# Final Report

## The Impact of Non-Native Predators on Pollinators and Native Plant Reproduction in a Hawaiian Dryland Ecosystem

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This Final Report describes our research project that examined impacts of non-native invasive predators on pollination and native plant reproduction in Hawai'i. We combined field observation, experimental manipulation, and laboratory analysis to examine interactions between eight focal native plants (endangered and common), insect pollinators (native and non-native), and predators. Synthesis of the data showed that invasive predator do affect pollinator-plant interactions both negatively and positively; however, most effects are negative. Rats and ants in particular had only negative effects on pollinator-plant interactions. Results can aid land managers in determining whether to control invasive predators in order to manage for pollination services and native plant reproduction.

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## Table of Contents

## List of Figures

## List of Tables



## List of Acronyms

| | |
|---|---|
| ANOVA | analysis of variance |
| ASR | Area of Species Recovery |
| CO1 | cytochrome oxidase 1 mitochondrial gene |
| INRMP | Integrated Natural Resources Management Plan |
| ISI | Index of Self-Incompatibility |
| IV | importance value (scaled or non-scaled) for flower visitor |
| KKE | Kīpuka Kālawamauna East fenced unit within PTA |
| MCMC | Markov chain Monte Carlo |
| NIP | non-native invasive predator |
| NRO | Natural Resources Office |
| OTU | Operational Taxonomic Unit |
| PLI | Pollen Limitation Index |
| PTA | U.S. Army Pōhakuloa Training Area |
| PVI | pollinator visitation importance |
| PWW | Pu'u Wa'awa'a Forest Reserve |
| SE | standard error |
| SHELOB | SERDP Hawai'i Ecosystem-Level Observation-Based model |
| TN | trap night for rodent trapping |
| USDA | United States Department of Agriculture |

## Keywords

*Argemone glauca*, *Apis mellifera*, Argentine ant, *Bidens menziesii*, black rat, conservation, dryland tropical ecosystem, *Dubautia linearis*, endangered species, *Haplostachys haplostachya*, Hawai‘i, house mouse, honey bee, Hymenoptera, insect, invasive species management, island endemics, Lepidoptera, *Linepithema humile*, *Mus musculus*, plant-animal interactions, plant breeding system assessment, plant reproduction, Pōhakuloa Training Area, predators, pollination, pollination trials, pollinator, *Rattus rattus*, rodents, *Sida fallax, Silene lanceolata, Stenogyne angustifolia, Tetramolopium arenarium, Vespula pensylvanica,* yellowjacket

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This study was approved by the U.S. Department of Agriculture, National Wildlife Research Center’s Institutional Animal Care and Use Committee (IACUC) as QA-2452. Findings and conclusions in this publication have not been formally disseminated by the U.S. Department of Agriculture and should not be construed to represent any agency determination or policy. Mention of trade names or commercial products in this Final Report are solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned.

## Abstract

Non-native species invasions can disrupt pollination services and result in negative impacts on native plant reproduction and genetic diversity. Such impacts are particularly acute for oceanic islands, which are well known for high endemism and unique biological diversity, but also particularly susceptible to invasions. Non-native invasive predators (NIPs) consume animal pollinators and can reduce pollinator populations, possibly eliminating entire pollinator guilds. Our research examined the impacts of NIPs on pollination and native plant reproduction in a tropical dryland ecosystem in Hawai‘i. We combined field observation, experimental manipulation, and laboratory analysis to examine interactions between eight focal native plants (endangered and common), insect pollinators (native and non-native), and NIPs.

Our findings show that flower visitation to focal plant species is mainly performed by non-native insect species, and that there may be differences in pollination dynamics between endangered and common plant species. NIPs in our system–specifically ants, rodents, and yellowjacket wasps–have been shown to consume insect groups that include important pollinators. We suppressed NIPs in treatment plots, and found spatial and temporal variability in suppression efficacy for each NIP species. Synthesis of the data showed that NIPs do affect pollinator-plant interactions both negatively and positively; however, most effects are negative. Rats and ants in particular had only negative effects on pollinator-plant interactions.

Results from our study have several management implications. First, current pollination differs for endangered versus common plant species in our study system. Pollinator management and conservation are likely important for managing endangered plant species. Second, pollinator interactions are localized in time and space, and can vary by time of year. It may be necessary to perform observations over repeated seasons and across multiple locations, in order to capture the spatial and temporal fluctuations. Third, NIPs can affect pollinator-plant interactions, and in most cases negatively. Controlling rats and ants likely would increase pollinator frequency, and potentially lead to more out-crossing for plant species. Finally, predator control treatments can be moderately effective at suppressing predator abundance or activity; however, the treatments require continuous effort. There is temporal and spatial variation in predator dynamics that must be taken into account when undertaking suppression.

## Objective

The principal objective of our research was to determine the impacts of non-native invasive predators (NIPs) on insect pollinators and pollinator services in an invaded tropical dryland ecosystem in Hawai‘i. NIPs consume animal pollinators and, by doing so, reduce pollinator populations and possibly eliminate entire pollinator guilds. Loss of pollination services due to NIPs likely is an important, although poorly understood, factor in both native plant conservation and management for long-term sustainability of native island ecosystems. In our study, we (1) identified current insect pollinators and pollination effectiveness for focal native plant species, (2) examined diets of study site NIPs to identify direct interactions with insect pollinators, (3) applied common NIP control methods to experimentally determine their effectiveness at both reducing NIP populations and NIP impacts on pollinator-plant interactions, and (4) analyzed the data using Bayesian hierarchical models to identify the direct and indirect relationships between NIPs, insect pollinators, and native plants. This project was carried out at the U.S. Army Pōhakuloa Training Area (PTA) on Hawai‘i Island. Pollination is a critical ecosystem process and this study aligns with the Department of Defense's ecosystem-based approach to managing its training lands with the goal of ensuring that native species and their associated habitat are protected and restored.

Our focal plant species included four endangered species (*Haplostachys haplostachya*, *Silene lanceolata*, *Stenogyne angustifolia*, and *Tetramolopium arenarium*) and four common native species (*Argemone glauca*, *Bidens menziesii*, *Dubautia linearis*, and *Sida fallax*) at PTA. The focal study site NIPs include rodents (*Mus musculus*, *Rattus rattus*), ants (*Linepithema humile*, *Tapinoma melanocephalum*), and yellowjacket wasp (*Vespula pensylvanica*).

For this final report, we summarize the overall project conducted from 2014–2019, including: 1) identification of primary pollinators for our focal plant species, 2) assessment of baseline pollination effectiveness, 3) measurement of NIP abundance/activity before and after experimental treatments, 4) examination of NIP diets, and 5) modeled interactions between NIPs, primary pollinators, and focal plant species. Results have led to the identification of insect flower visitor taxa for our focal plant species, identification of the same insect flower visitor taxa in NIP diets, analyses of pollination effectiveness for our plant species, determination of efficacy of NIP control methods, and analyses of relationships between NIPs, insect pollinators, and native plants. We found that NIPs affected pollinator-plant interactions both negatively and positively, however most effects were negative. These results suggest that NIPs are affecting pollinator-plant dynamics in the Hawaiian dryland system, and that predator control may benefit pollination of native plants.

## Technical Approach

In order to gain a more complete understanding of current pollination services in invaded landscapes, we combined field observation, experimental manipulation, and laboratory analysis to examine interactions between native plants, pollinators (native and non-native), and non-native invasive predators (NIPs). We established three primary technical objectives for the project:

1. Identify current pollinators for focal native plant species and measure pollination effectiveness in an invaded tropical dryland ecosystem in Hawai‘i.
2. Examine diets of NIPs to determine interactions with pollinators.
3. Assess the impacts on pollination services with regard to common management actions used by Department of Defense natural resource managers to control non-native species.

### Study area and focal species

Pōhakuloa Training Area (PTA) is the largest U.S. Army holding in the State of Hawai‘i and encompasses approximately 53,750 hectares in the saddle region between Mauna Kea, Mauna Loa, and Hualālai volcanoes on the island of Hawai‘i. PTA contains part of a remnant sub-alpine tropical dryland ecosystem and supports 20 federally designated threatened and endangered plant species, 5 of which occur exclusively in PTA (*Festuca hawaiiensis*, *Isodendrion hosakae*, *Kadua coriacea*, *Schiedea hawaiiensis*, and *Tetramolopium arenarium*) (Pōhakuloa Natural Resources Office, pers. comm. November 2016). Land cover is a mix of native Hawaiian plant communities plus barren lava, anthropogenically-disturbed areas, and grassland dominated by invasive fountain grass (*Pennisetum setaceum*). The 2010 PTA Implementation Plan outlines several management actions to protect threatened and endangered species on PTA; methods include plant propagation and outplanting, non-native plant control, survey protocols, ungulate control, large-scale fencing, rodent control, and invasive invertebrate control. However, outside of this project, the impact of NIPs on pollination services remains unexplored in both current research and management plans based on the information contained in the draft 2017 PTA Integrated Natural Resources Management Plan (INRMP), and the reproductive success of at-risk plant species under existing levels of NIP invasion is unknown.

Our field work occurred in Kīpuka Kālawamauna East (KKE) on PTA, a 794-hectare mix of grassland-shrubland and *Metrosideros polymorpha* (‘ōhi‘a) dominated woodland located at approximately 1,675 m in elevation. This area is fenced to exclude non-native invasive ungulates (primarily goat and sheep) and contains many threatened and endangered plant species. A road permits access to the area but the unit is closed to the public, which ensured that our experimental treatments were undisturbed by public activity. NIPs in KKE include house mouse, *Mus musculus*, Muridae; black rat, *Rattus rattus*, Muridae; Argentine ant, *Linepithema humile*, Formicidae; ghost ant, *Tapinoma melanocephalum*, Formicidae; and yellowjacket wasp, *Vespula pensylvanica*, Vespidae.

We focused on eight native plant species found within or adjacent to KKE. Four of the species are listed as federally endangered: honohono, *Haplostachys haplostachya*, Lamiaceae; *Silene lanceolata* (no common name), Caryophyllaceae; *Stenogyne angustifolia* (no common name), Lamiaceae; and *Tetramolopium arenarium* (no common name), Asteraceae. Permission to conduct research was obtained through US Fish and Wildlife Service Recovery Permit number

TE28360B-0 and Hawai‘i Department of Land and Natural Resources Permit for Threatened and Endangered Plant Species number P-201. The other four plant species are common throughout PTA: Hawaiian prickly poppy, pua kala, *Argemone glauca*, Papaveraceae; ko‘oko‘olau, *Bidens menziesii*, Asteraceae; na‘ena‘e, *Dubautia linearis*, Asteraceae; and ‘ilima, *Sida fallax*, Malvaceae.

**Experimental plots**

We established 20 plots to assess the effects of experimental removal of NIPs (rodents, ants, yellowjacket) on the insect pollinator community and pollination of focal plant individuals. Sixteen plots received predator control treatments (4 Rodent treatment; 4 Ant treatment; 4 Yellowjacket treatment; 4 All combined rodent/ant/yellowjacket treatment) and 4 Control plots remained untreated for reference (i.e., experimental control plots). Each experimental plot consisted of a 50 m x 50 m (0.25 ha) central core area nested within a 150 m x 150 m (2.25 ha) rodent and/or ant treatment area (Figure 1). Potted plants of our focal plant species were placed at the center of the central core area during the experimental treatments. Plot sizes were selected to protect the central core area from NIPs, based on previous research on these NIPs in Hawai‘i (rodents: Shiels 2010; ants: Krushelnycky et al. 2011; yellowjacket: Hanna et al. 2013). Twenty-five monitoring stations, spaced 25 m apart along one axis, were placed within the 2.25 ha plot in transects radiating outward from the plot center. NIPs as well as the general arthropod community were monitored at a subset of these stations within the plots.

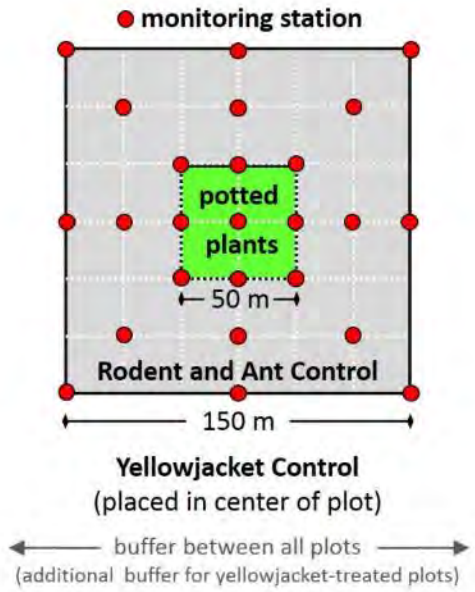

Figure 1. Experimental plot layout.

Plots were located at least 200 m apart with a wider buffer around yellowjacket and combined treatment plots. The insecticide used to control yellowjacket was placed in bait stations within the central 0.25 ha, and there was a minimum distance of 425 m between the yellowjacket treatment core areas and the edges of any rodent, ant, or control plots. There was a minimum distance of 400 m between the yellowjacket treatment and the combined treatment plots (from the treatment core area to the adjacent plot).

Field plots were situated based on an initial survey for NIPs in June 2014. We found rodents and wasps to be widespread within KKE; however, ant populations were patchy. The Argentine ant (*L. humile*) was in approximately one-half of the fenced unit while the ghost ant (*Tapinoma melanocephalum*) was in approximately one-third of the unit. The two ant species did not appear to overlap. The ant *Cardiocondyla* cf. *kagutsuchi* was found in low numbers in some localized areas within the fenced unit as well. This NIP survey information along with buffer distance criteria, proximity to access roads, and distribution of focal plant species were used to select locations for field plots.

We blocked our field plots into four blocks of five plots in order to account for habitat differences and also to maximize the area with ants that we could use in our project. The plots in each block included the Rodent plot, Ant plot, Yellowjacket plot, All plot, and Control plot.

Blocks 1 and 2 were within the grassland-shrubland habitat while blocks 3 and 4 were within the woodland habitat. Three blocks were in areas with the Argentine ant; the fourth block was in an area with the ghost ant.

We arranged the five plots in each block to fit in the available habitat type and to include Argentine ants (for blocks 1-3) and ghost ants (for block 4). Plot buffers were included in the arrangement, with larger buffers for the plots receiving yellowjacket treatment. Once we arranged the plots, we randomly assigned the treatments—one randomization for the Yellowjacket and All plots and a second randomization for the Rodent, Ant, and Control plots (Figure 2). The double randomization was performed to account for the differences in buffer distances between plots.

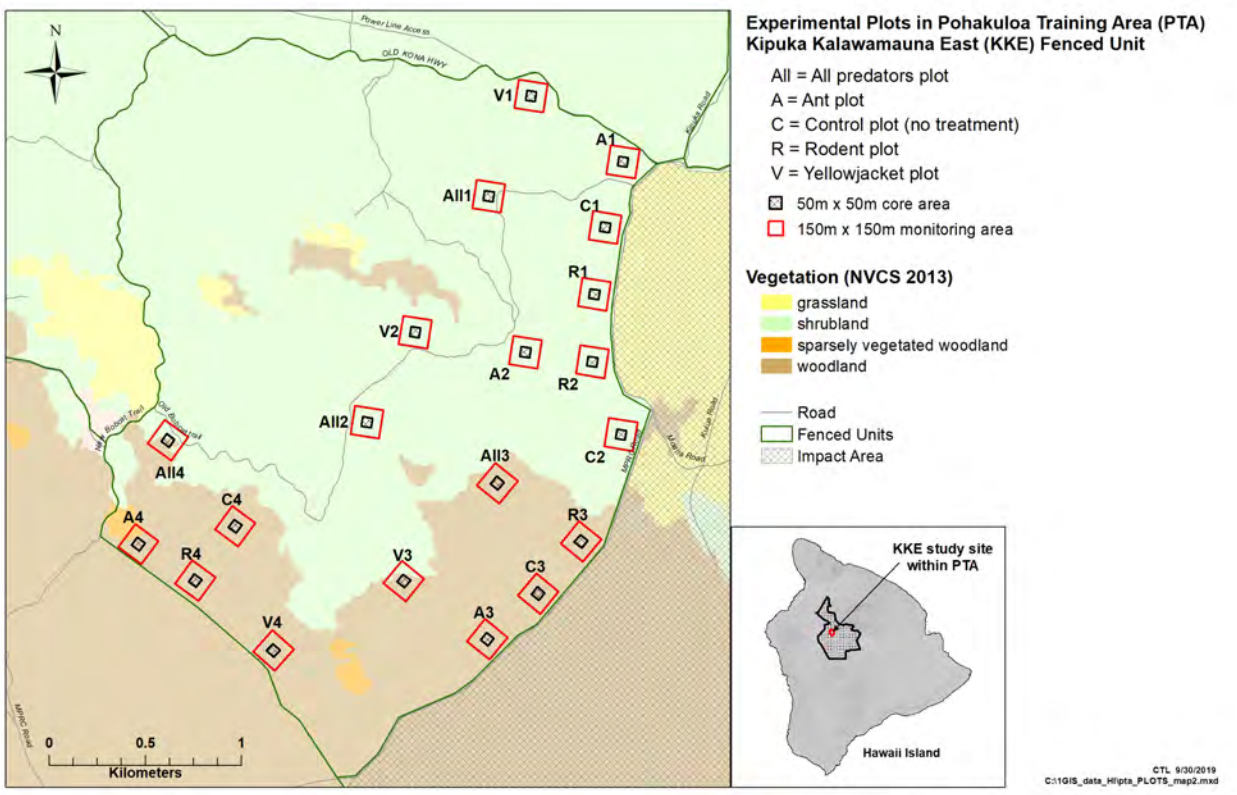


Figure 2. Experimental plots within Kīpuka Kālawamauna East fenced unit at PTA, Hawai‘i Island.

**Baseline flower visitation data collection**

To evaluate the effect of our experimental treatments on pollinator communities in KKE, it was first necessary to evaluate the current pollination regime in the site as an assessment of baseline pre-experiment conditions. We used flower visitation observations conducted March 2015–February 2016 to determine the identity of current flower visitors (all of which were insects) for each of our focal native plant species, as well as the rate of visitation exhibited by each insect taxon. This enabled us to construct partial pollination networks for the site (limited to the eight focal plant species but comprehensive for those plants). Our observations occurred on the focal plant species, when in flower, that were established within and adjacent to our 20 plots.

Potential pollinators were recorded as flower visitors observed to contact the reproductive structures of the plant species (Figure 3). The rate of visitation was calculated as a combination of the frequency of visits by a given visitor taxon and the number of flowers visited by each individual during a visit. Observations followed a systematic protocol (after Aslan et al. 2013). Each observation lasted 180 minutes, divided into 10-minute blocks. The first minute of each observation block was devoted to scan sampling, wherein the observer scanned all visible flowering plant individuals from a fixed observation point. All visitors interacting with flowers in any way were recorded during this scan. The data from scans repeated throughout each observation and across observations were analyzed to provide an estimate of the average number of individuals of each visitor species interacting with each plant species per unit time. For the remaining nine minutes of the observation block, the observer conducted focal individual observations, following individual visitors for as long as they were present and visible or until 180 seconds had elapsed. During focal individual observations, the recorder noted visitor behaviors, including the number of flowers and number of plants visited, nectar and pollen collection, nectar robbing, and pollen transport. These data permitted calculation of the average number of flowers and plants with which each visitor taxon interacted when present.

Figure 3. Photos of flower visitors (left to right): *Lasioglossum* sp. on *Stenogyne angustifolia*, *Mestolobes* sp. on *Tetramolopium arenarium*, *Hylaeus* sp. on *Argemone glauca*, *Apis mellifera* on *Sida fallax*.

The frequency of visitation (i.e., number of individuals of each visitor species present interacting with observed plants per unit time) and number of flowers visited by each individual (divided by the total number of open flowers available and expressed per unit time) were then combined multiplicatively to assess pollinator visitation importance (PVI) (Aslan 2015; modified from Renne et al. 2000). PVI is an index that identifies the most important visitors as those that are present most consistently within the flowering stand or those that visit large numbers of flowers when they are present. The PVI index is calculated as a combination of rates, so it standardizes flower visitor activities by a time unit (generally per minute). The use of this index allows comparison of complex visitation regimes with numerous visitor taxa and variable visit strategies, including flower visitors present in large numbers (e.g., European honey bee, *Apis mellifera*) and visitors that may be fewer in number but are highly faithful to a particular focal plant species, working its flowers and visiting multiple individuals within a flowering cluster when present. In analysis, we calculated the importance value of each visitor individual interacting with flowers of each of our focal plant species and divided the resulting importance values by the highest importance value for that plant species to develop a *scaled importance value* for each flower visitor species; via this method, the most important visitor to each plant species receives an importance value of 1.0, and all other visitors are assigned an importance that is a proportion of that top importance value.

Systematic pollinator observations were performed three times per week for each native plant species, from March 2015–February 2016, whenever the plant species was in flower. (Note that some of our focal species, such as *S. fallax*, flower nearly continuously throughout the year, while others, such as *D. linearis*, exhibit a much more limited flowering season and the total number of observations performed on such species was thus necessarily limited as well.) Each week, each plant was observed in the morning (start time between 0600 and 0900), midday (start time between 1000 and 1200 hours), and afternoon (start time between 1300 and 1500). A small number of additional nighttime observations, lasting two hours per session, were conducted using night-vision goggles (3 sessions for *H. haplostachya*, 8 sessions for *S. lanceolata*, 2 sessions for *S. angustifolia*; these evening observations began between 1830 and 1900 hours, but no flower visitation was detected during them). During each hour of systematic visitation observation, observers took a mandatory 10-minute break to avoid observer fatigue; a 180-minute observation period therefore yielded 150 minutes of visitation observation data.

In all, during the first year of the project, we observed *H. haplostachya* for 60.67 hours, *S. lanceolata* for 116.67 hours, *S. angustifolia* for 120.67 hours, *T. arenarium* for 35.17 hours, *A. glauca* for 55.67 hours, *B. menziesii* for 70.67 hours, *D. linearis* for 57.67 hours, and *S. fallax* for 59.17 hours. These quantities of observation time considerably exceed typical pollination visitation observation durations in published literature (e.g., Schemske and Bradshaw 1999; Thompson 2001; Albrecht et al. 2012).

During this first complete year of baseline data collection, we opportunistically selected plants of each species for observation, out of the existing populations of the eight focal plant species within KKE. Many such plants are naturally-occurring within the fenced study area, and others have been outplanted over the years as components of active, species-specific conservation efforts that have been undertaken by natural resources professionals at PTA. We observed plant individuals that were in flower at the time of the randomly-assigned observation period, performing observations throughout and sometimes just outside of KKE. Although some species exhibited very long flowering seasons (e.g., *B. menziesii*, *S. fallax*, *S. angustifolia*), the flowering of any individual plant lasted only a fraction of that total time, requiring us to frequently shift observation locations from week to week. Most individuals of *T. arenarium*, a federally-listed endangered species, were located to the north of KKE inside designated and fenced Area of Species Recovery 8N (ASR 8N) (located within ~500 m straight-line distance from the northern boundary of KKE). Individuals of the endangered *H. haplostachya* were located in ASR 8N, and other populations were located in the center of KKE itself. The endangered *S. lanceolata* occurred near the center of KKE, as well as in a patch located along the western edge of the study area. The endangered *S. angustifolia* occurs in low numbers across all of KKE, and observations ranged across the full study area.

The relatively common endemic species *B. menziesii* is located along the northern, central, and northeastern portions of KKE. *Dubautia linearis* occurs largely in the southern portion of the study area, and also within ASR 8N. *Argemone glauca* occurs in very low density across the full study area and *S. fallax* occurs commonly across the full study area, and observations utilized all parts of the study area as opportunistically available. As a result of the diverse spatial occurrence of flowering plants of the eight species at any given time, observations utilized the full study area and adjacent and nearby conservation plots, so observation results and analyses are applicable to the full study area rather than to any particular experimental plot.

As an additional consequence of the use of naturally-occurring plant individuals, observable plants could not be standardized by size or number of open flowers. Our protocol

required that observers record the total number of flowering individuals and total number of open flowers during each observation, so that visitor numbers could be divided by total number of available flowers to incorporate the likely effect of overall flowering stand attractiveness.

For *S. angustifolia* only, because of the extremely low number of flower visits observed for wild plants in KKE, we conducted an additional 20.5 hours of observations in spring 2016 at a lower-elevation site to the west of the PTA where *S. angustifolia* has been experimentally outplanted (Pu'u Wa'awa'a Forest Reserve, PWW) and where bee activity has been anecdotally reported to be higher than at the PTA. Start times of three-hour observations for this supplementary effort ranged from 10:08 a.m. to 4:04 p.m.

No observations were recorded in inclement weather. Observations continued throughout the year since at least some flowers were always available, but the diversity of open flowers was lowest in winter (December-January).

**Manual pollination treatments**

We carried out manual pollination treatments in 2015–2017 to evaluate the dependence of each plant species on outcrossing via pollinators. For each plant species, we administered the following treatments to flowers: bagging to prevent outcrossing while flowers were in bud stage; bagging in bud stage with hand-pollination in female phase to act as a bagging control; hand-supplementation with pollen in female phase; and an unmanipulated control treatment (Figure 4). Bagging prevented visitation by pollinators and therefore indicated whether plants were capable of setting fruit and seed via self-fertilization. A bag control indicated whether the bagging treatment itself delivered sufficient trauma to flowers to cause their failure, independent of selfing. Hand-supplementation of pollen enabled estimation of the maximum seed set possible for a species assuming maximum pollen delivery. The unmanipulated control enabled estimation of average fruit and seed production under normal, ambient pollination conditions.

Figure 4. Pollination treatments on *Dubautia linearis*.

We attempted to administer each treatment to three separate flowers on each of ten plants per plant species. At times, however, treatments were unsuccessful (e.g., flowers wilted immediately after treatment, possibly due to disturbance like strong wind or rain, or chance and without a clear link to a treatment effect, and could not be replaced) or no plants with at least 30

open flowers in treatable flower phase could be found in the study area. In such cases, we attempted to administer the full complement of treatments to each treated plant, but a smaller number of flowers per treatment were utilized. All flowers receiving a particular treatment within a plant individual were treated as subsamples in analyses. When treatments failed and could not be replaced, treatments on individual plants became unbalanced. Fruits were collected when ripe and the proportion of flowers that set fruit and number of seeds per fruit were calculated for each species and all treatments.

Due to limited availability of open flowers at any given time, flower treatments were administered to flowering plants repeatedly throughout the first three years of the project to accumulate sufficient sample size of treated flowers to enable analyses.

Administration of flower treatments required techniques tailored to the morphology of the flowers of each of the species, and each presented different challenges. Several of our focal species have small or delicate flowers, requiring the construction of bags from lightweight wedding tulle fabric or large plastic straws. Attachment of these bagging structures was difficult, particularly for flowers with highly reduced pedicels (*H. haplostachys*), extremely delicate flowers (*T. arenarium*), extremely sticky surfaces (*S. lanceolata*), small buds expanding to wide flowers (*B. menziesii*, *H. haplostachys* and *S. angustifolia*), delicate pedicels *(B. menziesii* and *S. fallax*), and sharp spines (*A. glauca*). Heavy winds across the site also made treatments difficult, tearing off bags that were loosely attached and sometimes tearing off flowers within bags if the bags were firmly attached.

To analyze fruit set and seed set from treated flowers, we calculated the proportion of flowers setting fruit for each flower treatment for each plant species and counted the number of filled seeds produced for each flower treatment. We analyzed fruit set and seed set separately for each species. For each analysis, we tested analysis of variance (ANOVA) assumptions (note: to test normality, we used quantile-quantile plots, recommended when sample size is limited (Wood 2010)). When assumptions were met, we used two-way ANOVA, with plant as a blocking factor, to examine whether fruit and seed set varied by flower treatment. For models with significant *p*-values, we used pairwise t-tests to determine which treatment pairs differed significantly. For plants with fruit set or seed set data that failed to meet ANOVA assumptions, we used non-parametric Kruskal-Wallis tests to determine whether significant differences among treatments were evident, and the Dunn's multiple comparisons test to determine which pairs of treatments exhibited significant differences. Analyses were performed in R version 2.14.1, using the packages "stats" and "FSA" (R Core Team, 2012).

**Predator treatments**

In the NIP treatment plots (4 Rodent, 4 Ant, 4 Yellowjacket, 4 All), suppression methods included snap-trapping for rodents, granular formicide for ants, and insecticide-laced bait for yellowjacket. NIP monitoring was performed immediately before and after treatments in treatment and control plots to assess the efficacy of treatments. We used suppression treatments that were identified in the draft 2017 PTA INRMP for rodents and ants, while suppression of yellowjacket was not mentioned in the plan. We also monitored NIPs for one year prior to beginning treatments to better account for NIP spatial and temporal variability among plots.

For rodent control, two types of snap-traps were used to account for the body size difference between rats and mice and activities were approved under the USDA Wildlife Services Institutional Animal Care and Use Committee Study Protocol number QA-2452. To

account for daily movements of rodents (Shiels 2010), mouse snap-traps were spaced approximately 12.5 m apart in a grid across the 2.25-ha plot and rat traps were placed along the same grid but spaced every 25 m. There were 169 mouse traps and 49 rat traps in each plot receiving rodent treatment. All snap-traps were placed in corrugated plastic boxes to reduce the chances of trapping non-target vertebrates such as birds (Pender et al. 2013; Figure 5).

Figure 5. Rat (left) and mouse (right) trap-boxes containing Victor snap-traps.

Traps in each plot were checked and re-baited daily for the first week, every other day for the second week, and then weekly thereafter and for the duration of our experimental treatment. Trapping began in April 2016 and commenced in each block on a rolling basis: block 1 (4/4/2016), block 3 (4/18/2016), block 2 (4/25/2016), and block 4 (5/16/2016). Rodent monitoring was conducted in Rodent plots, All plots, and Control plots just prior to treatment initiation and approximately each 2-3 months thereafter. All rodent monitoring occurred at the interior 17 monitoring stations in the plots, which were along the diagonal transects of the plot plus the perimeter of the 50 m x 50 m core area. Rodent trapping and monitoring did not occur in blocks 2 and 3 in July 2016 because of unexploded ordnances in the plots, but trapping and monitoring resumed in August 2016 in these plots. Rodent trapping ended in January 2018, and post-experimental monitoring occurred for 3 months afterwards in the Rodent plots, All plots, and Control plots.

Based on rodent monitoring data, we modified the weekly rodent trapping effort for the duration of the rodent trapping. For example, in block 1 in June 2016, blocks 2-3 in August 2016, and block 4 in September 2016, “reduced” trapping occurred for one week followed by “perimeter” trapping for two consecutive weeks. Reduced trapping involved setting and checking the traps spaced 25 m apart and along the perimeter (73 mouse traps and 49 rat traps). Perimeter trapping involved setting and checking the traps along the perimeter only (48 mouse traps and 24 rat traps). Trapping effort in each plot was increased by performing “reduced” trapping for

consecutive weeks if monitoring data show increased rodent activity during periods of "perimeter" trapping. This adaptive management strategy had the goal of maximizing rodent suppression while being as efficient as possible with efforts required of our field staff.

To reduce ant populations, granular formicide bait (Maxforce) was applied by hand using "whirlybird" spreaders throughout the 2.25-ha plot. Bait was applied 6 times over the course of the study, during the weeks of the following dates: 6/27/2016, 11/14/2016, 3/6/2017, 7/26/2017, 11/1/2017, and 1/1/2018. Pre-treatment monitoring was conducted in Ant plots, All plots, and Control plots one week prior to each application of bait, and post-treatment monitoring occurred one to two weeks after each application of bait. Monitoring occurred at 13 stations in the plot, along the diagonal transects of the plot. A final application of bait was conducted in January 2018, and post-experimental monitoring occurred for 3 months afterwards in the Ant plots, All plots, and Control plots.

Yellowjackets were targeted with fipronil insecticide and activities were approved under the Hawai‘i Department of Agriculture Experimental Use Permit number EUP-16-01. Bait was applied twice each calendar year. Nine bait stations were placed in the central 50 m x 50 m area in each treatment plot, and were constructed to allow yellowjacket workers to access the fipronil-laced bait inside the station through entry and exit holes. Heptyl butyrate was used to attract the workers to the bait stations. Canned chicken was used as the bait and fipronil was mixed into the meat, which was taken back to the yellowjacket nest by the workers. Bait stations were set up in the morning and taken down in the afternoon after approximately 8 hours. Stations were monitored throughout the day to assess yellowjacket activity. Yellowjacket treatments were applied 6/30/2016–7/1/2016, 12/15–12/16/2016, 6/26–27/2017, and 11/20–21/2017. Pre-treatment monitoring was conducted in Yellowjacket plots, All treatment plots, and Control plots one week prior to application of bait. Post-treatment monitoring was conducted two weeks after treatment application. Monitoring occurred at 9 stations in every plot within the block plus at additional stations up to 500 m away from the plot center, placed in 100 m increments. The 9 monitoring stations within plots were at the corners of the 2.25-acre plot, the corners of the 50 m x 50 m core area, and the center of the plot. Block 1 did not receive yellowjacket treatment after the first application, due to low numbers of yellowjackets in the pre-monitoring data. Post-experimental monitoring occurred for 3 months after the final application of insecticide in the Yellowjacket plots, All plots, and Control plots.

We analyzed efficacy of predator suppression methods by comparing data from treatment plots relative to untreated control plots. Rodent analyses were performed in R version 2.14.1, using the function "aov" (R Core Team, 2012). Ant and yellowjacket analyses were performed in JASP version 0.8.5.1.

**Predator and arthropod monitoring within plots**

The relative abundance of focal NIPs in all plots were monitored in 2015–2018 throughout the full duration of the field study (1 year prior to experimental treatments + approximately 15 months during treatments + 3 months after the end of experimental treatments). In each plot, NIPs were monitored at all or a sub-set of the 25 monitoring stations situated along transects radiating outward from the center of the plot. Effectiveness of NIP treatments were assessed by comparing data between treatment and untreated control plots, with the expectation that treatment effect will decrease with greater distance from the center of each plot.

Rodent activity was monitored using plastic tracking tunnels (50 cm x 10 cm x 10 cm) (Pender et al. 2013). Each tracking tunnel contained peanut butter bait in the center of an inked tracking card that was left out for 4 days to allow rodents and other animals to leave footprints on the cards (Pender et al. 2013; Figure 6). Footprints were identified and recorded when tracking cards were picked up from the tunnels. Ants were monitored using 7.6 cm x 6.4 cm index cards baited with a 40% tuna–60% corn syrup mixture. Cards were set on the ground in a shaded location and left in place for approximately 1 hour, after which they were photographed and collected (after Krushelnycky et al. 2011). Foraging ants attracted to the cards were identified in the field and later verified and counted in the office from the photographs. Yellowjacket abundance was monitored using heptyl butyrate traps left out for four days (after Foote et al. 2011; Figure 7). The number of individual yellowjackets in each trap were counted in the field on the fourth day. Statistics for analyses were proportion of stations that had rodent activity in each plot; number of ants in each plot; and number of yellowjackets in each plot. We analyzed NIP activity or abundance levels in treatment and control plots throughout the full duration of the project, in order to quantify the efficacy of NIP suppression methods over the course of treatment.

Figure 6. Baited and inked tracking card being placed into a tracking tunnel.

Figure 7. Yellowjacket trap with heptyl butyrate attractant inside the vial.

We also monitored components of the general native and non-native arthropod community in all plots by sampling vegetation, leaf litter, and sticky traps at five of the monitoring stations within each plot. Vegetation was sampled from two branches of each of four plants (chosen from a list of six common plant species in KKE) at each station, and collecting all arthropods that fell into a net held over the branches. Leaf litter was sampled by taking 1 cup (237 ml) of leaf litter from under each of four plants (chosen from a list of six common plant species in KKE) at each station and mixing thoroughly in a plastic bag, then subsampling 1 cup from the mixture. The mixed litter was taken back to the laboratory and processed through Tullgren funnels for 48-72 hours in order to extract arthropods. Sticky traps were hung on

vegetation at the monitoring stations (one per station) and left out for four days. Samples from the stations within each plot were pooled for our statistical analyses, and so each monitoring station was a sub-sample for the plot. The arthropod monitoring allowed us to evaluate potential broader impacts of our NIPs by comparing broad arthropod communities (including larval and adult individuals, flighted and non-flighted taxa) between treatment and control plots, and also to identify other non-focal predators such as spiders that may be important in the system.

In addition to the quantitative arthropod sampling methods, we periodically sampled nocturnal and flighted arthropods qualitatively through light traps and malaise traps from April 2015 through January 2016. Traps were placed in the center of each plot (one per plot) every other month, with trap type rotating each month (e.g., light traps in months one and three, malaise traps in months two and four). Light traps were left out overnight and malaise traps were left out for four days at a time. Malaise traps were set out at the end of our experiment in December 2017, and left out for four days at a time in the center of each plot (one trap per plot).

Bee nest blocks were constructed by drilling holes in 7.6 cm x 10.2 cm x 15.2 cm (3 in x 4 in x 6 in) balsa wood blocks. Holes varied in size from 0.3175 cm (0.125 in) to 0.635 cm (0.25 in) and were drilled so that the depth of the hole was 10.2 cm. Holes were drilled in a grid pattern and labeled with columns and rows. Nest blocks were placed at 5 monitoring stations in each plot (4 at the corners of the 50 m x 50 m central area and 1 in the plot center). Blocks were checked opportunistically to assess colonization of the blocks by native or non-native solitary bees.

*Pre-experimental baseline monitoring*

We started baseline monitoring of ants, yellowjackets, and arthropods in March 2015; rodent monitoring began in April 2015. All plots were monitored monthly by block from March 2015 through September 2015, and then every six weeks through March 2016. The monitoring schedule was semi-randomized, so that the same block was not monitored on the same dates each month. Access to the field site was sometimes denied, due to severe weather or unexploded ordnances in the plots, and blocks were not monitored during these times. Due to the amount of field collecting and intense physical nature of the work, our initial field schedule was not sustainable for the health and safety of our field crew. We eased our effort so that each plot was monitored every six weeks instead of every four weeks from October 2015 through March 2016, and modified the number of monitoring stations visited in each plot.

Rodents were monitored at the interior 17 monitoring stations in every plot during April 2015–March 2016. Ants were monitored at all 25 monitoring stations in every plot during March 2015–September 2015 and then monitored at a subset of 13 stations in every plot during October 2015–March 2016. The 13 monitoring stations were in diagonal transects through the plot. Yellowjackets were monitored at 9 monitoring stations in every plot during March 2015–March 2016. The 9 monitoring stations were at the corners of the plot, the corners of the 50 m x 50 m core area, and the center of the plot. See Figure 1 for general layout of monitoring stations within each plot.

*Experimental treatment and post-experimental monitoring*

After all predator treatments were fully implemented, we monitored NIPs and arthropods starting in September 2016 through to the end of our predator exclusion experiment in January

2018. We monitored one block of five plots each month, as the majority of field time was spent on weekly tasks such as maintenance of predator treatments, watering of potted plants within every plot, and observations on potted plants within every plot. Rodents were monitored at 17 stations in the Rodent plot, All plot, and Control plot within the block. The 17 monitoring stations were along the diagonal transects of the plot plus the perimeter of the 50 m x 50 m core area. Ants were monitored at 13 stations in all plots within the block, along the diagonal transects of the plot. Yellowjackets were monitored at 9 stations in all plots within the block plus at additional stations which were up to 500 m away from the plot center, placed in 100 m increments. The 9 monitoring stations within plots were at the corners of the 2.25-acre plot, the corners of the 50 m x 50 m core area, and the center of the plot.

We conducted 3 months of post-experimental monitoring after the end of predator treatments, from February through April 2018. Each block was monitored monthly. Rodents were monitored at 17 stations along the diagonal transects in the Rodent plots, All plots, and Control plots. Ants were monitored at 13 stations along the diagonal transects in the Ant plots, All treatment plots, and Control plots. Yellowjackets were monitored at 9 stations in the Yellowjacket plots, All plots, and Control plots, plus at additional stations up to 500 m away from the plot center, placed in 100 m increments. The 9 monitoring stations within plots were at the corners of the 2.25-acre plot, the corners of the 50 m x 50 m core area, and the center of the plot.

**Diet analyses to determine direct interaction between NIPs and pollinators**

Whole body, mouth, or fecal contents of NIPs were screened for insect prey DNA to determine the types of insect pollinators consumed by NIPs at our site. Field collection of NIPs for such dietary analysis included quarterly trapping/sampling to account for seasonal influences on NIP diets. Rats and mice were trapped along seven transects that were positioned in the buffer zones between treatment plots, and such trapping occurred on the following dates in 2015: May 26-June 12, September 8-17, and December 1-3. Rats and mice were also collected during the first weeks of our NIP suppression (April 4-20, 2016), which concluded our year-long collection of rodents for diet assessment.

To detect prey items consumed by Argentine ants, pooled samples of ants were taken. Colonies of ants were located by overturning rocks, and a sample of at least 100 ants was collected from each colony into a vial using an aspirator. Whenever possible, colonies containing brood (eggs and larvae) were sampled. Ant samples were kept alive on ice until transported back to the laboratory. Ants were collected on five occasions spread over the course of 15 months to capture potential temporal variation in diet: May 2015 and March, April, May, and September 2016.

Yellowjackets carry prey items back to their nests in their mouths as “prey balls,” which typically consist of individual arthropods. Therefore, we sampled yellowjacket prey items by first surveying our research plots and adjoining areas to locate yellowjacket nests. We then captured workers as they entered the nest using an apparatus modified from that described by Gambino (1992). A tubular plastic pipe was placed over the entrance of the nest and sealed using aerosol expanding foam (Figure 8). To capture a sample of yellowjackets entering the nest, a plastic bag partially filled with 95% ethanol was inserted into the entrance and left in place for 20 minutes. Workers returning to the nest flew into the ethanol, preserving both the wasps and their prey items. Although yellowjacket individuals were seen throughout our study plots, we

were only able to locate one yellowjacket nest due to the cryptic nature of the nests in the broken, rugged terrain at our field site. Therefore, all prey items originated from the same nest in block 4. This nest was fitted with the collecting apparatus in October 2015, and samples were taken in October and December 2015, and January, March, and April 2016.

Figure 8. Collection of yellowjacket prey items using a plastic pipe apparatus.

Once all NIPs were obtained from our field site, they were kept cold until they could be frozen and processed in the laboratory. Individual yellowjacket prey items were sorted from samples and identified to order based on morphology, when possible. DNA was then extracted from each prey ball using standard extraction kits (Qiagen DNeasy Blood and Tissue Kit). The "barcoding" region of the mitochondrial gene cytochrome oxidase 1 (CO1) was amplified using the universal polymerase chain reaction primers LCO1490 and HCO2198 (Folmer et al. 1994), and sequences were obtained by Sanger sequencing. Pooled ant samples (30 total, each consisting of randomly selected 100 worker ants and up to 100 larvae) and fecal contents of individual rodents (30 mice and 30 rats) were screened using high throughput DNA sequencing of the barcoding region of the CO1 gene, and this work was contracted out to Jonah Ventures LLC (Boulder, CO). Sequences for all sample types were then queried against NCBI Genbank and the Barcode of Life Database (BOLD) to assign species or Operational Taxonomic Units (OTU). The OTUs then enabled us to match the NIP-consumed prey with the flower visitors that we observed on our target plant species in the field, or with other arthropod groups.

**Propagation of focal plant species in greenhouse**

Beginning in early spring 2014, we planted seeds of our eight focal plant species in a greenhouse at the University of Hawai‘i Experimental Station in Volcano, Hawai‘i Island (Figure 9). Seeds were collected from wild plants at PTA for *A. glauca*, *B. menziesii*, *D. linearis*, and *S. fallax*. Seeds were obtained directly from PTA NRO for *S. lanceolata* and *T. arenarium*. Additionally, we planted cuttings of *H. haplostachya* and *S. angustifolia* (all obtained from PTA NRO). We used a mixture of commercial potting mix, perlite, vermiculite and cinders in our greenhouse. Plants were sown in flats initially and transplanted to successively larger pots as they grew. They were fed foliar or liquid Miracle-Gro plant food. On-contact mildew and pest controls included potassium bicarbonate (Kaligreen), Safer Brand insecticidal soap, M-Pede insecticidal soap and neem oil products. Marathon, a granular systemic pest control product was applied once (in 2015) to the top of the soil in pots of *H. haplostachya*, *S. lanceolata*, and *T. arenarium* to control aphids and whiteflies that were killing young plants.


Figure 9. Propagation of focal plants in the greenhouse.

Our aim was to grow the plant individuals to flowering within one year so that they could be placed in our field plots once our predator treatments were in place and pollination within each treatment could be monitored. Accordingly, we offered plants ideal watering and nutrient conditions, to encourage rapid growth and maturity. Due to the delay of field data collection because of hiring difficulties, plant species had almost two years to grow in the greenhouse. Greenhouse growth required thrice-weekly watering, construction of shade material for temperature moderation (because the greenhouse is located at a lower and thus hotter elevation than KKE), control of powdery mildew and aphids, and careful plant cleaning to remove any pathogens or fungi before they could be placed into the field.

Plants with rapid growth from seed to flowering included *B. menziesii* and *S. fallax*. *Haplostachys haplostachya* and *S. angustifolia* grew from cuttings and flowered within months, soon after rooting. Several *A. glauca* individuals flowered and then died or became dormant while still in the greenhouse (i.e., before baseline field data collection was completed and field experimental treatments initiated), so that by the time our treatments were in place and our first round of potted plants transferred to the field, only a small number of *A. glauca* individuals were evidently alive and none were in flower. We were unable to successfully sow additional seeds of *A. glauca* in order to obtain flowering individuals for our field experiments, and so this species was not included in our potted plant observations. For most of the first year, only a very small number of *T. arenarium* seedlings grew from seed (three of which became flowering

individuals). Similarly, only a small number of *S. lanceolata* seedlings grew from seed, and fewer than a half-dozen became flowering individuals. Following the cold of winter (early 2016), however, both species exhibited a rapid increase in germination, resulting in several dozen healthy plants of each species approaching maturity by the fall of 2016.

To prepare plants for field pollination trials, watering was reduced to once or twice per week, depending on local temperatures and visual inspection of pot dryness. Plants were treated with pest controls to ensure that no greenhouse pathogens would be transferred to the field with pots. Pots were transferred in vans or enclosed truck bed to KKE and placed in a staging area. At the staging area, the common plant species were repotted inside of white-painted 3-gallon pots to reduce the solar heating associated with black-colored pots (Figure 10). For the endangered species, the greenhouse pots containing *H. haplostachya*, *S. angustifolia*, and *T. arenarium* were placed inside the white-painted 3-gallon pots with potting material in order to minimize transplant shock. *Silene lanceolata* was transplanted into white-painted 1.5-gallon pots in the greenhouse and then taken to KKE for placement in the plots. The timing of transfer from greenhouse to KKE varied for the plant species, depending on flowering. Transfer began in July 2016 and was completed in November 2016 for all species except *A. glauca*.

Figure 10. Potted plants staged outside treatment plots at our study site at PTA, Hawai‘i Island.

**Flower visitation data collection on potted plants**

Potted flowering plants were distributed among the 20 study plots and placed as much as possible in sheltered locations such as small depressions in the landscape where they would receive some protection from desiccating sun and wind. We aimed to place 8 flowering plant individuals of each species in each plot, but were limited by the number of individuals that were concurrently in flower for some species at the time of plant placement (July-November 2016). When numbers were limited, we distributed plants evenly amongst plots so that each plot

received the same number of flowering individuals of a given species, ranging from 2 to 8. Each individual had multiple flowers, and pots for a given species were clumped spatially within the plot such that the full collection of flowers of each species represented the attracting element for visiting pollinators, which are likely to respond to number of flowers rather than number of plants. We therefore aimed to place an approximately equal "attractant" in each plot by matching up plants according to the number of open flowers they exhibited, such that plants with an unusually small number of flowers were paired with plants with unusually large numbers.

For *B. menziesii*, individuals had grown so tall and spindly within shared pots in the greenhouse that it was often not possible to separate them into individual pots without damaging their stems, and we therefore decided to leave some in shared 3-gallon pots in the field, reasoning that the attractant pool of flowers was similar in such a case (from a pollinator perspective) as several plant individuals in separate pots placed close together. The stems and roots of *S. fallax* also were entwined in shared greenhouse pots, and we likewise placed multiple individuals in shared 3-gallon pots in the field. Due to the small size of the *S. lanceolata* and *T. arenarium* plant individuals, we placed three individuals in one shared pot for each species (1.5-gallon pot for *S. lanceolata*; 3-gallon pot for *T. arenarium*). We did not have any flowering potted individuals of *A. glauca* for placement in the field, despite our best efforts to propagate them in the greenhouse.

Plants placed in the field were watered weekly with water containing Miracle-Gro, transported in backpack sprayers. Pollinator visitation observations were performed weekly in plots, and each observation included monitoring of ongoing flowering rates, plant condition, and visitation of potential pollinators to all currently flowering plant individuals. As plants finished their first flowering round and reverted to a purely vegetative state, we maintained them in the field with watering, and monitored their condition until they re-entered flowering.

Flower visitation observations began on our potted plants in August 2016 and continued through November 2017. Our protocols for these observations were somewhat different for the potted plants than for the wild individuals observed during baseline data collection. The baseline data collection combined with flower treatments had provided us with an initial assessment of pollinator communities and effectiveness prior to the initiation of our predator controls. Now that all predator controls were in place, we tracked pollination of potted plants to evaluate whether pollination was altered by a sharp reduction in NIP presence. Due to the constant and numerous personnel duties (i.e., our crew time was now distributed between intensive predator control maintenance, monitoring of predators and of non-target response, potted plant watering, and pollinator visitation observations every week) it became necessary to adopt a community-scale approach to pollinator visitation assessment. Rather than watching a single plant species at a time, observers watched up to three flowering potted plant species simultaneously while observing at a particular plot. They continued to use the combined scan sampling and focal individual observation methods described above, but now recorded the plant species with which each observed flower-visiting insect interacted. If a visitor moved from one plant species to another, the move was recorded as the beginning of a separate visitation event. All flowering individuals and numbers of open flowers were counted and recorded during each observation period so that rates of visitation could still be calculated based on number of available flowers of each focal plant species for each plot treatment. Observations were conducted in each plot at least once per month throughout the experimental period, such that all blocks were observed five times per month. As during baseline data collection, observers selected the order of plots for observation in a haphazard fashion and observed all plots at all times of day.

## Data synthesis

### *Experimental time periods*

The flower visitation data collection on potted plants during experimental predator control treatments spanned August 2016-November 2017. To capture seasonal and treatment-induced variation in predators and pollinators across this time period, as well as to account for the timing of all monitoring, treatments, and pollinator observations, we divided the experimental analysis period into three approximately half-year periods: August 2016-January 2017, February 2017-June 2017, and July 2017-November 2017.

### *SERDP Hawai'i Ecosystem-Level Observation-Based (SHELOB) statistical model*

We used Bayesian hierarchical models to determine the tri-trophic relationships between our focal native plant species, insect pollinators, and NIP species, from our experimental treatment data. The SERDP Hawai'i Ecosystem-Level Observation-Based (SHELOB) model contains two levels. Level one estimates NIP frequencies at each plot during each time period (20 plots over 3 time periods). Level two estimates the relationships between NIP (independent variables) and the presence/absence of pollinator species group interactions with six of our eight plant species (dependent variables). Pollinator species groups were determined by the flower visitation data, and based on general taxonomic classification. Two of our eight plant species were not included in the model: *A. glauca* was not in the plots during the experimental period (due to lack of potted individuals) and *S. angustifolia* had few pollinator interactions (during both the experimental observations on potted plants and the baseline pollination observations on wild plants).

While the multi-trophic SHELOB model accounts for many interactions between NIP, pollinators, and plants, it does not cover all potential non-native predators that could affect pollination. For example, mongoose, feral cats, and game birds are all found at PTA, and all potentially consume pollinator species (Cole et al. 1995, Mostello and Conant 2018). Our study represented a massive monitoring effort, and we incidentally recorded the presence of mongoose, feral cats, and game birds in the rodent tracking tunnels, but these species were not targeted by our methods and data were not robust enough to include within the model. Additionally, our seed set experiments did not yield robust enough results to include pollinator effects on seed set within the model. We instead analyze the effects of NIP on pollinators in the context of pollen limitation and self-incompatibility.

Values of predators within a plot for a time period were treated as frequencies, due to our data collection methods. Rat and mouse abundances were impossible to calculate because of the tracking tunnel method of monitoring. Instead, we calculated a presence/absence-based frequency measure, with observational unit at the level of the plot. To match the rat and mouse data, we converted ant and yellowjacket wasp data to a frequency measure based on proportion out of maximum value recorded for the species. For ant and wasp data, the observation unit was at the level of the monitoring station. We modeled frequency of NIP within each plot for each of the three half-year periods with a binomial distribution, where "trials" were the total number of monitoring stations within a plot (rodents) or the maximum number of ants or wasps found across all plots at each monitoring station.

Ants and yellowjackets were monitored in every plot, but due to the high field crew effort required and finite crew work time, rats and mice were only monitored in Rodent, All, and Control plots. During the pre-experimental phase of the project, rodents were monitored in all plot types. We compared pre-experimental frequencies of rodents in Ant and Yellowjacket plots to those in (untreated) Rodent, All, and Control plots. Correlations between the frequencies of rodents in Ant and Yellowjacket plots and in Rodent, All, and Control plots were low (all <0.7, most <0.5), but overall the best correlations for both Ant and Yellowjacket plots were with Control plots. We therefore used our estimated mean frequencies of rodents within Control plots for the estimated frequency of rodents in Ant and Yellowjacket plots within the SHELOB model.

While treatments were effective, stochasticity of NIP across the landscape made it so that abundances or frequencies of predators in treated plots were at times higher than in untreated plots. For example, the Control plot within block 3 naturally had a low abundance of Argentine ants, even when compared to Ant or All plots within block 3. Instead of using plot treatment as a categorical variable, we therefore modeled predator frequencies as continuous variables across all plot/block combinations to account for this natural variability of NIP.

We transformed pollinator PVI data into presence/absence data by converting any PVI into '1' and any lack of interaction between a pollinator species group and plant species into a '0'. Pollinator observations were performed in 180-minute observation periods so data were transformed for every 180-minute observation period within each half year period within each plot. We performed binomial regression with a logit link on each pollinator species group for each plant species, using mean frequencies of rats, mice, ants, and wasps as independent variables. This means that for each combination of pollinator species group and plant species separately, we estimated the relationships with rat, mouse, ant, and yellowjacket frequencies for that combination. The number of trials in the binomial distribution for each pollinator species was the number of 180-minute observation periods for each plot within each half year period. No flower visitors belonging to the honey bee, non-syrphid fly, or non-*Vespula* wasp groups were observed on *S. lanceolata* so these interactions were left out of the analysis. We additionally ran the same analysis but combined all pollinator observations for all plant species across a given pollinator species for the three half-year periods, to determine an average effect of NIP on pollinator species.

We fit the analyses in JAGS (Plummer 2003) via the R packages 'rjags' version 4-8 (Plummer et al. 2018) and 'R2jags' (Su and Yajima 2015). We used uninformative priors for all stochastic nodes. We ran three Markov chain Monte Carlo (MCMC) chains for each analysis, for 500,000 iterations, and we used the Gelman-Rubin convergence statistic to check for convergence between and within all chains by ensuring that its values were $\geq 1$ and $< 1.1$ (Gelman and Rubin 1992).

## Results and Discussion

### Flower visitation observations for baseline data collection year

During our baseline data collection year, we performed 55.67 hours of observations of flower visitation for *A. glauca*. Observations took place opportunistically when flowering individuals were found and occurred during the following months: January, March, June, July, August, November, and December. We performed 70.67 hours of observations of flower visitation for *B. menziesii*, with observations occurring in March, June, July, August, September, October, November, and December. We performed 57.67 hours of observations of flower visitation for *D. linearis*, with observations occurring in September-December. For *H. haplostachya*, we performed 60.67 hours of observations in March, April, May, June, July, October, and November. For *S. fallax*, we performed 59.17 hours of flower visitation observations in January, March, April, May, July, August, September, and October. We performed 116.67 hours of observations for *S. lanceolata* in January, February, March, April, June, July, November, and December. For *T. arenarium*, we performed 35.17 hours of flower visitation observations in March, May, June, and July. For *S. angustifolia*, we performed 120.67 hours of observations in January, March, April, May, June, July, August, and October.

Across all observations, the large majority of flower visitors were either known non-natives or of insufficient resolution to determine origin (native or non-native) (i.e., recorded at order or family levels where the taxon is known to include both native and non-native representatives in the study area) (Figure 11). Indeed, the only known native flower visitors we observed in systematic observations were the moth *Orthomecyna* sp. (Lepidoptera: Crambidae) and butterfly *Udara blackburni* (Lepidoptera: Lycaenidae), and only *Orthomecyna* was a primary visitor to any of our focal endemic plant species (*T. arenarium*). Otherwise, both natives were minor members of the visitor community (Figure 12), with importance values less than 10% of the top flower visitor for each plant species. Taxa with uncertain origin included unidentified micromoths (Lepidoptera), unidentified wasps (Hymenoptera), *Hylaeus* sp. bees, and unidentified beetles (Coleoptera). All other taxa were known non-natives.

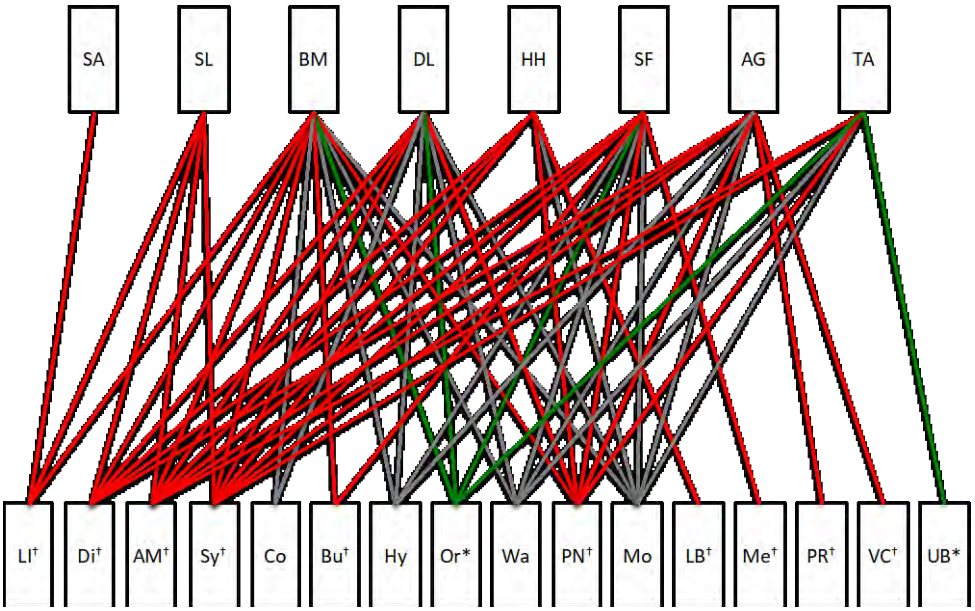

Figure 11. Networks displaying observed interactions between flower visitors and the eight focal native plant species in this study. Green connectors* = native flower visitors. Gray connectors = flower visitors of indeterminate nativity. Red connectors† = non-native flower visitors. Plants appear in the top row: SA = *Stenogyne angustifolia*, SL = *Silene lanceolata*, BM = *Bidens menziesii*, DL = *Dubautia linearis*, HH = *Haplostachys haplostachya*, SF = *Sida fallax*, AG = *Argemone glauca*, and TA = *Tetramolopium arenarium*. (A) Full network, containing all observed interactions. Flower visitors appear in the bottom row: LI = *Lasioglossum impavidum*, Di = Diptera (unspecified), AM = *Apis mellifera*, Sy = Syrphidae, Co = Coleoptera (unspecified), Bu = Butterfly (unspecified), Hy = *Hylaeus* sp. (unspecified), Or = *Orthomecyna* sp., Wa = Wasp (unspecified), PN = *Pachodynerus nasidens*, Mo = Moth (unspecified), LB = *Lampides boeticus*, Me = Megachilidae (unspecified), PR = *Pieris rapae*, VC = *Vanessa cardui*, and UB = *Udara blackburni*. Network was constructed in R version 2.14.1, using the package "bipartite" (R Core Team, 2012).

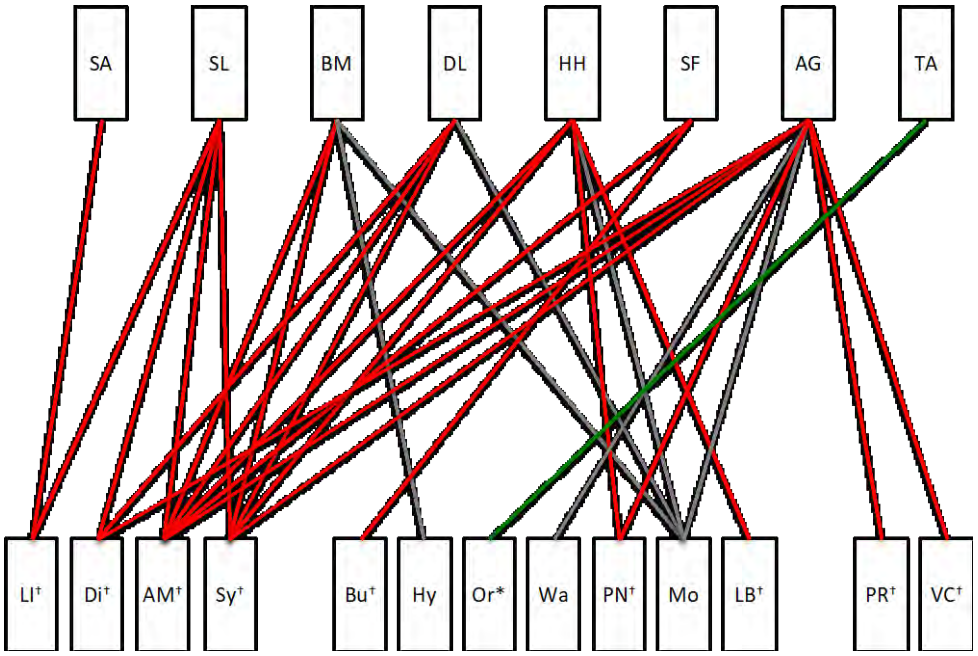

Figure 12. Primary network, containing just those interactions ≥25% as important as the most important interaction for each plant species. Green connectors* = native flower visitors. Gray connectors = flower visitors of indeterminate nativity. Red connectors† = non-native flower visitors. Plants appear in the top row: SA = *Stenogyne angustifolia*, SL = *Silene lanceolata*, BM = *Bidens menziesii*, DL = *Dubautia linearis*, HH = *Haplostachys haplostachya*, SF = *Sida fallax*, AG = *Argemone glauca*, and TA = *Tetramolopium arenarium*. (A) Full network, containing all observed interactions. Flower visitors appear in the bottom row: LI = *Lasioglossum impavidum*, Di = Diptera (unspecified), AM = *Apis mellifera*, Sy = Syrphidae, Co = Coleoptera (unspecified), Bu = Butterfly (unspecified), Hy = *Hylaeus* sp. (unspecified), Or = *Orthomecyna* sp., Wa = Wasp (unspecified), PN = *Pachodynerus nasidens*, Mo = Moth (unspecified), LB = *Lampides boeticus*, Me = Megachilidae (unspecified), PR = *Pieris rapae*, VC = *Vanessa cardui*, and UB = *Udara blackburni*. Network was constructed in R version 2.14.1, using the package "bipartite" (R Core Team, 2012).

In all, we recorded nine taxa visiting *A. glauca*, with *Apis mellifera* (the European honey bee) as the most important visitor (scaled importance value (IV) = 1.0), and micromoths as the second most important visitors (scaled IV = 0.67). Other visitors included syrphid flies (Syrphidae) (scaled IV = 0.22), painted lady butterflies (*Vanessa cardui*) (scaled IV = 0.19), unidentified Diptera (scaled IV = 0.19), the cabbage white butterfly (*Pieris rapae*) (scaled IV = 0.18), unidentified wasps (scaled IV = 0.14), the keyhole wasp (*Pachodynerus nasidens*) (scaled IV = 0.03), and *Hylaeus* sp. bees (scaled IV = 0.01).

We recorded 11 taxa visiting *B. menziesii*, with *A. mellifera* as again the most important visitor (scaled IV = 1.0), and micromoths (Lepidoptera) as the second most important (scaled IV = 0.37). *Hylaeus* sp. bees were a close third (scaled IV = 0.35). Other visitors included Syrphidae (scaled IV = 0.23), unidentified Diptera (scaled IV = 0.11), *Orthomecyna* sp. (scaled IV = 0.09), unidentified wasps (scaled IV = 0.06), *P. nasidens*) (scaled IV = 0.02), *Lasioglossum impavidum* (scaled IV = 0.01), unidentified butterflies (scaled IV = 0.01), and unidentified beetles (scaled IV = 0.001).

We observed a total of nine taxa visiting *D. linearis*, with Syrphidae as the most important visitor (scaled IV = 1.0), unidentified Diptera as the second most important (scaled IV = 0.90), and *A. mellifera* as the third most important (scaled IV = 0.90). Other visitors included micromoths (scaled IV = 0.30), unidentified wasps (scaled IV = 0.15), *Orthomecyna* sp. (scaled IV = 0.03), *Hylaeus* sp. bees (scaled IV = 0.007), unidentified beetles (scaled IV = 0.005), and *P. nasidens* (scaled IV = 0.0006).

For *S. fallax*, we observed a total of 10 flower visitor taxa: unidentified butterflies were most important (scaled IV = 1.0), followed by *A. mellifera* (scaled IV = 0.54). All other visitors exhibited very low importance and included Megachilidae (scaled IV = 0.06), *Hylaeus* sp. bees (scaled IV = 0.05), micromoths (scaled IV = 0.008), unidentified wasps (scaled IV = 0.008), *P. nasidens* (scaled IV = 0.006), Syrphidae (scaled IV = 0.003), *Orthomecyna* sp. (scaled IV = 0.0004), and unidentified Diptera (scaled IV = 0.0002).

The endangered plant species received fewer visitor taxa and were less likely to be visited by natives, compared with the common native plants in this study. For *H. haplostachya*, we observed a total of six flower visitor taxa: *P. nasidens* was most important (scaled IV = 1.0), followed by micromoths (scaled IV = 0.65), Syrphidae (scaled IV = 0.50), *A. mellifera* (scaled IV = 0.48), the bean butterfly (*Lampides boeticus*) (scaled IV = 0.47), and unidentified Diptera (scaled IV = 0.01). We observed only four visitor taxa for flowers of *S. lanceolata*: *A. mellifera* (scaled IV = 1.0), *L. impavidum* (scaled IV = 0.40), unidentified large Diptera (scaled IV = 0.36), and Syrphidae (scaled IV = 0.27). For *T. arenarium*, there were seven flower visitor taxa: *Orthomecyna* sp. (scaled IV = 1.0), unidentified wasps (scaled IV = 0.008), *P. nasidens* (scaled IV = 0.008), micromoths (scaled IV = 0.008), *A. mellifera* (scaled IV = 0.003), Syrphidae (scaled IV = 0.003), and *Udara blackburni* (scaled IV = 0.001).

In 120.67 hours of observation (724 10-minute observation blocks) at *S. angustifolia* flowers at the PTA, we observed just a single visitor: a non-native *Lasioglossum impavidum* bee. In all, 640 open flowers of *S. angustifolia* were observed at the PTA in the course of those 724 observation blocks. In 20.5 hours of observation at PWW, we observed two visiting *Lasioglossum impavidum* and six non-native *Ceratina cf. dentipes*. In all, we observed 281 open flowers at PWW. The non-scaled importance value (IV) for *L. impavidum* at the PTA was 0.000066, whereas the IV for *L. impavidum* at PWW was 0.014, and the IV for *C. cf. dentipes* at PWW was 0.032.

The baseline year of visitation observations provided evidence that all focal plant species were receiving floral visitations under ambient, pre-experimental conditions within the study site. The number of visitor taxa per plant species ranged from 1 to 11. Non-native insects were responsible for the large majority of this visitation. Particularly important flower visitors included *A. mellifera* (the most important visitor for *A. glauca*, *B. menziesii*, and *S. lanceolata*), syrphid flies (the most important visitors for *D. linearis*), *P. nasidens* (the most important visitor for *H. haplostachya*), butterflies (unidentified) (the most important visitors for *S. fallax*), *Orthomecyna* sp. (the most important visitor for *T. arenarium*), and *L. impavidum* (the most important visitor for *S. angustifolia*). Of these visitors, only *Orthomecyna* sp. is a known native species. In combination with our pollination treatment results (below), this suggests that pollination as an ecological function is active within the study site, but that under non-treated conditions endemic plants are largely dependent upon non-native insects for outcrossing.

The importance of non-native species as pollinators has been explored in several systems (e.g., Carmo et al. 2004; Celebrezze and Paton 2004; Cayuela et al. 2011; Aslan et al. 2013). Non-native birds have emerged as important replacements for extinct native pollinators in

Hawai‘i and New Zealand, for example (Anderson 2003; Aslan et al. 2013). When non-natives are providing an important mutualistic service such as pollination, native species may become dependent on non-native partners, and efforts to eliminate non-natives could harm native species. In other cases, non-native pollinators have exhibited competitive interactions with native pollinators, reducing the success of native plants and pollinators alike (e.g., Martins et al. 2013). When non-native pollinators perform a high level of pollen transfer for a particular species, it may be that the direction, timing, distance, or quantity of gene flow are affected (Dohzono and Yokoyama 2010; Aslan et al. 2014, 2016). Many non-natives are extremely generalist, carrying pollen from a large number of plant species; therefore, the probability of transferring heterospecific pollen to a particular plant or of wasting pollen by failing to deposit it on a conspecific may be high (Johnson and Steiner 2000). Such qualitative and quantitative impacts of a shift from native to non-native pollinators have been very little explored in ecology. However, via the above mechanisms, non-native pollinators could impact the evolutionary trajectory, phenology, and relative abundance of native plant species. Our results suggest that these factors may warrant further research in our study area. For two of our endangered plant species (*S. lanceolata* and *S. angustifolia*), only non-native insects were observed visiting flowers during this first year. The long-term implications of such a wholesale shift to pollination mediated by non-natives are wholly unknown for these species.

**Pollination treatments**

Overall, a total of 38 flowers were treated on 12 *A. glauca* plants. Mean seeds produced per flower for *A. glauca* was 151.03 ± 36.79 for bagged flowers, 253.89 ± 53.86 for bagged control flowers, 249.04 ± 41.23 for hand-supplemented flowers, and 138.28 ± 43.54 for unmanipulated flowers. A Kruskal-Wallis test revealed significant differences between treatments (Kruskal-Wallis test, chi-square = 9.53; $p = 0.0230$), with seed production differing significantly between unmanipulated and hand-supplemented flowers ($p = 0.033$; Dunn’s test for multiple comparisons) (Figure 13).

A total of 91 flowers on 12 *B. menziesii* plants were treated, with seed set per flower differing significantly by treatment (Kruskal-Wallis test, chi-square = 30.86; $p < 0.0001$). Unmanipulated flowers set significantly more seed than bagged flowers ($p < 0.0001$; Dunn’s test for multiple comparisons) and bagged control flowers ($p = 0.029$; Dunn’s test for multiple comparisons). Hand-supplemented flowers produced more seed than bagged flowers ($p = 0.0002$; Dunn’s test for multiple comparisons). Mean seeds produced per flower for *B. menziesii* was 0.16 ± 0.24 for bagged flowers, 1.00 ± 0.71 for bagged control flowers, 2.87 ± 0.47 for hand-supplemented flowers, and 3.61 ± 0.34 for unmanipulated flowers (Figure 13).

For *D. linearis*, a total of 149 flowers on 14 plants were treated, with seed set per flower differing significantly by treatment (Kruskal-Wallis test, chi-square = 24.69; $p < 0.0001$). Unmanipulated flowers set significantly more seed than bagged flowers ($p < 0.0001$), as did bagged control flowers ($p = 0.0023$). Hand-supplemented flowers also set significantly more seed than bagged flowers ($p = 0.035$) (Dunn’s tests for multiple comparisons). Mean seeds produced per flower for *D. linearis* was 0.56 ± 0.28 for bagged flowers, 2.13 ± 0.36 for bagged control flowers, 1.78 ± 0.31 for hand-supplemented flowers, and 2.72 ± 0.33 for unmanipulated flowers (Figure 13).

A total of 82 flowers were treated on 18 *S. fallax* plants, with seed set per flower differing significantly by treatment (Kruskal-Wallis test, chi-square = 43.53; $p < 0.0001$). Hand-

supplemented flowers set significantly more seed than bagged flowers ($p < 0.0001$), as did unmanipulated flowers ($p < 0.0001$) and bagged control flowers ($p = 0.011$). Hand-supplemented flowers also set significantly more seed than bagged control flowers ($p = 0.040$) (Dunn's tests for multiple comparisons). Mean seeds produced per flower for *S. fallax* was $3.63 \pm 0.48$ for bagged flowers, $5.73 \pm 0.11$ for bagged control flowers, $6.42 \pm 0.14$ for hand-supplemented flowers, and $5.89 \pm 0.20$ for unmanipulated flowers (Figure 13).

For *H. haplostachya*, a total of 105 flowers on 23 plants were treated, with seed set per flower differing significantly by treatment (Kruskal-Wallis test, chi-square = 76.97; $p < 0.0001$). Both bagged and bagged control flowers set significantly fewer seeds than unmanipulated flowers ($p < 0.0001$ and $p = 0.015$, respectively; Dunn's tests for multiple comparisons). Hand-supplemented flowers set significantly more fruit than bagged ($p = 0.0002$) or bagged control ($p = 0.0002$) flowers (Dunn's tests for multiple comparisons). Hand-supplemented flowers set significantly more seed than unmanipulated ($p < 0.0001$), bagged ($p < 0.0001$) or bagged control ($p < 0.0001$) flowers. Mean seeds produced per flower for *H. haplostachya* was $0.23 \pm 0.16$ for bagged flowers, $0.50 \pm 0.29$ for bagged control flowers, $2.84 \pm 0.19$ for hand-supplemented flowers, and $1.22 \pm 0.28$ for unmanipulated flowers (Figure 13).

A total of 55 flowers on 28 *S. lanceolata* plants were treated, with no significant difference among treatments in seed set per flower. Mean seeds produced per flower for *S. lanceolata* was $51.20 \pm 2.17$ for bagged flowers, $30.00 \pm 3.29$ for bagged control flowers, $59.49 \pm 5.50$ for hand-supplemented flowers, and $53.14 \pm 2.19$ for unmanipulated flowers (Figure 13).

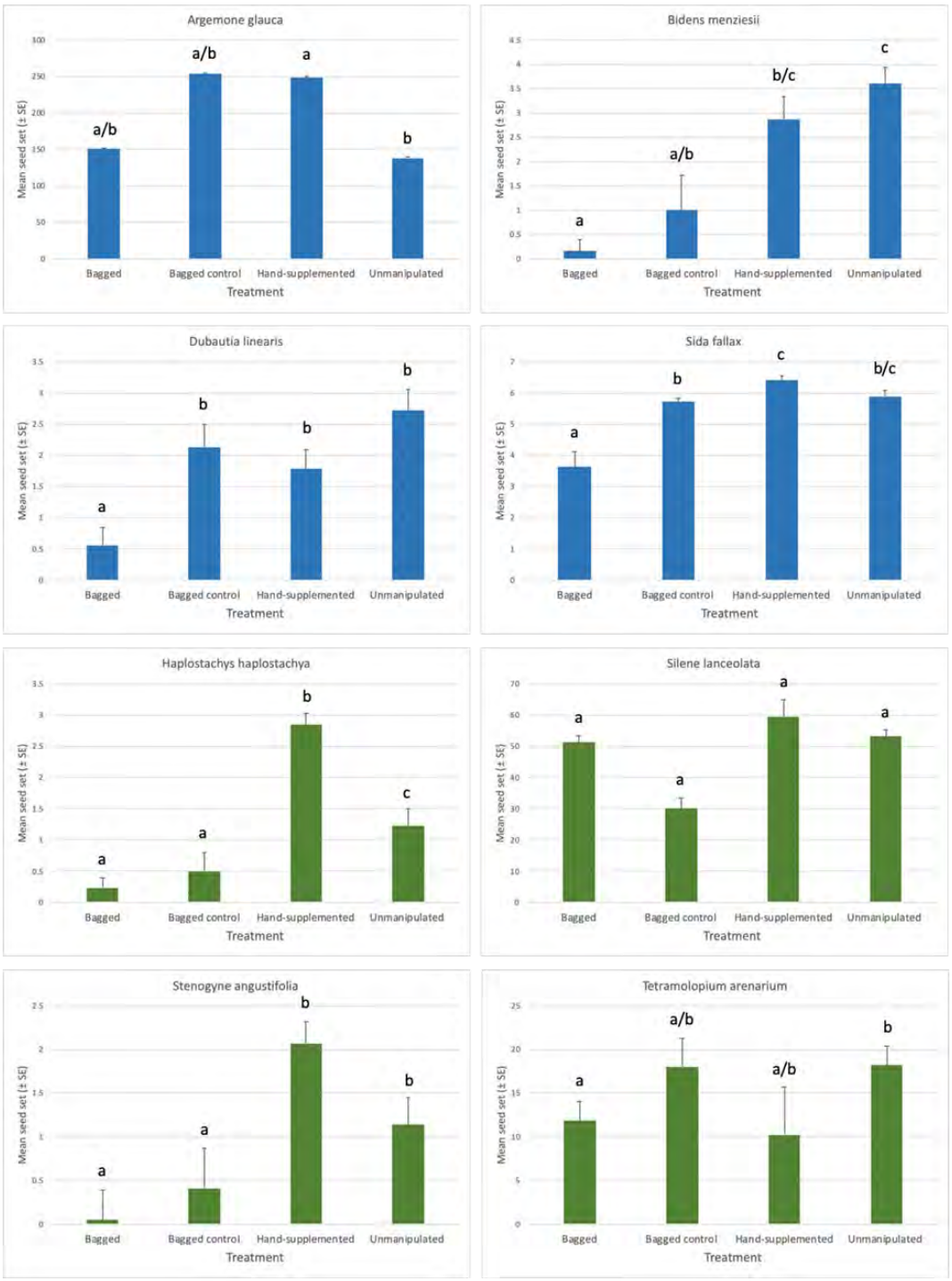


Figure 13. Treatment results at the end of the first year of pollination treatments. Seed production per flower are provided for: a) *Silene lanceolata*, b) *Sida fallax*, c) *Tetramolopium arenarium*, d) *Bidens menziesii*, e) *Haplostachys haplostachya*, f) *Dubautia linearis*, g) *Stenogyne angustifolia*, and h) *Argemone glauca*. Blue graphs (the top four) are common endemic plant species, and green graphs (the bottom four) are endangered plant species. Treatments included open or unmanipulated flowers, hand-supplementation with pollen, bagged self-fertilized flowers, and bagged control flowers bagged as buds and hand-cross-pollinated when open. Letters above bars correspond to significant differences between treatments for that species.

For *S. angustifolia*, pollination treatments were administered to 55 flowers on 28 plants, with seed set per flower differing significantly by treatment (Kruskal-Wallis test, chi-square = 20.43; p = 0.0001). Both bagged and bagged control flowers set significantly fewer seeds than unmanipulated flowers (p = 0.0026 and p = 0.024, respectively; Dunn's tests for multiple comparisons). Hand-supplemented flowers set significantly more fruit than bagged (p = 0.0008) or bagged control (p = 0.0072) flowers (Dunn's tests for multiple comparisons). Mean seeds produced per flower for *S. angustifolia* was 0.05 ± 0.34 for bagged flowers, 0.41 ± 0.46 for bagged control flowers, 2.07 ± 0.25 for hand-supplemented flowers, and 1.14 ± 0.31 for unmanipulated flowers (Figure 13).

For *T. arenarium*, a total of 91 flowers on 10 plants were treated, with significant differences in seed production per flower among treatments (Kruskal-Wallis test, chi-square = 9.77, p = 0.021). Unmanipulated flowers set significantly more fruit than bagged flowers (p = 0.039). Mean seeds produced per flower for *T. arenarium* was 11.84 ± 2.17 for bagged flowers, 18.00 ± 3.29 for bagged control flowers, 10.24 ± 5.50 for hand-supplemented flowers, and 18.18 ± 2.19 for unmanipulated flowers (Figure 13).

All plant species produced some seed when bagged to exclude outcrossing, indicating some degree of self-compatibility for each species. Index of Self-Incompatibility (ISI) values between 0.0 and 0.2 are considered to indicate self-incompatibility; values between 0.2 and 1 are considered to indicate partial self-compatibility; and values of 1 are considered to indicate self-compatibility (Zapata and Arroyo 1978). ISI values calculated from our pollination treatment data were 0.80 for *A. glauca*, 0.43 for *H. haplostachya*, 0.24 for *B. menziesii*, 0.29 for *D. linearis*, 0.66 for *S. fallax*, 0.56 for *S. lanceolata*, 0.18 for *S. angustifolia*, and 0.54 for *T. arenarium*. The Pollen Limitation Index (PLI) indicates the amount to which ambient pollen transfer is limiting reproduction; a value of 0 indicates no pollen limitation and a value of 1 indicates complete pollen limitation (Larson and Barrett 2000). Results were 0.44 for *A. glauca*, 0.57 for *H. haplostachya*, 0.08 for *S. fallax*, 0.11 for *S. lanceolata*, and 0.45 for *S. angustifolia*. The highest pollen-limitation values were exhibited by the endangered species *H. haplostachya* and *S. angustifolia* and the common native *A. glauca*. The endangered species *S. lanceolata*, which also demonstrated no significant decrease in seed set when outcrossing was prevented, exhibited a very low PLI of 0.11, suggesting that much of the seed production for this species may occur via autogamy. For all of the Asteraceae species we examined, PLI was negative (−0.78 for *T. arenarium*, −0.26 for *B. menziesii*, −0.53 for *D. linearis*), implying stigmatic damage during hand-supplementation treatments (see below; Young and Young, 1992). Asteraceae flowers are composites, comprising concentric rings of florets that open in sequence. This means that only a fraction of the florets within any particular composite are pollen-receptive at any given time; in spite of our attempts to be gentle, when we applied pollen to the composite we may have damaged immature florets or removed developing pollen tubes from older florets--a common problem in pollination ecology studies. The flowers of all three of our focal asters are very small and it was often difficult to find large quantities of pollen to apply.

Our flower treatment results demonstrate at least some level of self-compatibility for all of our plant species, implying that they are capable of producing fruits and seeds even in the absence of all pollination. Self-compatibility may be critical for the survival of a population when pollinators are rare or individual plants are rare across a landscape. Island endemics are known to exhibit a high rate of self-compatibility, thought to be a result of the importance of self-compatibility to the earliest colonizers of a particular island, since it removes the need to arrive along with a mate (Baker 1955). The self-compatibility we observed for our focal species

limits their dependence on pollinators in general. At the same time, bagging decreased fruit or seed set for all of our focal plants, indicating a likely cost to self-fertilization and emphasizing the importance of outcrossing in this system.

Our PLI results suggest that most of our plants are producing similar amounts of seed from ongoing, ambient pollination as from hand-supplementation, suggesting low pollen limitation in this system. This suggests that many of the flower visitors we observed are likely successfully transferring pollen, and that these plant species are experiencing active pollination in this ecological community in spite of the heavy dominance of non-native pollinators.

**NIP species composition, relative abundance, and seasonality**

*Rodent populations and dynamics*

Although four rodent species (all non-native) reside on Hawai‘i Island, trapping at our study site revealed that just two species are present: *Rattus rattus* (black, roof, or ship rat), and *Mus musculus* (house mouse). During our pre-experimental quarterly trapping (May 2015-April 2016) where we obtained rodents to extract fecal contents for diet analysis, we trapped 166 rodents, of which 60 were black rats and 106 were house mice. The total trapping effort for this period was 450 trap nights for rat trapping and 988 trap nights for mouse trapping. A trap night (TN) is a single trap set for one night. The quarterly trapping results were: Quarter 1 (June): 4.7 rat/100 TN, 0.7 mice/100 TN; Quarter 2 (September): 30.6 rat/100 TN, 20.8 mice/100 TN; Quarter 3 (December): 16.7 rat/100 TN, 32.3 mice/100 TN; Quarter 4 (April): 0.036 rat/100 TN, 0.015 mice/100 TN. Although the ratio of rats to mice differed seasonally and between grassland-shrubland areas (near block 1 and 2) and woodland areas (near block 3 and 4), we observed that mice generally outnumber rats at our study site by a ratio of roughly 2:1. Our monthly tracking tunnel measurements that occurred prior to NIP removal/suppression indicated that rats and mice are present in all 20 study plots on a year-round basis (Figure 14). While July through November had the highest rat tracking during the year, the lowest periods of rat activity were during March and April. Whereas May and June tended to be the months of lowest mouse activity, they steadily became more active in subsequent months, and reached peak activity during December through March (Figure 14). As expected, the plots in grassland-shrubland habitat (blocks 1 and 2) tended to have the most mouse activity, whereas rats tended to dominate the woodland plots (blocks 3 and 4) (Figure 14). Tracking tunnel monitoring also revealed the presence of mongoose (*Herpestes auropunctatus*), which is a predator of rodents, within all blocks at our study site (Figures 15, 16).

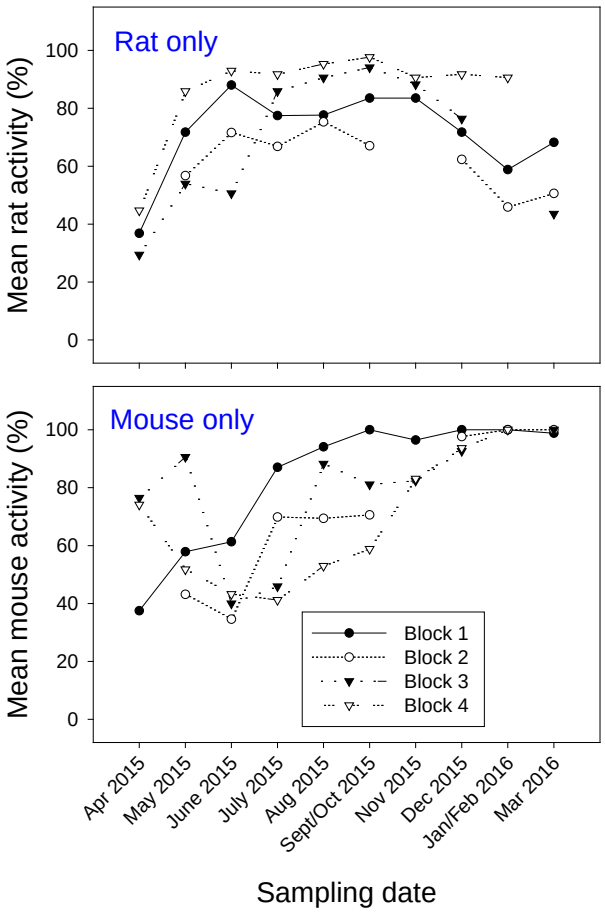


Figure 14. Rat (*Rattus rattus*) and mouse (*Mus musculus*) activity resulting from tracking tunnels deployed monthly April 2015–March 2016 in all 20 plots (i.e., 5 plots x 4 blocks) prior to NIP-suppression treatments.

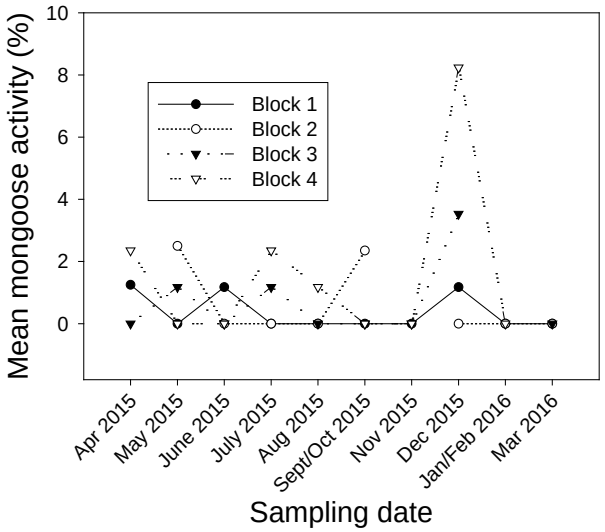


Figure 15. Mongoose activity resulting from tracking tunnels deployed monthly April 2015–March 2016 in all 20 plots (i.e., 5 plots x 4 blocks) prior to NIP-suppression treatments.

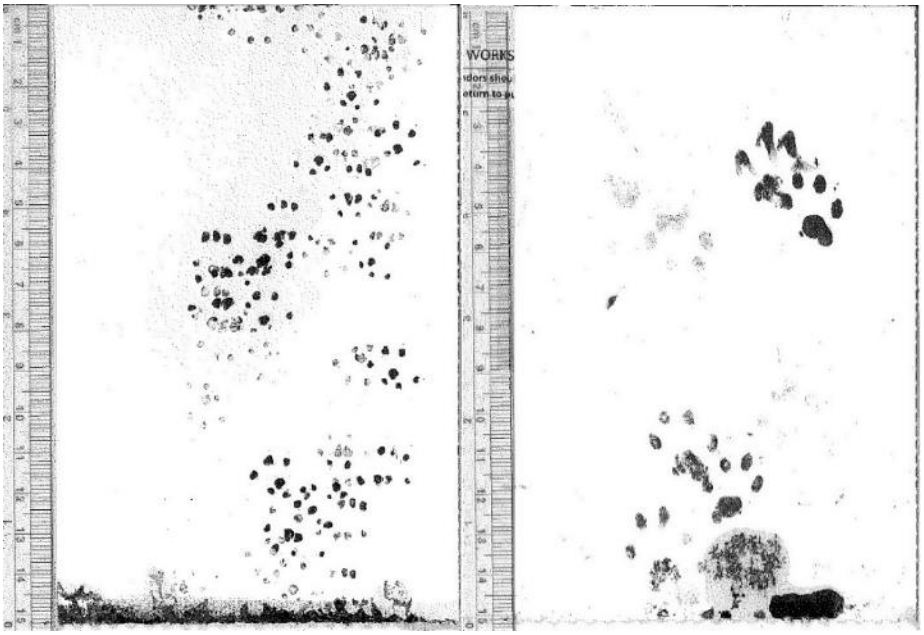

Figure 16. Tracking cards with rat (*Rattus* sp.) tracks (left) and mongoose (*Herpestes auropunctatus*) tracks (right), recovered from tracking tunnels.

We found several consistencies between our rodent abundance and seasonality findings with those of other studies within and outside of Hawai‘i. Black rat abundances in montane forests on Oahu (8-14 rat/100 trap nights; Shiels 2010) and Maui (8-14 rat/100 TN; Sugihara 1997) were within the range of those during the first three quarters of trapping at PTA (4-31 rat/100 TN; May-December 2015); the April 2016 trapping abundance at PTA was likely very low (e.g., 0.036 rat/100 TN) because of the very large number of traps deployed to reduce rodents in treatment plots. Other studies of black rats from North Island, New Zealand, found that abundances ranged from 5-35 rats/100 TN (Dowding and Murphy 1994; Wilson et al. 2007). House mouse abundances in our study ranged widely (1-31 mice/100 TN; May-December 2015), and certainly were within the range of past studies near PTA (9-16 mice/100 TN; Banko et al. 2002), in Maui wet forest (3-8 mice/100 TN; Sugihara 1997), in subantarctic New Zealand (7 mice/100 TN; Harper 2010), disturbed planation understory (10 mice/100 TN; King et al. 1996), and logged native forest (4 mice/100 TN; King et al. 1996) in North Island, New Zealand.

In mesic forests on Oahu, black rats were in greatest abundance from August through November (Shiels 2010), thereby roughly matching the black rat peak abundances of July through November at PTA. However, peak house mice abundance/activity in Oahu forest varied from year-to-year, as some years it was during the low at PTA (March-April), and other years it was closer to the December through March peak period (Shiels 2010) that we recorded. In a 1-year study on Maui, August was the peak mouse abundance and February was the peak black rat abundance (Shiels et al. 2017). The seasonal differences in rodent activity and abundance among studies in Hawai‘i may result from a range of site-specific factors including those that are both biotically and abiotically mediated. For example, house mouse abundance correlated with rainfall in crop fields in Australia (Singleton 1989), and dramatic seasonal increases in rat and mouse populations were explained by increased litter arthropods that fed on fallen flowers of the dominant tree in New Zealand (Fitzgerald et al. 1996). The presence of mongoose, and their

potential increase in activity in December, did not seem to directly correlate with either rat or mouse activity within plots or blocks at PTA.

*Ant populations and dynamics*

There are an estimated 57 species of ants (all non-native) in the Hawaiian Islands. In our study plots, we detected three species: the Argentine ant (*Linepithema humile*), the ghost ant (*Tapinoma melanocephalum*), and the less abundant *Cardiocondyla* cf. *kagutsuchi*. Our baseline ant surveys that began in early 2015 showed that the Argentine ant was most abundant and ubiquitous in blocks 1 and 2, had a spotty distribution in block 3, and was absent from block 4. The ghost ant was only found in block 4, and was the dominant ant species there. *Cardiocondyla* cf. *kagutsuchi* was present in parts of blocks 3 and 4, but was never abundant, and was unlikely to significantly affect arthropod communities. Abundance of Argentine ants and ghost ants varied by sampling event, but did not show a strong seasonal pattern (Figure 17), in contrast to yellowjackets (Figure 18).

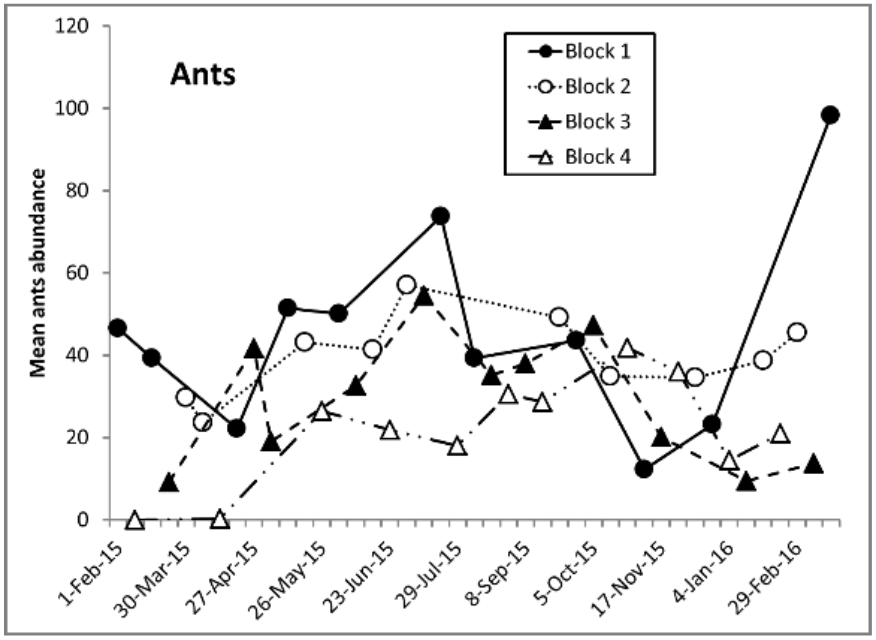

Figure 17. Fluctuation in abundance of ants (*Linepithema humile* in blocks 1-3 and *Tapinoma melanocephalum* in block 4) over approximately one year prior to treatment, expressed as the mean number of ants observed on cards baited with tuna and corn syrup (pooled across all plots in each block).

*Yellowjacket population and dynamics*

Abundance of yellowjacket wasps varied both temporally and spatially across our study sites between March 2015 and March 2016. Wasps were most abundant in blocks 3 and 4, which are characterized by *Metrosideros polymorpha* woodland, and were least abundant in blocks 1 and 2, dominated by grassland-shrubland. In all plots, wasp abundance peaked during the summer months (June to August) and was lowest in the spring (Figure 18).

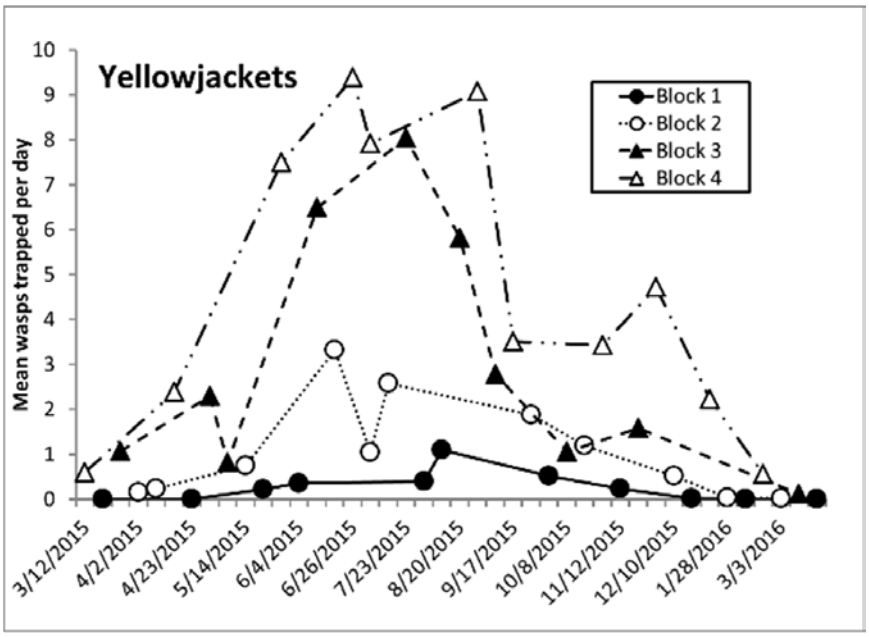

Figure 18. Fluctuation in abundance of yellowjacket wasps (*Vespula pensylvanica*) over the course of the study (prior to treatment), expressed as the mean number of wasps captured in traps baited with heptyl butyrate (pooled across all plots in each block). Blocks 1 and 2 had consistently fewer wasps than blocks 3 and 4. Wasp numbers were highest during the summer and lowest during the winter.

These fluctuations mirror seasonal trends previously found at various sites on Maui and Hawai‘i Island (Gambino and Loope 1992); wasp populations at most sites peaked between August and October, and were lowest in the winter and spring months, though some drier sites (Kipuka Nene and Ka‘u Desert) showed a different pattern, with wasp numbers peaking in November and December. Most yellowjacket nests in Hawai‘i die off during the winter. New queens are produced in the fall, and these mate, disperse and overwinter before founding new colonies in the spring. However, a small percentage of nests do overwinter in Hawai‘i (Gambino and Loope 1992).

**Efficacy of NIP suppression**

*Rodent suppression*

Suppression of the three types of NIP (rodents, ants, yellowjacket) started within the treatment plots in April 2016. The 150 m x 150 m rodent trapping grids to reduce or eliminate rat and mouse populations were activated on April 4, 2016, and the initial 2 weeks of trapping in a plot resulted in the bulk of the total rodents trapped in the All plots (receiving all combined predator control treatments) and Rodent (R) plots (receiving only rodent control treatment) (Figure 19). The grassland-shrubland of block 1 (plots All1 and R1) had the greatest take of rodents, whereas the woodland of block 4 (plots All4 and R4) had the lowest take of rodents. In all the plots and blocks, mice made up the majority of rodent take (Figure 19). When rodent kills at the 8 rodent treatment plots (i.e., All and R plots in every block) were combined for April-August 2016, we trapped 763 mice and 123 rats. There was little evidence of non-target

vertebrates trapped during our study, but four non-native game birds (*Francolinus erckelii*) and two mongooses were killed by our traps during the April 2016-January 2018 rodent trapping period. We documented many cockroaches (128) and some slugs (13) springing rodent traps, as evidenced by their remaining carcasses in the traps. Evidence of mongoose, and perhaps other rodents, scavenging dead carcasses from traps was also common.

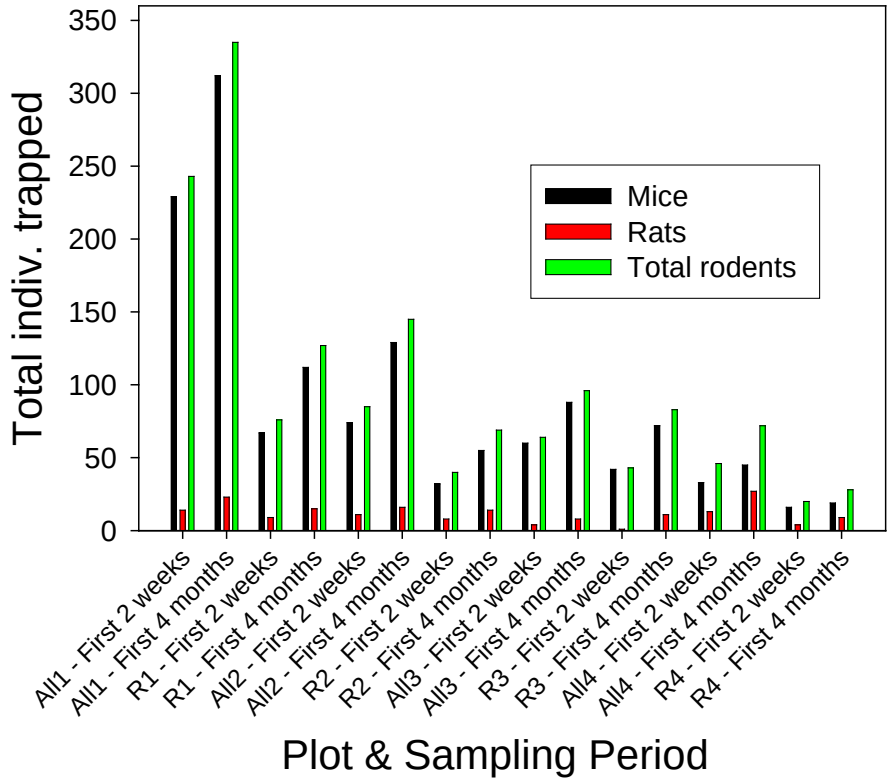


Figure 19. Total rodents trapped in All treatment plots (n = 4) and Rodent (R) treatment plots (n = 4) during the first four months (April–July 2016) of activating the rodent snap-trapping grids (150 x 150 m each). Number after plot type on horizontal axis refers to the block.

Tracking tunnels are used to measure the efficacy of our rodent trapping, and results from the tracking tunnels in July-October 2016 demonstrate that our snap-trapping grids initially were highly effective at suppressing rodents. In the first four months, our trapping regime generally reduced rodent activity to ≤20% tracking, whereas the no treatment (i.e., experimental control) plots where rodent suppression did not occur maintained >80% total rodent tracking (Figure 20). A study in Oahu mesic forest revealed that a 70% reduction in rat tracking (and thus maintaining rat tracking at <20%) from large-scale rodent trapping that used similar methods as ours at PTA resulted in a significant decrease in seed predation by black rats of an endangered plant (*Cyanea superba*; Pender et al. 2013). Therefore, we expected that the levels for which we were suppressing rats and mice in treatment plots at PTA should be sufficient to reveal biological responses, if they were indeed present.

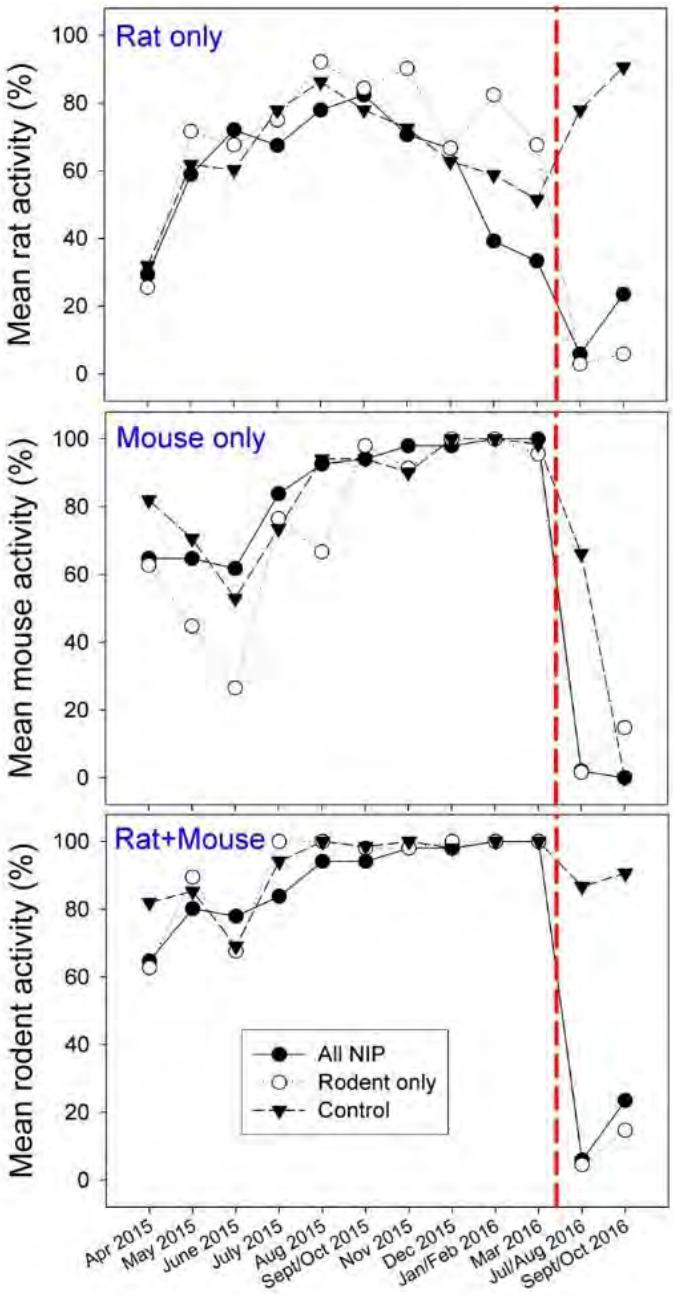


Figure 20. Rat, mouse, and rat+mouse activity identified using baited tracking tunnels in PTA plots. “All NIP” and “Rodent only” plots (n = 4 each) received rodent suppression via 150 x 150 m snap-trapping grids; “Control” plots (n = 4) did not have any rodent trapping or suppression. The red dashed line indicates when the rodent snap-trapping grids were activated.

After initially suppressing rodent populations for the first four months (August through November 2016), we found it challenging to keep the mouse populations below our 20% tracking index goal. Thus, we determined that both rats and mice could be effectively suppressed (<20% detection in tracking tunnels) for ~4 months after trapping grids were collectively active; yet only rat, and not mouse, suppression was sustainable thereafter.

Following statistical analysis, we found that our rat trapping technique significantly reduced rat tracking in the rodent treatment and the combined treatment relative to the untreated control (ANOVA: $F_{2,93} = 64.9$, $P < 0.00001$). In fact, all sampling periods had average rat tracking <20% (Figure 21). Rat tracking also significantly differed over time when averaged across treatment (ANOVA: $F_{10,93} = 5.1$, $P < 0.00001$), and there was no significant treatment x time interaction (ANOVA: $P = 0.108$). There appeared to be a natural spike in rat tracking during the September-November 2016 sampling, and the greatest lull in rat tracking occurred in March-May 2017; however, rat seasonal spikes and lulls did not appear to be predictable seasonally because the same patterns were not observed in the untreated control plots during subsequent years (Figure 21).

Once rat trapping ceased in January 2018, we monitored rat incursion into the plots for the subsequent three months. Interestingly, rat tracking did not recover to pre-experimental levels within three months (Figure 21). In an Oahu forest, rat tracking reached 33% 8 months following ceasing long-term rat trapping that had kept rat populations suppressed to <20% (Shiels et al. 2019). Although many factors are likely to affect rat recolonization into a trapping grid once trapping ceases, the size of the trapping grid and the duration for which it is active and inactive will certainly be key factors. In contrast to mice, grids of snap-traps appear to be a sustainable method to maintain suppressed rat populations at <20% tracking at PTA, assuming field labor can support the weekly or biweekly trap servicing.

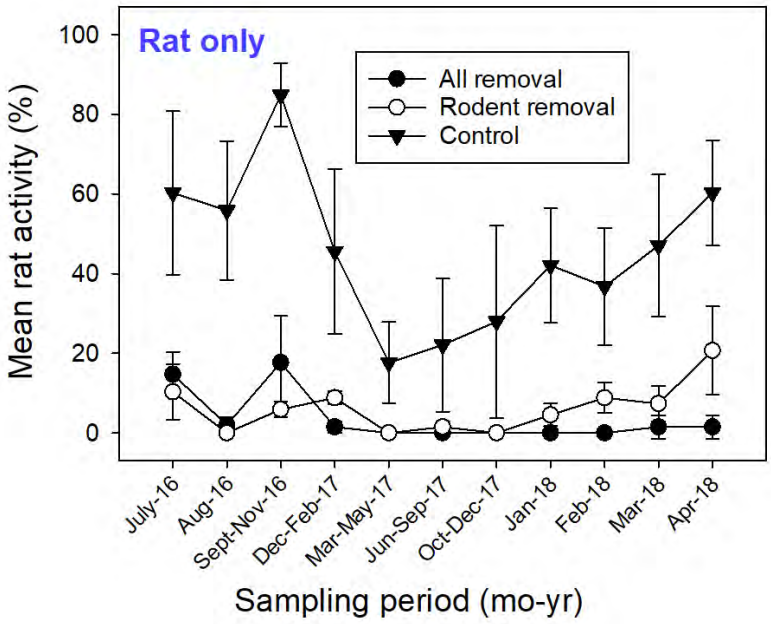


Figure 21. PTA rat tracking tunnel results in the rodent treatment (Rodent removal) and combined treatment (All [rodents+ants+yellowjackets] removal), and the untreated Control or reference plots where rodents were not suppressed. Each data point represents an average of n = 3 or 4 treatment plots that each had a percentage of the 17 tracking tunnels per plot where rats foot tracks were present. All time periods shown are during the treatment period. Note that rat activity (%) in all plots were ~80% tracking during the pre-experimental baseline period that occurred prior to July 2016; the post-experimental period was February–April 2018.

Following statistical analysis, we found that our mouse trapping technique significantly reduced mouse tracking in the rodent treatment and the combined treatment relative to the untreated control (ANOVA: $F_{2,93} = 25.7$, $P < 0.00001$). Mouse tracking significantly differed over time when averaged across treatment (ANOVA: $F_{10,93} = 10.5$, $P < 0.00001$), and there was no significant treatment x time interaction (ANOVA: $P = 0.094$). Although we indeed reduced mouse populations relative to the untreated control plots, we were not able to maintain the mouse populations at target levels for suspected conservation purposes (<20%; Pender et al. 2013), aside from the first four months following suppression (Figure 22). A similar result was found in an Oahu forest where rat snap-traps and Goodnature A24 self-setting rat traps were installed for long-term rodent suppression; house mice were not able to be suppressed below 20% tracking during any month annually aside from in January and two months immediately following application of rodenticide baits (Shiels et al. 2019). Because black rats and house mice coexist in many of the same habitats in Hawai‘i, there is likely to be competitive interactions between these two rodent species (Shiels et al. 2013). Seasonal trends in rodent abundance may also show evidence of competition. In our study at PTA, and as a mirror opposite to the spike in rat abundance in September-November 2016, house mouse abundance simultaneously plummeted naturally during this period.

Once mouse trapping ceased in January 2018, we monitored mouse incursion into the plots for the subsequent 3 months. Mouse tracking had recovered to pre-experimental levels within 3 months in some plots, but not in others (Figure 22).

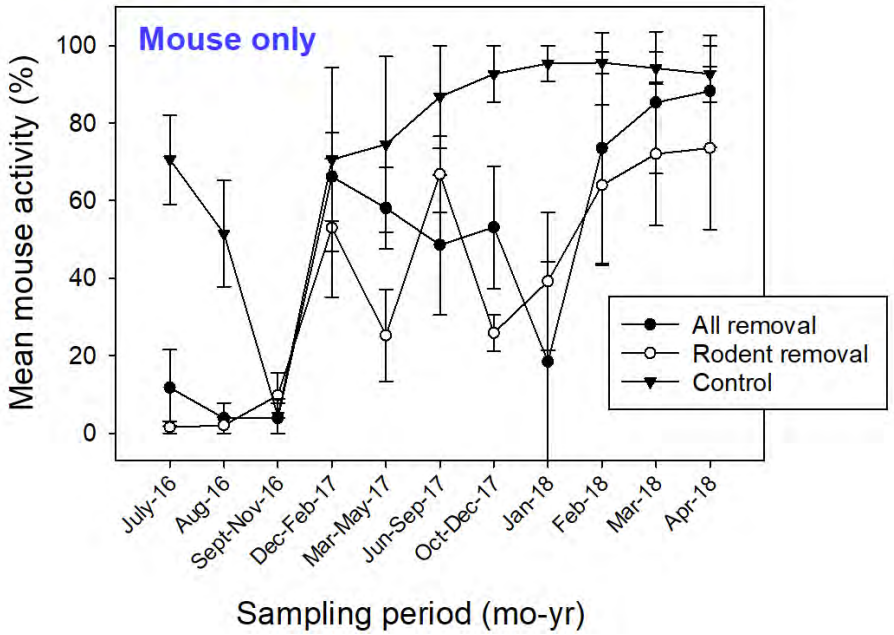


Figure 22. Results for house mouse tracking tunnels at PTA in the rodent treatment (Rodent removal) and combined treatment (All [rodents+ants+yellowjackets] removal), and the untreated Control or reference plots where rodents were not suppressed. Each data point represents an average of n = 3 or 4 treatment plots that each had a percentage of the 17 tracking tunnels per plot where mouse foot tracks were present. Note that mouse activity (%) in all plots were ~80% tracking during the pre-experimental baseline period that occurred prior to July 2016; the post-experimental period was February–April 2018.

The most likely reasons that we believe our methodology was inadequate to maintain house mouse populations below 20% tracking include: habituation to traps, non-target trap interference, and the high abundance of mice at the site. Trail camera evidence revealed that mice became habituated to traps in some cases, leading to trap avoidance, and some non-target animals interfered with mouse traps. Non-target interference was mostly due to game birds, mongooses, and feral house cats. These animals would either trigger the mouse traps strategically to avoid injury, and then eat the peanut butter bait; or in many cases the mongooses and feral cats that we observed would scavenge the rodent carcasses off the traps. In either scenario, the trap was generally non-functional because it was triggered or the predator scent left on the trap and trap box was a deterrent for rodents. Although rodent home-ranges tend to decrease with increased population density, the 12.5 m trap spacing (established based on Shiels 2010) may have been insufficient for plots with such high mouse abundances (especially blocks 1 and 2). However, we felt that re-setting the abundance of rodent traps on a weekly basis was already difficult to maintain with our field crew of five full-time staff. Therefore, it was unlikely that increasing trap servicing intervals or the numbers of traps in a plot would have helped reduce mice more than we demonstrated in our study.

In areas like PTA with high house mouse populations, grids of snap traps may not be a sustainable management technique for long-term house mouse control and protection of natural resources from pest house mice. Use of automated self-resetting rat traps (i.e., Goodnature A24s, which are popular for rodent control for conservations purposes in Hawai‘i and elsewhere), are also not recommended for long-term house mice control in Hawaiian natural areas (see Shiels et al. 2019). Instead, house mice can be successfully suppressed to very low levels (much less than 20% tracking) by using diphacinone anticoagulant rodenticide bait, such as used in Shiels et al. (2019). However, many land managers (including those at PTA) disfavor or do not allow the use of pesticides on their property. Alternatively, mouse repeater traps, like those used at Ka‘ena Point on Oahu, are a non-toxicant method that has shown promise for house mouse population suppression in Hawai‘i (Young et al. 2013).

*Ant suppression*

Formicidal bait (Maxforce Complete Insect Bait, 1% hydramethylnon) was used to suppress both Argentine ants and ghost ants in our Ant and All plots. Bait was applied six times during the treatment period between June 2016 and January 2018. Ant abundance was monitored by counting the number of foragers visiting cards baited with a tuna and corn syrup mixture.

Efficacy of Maxforce treatments against Argentine ants (blocks 1, 2, and 3) was evaluated separately from efficacy against ghost ants (block 4). During the pre-experimental period Argentine ants were significantly more abundant in the plots designated as treatment plots than in plots designated as untreated controls (ANOVA: $F_{2, 52} = 6.866$, $p = 0.002$). In contrast, throughout the experimental period, treated plots had significantly fewer ants than untreated plots (ANOVA: $F_{2, 108} = 7.493$, $p < 0.001$), indicating that treatments effectively suppressed Argentine ants (Figure 23). However, Argentine ants were not always suppressed to near-zero levels, sometimes rebounding soon after treatments. Frequent treatments were needed to maintain suppression.

Maxforce treatments were not as effective against ghost ants (block 4), though they did achieve some suppression. During the pre-experimental period, there were no significant differences in ant abundance among plots in block 4 (ANOVA: $F_{2, 12} = 1.333$, $p = 0.300$). During

the experimental period, there was a significant effect of treatment overall (ANOVA: $F_{2, 26}$ = 5.463, p = 0.010) (Figure 24). Post-hoc Tukey tests revealed that the All plot had fewer ants than the Control plot (p = 0.008), but the Ant plot did not differ significantly from the Control plot (p = 0.282). As with Argentine ants, ghost ant populations rebounded soon after treatment, so frequent bait application was necessary to maintain suppression.

When treatments must be applied frequently to maintain suppression, impacts on non-target species are important to consider. Granular formicidal baits, such as Maxforce Granular Insect Bait, are most likely to impact ground-dwelling scavengers that may consume the bait. Non-target arthropod groups likely to be impacted by granular baits include isopods, springtails, earwigs, roaches, and crickets. Most pollinators are unlikely to be directly affected by Maxforce bait, with the exception of perhaps certain flies or moths whose larvae are ground-dwelling scavengers.

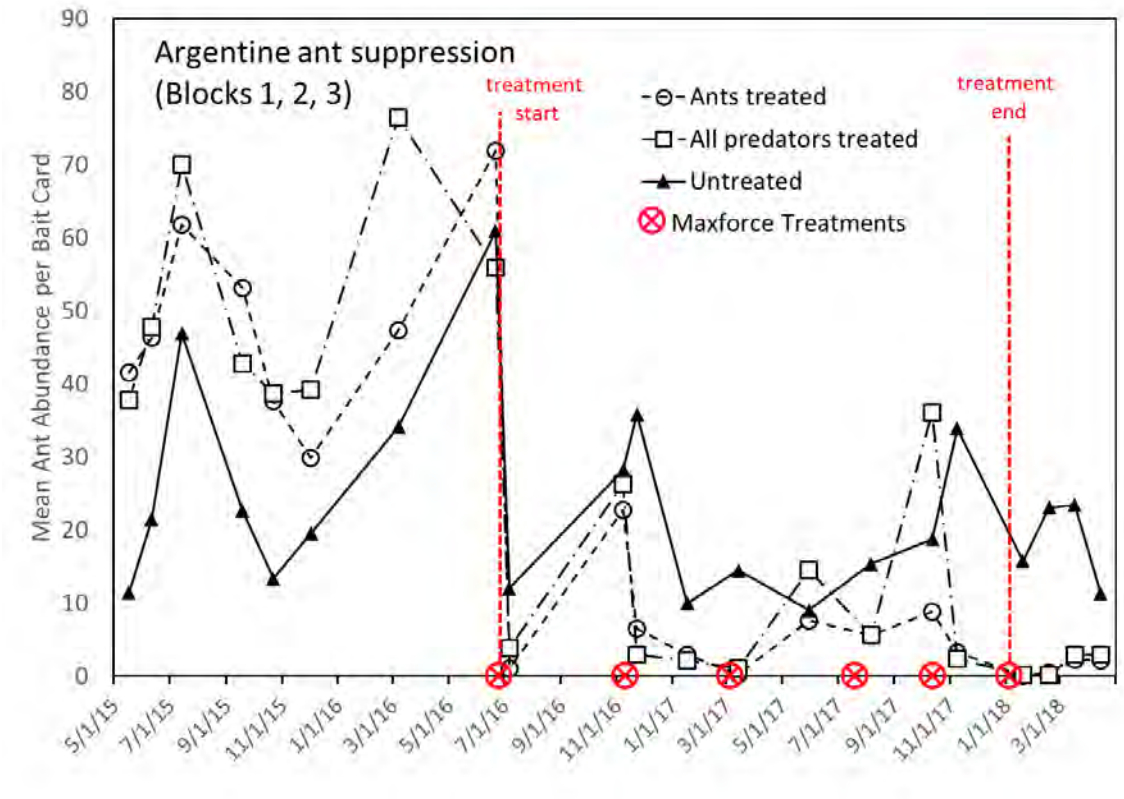


Figure 23. Suppression of Argentine ants (*Linepithema humile*) with Maxforce Granular Insect Bait. During the pretreatment period, ant numbers were significantly higher in plots designated as treatment plots compared to untreated plots. Following treatment, ant numbers were significantly lower in Ant and All plots compared to Control plots, indicating that suppression was effective.

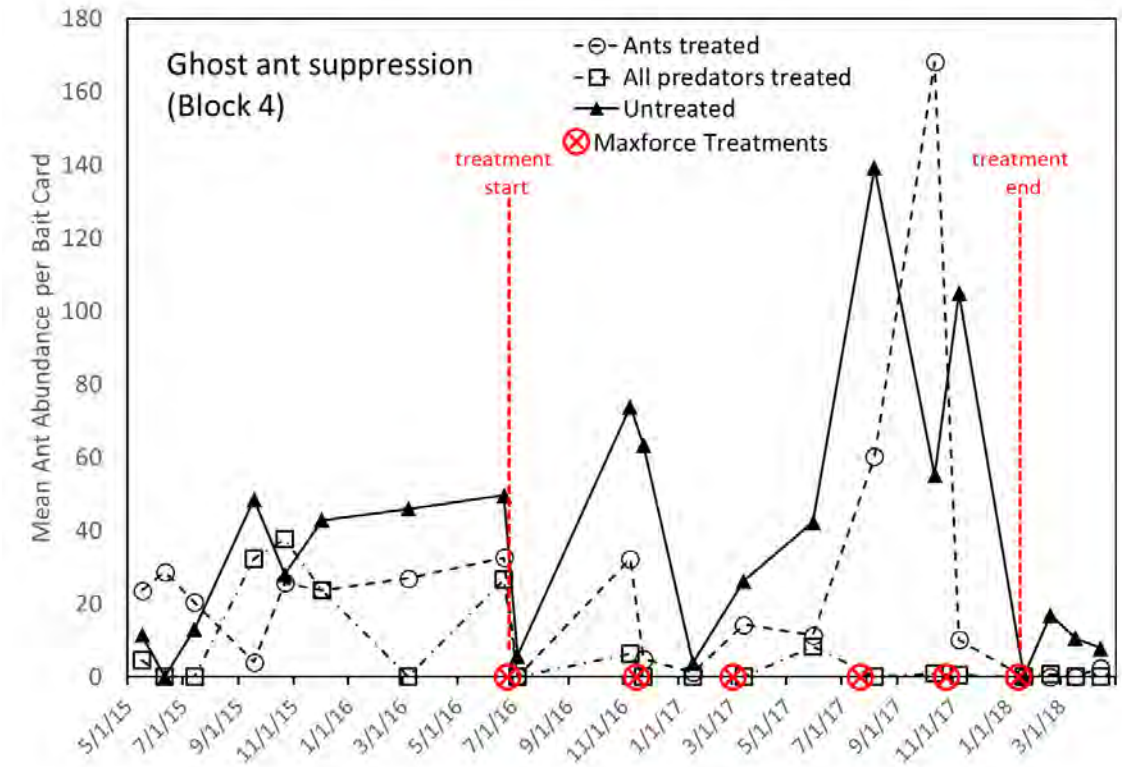


Figure 24. Suppression of ghost ants (*Tapinoma melanocephalum*) with Maxforce Granular Insect Bait. During the pretreatment period, there were no significant differences among the three different plots. Following treatment, ant numbers were significantly lower in the All plot compared to the Control plot, but were not significantly lower in the Ant plot.

*Yellowjacket suppression*

Yellowjacket wasps were treated in the Yellowjacket and All plots from June 2016 to November 2017. Yellowjacket abundance was monitored using traps baited with the attractant heptyl butyrate and set for four days at a time. Block 1 was excluded from analyses, since this block had naturally low numbers of yellowjackets, and was not treated after the first treatment.

During the pre-experimental period, yellowjacket abundance did not differ significantly among plot treatments (ANOVA: $F_{2, 76} = 2.962$, $p = 0.058$). During the treatment period, yellowjacket numbers were significantly lower in treated plots (ANOVA: $F_{2, 108} = 5.905$, $p = 0.004$). Post-hoc Tukey tests revealed that wasp numbers in Control plots were significantly higher than those in both Yellowjacket plots ($p = 0.004$) and All plots ($p = 0.031$). However, although these differences were statistically significant, suppression was not long-lasting, nor were wasp populations controlled at levels we considered to be adequate. Treated plots continued to have spikes in wasp numbers even while treatments were ongoing, for the most part mirroring population fluctuations in the untreated plots (Figure 25).

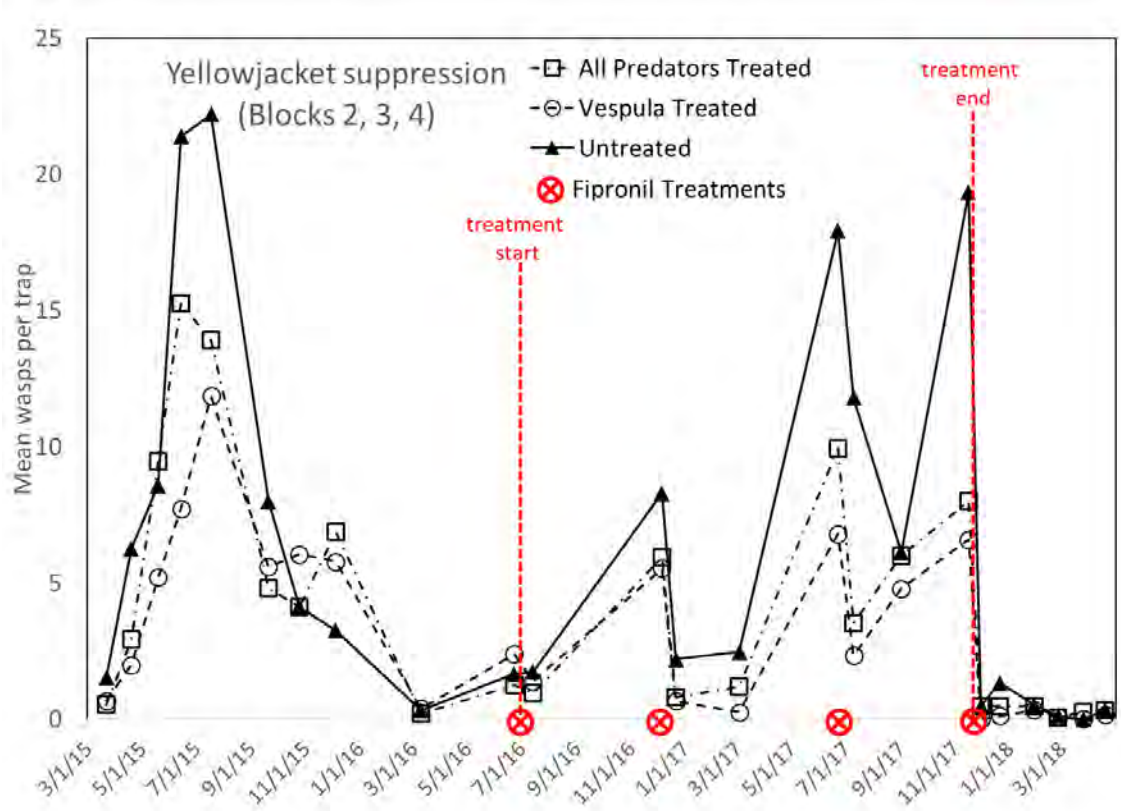


Figure 25. Yellowjacket abundance in traps baited with heptyl butyrate throughout the study period. Although wasp abundance was significantly lower in treated plots than in untreated plots after treatments were initiated, any suppression was slight and was not sustained for long periods.

It is unclear why yellowjacket treatments were not very effective. In plots where wasps were abundant, foraging wasps quickly found the toxic baits during treatments, and in many cases, wasps collected virtually all of the available bait, presumably taking it back to share with nestmates. Over the course of the study, only one yellowjacket nest was detected near our experimental plots (in block 4), and this nest died within days of the first fipronil treatment, providing some evidence that the baits were effective. It is possible that although nests in the vicinity of our treated plots were killed off, wasps continued to fly into the area from beyond the radius of efficacy of the baits. *V. pensylvanica* is known to have a long foraging distance, and it is thought that this foraging distance is greater in dry forests compared to wet forests (Gambino and Loope 1992).

**Analysis of NIP diets**

*Rodent prey items*

Fecal pellets from 30 mice and 30 rats were submitted for extraction and high-throughput sequencing, which yielded a total of 8,239 sequence reads. Sequence reads from high throughput sequencing are still in the process of being analyzed to classify OTUs to lower taxonomic levels. Rat fecal pellets yielded more sequence reads on average (209 reads per pellet) than mouse

pellets (86 reads per pellet), which might be expected due to the larger size of rat pellets. Sequences were classified into 185 OTUs, which were matched to the most similar sequences in reference libraries. The results are summarized by arthropod order in Figure 26. Based on the number of sequence reads, which can be considered to be roughly proportional to the biomass of prey items consumed, both rats and mice fed primarily on Lepidoptera (moths and butterflies) and Diptera (flies), both of which include important groups of pollinators. Lepidoptera accounted for the largest proportion (42%) of arthropod DNA in mouse pellets, while Diptera was the largest component (43%) in rat pellets. Hymenoptera (bees, wasps, and ants) accounted for only a small percentage of sequence reads in both species, and honey bees, yellowjacket wasps, and ants were not detected in any of the rodent fecal samples. However, this result may have ensued from the primer used in the DNA sequencing and we currently are exploring further analyses for identifying Hymenoptera taxa in the prey items. Araneae (spiders) accounted for a substantial proportion of arthropod DNA detected in both mice (13%) and rats (12%), as did Hemiptera (true bugs and hoppers), which accounted for 13% in mice feces and 14% in rat feces. The large proportion of the arthropod diet accounted for by caterpillars in our study is similar to other rodent diet studies in Hawai‘i where caterpillars accounted for >3% of the total black rat diet and >50% of the total house mouse diet (Shiels et al. 2013).

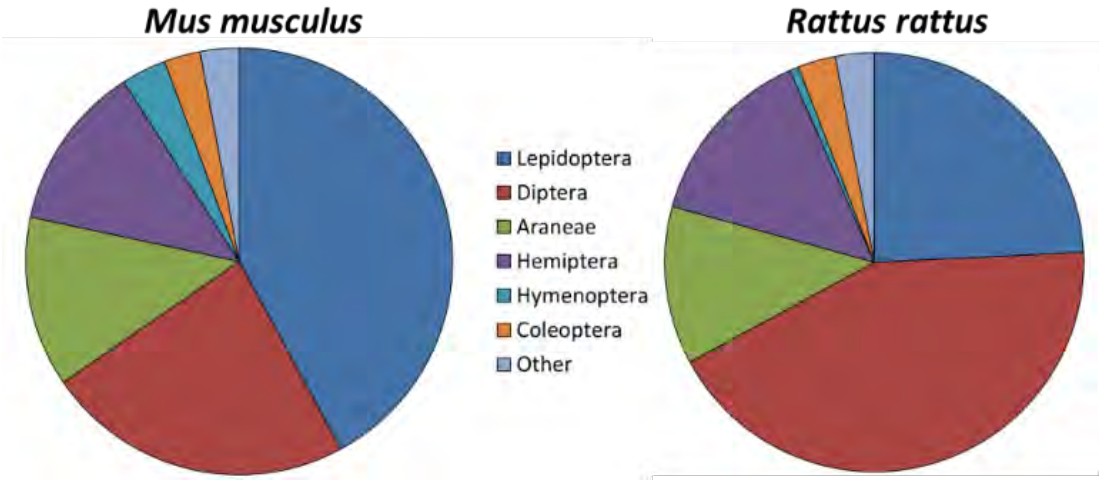


Figure 26. Proportion (by arthropod order) of CO1 sequence reads detected in mouse and rat fecal pellets by high throughput DNA sequencing.

*Ant prey items*

Pooled ant samples from 30 colonies were also submitted for high-throughput sequencing and are still in the process of being analyzed to classify OTUs to lower taxonomic levels. Each sample consisted of 100 worker ants and a variable number of larvae, up to 100 (dependent on how many larvae were present in nests). After excluding sequence reads identified as Argentine ant DNA, ant samples yielded a total of 67,627 reads that were identified as arthropod DNA. These reads were classified into 416 OTUs that were matched to the most similar sequences in online sequence databases. Taxonomic distribution of sequence reads is illustrated in Figure 27. Hemiptera (true bugs and hoppers, 23%), Acari (mites, 20%), and Diptera (flies, 17%) accounted for the majority of reads. Lepidoptera (moths and butterflies, 8%) and Hymenoptera (bees and ants, 5%) accounted for a smaller proportion of reads. Again, the result for Hymenoptera may

have ensued from the primer used in the DNA sequencing and we currently are exploring further analyses for identifying these taxa in the prey items.

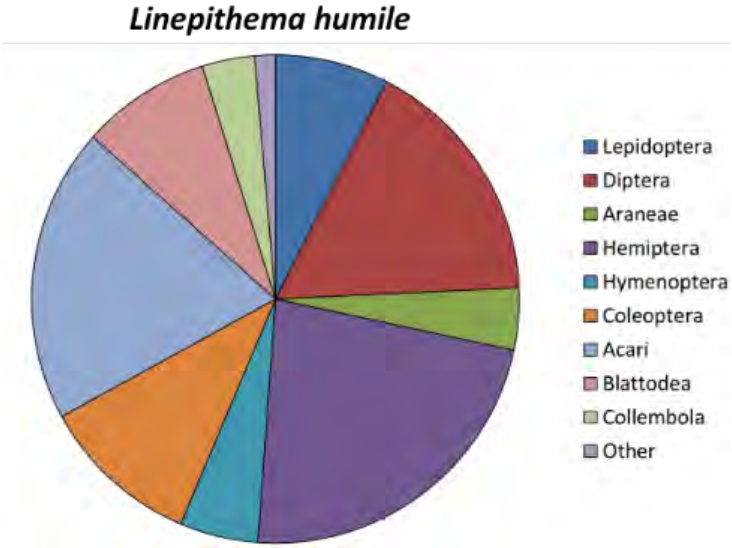


Figure 27. Proportion (by arthropod order) of CO1 sequence reads detected in Argentine ant samples (N=30) by high-throughput DNA sequencing. Sequence reads identified as Argentine ant DNA were excluded from the total.

*Yellowjacket prey items*

In all, 118 prey balls or fragments were recovered from nine separate samples of workers returning to the nest in block 4. From 83 of these, we successfully extracted and amplified the barcoding region of CO1 and produced good quality sequences that could be queried against existing databases. Taxonomic distribution of prey items is illustrated in Figure 28. By far, most prey fragments belonged to the order Hemiptera (true bugs and hoppers, 47%), and most of these were introduced plant bugs (Miridae), spittlebugs (Cercopidae), or native psyllids (*Trioza* spp.). Moths were the second most frequently encountered prey item, with 22% of fragments belonging to the order Lepidoptera. These included native moths in the genera *Carposina* (in four separate samples) and *Eccoptocera*, as well as an introduced tortricid moth, *Cryptophlebia illepida*. Physical examination of prey balls revealed that the majority of these were captured as caterpillars. Diptera (8%) and Hymenoptera (7%) were less well represented in yellowjacket prey items, but they did include known pollinators such as the honey bee *Apis mellifera* (present in two separate sampling events) and the hover fly *Allograpta exotica* (present in one sampling event). In two samples, some prey balls consisted of *Vespula pensylvanica* itself, indicating intraspecies predation or scavenging.

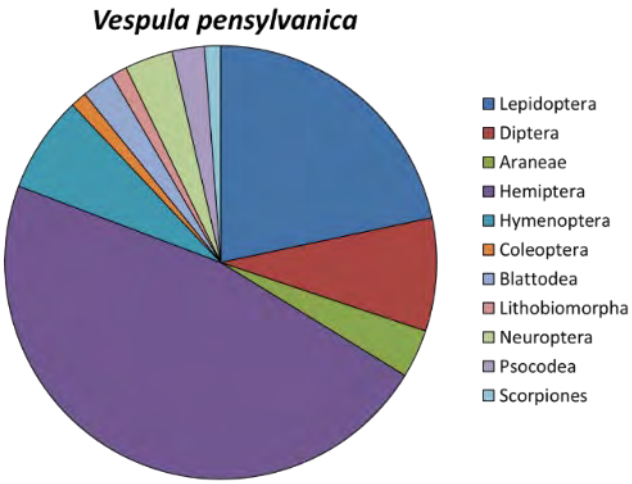


Figure 28. Proportion (by arthropod order) of prey fragments (N=83) collected from foraging yellowjacket wasps as they returned to their nest. Fragments were identified by Sanger sequencing the CO1 region.

Our results were similar to previous findings on yellowjacket prey items. Yellowjacket prey items have been identified in wet and mesic forest and shrubland in Hawai‘i Volcanoes and Haleakala National Parks using both morphological characters (Gambino 1992) and DNA sequencing (Wilson et al. 2009). These studies found roughly similar types of prey items to our own, though the composition by order differed among sites. In particular, we found that Hemiptera made up a higher proportion of yellowjacket diets at PTA than was previously reported for other montane sites in Hawai‘i. These differences likely reflect differences in prey availability in different habitat types (dry forest vs. wet and mesic forest), but might also reflect identification bias in morphological studies. Gambino (1992) reported that fewer than 20% of the prey items collected could be identified to order. Our success rate using DNA barcoding was considerably higher; we successfully obtained sequences from 70% of collected fragments, and all of these were classified to at least the order level, with 77% identified to the genus or species level. In our study, 56.6% of identified prey items were nonnative, 21.7% were native, and 21.7% were of undetermined origin.

**Arthropod monitoring**

From March 2015 through March 2016, we collected a total of 950 vegetation samples, 950 leaf litter samples, 1050 sticky traps, 101 malaise trap samples, and 81 light trap samples during the pre-experimental baseline period. Malaise and light trap sampling were not consistent due to weather, equipment failure, and predation of samples in the field by rodents or other predators. Bee nest blocks did not attract any nesting bees, likely due to the wood block material which swelled and partially filled the drilled holes. We discontinued the malaise and light trap sampling along with the bee nest blocks after the pre-experimental period. From September 2016 through April 2018, we collected a total of 875 vegetation samples, 875 leaf litter samples, and 875 sticky traps during the experimental treatment and post-experimental periods. Samples were sorted and identified to order or lower taxonomic level.

We employed a variety of methods to try to capture the diversity of arthropods at the site, with the realization at the outset that a comprehensive cataloging of arthropod species was

beyond the purpose and scope of this project. One of the methods, the balsa wood bee nest blocks, was an experimental approach suggested by an expert on solitary bees (Jason Graham, pers. comm. January 2015) but we were unsure if colonization by solitary bees would occur. We did not modify the blocks, and there were not any other proven methods to attract nesting bees. Sticky traps were also used to assess solitary bees, however very few specimens were recorded. Other sampling methods such as the malaise and light traps were intended to be more qualitative in nature rather than quantitative, based on our sampling scheme of one trap in each plot. Leaf litter samples were extremely time consuming to sort, and the majority of samples have not been processed.

**Flower visitation observations on potted plants**

During our treatment period, we performed 165.67 hours of observations of flower visitation for potted *B. menziesii* in our treatment plots, 42.83 hours of observations of flower visitation for potted *D. linearis* (which flowers in only one season per year), 114.00 hours of observation for potted *H. haplostachya*, 189.83 hours of observation for potted *S. fallax*, 76.67 hours of observations for potted *S. lanceolata*, 111.00 hours of observation for potted *T. arenarium*, and 137.17 hours of observation for potted *S. angustifolia*. Potted A. *glauca* plants flowered prior to the experimental treatment period and then senesced, leaving the experiment with too few individuals to include in the treatment period visitation observations.

Pollinator visitation importance was calculated as for the baseline data collection. A total of 11 taxa visited flowers of *B. menziesii* during the experimental period. The most important visitors to *B. menziesii* (those with the highest PVI in at least one treatment plot) included *P. aurifer*, *A. mellifera*, and *Allograpta* sp. in Rodent treatment plots. In Ant treatment plots, the most important visitors included *Orthomecyna* sp. and *A. mellifera*. In Yellowjacket treatment plots, the most important visitors included *A. mellifera*, *Orthomecyna* sp., and *Hylaeus* sp. In All treatment plots, the most important visitors included Diptera sp., *P. nasidens*, and *Allograpta* sp. In Control plots, the most important visitors included *P. nasidens*, *Orthomecyna* sp., *A. mellifera*, and *P. aurifer*.

A total of 8 taxa visited flowers of *D. linearis* during the experimental period. The most important visitors to *D. linearis* (those with the highest PVI in at least one treatment plot) included *A. mellifera*, and Diptera sp. in Rodent treatment plots. In Ant treatment plots, the most important visitors included *A. mellifera* and *Hylaeus* sp. In Yellowjacket treatment plots, the most important visitors included *A. mellifera* and *Allograpta* sp. In All treatment plots, the most important visitors included *A. mellifera* and *L. impavidum*. In Control plots, the most important visitor was *A. mellifera*.

A total of 9 taxa visited flowers of *H. haplostachys* during the experimental period. The most important visitors to *H. haplostachys* (those with the highest PVI in at least one treatment plot) included *A. mellifera*, *P. rapae*, and *U. blackburni* in Rodent treatment plots. In Ant treatment plots, the most important visitors included *A. mellifera*, *Allograpta* sp., and Diptera sp. In Yellowjacket treatment plots, the most important visitor was *P. rapae*. In All treatment plots, the most important visitors included *Allograpta* sp., *A. mellifera*, and Diptera sp. In Control plots, the most important visitor was *Allograpta* sp.

A total of 14 taxa visited flowers of *S. fallax* during the experimental period. The most important visitors to *S. fallax* (those with the highest PVI in at least one treatment plot) included *P. rapae* and *A. mellifera* in Rodent treatment plots. In Ant treatment plots, the most important

visitors included *A. mellifera*, *Allograpta* sp., and *Hylaeus* sp. In Yellowjacket treatment plots, the most important visitors were *Allograpta* sp. and *A. mellfera*. In All treatment plots, the most important visitor was *A. mellifera*. In Control plots, the most important visitors were *Allograpta* sp., Diptera sp., and *A. mellifera*.

A total of 3 taxa visited flowers of *S. lanceolata* during the experimental period. The most important visitor to *S. lanceolata* (those with the highest PVI in at least one treatment plot) was *Allograpta* sp.in Rodent treatment plots. In Ant treatment plots, the most important visitors included *Allograpta* sp. and *P. rapae*. In Yellowjacket treatment plots, no visitors were recorded interacting with flowers. In All treatment plots, the most important visitors were *Allograpta* sp. and *P. rapae*. In Control plots, the most important visitors were *L. impavidum* and *Allograpta* sp.

A total of 11 taxa visited flowers of *T. arenarium* during the experimental period. The most important visitors to *T. arenarium* (those with the highest PVI in at least one treatment plot) included Diptera sp., *Orthomecyna* sp., and *Allograpta* sp. in Rodent treatment plots. In Ant treatment plots, the most important visitors included *L. impavidum*, *Allograpta* sp., and *Hylaeus* sp. In Yellowjacket treatment plots, the most important visitors were Allograpta sp. and Diptera sp. In All treatment plots, the most important visitors were *Orthomecyna* sp., Diptera sp., *Allograpta* sp., and *P. nasidens*. In Control plots, the most important visitors were *Orthomecyna* sp., *A. mellifera*, and *Allograpta* sp.

Just two taxa visited flowers of *S. angustifolia* during the experimental period. In one Ant treatment plot, a potted plant was visited by an individual of *Allograpta* sp. In one Control plot, a *Hylaeus* sp. bee visited the potted plants.

Most of our plant species interacted with a higher diversity of flower visitors during the treatment period than during the baseline analysis. This may partially have been due to habitat: our potted plants were placed in plots across both grassland and woodland portions of the study area, thus exposing them to potential pollinators across both habitat types. To move beyond habitat type and examine the influence of predator controls and density on pollinator communities, we imported all treatment period flower visitation data into the SHELOB model.

**SHELOB model**

*Plant-pollinator networks in experimental time periods*

For the SHELOB model, we classified six pollinator species groups known to perform frequent, legitimate pollen transfer: solitary bees (primarily *Hylaeus* spp. and *Lasioglossum* spp), honey bee (*Apis mellifera*), moths and butterflies (primarily Crambidae spp., *Lampides boeticus*, *Orthomecyna* spp., *Pieris rapae*, and *Udara blackburni*), non-syrphid flies (primarily *Dioxyna sororcula* and Muscidae spp.), syrphid flies (*Allograpta* spp.), and wasps excluding *Vespula pensylvanica* (primarily *Pachodynerus nasidens* and *Polistes aurifer*; hereinafter, "non-*Vespula* wasps"). During the entire experimental treatment, the most important pollinator species groups were syrphid flies (for *D. linearis*, *H. haplostachya*, *S. lanceolata*, and *S. angustifolia*), honey bees (for *S. fallax*), and non-*Vespula* wasps (for *B. menziesii*). The honey bee group consisted of only *Apis mellifer*a, which are non-native in Hawai'i, and the other groups are composed of both native and non-native species.

There was variation in the pollination networks across the three experimental time periods (Figure 29). This variation partly stems from plant phenology, as well as the difficulty of observing plant-pollinator interactions because these events are rare for some species. *Dubautia*

*linear*is flowers seasonally in the fall, and so did not experience any pollination events during the February–June experimental time period. Over the entirety of the experimental treatment, *H. haplostachya*, *S. angustifolia*, and *S. lanceolata* experienced pollination events in only a few plot/time period combinations. *Haplostachys haplostachya* had no pollination events observed in eight plots, and *S. lanceolata* had no pollination events observed in twelve plots. *Stenogyne angustifolia* had pollinator interactions in only three plot/time period combinations during the experimental treatment, and these pollination events took place in either ant treatment plots (February–June 2017, block 3, syrphid fly and July–November 2017, block 2, syrphid fly) or an untreated control plot where ant populations were naturally as low as the treatment plots (August 2016–January 2017, block 3, solitary bee).

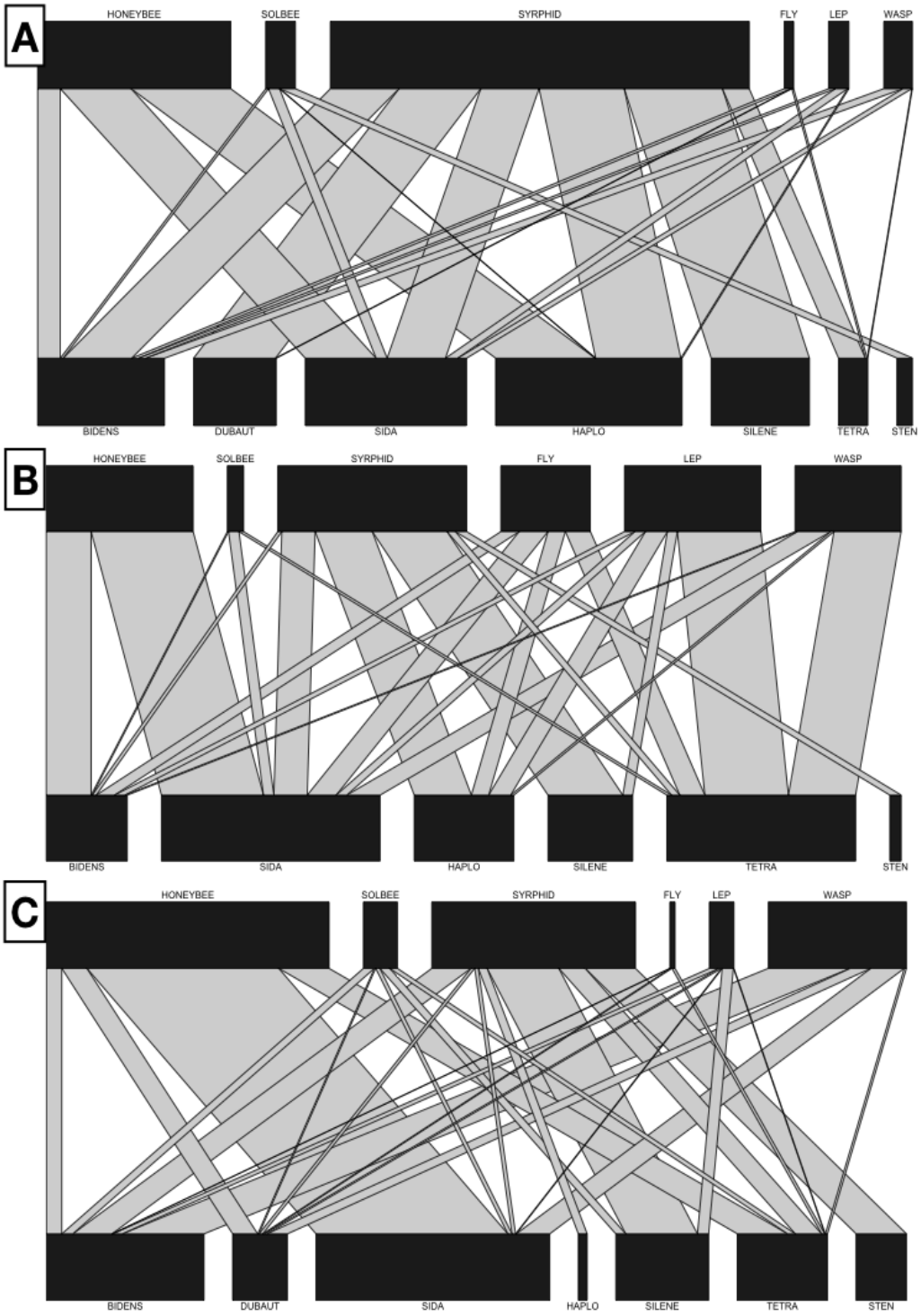


Figure 29. Plant-pollinator networks averaged across all plots and blocks for A) August 2016–January 2017, B) February–June 2017, and C) July–November 2017. Upper level: FLY = non-syrphid dipterans, LEP = butterflies and moths (lepidopterans), HONEYBEE = honey (social) bees, SOLBEE = solitary bees, SYRPHID = syrphid flies, WASP = non-*Vespula* wasps. Lower level: BIDENS = *B. menziesii*, DUBAUT = *D. linearis*, HAPLO = *H. haplostachya*, SIDA = *S. fallax*, SILENE = *S. lanceolata*, STEN = *S. angustifolia*, and TETRA = *T. arenarium.*

*Predator frequencies at PTA*

Frequencies of our target NIPs at PTA varied by experimental block (Figure 30). Yellowjacket frequencies in particular generally displayed higher frequencies in woodland blocks (3 and 4) than in grassland/shrubland blocks (1 and 2) for all treatment types. Ant frequencies demonstrated the opposite trend in that they generally displayed higher frequencies in grassland/shrubland blocks (1 and 2) than in woodland blocks (3 and 4). Both rats and ants demonstrated a clear treatment effect (Figure 30).

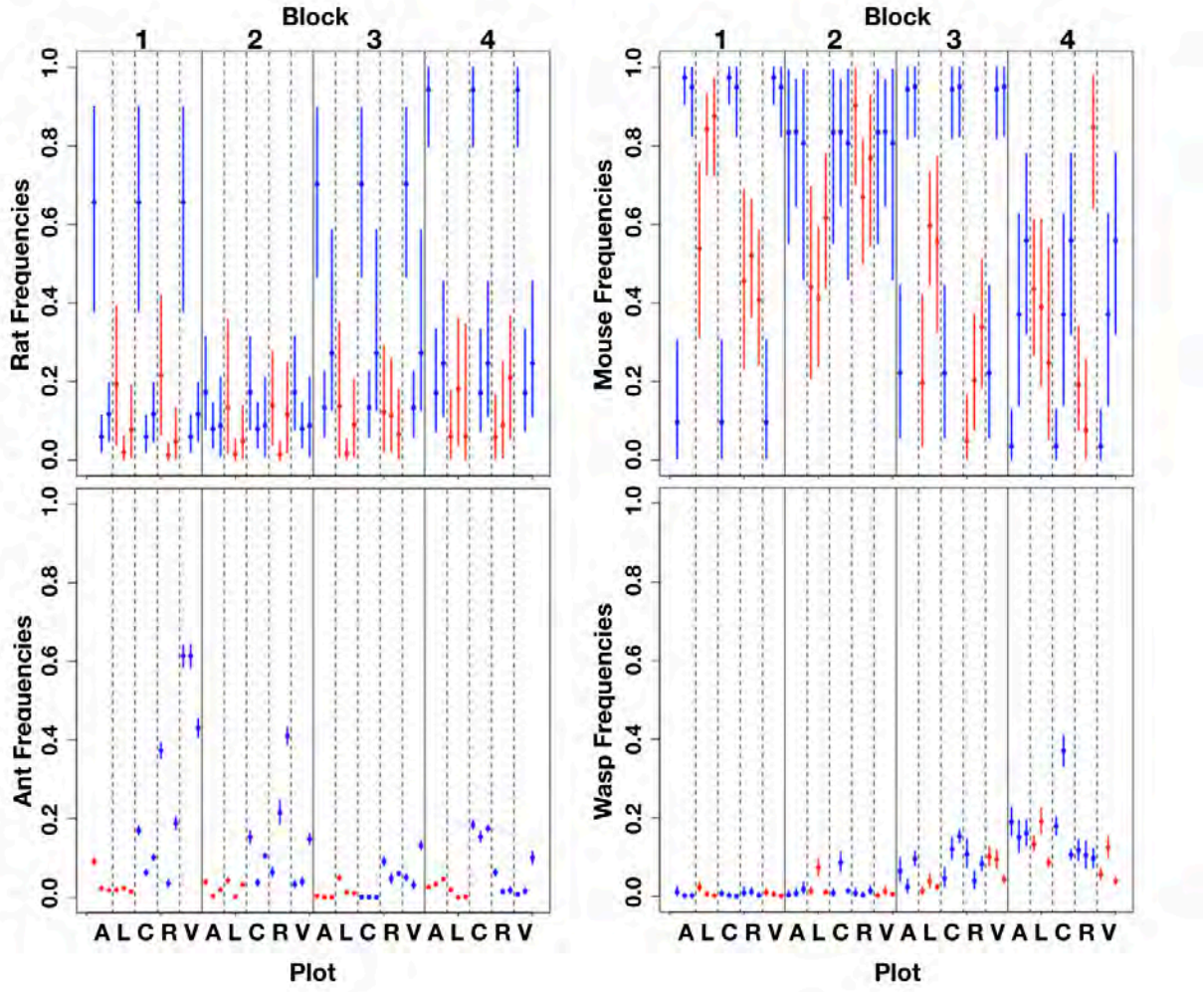


Figure 30. NIP frequencies across all blocks (labeled at the top), plots (labeled at the bottom), and time periods (within each block/plot combination: left = August 2016–January 2017, middle = February–June 2017, right = July–November 2017). Red represents treatment plots (Rodent and All plots for rats, top left, and mice, top right; Ant and All plots for ants, bottom left; Yellowjacket and All plots for yellowjackets, bottom right), and blue represents non-treatment plots, including Control plots. Ant plots are labeled with "A", All plots are labeled with "L", Control plots are labeled with "C", Rodent plots are labeled with "R", and Yellowjacket plots are labeled with "V." Dots represent mean frequencies and bars represent 95% credible intervals around the mean. Credible intervals for ant and yellowjacket data are smaller than rodent data

because rodent frequency at each plot was estimated based on a single data point but ant and yellowjacket frequency at each plot were both estimated based on multiple data points (monitoring stations).

*NIP-Pollinator frequency relationships by individual plant species at PTA*

The SHELOB model analyzed relationships between NIP frequencies and pollinator frequencies for six of our eight focal plant species. *Argemone glauca* and *S. angustifolia* were excluded from analysis due to no presence in the treatment plots and very low flower visitation, respectively.

We found significant negative relationships between the frequency of NIPs and the frequency of flower visitation by at least one of the six pollinator species groups for four of the six plant species (Figure 31). We found negative relationships between NIPs and nearly every pollinator species group for *H. haplostachya, S. fallax,* and *T. arenarium*. Solitary bees had a negative relationship with ants on one of the six plant species, honey bees with ants on one of the six plant species, moths and butterflies with rats on two of the plant species, non-syrphid flies with rats on three of the six plant species, syrphid flies with mice on two of the six plant species, and non-Vespula wasps with rats on two of the six plant species. There was at least one negative relationship between rats and one of the pollinator groups for *H. haplostachya, S. fallax,* and *T. arenarium*. There was at least one negative relationship with ants and at least one of the pollinator groups for *S. fallax* and *T. arenarium*.

| | Bidens | Dubautia | Haplostachys | Sida | Silene | Tetramolopium |
|---|---|---|---|---|---|---|
| Solitary Bees | ns | ns | Mice (-) | Wasps (-) | ns | Ants (-) |
| Honey Bees | ns | ns | ns | Ants (-)<br>Wasps (+) | | Mice (+) |
| Moths & Butterflies | ns | ns | Rats (-) | ns | ns | Rats (-) |
| Non-syrphid flies | ns | ns | Rats (-) | Rats (-) | | Rats (-)<br>Ants (-) |
| Syrphid flies | Mice (-) | ns | ns | Mice (-)<br>Wasps (-) | ns | ns |
| Non-*Vespula* wasps | Wasps (-) | Mice (+) | Rats (-) | Rats (-) | | Ants (-) |

Figure 31. NIP-Pollinator frequency relationships by plant species. Relationships between NIPs and flower visitation. Significant relationships between NIPs and flower visitation by a given pollinator species group on a given plant species are denoted with the name of the NIP and

direction is denoted in parentheses. Negative relationships are in red font, and positive relationships are in blue font. NS = No significant relationship between NIPs and flower visitation on a given plant species by a given pollinator species group. Dark squares = No pollinator events recorded between the pollinator species group and plant species during the experimental treatment period. Bidens = *B. menziesii*, Dubautia = *D. linearis*, Haplostachys = *H. haplostachya*, Sida = *S. fallax*, Silene = *S. lanceolata*, and Tetramolopium = *T. arenarium*.

Across all 36 possible interactions between pollinator groups and plant species, rats had a negative relationship with 7 of the interactions, ants with 4 of them, yellowjackets with 3 of them (and a positive relationship with 1 of them), and mice with 3 of them (and a positive relationship with 2 of them). Our results suggest that reducing populations of rats, the NIP with the most negative associations with pollinator species groups across all plant species, would increase the pollinator frequency of specific groups on three of the six focal plant species that we analyzed, including the endangered *H. haplostachya* and *T. arenarium*. Reducing populations of ants, the NIP with the second most negative associations with pollinator species groups across all plant species, would increase the pollinator frequency of specific groups on two of the six focal plant species, including the endangered *T. arenarium*. Previous work has suggested that Argentine ants at Haleakala negatively impact native *Hylaeus* bees (Cole et al. 1992). Our results are consistent with this in that we found a negative relationship between ant activity and visitation rates of solitary bees to *T. arenarium*.

We calculated effect sizes for the relationships between pollination interactions and NIP (Figure 32). Effect sizes in this case quantify how much we expect the pollinator visitation frequency to increase (assuming a negative relationship between NIP and pollinator group) if we were to decrease the NIP frequency from 0.6 to 0. NIP frequency of 0.6 was the untreated average frequency for mice and ants in our experimental plots (rats were 0.3 and wasps were 0.5). NIP frequency of 0 would represent eradication of the predator species. The increases in pollinator visitation frequency when lowering NIP frequency from 0.6 to 0 were positive but uncertain (95% CI: <0.01, >0.90 for all pollinator groups) for many plant species-pollinator interactions: mice on solitary bees visiting *H. haplostachya*, rats on moths and butterflies visiting *H. haplostachya* and *T. arenarium*, rats on non-syrphid flies visiting *S. fallax* and *T. arenarium*, and ants on non-*Vespula* wasps visiting *T. arenarium*. Eradication of mice, rats, and ants could produce up to a >90% increase in pollinator visitation of the specified pollinators for the specified plant species, although effects could be much smaller. Mean increases in non-syrphid flies and honey bee pollination of *S. fallax* with rat and ant eradication, respectively, were predicted to be notably larger than any other positive effect sizes.

Other NIP eradications were predicted to have a smaller maximum increase in pollination. The effect of eradicating ants on solitary bees visiting *T. arenarium* (95% CI: <0.01, 0.45), eradicating rats and ants on non-syrphid flies visiting *H. haplostachya* (95% CI: <0.01, 0.54) and *T. arenarium* (95% CI: <0.01, 0.49), respectively, and eradicating yellowjackets on non-*Vespula* wasps visiting *B. menziesii* (95% CI: <0.01, 0.45) could all produce up to an ~50% increase in these pollinator visitations. The effect of NIP eradications on other plant-pollinator interactions had a smaller maximum effect: mice on syrphid flies visiting *B. menziesii* and *S. fallax*, rats on non-*Vespula* wasps visiting *H. haplostachya* and *S. fallax*, and yellowjackets on solitary bees and syrphid flies visiting *S. fallax*.

Negative effect sizes, where decreasing a NIP was predicted to result in a decrease in pollinator visitation frequency, were uncertain but could decrease pollinator visitation up to

>90% (95% CI: >-0.01, <-0.90 for all pollinator groups) for three plant species-pollinator interactions: mice on non-*Vespula* wasps visiting *D. linearis*, yellowjackets on honey bees visiting *S. fallax*, and mice on honey bees visiting *T. arenarium*. The largest mean negative effect size was the reduction in honey bee pollination of *S. fallax* predicted to occur with the eradication of yellowjackets.

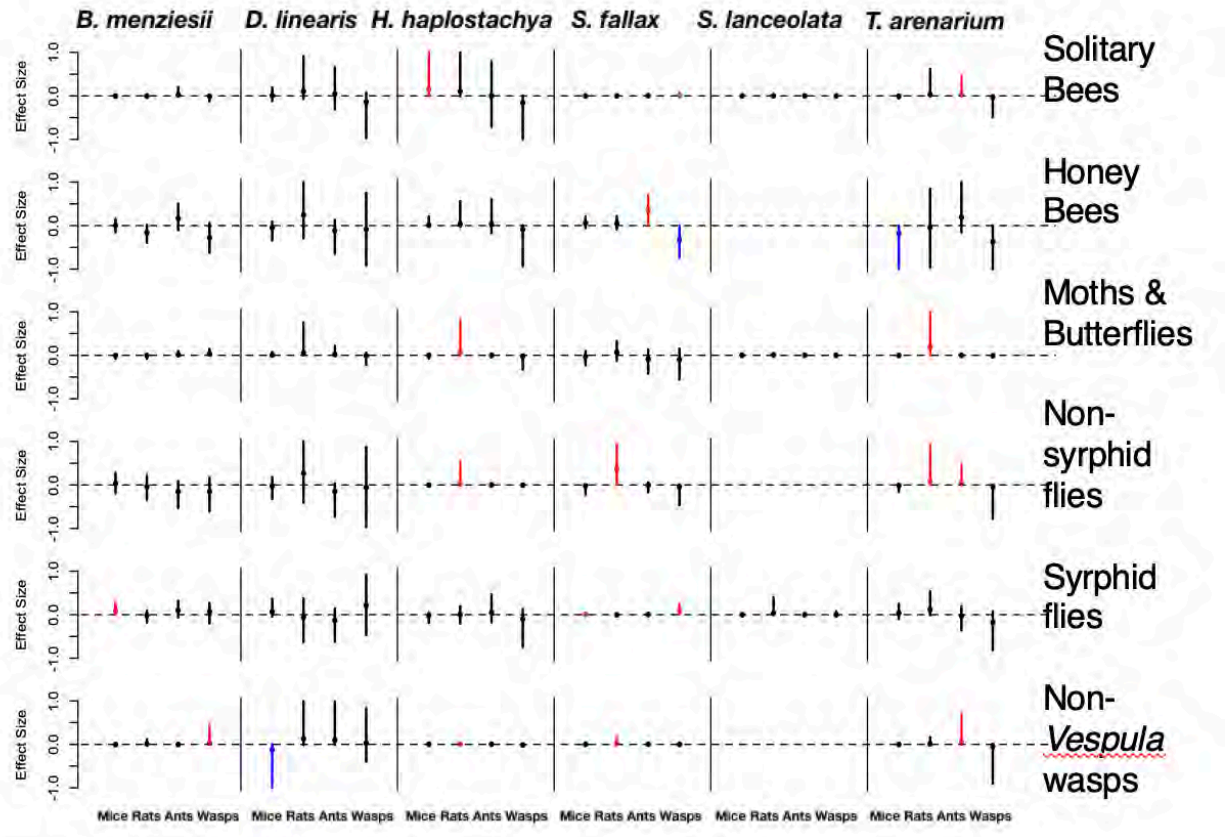


Figure 32. Effect sizes for the relationships between NIPs and pollination interactions between pollinator (row) and plant (column) species. Effect sizes were calculated as the change in pollinator frequency on a given plant species when a NIP frequency was reduced from 0.6 to 0. Positive effect sizes are increases in pollinator visitation frequency with the reduced NIP frequency, given a negative relationship (red) between NIPs and pollinators. Negative effect sizes are decreases in pollinator visitation frequency with the reduced NIP frequency, given a positive relationship (blue) between NIPs and pollinator. Effect sizes were calculated by back-transforming the regression equations for each pollinator group, using the posterior distributions as intercept and slope estimates, and holding the non-target NIPs constant at a frequency of 0.5 (the untreated average across all NIPs). Dots denote mean effect sizes and associated bars are the 95% confidence interval around the mean.

We multiplied the mean effect size for each NIP on each pollinator-plant species combination by the calculated PVI for that pollinator-plant species combination to get an index from 0 to 1 of how much impact the eradication of an NIP would have on the reproduction of each plant species (Table 1). The two highest index values are for ants on (honey bees visiting)

*S. fallax* and rats on (moths and butterflies visiting) *T. arenarium*. This indicates that reducing rats would have the greatest effect on T. arenarium reproduction and reducing ants would have the greatest effect on S. fallax reproduction.

Table 1. Effect size of NIP on pollinator visitation frequency on each plant species multiplied by PVI. Significant negative relationships are bolded and in red font. Values for negative relationships are positive because the effect size is calculated as the mean increase in frequency of pollinator visitation on a given plant species, if NIP frequencies were reduced from 0.6 to 0. Bidens = *B. menziesii*, Dubautia = *D. linearis*, Haplostachys = *H. haplostachya*, Sida = *S. fallax*, Silene = *S. lanceolata*, and Tetramolopium = *T. arenarium*

| | | Bidens | Dubautia | Haplostachys | Sida | Silene | Tetramolopium |
|---|---|---|---|---|---|---|---|
| Solitary Bees | Mice | 0.000 | 0.000 | **0.001** | 0.000 | 0.000 | -0.001 |
| | Rats | 0.000 | 0.004 | 0.000 | 0.000 | 0.001 | 0.012 |
| | Ants | 0.009 | 0.002 | 0.000 | 0.000 | 0.000 | **0.024** |
| | Wasps | -0.001 | -0.003 | 0.000 | **0.003** | 0.000 | -0.005 |
| Honey Bees | Mice | 0.002 | -0.027 | 0.011 | 0.085 | | **-0.192** |
| | Rats | -0.132 | 0.141 | 0.036 | 0.067 | | -0.035 |
| | Ants | 0.137 | -0.075 | 0.041 | **0.479** | | 0.280 |
| | Wasps | -0.222 | -0.049 | -0.060 | **-0.367** | | -0.353 |
| Moths & Butterflies | Mice | -0.002 | 0.000 | -0.001 | -0.002 | 0.000 | -0.004 |
| | Rats | -0.002 | 0.005 | **0.018** | 0.005 | 0.004 | **0.458** |
| | Ants | 0.005 | 0.002 | 0.000 | -0.005 | 0.001 | -0.004 |
| | Wasps | 0.022 | 0.000 | -0.003 | -0.006 | 0.000 | -0.010 |
| Non-syrphid flies | Mice | 0.008 | -0.001 | 0.000 | -0.002 | | -0.005 |
| | Rats | -0.010 | 0.012 | **0.009** | **0.044** | | **0.063** |
| | Ants | -0.035 | -0.007 | 0.001 | -0.001 | | **0.044** |
| | Wasps | -0.033 | -0.001 | -0.001 | -0.003 | | -0.017 |
| Syrphid flies | Mice | **0.118** | 0.093 | -0.025 | **0.002** | -0.004 | 0.038 |
| | Rats | -0.019 | -0.070 | -0.002 | -0.001 | 0.079 | 0.136 |
| | Ants | 0.133 | -0.181 | 0.084 | 0.001 | 0.001 | -0.030 |
| | Wasps | 0.112 | 0.271 | -0.104 | **0.059** | 0.030 | -0.179 |
| Non-*Vespula* wasps | Mice | -0.004 | **-0.002** | 0.000 | 0.000 | | -0.001 |
| | Rats | 0.103 | 0.004 | **0.000** | **0.033** | | 0.014 |
| | Ants | -0.002 | 0.002 | 0.000 | 0.001 | | **0.082** |
| | Wasps | **0.074** | 0.001 | 0.000 | 0.000 | | -0.026 |

*Stenogyne angustifolia*, one of the four federally endangered plant species within our study, only had observed pollination events in plots with either experimentally lowered or naturally very low ant frequencies. The species only had four pollination events within the experimental treatment period, one each by a syrphid fly, solitary bee, homoptera, and hemiptera, so any interpretation of these data should be treated with caution. However, although there is no possible statistical analysis for this species, these pollination events for *S. angustifolia* only occurred when ant frequencies were low, which tentatively suggests that reducing ant populations may increase pollination. In fact, the presence of Argentine ants may be reducing pollination by one of the observed visitors, solitary (*Hylaeus*) bees (Cole et al. 1992). In the pollination effectiveness experiments, *S. angustifolia* was highly self-incompatible and highly pollen limited. Therefore, reducing ant populations, if it increases pollination, would increase the reproductive success of this endangered species.

*NIP-Pollinator frequency relationships across plant species combined*

NIP effects on pollinator groups across all six plant species combined had high levels of variation, reflected in large confidence intervals around the mean relationship. Because of this high level of variation, confidence intervals overlapped zero for all NIP-pollinator interactions with one exception: a positive relationship between yellowjackets and honey bees (Figure 33). This result shows the importance of taking a species-by-species approach for plants when considering the effects of predators, as the effects may not be uniform. A community-based approach would potentially mask any significant relationships between NIPs and pollinators.

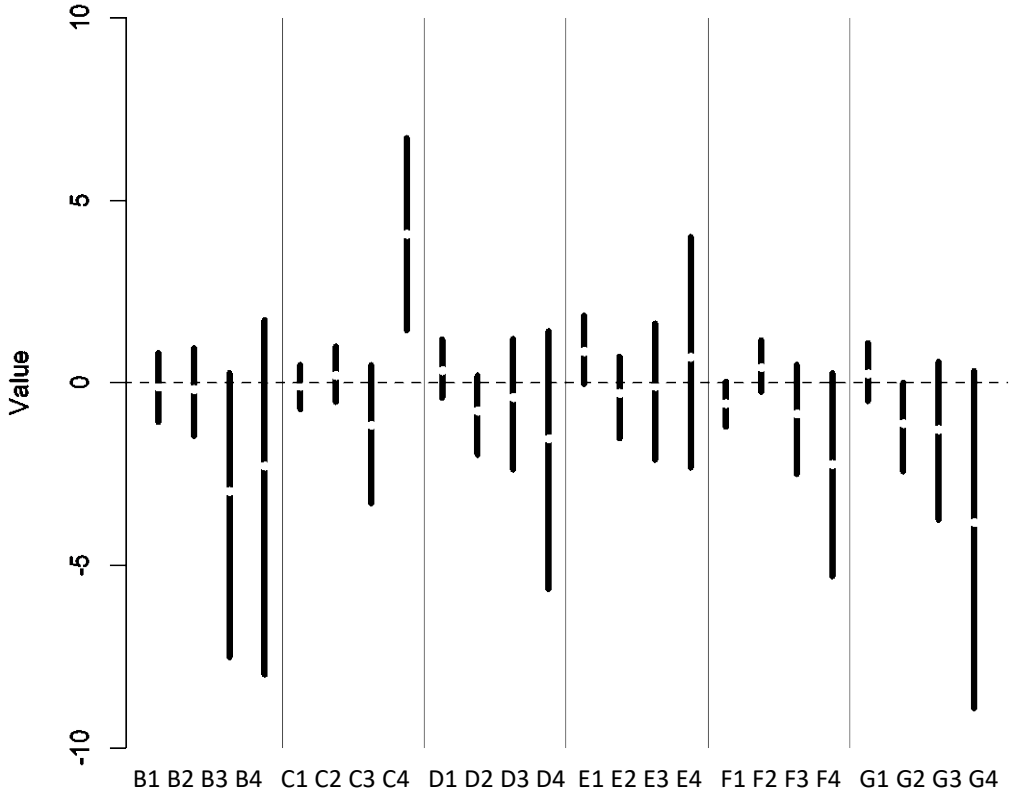


Figure 33. Mean (white dot) and 95% credible intervals of the slope values for the effects of NIP on pollinator species, across all plant species. B slope values are the effects of NIPs on solitary bees, C slope values are the effects of NIPs on honey bees, D slope values are the effects of NIP on Lepidopterans, E slope values are the effects of NIP on non-Syrphid Dipterans, F slope values are the effects of NIPs on Syrphids, and G slope values are the effects of NIP on non-*Vespula* wasps. NIPs are coded as follows: 1 = mice, 2 = rats, 3 = ants, 4 = yellowjackets.

## Conclusions

We investigated the relationship between NIPs, insect pollinators, and native plants (both endangered and common species) at PTA. We identified the direct interaction between insect flower visitor species and focal native plant species through flower visitation observations (Technical Objective 1). We confirmed the direct interaction between the study site NIPs and insect flower visitors through diet analyses (Technical Objective 2). Through NIP suppression, we analyzed the indirect effect of NIPs on plant pollination and found significant negative relationships between NIPs and pollinators (Technical Objective 3).

From our study, there are several management relevant results that we want to highlight. First, current pollination differs for the endangered versus common plant species in our study system. Endangered species have fewer flower visitor species than common species, and more of the endangered species are pollen limited. For most of the endangered species, additional pollen and outcrossing improves seed set (Fig. 13). Taken together, this indicates that pollinator management and conservation are important for endangered plant species.

Second, pollinator interactions are localized in time and space, and can vary by time of year. Pollinator observations are time- and labor-intensive, but to capture the spatial and temporal fluctuations, particularly for endangered species that are nearly or entirely self-incompatible, it may be necessary to perform observations over repeated seasons and across multiple locations. Pollinator networks during the experimental treatment period also differed from those derived from wild plant observations, with most plant species exhibiting a different 'most important pollinator species' in the experimental treatment period than during the wild plant observations. One explanation for this finding is that pollinator importance may be strongly tied to local conditions, and also vary in time or space.

Third, NIPs can affect the pollinator-plant interaction, and in most cases negatively. Rats and ants had only negative associations with pollinator species groups, as well as the greatest number of negative associations, including for the endangered *H. haplostachya* and *T. arenarium*. Controlling rats and ants likely would increase pollinator frequency, and potentially lead to more out-crossing for plant species.

Finally, predator control treatments can be moderately effective at suppressing predator abundance or activity; however, the treatments require continuous effort. Black rat but not house mouse can be effectively suppressed using snap-traps, although again it takes continuous effort. Formicidal bait can be used to suppress Argentine ants, although effects are short-lived. Yellowjackets can be suppressed by fipronil, but abundances may still be above an acceptable threshold. Overall, there is temporal and spatial variation in predator dynamics that must be taken into account when undertaking suppression.

PTA land managers, specifically the Natural Resources Office staff, were regularly updated on our research by way of field activity reports (approximately every 2 to 4 weeks). Our project was included under External Stakeholders in the draft 2017 PTA INRMP, which states that "The findings will guide future land management decisions involving invasive predators at PTA." As part of our technical transfer, we created a User's Guide which includes options for

identifying insect visitation to flowering plants and determining plant breeding systems; a brief guide to common insect pollinator taxa in dryland Hawai‘i; efficacy of predator suppression methods, cost analyses, and alternative suppression methods; comparison of diet analysis methods; propagation of our focal plant species in the greenhouse; and results from our research study. We provided a workshop based on the User’s Guide in August 2019 to PTA NRO staff as well as to other land managers and interested parties.

## Literature Cited


Aizen, M.A., and L. D. Harder. 2007. Expanding the limits of the pollen-limitation concept: effects of pollen quantity and quality. Ecology 88:271-281.

Albrecht, M., B. Schmid, Y. Hautier, and C. B. Müller. 2012. Diverse pollinator communities enhance plant reproductive success. Proceedings of the Royal Society B 279:4845–4852.

Armbruster, P., and D. H. Reed. 2005. Inbreeding depression in benign and stressful environments. Heredity 95:235-242.

Anderson, S. H. 2003. The relative importance of birds and insects as pollinators of the New Zealand flora. New Zealand Journal of Ecology 27:83–94.

Aslan, C. E., E. S. Zavaleta, B. Tershy, D. Croll, and R. H. Robichaux. 2013. Imperfect replacement of native species by non-native species as pollinators of endemic Hawaiian plants. Conservation Biology 28:478-488.

Aslan, A., P. Hart, J. Wu, and C. E. Aslan. 2014. Evaluating the qualitative effectiveness of a novel pollinator: a case study of two endemic Hawaiian plants. Biotropica 46:732–739.

Aslan, C. E. 2015. Pollination of the endangered Arizona hedgehog cactus (*Echinocereus arizonicus*). The American Midland Naturalist 173:61–72.

Aslan, C. E., C. T. Liang, B. Galindo, K. Hall, and W. Topete. 2016. The role of honey bees as pollinators in natural areas. Natural Areas Journal 36:478–488.

Baker, H. G. 1955. Self-compatibility and establishment after "long-distance" dispersal. Evolution 9:347–349.

Banko, P.C., P.T. Oboyski, J.W. Slotterback, S.J. Dougill, D.M. Goltz, L. Johnson, M.E. Laut, and T.C. Miller. 2002. Availability of food resources, distribution of invasive species, and conservation of a Hawaiian bird along a gradient of elevation. Journal of Biogeography 29: 789-808.

Carmo, R. M., E. V. Franceschinelli, and F. A. Silveira. 2004. Introduced honeybees (*Apis mellifera*) reduce pollination success without affecting the floral resource taken by native pollinators. Biotropica 36:371–376.

Cayuela, L., S. Ruiz-Arriaga, and C. P. Ozers. 2011. Honeybees increase fruit set in native plant species important for wildlife conservation. Environmental Management 48:910–919.

Celebrezze, T., and D. C. Paton. 2004. Do introduced honeybees (*Apis mellifera*, Hymenoptera) provide full pollination service to bird-adapted Australian plants with small flowers? An experimental study of *Brachyloma ericoides* (Epacridaceae). Austral Ecology 29:129–136.

Cole, F. R., A. C. Medeiros, and L. L. Loope. 1992. Effects of the Argentine ant on arthropod fauna of Hawaiian high-elevation shrubland. Ecology 73: 1313-1322.

Cole, F.R., L.L. Loope, A.C. Medeiros, J.A. Raikes, and C.S. Wood. 1995. Conservation implications of introduced game birds in high-elevation Hawaiian shrubland. Conservation Biology 9: 306-313.

Dohzono, I., and J. Yokoyama. 2010. Impacts of alien bees on native plant-pollinator relationships: a review with special emphasis on plant reproduction. Applied Entomology and Zoology 45:37–47.

Dowding, J.E., and E.C. Murphy. 1994. Ecology of ship rats (*Rattus rattus*) in a kauri (*Agathis australis)* forest of Northland, New Zealand. New Zealand Journal of Ecology 18:19-27.

Fitzgerald, B.M., M.J. Daniel, A.E. Fitzgerald, B.J. Karl, M.J. Meads, and P.R. Notman. 1996. Factors contributing to fluctuations in house mice (*Mus musculus*) populations in hard beech (*Northofagus truncata*) forest. Journal of the Royal Society of New Zealand 26:237-249.

Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3:294-299.

Foote, D., C. Hanna, C. King, and E. B. Spurr. 2011. Efficacy of fipronil for suppression of invasive yellowjacket wasps in Hawaii Volcanoes National Park. Technical report HCSU-028, Hawaii Cooperative Studies Unit, University of Hawaii at Hilo, Hilo, HI, 20 pp.

Gambino, P. 1992. Yellowjacket (*Vespula pensylvanica*) predation at Hawaii Volcanoes and Haleakala National Parks: Identity of prey items. Proceedings of the Hawaiian Entomological Society 31:157-164.

Gambino, P. and L. Loope. 1992. Yellowjacket (*Vespula pensylvanica*) biology and abatement in the National Parks of Hawaii. University of Hawaii Cooperative National Park Resources Studies Unit Technical Report:i-v, 41 pp.

Gelman, A., and D. B. Rubin. 1992. Inference from Iterative Simulation Using Multiple Sequences. Statistical Science 7:457–472.

Hanna, C., D. Foote, and C. Kremen. 2013. Invasive species management restores a plant–pollinator mutualism in Hawaii. Journal of Applied Ecology 50:147–155.

Harper, G.A. 2010. Habitat use by mice during winter on subantarctic Auckland Island. New Zealand Journal of Ecology 34:262-264.

Herrera, C.M. 1987. Vertebrate-dispersed plants of the Iberian Peninsula: a study of fruit characteristics. Ecological Monographs 57:305-331.

Johnson, S. D., and K. E. Steiner. 2000. Generalization versus specialization in plant pollination systems. Trends in Ecology and Evolution 15:140–143.

King, C.M., J.G. Innes, M. Flux, and M.O. Kimberley. 1996. Population biology of small mammals in Pureora Forest Park: 2. The feral house mouse (*Mus musculus*). New Zealand Journal of Ecology 20:253-269.

Krushelnycky, P.D., W. Haines, L. Loope and E. Van Gelder. 2011. The Haleakala Argentine ant project: a synthesis of past research and prospects for the future. Pacific Cooperative Studies Unit Technical Report 173. University of Hawai‘i at Mānoa, Department of Botany. Honolulu, HI. 127 pp.

Larson, B. M., and S. C. Barrett. 2000. A comparative analysis of pollen limitation in flowering plants. Biological Journal of the Linnean Society 69:503–520.

Martins, A. C., R. B. Gonçalves, and G. A. Melo. 2013. Changes in wild bee fauna of a grassland in Brazil reveal negative effects associated with growing urbanization during the last 40 years. Zoologia (Curitiba) 30:157–176.

Mostello, C. S., and S. Conant. 2018. Diets of native and introduced apex predators in Hawai‘i. Pacific Conservation Biology 24:25-34.

Pender, R. J., A. B. Shiels, L. Bialic-Murphy, and S. M. Mosher. 2013. Large-scale rodent control reduces pre- and post-dispersal seed predation of the endangered Hawaiian lobeliad, *Cyanea superba* subsp. *superba* (Campanulaceae). Biological Invasions 15:213–223.

Plummer, M. 2003. JAGS: A program for analysis of Bayesian graphical models using Gibbs sampling. Proceedings of the 3rd International Workshop on Distributed Statistical Computing (DSC 2003) March, pp. 20–22.

Plummer, M., A. Stukalov, and M. Denwood. 2018. Rjags: Bayesian graphical models using MCMC. R package version 4-8. https://CRAN.R-project.org/package=rjags.

R Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/

Renne, I. J., S. A. Gauthreaux, Jr, and C. A. Gresham. 2000. Seed dispersal of the Chinese tallow tree (*Sapium sebiferum* (L.) Roxb.) by birds in coastal South Carolina. The American Midland Naturalist 144:202–215.

Schemske, D. W., and H. D. Bradshaw. 1999. Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). Proceedings of the National Academy of Sciences 96:11910–11915.

Shiels, A. B. 2010. Ecology and impacts of introduced rodents (*Rattus* spp. and *Mus musculus*) in the Hawaiian islands. PhD Dissertation, University of Hawaii-Manoa.

Shiels, A. B., C. A. Flores, A. Khamsing, P. D. Krushelnycky, S. M. Mosher, and D. R. Drake. 2013. Dietary niche differentiation among three species of invasive rodents (*Rattus rattus*, *R. exulans*, *Mus musculus*). Biological Invasions 15:1037-1048.

Shiels, A.B., A.C. Medeiros, E.I. von Allmen. 2017. Shifts in an invasive rodent community favoring black rats (*Rattus rattus*) following restoration of a native forest. Restoration Ecology 25: 759-767.

Shiels, A.B., T. Bogardus, J. Rohrer, and K. Kawelo. 2019. Effectiveness of snap and A24-automated traps, and broadcast anticoagulant bait, in suppressing commensal rodents in Hawaii. Human-Wildlife Interactions 13:226-237.

Singleton, G.R. 1989. Population dynamics of an outbreak of house mice (*Mus domesticus*) in the mallee wheatlands of Australia—a hypothesis of plague formation. Journal of Zoology 219:495-515.

Su, Y.-S., and M. Yajima. 2015. R2jags: Using R to Run 'JAGS'. R package version 0.5-7. https://CRAN.R-project.org/package=R2jags.

Sugihara, R.T. 1997. Abundance and diets of rats in two native Hawaiian forests. Pacific Science 51:189-198.

Thompson, J. D. 2001. How do visitation patterns vary among pollinators in relation to floral display and floral design in a generalist pollination system? Oecologia 126:386–394.

Wilson, D.J., M.G. Efford, S.J. Brown, J.F. Williamson, and G.J. McElrea. 2007. Estimating density of ship rats in New Zealand forests by capture-mark-recapture trapping. New Zealand Journal of Ecology 31:47-59.

Wilson, E. E., L. M. Mullen, and D. A. Holway. 2009. Life history plasticity magnifies the ecological effects of a social wasp invasion. Proceedings of the National Academy of Sciences of the United States of America 106:12809-12813.

Wood, S. N. 2010. Statistical inference for noisy nonlinear ecological dynamic systems. Nature 466:1102–1104.

Young, H. J., and T. P. Young. 1992. Alternative outcomes of natural and experimental high pollen loads. Ecology 73: 639–647.

Young, L.C., E. A. VanderWerf, M.T. Lohr, C.J. Miller, A.J. Titmus, D. Peters, and L. Wilson. 2013. Multi-species predator eradication within a predator-proof fence at Ka'ena Point, Hawai'i. Biological Invasions 12:2627–2638.

Zapata, T.R., and M. T. K. Arroyo. 1978. Plant reproductive ecology of a secondary deciduous tropical forest in Venezuela. Biotropica 10:221-230.