, imidacloprid and several other pesticides not used in this study were also detected in the water samples. Indoxacarb was detected in only one sea water sample, at a concentration of 4 ppt (Table 3-15).

Table 3-14. Pesticide residues in soil sampled in the YCA efficacy plots.

Treatment	days post application	dinotefuran residue (ng/g)[1]	indoxacarb residue (ng/g)[1]
0.05% dinotefuran 55 L/ha	-9	0.00	0.00
	10	1.51	0.00
	31	1.41	0.00
	90	0.45	0.00
0.005% dinotefuran 55 L/ha	-9	0.00	0.00
	10	0.18	0.00
	31	0.68	0.00
	90	0.02	0.00
0.05% indoxacarb 55 L/ha	-9	0.00	0.00
	10	0.00	4.21
	31[2]	0.00	0.33
	90[3]	0.00	5.10
0.005% indoxacarb 55 L/ha	-9	0.00	0.00
	10	0.00	0.05
	31	0.00	2259.65
	90	0.00	0.00

[1]Method detection limit = 0.05 ng/g dry weight.
[2]31 days after the second bait application, but 46 days after the first application
[3]90 days after the second bait application, but 105 days after the first application

Table 3-15. Pesticide residues in water sampled near the YCA efficacy plots.

Sampling point	days post application	dinotefuran residue (µg/L)[1]	indoxacarb residue (µg/L)[1]
Freshwater 1	-9	0.000	0.000
	10	0.001	0.000
	31	0.000	0.000
Freshwater 2	-9	0.000	0.000
	10	0.001	0.000
	31	0.002	0.000
Freshwater 3	-9	0.000	0.000
	10	0.000	0.000
	31	0.000	0.000
Seawater 1	-9	0.006	0.000
	10	0.001	0.000
	31	0.001	0.000
Seawater 2	-9	0.000	0.000
	10	0.015	0.000
	31	0.015	0.000
Seawater 3	-9	0.000	0.000
	10	0.001	0.000
	31	0.003	0.004

[1]Method detection limit = 0.002 µg/L.

Residues of indoxacarb, thiamethoxam, and two thiamethoxam metabolites (thiamethoxam urea and clothianidin) in plant tissues sampled in the AA efficacy plots treated with polyacrylamide granules at HALE are reported in Table 3-16. Thiamethoxam (and metabolite) residues in plant tissues composited across entire plots were less than or equal to 0.21 ng/g, or ppb. Indoxacarb residues in plant tissues were higher than those of thiamethoxam, up to approximately 150 ppb, even accounting for the 100-fold difference in formulation concentration (Table 3-16).

Residues of thiamethoxam and metabolites in soil samples from AA efficacy plots were also very low, with all measurements less than 0.2 ppb (Table 3-17). Indoxacarb residues in soil samples were lower than in plant tissues, being less than 8 ppb in all samples tested (Table 3-17).

Table 3-16. Pesticide residues in plant tissues sampled in the AA efficacy plots. Concentrations reported in terms of fresh plant tissue weight.

Treatment	**days post application**	**indoxacarb residue (ng/g)[1]**	**thiamethoxam residue (ng/g)[2]**	**thiamethoxam urea residue (ng/g)[3]**	**clothianidin residue (ng/g)[4]**
0.05% indoxacarb 55 L/ha	-8	0.00	0.00	0.00	0.00
	5	12.31	0.00	0.00	0.00
	10	148.88	0.00	0.00	0.00
	30	5.76	0.00	0.00	0.00
0.05% indoxacarb 25 L/ha	-8	0.00	0.00	0.00	0.00
	5	95.51	0.00	0.00	0.00
	10	1.73	0.00	0.00	0.00
	30	83.94	0.00	0.00	0.00
0.0005% thiamethoxam 55 L/ha	-8	0.00	0.00	0.00	0.00
	5	0.15	0.05	0.00	0.00
	10	0.27	0.00	0.00	0.00
	30	0.00	0.00	0.00	0.00
0.0005% thiamethoxam 25 L/ha	-8	0.00	0.00	0.00	0.00
	5	0.00	0.21	0.13	0.00
	10	0.00	0.00	0.00	0.00
	30	0.00	0.00	0.00	0.00

[1]Method detection limit = 0.18 ng/g dry weight.
[2]Method detection limit = 0.3 ng/g dry weight.
[3]Method detection limit = 0.1 ng/g dry weight.
[4]Method detection limit = 0.09 ng/g dry weight.

Table 3-17. Pesticide residues in soil sampled in the AA efficacy plots.

Treatment	days post application	indoxacarb residue (ng/g)[1]	thiamethoxam residue (ng/g)[1]	thiamethoxam urea residue (ng/g)[1]	clothianidin residue (ng/g)[1]
0.05% indoxacarb 55 L/ha	-8	0.00	0.00	0.00	0.00
	10	1.15	0.00	0.00	0.00
	30	4.15	0.00	0.00	0.00
	90	2.54	0.00	0.00	0.00
0.05% indoxacarb 25 L/ha	-8	0.00	0.00	0.00	0.00
	10	7.70	0.00	0.00	0.00
	30	2.54	0.00	0.00	0.00
	90	1.91	0.00	0.00	0.00
0.0005% thiamethoxam 55 L/ha	-8	0.00	0.00	0.00	0.00
	10	0.00	0.00	0.00	0.00
	30	0.00	0.08	0.15	0.00
	90	0.00	0.02	0.00	0.00
0.0005% thiamethoxam 25 L/ha	-8	0.00	0.00	0.00	0.00
	10	0.00	0.00	0.00	0.00
	30	0.00	0.05	0.04	0.00
	90	0.00	0.00	0.00	0.00

[1]Method detection limit = 0.05 ng/g dry weight.

DISCUSSION

Aspects related to efficacy

Drying rates and period of attractiveness

The WSG granules dried more quickly than anticipated under all three scenarios tested. Prior studies reported mean T50 times (time until 50% water loss) of roughly 2 to 15 hours or longer for polyacrylamide crystals or alginate beads, depending on relative humidity and substrate conditions (Buczkowski et al. 2014a, Rust et al. 2015, Tay et al. 2017). In comparison, the WSG tested here exhibited median T50 times of under 2 hours in almost all cases. The prior studies may have underestimated rates of evaporation under field conditions because they either used small clumps of granules, which would typically break apart into more rapidly drying individual granules upon impact if they are broadcast, or were conducted in desiccation chambers lacking wind and solar exposure. The results in this study suggest that if T50 times are a reliable indicator of period of attractiveness, WSG should have surprisingly short periods of activity under field conditions. This should be especially true for alginate beads and TVP, which exhibited much shorter median T50 times than polyacrylamide, and also had a much narrower range of T50 times in a typical batch of granules. The poorer water retention performance of alginate beads was related to their smaller size, whereas TVP lost water more rapidly per unit size than polyacrylamide.

Despite this poor predicted performance, all three WSG types yielded good results in field efficacy trials (see below). This discrepancy may indicate that 1) ants continue to feed substantially on WSG even after 50% water loss, 2) that a sufficient portion of broadcast granules fall in shaded or other sheltered locations that slow evaporation rates, 3) that consumption of the bait prior to the T50 time is sufficient to achieve good control, or 4) a combination of these is true. Nevertheless, any provisions that slow rates of evaporation, such as treatment under humid conditions or in the late afternoon for nocturnally active ants, could be expected to increase efficacy.

Bait preference among WSG

There was not much evidence of strong preference for any of the WSG types for any of the ant species tested. The rate of decline in attractiveness of the WSG was faster for AA than YCA, perhaps because of faster drying under drier conditions at HALE. The increasing recruitment over time for LFA was unexpected, but may suggest that WSG will have a longer period of activity with LFA in humid regions like Puna. Overall, the trials suggest that all three WSG types should work well as carriers of the sucrose bait from the perspective of palatability.

Pesticide repellency

The pesticide repellency trials suggest that YCA is quite sensitive to thiamethoxam, while indoxacarb and dinotefuran are not repellant to YCA until concentrations are relatively high. This is consistent with poor results using thiamethoxam and good results using dinotefuran against YCA on Johnston Atoll (Peck et al. 2016). In contrast, AA appear to be quite sensitive to dinotefuran, but exhibited much lower repellency to indoxacarb and thiamethoxam. Good results with thiamethoxam have previously been obtained for AA in California and South Africa (Buczkowski et al. 2014b, Rust et al. 2015, Boser et al. 2017). Based on the repellency results, efficacy tests for YCA in Hawai‘i focused on formulations with indoxacarb and dinotefuran, whereas efficacy tests for AA focused on formulations with indoxacarb and thiamethoxam. Repellency tests with LFA suggest that indoxacarb, and possibly dinotefuran, would be good candidate AI’s with which to conduct efficacy tests because of relatively low repellency, whereas LFA exhibited strong repellency towards thiamethoxam.

Efficacy of WSG for controlling ants

The first AA efficacy trial suggested that both thiamethoxam at 0.0005% concentration and indoxacarb at 0.05% concentration are highly effective at reducing ant densities: numbers dropped sharply (>98%) after a single application in all six plots testing these formulations. Although reductions in plots treated with indoxacarb at the lower concentration of 0.005% were not significantly different from the other two formulations, the number of replications with each formulation, and thus statistical power to detect differences, was low. The average reduction of 87% across the three 0.005% indoxacarb plots may represent substantial differences in control relative to the other two formulations. Although mean reduction increased to 94% after the second application, the lower concentration indoxacarb formulation may still be a less effective option.

The second AA efficacy trial confirmed that a 0.05% indoxacarb formulation is highly effective in reducing AA numbers. Three replicate plots of this formulation all yielded good control. This trial also found that a reduction of the application rate by over 50% (25 L/ha vs. 55 L/ha) did not decrease the effectiveness of this formulation. The latter result indicates that less materials, including pesticide, may be used, depending on the situation and management goal. In contrast, the 0.0005% thiamethoxam formulation yielded significantly poorer control of AA, averaging about 80% reduction in ant numbers, than the indoxacarb formulation, regardless of application rate. This result conflicted with results in the first trial, in which all three plots treated with 0.0005% thiamethoxam reduced AA numbers by over 95%. The reason for this discrepancy is unclear, but could be related to spatial variation in ant densities and/or dietary needs. The plots treated with thiamethoxam in the second trial had relatively lower ant densities prior to treatment, which may have influenced their foraging behavior and hence their response to the bait. The combined efficacy results from both trials suggest that the 0.0005% thiamethoxam formulation may yield good but somewhat inconsistent results for AA, relative to 0.05% indoxacarb.

For YCA, both concentrations of dinotefuran tested in the first trial (0.05% and 0.005%) yielded good results, with >90% reductions in ant numbers with a single application. Multiple applications would be necessary to achieve eradication, and this was not tested here. Dinotefuran has been applied previously in polyacrylamide granules at 0.05% concentration at Johnston Atoll, where eradication is the goal, with highly promising results to date (Peck et al. 2016, 2017; S. Plentovich, USFWS, pers. comm.). The results from the present study suggest that a concentration of 0.005% may be similarly effective for YCA. Reductions in ant numbers using 0.005% dinotefuran averaged 94.5% in the first efficacy trial and 88.1% in the second efficacy trial, when applied at 55 L/ha. When this formulation was applied at the lower rate of 25 L/ha, efficacy dropped slightly to 84.5% reduction in ant numbers following treatment. Reducing dinotefuran concentration even further to 0.0005% generally yielded poorer results, especially at the lower application rate of 25 L/ha (66.3% reduction in ant numbers following treatment). Collectively, the two trials suggest that reducing dinotefuran concentration below 0.005% will reduce the level of YCA control to some degree. The 0.0005% dinotefuran formulation therefore appears to be less effective against YCA than the same concentration of thiamethoxam against AA. This difference may result from differences in the two neonicotinoid compounds, from differences in the biology of YCA and AA, or from both factors.

For YCA, the lower application rate of 25 L/ha provided statistically similar control to the higher rate of 55 L/ha across two dinotefuran formulations tested (0.005% and 0.0005%), however, a larger sample size may have found these to be statistically different. Overall, the two efficacy trials suggest a progressively lower level of YCA control with decreasing amounts of dinotefuran applied. The optimal concentration and application rate will therefore depend in part on whether the management goal is ant suppression or eradication.

In contrast, indoxacarb formulations generally performed more poorly against YCA. While the higher concentration indoxacarb formulation (0.05%) tested was not significantly different from the dinotefuran formulations, the mean percent reduction in ant numbers (75.8%) after a single application was substantially lower, and would likely be significantly different from the dinotefuran formulations with a larger sample size. A second application of the 0.05% indoxacarb formulation yielded generally similar results. A clear knock-down effect occurred immediately after treatment, but ant numbers rebounded relatively quickly. Relative to the dinotefuran formulations, indoxacarb was apparently not able to kill enough ants to prevent

renewed active foraging from surviving nests and/or rapid recolonization by nests outside the plots. The poorer indoxacarb results with YCA relative to AA may be attributed in part to extremely high densities of YCA at the study site, or perhaps to the larger size of this ant or other biological differences between the species.

Neither of the efficacy trials that tested different WSG granule types found significant differences in their performance, in terms of reductions in numbers of either AA or YCA. However, mean percent reductions were inversely related to drying rates of the granule types for both ant species following most applications, with reductions generally following the pattern of highest with polyacrylamide, intermediate with alginate, and lowest with tvp. This may suggest that there are small differences in efficacy among the granule types that could be tied to differences in drying rates and hence longevity of attractiveness. Yet, such differences were minor, particularly for the most effective AI formulations, perhaps owing to the reasons enumerated in the section on drying rates above. Overall, the efficacy trials indicate that all three types of WSG can successfully deliver sugar water baits laced with pesticides to ants, and yield good results when formulated at the right concentrations with the right AI.

Other considerations

Polyacrylamide was by far the easiest and cheapest WSG type to use. Whereas only 20 g of polyacrylamide crystals are needed to absorb 1 L of sucrose bait, at least 350 g of TVP is needed to absorb the same volume. For a single application at the higher rate used in this study (55 L bait/ha), this translates to approximately $32/ha for the polyacrylamide crystals used in this study, compared to approximately $183/ha for the TVP used (including shipping). The additional weight and volume of the TVP carrier is another disadvantage. Alginate beads cost approximately $240/ha for materials, but that does not include the considerable labor time needed to produce them. In their current state of development, alginate beads also need to be manufactured fresh for each application, have a short shelf life once manufactured, and present additional logistical challenges compared to the other two granule types.

The main advantage of both the alginate beads and TVP over polyacrylamide is their biodegradable characteristics. Alginate beads disintegrate rapidly in the field, and TVP can be expected to break down fairly quickly as well. The longevity of polyacrylamide granules in the field is unknown, but is clearly longer than the other two granule types, and some of the degradates of polyacrylamide are deemed toxic (Tay et al. 2017). The longer persistence of polyacrylamide granules, however, could increase their efficacy for ant control if they can reabsorb moisture from the environment after their initial application and desiccation, and thereby regain some activity (Peck et al. 2016).

Non-target risks

Attraction to and consumption of WSG baits by non-target insects

The video observation data suggest that WSG granules that fall to the ground pose relatively low risk to common pollinating insects, as there were few visits by bees, moths and hover flies to baits placed on the ground. However, baits on the ground do attract flies, especially the TVP bait which has a strong odor. WSG that lodge in the vegetation near flowers, in contrast,

pose a much higher risk to pollinating insects. Granules located immediately adjacent to flowers attracted many visits by bees, including native and non-native solitary bees and non-native honey bees. Moths and wasps were also relatively common visitors to the granules. When discovered, these insects clearly fed on the granules, in many cases extensively. However, the fact that granules were visited more often on plant species that received higher rates of flower visitation suggests that insects discovered the granules not because they were strongly attractive, but because they were located near a primary source of attraction (flowers). This might suggest that the vast majority of granules that lodge in vegetation at some distance from flowers will not often be discovered by pollinators and other flying insects, which could be tested with further observations.

The latter hypothesis is supported by the results of the non-target broadcast plots. Frequency of consumption of the broadcast baits, as judged by detection of the protein marker placed in the baits, was low among most insect groups including bees and other common pollinators. This likely occurred because most of the broadcast granules were observed to fall to the ground, and relatively little lodged near flowers. Several taxa, however, appeared to find and consume the baits much more readily, in particular the recently detected non-native wasp in the genus *Bembecinus*. Hence, some mortality of non-target insects, including native species, through direct consumption of baits can inevitably be expected. The magnitude of this non-target risk to pollinators may be reduced by broadcasting the baits, when possible, in such a way as to minimize lodging in vegetation frequented by flower-visiting insects. The ability to do this will vary according to habitat type. All of the non-target broadcast plots were located in areas with relatively little flowering ground-cover vegetation. In areas supporting abundant flowering ground-cover, discovery of WSG by pollinators may be substantially higher.

Attraction to and consumption of WSG baits by birds

Efforts to assess attraction to WSG baits by birds were inconclusive. It is safest to assume that some types of birds will consume at least some of the bait, even if they are not strongly attracted to it. Chickens were observed to eat some of the baits in the bait preference tests, and crows feed on polyacrylamide baits used in Australia (B. Hoffmann, CSIRO Australia, pers. comm.). Risk of WSG baits to birds is therefore best assessed by the toxicity of the active ingredients used in them. Although completely speculative at this point, the stronger odor and organic nature of TVP granules might make them more attractive to birds than the polyacrylamide or alginate granules.

Indirect exposure via pesticide residues

Residues of the two neonicotinoid pesticides tested, dinotefuran and thiamethoxam, were generally relatively low in and around the field efficacy plots. Residues tended to be highest in plant tissues, and were higher when bait formulations used higher concentrations of the AI. The highest plant tissue residues were measured in plots treated with the 0.05% dinotefuran formulation. These values, on the order of <10 ppb, are much lower than values reported from direct neonicotinoid application methods such as seed treatments (e.g. approximately 5-50 ppm of clothianidin at peak in shoots of corn seedlings, Alford and Krupke 2017; 12.4 ppm of imidacloprid in sugar beet leaves, Bonmatin et al. 2015), sprays (e.g. up to 182 ppm for single application spray on tomato, Karmakar and Kulshrestha 2009), or soil drenches, which generally

result in higher tissue residue values than seed treatments (Pisa et al. 2015). However, these values are of similar orders of magnitude as values reported in pollen and nectar of wildflowers growing near crop fields treated with neonicotinoids. For example, Botias et al. (2015) measured residue values of <0.12 to >86 ppb of four neonicotinoids in pollen and <0.03 to 1.80 ppb of the same compounds in nectar of wildflowers. Similarly, Mogren and Lundgren (2016) measured mean concentrations of clothianidin in tissues of wildflowers to range from approximately 1 to 14 ppb in leaves, 0.2 to 1.6 ppb in nectar, and 25 to 42 ppb in bee bread (bee-processed pollen). In a review, Sanchez-Bayo and Goka (2014) reported average dinotefuran residue values of 45 ppb in plant pollen and 14 ppb in nectar. No residues could be detected in the nectar samples collected in the WSG efficacy plots. Because residues in pollen can be higher than in other tissues (Mogren and Lundgren 2016), however, the residues reported for composite plant tissues or for nectar may underestimate concentrations occurring in plant pollen in the treated plots.

The higher neonicotinoid concentrations measured in plant tissues in this study are generally considered of low risk to honey bees on the basis of acute oral or topical toxicity (Sanchez-Bayo and Goka 2014, Botias et al. 2015), but are within the range of values that have been suggested to cause a variety of sub-lethal impacts on honey bees (Pisa et al. 2015, Mogren and Lundgren 2016). Solitary bees may be more sensitive to neonicotinoids than honeybees, although fewer data exist on this topic (Pisa et al. 2015). As lower concentrations of the two neonicotinoid pesticides are formulated in WSG baits, such risks presumably decrease. Formulations that used 0.005% or 0.0005% AI had composite plant tissue residue values of <2.5 ppb and <0.25 ppb, respectively.

Neonicotinoid residues in soil samples were generally lower than in plant tissue samples, all being <2 ppb, likely reflecting their high water solubility. Insects coming into contact with neonicotinoids in the soil may be adversely affected, depending on the concentration. For example, Lepidoptera pupating in the soil may have been impacted by imidacloprid soil drenches and soil injections in a study by Dilling et al. (2009), although the soil concentrations were not measured and presumably were substantially higher than the levels reported here. Similarly, direct exposure of crabronid wasp prepupae to “field-realistic concentrations” of imidacloprid and thiamethoxam severely reduced survival and rates of adult eclosion (Heneberg et al. 2020). However, the application rates used in the latter study (126 to 1184 ng/cm^2 in laboratory plate wells) are difficult to relate to the soil concentrations measured in the WSG field plots.

Dinotefuran concentrations of up to 2 ppt and 15 ppt were measured in freshwater and sea water samples, respectively, near the YCA efficacy plots at JCNWR. Dinotefuran is reported to be practically non-toxic to freshwater and estuarine/marine fish (EC50 > 99.1 ppm for several fish) and freshwater invertebrates (EC50 > 968.3 ppm for *Daphnia magna*) (EPA 2004). Dinotefuran is classified as highly toxic to estuarine/marine invertebrates based on the EC50 of 0.79 ppm for the mysid shrimp *Mysidopsis bahia* (EPA 2004). However, the latter value is more than 10^4 times higher than the highest concentration measured in the water samples in the present study.

Surprisingly, indoxacarb residues were considerably higher than residues of either neonicotinoid in plant tissues, even after accounting for differences in bait formulation concentrations. This was true in efficacy plots conducted at both JCNWR and HALE. Because of the higher solubility of neonicotinoids, the opposite pattern was expected. Indoxacarb is not generally reported to be a systemic pesticide, and it is possible that the residues measured in plant tissues resulted in part from residual bait that dried on leaf surfaces when granules impacted vegetation before falling to the ground during the broadcast applications. On the other

hand, Sanchez-Bayo and Goka (2014) reported average indoxacarb residue values of 108 ppb in plant pollen, which is in line with concentrations measured for composite plant tissues in the present study.

Impacts of indirect exposure of pollinators to indoxacarb via plant tissue residues are not well documented in comparison to neonicotinoids, but acute contact and oral toxicities of indoxacarb to honey bees are reported to be 0.59 and 16 µg per bee, respectively, which are considerably higher than the same values for dinotefuran (0.049 and 0.022 µg per bee for contact and oral toxicity, respectively) or for thiamethoxam (0.02 and 0.005 µg per bee for contact and oral toxicity, respectively) (Sanchez-Bayo and Goka 2014). Indoxacarb is considered highly toxic to bees by contact, but practically non-toxic by dietary intake (CDPR 2006).

Except for one soil sample that had a value of over 2 ppm, likely because it contained one or more undegraded water storing granules, indoxacarb residues in soil were less than 8 ppb at both sites. This was also unexpected, given that indoxacarb has a relatively low water solubility and high tendency to become immobile in soil (CDPR 2006).

Indoxacarb was detected in only one sea water sample, at a concentration of 4 ppt. This is considerably lower than the acute toxicity values (LC50) for a variety of freshwater and estuarine/marine fish and invertebrates (all > 0.024 mg/L, or 24 ppb; CDPR 2006).

The pesticide residue values reported here should not be taken as precise estimates or predictions for future bait applications, because pesticide behavior and persistence in the environment is dependent on many complex factors. Rates of uptake and degradation of systemic pesticides can differ strongly among plant growth forms and species, for example (Bonmatin et al. 2015). Similarly, movement of pesticides in ground water depends on timing and size of rainfall events, as well as other edaphic and hydrological factors (Bonmatin et al. 2015). Degradation rates also depend on degree of exposure to sun and water, soil properties, and on other factors (Bonmatin et al. 2015). The residues measured in the present study for the pesticides tested should therefore be viewed as only approximate guidelines for their behavior in the environment when broadcast in WSG baits.

ACKNOWLEDGMENTS

Matching funding for portions of this work was provided by the Hawai‘i Department of Agriculture and the Hawai‘i Invasive Species Council. Maria Aihara-Sasaki supervised work conducted under Hawai‘i experimental use permits and assisted with ant surveys. Molly O’Grady assisted with ant surveys and experimental bait applications. Michelle Montgomery and the Hawai‘i Ant Lab conducted the studies involving little fire ants. Daniel Snow of the Water Sciences Laboratory at the University of Nebraska performed the pesticide residue analyses. Max Gatlin, Isabel Hernandez, Miho Maeda, Taryn Nakamura, Marie Pagkalinawan, Lois Taylor and Lesley Umeda reviewed the non-target video footage and/or sorted non-target arthropod samples. James Hagler provided much helpful advice on the protein marking and ELISA methodology, and Mike Melzer provided additional advice, materials, and the use of his lab to conduct the ELISA analysis. Jia-Wei Tay provided alginate beads for initial study components. Bob Peck gave helpful advice on hydrogel methods. Karl Magnacca collected picture-winged flies for bait attraction trials. The Rubinoff lab at the University of Hawai‘i at Mānoa kindly identified a *Hyposmocoma* species. Regulatory oversight and guidance on experimental pesticide use was provided by the Hawai‘i Department of Agriculture Pesticides Branch. Land access and permitting was generously provided by Schofield Barracks Army Base, Marine Corps Base Hawai‘i, Pacific Missile Range Facility, Haleakalā National Park, James Campbell National Wildlife Refuge, the US Fish and Wildlife Service, the Hawai‘i Department of Land and Natural Resources Natural Area Reserve System and Division of State Parks. Permitting, access and field logistics were greatly facilitated by Kapua Kawelo, Karl Magnacca, Lance Bookless, Cathy Tarutani-Weissman, Greg Takeshima, Cynthia King, Chris Miller, Jeff Burgett, Glenn Klingler, Bethany Chagnon, Kelly Goodale, Linette Makua, and Brooke McFarland.

LITERATURE CITED

Alford, A., and C.H. Krupke. 2017. Translocation of the neonicotinoid seed treatment clothianidin in maize. PLoS ONE 12: e0173836.

Bonmatin, J.M., C. Giorio, V. Girolami, D. Goulson, D.P. Kreutzweiser, C. Krupke, M. Liess, E. Long, M. Marzaro, E.A.D. Mitchell, D.A. Noome, N. Simon-Delso, and A. Tapparo. 2015. Environmental fate and exposure; neonicotinoids and fipronil. Environmental Science and Pollution Research 22: 35-67.

Boser, C. L., C. Hanna, K. R. Faulkner, C. Cory, J. M. Randall and S. A. Morrison. 2014. Argentine ant management in conservation areas: results of a pilot study. Monographs of the Western North American Naturalist 7:518–530.

Boser, C.L., C. Hanna, D.A. Holway, K.R. Faulkner, I. Naughton, K. Merrill, J.M. Randall, C. Cory, D.-H. Choe, and S.A. Morrison. 2017. Protocols for Argentine ant eradication in conservation areas. Journal of Applied Entomology doi:10.1111/jen.12372.

Botías, C., David, A., Horwood, J., Abdul-Sada, A., Nicholls, E., Hill, E. and Goulson, D. 2015. Neonicotinoid residues in wildflowers, a potential route of chronic exposure for bees. Environmental Science & Technology 49: 12731-12740.

Buczkowski, G., and G.W. Bennett. 2006. Dispersed central-place foraging in the polydomous odorous house ant, *Tapinoma sessile* as revealed by a protein marker. Insectes Sociaux 53: 282-290.

Buczkowski, G., and P. Krushelnycky. 2012. The odorous house ant, *Tapinoma sessile*, as a new temperate-origin invader. Myrmecological News 16: 61-66.

Buczkowski, G., E. Roper, and D. Chin. 2014a. Polyacrylamide hydrogels: an effective tool for delivering liquid baits to pest ants (Hymenoptera: Formicidae). Journal of Economic Entomology 107: 748-757.

Buczkowski, G., E. Roper, D. Chin, N. Mothapo, and T. Wossler. 2014b. Hydrogel baits with low-dose thiamethoxam for sustainable Argentine ant management in commercial orchards. Entomologia Experimentalis et Applicata 153: 183-190.

CDPR. 2006. Environmental fate of indoxacarb. California Department of Pesticide Regulation, Environmental Monitoring Branch. 20 pp.

DeGrandi-Hoffman, G., and J. Hagler. 2000. The flow of incoming nectar through a honey bee (*Apis mellifera* L.) colony as revealed by a protein marker. Insectes sociaux 47: 302-306.

Dilling, C., P. Lambdin, J. Grant, and R. Rhea. 2009. Community response of insects associated with Eastern Hemlock to imidacloprid and horticultural oil treatments. Environmental Entomology 38: 53-66.

EPA. 2004. Pesticide Fact Sheet for Dinotefuran. 63 pp.

Giambelluca, T.W., Q. Chen, A.G. Frazier, J.P. Price, Y.-L. Chen, P.-S. Chu, J.K. Eischeid, and D.M. Delparte, 2013. Online Rainfall Atlas of Hawai'i. *Bull. Amer. Meteor. Soc.* 94, 313-316, doi: 10.1175/BAMS-D-11-00228.1.

Graham, J.R. 2018. Recovering populations of Hawaiian yellow-faced bees. 2017 Year-end Report for Hawaii Department of Land and Natural Resources, Division of Forestry and Wildlife.

Graham, J.R., and C.B.A. King. 2016. Developing captive propagation methodologies for five native Hawaiian yellow-faced bee species: a novel approach to endangered species mitigation. Department of Defense Legacy Program Project 13-104 Final Report.

Gruner, D.S., R.A. Heu, and M.E. Chun. 2003. Two ant species (Hymenoptera: Formicidae) new to the Hawaiian Islands. Bishop Museum Occasional Papers 74: 35-40.

Hagler, J.R. 1997. Field retention of a novel mark-release-recapture method. Environmental Entomology 26: 1079-1086.

Hagler, J.R., S.E. Naranjo, S.A. Machtley, and F. Blackmer. 2014. Development of a standardized protein immunomarking protocol for insect mark-recapture dispersal research. Journal of Applied Entomology 138: 772-782.

Hartley, S., P.D. Krushelnycky and P.J. Lester. 2010. Integrating physiology, population dynamics and climate to make multi-scale predictions for the spread of an invasive insect: the Argentine ant at Haleakala National Park, Hawaii. Ecography 33: 83-94.

HHFP (Helber Hastert & Fee Planners, Inc.). 2010. Integrated Natural Resources Management Plan, Pacific Missile Range Facility. Prepared for the Commander, Navy Region Hawaii. 270 pp.

Heneberg, P., P. Bogusch, A. Astapenkova, and M. Rezac. 2020. Neonicotinoid insecticides hinder the pupation and metamorphosis into adults in a crabronid wasp. Scientific Reports 10: 7077, doi.org/10.1038/s41598-020-63958-w

Hoffmann, B.D., G.M. Luque, C. Bellard, N.D. Holmes, and C.J. Donlan. 2016. Improving invasive ant eradication as a conservation tool: a review. Biological Conservation 198: 37-49.

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

Hopper, D.R. 2002. The reproductive biology and conservation of the endangered, Hawaiian legume, *Sesbania tomentosa*, with emphasis on its pollination system. PhD Dissertation, University of Hawaii at Manoa.

Karmakar, R., and G. Kulshrestha. 2009. Persistence, metabolism and safety evaluation of thiamethoxam in tomato crop. Pest Management Science 65: 931-937.

Krushelnycky, P.D. 2015. Ecology of some lesser-studied introduced ant species in Hawaiian forests. Journal of Insect Conservation 19: 659-667.

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

Krushelnycky, P.D., L.L. Loope, and N.J. Reimer. 2005. The ecology, policy, and management of ants in Hawaii. Proc. Hawaii Entomol. Soc. 37: 1-25.

Krushelnycky, P.D., Ogura-Yamada, C.S., Kanegawa, K.M., Kaneshiro, K.Y. and Magnacca, K.N. 2017. Quantifying the effects of an invasive thief ant on the reproductive success of rare Hawaiian picture-winged flies. Biological Conservation 215: 254-259.

Lach, L., and L.M. Hooper-Bùi. 2010. Consequences of Ant Invasions. pp. 261-286, *In* L. Lach, C.L. Parr, and K.L. Abbott (eds.), Ant Ecology. Oxford University Press, Oxford, UK.

Magnacca, K.N. 2007. Conservation status of the endemic bees of Hawaii, *Hylaeus* (*Nesoprosopis*) (Hymenoptera: Colletidae). Pacific Science 61: 173-190.
Magnacca, K. 2014. Chapter 5: *Drosophila* Species Management. In OANRP 2014 Year-End Report.

Magnacca, K. 2017. MCBH Kaneohe *Hylaeus* Survey, November 2016 and May 2017. Unpublished report.

Mallipudi, N.M., S.J. Stout, A. Lee, and E.J. Orloski. 1986. Photolysis of Amdro fire ant insecticide active ingredient hydramethylnon (AC 217,300) in distilled water. J. Agric. Food Chem. 34: 1050-1057.

Mogren, C.L., and J.G. Lundgren. 2016. Neonicotinoid-contaminated pollinator strips adjacent to cropland reduce honey bee nutritional status. Scientific Reports 6: 29608.

NPIC. 2002. Hydramethylnon, General Fact Sheet. National Pesticide Information Center, 5 pp.

Ogura-Yamada, C.S., and P.D. Krushelnycky. 2016. Testing the attractiveness and efficacy of baits for the monitoring and control of the thief ant, *Solenopsis papuana*. Proc. Hawaii Entomol. Soc. 48: 95-108.

Ogura-Yamada, C.S., and P.D. Krushelnycky. 2020. The effects of the invasive thief ant, *Solenopsis papuana*, on ground-dwelling invertebrates in mesic forests of Hawai‘i. Journal of Insect Conservation 24: 151-162.

Peck, R.W., P.C. Banko, K. Donmoyer, S. Kropidlowski, and A. Pollock. 2016. Efforts to eradicate Yellow Crazy Ants on Johnston Atoll: results from crazy ant strike team IX, December 2014-June 2015. Technical Report HCSU-067, Hawaii Cooperative Studies Unit, University of Hawaii at Hilo.

Peck, R.W., P.C. Banko, K. Donmoyer, K. Scheiner, R. Karmi, and S. Kropidlowski. 2017. Efforts to eradicate Yellow Crazy Ants on Johnston Atoll: results from crazy ant strike team X, XI and XII (June 2015-December 2016). Technical Report HCSU-081, Hawaii Cooperative Studies Unit, University of Hawaii at Hilo.

Pisa, L.W., V. Amaral-Rogers, L.P. Belzunces, J.M. Bonmatin, C.A. Downs, D. Goulston, D.P. Kreutzweiser, C. Krupke, M. Liess, M. McField, C.A. Morrissey, D.A. Noome, J. Settele, N. Simon-Delso, J.D. Stark, J.P. Van der Sluijs, H. Van Dyck, and M. Wiemers. 2015. Effects of neonicotinoids and fipronil on non-target invertebrates. Environmental Science and Pollution Research 22: 68-102.

Plentovich, S. 2010. Survey of invasive ant species within Makua and Oahu Implementation plan management units, Oahu, Hawaii 2004 – 2009. Report to Oahu Army Natural Resources Program, Schofield Barracks, Hawaii.

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

Plentovich, S., J.R. Graham, W.P. Haines, and C.B.A. King. 2021. Invasive ants reduce nesting success of an endangered Hawaiian yellow-faced bee, Hylaeus anthracinus. NeoBiota 64: 137-154.

Reimer, N.J. 1994. Distribution and impact of alien ants in vulnerable Hawaiian ecosystems, pp. 11–22. *In* D.F. Williams (ed.), Exotic ants: biology, impact, and control of introduced species. Boulder, Colorado: Westview Press.

Rust, M.K., A. Soeprono, S. Wright, L. Greenberg, D.-H. Choe, C.L. Boser, C. Cory, and C. Hanna. 2015. Laboratory and field evaluations of polyacrylamide hydrogel baits against Argentine ants (Hymenoptera: Formicidae). J. Econ. Entomol. 108: 1228-1236.

Sanchez-Bayo, F., and K. Goka. 2014. Pesticide Residues and Bees – A Risk Assessment. PLoS ONE 9: e94482.

Sharaf, M.R., D. Gotzek, B. Guénard, B.L. Fisher, A.S. Aldawood, H.M. Al Dhafer, and A.A. Mohamed. 2020. Molecular phylogenetic analysis and morphological reassessments of thief ants identify a new potential case of biological invasions. Scientific Reports 10: 12040.

Snow, D. D., T. Damon-Powell, S. Onanong and D. A. Cassada. 2013. Sensitive and simplified analysis of natural and synthetic steroids in water and solids using on-line solid phase extraction and microwave-assisted solvent extraction coupled to liquid chromatography tandem mass

spectrometry atmospheric pressure photoionization. Analytical Bioanalytical Chemistry 405: 1759-1771.

Tay, J.-W., M.S. Hoddle, A. Mulchandani, and D.-H. Choe. 2017. Development of an alginate hydrogel to deliver aqueous bait for pest ant management. Pest Management Science 73: 2028-2038.

USEPA. 1986. Guidelines for establishing test procedures for the analysis of pollutants — Definition and Procedure for the Determination of the Method Detection Limit—Revision 1.11. Electronic Code of Federal Regulations Title 40: Protection of Environment (Part 136), Appendix B to Part 136—Definition and Procedure for the Determination of the Method Detection Limit—Revision 131.111.

USFWS. 2006. Endangered and threatened wildlife and plants; determination of status for 12 species of picture-wing flies from the Hawaiian Islands. Department of the Interior, Fish and Wildlife Service. 50 CFR Part 17, Federal Register Vol. 71, No. 89.

USFWS. 2016. Endangered and threatened wildlife and plants; endangered status for 49 species from the Hawaiian Islands. Department of the Interior, Fish and Wildlife Service. 50 CFR Part 17, Federal Register Vol. 81, No. 190.

Vander Meer, R.K., D.F. Williams, and C.S. Lofgren. 1982. Degredation of the toxicant AC 217,300 in Amdro imported fire ant bait under field conditions. J. Agric. Food Chem. 30: 1045-1048.

Vanderwoude, C., M. Montgomery, H. Forester, E. Hensley, and M.K. Adachi. 2016. The history of little fire ant *Wasmannia auropunctata* Roger in the Hawaiian Islands: spread, control, and local eradication. Proc. Hawaii Entomol. Soc. 48: 39-50.

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

Wilson, E.O. 1996. Hawaii: a world without social insects. Bishop Museum Occasional Papers 45: 3-7.

Zimmerman, E.C. 1970. Adaptive radiation in Hawaii with special reference to insects. Biotropica 2: 32-38.