Technical Report HCSU-040

DISTRIBUTION OF INVASIVE ANTS AND METHODS FOR THEIR CONTROL IN HAWAI`I VOLCANOES NATIONAL PARK

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TABLE OF **C**ONTENTS

LIST OF TABLES

LIST OF FIGURES

EXECUTIVE SUMMARY

The first invasive ants were detected in Hawai`i Volcanoes National Park (HAVO) more than 80 years ago. Ecological impacts of these ants are largely unknown, but studies in Hawai`i and elsewhere increasingly show that invasive ants can reduce abundance and diversity of native arthropod communities as well as disrupt pollination and food webs. Prior to the present study, knowledge of ant distributions in HAVO has primarily been restricted to road- and trail-side surveys of the Kīlauea and Mauna Loa Strip sections of the park. Due to the risks that ants pose to HAVO resources, understanding their distributions and identifying tools to eradicate or control populations of the most aggressive species is an important objective of park managers. We mapped ant distributions in two of the most intensively managed sections of the park, Mauna Loa Strip and Kahuku. We also tested the efficacy of baits to control the Argentine ant (*Linepithema humile*) and the big-headed ant (*Pheidole megacephala*), two of the most aggressive and ecologically destructive species in Hawai`i. Efficacy testing of formicidal bait was designed to provide park managers with options for eradicating small populations or controlling populations that occur at levels beyond which they can be eradicated.

Within the Mauna Loa Strip and Kahuku sections of HAVO we conducted systematic surveys of ant distributions at 1625 stations covering nearly 200 km of roads, fences, and transects between August 2008 and April 2010. Overall, 15 ant species were collected in the two areas, with 12 being found on Mauna Loa Strip and 11 at Kahuku. *Cardiocondyla kagutsuchi* was most widespread at both sites, ranging in elevation from 920 to 2014 m, and was the only species found above 1530 m. Argentine ants and big-headed ants were also found in both areas, but their distributions did not overlap. Surveys of Argentine ants identified areas of infestation covering 560 ha at Mauna Loa Strip and 585 ha at Kahuku. At both sites, upper boundaries of big-headed ants coincided with lower boundaries of Argentine ants. Significantly, *Wasmannia auropunctata* (little fire ant) was not detected during our surveys.

Formicidal baits tested for controlling Argentine ants included Xstinguish™ (containing fipronil at 0.01%), Maxforce® (hydramethylnon 1.0%), and Australian Distance® (pyriproxyfen 0.5%). Each bait was distributed evenly over four 2500 m^2 replicate plots. Applications were repeated approximately four weeks after the initial treatment. Plots were subdivided into 25 subplots and ants monitored within each subplot using paper cards containing tuna bait at approximately one week intervals for about 14 weeks. All treatments reduced ant numbers, but none eradicated ants on any of the plots. Xstinguish™ produced a strong and lasting effect, depressing ant abundance below 1% of control plot levels within the first week and for about eight weeks afterward. Maxforce® was slower to attain maximum effectiveness, reducing ants to 8% of control levels after one week and 3% after six weeks. Australian Distance® was least effective, decreasing ant abundance to 19% of control levels after one week with numbers subsequently rebounding to 40% of controls at four weeks and 72% at 10 weeks. In measurements of the proportion of bait cards at which ants were detected, Xstinguish™ clearly out-performed Maxforce®, reaching a minimum detection rate of 3% of bait cards at one week compared to a low of 19% for Maxforce® two weeks following the second treatment. Although ant abundances were dramatically reduced on Xstinguish™ plots, it is not currently registered for use in the USA. Our results suggest that ant abundance can be greatly reduced using registered baits, but further research is needed before even small-scale eradication of Argentine ants can be achieved.

Formicidal baits tested to control big-headed ants included Amdro® (hydramethylnon 0.75%), Xstinguish™ (fipronil 0.01%), Extinguish® Plus (a blend of hydramethylnon 0.365% and S-methoprene 0.25%), and Australian Distance® Plus (hydramethylnon 0.365% and pyriproxyfen 0.25%). Application methods were the same as used for Argentine ants, with baits being applied on two occasions (approximately four weeks apart) on four 2500 m² replicate plots. All four baits reduced populations to below 2% of control plot levels within one week of treatment. Amdro® was particularly effective as no ants were detected on two of the four Amdro® plots immediately following treatment. Suppression was long-lived in three of the treatments; Amdro®, Australian Distance® Plus, and Extinguish® Plus all maintained ant abundances at levels less than 1% of control plots over 12 weeks of study. In contrast, ant abundances in Xstinguish™ plots rose to 7% of control plots after four weeks and 20% after 10 weeks. Our results corroborate other recent studies indicating that small populations of big-headed ants can be controlled in natural areas using products registered in the USA.

GENERAL INTRODUCTION

Globally, invasive ants are destructive, habitat-altering organisms in many habitats that they invade. Because no clear evidence indicates that ants existed in the Hawaiian Archipelago prior to human contact, Hawaiian plants and animals may be uniquely vulnerable to their impacts by virtue of having evolved with few or none of these extraordinarily powerful and nearly ubiquitous ecosystem transformers. When highly predaceous ant species invade, native arthropods are often exterminated quickly, and plants may be deprived of their native pollinators (Perkins 1913, Cole *et al*. 1992). Hawaiian arthropods are renowned for unprecedented and spectacular examples of adaptive shifts, flightlessness, and adaptive radiation, and whole assemblages of native species lack effective defenses against many invasive ant species (Zimmerman 1948, Howarth and Mull 1992). Even native predators, such as spiders, are preyed upon by ants (Gillespie and Reimer 1993).

Since Western contact, nearly 60 ant species have been reported to have become established in the Hawaiian Islands (Reimer 1994, Krushelnycky *et al*. 2005b, Paul Krushelnycky personal communication). The date that ants were first introduced to Hawai`i is unknown, but at least one species had been established by 1825 (Bloxam 1925). Based on an extensive collection of Hawaiian arthropods made by R. C. L. Perkins in the 1890s, Forel (1899) listed 20 species of ants when summarizing the family Formicidae in *Fauna Hawaiiensis*, showing that a wide variety of ants had become established in the islands by that time. Unfortunately, the number of ant species in Hawai`i continues to grow, with the little fire ant (*Wasmannia auropunctata*) (Conant and Hirayama 2000) and *Tapinoma sessile* (Krushelnycky 2010) being among the most recent invaders. In general, dominant components of the Hawaiian ant fauna are comprised of highly vagile species that have widespread distributions within the Pacific Basin. Most of these species are tropical in origin and are restricted to elevations below 1500 m, but several species are found above 2000 m on the islands of Maui and Hawai`i (Reimer 1994, Wetterer *et al*. 1998, Krushelnycky 2010). Most of the Hawaiian ant fauna are found in dry and mesic forests, but a few species are able to penetrate native wet forests. Globally, *Linepithema humile* (hereafter Argentine ant) and *Pheidole megacephala* (hereafter big-headed ant) are recognized to be among the most ecologically destructive invasive ant species (Holway *et al*. 2002). Both of these ants are found in Hawai`i Volcanoes National Park (HAVO), but their distributions and impacts are poorly known.

Impacts of Argentine Ants and Big-headed Ants on Arthropod Communities
Argentine ants and big-headed ants share several characteristics that make them effective invaders of Hawaiian habitats: they are omnivorous; their colonies, which are comprised of multiple queens, are tolerant of other colonies and sometimes coalesce into supercolonies; and they are readily dispersed by man (Holway *et al*. 2002). Both species are highly aggressive and their expansive colonies allow them to dominate entire habitats and communities of invertebrates, profoundly impacting ecosystem structure and function.

In many areas of the world outside of Hawai`i, Argentine ants competitively displace native species of ants, particularly those with foraging behaviors similar to their own (Human and Gordon 1997, Holway 1998, Suarez *et al*. 1998, Sanders *et al*. 2001, Carpintero *et al*. 2005, Rowles and O'Dowd 2007), although at least some native ants appear relatively resistant to invasion (Wetterer *et al*. 2006). Where Argentine ants do impact native ants, they tend to reduce species richness and abundance, but they can also affect higher trophic levels by reducing populations of native ants that are important prey to specialized reptilian insectivores (Suarez *et al*. 1998, Suarez and Case 2002), by disrupting seed-dispersing mutualisms involving native ants (Christian 2001, Carney *et al*. 2003), or by competing with birds for nectar resources (Lach 2005).

Argentine ants have also been associated with reduced numbers of non-ant arthropods. For example, in northern California, lower abundances of several groups of arthropods, including Collembola (springtails), Diptera (flies), and Araneae (spiders), were found in invaded areas compared to nearby uninvaded areas (Human and Gordon 1997). In contrast, the diversity and abundance of ground-dwelling arthropods did not differ between invaded and uninvaded sites in another northern California study, indicating that Argentine ants had no negative impact or an impact that differed from that of the native ant species they displaced (Holway 1999). In Hawai`i, Argentine ants have been found to have a significant impact on a variety of arthropod taxa. Within subalpine habitats of Haleakalā National Park (HALE), Maui, Argentine ants were found to reduce overall diversity as well as abundances of several arthropod groups, including carabid beetles, lycosid spiders, noctuid moths, and ground-nesting bees (Cole *et al*. 1992, Krushelnycky 2007). The loss of native *Hylaeus* bees and *Agrotis* moths, both important pollinators of native plants, may affect the population structure of some plant species. Significantly, Argentine ants tend to have a greater impact on native species compared to non-native species (Krushelnycky 2007, Liebherr and Krushelnycky 2007). Because Argentine ants are still spreading within HAVO, much concern exists that they may impact or extirpate arthropod species currently found within ant-free habitats.

Established populations of Argentine ants were first reported in Hawai`i in 1940 at the Fort Shafter military facility in Honolulu (Zimmerman 1941). Since then they have been recorded from all of the high islands (Nishida 2002), but now they may be restricted to the islands of Kaua`i, Maui, and Hawai`i, where they are generally limited to dry and mesic habitats above 1000 m elevation (Krushelnycky *et al*. 2005b, U.S. Geological Survey unpublished data). Their distribution at lower elevations may be truncated by competition with big-headed ants and *Anoplolepis gracilipes* (yellow crazy ants; Fluker and Beardsley 1970), and at higher elevations they appear limited by cold temperatures or high rainfall, but are capable of surviving in alpine environments when conditions are favorable (Krushelnycky *et al*. 2005a). Climate models created for HALE suggest that approximately 75% of subalpine shrubland and aeolian areas are potential habitat for Argentine ants (Krushelnycky *et al*. 2005a).

Similar to the Argentine ant, the big-headed ant negatively influences native arthropod abundances in areas it infests. Several studies have documented significant reductions in the richness and abundance of native ants following invasion by big-headed ants (Haskins and Haskins 1988, Vanderwoude *et al*. 2000, Hoffmann 2010), and declines of other native arthropods, including spiders, pseudoscorpions, and larval insects, have also been noted (Hoffmann *et al*. 1999).

In Hawai`i, big-headed ants had disrupted native arthropod communities long before the arrival of Argentine ants. More than 100 years ago, Perkins recognized the devastating impact that alien ants were having on the native Hawaiian arthropod fauna over broad areas of lowland habitat. He was clearly pained by the impacts of big-headed ants, observing that "no native coleopterous insect [beetle] can resist this predator, and it is practically useless to attempt to collect where it is well established" (Perkins 1913). Originating in Africa (Wilson and Taylor 1967), the big-headed ant was widespread in Hawai`i by the time it was formally documented by Perkins (1913).

Recent work has revealed continuing impacts of big-headed ants on Hawaiian arthropods. For example, Gillespie and Reimer (1993) surveyed tetragnathid spiders and ants in native and disturbed forests on all of the main Hawaiian Islands and found spiders to be conspicuously absent from some sites occupied by big-headed ants, suggesting that the ant had a role in excluding the spiders. Similarly, LaPolla *et al*. (2000) found no geographic overlap between big-headed ants and native crickets on Kaua`i, leading them to conclude that this ant was responsible for the decline of the crickets. Additionally, on the island of Hawai`i, Gagné (1979) found a correlation between the prevalence of ants, particularly big-headed ants, and the paucity of native arthropods on the dominant canopy-forming tree species, `ōhi`a (*Metrosideros polymorpha*), at low elevation. The effects of big-headed ants and other ant species on native arthropod populations cascade through food webs and have contributed to the decline of Hawaiian forest birds generally and to feeding specialists in particular (Banko and Banko 1976, Wetterer *et al*. 1998, Banko and Banko 2009). Pollination webs also appear to be affected by big-headed ants as these ants prevent native *Hylaeus* bees from visiting flowers (Howarth 1985, Lach 2008).

Formicidal Baits Used to Control Argentine and Big-headed Ants
Broadcast application of formicidal (Formicidae = the ant family within the order Hymenoptera) baits has been the most effective method of controlling ants in field studies. Baits are collected by foraging worker ants and returned to the nest where they are ideally shared with the queen, her brood, and worker ants via trophyllaxis (essentially, regurgitation). To effectively control ant colonies, bait must be attractive to foraging ants, the toxicant must be non-repellant, and the toxicant must not kill the ant before it can return with it to the nest. Most baits are composed of an attractant to which foraging ants are drawn and a toxicant designed to kill the queen, her brood, and the workers. Because many ant species respond differently to the attractants, much research emphasis is placed on improving attractiveness of baits to individual species.

Hydramethylnon, a metabolic inhibitor of ATP formation, has been used to control a variety of ants, including Argentine ants and big-headed ants. Amdro® combines hydramethylnon (at 0.75%) with soybean oil on corn grit, and is highly attractive to big-headed ants. Amdro® has been used successfully to eradicate big-headed ants from 30 ha of infested land in northern Australia (Hoffmann and O'Connor 2004) as well as offshore islets in Hawai`i (Plentovich *et al*. 2009). In contrast, Maxforce® combines hydramethylnon (0.9%) with protein bait (ground

silkworm pupae) and is much more attractive than Amdro[®] to Argentine ants. The use of Maxforce[®] to control Argentine ants in subalpine habitats in HALE has resulted in dramatic decreases in both worker and nest abundance, but it failed to eradicate the species. Weather conditions leading to molding and rapid degradation of the bait may have reduced its efficacy (Krushelnycky and Reimer 1998).

The neurotoxin fipronil has been developed as a toxin-based alternative to hydramethylnon. Produced in New Zealand, Xstinguish™ combines fipronil (0.01%) with a protein/sucrose matrix and has been found to be effective against both Argentine ants and big-headed ants. Fipronil has gained much recent attention due to its success in controlling large populations of *Anoplolepis gracilipes* on Christmas Island (Green *et al*. 2004). Fipronil is considered to be more effective at killing queens than hydramethylnon, thus providing a greater chance of eradication (Hooper-Bui and Rust 2000).

In contrast to toxins, insect growth regulators (IGRs) affect the endocrine system of arthropods by acting to prevent larvae from developing into adults. Without the development of brood, adults are not replaced when they die and the colony is gradually extirpated. Although IGR baits are slower to work than acute, quick-acting toxins (6–8 weeks vs. 1–2 weeks), they may be more effective at eradicating the colony since the bait has more potential to become distributed among all members of the colony (Oi *et al*. 2000). The IGR bait Australian Distance[®] (5 g/kg pyriproxyfen on corn grit) has been found to be attractive to big-headed ants in Australia and may also be effective at killing Argentine ants (G. Webb, Sumitomo Chemical, personal communication). The baits Extinguish[®] Plus and Australian Distance[®] Plus combine an IGR with hydramethylnon (0.365% in both baits). Extinguish[®] Plus incorporates the IGR S-methoprene (0.25%) while Australian Distance[®] Plus utilizes pyriproxyfen (0.25%).

Overall Objectives
The primary goals of this study were to determine the distributions of all species of ants within the Mauna Loa Strip and Kahuku sections of HAVO and to test the efficacy of baits designed to eradicate or control populations of Argentine ants and big-headed ants, the species considered to be the greatest threats to HAVO natural resources. Mauna Loa Strip and Kahuku sections of HAVO were identified by park management as key areas in which to focus surveys of invasive ants (Rhonda Loh, Hawai`i Volcanoes National Park, personal communication). Although it was hoped that well-designed and carefully-implemented trials would demonstrate the efficacy of registered baits in controlling ant infestations in small, highly-sensitive ecological areas, it was also thought that emerging technologies might prove more effective in controlling ant populations at both small and large scales. Testing the efficacy of emerging methods is an important first step in the process to register new baits to protect native species and ecosystems from invasive ants. In this regard, therefore, an important goal of our research was to demonstrate how registered baits might be most effectively applied under local conditions and to identify novel management tools that might warrant registration.

CHAPTER 1. ANTS OF THE MAUNA LOA STRIP AND KAHUKU SECTIONS OF HAWAI`I VOLCANOES NATIONAL PARK

Introduction
Ants have been known from Hawai`i Volcanoes National Park (HAVO) for more than 80 years. The earliest documented detections within the park seem to have been made in 1934 by Otto Swezey, who noted several species at Nāpau Crater, including *Pheidole megacephala* (hereafter

big-headed ants), *Hypoponera opaciceps*, *Nylanderia* (=*Paratrechina*) *bourbonica*, and *Cardiocondyla emeryi* (personal communication by HAVO biologist C. Davis cited in Medeiros *et al*. 1986). Additional unpublished records by Davis identified *Technomyrmex albipes* from Kīpuka Kī in 1944 (Medeiros *et al*. 1986). In an extensive statewide survey of ants that included three sites along Mauna Loa Road (Kīpuka Kī fire cache and sites at 4100 ft [1250 m] and 5000 ft [1524 m] elevations) and one at Nāulu picnic area, Huddleston and Fluker (1968) recorded *H. opaciceps*, *Tetramorium bicarinatum* (= *T. guineense*), *N. bourbonica*, and *Plagiolepis alluaudi*. The first study documenting landscape patterns of ants in the park was that of Gagné (1979), who systematically surveyed arthropod communities within the canopy of `ōhi`a and koa over an elevation gradient ranging from 15 m to 2400 m elevation on Mauna Loa Volcano. Overall, Gagné reported big-headed ants and *P. allaudi* at the two lowest elevation sites (15 m and 76 m elevation) and *Linepithema humile* (hereafter Argentine ant) at all eight sites at and above 1190 m elevation. More ant-focused surveys within the park revealed the diversity of ants to be considerably greater than previously known. An assessment of ants in HAVO by Medeiros *et al*. (1986) yielded 12 species. That study summarized distributions of three highly aggressive ant species, the Argentine ant, the big-headed ant, and *Anoplolepis gracilipes* (= *A. longipes*; yellow crazy ant) and highlighted their potential impacts on the park's ecosystems. That publication was also important in that it showed significant negative impacts of Argentine ants on native arthropod communities within Haleakalā National Park on Maui. More recently, road and trail surveys within HAVO in the 1990s by Jorgensen (unpublished data cited in Wetterer 1998) and Wetterer (1998) increased the list of known ant species to 20. Overall, these studies laid the foundation for our current knowledge of ants in the park.

Although knowledge of invasive ants and their distributions within HAVO has expanded considerably over the past few decades, significant gaps still exist in our understanding of their distributions and the habitats in which they are found. The objective of this study was to fill some of these gaps by identifying and mapping distributions of ants within the Mauna Loa Strip and Kahuku sections of the park. These two sections were identified by park resource managers as priority areas for ant surveys because of their high ecological value. The Mauna Loa Strip has been the centerpiece for mid- and high-elevation habitats since the park's inception in 1916, and it harbors several key ecological communities including those at Kīpuka Puaulu and Kīpuka Kī. The Mauna Loa Strip has been free from disturbance by ungulates for more than 30 years and is undergoing much natural regeneration. In addition, recent management efforts on the Mauna Loa Strip have resulted in restoration of several plant communities and the control of exotic weeds. In contrast to this area, the Kahuku section, formerly operated as a private cattle ranch, was obtained by the park only in 2003, and it is currently being managed to be free of all cattle and other ungulates. Much habitat below 1500 m elevation was highly degraded by grazing, but large-scale habitat restoration is planned to begin once ungulates have been removed. Unlike the situation for the Mauna Loa Strip, the distribution of ants at Kahuku was never investigated. Significantly, each of these sections of the park contains high-elevation montane and subalpine habitats in which ants may not yet have become established. This study was designed to detect all ant species but with focus on identifying the most aggressive and ecologically destructive species, particularly the Argentine ant and the big-headed ant.

Methods
Areas surveyed
Mauna Loa Strip: Mauna Loa Strip covers much of the eastern part of Mauna Loa Volcano and lies between Keauhou (formerly Keauhou Ranch) to the east and Kapāpala Ranch to the west. While the Mauna Loa Strip could be loosely defined as running all the way to the summit of

Mauna Loa at 4170 m elevation, the area included in this study ranged between approximately 1200 and 2010 m elevation, a gradient that encompasses the length of Mauna Loa Strip Road (Figure 1.1). This part of Mauna Loa Strip is dominated by dry and mesic habitats, with rainfall generally decreasing from east to west and from lower to higher elevation. Mauna Loa Strip includes Kīpuka Puaulu and Kīpuka Kī, critical habitats for several endangered plant species (Pratt *et al.* 2010). Like much of Mauna Loa, this area is heavily dissected by lava flows of varying age, ranging from approximately 200 to over 5000 years old (Sherrod *et al.* 2007). Among the factors affecting the composition and structure of the vegetation community on Mauna Loa Strip are elevation, rainfall, and type and age of substrate, including kīpuka, which are islands of older substrate and vegetation that were not covered by subsequent lava flows. Kīpuka Puaulu and Kīpuka Kī are unique in the Mauna Loa Strip because they contain relatively deep, well-developed soils that support a diverse plant community and a tall, generally closed-canopy forest (Mueller-Dombois and Lamoureux 1967). The Broomsedge Burn, a 400 ha area of grassy woodland that burned in 2000 between Highway 11 and Kīpuka Puaulu, composes most of the portion of the Mauna Loa Strip that occurs on Kīlauea Volcano.

Kahuku: Encompassing about 47,000 ha, the Kahuku Unit of HAVO extends along Mauna Loa's southwest rift zone from Highway 11 to near the summit of the mountain. Our survey took place between approximately 590 and 2000 m elevation within the southern section that is bounded by the Ka`ū Forest Reserve to the east and the community of Hawaiian Ocean View Estates to the west (Figure 1.1). At about 4.3 km across at its narrowest point, Kahuku displays a steep moisture gradient that becomes progressively drier from east to west and includes wet, mesic and dry habitats (Jacobi 1989). The area has a long history of cattle grazing and has been home to wild mouflon sheep (*Ovis gmelini musimon*) for more than 40 years (Hess *et al.* 2006). Impacts of these ungulates on the vegetation have been profound and have resulted in a forest understory largely devoid of native plants. Former cattle paddocks are located primarily east of the main road on relatively moist substrates that support dense carpets of forage grass. Lava flows in this area range from historic age to more than 3000 years old (Sherrod *et al.* 2007).

Survey protocols
Mauna Loa Strip: On the Mauna Loa Strip, surveys were conducted between August 2008 and April 2010. Ants were primarily surveyed along parallel east-west oriented transects placed at 250 m intervals, but surveys also were conducted along roads and boundary fences. Above about 1250 m elevation, we focused our survey in the eastern portion of the strip due to the transit of the road through this area and its likely influence on ant distributions. Transects in this area extended from the fence on the eastern boundary of the park to between 500 and 1000 m west of the road. Several points along some transects were not sampled due to extensive, nearly impenetrable thickets of vegetation. Surveys were not conducted in the more remote and difficult to access western portion of the strip, where there were fewer potential routes of human-mediated ant dispersal.

A sampling protocol establishing survey points at 100-m intervals along transects, roads, and fences was developed using Hawth's Analysis Tools (Spatial Ecology.com) for ArcGIS prior to the survey. Hawth's Analysis Tools allows points to be generated at fixed distances along pre-determined lines identified within GIS (geographic information system) layers. These points were then loaded onto Garmin GPSmap 60CSx gps (global positioning system) units, and stations were surveyed for ants.
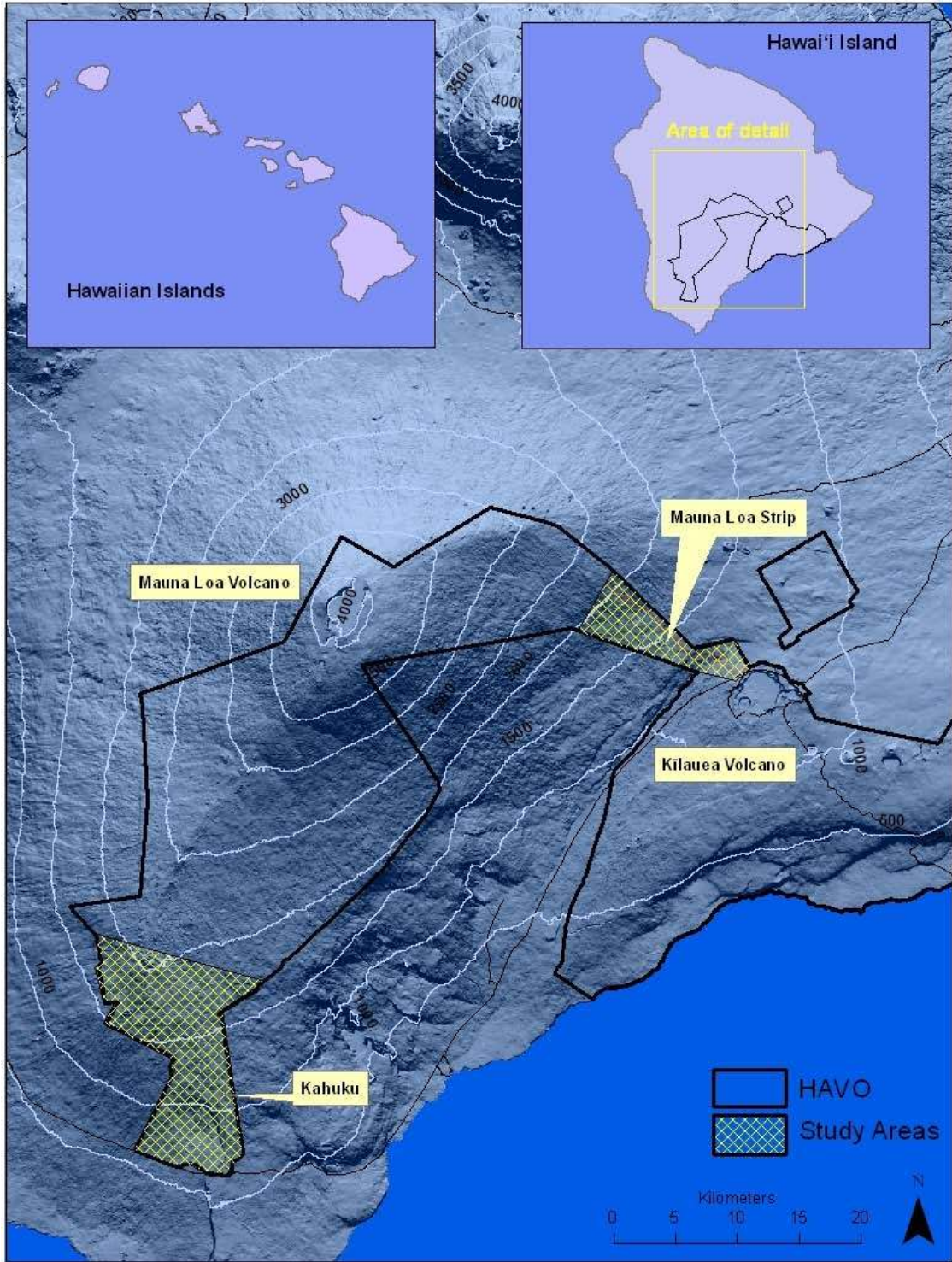
Figure 1.1. Mauna Loa Strip and Kahuku sections of Hawai`i Volcanoes National Park (HAVO)

Ants were surveyed at each station by visually searching for 5 person-minutes and by monitoring baits (a dollop of 50:50 mixture of tuna and corn syrup) placed on 5 x 7 cm cards

that were placed on the ground. Surveys were generally conducted by teams of two people: one person applied bait and flagged its location while the second person checked baits and hand searched for ants approximately one hour later. Hand searching generally consisted of looking for ants under rocks and coarse woody debris and by exploring soil, litter, and bark surfaces.

Kahuku: Road-based and grid-based ant surveys were conducted at Kahuku. Road surveys were designed to determine the general, large-scale distribution of ants. Once Argentine ants and big-headed ants were detected, we implemented focused, grid-based surveys to identify population boundaries of those species. For big-headed ants, only the upper elevation boundary was delimited because concern for their spread is primarily toward higher elevations. Road surveys took place between 16 October–14 November 2008 and population boundaries of Argentine ant and big-headed ants were determined 21 April–14 May 2010.

Road surveys took place along paddock and access roads at stations located 100 to 200 m apart. Nearly all roads within this section of Kahuku were surveyed. In addition, the east-west road running above the Ka`ū Forest Reserve was surveyed eastward for approximately 4.2 km from the northeast corner of the park boundary (at approximately 1600 m elevation). Similarly, the road along the fence line above Hawaiian Ocean View Estates, Manukā Natural Area Reserve, and The Nature Conservancy's Kona Hema Preserve was surveyed for about 12 km beyond the gate entering the park from the northeast corner of Hawaiian Ocean View Estates. The main road running from the park entrance upslope to the northeast corner of the study area is primarily gravel-based whereas all other roads generally lie over compacted soil or exposed lava rock.

Where Argentine ants and big-headed ants were detected along roads and fence lines, we conducted intensive surveys over a 100 x 100 m sampling grid to identify population boundaries. A station was considered to represent the population boundary when ants were not found at one or more adjacent stations outward from that point. The sampling grid was established using Hawth's Analysis Tools. We searched for ants at each station along roads and on grids using the same methods as those used on the Mauna Loa Strip.

Results

In total, 1625 stations covering nearly 200 km of roads, fence lines, and transects were surveyed for ants within the Mauna Loa Strip and Kahuku sections of HAVO (Table 1.1). In addition, 183 stations were systematically surveyed at Kahuku to delimit population boundaries of Argentine ants and big-headed ants (Table 1.2). Overall, 15 species of ants were collected in the two areas, with 11 being found on Mauna Loa Strip and 10 at Kahuku (Table 1.3). Six species were common to both areas, five species were found only on Mauna Loa Strip, and four species were detected only in Kahuku.

Mauna Loa Strip

Ants were detected at 320 (30%) of 1064 stations on the Mauna Loa Strip (Figure 1.2). *Cardiocondyla kagutsuchi* was the most widespread, ranging from 1178 m elevation on the lowest transect to 2014 m elevation at the top of Mauna Loa Road (Figure 1.3, Table 1.3), and it was the only species found above 1530 m elevation. Overall, this ant was found at 61 stations and on 18 of 29 transects. *C. kagutsuchi* was typically found in areas consisting of rocky substrates and relatively open vegetation structure.

Table 1.1. Survey effort (number of stations and kilometers [km] surveyed) along roads, fence lines, and transects in the Mauna Loa Strip and Kahuku sections of Hawai`i Volcanoes National Park.

Location	Road		Fence		Transect	
	Stations	Transect (km)	Stations	Transect (km)	Stations	Transect (km)
Mauna Loa Strip	120	21.4	117	10.7	827	79.8
Kahuku	561	74.2	ns[1]	ns	ns	ns

[1] ns = not sampled

Table 1.2. Survey effort (number of stations and kilometers [km] surveyed) to delimit population boundaries of Argentine ants and big-headed ants in the Kahuku section of Hawai`i Volcanoes National Park.

Argentine ant boundary		Big-headed ant boundary	
Stations	Transect (km)	Stations	Transect (km)
145	10	37	2.3

Table 1.3. Highest elevation (meters [m]) recorded and year first reported in Hawai`i for ants detected in the Mauna Loa (ML) Strip and Kahuku sections of Hawai`i Volcanoes National Park, 2008–2010.

Species	Number of stations occupied		Highest elevation detected (m)		Year first reported in Hawai`i[a]
	ML strip	Kahuku	ML strip	Kahuku	
Anoplolepis gracilipes	2	1	1179	621	1952
Cardiocondyla emeryi	2	0	1213	—	1943
Cardiocondyla kagutsuchi	61	65	2014	1589	1967
Cardiocondyla minutior	3	0	1187	—	1893
Cardiocondyla obscurior	0	1	—	1540	1994
Cardiocondyla wroughtonii	2	1	1335	1133	1893
Hypoponera opaciceps	2	0	1444	—	1899
Linepithema humile	210	121	1485	1557	1940
Nylanderia bourbonica	32	18	1463	1442	1879
Paratrechina longicornis	3	0	1304	—	1899
Pheidole megacephala	14	80	1345	1371	1879
Plagiolepis alluaudi	0	1	—	654	1913
Tapinoma melanocephalum	0	1	—	744	1899
Tetramorium bicarinatum	3	0	1492	—	1879
Tetramorium simillimum	0	5	—	940	1934

[a] summarized in Krushelnycky *et al*. (2005b)
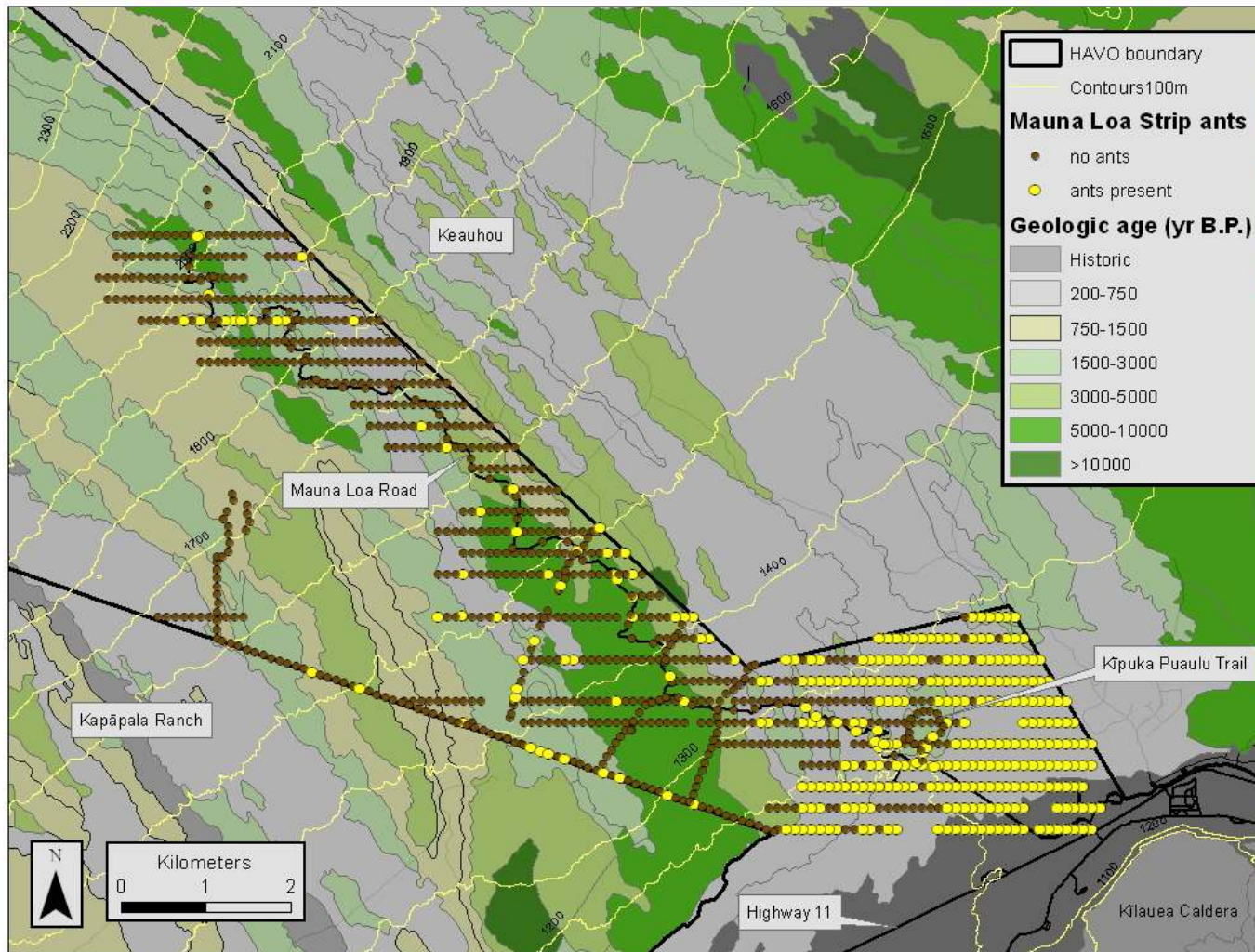
Figure 1.2. Survey stations at which one or more ant species were detected on the Mauna Loa Strip section of Hawai`i Volcanoes National Park (HAVO), 2008–2010. Brown dots represent survey stations at which no ants were detected.

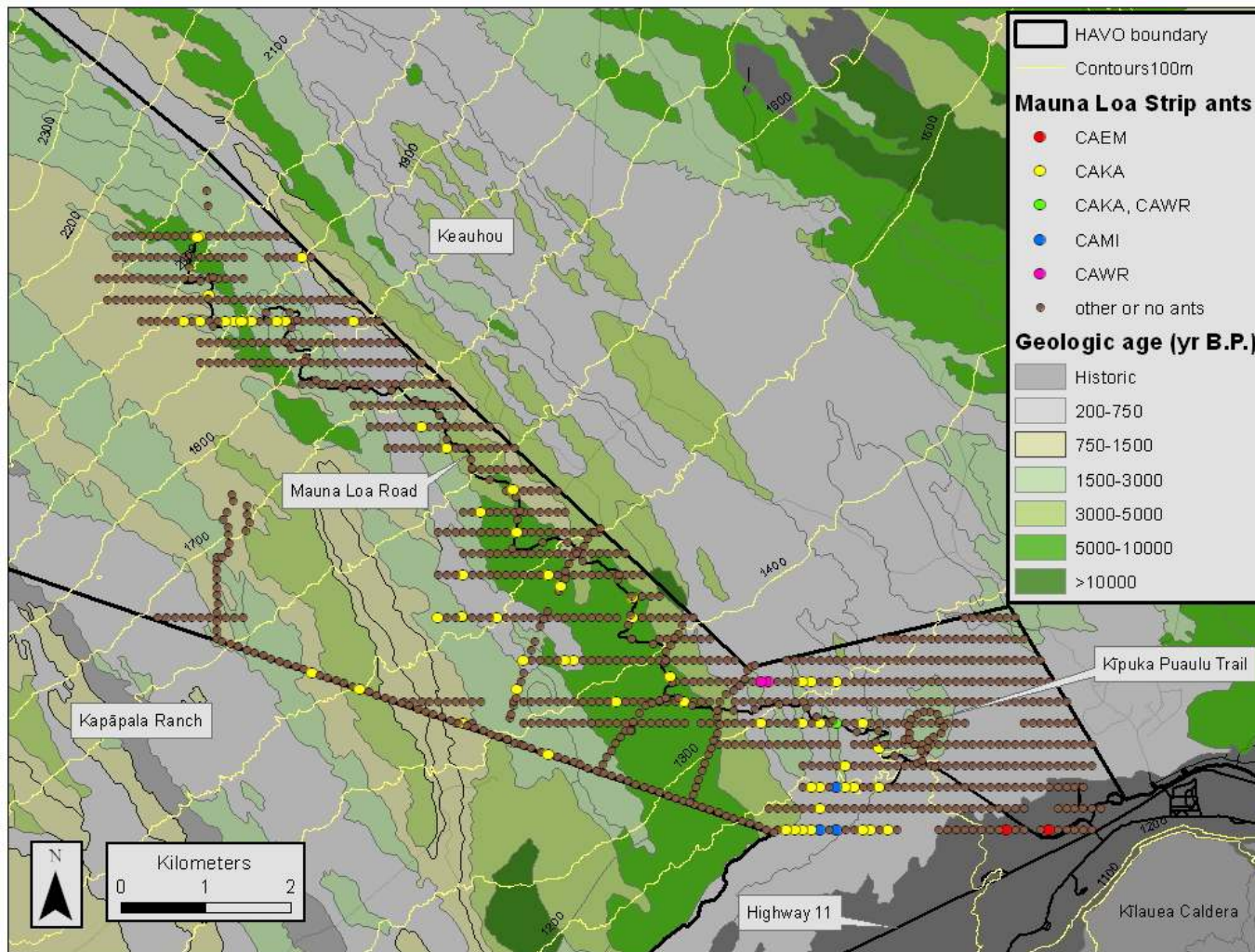Figure 1.3. Distribution of *Cardiocondyla emeryi* (CAEM), *C. kagutsuchi* (CAKA), *C. wroughtonii* (CAWR), and *C. minutior* (CAMI) on the Mauna Loa Strip section of Hawai`i Volcanoes National Park (HAVO), 2008–2010. Brown dots represent survey stations at which these ants were not detected.

In contrast to the widespread but patchy distribution of *C. kagutsuchi*, Argentine ants formed a large, apparently contiguous population that covered much of the lower section of Mauna Loa Strip (Figure 1.4). This ant was uncommon within Kīpuka Puaulu, but the population extended to the north, south, and east of the kīpuka and was found at 210 stations along transects, roads, or fence lines. Within the apparent boundary of the population, ants were detected at 188 (89%) of 211 stations and covered an area of approximately 560 ha. Few other ant species were collected at stations where Argentine ants were found.

Argentine ants occupied habitat consisting mainly of open canopy ʻōhiʻa (*Metrosideros polymorpha*) forest dominated by grass (e.g., the Broomsedge Burn; Figure 1.5), but they also were found in relatively closed-canopy koa (*Acacia koa*) forest with a grassy understory in the area north and northeast of Kīpuka Puaulu. Within this stand of koa, ants were typically detected on downed logs that extended above the dense grass. Argentine ants were found on only 3 of 19 stations along the loop trail within the southeastern section of the kīpuka and within about 350 m of the trailhead. However, they were detected at several stations along the transect passing through the northern part of the kīpuka. Argentine ants also were distributed upslope along the eastern margin of the park to about 1495 m elevation, near the intersection of Power Line Road and the eastern boundary fence (Figure 1.4).

Big-headed ants were detected at several stations just west of Mauna Loa Road on the lowest transect (about 1200 m elevation) and at four stations along the western boundary fence to as high as 1345 m elevation (Figure 1.4).

Several other ant species were relatively uncommon or patchily distributed on the Mauna Loa Strip, including *C. emeryi*, *C. minutior*, *C. obscurior*, *C. wroughtonii*, *Anoplolepis gracilipes*, *Hypoponera opaciceps*, *Nylanderia bourbonica*, *Paratrechina longicornis*, and *Tetramorium bicarinatum* (Figures 1.3, 1.4, and 1.6). *Nylanderia bourbonica* was most abundant among these species, being found at 32 stations primarily along or east of Mauna Loa Road below 1275 m elevation. None of the other species were found at more than three stations.

Kahuku
All 10 species of ants identified at Kahuku were collected during the general survey along roadsides (Table 1.3). Overall, ants were detected at 177 of 561 stations, primarily those restricted to the main Kahuku access road or habitats west of the road with only a few occurrences on roads within the grass-dominated paddock area between the eastern boundary of the park and the main road (Figure 1.7).

As found on Mauna Loa Strip, *C. kagutsuchi* was the most widespread ant species found at Kahuku, ranging in elevation from 920 m in the paddock area just off the main access road to 1590 m in the uppermost tip of Manukā Natural Area Reserve located at the northwestern limit of the survey area (Figure 1.8, Table 1.3). *Cardiocondyla kagutsuchi* was found at two adjacent stations near the eastern boundary of the park at about 1220 m elevation, but those stations were located on a narrow tongue of lava of historic age extending into the grassy habitat that dominated that part of Kahuku.

A contiguous population of Argentine ants was found directly above and to the northeast of the Hawaiian Ocean View Estates (HOVE) subdivision that borders the park on the west (Figure 1.9). From the northeast corner of the subdivision, the Argentine ant population extended roughly 1.4 km east, 1.1 km north, and 4.0 km northwest. In total, this population covered about 585 ha of park land at Kahuku. Ants were primarily restricted to the older lava flows and
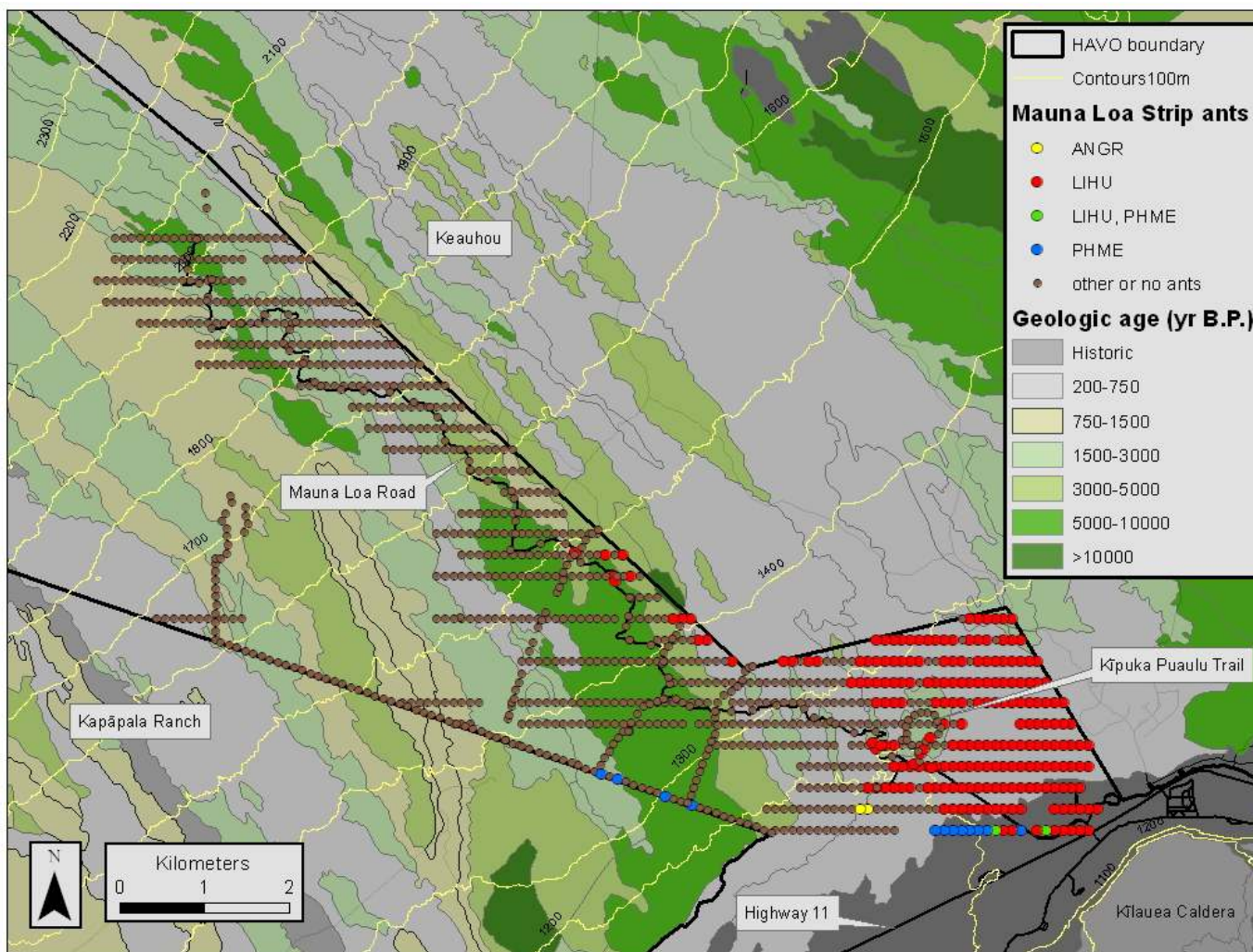
Figure 1.4. Distribution of *Anoplolepis gracilipes* (ANGR), Argentine ants (*Linepithema humile* [LIHU]), and big-headed ants (*Pheidole megacephala* [PHME]) on the Mauna Loa Strip Section of Hawai`i Volcanoes National Park (HAVO), 2008–2010. Brown dots represent survey stations at which these ants were not detected.

Figure 1.5. Typical habitat for Argentine ants in the grass-dominated Broomsedge Burn area in the lower part of the Mauna Loa Strip section of Hawai`i Volcanoes National Park.

cinder that supported vegetation with few ants found on the young, historic-aged `a`ā flows that dissected the area. The few points where Argentine ants were detected on young flows were generally close to the flow edge and contained small patches of vegetation. The eastern part of the population was found primarily on a pāhoehoe flow that was 1500–3000 years old, while the western part of the population was distributed on cinder-dominated substrate that was 750–1500 years old. The northwestern boundary of the population abutted a lobe of historic-aged `a`ā (Figure 1.10). The population ranged in elevation from about 1190 m to 1560 m. The upper boundary of the population was not clearly delimited, but ants were not likely to have inhabited the sparsely vegetated substrate about 1 km above the northern boundary of HOVE.

A population of big-headed ants was found directly south of the Argentine ants, and it extended down slope to at least 760 m elevation (Figure 1.9). The focused grid survey along the upper boundary of big-headed ants indicated no overlap with Argentine ants. Few big-headed ants were found on the main Kahuku access road to the east of the main population, suggesting that most of the population was distributed to the west of the main road. Lower boundaries of the population were not determined, but our results indicated the population encompassed at least 825 ha. This population was apparently centered on a 1500–3000 year-old pāhoehoe flow that supported scattered `ōhi`a but with much exposed rocky ground (Figure 1.11). No big-headed ants were found on the few survey points located on the historic-aged `a`ā flow on the western border of the population, suggesting that the flow was a barrier to westward expansion.

Nylanderia bourbonica was found at numerous stations along a 4-km segment of the main Kahuku access road, where its range extended to as low as 700 m elevation. Along with *C. kagutsuchi*, it was detected in the paddock area east of the main road, where it was found at approximately 1040 m elevation at three adjacent stations (Figure 1.12).

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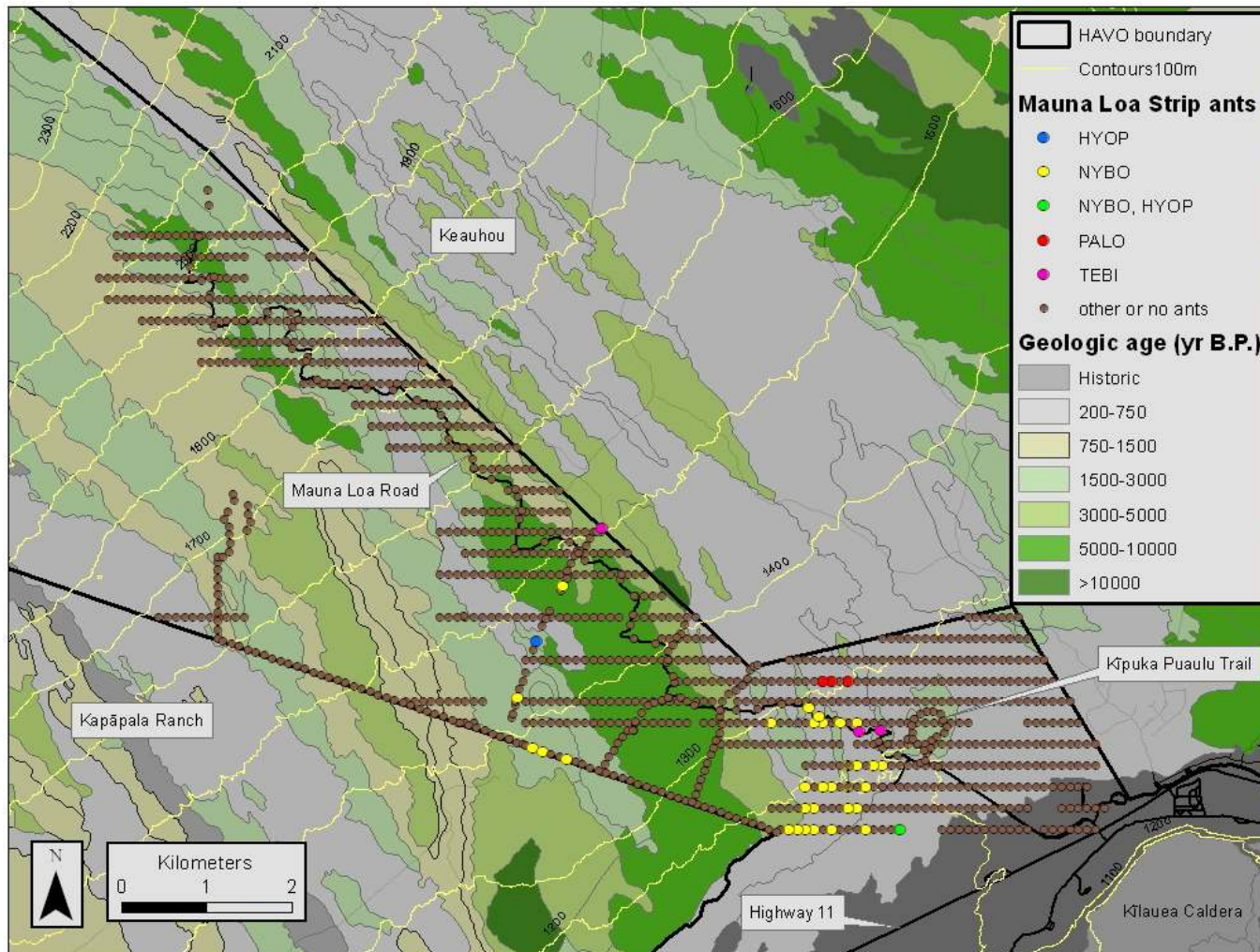
Figure 1.6. Distribution of *Hypoponera opaciceps* (HYOP), *Nylanderia bourbonica* (NYBO), *Paratrechina longicornis* (PALO), and *Tetramorium bicarinatum* (TEBI) on the Mauna Loa Strip section of Hawai`i Volcanoes National Park (HAVO), 2008–2010. Brown dots represent survey stations at which these ants were not detected.
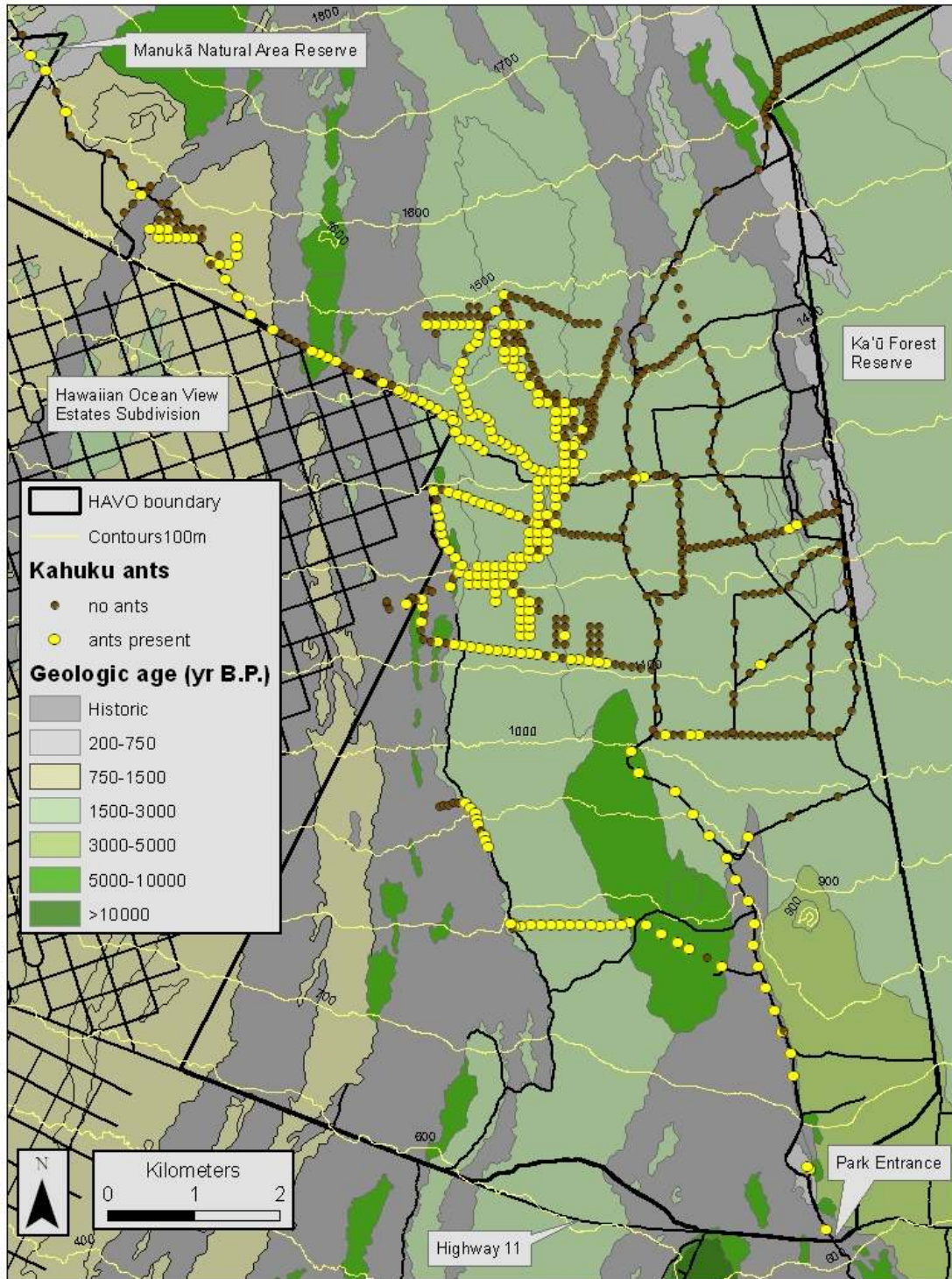
Figure 1.7. Survey stations at which one or more ant species were detected on the Kahuku section of Hawai`i Volcanoes National Park (HAVO), 2008–2010. Brown dots represent survey stations at which no ants were detected.
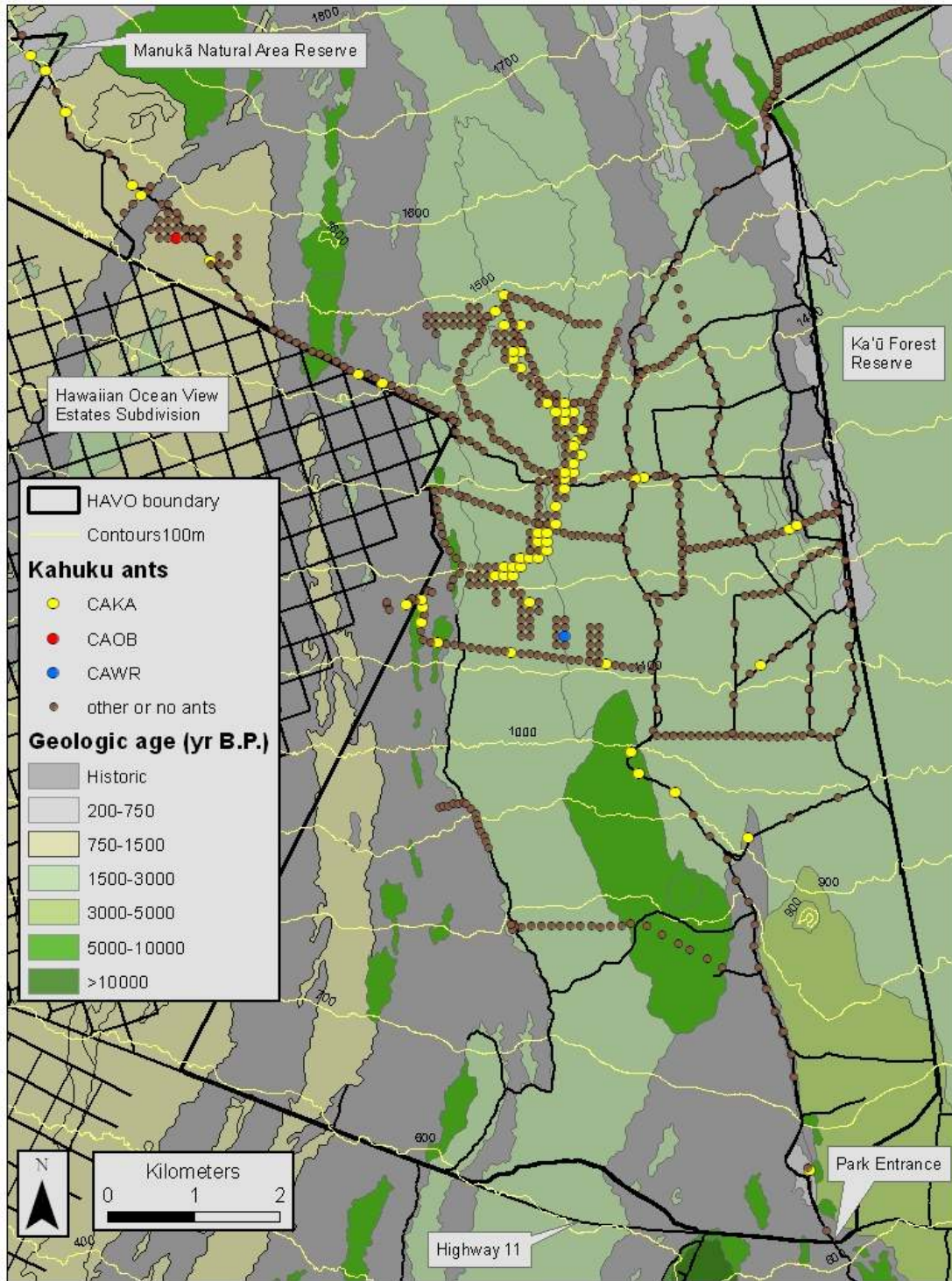
Figure 1.8. Distribution of *Cardiocondyla kagutsuchi* (CAKA), *C. obscurior* (CAOB), and *C. wroughtonii* (CAWR) on the Kahuku section of Hawai`i Volcanoes National Park (HAVO), 2008–2010. Brown dots represent stations at which these ants were not detected.
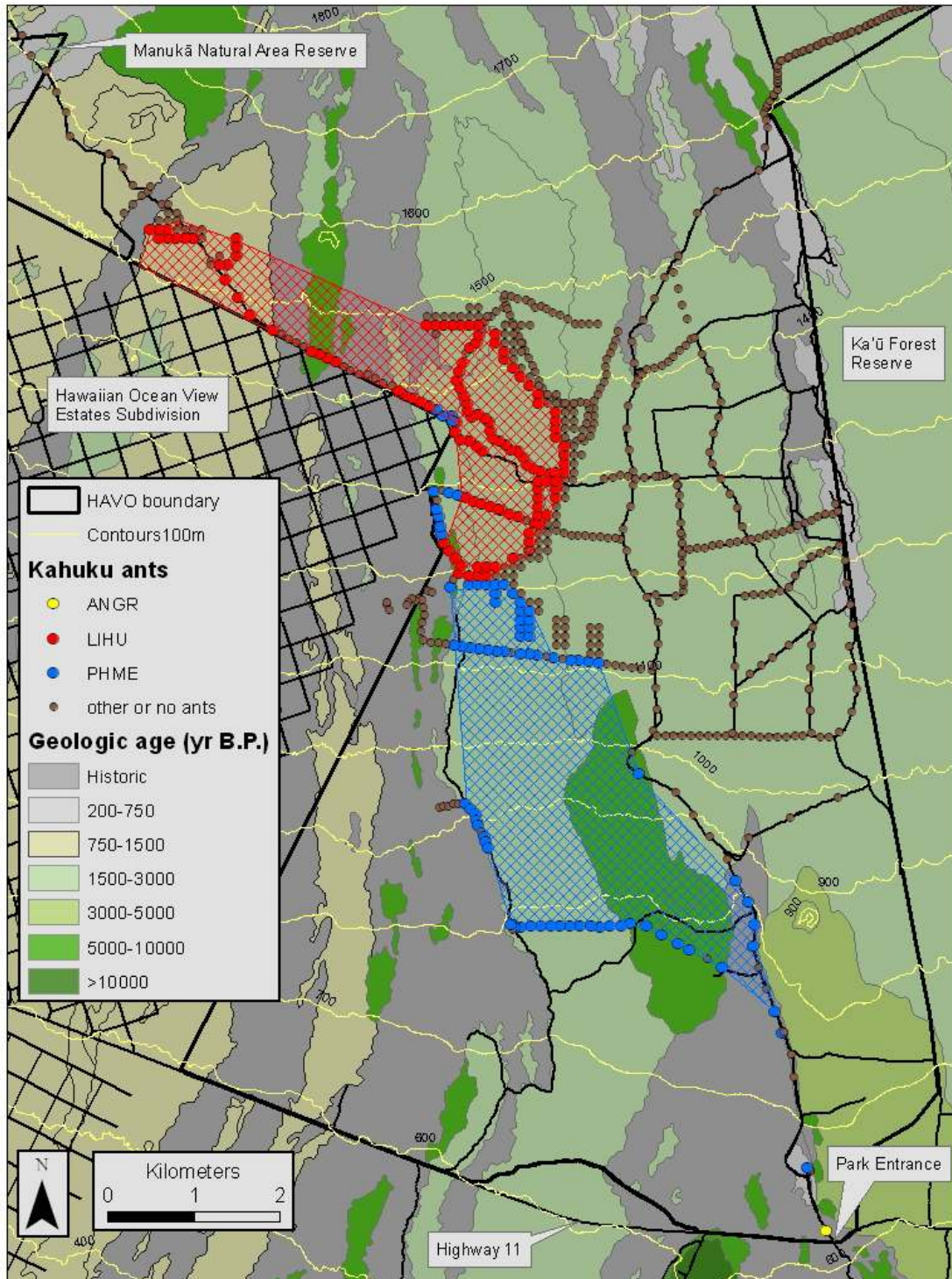
Figure 1.9. Distribution of *Anoplolepis gracilipes* (ANGR), Argentine ants (*Linepithema humile* [LIHU]), and big-headed ants (*Pheidole megacephala* [PHME]) on the Kahuku section of Hawai`i Volcanoes National Park (HAVO), 2008–2010. Estimated distributions of Argentine ants and big-headed ants are indicated by red and blue cross-hatching, respectively. Brown dots represent stations at which these ants were not detected.

Figure 1.10. Location of the western margin of the Argentine ant population along the road above Hawaiian Ocean View Estates Subdivision on the Kahuku section of Hawai`i Volcanoes National Park. Cinder (light substrate in foreground) supported ants while the adjacent historic-age `a`ā flow (dark substrate in background) did not support ants.

Figure 1.11. Typical habitat supporting big-headed ants on the Kahuku section of Hawai`i Volcanoes National Park. Note the heavy grazing by ranch and feral ungulates in the understory.

Figure 1.12. Distribution of *Nylanderia bourbonica* (NYBO), *Tetramorium simillimum* (TESI), *Plagiolepis alluaudi* (PLAL), and *Tapinoma melanocephalum* (TAME) on the Kahuku section of Hawai`i Volcanoes National Park (HAVO), 2008–2010. Brown dots represent stations at which these ants were not detected.

The only detection of *Plagiolepis alluaudi* in Kahuku was in disturbed habitat surrounding the building compound near the park entrance (Figure 1.12). It occurred here with big-headed ants and *C. kagutsuchi*.

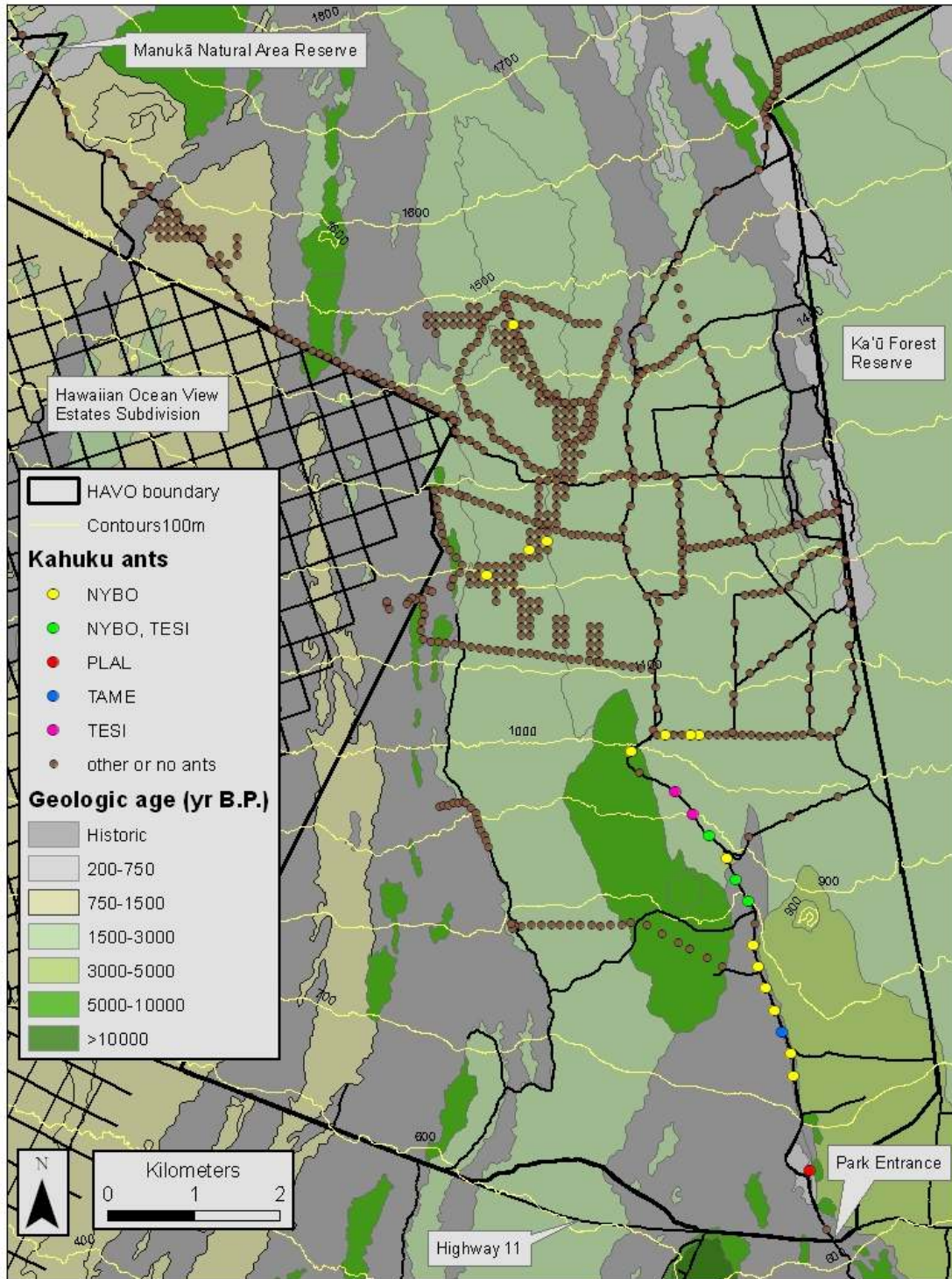Five other ant species were rarely detected at Kahuku, suggesting that their distribution was highly localized or that their numbers were very low within the areas surveyed. The five rare species included three species of *Cardiocondyla* (*C. emeryi*, *C. obscurior*, and *C. wroughtonii*), *Tapinoma melanocephalum*, *Tetramorium simillimum*, and *A. gracilipes* (Figures 1.8 and 1.12). *Anoplolepis gracilipes* was found only near the gate at the park entrance.

No ants were found along the rocky road running northeast above Ka`ū Forest Reserve, nor at the Nēnē Cabin located along the road at approximately 1280 m elevation and 12 km east of the northwest corner of the forest reserve. Similarly, no ants were found along the road running northwest through Kahuku beyond the point where the road re-enters the park after passing through the tip of Manukā Natural Area Reserve. Roads through these two remote stretches of Kahuku transit mostly between 1600 and 1700 m elevation.

Discussion

This study provided a snapshot in time of ant distributions within the Mauna Loa Strip and Kahuku sections of HAVO. The survey methods we used were well-suited to identifying distributions of the most aggressive species, but the methods may have underestimated the presence of species that do not recruit strongly to bait, have cryptic habits, or sustain small population densities. A more intensive survey using methods that target small, litter-dwelling ants probably would have detected several additional species. Nevertheless, managers have for the first time a comprehensive understanding of the distributions of the dominant ant communities in these two important areas of the park.

Thirteen of 15 species detected were previously known from HAVO (Wetterer 1998). The two species representing new records, *C. obscurior and C. wroughtonii*, were infrequently collected at both sections of the park. Like most *Cardiocondyla*, little is known about the biology of these two species, but if they are similar to other members of the genus, they likely maintain relatively small, polygynous (multiple queens) nests, share foraging territories with other nests, and feed on small living and dead arthropods (Creighton and Snelling 1974, Heinze *et al*. 2006).

Several species were collected for the first time on the Mauna Loa Strip, including *C. minutior* (previously erroneously referred to as *C. nuda*), *C. emeryi*, *T. bicarinatum*, and *P. longicornis*. Each of these species had been considered to be restricted to "lowlands" in HAVO and had previously been found only below 900 m elevation (Wetterer 1998). The highest elevations where we collected these ants ranged from 1187 m for *C. minutior* to 1492 m for *T. bicarinatum*. In addition, we collected six of eight species that Wetterer (1998) considered to be "cold-tolerant" (found above 900 m elevation) well above 1200 m elevation, including the Argentine ant, big-headed ant, *C. kagutsuchi*, *H. opaciceps*, *A. gracilipes* (= *longiceps*), and *Nylanderia* (= *Paratrechina*) *bourbonica*. The only two "cold-tolerant" species not collected on the Mauna Loa Strip were *Hypoponera punctatissima* and *Nylanderia* (= *Paratrechina*) *vaga*.

The addition of new species to the list known from HAVO does not necessarily mean that there are more ant species now than in the recent past. While the list is expected to grow as ants increase their range in Hawai`i and as additional species invade the islands, some ants may have been undetected in the park for a long time and are only now being found as sampling efforts increase. Additional overlooked species might be expected as surveys deviate from roads

and trails and employ more intensive sampling methods. The patchy distribution of *C. obscurior* and *C. wroughtonii* and their cryptic nature, which is characteristic of the genus, suggest that they could have been missed in earlier surveys. This is particularly likely for *C. wroughtonii*, which has been in Hawai`i since at least 1893 (Seifert 2003). In contrast, *C. obscurior* was first detected in Hawai`i in 1994 (Seifert 2003) and its range may still be expanding. Significantly, *Wasmannia auropunctata* (little fire ant), discovered for the first time in Hawai`i near Hilo in 1999 (Conant and Hirayama 2000) and now widespread over much of the lower elevation windward habitat on Hawai`i Island, has not been found in HAVO. This species is of particular concern because it contains a sting and is capable of inflicting severe pain to humans and their pets. *Wasmannia auropunctata* appears restricted to warmer temperatures and has thus far not been found in HAVO.

Big-headed ant
Of all the ants detected in this study, the big-headed ant has surely had the greatest impact on Hawaiian ecosystems. In the late 1800s, Perkins (1913) recognized the profound effect that big-headed ants were having on native arthropod communities within low elevation habitats in Hawai`i as he found it difficult to find many native insects in areas occupied by this ant. More recent studies investigating ant impacts in Hawai`i further implicate big-headed ants in the decline of several groups of native arthropods (Gagné 1979) including spiders (Gillespie and Reimer 1993) and crickets (LaPolla *et al.* 2000). Unfortunately, big-headed ants now occupy much low elevation dry and mesic habitat throughout the main islands of the Hawaiian Archipelago.

Several factors may limit the distribution of big-headed ants in HAVO, but climate and competition with even more aggressive species are likely most important, particularly at the higher end of their elevation range. Wetterer (1998) considered big-headed ants to be a "lowland species," generally restricted to habitats below 900 m elevation, and he attributed his finding populations at 1200–1220 m around geothermal areas and buildings within the park to heat sources unique to these "habitat islands." Subsequently, Wetterer *et al.* (1998) and Krushelnycky and Gillespie (2008) found big-headed ants as high as 1770 m and 1880 m, respectively, on the slope of Mauna Kea Volcano, suggesting tolerance to conditions colder than previously recognized. Relatively warm drainage winds that descend the leeward slopes of Mauna Kea may contribute to this apparent distributional anomaly, but big-headed ants may also be able to colonize elevations higher than previously thought possible. Our finding of big-headed ants at 1345 m on Mauna Loa Strip and 1371 m at Kahuku further indicate this ant's ability to colonize relatively high elevation habitats within the park.

The fact that we did not find big-headed ants interior to the western boundary fence of the Mauna Loa Strip suggests that at this elevation (1220–1345 m) big-headed ants may be restricted to the disturbed habitat found on Kapāpala Ranch adjacent to the park. The open nature of the pasture land likely results in relatively warmer ground-level day-time temperatures that may allow these ants to persist at elevations that are marginal to survival. Alternatively, the ants along the fence may represent an invading front that has not yet penetrated the park in that area. Future surveys would be required to determine whether big-headed ants are moving into HAVO from the ranch. In addition, big-headed ants were detected at numerous stations during an informal survey along about 2.5 km of the park boundary fence beginning at the eastern corner of Kapāpala Ranch and paralleling Highway 11.

Like most ant species in Hawai`i, big-headed ants appear unable to colonize wet forest habitats. Even at low elevations, numerous studies have failed to find this ant in landscape-level surveys in Hawai`i (Huddleston and Fluker 1968, Gillespie and Reimer 1993, Wetterer 1998, LaPolla *et al*. 2000).

The second factor that may limit distributions of big-headed ants in HAVO is competitive interactions with Argentine ants. Several studies have found antagonistic interactions between these two species, resulting in discrete, non-overlapping territories (Huddleston and Fluker 1968, Fluker and Beardsley 1970). Although the outcome of these competitive interactions may not be predictable, and are likely influenced by many abiotic and biotic factors, Argentine ants are often competitively superior to big-headed ants, even to the extent of nearly displacing big-headed ants from areas that they have inhabited for over 100 years (Crowell 1968, Wetterer and Wetterer 2004). In Hawai`i, Argentine ants appear to exclude big-headed ants at higher elevations where lower temperatures probably favor more cold-tolerant Argentine ants. Our results support this pattern of mutual exclusion as we generally found well-defined boundaries between populations of these two species. On the Mauna Loa Strip, Argentine ants probably limit north-eastward expansion of big-headed ants into the Broomsedge Burn, while at Kahuku big-headed ants are probably limited at higher elevations by both temperature and Argentine ants, as their population abuts that of Argentine ants at about 1371 m.

Argentine ant
Although big-headed ants had early impacts on Hawaiian ecosystems, the highly aggressive Argentine ant likely poses a greater threat today to natural resources in HAVO. The Argentine ant is one of only a few species of ants in Hawai`i capable of invading dry and mesic montane and subalpine habitats. These habitats are among the least disturbed in the park, and they support relatively intact plant and invertebrate communities as well as provide feeding and nesting habitat for native forest birds (Stone and Pratt 2002) and nesting habitat for the endangered `ua`u, or Hawaiian petrel (*Pterodroma sandwicensis*; Simons and Hodges 1998). Arthropods potentially impacted by Argentine ants are numerous and include ground-nesting *Hylaeus* bees (Cole *et al*. 1992), which are important pollinators of the endangered Mauna Loa silversword (*Argyroxiphium kauense*) and some other rare native plant species.

Argentine ants have been present in HAVO for at least 40 years. Gagné (1979, 1981) reported this ant to be widespread on the Mauna Loa Strip during the 1970s, having collected it at all eight sites he sampled between 1190 and 2400 m elevation during his survey of arthropod communities within canopies of `ōhi`a and koa. Subsequently, Medeiros *et al*. (1986) found Argentine ants at several locations between Kīpuka Nēnē (970 m) and Kīpuka Puaulu (1220 m), although they failed to detect them at 1480, 1780, and 2010 m elevation along Mauna Loa Road. Prior to our study, Argentine ants had not been reported from the Kahuku section of the park.

Considering that we did not find Argentine ants above 1485 m elevation on the Mauna Loa Strip, the report of these ants at four sites above that elevation (1600, 2030, 2130, and 2400 m) by Gagné (1979, 1981) is difficult to explain. It is possible that we missed detecting these ants because we only sampled on the ground (as opposed to sampling in trees, as was done by Gagné), but that is unlikely because Argentine ants generally nest under rocks, and when present, are generally conspicuous on the ground and recruit aggressively to bait. If, as seems to be the case, Argentine ants no longer inhabit the upper Mauna Loa Strip, it may represent the first record of a significant contraction of range for this species in Hawai`i. Krushelnycky *et*

al. (2005a) reported small-scale fluctuations in a portion of the range of Argentine ants near Hosmer's Grove in HALE, but the area where that population contracted was at the margin of habitable territory. Other than parts of the young Ke`āmoku Lava Flow that lack vegetation, the upper portion of Mauna Loa Strip contains habitat that seems favorable to Argentine ants, making contraction of their range due to environmental conditions doubtful. Alternatively, there may have been an error in Gagné's report of Argentine ants from sites above 1600 m elevation.

Gagné's results aside, the Argentine ants on Mauna Loa Strip apparently represent the western portion of a much larger, likely contiguous population. Argentine ants range east of HAVO into the Volcano Golf and Country Club Subdivision and Volcano Village, and they range north at least to the southwestern edge of Keauhou (U.S. Geological Survey unpublished data). The eastern limit of this population is probably determined by high rainfall and closed-canopy forest, but more favorable mesic habitat is available for a considerable distance to the north. Surveys on Keauhou would be needed to identify the northern boundary of this population. Argentine ants also have the potential to colonize westward across the Mauna Loa Strip into Kīpuka Kulalio and Kīpuka Mauna`iu, but they may be limited by food resources on the young, sparsely-vegetated lava flows that dissect the area.

Although our surveys indicated that the Argentine ant population at Kahuku was restricted to an area of about 585 ha above and to the northeast of HOVE subdivision, they may expand unimpeded eastward towards the Ka`ū Forest Reserve and westward above the Manukā Natural Area Reserve. Recent, informal roadside surveys within HOVE revealed that this population occupied an area at least as large as they occupied within the park, and Argentine ants were detected along the western edge of the subdivision (U.S. Geological Survey unpublished data), suggesting that they may have already penetrated mesic forest of the Manukā Natural Area Reserve.

Rate of spread of Argentine ants: The rate at which Argentine ants spread over the landscape is likely affected by many factors including the availability of food and nest sites, temperature, rainfall, and competition with other ants. Argentine ants lack winged dispersal, so populations expand by budding, whereby a queen and several worker ants walk into adjacent, generally unoccupied habitat and establish new nests. Dispersal by budding is relatively slow, but typically results in complete occupation of suitable habitat.

One of the best studies documenting the spread of Argentine ants was conducted in Haleakalā National Park, Maui, where two non-contiguous populations, centered at approximately 2100 and 2750 m elevation, were monitored for nearly 20 years (Krushelnycky *et al.* 2005a). The rate at which these two populations spread in this cool environment ranged from 18 m/year (over a seven-year period) to 157 m/year (over a two-year period) with an overall average of about 62 m/year. We would expect Argentine ants to have faster rates of dispersal in Kahuku and the Mauna Loa Strip due to warmer temperatures prevailing at these lower elevations (Krushelnycky *et al.* 2005a, Hartley *et al.* 2010).

Potential range of Argentine ants in the park: It is difficult to predict the potential range of any invasive species, but populations of Argentine ants persisting as high as 2640 m elevation on Mauna Kea (Wetterer *et al.* 1998) and 2850 m elevation on Haleakalā (Krushelnycky *et al.* 2005a) suggest that this species is capable of expanding into considerably higher habitats than what are currently occupied at HAVO. In these relatively cold, subalpine environments, Argentine ants are likely able to persist because sparser vegetation results in high levels of solar radiation reaching the ground and warming the substrate.

Like temperature, rainfall has a strong limiting influence on Argentine ant distributions. At HALE, 1600 mm of average annual rainfall is apparently the threshold restricting advancement of the ants into wetter, windward habitats (Krushelnycky *et al*. 2005a). Tolerance to rainfall may be greater at low elevation, however, as mean annual rainfall experienced by Argentine ants at 1300 m elevation along the eastern edge of Mauna Loa Strip is approximately 2000 mm per year (Giambulluca *et al*. 2011). Argentine ants experience even greater rainfall amounts in the Volcano Golf Course subdivision and parts of Volcano Village. We did not survey the wet, closed-canopy forest comprising the `Ōla`a Tract of HAVO, but ants have never been reported there, and it is highly unlikely that Argentine ants would be able to survive in that habitat.

How much of HAVO are Argentine ants capable of colonizing? Assuming that colonization is possible on all mesic and dry habitats (Jacobi 1989) located between 1200 and 2900 m elevation on substrates older than historic-aged flows, Argentine ants could potentially occupy approximately 85,000 ha of park land, an area more than 70 times that which they currently occupy. Furthermore, Argentine ants could occupy an additional 20,000 ha of habitat between the Kahuku and Mauna Loa sections of the park on state and private land. Clearly, the range currently occupied by Argentine ants is considerably less than the amount of habitat available to them in and adjacent to the park.

Other potential high elevation ant species
Most ant species in Hawai`i are restricted to lower elevations, but several species collected in our study are "cold tolerant" (Wetterer 1998) and likely to expand their range in HAVO. These include Argentine ants, *C. kagutsuchi* (probably previously erroneously identified as *C. venustula*), *N. (=Paratrechina) bourbonica*, and *H. opaciceps*.

Cardiocondyla kagutsuchi and *H. opaciceps* are both cryptic species that form relatively small nests generally located under rocks or downed wood. The ecological impacts of these species are likely to be considerably less than that of more aggressive species due to their smaller sizes and lower population abundances. *Cardiocondyla kagutsuchi* appears to be widespread at higher elevations on Hawai`i Island and has been found on the western slope of Mauna Kea at 2430 m elevation (Wetterer *et al*. 1998) as well as at about 1900 m at Hakalau Forest National Wildlife Refuge (NWR) on the windward side of the island (Peck and Banko 2012). Both *C. kagutsuchi* and *H. opaciceps* were recently detected in subalpine habitat above 2100 m on Maui (Krushelnycky 2010) although both species have existed there for at least 45 years (Huddleston and Fluker 1968). *Nylanderia bourbonica* may be a greater threat to park ecosystems because workers of this species are relatively large and often search out and aggressively attack smaller insects at distances five or more meters from nests (Trager 1984). They also recruit to and defend carbohydrate sources such as nectar and honeydew secreted from non-native homopteran insects. Such rich energy sources often support large colonies capable of consuming great numbers of arthropod prey. In addition, *N. bourbonica* is notable for ants in Hawai`i as it is capable of colonizing some forested habitats. For example, Wetterer (1998) found *N. bourbonica* at two sites in "undisturbed forest" along Crater Rim Trail in HAVO. Nevertheless, the extent to which this species can penetrate native forest is unclear, as LaPolla *et al*. (2000) found *N. bourbonica* at Kalōpā State Recreational Area (approx. 600 m elevation) "at the edge of the woods, but none inside the woods" and Reimer (1994) found it in "disturbed montane habitats such as roadside and urban developments, but never in undisturbed sites." Similarly, recent surveys of wet forest on the Kona Unit of Hakalau Forest NWR found *N. bourbonica* along gravel roads at elevations ranging from 777 to 930 m elevation (Haines and Foote 2005, Peck and Banko 2012).

Controlling invasive ants and preventing their spread
Eradicating Argentine ants throughout their 1100-ha range in HAVO would be extremely challenging using the control measures that are currently available. Furthermore, ant populations extending onto adjacent land outside the park would likely act as a source of continuous incursion unless a collaborative, area-wide approach among land owners was undertaken. On the other hand, preventing or slowing the spread of Argentine ants into new habitats within the park would be a more feasible management objective until effective, landscape-level eradication tools are developed.

To facilitate the control of ant populations, natural barriers such as lava flows or patches of inhospitable habitat could be used as "lines in the sand" representing boundary limits at which ant dispersal would be stopped. For example, the historic-aged `a`ā flow that enters the northwestern section of HOVE (Figures 1.7 and 1.8) could be utilized as a natural barrier to Argentine ant dispersal into western Kahuku because it is nearly devoid of vegetation and likely supports few, if any, ants. Occasional application of toxic bait along the eastern side of the flow would greatly reduce the chance of ants building up populations large enough to facilitate dispersal westward across the flow. If ant nests are detected on the western side of the flow, then aggressive application of toxic bait might still eliminate them. Additional treatment along the boundary fence with HOVE may be necessary if ants cross the flow within the subdivision and begin to disperse to higher elevations.

The eastern boundary of the Kahuku Argentine ant population is much longer and is not constrained by a natural barrier, probably making control more difficult. Nevertheless, a 50-m swath of treatment periodically applied along the population front might reduce population densities sufficiently to prevent the spread of ants into new areas.

Trials testing the efficacy of toxic bait to control Argentine ants indicate that Maxforce[®] is the most effective bait commercially available, killing 88–97% of worker ants within seven days and about 50% of nests (queen ants and developing brood) after two weeks (Chapter 2 in this report, Krushelnycky and Reimer 1998). A second application of Maxforce[®] within 30 days can further reduce worker ant abundances, but has not been shown to eradicate ants or nests from treatment plots (Chapter 2 in this report, Krushelnycky and Reimer 1998). Following two treatments, worker ant abundance was relatively slow to recover, returning to only 26% of levels in control plots after 120 days. Experimental trials using fipronil-based Xstinguish™ bait were slightly more effective than Maxforce[®] at reducing numbers of worker ants (99% reduction), but the product is not registered for use in the USA and is therefore not available for landscape-level application.

No tests have been conducted to determine the frequency at which Maxforce[®] should be applied to prevent the spread of Argentine ants in a landscape setting, but considering the relatively slow rate at which ant abundances recovered in experimental plots, a treatment schedule of two to four times per year may be sufficient (the Maxforce[®] label restricts the number of applications to no more than four per year). Monitoring population boundaries between applications would be important for informing decisions about when to reapply treatments. Maxforce[®] is formulated in granules and is applied most effectively by broadcasting the bait using a hand-held fertilizer spreader. The recommended rate indicated on the product label for broadcast application is 1.7 kg/ha. A similar strategy to control Argentine ants could be developed for the Mauna Loa Strip section of the park, perhaps using Mauna Loa Road as the line at which to stop the spread of ants.

Preventing the spread of Argentine and other ants is an important way of protecting sensitive ecological areas. Ants can be spread in a variety of ways, including in vehicles, backpacks, field equipment, potting material, fill for road construction, and on live and dead plants. Ants are notoriously attracted to human food, so preventing access to food that is destined to be transported to other areas of the park is critical for preventing their spread. Because ants can nest in the undercarriage and in beds of pickups and other vehicles, and colonization can occur quickly, the potential for dispersing ants is significantly greater if vehicles are parked in ant-infested areas. Controlling ants where vehicles are parked would greatly reduce opportunities for transporting ants. Monitoring vehicle contamination using bait (Spam®, tuna, peanut butter, or jam) prior to driving to upper elevations at Kahuku and the Mauna Loa Strip would also greatly reduce the risk of transporting ants into these sensitive areas.

CHAPTER 2. EFFICACY OF BAITS CONTAINING HYDRAMETHYLNON, FIPRONIL, AND PYRIPROXYFEN TO CONTROL ARGENTINE ANTS (*LINEPITHEMA HUMILE*) IN HAWAI`I VOLCANOES NATIONAL PARK

Introduction
The Argentine ant (*Linepithema humile*) is notoriously difficult to control in natural environments. Aqueous sugar solutions containing boric acid (Klotz *et al*. 1998) or borate (Greenberg *et al*. 2006) have proven effective at reducing ant numbers, but since the solution must be dispensed in bait stations, it may be impractical for landscape level applications. In contrast, granular baits can be distributed more easily over large areas, but few baits specifically targeting Argentine ants have been tested at the landscape level. The largest trial to date was an aerial application of hydramethylnon-based bait to more than 86 ha at Haleakalā National Park (HALE) in an attempt to suppress an advancing front threatening a fragile subalpine ecosystem (Krushelnycky *et al*. 2011). While the application greatly decreased ant abundances, it did not prevent their spread. Smaller-scale field trials using the same baits yielded similar results (Krushelnycky and Reimer 1998). From this experience, therefore, it is evident that land managers in Hawai`i lack effective tools for containing Argentine ants.

While it may not currently be possible to eradicate large-scale populations of Argentine ants, management goals may often include preventing the establishment of populations in areas free of ants and eradicating or reducing populations in areas of high ecological value or sensitivity. This study was designed to assess the effectiveness of three baits in eradicating relatively small populations of Argentine ants in Hawai`i Volcanoes National Park (HAVO). Two baits contained acute toxins and one bait acted hormonally to disrupt the development of eggs and larvae.

Methods
Study site
This study took place at 1200 m elevation in the Broomsedge Burn ($19^0 25'59.6"$ N, $155^0 17'35.2"$ W) area of HAVO, an area of over 400 ha of mesic to dry `ōhi`a woodland that burned on 30 June 2000. Prior to the fire, the understory consisted of a variety of native shrubs but was dominated by the invasive, fire-tolerant broomsedge (*Andropogon virginicus*). Approximately two years following the fire, broomsedge regained dominance of the community (Loh *et al*. 2007). Ant distribution prior to the fire was unknown, but by 2005 Argentine ants were found to inhabit nearly 50% of the burned area (U.S. Geological Survey unpublished data). Several other ant species, including big-headed ants (*Pheidole megacephala*), *Anoplolepis gracilipes*,

Nylanderia bourbonica, and an unidentified *Cardiocondyla* were also found in the study area, but maintained distributions that generally did not overlap with Argentine ants.

Formicidal treatments

The efficacies of three baits were compared in this study: Maxforce®, containing hydramethylnon at 1.0% (Bayer Environmental Science, Montvale, NJ); Xstinguish™, containing fipronil at 0.01% (Bait Technology, Auckland, New Zealand); and Australian Distance®, containing pyriproxyfen at 0.5% (Sumitomo Chemical, Chatswood, Australia). Hydramethylnon is an acute toxin that causes death by inhibiting ATP production, and in Maxforce® it is incorporated into ground silkworm pupae to produce high-protein bait that is very attractive to Argentine ants (Forschler and Evans 1994, Krushelnycky and Reimer 1998). Maxforce® is the standard bait used against Argentine ants in the USA. Although Maxforce® was not found to eradicate Argentine ants in experimental plots at HALE (Krushelnycky and Reimer 1998), we included it in our study because it was the most effective bait available commercially in the USA, and therefore, could be used for comparison to the other baits. Xstinguish™ utilizes fipronil, also an acute toxin but one that disrupts nervous system function (Cole *et al*. 1993). The matrix of Xstinguish™ is comprised of both protein and carbohydrate (Stanley 2004) and is also highly attractive to Argentine ants. In Australian Distance®, the insect growth regulator (IGR) pyriproxyfen is incorporated into a matrix consisting of defatted corn grit saturated with soybean oil. Through hormonal action, pyriproxyfen leads to atrophy of ovaries in egg-producing queens and disrupts the development of larvae and pupae (Reimer *et al*. 1991). The Australian Distance® differs from Distance® manufactured in the USA (Valent Corporation, Walnut Creek, CA) by the addition of a proprietary ingredient to enhance the attractiveness of the bait to Argentine ants (G. Webb, personal communication). Corn-based baits are generally not very attractive to Argentine ants, but small-scale trials using bait without the IGR suggested that it was attractive to the ants in our study area (U.S. Geological Survey unpublished data). Xstinguish™ and Australian Distance® from Australia are not registered for use in the USA and were tested under a State of Hawai`i Experimental Use Permit.

Application methods

The study was conducted as a randomized, complete block design in which blocks were located 100–200 m apart. Each block consisted of four contiguous 50 x 50 m plots (plus 4-m buffer areas around each plot) of which three received bait treatments and one acted as an untreated control. Each plot was subdivided into 25 cells measuring 10 x 10 m to facilitate even application of bait and consistent monitoring of ant abundance over the entire plot. Baits were applied between 10 October and 16 November 2006 during the morning (0800–1100 hours) on days without rain. Applications were repeated approximately 30 days after the initial treatment. To minimize the immigration of ants from outside the plots, the 4-m-wide buffer area around each treated plot received a standard dosage of bait applied within the plot. The buffer areas were re-treated simultaneously with plot re-applications.

Formicidal baits were applied at label rates: Maxforce® at 1.7 kg/ha, Xstinguish™ at 3 kg/ha, and Australian Distance® at 4 kg/ha (Table 2.1). Xstinguish™ was available in a paste-like form within 325 g caulking-style tubes. Because we found it difficult to apply in accurately measured quantities, the paste was transferred into 60-ml plastic syringes for dispensing. Five people walked in parallel applying small dollops at one to two meter intervals. Since initial results suggested that this bait was highly effective, two of the four plots were randomly selected to be "spot-treated" on the second application. That is, Xstinguish™ was only applied to cells in which

Table 2.1. Formicidal baits tested against Argentine ants in Hawai`i Volcanoes National Park. Maxforce® is registered for use in the USA, but Xstinguish™ and Australian Distance® are not.

Bait	Active ingredient	Mode of action	Formulation	Application rate
Maxforce®	Hydramethylnon 1.0%	acute toxin	granular	1.70 kg/ha
Xstinguish™	Fipronil 0.01%	acute toxin	paste	3.00 kg/ha
Australian Distance®	Pyriproxyfen 0.5%	IGR	granular	4.00 kg/ha

IGR = insect growth regulator

ants were detected on at least one occasion following the first treatment. Since Xstinguish™ was the most labor-intensive bait to apply, requiring approximately twice the effort to dispense, it was deemed important to determine if application effort could be reduced through spot treatment. Both Maxforce® and Australian Distance® are granular formulations and were applied by hand using a "salt shaker" method (a 120 ml plastic cup with a lid containing multiple holes large enough for bait to fall through when shaken) with two people walking side-by-side through each cell.

Ant abundance and distribution were monitored in each plot before and after treatment using a small amount of canned tuna (drained of most of its oil) placed onto 5 x 7 cm paper cards. The backs of the cards were plasticized with packaging tape to prevent oil from soaking through the cards and attracting ants to the underside. In all plots, except those receiving Xstinguish™ treatment, one bait card was randomly placed within each of the 25 cells in each plot; since Xstinguish™ appeared to be highly effective at the first monitoring date, two bait cards were subsequently placed in each cell to maximize the likelihood of detecting ants. Baits cards were available to ants for one hour, after which all ants were counted on the card. Abundance was calculated as the mean number of ants per card per plot. Calculating the proportion of cards at which ants were observed (including ants under or within 1 m of the bait cards) provided us with an index of occurrence and indicated how ants were distributed across the 25 cells of each plot. Ant abundance and detection were monitored weekly for the first 12–14 weeks, and then biweekly for a total of about 25 weeks. The number of days of monitoring at plots sometimes differed among blocks, so monitoring dates were rounded to the nearest week. For graphic presentation, the dataset was truncated at 15 weeks.

Statistical analysis
Pre-treatment mean ant abundance was compared among plots using a randomized block analysis of variance (ANOVA). Mean ant abundances in plots following treatment did not conform to assumptions of parametric analyses due to very small numbers of individuals in most plots, so the overall differences in mean ant abundance following treatment were tested with Kruskal-Wallis analyses. Post-treatment tests were performed after the first treatment (week 1), before and after the second treatment (weeks 4 and 5, respectively), and nine weeks following the initial treatment.

The proportion of bait cards at which ants were detected on each plot was modeled as a binomial distribution. To control for spatial heterogeneity, we fit a generalized linear mixed model that nested pesticide treatment and treatment time (prior to treatment and in the same four post-treatment periods listed above) within blocks as a random factor. The block factor, despite its insignificant effect on the response, was included in each model as a means of accounting for site-to-site differences. There were no significant interactions of block by

treatment or block by time, indicating similar treatment effects across sites and over time. The dispersion factor of the binomial model was relatively small (4.2) indicating slight over-dispersion. Significant factors all had p-values well below our 0.05 significance threshold, so the slight under-estimate of variance would not have affected our conclusions.

Values presented are means (± SEM, standard error of the mean) of the four replicate plots for each treatment and the control. Significant differences for all tests were recognized at α = 0.05.

Results
Abundance of ants at cards
There was no significant difference in ant abundance among treatment or control plots prior to the application of the baits ($F_{3,15}$ = 1.8, P = 0.37). The mean value of the four control plots was 78% higher during pre-treatment measurements than at one week following the first treatment, but much of this difference was due to a single plot on which ants were 2.9 times more abundant than the mean of the other three plots prior to treatment (67 vs. 23 ants/card). The high pre-treatment value found on this one plot is viewed as anomalous because this plot was generally not found to have more ants than the other control plots on subsequent post-treatment dates. Ignoring the value for this single plot, pre-treatment abundance was 21% higher than one week post-treatment (23 vs. 19 ants/card).

All three baits had strong impacts on Argentine ants, greatly reducing their abundance at bait cards (Figure 2.1). There was a significant overall treatment effect at each post-treatment date (P< 0.05). Xstinguish™ had the strongest immediate impact, decreasing numbers to < 1% of control levels at one week. This level of suppression persisted for about eight weeks. After 12–14 weeks, ant abundance in Xstinguish™ plots ranged from 3–8% of control levels, indicating a long-lasting effect with minimal recovery from surviving nests or immigration from outside the plots. Ant abundance in Xstinguish™ plots was significantly lower than in control plots through nine weeks.

Results from spot-treating two of four plots with Xstinguish™ during the second application indicated that the full-plot treatment was not more effective than the spot treatment. Ant abundance one week following treatment in fully treated plots was reduced to 31% of pre-treatment levels compared to 11% in spot-treated plots. Two weeks after treatment ant abundance was at 38% of pre-treatment levels in fully treated plots and 6% in spot-treated plots. Overall, ants were rarely detected prior to the second treatment in any of the Xstinguish™ plots (mean = 0.64 ants/bait card) and were not eradicated either by spot treatment or full-plot treatment.

Maxforce® reduced ant abundance to about 8% of control levels after one week and 3% of control levels at six weeks (Figure 2.1). Subsequently, however, ants in Maxforce® plots increased to 21% of control levels at 12 weeks.

Australian Distance® initially reduced ant abundance to 19% of control levels after one week, but abundances rebounded to 40% of control levels after four weeks. Ant numbers then dropped to 15–18% of control levels during weeks 5–8 but increased to 31% by week 9 and 72% by week 10 (Figure 2.1).

Frequency of occurrence of ants at bait cards
In control plots, the proportion of bait cards at which ants were detected remained relatively constant over the course of the study, with ants found on an average of 90% of the cards
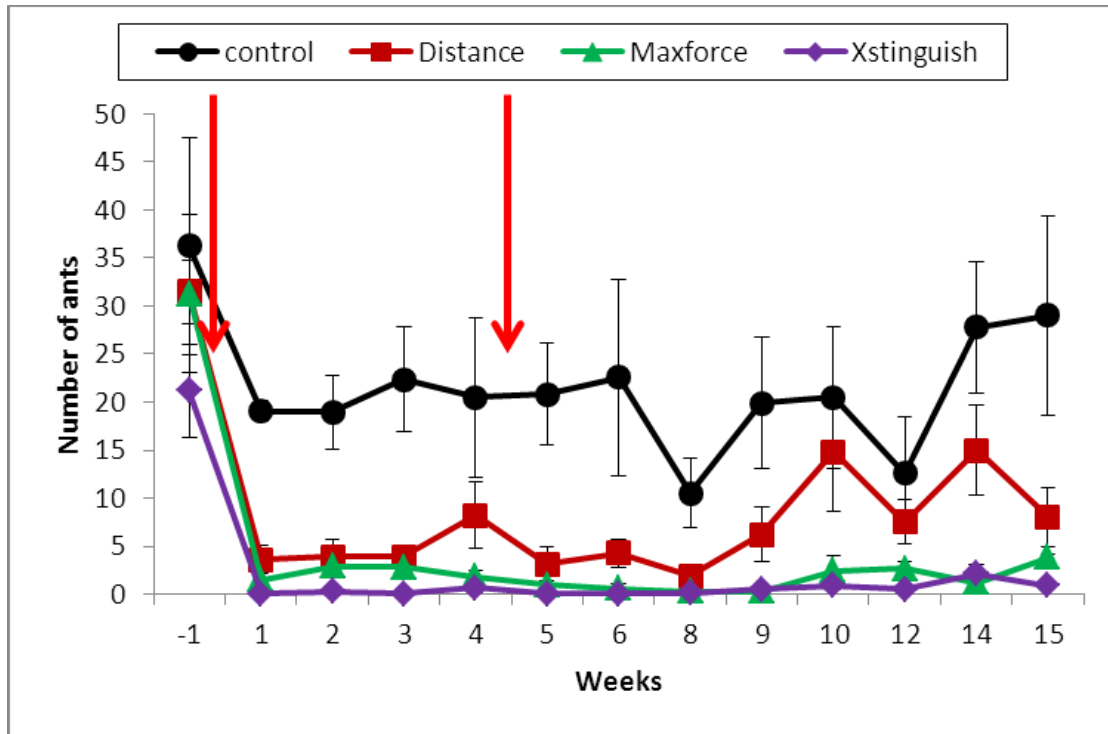
31

Figure 2.1. Mean (± SEM) number of Argentine ants detected at bait cards prior to application of insecticide treatments and over the following 15 weeks of the study. The red arrows indicate the approximate dates of the first and second treatments.

(Figure 2.2). There were no significant differences between treatment or control plots prior to application of the baits ($P > 0.10$).

In general, trends in ant frequency of occurrence and abundance were similar and were lowest in Xstinguish™ plots, where ants were recorded at 4% of control levels after one week and 10% by eight weeks (Figure 2.2). As reflected by the low average number of ants per card (Figure 2.1), only one or two ants were found on 79% of those cards containing ants over the first eight sample dates. Frequency of occurrence increased faster than ant abundance, however, and ants were found at 25% of the cards at 9 weeks and at 38% of the cards at 12 weeks. The proportion of cards with ants in Xstinguish™ plots was significantly lower than in control plots on all dates compared ($P < 0.001$).

The frequency of occurrence of ants at cards in Maxforce® plots was considerably higher than in Xstinguish™ plots, with ants being found at 35% of cards after one week and 47% of cards after two weeks (Figure 2.2). Detection rates remained at similar levels for the first five weeks before dropping to a low of 19% two weeks following the second treatment. Detection rates increased steadily over the next nine weeks before reaching a rate of 60% at week 15. The proportion of cards with ants in Maxforce® plots was significantly lower than in control plots on all dates ($P < 0.001$ at weeks 1, 5, and 9; $P < 0.01$ at week 4).
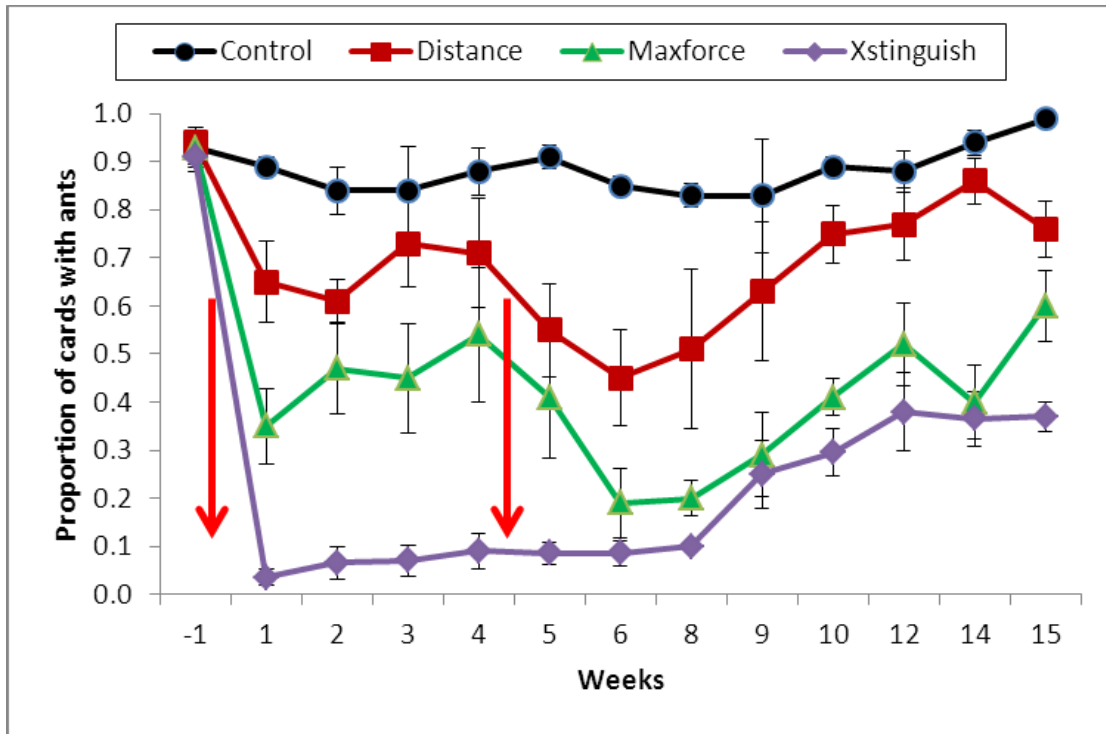
Figure 2.2. Mean (± SEM) proportion of bait cards at which Argentine ants were detected prior to application of insecticide treatments and over the following 15 weeks of the study. The red arrows indicate the approximate dates of the first and second treatments.

In Australian Distance® plots, detection rates were higher but followed a trend similar to that seen on Maxforce® plots. Detection rates ranged from 65–71% during the first four weeks following the initial treatment before decreasing to a low of 45% after six weeks. By week 14, ants were detected at 86% of the cards. Detection rates in Australian Distance® plots differed significantly from control plots at week 1 ($P < 0.05$) and week 5 ($P < 0.001$).

Discussion

All three treatments significantly reduced numbers of Argentine ants at bait cards, but none eradicated ants on any of the treatment plots. These results are largely consistent with previous studies (Klotz *et al*. 1998, 2000, Krushelnycky and Reimer 1998, Hooper-Bui and Rust 2000, Silverman and Roulston 2001, Greenberg *et al*. 2006) and underscore the difficulties associated with eradicating this species, even on a relatively small scale.

The fipronil-based Xstinguish™ was highly effective at reducing ant abundance, both in terms of numbers on bait cards and the proportion of cards occupied. The fact that ant numbers in Xstinguish™ plots remained at levels < 1% of the control plot for about eight weeks suggests that the vast majority of brood and workers were killed. It is not clear whether the few ants detected at bait cards were associated with small, surviving nests or whether they were individuals that had not consumed toxic levels of fipronil and were simply wandering the plots in search of nests that had been extirpated. It is also possible that some nests survived treatment but that the surviving ants subsequently switched food preferences and were avoiding the rich protein and fat tuna baits. Argentine ants in our study site frequently nested in inconspicuous

voids in soil or lava rock, making it difficult to locate small nests. Curiously, individual ants observed leaving bait cards often walked in near-random directions, suggesting an absence of a pheromone trail leading to a nest.

Maxforce® was the only bait that resulted in a steady decrease in ant abundance at cards over time, and by about eight weeks it had achieved results approaching those found on Xstinguish™ plots. While ants eventually declined to very low densities, their lowest detection rate (19% of bait cards) was still more than twice that found on Xstinguish™ plots within the first six weeks following initial treatment. These results suggest that surviving ants were more widely scattered over the Maxforce® plots than on Xstinguish™ plots. Overall, our results were similar to those of Krushelnycky and Reimer (1998), where Maxforce® reduced ant abundances about 96% after two days and 92% after two months in subalpine habitats at HALE. Within the Maui study sites, however, Argentine ants generally nested under rocks, allowing evaluation of nest survival even at low densities. As a result, Krushelnycky and Reimer (1998) were able to show that about 50% of nests treated with Maxforce® at 4.5 kg/ha (equivalent to our double-dosage treatment) were still active two weeks following treatment. Subsequent studies by Krushelnycky *et al.* (2011) comparing Maxforce® to other baits corroborated our results of high worker mortality following treatment; in addition, they were able to document low-level survival of nests on treatment plots.

The relatively strong, immediate reduction in ants in the Australian Distance® plots was unexpected, since pyriproxyfen and other IGR baits generally have a delayed effect on ant abundance by preventing production of eggs and development of brood (Reimer *et al.* 1991). Our results, however, indicate that Australian Distance® was toxic to the ants. Although little work has been performed testing IGR baits on Argentine ants, trials of pyriproxyfen (2%) on big-headed ants resulted in a significant reduction in worker abundance nine weeks following treatment, when pupal emergence was insufficient to replace dying workers (Reimer *et al.* 1991). In that study, both egg production by queens and brood survival decreased by more than 95% three weeks following exposure to the IGR. Direct toxic effects of pyriproxyfen were attributed to the death of workers of pharaoh ants (*Monomorium pharaonis*; Vail and Williams 1995), but not until six weeks following treatment, considerably later than in our study.

We did find a reduction in ant abundance in Australian Distance® plots starting about five weeks after the first application, but it is not clear whether this was an IGR effect from the first application or a toxic effect from the second application, which occurred about one week earlier. The timing of this decline is generally consistent with an IGR effect if workers dying a natural death are no longer replaced by adults emerging from pupae, but it is also consistent with toxic exposure to the second application. Either way, the reduction was relatively small (a decrease of about 23%) and short-lived, as abundance began to increase after eight weeks.

It has been suggested that protein baits are more attractive to Argentine ants during spring and summer, when brood are most actively being produced and protein is in greatest demand (Krushelnycky and Reimer 1998, Rust *et al.* 2000). Although brood are produced year-round on our study site (U.S. Geological Survey unpublished data), patterns of nest development and proximate cues influencing brood production are unknown. On average, seasonal differences in climate are weak at our sites, but mean daily temperature is slightly lower and rainfall slightly higher during the winter months (Giambelluca *et al.* 1986). Stronger seasonal changes in temperature resulted in fewer ants during winter months at 2200 m elevation in HALE (Krushelnycky and Reimer 1998, Krushelnycky *et al.* 2004), whereas ant numbers at our HAVO

sites began increasing in January (approximately 100 days following initial treatment) and continued to increase as the winter progressed, apparently due to brood development. Limited comparative testing of Maxforce® during July 1996 and February 1997 at HALE resulted in no differences in effectiveness, suggesting time of year did not affect efficacy of the bait (Krushelnycky *et al.* 2011). Regardless, it is unclear whether the baits we tested would be more effective at other times of the year. Perhaps it is significant that Xstinguish™ is comprised of both proteins and carbohydrates (Stanley 2004), which may make it attractive to Argentine ants at all times of the year.

Although we did not eradicate Argentine ants in any of our plots, our results indicate that Xstinguish™ was highly effective at reducing their abundance and distribution. This suggests that Xstinguish™ could be an effective tool for controlling the Argentine ant on a small scale. While the high moisture content of Xstinguish™ paste likely contributed to its attractiveness to Argentine ants, it required approximately twice the effort of applying granules of Maxforce® or Australian Distance®. Decreasing the application density of Xstinguish™ would save time, but additional testing of efficacy would be required. Nevertheless, Xstinguish™ is not registered for use in the USA, preventing its application beyond experimental use. Our results corroborate those of Krushelnycky and Reimer (1998), indicating that Maxforce®, the bait most commonly used to control the Argentine ant in the USA, can effectively suppress ant abundance in field settings but is unlikely to lead to eradication.

CHAPTER 3. EFFICACY OF BAITS CONTAINING HYDRAMETHYLNON, FIPRONIL, AND HYDRAMETHYLNON COMBINED WITH S-METHOPRENE AND PYRIPROXYFEN TO CONTROL BIG-HEADED ANTS (*PHEIDOLE MEGACEPHALA*) IN HAWAI`I VOLCANOES NATIONAL PARK

Introduction

Big-headed ants (*Pheidole megacephala*) have a long history of invading tropical areas and today are among the most widespread invasive ants in the world (Forel 1899, Haskins and Haskins 1988, Holway *et al.* 2002). Several studies have illustrated their devastating effect on native ecosystems that they invade (Perkins 1913, Hoffmann *et al.* 1999, Vanderwoude *et al.* 2000). Because big-headed ants effectively defend many disease-carrying homopteran insects from their natural enemies, they also have indirectly become agricultural pests. Following the ban of chlorinated hydrocarbon-based pesticides such as DDT that were used to control ants and many other insect pests, considerable effort went into searching for alternative pesticides that would be equally effective at controlling undesirable insects. Several early studies investigating the efficacy of alternative insecticides to control big-headed ants were conducted in Hawai`i where this ant was a major pest of pineapple. On Hawaiian pineapple, big-headed ants are effective defenders of *Dysmicoccus* mealybugs responsible for transmitting the virus that causes a devastating wilt disease (Beardsley *et al.* 1982, Gunasinghe and German 1989). These studies found hydramethylnon to be an effective pesticide for controlling big-headed ants in field plots (Reimer and Beardsley 1990), but also found the insect growth regulators (IGRs) fenoxycarb and pyriproxyfen to significantly reduce the production of eggs by queen ants and the maturation of brood, illustrating their potential for controlling ants in the field (Reimer and Beardsley 1990, Reimer *et al.* 1991). Recent eradication of several populations of big-headed ants in northern Australia using hydramethylnon underscores the effectiveness of this pesticide in natural areas (Hoffmann 2011).

While hydramethylnon has proven effective at eradicating or controlling big-headed ants, it readily photo-degrades into relatively benign metabolites under field conditions and thus may not be the ideal pesticide in some natural areas. Hydramethylnon has a half-life of about 12 hours when exposed to sunlight and was found to be ineffective at controlling red imported fire ants (*Solenopsis invicta*) after 12–30 hours (Vander Meer *et al*. 1982). In contrast, the IGR, pyriproxyfen, that was incorporated into Distance® ant bait registered for use in the USA (Valent Corp., Walnut Creek, CA), was found to have a half-life of seven to nine days in sunlight (Taniguchi *et al*. 2003), suggesting that it might provide a longer period of activity than hydramethylnon in environments with high levels of solar radiation.

The goal of our study was to test the efficacy of Amdro® containing hydramethylnon with several other baits, including two that utilize a mixture of hydramethylnon with IGRs, to control big-headed ants in Hawai`i Volcanoes National Park (HAVO). Additionally, we tested fipronil-based Xstinguish™ ant bait because it proved highly successful against Argentine ants (*Linepithema humile*; Chapter 2 in this report) and was found to be attractive to foraging big-headed ants in preliminary trials (U.S. Geological Survey unpublished data).

Methods
Study site
This study was conducted within the Broomsedge Burn area of HAVO ($19^0 25'47"$ N, $155^0 17'51"$ W) at 1190 m elevation. Treatment plots were located 750 m southwest of the location where formicidal baits were tested against Argentine ants (see Chapter 2 of this report for a brief description of the area).

Formicidal treatments
We tested the efficacy of four baits against big-headed ants: two experimental baits not registered for use in the USA and two baits that are registered (Table 3.1). The experimental baits included Australian Distance® Plus (Sumitomo Chemical, Epping, NSW, Australia), which is a combination of pyriproxyfen (0.25%) and hydramethylnon (0.365%) in a proprietary formulation of corn, soybean oil, and fishmeal, and Xstinguish™ (Bait Technology, Auckland, New Zealand), which mixes fipronil (0.01%) into a matrix of protein and sucrose and is formulated in a paste-like consistency. To our knowledge, neither of these baits has been tested against big-headed ants, but preliminary trials we conducted revealed strong attraction to both baits (U.S. Geological Survey unpublished data).

Table 3.1. Formicidal baits tested against big-headed ants in Hawai`i Volcanoes National Park. Amdro® and Extinguish® Plus are registered for use in the USA, but Australian Distance® Plus and Xstinguish™ are not.

Bait	Active ingredient	Mode of action	Formulation	Application rate
Amdro®	Hydramethylnon 0.75%	acute toxin	granular	1.24 kg/ha
Extinguish® Plus	Hydramethylnon 0.365% & S-methoprene 0.25%	acute toxin & IGR	granular	1.24 kg/ha
Australian Distance® Plus	Hydramethylnon 0.365% & Pyriproxyfen 0.25%	acute toxin & IGR	granular	4.00 kg/ha
Xstinguish™	Fipronil 0.01%	acute toxin	paste	3.00 kg/ha

IGR = insect growth regulator

The two registered baits tested were Amdro®, containing hydramethylnon (0.73%; Ambrands, Atlanta, GA), and Extinguish® Plus, containing a mixture of hydramethylnon (0.365%) and S-methoprene (0.25%; Wellmark International, Schaumberg, IL; Table 3.1). Amdro® is the standard bait used against big-headed ants internationally. Extinguish® Plus is widely used to control red imported fire ants (*Solenopsis invicta*) but may also prove effective in controlling big-headed ants. Hydramethylnon and fipronil are acute, fast-acting toxins while pyriproxyfen and S-methoprene are relatively slow-acting IGRs, chemicals that disrupt endocrine function, egg production, and development of larvae (Reimer *et al*. 1991). It was anticipated that the combination of an acute toxin with an IGR might prove more effective than either ingredient alone.

Application methods
Using the same experimental design as was used in the Argentine ant control trials (Chapter 2 in this report), the big-headed ant control study was conducted as a randomized complete block design in which four blocks were located 100–200 m apart. Each block consisted of four 50 x 50 m treatment plots and one 50 x 50 m untreated control plot. The control plot was established at a distance of 20 m from treatment plots. The four treatment plots within each block were randomly assigned treatment by one of the four baits. A treated 4-m-wide buffer was created around the perimeter of each treatment plot to minimize immigration of ants into the plots. The buffers around each plot were treated with their respective baits, and the treatment of buffer areas between plots was split between the two respective bait types (2-m strips for each bait).

Each plot was subdivided into 25 cells measuring 10 x 10 m each to facilitate even application of bait and consistent monitoring of ant abundance over the entire plot. Pesticides were applied on days without rain between 28 August and 14 September 2007. Pesticide applications were repeated about 30 days after the initial treatment.

Baits were applied at the rate indicated on their label (Amdro®, Extinguish® Plus, and Xstinguish™) or at the rate recommended by the manufacturer (Australian Distance® Plus; Table 3.1). Maxforce®, Extinguish® Plus, and Australian Distance® Plus were formulated as granules and were applied using a "salt shaker" method with two people distributing pre-measured quantities of bait in concert within each cell. Xstinguish™, which was formulated as a paste, was transferred into 60-ml plastic syringes and dispensed onto each plot. To apply the bait, five people walked in parallel throughout the plot dispensing dollops of bait at one to two meter intervals. Because our supply of Xstinguish™ was limited to the amount necessary to treat the plots, Amdro® was used to treat the buffer area around Xstinguish™ plots.

Ant abundance and distribution were monitored within each plot using a small amount of canned tuna (drained of oil) placed on 5 x 7 cm plasticized index cards. One card was placed in the center of each of the 25 cells within each plot for the first two monitoring sessions (pre-treatment, week 1) and two cards were placed within each cell thereafter (except for control plots) to increase the odds of detecting ants when in low abundance. Ants were monitored weekly for the first two weeks following the application of the first treatment and biweekly thereafter for a total of 12 weeks following initial treatment. During each monitoring survey, tuna baits were allowed to attract ants for approximately one hour before counting all ants on the bait cards for an index of abundance (mean number of ants/card). The presence or absence of ants on, under, or within one meter of the bait cards was used to calculate the frequency of occurrence (proportion of cards with ants) and the spatial distribution of ants among the 25 cells of each plot.

Statistical analysis
Statistical analyses followed those used for testing the efficacy of baits to control Argentine ants (Chapter 2 in this report). Responses to formicidal treatment were measured using the abundances of ants at bait cards and were evaluated prior to treatment using a randomized block analysis of variance (ANOVA) and post-treatment using Kruskal-Wallis tests. In contrast, the effects of baits as determined by the proportion of bait cards at which ants were detected were tested using a general linear mixed model nesting pesticide treatment and treatment time. For both metrics of ant abundance, treated plots were compared to control plots on the following dates: one week prior to treatment, one week after the initial treatment (week 1), one week prior to the second treatment (week 4), two weeks following the second treatment (week 6), and eight weeks following the initial treatment (four weeks after the second treatment). There were no significant interactions of block by treatment or block by time, indicating similar treatment effects across sites and over time. The dispersion factor of the binomial model was relatively small (2.2), indicating slight over-dispersion but likely too small to influence our conclusions.

Values presented are means (± SEM) of the four replicate plots for each treatment and the control. Significant differences for all tests were recognized at $\alpha = 0.05$.

Results
Abundance of ants at cards
There were no significant differences in ant abundance among treatment or control plots prior to application of formicidal baits ($F_{4,19} = 0.73$, df = 4, $P = 0.59$; mean = 57.3 ± 3.3 ants/card). Mean ant abundance in control plots increased slightly after one week (64.5 ± 9.4 vs. 49.1 ± 4.1) but generally decreased steadily over the course of the study (Figure 3.1). By week 12, ant abundance in control plots was at 40% of that found during the pre-treatment measurement.

All four baits resulted in a fast and long-lasting reduction in ant abundance relative to controls (Figure 3.1), leading to significant overall treatment effects on all post-treatment sampling dates ($P < 0.05$). Ant abundances were reduced to < 2% of control levels one week following the first treatment on all treatment plots. Amdro® had the strongest initial impact with ant abundance reduced to < 1% of control levels and no ants detected on two of the four plots. Suppression of ants was particularly long-lived for Amdro®, Australian Distance® Plus, and Extinguish® Plus as ant abundances remained at levels < 1% of levels in control plots over the course of the study; ant suppression in Xstinguish™ plots was shorter as ant abundances increased to 7% of control levels after four weeks and 20% after ten weeks. The second application of insecticidal baits further reduced ant numbers in all four treatments. No ants were detected in Amdro® and Australian Distance® Plus plots one week following the second application (six weeks following the initial application). Very small numbers of ants were detected in Amdro® and Australian Distance® Plus plots at week 8 (0.02 and 0.01 ants/card, respectively).

Frequency of occurrence of ants at bait cards
Overall, ants were detected at 96% of the baits cards in all plots prior to application of formicidal baits (Figure 3.2). The percent of cards at which ants were detected in control plots decreased somewhat over the course of the study, ranging from 96% prior to application of baits to 78% after 12 weeks. There was no significant difference among treatment or control plots prior to application of the baits ($P > 0.10$).
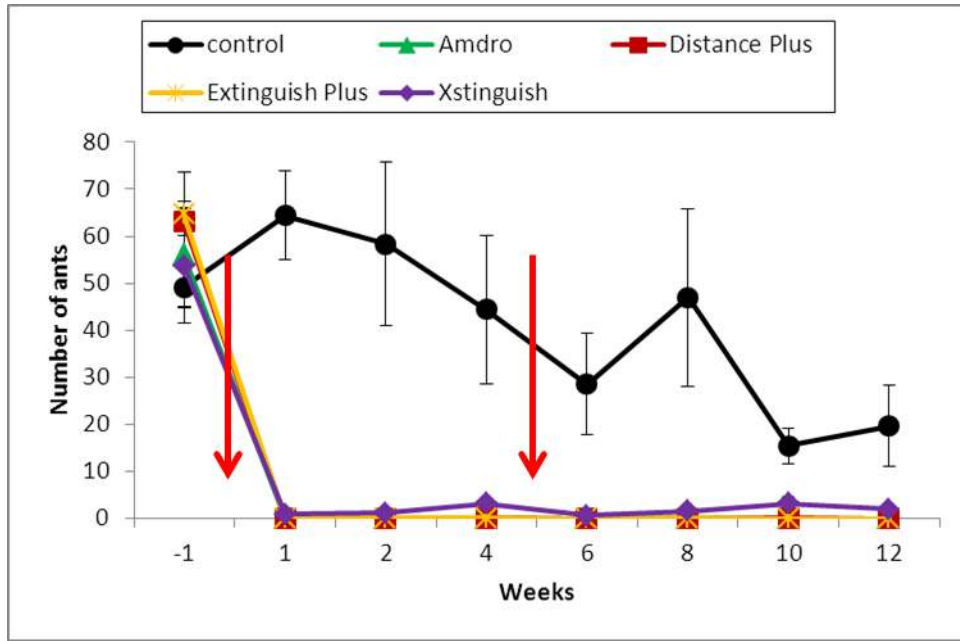
Figure 3.1. Mean (± SEM) number of big-headed ants detected at bait cards prior to application of insecticide treatments and over the following 15 weeks of the study. The red arrows indicate the approximate dates of the first and second treatments.
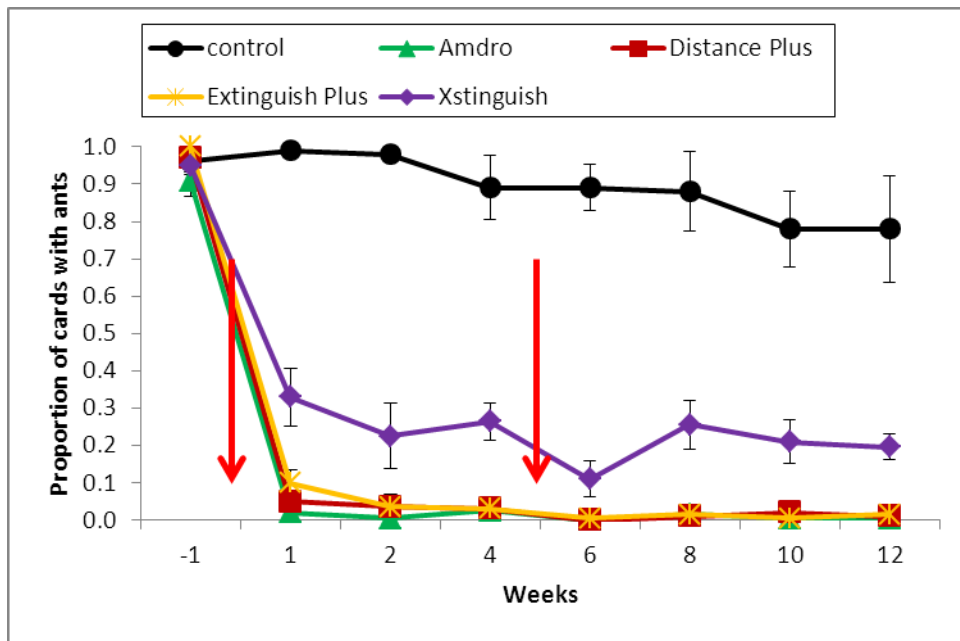

Figure 3.2. Mean (± SEM) proportion of bait cards at which big-headed ants were detected prior to application of insecticide treatments and over the following 15 weeks of the study. The red arrows indicate the approximate dates of the first and second treatments.

Trends in the frequency of occurrence of ants at bait cards generally paralleled trends of ant abundance. The greatest reduction in frequency of occurrence relative to controls after one week was found for Amdro® (2% of the control level), followed by Australian Distance® Plus (5%), Extinguish® Plus (10%), and Xstinguish™ (33%). For the remainder of the study, frequency of occurrence remained below 4% of control levels for Amdro®, Australian Distance® Plus, and Extinguish® Plus. In contrast, ants were found at 22–30% of bait cards on Xstinguish™ plots after one week, although a temporary decrease to 12% of the control level was detected a week after the second treatment in week 6. Frequency of occurrence on treatment plots was significantly lower than it was on control plots on all sampling dates ($P <$ 0.05).

Discussion
Our results show that the abundance of big-headed ants can be consistently and significantly reduced across replicated plots using both commercially available and non-registered formicidal baits. Although our inability to eradicate ants from the plots suggests that the treatments were not fully effective, it is possible that the small size of the plots resulted in the detection of foraging ants associated with nests located outside the treatment area. We were unable to directly measure the impact of the baits on the survival of egg-producing queens or the fate of developing brood because nests were generally located within cracks of the pāhoehoe lava substrate that dominated the study area, making them unobservable.

Baits using hydramethylnon alone or in combination with an IGR were highly effective, reducing ant abundance to 1% of control levels for at least 12 weeks following the initial application of bait. Nevertheless, Amdro® was slightly more effective at killing worker ants, given that their frequency of occurrence at bait cards was equal to or lower than that found on Extinguish® Plus or Australian Distance® Plus plots on six of seven post-treatment monitoring dates. The slightly stronger effect of Amdro® compared to Extinguish® Plus and Distance® Plus may have been due to its higher concentration of hydramethylnon (0.75% vs. 0.365% in Extinguish® Plus and Distance® Plus).

It was unclear whether there was a detectable influence of the IGRs S-methoprene (Extinguish® Plus) and pyriproxyfen (Australian Distance® Plus) on ant abundances. We would have expected the IGRs to have gradually reduced numbers of ants on the plots as workers died of natural causes and were not replaced by brood maturing in the nests. The number of ants on Extinguish® Plus and Australian Distance® Plus plots was slightly lower at week 5 compared to weeks 2 and 4, as expected from an IGR, but the week 5 monitoring date immediately followed the second application of all baits, thus making it difficult to distinguish between the fast effects of hydramethylnon and the slow effects of IGRs. Moreover, the trend in ant abundance on IGR plots paralleled that found on Amdro® plots, which further obscured any possible effects of the IGRs. It is likely that the strong effect of hydramethylnon masked any influence of the IGRs.

Our results are consistent with other recent studies showing that hydramethylnon can be used to control or eradicate relatively small populations of big-headed ants in natural areas. In northern Australia, more than 30 populations of big-headed ants, ranging in size from 0.001 to 5 ha, were eradicated with Amdro®, generally following only a single application of bait (Hoffmann and O'Connor 2004, Hoffmann 2010, 2011). Similarly, Plentovich *et al*. (2009) were able to eradicate big-headed ants from Moku`auia Islet off the northwestern coast of O`ahu, Hawai`i, with a single application of Amdro®. Additionally, in a pineapple plantation that had

recently been removed from production, Amdro® was used to eliminate big-headed ants from three 1.4 ha plots a week following treatment (Reimer and Beardsley 1990).

The fipronil-based Xstinguish™ was the least effective bait at controlling big-headed ants. Ant abundance was reduced to 2% of control plots one week following treatment, but numbers increased to 21% by 10 weeks, suggesting that numerous nests survived treatment. Fipronil has been found to be highly effective at killing other ants, including Argentine ants (Chapter 2 in this report, Harris 2002) and yellow crazy ants (*Anoplolepis gracilipes*; Green *et al.* 2004), and also would be expected to be highly toxic to big-headed ants. Even so, it is possible that the protein and sucrose-based paste matrix used to carry the fipronil in Xstinguish™ (Stanley 2004) was less attractive to foraging big-headed ants than the soybean oil-impregnated corn-grit granules used in the other baits. Alternatively, the concentration of fipronil in Xstinguish™ may have been a slight deterrent to the ants, resulting in a lower level of food collection or sharing in the nest.

While hydramethylnon is known to degrade quickly when exposed to sunlight, clearly it remained active on the study plots long enough to strongly affect ants. Considering our plots received little shade from trees or tall shrubs (see Chapter 1, Figure 1.4 for a photograph of the study area), there should be little concern for applying Amdro® in similar open habitats in HAVO. In managed areas where there is constant ant incursion from nearby untreated areas, protecting hydramethylnon bait from sun and weather could extend its activity and reduce the frequency of reapplication. This was illustrated by Taniguchi *et al.* (2003), who extended the full effectiveness of Amdro® in the field to 12 weeks by preventing its exposure to sun and rain through the use of protective bait stations. While effective, bait stations may not be practical for large scale applications as their recommended spacing was one per 15.2 m^2, which required 658 bait stations per ha.

In summary, our results indicate that all three baits containing hydramethylnon are highly effective at controlling big-headed ants. Amdro® was slightly more effective than Extinguish® Plus or Australian Distance® Plus immediately following treatment and after 12 weeks. In contrast, Xstinguish™, containing fipronil, performed relatively poorly compared to the other baits. The number of cases in which Amdro® has successfully been used to eradicate populations of big-headed ants is rapidly expanding, indicating the effectiveness of this bait under field conditions. Although both Amdro® and Extinguish® Plus are registered for use in the USA, the slightly better performance of Amdro® in our study, coupled with its proven performance in natural areas, suggests it would be a viable option for controlling big-headed ants in HAVO.

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