User's Guide

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

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14. ABSTRACT
This User's Guide shares findings from a four-year research study (2015 -2019) titled "The Impact of Non-Native Predators on Pollinators and Native Plant Reproduction in a Hawaiian Dryland Ecosystem." The guide provides information to land and natural resource managers on: i) pollination of native plants by native and non-native insects, ii) interactions among non-native invasive predators and insect pollination of native plants, and iii) methods and experimental designs for measuring plant-pollinator interactions and predator suppression.

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I. Introduction and Purpose of Guide

Our purpose in composing this User's Guide is to provide information to land and natural resource managers on: i) pollination of native plants by native and non-native insects, ii) interactions among non-native invasive predators (NIPs) and insect pollination of native plants, and iii) methods and experimental designs for measuring plant-pollinator interactions and NIP suppression. We share findings from our four-year research study (2015–2019) titled "The Impact of Non-Native Predators on Pollinators and Native Plant Reproduction in a Hawaiian Dryland Ecosystem," conducted at U.S. Army Pōhakuloa Training Area (PTA) on Hawai'i island. We aim to inform current and future management efforts in Hawai'i, as well as in other similar systems, that are concerned with invasive predators and their potential impacts to pollination services. In this User's Guide, we provide guidance for:

a. Determining insect visitation to target flowering plant species and assessing effective pollination (i.e., contact with flower reproductive parts, identification of pollen from captured insects).
b. Determining the breeding systems of target plants (i.e., extent a plant species relies on pollination versus self-fertilization).
c. Guide to common insect flower visitors.
d. General monitoring of arthropod communities.
e. Measuring NIP abundance/activity before and after experimental treatments.
f. Suppressing NIPs using traps and toxicants.
g. Determining NIP diets.
h. Propagating our focal plant species in the greenhouse.
i. Modeling the direct and indirect interactions between native plants, insect pollinators, and NIPs.
j. Managing for resilience.

Each main section in the User's Guide provides a summary and key findings ("In a Nutshell"), followed by further details on background information for the topic, general methods, and results and conclusions from our study.

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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 User's Guide is 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.

Funding for this project was awarded by the US Department of Defense's Strategic Environmental Research and Development Program (SERDP, project RC-2432). Due to our presentation of this Guide occurring prior to final data analysis, some interpretations and recommendations may change and therefore the reader is encouraged to request updates from the authors and also refer to the Final Report available at the SERDP website.

II. PTA Study Area, Focal Species, and Experimental Design

Study area

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 (*Festuca hawaiiensis*, *Isodendrion hosakae*, *Kadua coriacea*, *Schiedea hawaiiensis*, and *Tetramolopium arenarium*) occur exclusively in PTA (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, and the reproductive success of at-risk plant species under existing levels of NIP invasion is unknown.

Our field work at PTA occurred in Kīpuka Kālawamauna East (KKE), a 794-hectare mix of grassland-shrubland and *Metrosideros polymorpha* (‘ōhi‘a) dominated woodland located at approximately 1,675 m in elevation. This unit is fenced to exclude non-native invasive ungulates (primarily goats and sheep) and contains several endangered plant species, giving it high conservation value.

Native plant species

We focused on eight native plant species found within or adjacent to KKE. Four of the species are listed as federally endangered (permission to conduct research obtained through US Fish and Wildlife Service Recovery Permit number TE28360B-0; Hawai‘i Department of Land and Natural Resources Permit for Threatened and Endangered Plant Species number P-201):

Haplostachys haplostachya, honohono, Lamiaceae
Silene lanceolata, (no common name), Caryophyllaceae
Stenogyne angustifolia, (no common name), Lamiaceae
Tetramolopium arenarium, (no common name), Asteraceae

The other four species are common throughout PTA and dryland habitats:

Argemone glauca, pua kala, Hawaiian prickly poppy, Papaveraceae
Bidens menziesii, ko‘oko‘olau, Asteraceae
Dubautia linearis, kūpaoa, Asteraceae
Sida fallax, ‘ilima, Malvaceae

Non-native invasive predators

Our target predator species in KKE were:

Linepithema humile, Argentine ant, Formicidae
Mus musculus, house mouse, Muridae
Rattus rattus, black rat, Muridae
Vespula pensylvanica, yellowjacket wasp, Vespidae.

Experimental design

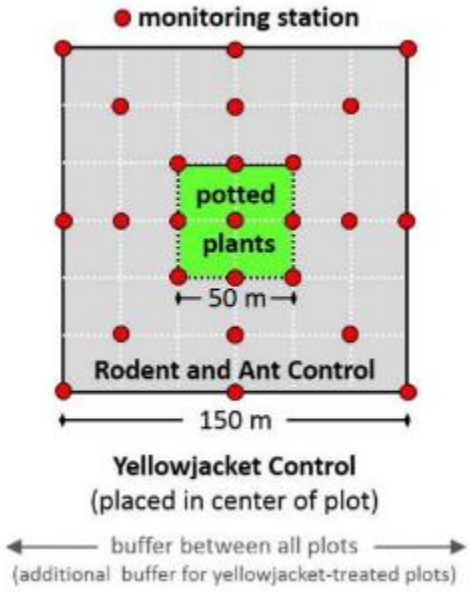

Figure 1. Experimental plot layout.

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 ant/rodent/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 within 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 these stations within the plots.

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 before and after treatments in treatment and control plots to assess the efficacy of treatments. Treatment methods and results are discussed in the predator-specific sections in this User's Guide.

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 area and the rodent, ant, and 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 predators 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. We found two other ant species in KKE, the big-headed ant (*Pheidole megacephala*) and *Cardiocondyla* cf. *venustula* (no common name), but they were found only in low numbers in localized areas within the fenced unit.

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). 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 treatment plots and a second randomization for the Rodent, Ant, and Control treatment plots (Figure 2). The double randomization was performed to account for the differences in buffer distances between plots.

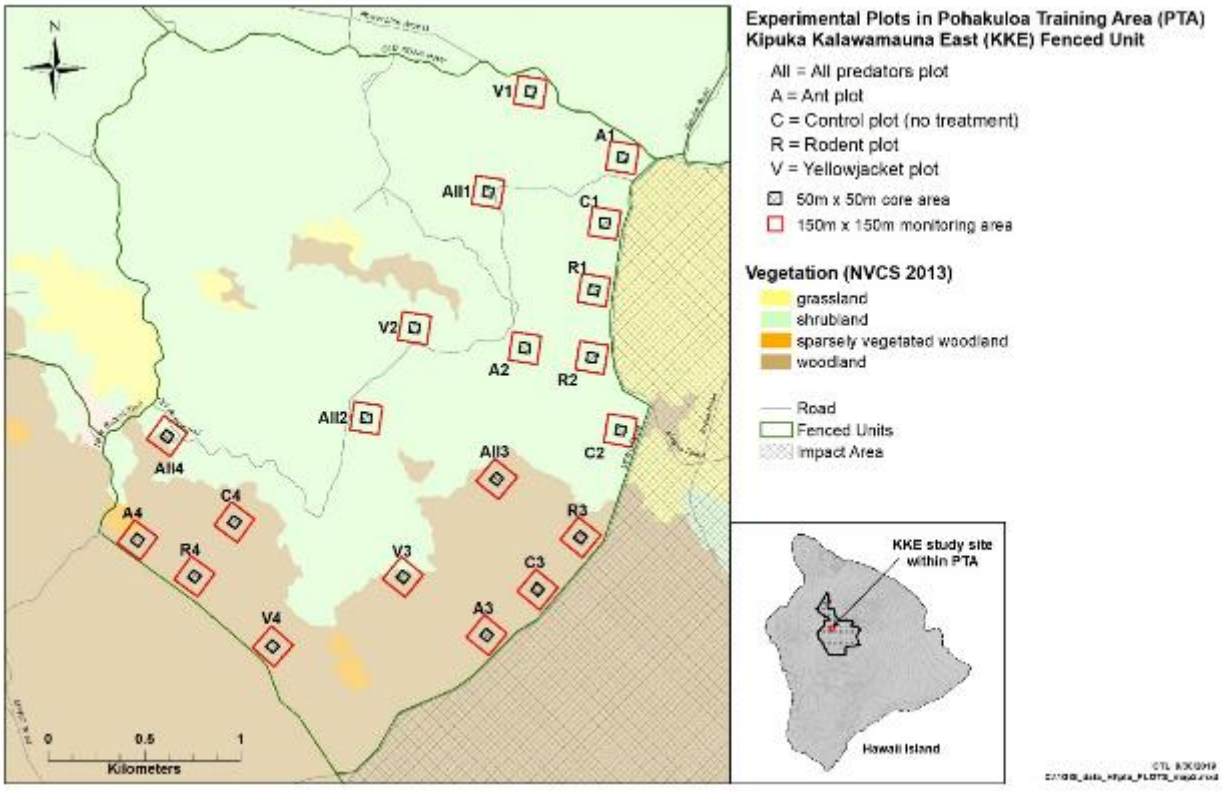

Figure 2. Experimental plots within Kīpuka Kālawamauna East fenced unit at PTA.

References:

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.

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.

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.

III. Flower Visitation Observations

In a Nutshell:

- Pollination is a highly idiosyncratic process, requiring research methods that are specifically tailored to the individual plant species.
- We used systematic observations to confirm and quantify flower visitation to our focal plant species at PTA.
- Out of all recorded flower visits, 85% were performed by non-native species, particularly the honeybee (*Apis mellifera*) and flies in the family Syrphidae.
- Endangered plant species interacted with a lower diversity of flower visitors than did common plant species.
- The flower visitor community in this system, although heavily dominated by non-native insects, appears to be interacting with multiple plant species, suggesting that non-native insects may thus be sustaining biotic interactions otherwise threatened with disruption in this island ecosystem.

General background

Identifying pollinators of target plants in any given system

Pollination is highly variable and can be difficult to predict and measure (Ollerton et al. 2009). A wide variety of invertebrates and vertebrates can be found at least occasionally on or within flowers, with the potential to contact and possibly carry pollen on their bodies. Opportunistic pollen transport, however, will have a low likelihood of leading to effective pollination unless the animal moves to another plant of the same species and contacts the second plant's reproductive structures while the pollen is still viable. As a result, certain animal groups known to exhibit behaviors that tend to increase the likelihood of such legitimate pollen transfer are generally considered to be the most important pollinators in any given ecosystem. These groups include: hummingbirds (Trochilidae), sunbirds (Nectariniidae), honeycreepers (Carduelinae), honeyeaters (Meliphagidae), nectarivorous bats (Chiroptera), moths and butterflies (Lepidoptera), bees and wasps (Hymenoptera), and some flies (Diptera) (e.g., Fenster et al. 2004, Rosas-Guerrero et al. 2014). There are also species in a number of other groups that incorporate nectar or pollen into their diets and are thus known to be effective pollinators, even if most of their relatives are not (e.g., *Zosterops japonicus*, Aslan et al. 2013; some lizards (Olesen and Valido 2003).

Flower "syndromes" are one method of generalizing across plant species in order to predict which pollinators or pollinator guilds are likely most important for any given plant. Syndromes describe the set of flower characteristics that tend to predict which functional groups of visitors are likely to both be attracted to the flower and to effectively transfer its pollen (Fenster et al. 2004). A classic example is hummingbird syndrome flowers, which include long red floral tubes, exserted anthers, and copious dilute nectar (Castellanos et al. 2003). Syndromes can be visibly distinct, to the point that researchers and managers alike may make assumptions about likely pollinators based on flower color, size, and shape. Evaluations of the

usefulness and accuracy of syndrome-based predictions have varied, with some authors finding less support for syndrome assumptions (e.g., Ollerton et al. 2009) and others finding that observed pollinator effectiveness aligns well with syndromes (e.g., Rosas-Guerrero et al. 2014). At the same time, even when predicted pollinators are known to effectively transfer pollen for a given plant, additional secondary pollinators may also be important (Rosas-Guerrero et al. 2014); as an example, honeybees (*Apis mellifera*) and birds can effectively pollinate the large, fleshy, aromatic white flowers of saguaro cactus (*Carnegiea gigantea*) although it fits a classic bat-pollination syndrome (C. Aslan, unpubl. data). The complexity of syndromes suggests that managers concerned about pollination of particular plant species should use caution in predicting which organisms are most important as visitors for those plants; it may be that flowers can be visited by a greater diversity of potential pollinators than their appearance suggests.

Pollinator guilds vary by geography, as well. Bees, for example, which appear to be one of the most effective pollinator groups worldwide, peak in diversity at subtropical and dry latitudes, whereas vertebrate pollinators such as birds and bats are most diverse in the tropics (Ollerton et al. 2017). Flies are dominant pollinators at the highest (Arctic) latitudes and bumblebees across the rest of the northern hemisphere (Ollerton et al. 2017). Bird pollinators include hummingbirds in the New World, sunbirds in Africa and Asia, and honeyeaters in Australia (Ollerton et al. 2017). On islands, generalist pollinators (i.e., able to interact with a diversity of plant species) are most common (Kaiser-Bunbury et al. 2010), likely because the relatively low diversity of species able to disperse to many islands tends to result in species occupying a broad diversity of open niches following island establishment (Scott et al. 2003).

Non-native species are important pollinators in several systems (e.g., Carmo et al. 2004; Celebrezze and Paton 2004; Cayuela et al. 2011; 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 exhibit competitive interactions with native pollinators, reducing the success of native plants and pollinators alike (e.g., Martins et al. 2013). When non-native pollinators are responsible for a lot of pollen transfer for a particular plant species, it may be that the direction, timing, distance, or quantity of gene flow are affected (Dohzono and Yokoyama 2010; Aslan et al. 2014). Since they lack coevolutionary history where they are introduced, many non-natives are extremely generalist. They therefore carry pollen from a large number of plant species. The probability of non-native pollinators transferring heterospecific pollen to a particular plant or wasting pollen by failing to deposit it on a conspecific may be high (Johnson and Steiner 2000). Through these mechanisms, non-native pollinators could impact the evolutionary trajectory, phenology, and relative abundance of native plant species.

Pollen load assessment

Pollination biologists also infer pollination by examining its outcomes rather than direct observation of interactions. This may involve capture of likely pollinators and examination of their bodies for pollen loads (i.e., transported pollen) or examination of stigmatic surfaces

following exposure of flowers to pollinators. Examination of pollen loads is an important piece of the puzzle when determining if flower visitors are also pollinators. Organisms that visit flowers but carry no pollen on their bodies may be nectar robbers or predators rather than pollinators. To examine pollen loads on birds or bats, individual animals can be captured using mist nets as long as researchers/managers have appropriate training and approval from an Institutional Animal Care and Use Committee (IACUC). Scotch tape can be used to lightly blot the animal's face, forehead, chin, and breast to remove any loose debris, including pollen grains. The tape can then be placed on a microscope slide and later examined for the presence of pollen. To examine pollen loads on insects, individual animals can be captured using sweep nets, aspirators, or vials. Many insects instinctively move upward when covered from above, so lowering a glass or plastic vial over the top of a foraging insect is often successful, as the animal will fly or crawl upward into the vial. Once animals are collected, their bodies (particularly the head, face, proboscis, and legs) can be swabbed with a cube of fuchsin gel (Kearns and Inouye 1993). [To prepare the gel, mix 175 mL distilled water with 150 mL glycerine and 50 gelatin and bring to a boil. Slowly sprinkle in a small scoop (<1/8 tsp) of crystalline basic fuchsin stain. The mixture will darken as additional crystals are added; add until the desired magenta color. Gel can be kept in refrigerated indefinitely.] Any pollen grains attached to the animal will adhere to the gel. The gel cube can then be melted onto a microscope slide using (in the field) a cigarette lighter or by being placed in warm sunlight on a flat surface. A coverslip should be applied to the gel prior to melting to protect the surface from additional debris. If the insects are active and may escape, captured insects can be kept in vials returned to the lab and the gel slides prepared there. However, pollen grains may detach from the insects and adhere to the walls of the vial in transport, and it may be necessary to swab the full vial to capture these grains and transfer them to the slide. Even this swabbing may fail to pick up all pollen grains from the vial. Gel slides should be kept refrigerated for storage to prevent mold from growing on the gel.

When insects are too mobile to handle easily (or for stinging insects such as honeybees), captured individuals can be cooled in a refrigerator or cold cooler until they become torpid. At this point, they can be swabbed with fuchsin gel as described above. Some animals will revive as they warm up, and could perhaps be re-released; however, it is important to note that the swabbing process may be rough for delicate insect bodies, and captured individuals may not survive. If potential pollinators are themselves rare or threatened, capture and swabbing may not be desirable, due to the risks it poses to the individual insects.

Methods

Quantifying flower visitation for target plant species

Direct observation is the most precise method of determining which flower visitors are present and interacting with focal plant species flowers in a target system (Figure 3). (Below, we also discuss the benefits and drawbacks of alternative approaches including video cameras and pollen load analysis.) Systematic flower visitation observations involve collection of flower visitation data according to predetermined protocols intended to minimize bias associated with observer, weather, time of day, condition of flowering plants, etc. Collected data include the

richness and types of flower visitor species recorded as well as the rate of visitation. The rate of visitation should also be adjusted by factors that make it difficult to compare between sites and observation periods, including the number and condition of open flowers, temperature and precipitation, wind, time of day, date, etc. Adjusted visitation rates can be used to construct flower visitation networks depicting the observed relationships between flower visitors and flowering plant species. (Note that observations can be used to determine the list of flower visitors, but not every visitor is a pollinator. Pollination occurs when visitors transfer pollen between conspecific plant individuals. Assessment of pollen loads, pollen deposition on stigmas, and outcrossing rates following visitation are needed to confirm that visitors are acting as pollinators.)

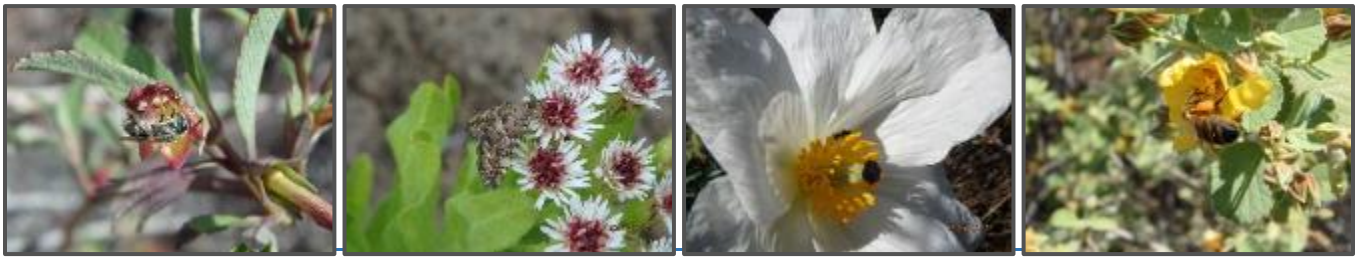

Figure 3. Photos of flower visitors (left to right): *Lasioglossum* sp. on *Stenogyne angustifolia*; *Mestolobes* sp. on *Tetramolopium arenarium*; *Hylaeus* sp. on *Argemone glauca*; *Apis mellifera* on *Sida fallax*.

Quantifying visitation at PTA

As an example of a systematic flower visitation observation protocol, we describe here the methods used in our four-year study of plants and pollinators at PTA (2015–2018); users may choose to adapt these methods for their own research purposes:

Observations followed a systematic protocol (after Aslan et al. 2013). Each observation lasted 180 minutes, divided into 10-minute blocks. To avoid fatigue, the protocol stipulated that the observer rest for ten minutes of every hour, so the 180-minute observation contained a total of 150 minutes of data collection. The first minute of each 10-minute observation block was devoted to a method called *scan sampling*, wherein the observer visually 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 observer also noted the number of visible flowers during each scan. The data from scans repeated throughout each observation and across observations were combined to provide an estimate of the mean number of individuals of each visitor species per visible flower of each plant species per minute.

For the remaining nine minutes of each 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 observer noted the number of flowers and number of plants visited. These data permitted calculation of the average number of flowers and plants with which each visitor taxon interacted when present. (During focal visitor observations, we also casually noted visitor behaviors that might be useful

in later assessment of their role as interactors, including nectar and pollen collection, nectar robbing, and pollen transport.) We opportunistically selected plants of each species for observation, out of the existing populations of the eight focal plant species included in the study. 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.

As a consequence of the use of naturally-occurring plant individuals in this study, observable plants could not be standardized by size or number of open flowers—a challenge common to many such field studies. 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. Thus, the presence of a higher number of flowers could be used to adjust the visitation rate to combine it more robustly with observations of sites or time periods with fewer flowers. No observations were recorded in rain. 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); not all flowering plants were observed during all months.

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 multiplied to provide an estimate of **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., *Apis mellifera*, European honey bee) 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.

Pollinator visitation importance (PVI) values tend to be extremely small, since the use of a per-minute and per-flower calculation, while enabling comparison between sites and observation periods, involves multiplying fractions by fractions. The resulting values may be difficult to interpret biologically. Therefore, in our analyses, we divided the importance value of each visitor taxon for each plant species by the highest importance value for that plant species. This resulted in 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 equal to their proportional importance, relative to that most important visitor species (e.g., Table 1).

When considering how often to perform observations and for how long, it is important to consider inherent traits of the system of interest. When focal plant or pollinator species are low in density, important flower visits may occur with great rarity. As an example, in the first year of our study at PTA, we observed a single flower visitor to *S. angustifolia* in over 120 hours

observation (Aslan et al. 2018). Published flower visitation observation protocols from some studies include very short observation durations (e.g., 5-minute windows totaling less than an hour per site), but these are unlikely to detect rare events. In some cases, the rare events are an important component of pollen transfer. For example, in a Sonoran Desert study, although bees visited a focal endangered cactus with four times as much frequency as hummingbirds, hummingbirds were responsible for nearly half of the seed production of the cactus, indicating that their importance was disproportional to their frequency (Aslan 2015). When rare events are of interest, investing a large amount of time in observations or employing supplemental methods like camera observations (discussed further below) may be necessary. On the other hand, if rare events are of less importance, such as if a study is aiming to determine the general types of flower visitors that are most common in an area or the role of invasive flower visitors in a community, it may be more efficient to devote shorter periods of time to observations and recognize that some rare interactions will go unrecorded.

In our PTA study, 180-minute 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. (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 limited number of additional nighttime observations, totaling 40 hours, 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 and ended between 2030 and 2200 hours, but no flower visitation was detected during them). In all, during the observation year, 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). Total observation time varied widely as a result of variation in flowering periods and thus availability of flowers for observations; some plants flower almost continuously over the course of the year while others have distinct flowering seasons.

Alternative observation approaches for special circumstances

In addition to standardized visitation observations, it may be necessary in some cases to investigate pollination at night or using inferential techniques. Nighttime visitation is extremely common on some flower types, including many desert and tropical species. Flowers that are pale in color and emit strong scent in the evening or close during daytime hours are often assumed to be visited by bats, moths, and other nighttime visitors (e.g., nocturnal mammals; Janson et al. 1981). Bat-visited flowers may be large and fleshy, and moth-visited flowers may

be tubular. Flower visitation can be observed at night using systematic visitation observations just like daytime visitation but likely require the use of infrared camera or night-vision goggles.

Night-vision goggles require human observation at the same effort as the daytime observations described above. Cameras can be difficult to use efficiently if flower visitors are particularly small and therefore unlikely to trigger a motion-activated device. This is unfortunately the case with many insects. In these circumstances, it becomes necessary to use video cameras and review footage, a process that can occupy a large number of human hours. In some cases, it may be possible to watch the footage at an increased number of frames per second, which can reduce the total human hours compared to real time observation over the same time period. However, for very small and very quick visitors, reviewing footage at an increased rate, since video frames are lost, could result in missed visitation events; therefore, any attempt to fast-forward requires caution. We recommend that efforts with cameras be trialed by first recording visitation using a camera while an observer is also watching the plant, in order to compare the number of events that are recorded to ensure that camera resolution and frames per second are appropriate for the system of interest. Another important consideration is that a camera can focus only on a relatively narrow field of view. It is unlikely to pick up very rare events due to the low probability that they will intercept that field of view. Finally, the resolution of cameras is rarely sufficient to identify cryptic or small visitors, especially if there are several similar species in an area. It may be possible to determine that a moth of a particular family has visited, for example, but it may not be possible to determine which species it was.

Camera use at PTA

At PTA, we used Hawk Eye High Definition Nature Cams purchased from Birdhouse Spy Cam (http://www.birdhousespycam.com). The camera has resolution of 700 tvl (television lines) and night vision capability. In addition to the cameras, the following equipment was needed for the video observations:

- Digital video recorder (DVR) and high-capacity memory cards (e.g., 128 GB).
- Power source for use in the field. Our setup included sealed car battery, car power inverter, power cord splitter (to split power from one outlet into 3-4 outlets), and analog mechanical timer (to turn power on/off at intervals).
- RCA to USB adapter cable (for connecting camera to tablet for visual focus during initial placement of camera in field).
- Waterproof storage cover/case for the entire camera set-up. We used small plastic storage bins and we cut out holes for the camera lens as well as for ventilation.
- USB fans for ventilating the storage case.
- High capacity hard drives to store video footage (e.g. 2 TB).

We recorded 417 hours of nighttime footage at the endangered plant species (*H. haplostachys, S. lanceolata*, *S. angustifolia*, and *T. arenarium*), in the combined treatment plot and untreated control plot in each block. Via footage review, we were able to detect 71

minutes of noctuid moth visitation to flowers, but could not determine which of the hundreds of possible noctuid species were present. Nevertheless, because our focal plant species were visited so rarely, confirmation of moth visitation helped to complete our understanding of the pollinator community interacting with these plants at PTA.

Pollen load assessment at PTA

In our PTA study, we attempted to collect ten individuals of each of the most important (i.e., non-rare) flower visitor taxa interacting with our focal plants. In practice, we were able to capture between 3 and 10 individuals of most visitor/plant combinations. We used fuchsin gel to examine pollen loads of all of these individuals. Following preparation in the field, all gel slides were examined under a Reichert Microstar IV microscope (Reichert Technologies, Depew, New York, USA) at 200x magnification. The gel stains pollen grain walls dark pink, so they are readily detected and counted under the microscope. Pollen counts per slide in our study ranged from zero to tens of thousands, with large counts occurring only on honeybee slides. We identified the pollen by morphotype (i.e., general shape and size), comparing each pollen grain to reference grains collected directly from plants and prepared using the same fuchsin gel techniques as were used for grains collected from animals.

Results

Analysis of pollination networks

To analyze flower visitation data for our focal plant species, we used observation data to calculate: 1) the average number of individuals of each visiting taxon per open flower per observation minute for each target plant species, 2) the total richness of visitor taxa per plant species, and 3) the average number of flowers probed per minute by each visitor taxon. We multiplied #1 and #3 together to generate an overall visitor importance value for each insect taxon/plant combination (after Renne et al., 2000; Aslan et al., 2013). This analysis gave us a complete list of the observed visitors for each plant species, ranked by their relative importance so that the most important visitors could be identified and compared among plant species. A taxon would be considered to have high importance if it visited the target plant frequently or probed a large number of flowers during each visit. To standardize importance values, we then set the importance value of the most important visitor for each target plant species equal to 1.0, and the importance values of all other visitors equal to their proportional value relative to that maximum. For each plant species, we considered all visitors at least 25% as important as the most important visitor to be primary visitors. We entered all importance values into a network matrix in the *bipartite* package in R version 2.14.1 (R Development Core Team 2012) to visualize the resulting full and primary networks (Figures 4, 5) using the following code:

```
>PTAnetwork<-read.csv(file.choose(),header=T,row.names=1) #loads the file to be analyzed
>PTAnetwork #visualizes the pollination matrix being analyzed
>str(PTAnetwork) #reads the structure of the network
>plotweb(PTAnetwork) #visualizes the graphical pollination network
```

>networklevel(PTAnetwork,index="ALLBUTDD") #generates the full set of metrics describing the network

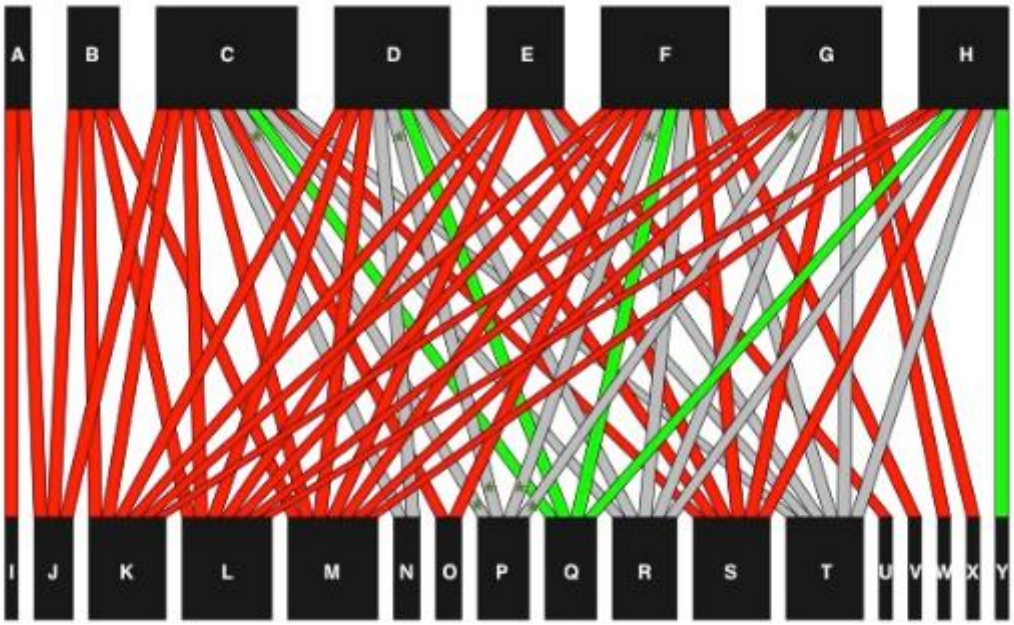

Figure 4. Network diagram displaying all observed interactions between plants and flower visitors. Green connectors = native flower visitors. Gray connectors = flower visitors of indeterminate provenance. Red connectors = non-native flower visitors. The green asterisks within connectors indicate a likely (though not certain) native flower visitor (*Hylaeus* sp.). Plants appear in the top row: A = *Stenogyne angustifolia*; B = *Silene lanceolata*; C = *Bidens menziesii*; D = *Dubautia linearis*; E = *Haplostachys haplostachya*; F = *Sida fallax*; G = *Argemone glauca*; H = *Tetramolopium arenarium*. Flower visitors appear in the bottom row: I = *Ceratina* cf. *dentipes*; J = *Lasioglossum impavidum*; K = Diptera (unspecified); L = *Apis mellifera*; M = *Allograpta exotica*; N = Coleoptera (unspecified); O = Butterfly (unspecified); P = *Hylaeus* sp. (unspecified); Q = *Orthomecyna* sp.; R = Wasp (unspecified); S = *Pachodynerus nasidens*; T = Moth (unspecified); U = *Lampides boeticus*; V = Megachilidae (unspecified); W = *Pieris rapae*; X = *Vanessa cardui*; Y = *Udara blackburni*.

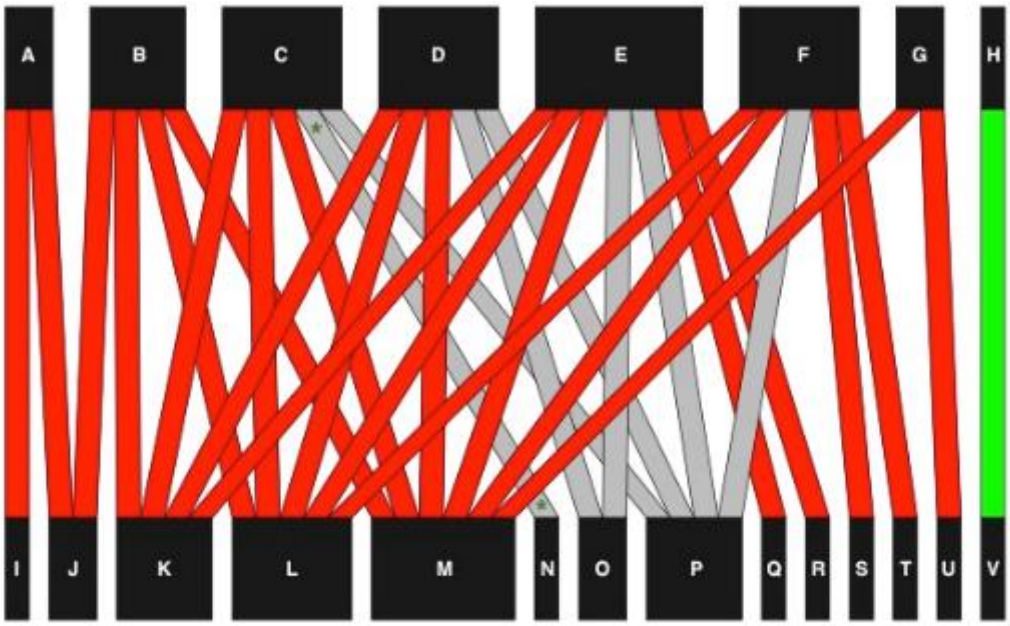

Figure 5. Network diagram displaying the primary observed interactions between plants and flower visitors. For each plant, only the most important visitor and all visitors with at least 10% of the importance value of the most important visitor are included. Green connectors = native flower visitors. Gray connectors = flower visitors of indeterminate provenance. Red connectors = non-native flower visitors. The green asterisks within connectors indicate a likely (though not certain) native flower visitor (*Hylaeus* sp.). Plants appear in the top row: A = *Stenogyne angustifolia*; B = *Silene lanceolata*; C = *Bidens menziesii*; D = *Dubautia linearis*; E = *Haplostachys haplostachya*; F = *Sida fallax*; G = *Argemone glauca*; H = *Tetramolopium arenarium*. Flower visitors appear in the bottom row: I = *Ceratina* cf. *dentipes*; J = *Lasioglossum impavidum*; K = Diptera (unspecified); L = *Allograpta exotica*; M = *Apis mellifera*; N = *Hylaeus* sp. (unspecified); O = Wasp (unspecified); P = Moth (unspecified); Q = *Pieris rapae*; R = *Vanessa cardui*; S = *Lampides boeticus*; T = *Pachodynerus nasidens*; U = Butterfly (unspecified); V = *Orthomecyna* sp.

The baseline year of visitation observations provided evidence that all focal plant species are receiving floral visitations under ambient conditions within the study site. The number of visitor taxa per plant species ranged from 2 to 11. Non-native insects are responsible for the large majority of this visitation. Particularly important flower visitors included the honeybee (*A*. *mellifera*) (the most important visitor for *A*. *glauca*, *B*. *menziesii*, and *S*. *lanceolata*), syrphid hoverflies (the most important visitors for *D*. *linearis*), the keyhole wasp *P*. *nasidens* (the most important visitor for *H*. *haplostachya*), butterflies (unidentified) (the most important visitors for *S*. *fallax*), the moth *Orthomecyna* sp. (the most important visitor for *T*. *arenarium*), and the sweat bee *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 endemic plants are largely dependent upon non-native insects for outcrossing.

Study conclusions

Our focal system at PTA is heavily dominated by non-native flower visitors (Aslan et al. 2019), and the implications of this level of invasion and species reorganization have been little explored in ecology. Non-native pollinators, by altering the qualitative and quantitative aspects of pollination (Herrera 1987, Aizen and Harder 2007), have the potential to impact native species' gene flow, phenology, evolutionary trajectory, recruitment, and spatial distribution. For two of our endangered plant species (*S. angustifolia* and *S. lanceolata*), only non-native insects were observed visiting flowers during the first year of baseline pollination observations, prior to initiation of our predator control treatments. The long-term implications of a wholesale shift to pollination mediated by non-natives are unknown for these species. As a group, the endangered plant species in this study were visited by fewer visitor taxa than were the common native plant species. This suggests that non-native insects may be sustaining biotic interactions otherwise threatened with disruption in this ecosystem. A thorough understanding of pollination is an important component of endangered plant conservation.

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IV. Pollination Treatments

In a Nutshell:

- Lack of sufficient pollination could contribute to reductions in populations, reproduction, and genetic diversity among plant species but has been little studied for our focal species.
- Manual pollination treatments were used to quantitatively assess the level of dependence of plant species on outcrossing via pollen transfer.
- All plant species produced some seed when experimentally bagged to exclude outcrossing, indicating self-compatibility for each species.
- Three of our focal plant species, including two endangered species, displayed moderate pollen limitation under ambient, unmanipulated conditions, suggesting that their seed set is not maximized by the current community of flower visitors.

General background

Determining the breeding system of target plants

Particularly when plants are rare or populations are declining, it may be of interest to learn whether pollination limitation, or lack of sufficient pollen transfer to enable the plant to achieve its full reproductive potential, could be contributing to their decline (Wilcock and Neiland 2002). Pollination limitation could occur when pollinators are themselves rare or when plants are few enough on the landscape that their flowers are too unattractive or unreliable to consistently receive effective visits from pollinators. Understanding whether pollination limitation is a concern in a given system requires understanding the plants' level of dependence on pollination, pollinators, and outcrossing. Some plants reproduce readily via autogamy, or self-fertilization, and therefore show only low dependence on pollination. Plants with wind- or water-mediated pollen transfer may have little or no dependence on pollinators. And plants vary in their dependence on gene flow among individuals or populations; for some plants and in some contexts, inbreeding carries few evident fitness drawbacks and outcrossing dependence therefore appears low.

Determining effective pollination

Effective pollination by an animal requires (a) visitation to a plant involving contact with pollen; (b) pollen adherence to the animal's body or deliberate collection by the animal; (c) visitation to another plant of the same species while carried pollen is still viable; (d) contact with the second plant's stigma; (e) deposition of pollen onto the second plant's stigma. Visitors may be ineffective pollinators if they fail to contact plant reproductive structures, fail to acquire pollen while interacting with a flower, or fail to readily reach a conspecific plant following pollen acquisition.

Observation of visitation and collection of pollen from the bodies of visitors are important indicators of effectiveness, by providing insight into points a) and b) above. To determine the frequency with which individuals of a given visitor deposit conspecific pollen on stigmas (points d) and e) above), single-visit effectiveness experiments are often advocated. In

these experiments, flowers are covered or bagged in bud stage. Once the flower has become receptive, the bag or cover is removed and a manager or researcher observes the flower until a visitor arrives and interacts with it. Once the visitor leaves, the manager replaces the flower cover until the flower senesces. Any fruit or seed production is assumed to be the result of the observed visit, permitting assessment of the effectiveness of that visitor as a pollinator.

Single-visit effectiveness experiments can work well for some flower types, but pose their own challenges. First, this approach is time-intensive and may be unsuccessful. To ensure that pollen transfer is not occurring among flowers on the same plant, flower covers must isolate single flowers, and this is physically difficult and time-consuming when flowers are very small or borne in tight inflorescences. When visitation is rare (as is commonly the case when plants are rare or the full community is highly impacted by environmental change), it is not uncommon for a researcher to apply flower covers to dozens of flowers (a time-consuming task) and then, when the flowers are receptive, wait by those flowers for a full day without observing a single visit. It may be necessary for a small group of managers or researchers to work together to observe enough flowers to capture data from rare events. An alternative option arises when a potential pollinator of interest is large-bodied relative to other flower visitors: in these cases, researchers or managers may cover flowers with loose mesh or cages to exclude the large visitors while allowing smaller visitors to access the flower. This approach allows researchers to separate the contribution of small vs. large visitors to overall flower reproductive success (e.g., Aslan 2015).

Single-visit effectiveness may also be challenging if flowers are protandrous, with mature pollen-producing male stamens present before the female stigma becomes receptive. A flower that is isolated and bagged throughout this male phase may retain a great deal of its own pollen by the time the stigma becomes receptive and the bag is removed. It will be difficult or impossible in this case to prevent the flower's own pollen from landing on the stigma, with the potential for self-fertilization. Determining how many of the resulting seeds are from self-fertilization vs. from the single pollinator visit would be impossible without expensive genetic paternity analyses. Emasculation of the flower (removal of stamens) just prior to male phase may avoid this problem, but can be difficult (or infeasible) in the field when flowers are small.

Depending on the research question at hand, a promising alternative to single-visit effectiveness experiments may be the use of fluorescent powder dye, applied to the anthers of flowers on one plant. After several hours or a day, researchers can use a UV-A light to examine the stigmas of nearby plants to determine whether dye transfer has occurred; such transfer would suggest that pollen is effectively moving within the system. Applying a single-visit effectiveness technique, wherein flower covers are removed from some flowers after other flowers have been dyed, could enable the detection of pollen movement if the dye appears on flowers following single visits. Additionally, when large pollinators such as honeybees pick up the dye in their foraging, it is often possible to observe it on their bodies even while they are moving past (pers. obs.) and thus to infer which plants they have visited. All of these methods, however, require a great investment of human hours due to the rare occurrence of a particular animal moving to and interacting with a particular pair of plants.

Methods

Plant breeding system assessment at PTA

Dependence on outcrossing and on pollinators is generally determined via experimental flower treatments (Kearns and Inouye 1993). Flower treatments may be used to evaluate plant reproductive success in the absence of all or some pollinators, in ambient or open pollination conditions, and following hand-supplementation with pollen. At PTA, we carried out manual pollination treatments to evaluate the dependence of each plant species on outcrossing via pollinators. For each plant species, we administered the following treatments: bagging individual flowers to prevent outcrossing while flowers were in bud stage; bagging individual flowers in bud stage followed by hand-pollination of flowers in female phase, to act as a control for the effect of the bag; hand-supplementation with pollen while the flower was in female phase; and an unmanipulated treatment to serve as a control for the remaining treatments (Figure 6). Bagging prevents visitation by pollinators and therefore can be used to determine whether plants are capable of setting fruit and seed via self-fertilization. A bag control can be used to determine whether the bagging treatment itself has delivered sufficient trauma to flowers to cause their failure or poor performance, independent of the effect of self-fertilization. Hand-supplementation of pollen enables estimation of the maximum seed set possible for a species assuming maximum pollen delivery. The unmanipulated control enables estimation of average fruit and seed production under normal, ambient pollination conditions and serves as a baseline comparison for all other treatments.

Figure 6. Pollination treatments on *Dubautia linearis*.

Administration of flower treatments requires techniques tailored to the morphology of the flowers of each of the species, and each of our species at PTA presented different challenges. Several of our focal species had extremely small or delicate flowers, requiring trials of various types of flower covers, including lightweight wedding tulle, large straws, plastic ketchup cups, window screen, and loose-weave ribbon. Attachment of 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 often tore off bags that were loosely attached and sometimes tore off flowers within bags if the bags were firmly attached. An additional challenge emerged from the fact that several of our plants are rare: in some cases, no plants with at least 30 open flowers in treatable flower phase could be found in the study area during a particular treatment round. 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. We were forced to innovate flower cover structures and attachment methods and also to repeat many treatments in order to obtain a minimal sample size for analysis. We aimed for minimal sample size for our focal endangered plant species, to avoid contributing substantially to reproductive failure for those plants. We therefore performed treatments on a small number of flowers/plants at a time (three flowers each on six plants) and repeated them as needed/possible once we were able to determine whether treatments had succeeded.

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 seeds produced for each flower treatment. For some species, counting seeds required a microscope. For each plant species, we analyzed flower treatment effects on seed set, defined as number of seeds produced per flower. Data did not meet assumptions of normality (based on quantile-quantile plots, used to evaluate normality due to limitations in sample size; Wood 2010), so we used non-parametric Kruskal-Wallis tests to examine differences among treatments and employed Dunn's multiple comparisons test to determine which pairs of treatments differed significantly. Multiple flowers receiving the same treatment on a given plant individual were treated as subsamples (=averaged) in these analyses. These analyses were performed in R version 2.14.1 (R Development Core Team 2012) using the following code:

```
> library(nlme) #loads the necessary R library
> TETAREseedset<-read.csv(file.choose(),header=T) #loads the data to be analyzed
> a<-TETAREseedset #renames the data file to "a" for simplicity
> View(a) #displays the data to be analyzed
> response<-a[,"SeedSet"] #identifies seed set as the response variable
> treatment<-a[,"Treatment"] #identifies the treatment variable
```

> kruskal.test(response~treatment) #performs a Kruskal-Wallis non-parametric test to determine whether seed set varies by treatment
> PT<-dunnTest(response ~ treatment,data=a,method="bh") #performs multiple comparisons using the Dunn's tests
> PT #displays the outputs of the Dunn's tests

We used seed set data to calculate the **Pollen Limitation Index (PLI)** for each focal plant species. PLI is calculated as 1−(*U*/*S*), where *U* = the proportional seed set of unmanipulated flowers and *S* = the proportional seed set of hand-supplemented flowers (Larson and Barrett, 2000). A PLI of 0 indicates no pollen limitation and a PLI of 1 indicates full pollen limitation. We also evaluated the level of self-incompatibility of each target species using the **Index of Self-Incompatibility (ISI)**. The ISI is calculated as *Self*/*Supp*, where *Self* = the mean seed production of bagged (i.e., self-pollinated) flowers and *Supp* = the mean seed production of hand-supplemented flowers. Values of ISI indicate whether flowers are self-incompatible (values ranging from 0.0–0.2), partially self-compatible (0.2–1.0), or self-compatible (1.0) (Zapata and Arroyo 1978).

Data were analyzed using the packages *nlme* (Pinheiro et al. 2018), *vegan* (Oksanen et al. 2017), and *FSA* (Ogle 2016) in the statistical software environment R version 2.14.1 (R Development Core Team 2012), with significance accepted at $p \leq 0.05$.

Results

Analysis of seed set at PTA

In our PTA study, all plant species produced some seed when bagged to exclude outcrossing, indicating partial self-compatibility for each species (Table 1). Index of Self-Incompatibility (ISI) values were 0.68 for *A. glauca*, 0.22 for *H. haplostachya*, 0.31 for *B. menziesii*, 0.49 for *D. linearis*, 0.66 for *S. fallax*, 0.42 for *S. lanceolata*, 0.55 for *T. arenarium*, and 0.19 for *S. angustifolia*. The Pollen Limitation Index (PLI) results, which are positive when hand-supplementation boosts fruit or seed production compared with unmanipulated controls, indicated that pollen limitation is not severe in the system: values 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*. 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 (Young and Young, 1992).

Table 1**.** Flower treatment results. Reproductive success was evaluated as seed set, defined as seeds produced per flower. Treatments included hand-supplementation with conspecific pollen to evaluate pollination limitation, bagging to evaluate dependence on outcrossing, a bagged control treatment to evaluate the effect of the bag on seed production, and an unmanipulated control to assess seed production under ambient pollination conditions. ***Endangered species.**

Plant spp. (*n* treated flowers/ *n* plants)	Significant treatment (Kruskal-Wallis)	Significant contrasts (Dunn's test of multiple comparisons)	Treatment	Mean seed set (± SE)
Argemone glauca (38/12)	$\chi^2 = 9.53$; $P = 0.0230$	Unmanipulated vs. supplemented ($P = 0.033$)	Bagged	151.03 ± 36.79
			Bagged control	253.89 ± 53.86
			Hand-supplemented	249.04 ± 41.23
			Unmanipulated	138.28 ± 43.54
Bidens menziesii (91/12)	$\chi^2 = 30.86$; $P < 0.0001$	Unmanipulated vs. bagged control ($P = 0.029$)	Bagged	0.16 ± 0.24
		Unmanipulated vs. bagged ($P < 0.0001$)	Bagged control	1.00 ± 0.71
		Supplemented vs. bagged ($P = 0.0002$)	Hand-supplemented	2.87 ± 0.47
			Unmanipulated	3.61 ± 0.34
Dubautia linearis (149/14)	$\chi^2 = 25.69$; $P < 0.0001$	Unmanipulated vs. bagged ($P < 0.0001$)	Bagged	0.56 ± 0.28
		Bagged control vs. bagged ($P = 0.0023$)	Bagged control	2.13 ± 0.36
		Supplemented vs. bagged ($P = 0.035$)	Hand-supplemented	1.78 ± 0.31
			Unmanipulated	2.72 ± 0.33
Sida fallax (82/18)	$\chi^2 = 43.53$; $P < 0.0001$	Bagged vs. supplemented ($P < 0.0001$)	Bagged	3.63 ± 0.48
		Unmanipulated vs. bagged ($P < 0.0001$)	Bagged control	5.73 ± 0.11
		Bagged control vs. bagged ($P = 0.011$)	Hand-supplemented	6.42 ± 0.14
		Supplemented vs. bagged control ($P = 0.040$)	Unmanipulated	5.89 ± 0.20
*Haplostachys haplostachya** (105/23)	$\chi^2 = 76.97$; $P < 0.0001$	Unmanipulated vs. bagged ($P < 0.0001$)	Bagged	0.23 ± 0.16
		Unmanipulated vs. bagged control ($P = 0.015$)	Bagged control	0.50 ± 0.29
			Hand-supplemented	2.84 ± 0.19
		Unmanipulated vs. supplemented ($P < 0.0001$)	Unmanipulated	1.22 ± 0.28
		Supplemented vs. bagged control ($P < 0.0001$)		
		Supplemented vs. bagged ($P < 0.0001$)		
*Silene lanceolata** (102/11)	None	None	Bagged	51.20 ± 2.17
			Bagged control	30.00 ± 3.29
			Hand-supplemented	59.49 ± 5.50
			Unmanipulated	53.14 ± 2.19
*Stenogyne angustifolia** (55/28)	$\chi^2 = 20.43$; $P = 0.0001$	Unmanipulated vs. bagged control ($P = 0.024$)	Bagged	0.05 ± 0.34
		Unmanipulated vs. bagged ($P = 0.0026$)	Bagged control	0.41 ± 0.46
		Supplemented vs. bagged control ($P = 0.0072$)	Hand-supplemented	2.07 ± 0.25
			Unmanipulated	1.14 ± 0.31
		Supplemented vs. bagged ($P = 0.0008$)		
*Tetramolopium arenarium** (91/10)	$\chi^2 = 9.77$; $P < 0.021$	Unmanipulated vs. bagged ($P = 0.039$)	Bagged	11.84 ± 2.17
			Bagged control	18.00 ± 3.29
			Hand-supplemented	10.24 ± 5.50
			Unmanipulated	18.18 ± 2.19

Study conclusions

Although all the plant species examined here are self-compatible (setting seed in the absence of outcrossing), outcrossing increased seed production significantly for six of the focal plant species, including three of the endangered species. Self-compatibility may shield a plant population from negative effects of pollinator loss by enabling flowers to continue to produce seed under uncertain pollination conditions. However, over multiple plant generations, the lack of gene flow among populations and the resulting increase in inbreeding could limit the adaptive capacity of plants relegated entirely or mostly to self-fertilization (Armbruster and Reed, 2005). In our study, open or unmanipulated flowers set more seed or fruit than bagged or self-fertilized flowers for most of our focal plant species, suggesting that effective outcrossing is indeed occurring in this system, under ambient conditions, for species varying in flower morphology and phenology. At the same time, three of our plants (*A. glauca*, *H. haplostachys*,

and *S. angustifolia*) exhibited pollen limitation, implying that their reproductive output would be higher with increased pollen transfer.

Pollen limitation could contribute to small population sizes by reducing reproductive rates, or could be a consequence of small population sizes by reflecting isolation among individuals. Without historical knowledge of the population sizes and distributions of these plant species in a system dominated by native pollinators, we cannot know whether the pollen limitation observed here is a consequence of environmental change and contributing to vulnerability of these species. Nevertheless, an understanding of current outcrossing rates, pollinator dependence, and pollen limitation may help conservation planning for the endangered species included in this study.

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V. Common Flower Visitors in Dryland Hawai'i

Common flower visitors in dryland Hawai'i

Insects account for the bulk of biodiversity in Hawaii, with about 5,400 described endemic species, and about 2,700 nonnative species now established (Nishida 2002). A subset of these species are flower visitors and potential pollinators of native and nonnative plants. Although it is beyond the scope of this document to provide a comprehensive identification guide for Hawaiian insects in general, we have included photos of some of the most common flower visitors encountered in our study. Other resources for identification of Hawaiian insects include the Insects of Hawai'i series, many of which are available online: https://scholarspace.manoa.hawaii.edu/handle/10125/1768/browse?type=title&submit_browse=Title

For identification of insects in general, a good resource is Borror and DeLong's Introduction to the Study of Insects (Johnson and Triplehorn 2005). This book is used as a textbook for college entomology courses, and includes keys to the family level for most insect orders.

Unless otherwise noted in the captions, the photos in this guide were taken by our staff or belong to the public domain. Note that not all of these photos were taken at PTA.

Hymenoptera (bees, wasps, and ants)

Family Apidae, *Apis mellifera* (European honey bee; Figure A). Overall, honey bees were the most frequent flower visitors observed in our study. Honey bees are not native to Hawai'i (nor North America), and although they may provide important pollination services, they may also compete with native pollinators due to their very high densities in some areas. Unlike our native bees (genus *Hylaeus*), honey bees are social bees, nesting in large colonies. These colonies are usually found in natural cavities in trees or crevices. At PTA, nests were frequently encountered in the bases of *Metrosideros* trees or in cracks in pāhoehoe lava flows.

Figure A. European honey bee, *Apis mellifera* on *Dubautia menzeisii*. Body length about 15 mm.

Family Colletidae, *Hylaeus* spp. (yellow-faced bees)

The widespread genus *Hylaeus*, referred to as yellow-faced bees due to the markings on the face of the males of many species (Figure B), includes about 60 species that are endemic to Hawaii. Hawaiian yellow-faced bees are small, solitary bees, and do not form social colonies. Instead, females build tubular nests in holes in dead twigs, bare ground, or under rocks. Adult bees are much smaller than honey bees, usually between 5-8 mm and predominantly black, though some species have reddish markings on their body or yellow banding on their legs. Yellow-faced bees visit flowers to collect nectar and pollen as a food source for themselves and their larvae. Seven species of Hawaiian *Hylaeus* were listed as endangered in 2016, but we did not observe any endangered *Hylaeus* during the course of our study. Note that there are a few nonnative species of Hylaeus established in Hawaii, including *H. strenuus* and *H. albonitens*. Identification resources for *Hylaeus* of Hawai‘i can be found in the Insects of Hawai‘i Volume 17 (Daly and Magnacca 2003) and here: http://www.starrenvironmental.com/resources/hylaeus/

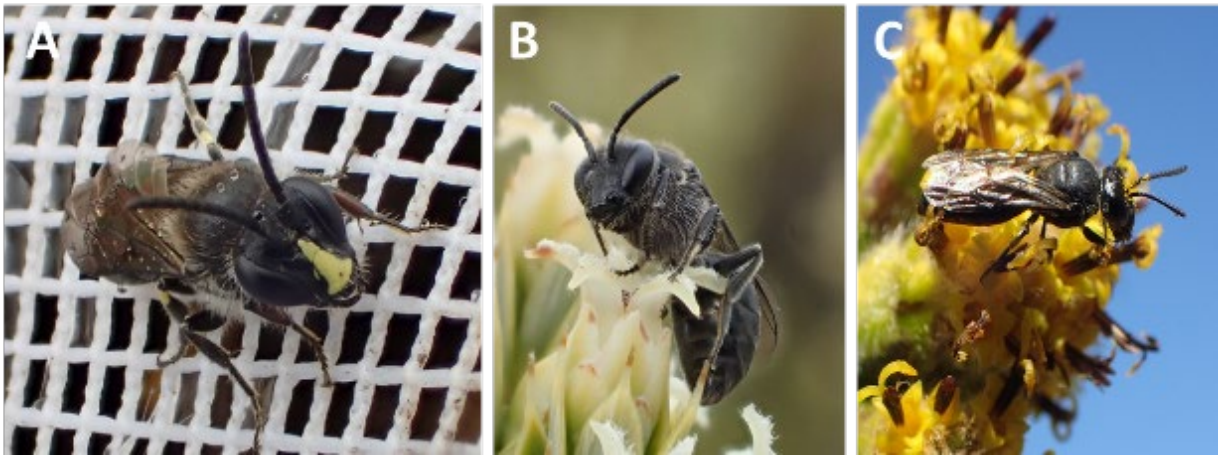

Figure B. Hawaiian yellow faced bees (*Hylaeus nivicola*) male (A) and female (B and C). These photographs were taken at Haleakalā on Maui. Body length about 7 mm.

Family Halictidae, *Lasioglossum* spp. (sweat bees)

Several species of sweat bee in the genus *Lasioglossum* have been introduced to Hawaii, and these were observed visiting native plants in PTA (Figure C). Sweat bees are similar in size and appearance to *Hylaeus*, but they are covered with a denser fuzz. Their bodies have a metallic coppery or green tinge to them, while *Hylaeus* spp. are mostly black. Their fuzz can make them appear grey.


Figure C. *Lasioglossum impavidum*, a nonnative sweat bee, visiting *Stenogyne angustifolia* at PTA. Body length about 6 mm.

Family Megachilidae (leafcutter bees)

There are several species of leafcutter bees established in Hawaii, though all of them are nonnative. Leafcutter bees get their name from their nest-building behavior. Female bees cut semicircular pieces out of the edges of leaves and use these to line their nests, which are built in crevices or holes. Leafcutter bees are about the same size as honey bees, but their bodies are slightly more squat, and the species present in Hawai‘i generally are covered with a dense golden or silvery fuzz.

Figure D. A nonnative leaf-cutter bee (*Megachile* sp.) on vegetation at PTA, roughly 14 mm.

Family Vespidae (vespid wasps)

Vespid wasps (including *Vespula pensylvanica*, one of our target NIPs) are important predators, hunting and scavenging on a diversity of other arthropods. Several nonnative species of vespid wasp were encountered in our study (Figure E). Endemic wasps in the genus *Nesodynerus* (Figure E(B)) also occur at PTA. These are similar in appearance to *Pachodynerus* but are typically predominantly black with less banding on the abdomen (if banding is present at all).

Figure E. Some vespid wasps that might be encountered in Hawai‘i dry forests. (A) the keyhole wasp *Pachodynerus nasidens*, roughly 13 mm (Photo by Steve Wells). (B) the endemic potter wasp *Nesodynerus nubicola* from Haleakalā, Maui, roughly 18 mm. (C) the paper wasp *Polistes aurifer*, roughly 20 mm. (D) the western yellowjacket *Vespula pensylvanica*, roughly 18 mm.

Lepidoptera (moths and butterflies)

Family Noctuidae (owlet moths, cutworms and armyworms)

The family Noctuidae is the most diverse family of Lepidoptera worldwide and is represented in Hawai‘i by a large number of native and nonnative species. Species in this family range from very small (a few millimeters in length) to very large, such as the black witch moth, *Ascalapha odorata*, which is common in Hawaii. Most noctuid moths, however, are mid-sized, between 15-30 mm in length with stout bodies covered in fuzzy scales. Noctuid moths are frequent flower visitors, but like many moths, most are nocturnal. Our infrared cameras detected noctuid moths visiting several of our focal plants at night, but unfortunately, the resolution of the cameras was not high enough to identify the moths to the species level.

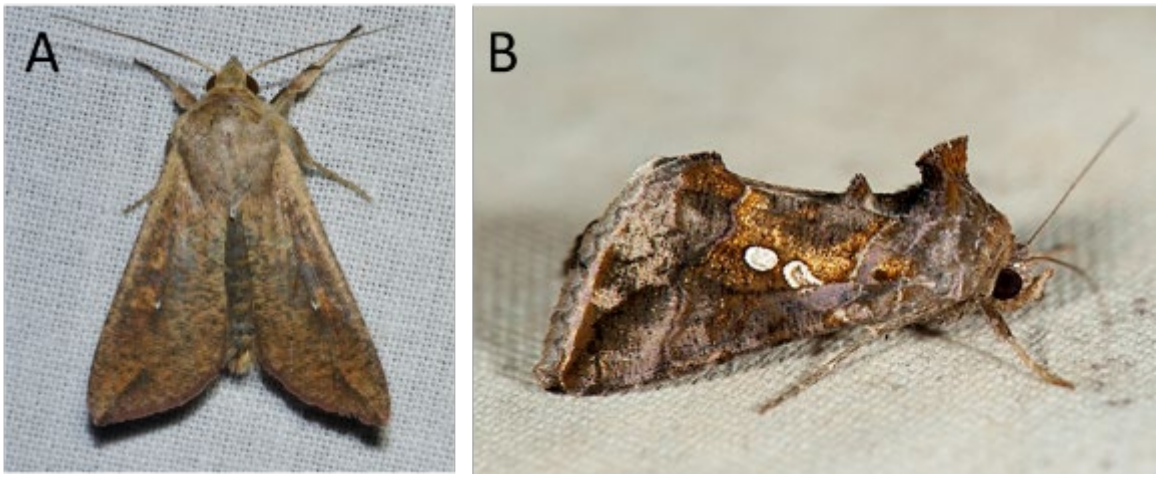

Figure F. Two nonnative noctuid moths that are common in Hawai‘i forests. (A) The fall armyworm moth, *Mythimna unipuncta* (Photo by A. Reago and C. McClarren). (B) The green garden looper, *Chrysodeixis eriosoma* (Photo by LiCheng Shih, cropped). Each is about 30 mm long.

Family Sphingidae (Hawk moths or sphinx moths)

Hawk moths are very large, fat-bodied moths that are active primarily at night (though sometimes at dusk or dawn). There are two genera of native hawk moths (*Hyles* and *Manduca*), and both are known to occur at PTA, though neither was observed visiting flowers in our study. Hawk moths can be important pollinators of plants, especially those with long, narrow, tubular flowers, as the hawk moth proboscis is often very long. *Manduca blackburni* is an endangered species, and has recently been documented breeding on tree tobacco (*Nicotiana glauca*) at PTA (PTA staff, personal communication).

Figure G. Native sphingid moths: (A) Blackburn’s sphinx moth, *Manduca blackburni* (body length about 90 mm) (B) A native sphinx moth, *Hyles calida* (body length about 55 mm), whose larvae feed on native plants in the coffee family.

Family Geometridae (geometer moths)

Like most other moths, geometer moths are primarily nocturnal. Larvae of geometer moths are “inchworms” or “loopers”, referring to their characteristic mode of movement. There are several genera of native Geometridae in Hawaii, in addition to many nonnative species. The genus *Eupithecia* includes many species whose larvae are predaceous. The endemic genus *Scotorythra* includes approximately 50 species, many of them undescribed. Some species, such as the koa looper (*S. paludicola*) undergo occasional population outbreaks, during which they may be more active visiting flowers during the daytime.

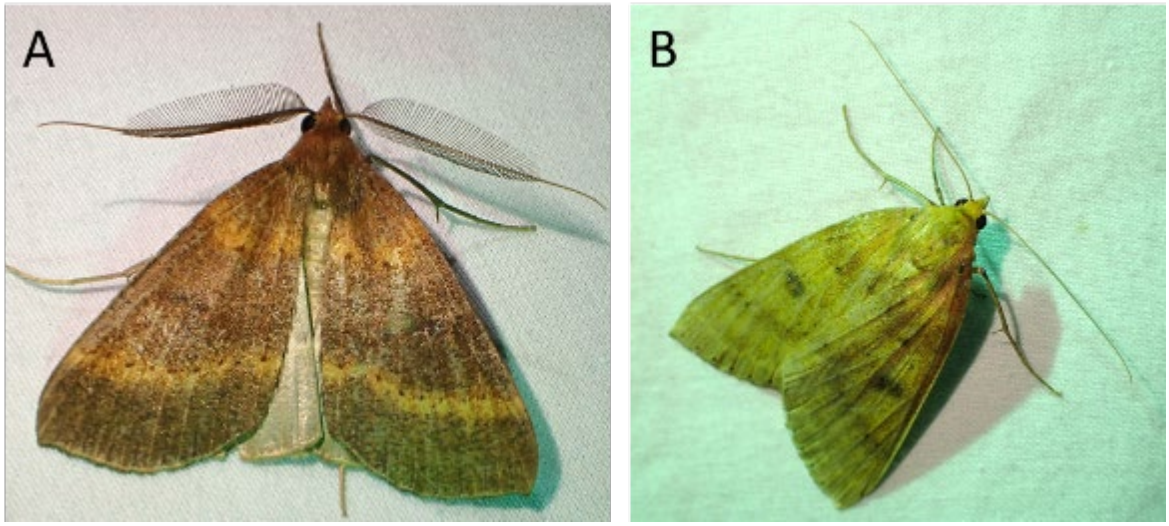

Figure H. The endemic genus *Scotorythra* includes many species whose caterpillars specialize to various degrees on native plants. Male *Scotorythra* (A) have distinct plumose antennae, while females (B) have filamentous antennae. Two different species of *Scotorythra* are illustrated here. Different *Scotorythra* spp. range in size, with body lengths between 15 mm and 50 mm.

Family Crambidae (grass moths)

Crambid moths include several endemic groups, in addition to many nonnative species. Most crambids are relatively small moths, less than 20 cm long, and they often have a streamlined, triangular shape. The moths most commonly observed visiting flowers during the day were *Mestolobes* spp. and *Orthomecyna* spp., both belonging to endemic Hawaiian genera that include both diurnal and nocturnal species.

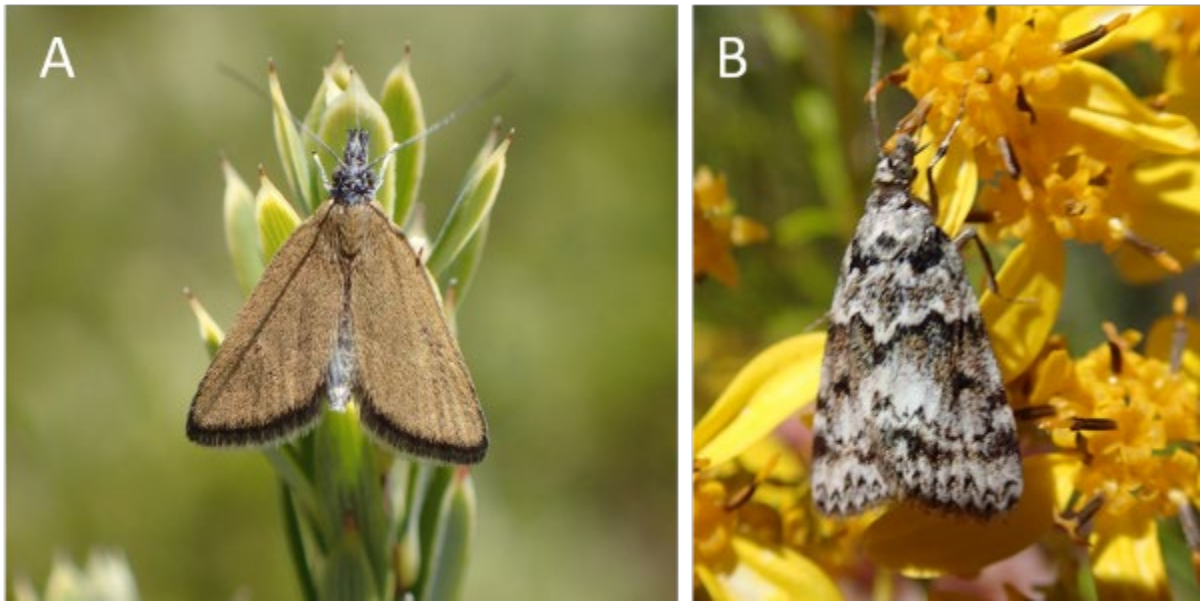

Figure I. Some native Crambidae that are common diurnal flower visitors. (A) *Mestolobes* sp. nr. *homalopa,* roughly 10 mm (from Haleakalā, Maui). (B) *Orthomecyna* sp. on *Bidens* flowers at PTA, roughly 15 mm.

Butterflies (Families Lycaenidae, Pieridae, and Nymphalidae)

Butterflies are common flower visitors and can be important pollinators for some plants. Because they are active during the day, and because there are a relatively small number of native and nonnative butterflies in Hawaii, they generally can be easily identified in the field. At PTA, we commonly observed four butterfly species visiting flowers in our plots, including the endemic Hawaiian blue, *Udara blackburni*. A fifth species, the red admiral, was not observed in our plots, but is sometimes common in dry forest habitat along the Saddle Road not far from PTA.

Figure J. Some butterflies commonly observed at PTA included: (A) the native Hawaiian blue *Udara blackburni* (Lycaenidae), roughly 18 mm; (B) the nonnative bean butterfly *Lampides boeticus* (Lycaenidae), roughly 20 mm (Photo by Z. Cebeci, cropped); (C) the nonnative cabbage white butterfly *Pieris rapae* (Pieridae), roughly 30 mm; and (D) the nonnative painted lady *Vanessa cardui* (Nymphalidae), roughly 35 mm in length. Another nymphalid, the nonnative red admiral *Vanessa atalanta* € was not observed in this study (roughly 40 mm in length).

Family Pterophoridae (plume-winged moths)

Plume-winged moths are small moths with very narrow wings held out at right angles from the body (Figure K). There are several nonnative plume-winged moths in Hawaii, some of which were introduced as biological control agents against *Lantana*. Plume-winged moths are sometimes active during the day, and can be common in high-elevation shrubland and grassland.

Figure K. A nonnative plume-winged moth *Stenoptilodes littoralis*, whose larvae feed on *Vaccinium* spp. (wingspan approximately 20 mm).

Miscellaneous Microlepidoptera

In addition to the above families of larger moths, there are numerous families of tiny moths referred to as Microlepidoptera, some of them only a few millimeters long. Small moths were often observed visiting flowers, but were generally too small to be identified in the field, so were simply recorded as Microlepidoptera.

Diptera (flies)

Family Syrphidae (hoverflies)

Hoverflies were some of the most common flower visitors in our study. These flies, which vary in size (5-20 mm) and shape, feed mainly on nectar and pollen as adults, and as their name suggests, they often hover around flowers. Many species have yellow bands, similar to bees and wasps. In Hawaii, several species of hoverfly are established, all of them nonnative. There are two species in genus *Allograpta* established in Hawaii: *A. obliqua* and *A. exotica*. These small species are very difficult to distinguish from one another in the field, so they were recorded as *Allograpta* sp. when encountered in our study. The larvae of many hoverflies are predaceous, feeding on aphids and other sedentary insects. *Toxomerus marginatus* is a similarly-sized hoverfly that can be distinguished from *Allograpta* spp. by the patterning on the dorsal side of the abdomen (Figure L(A) and L(B)). *Eristalis tenax* is a large hoverfly similar in size and overall appearance to a honey bee. Flies can be distinguished from honey bees by the fact that flies have a single pair of wings, while bees have two pairs of wings.

Figure L. Common hoverflies in dry forest ecosystems of Hawaii. (A) *Allograpta* sp. (roughly 7 mm) (B) *Toxomerus marginatus*, photo by M. McMasters, roughly 7 mm. (C) *Eristalis tenax,* roughly 14 mm.

Family Tephritidae (fruit flies and gall flies)

Although tephritid flies are not typically thought of as pollinators, the larvae of many species feed on developing seeds, so these species are frequently seen on and around flowers. In Hawaii, there are many endemic species in the genus *Trupanea* that specialize on native plants in the family Asteraceae. Some of these breed in flowerheads, while others are stem gallers. *Trupanea* flies are about the size of a common housefly, but their wings are covered in lacelike patterns.


Figure M. *Trupanea cratericola*, an endemic fly that breeds in the inflorescences of *Dubautia* spp. Body length about 6 mm.

Hemiptera (true bugs and hoppers)

Family Lygaeidae (seed bugs)

There are multiple genera of native and nonnative seed bugs in Hawaii, and these bugs can often be found on inflorescences, particularly on Asteraceae, where they feed on

developing seeds. The most commonly encountered seed bugs are in the genus *Nysius* spp. (Figure N), which includes both native and nonnative species. These bugs can be very abundant, especially at higher elevations in Hawaii, as the bugs can be blown up from lower elevations where they feed on weedy asters. Because they consume seeds, they are not generally seen as beneficial to plants, but it is possible that they play a role in pollination of native Asteraceae.

Figure N. *Nysius palor*, a nonnative seed bug (body length about 5 mm).

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(See also volumes 2-17 of Insects of Hawaii, by Zimmerman and various other authors covering various insect taxa)

VI. Monitoring of Arthropod Communities

In a Nutshell:

- Knowledge of the arthropod community and insect pollinators is necessary when investigating pollination dynamics and insect-plant interactions.
- In addition to NIP, habitat and seasonal variation affect arthropod and pollinator communities, and we sampled vegetation arthropods at KKE where arthropod family diversity, but not species diversity, differed between woodland and grassland habitats.
- Spiders were more abundant in the woodland than grassland habitat, and beetles and ants (particularly Argentine ant, *Linepithema humile*) were more abundant in the grassland than woodland habitat.
- Air temperature predicted Araneae, Coleoptera, and Hymenoptera abundance, and precipitation predicted Araneae and Coleoptera abundance.
- Understanding insect ecology enables better management of island communities that are comprised of both native and non-native species.

General background

Arthropod diversity and distributions are poorly known for Hawaiian dry forests, despite these areas being highly threatened and historically diverse. Monitoring and inventorying insects and other terrestrial invertebrates poses many challenges because these taxa are often small, cryptic, and difficult to identify. Additionally, invertebrate populations often exhibit extreme temporal fluctuations due to short lifespans, rapid generation times, and responses to biotic and abiotic factors. Specific drivers of these fluctuations are usually not well understood and the fluctuations can therefore be difficult to predict. Monitoring throughout the year can provide a more complete picture of invertebrate population dynamics. This information can be used by land managers in their assessments of targeted actions or of monitoring of outbreaks, particularly in imperiled ecosystems such as Hawaiian dry forests. Finally, understanding the native and non-native insect pollination community, as well as arthropod predators, is critical for understanding pollination limitations and dynamics for native and endangered plants.

We describe additional sampling techniques that were used to evaluate the arthropod community at KKE, with focus on the key arthropod predators (e.g., ants, spiders) and some likely pollinators (e.g., beetles, flies, moths). We present some results of understory arthropod monitoring throughout one year at KKE, the patterns in arthropod diversity and abundance in the two main habitat types (grassland-shrubland and woodland), and how these diversity and abundance metrics are related to climate variables.

Methods

General arthropod monitoring

We 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 by shaking two branches from 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 nets 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, then subsampling 1 cup from the mixture. The mixed litter was taken back to the office 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 list of six common plant species in KKE for both the vegetation and leaf litter sampling was C*henopodium oahuense, Dodonaea viscosa, Dubautia linearis, Leptecophylla tameiameiae, Sida fallax, Sophora chrysophylla*.

In addition to the quantitative arthropod samples, we periodically sampled nocturnal and flighted arthropods in a more qualitative fashion by using light traps and malaise traps on a few occasions. 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. We also placed bee boxes (i.e., wooden blocks with various-sized holes drilled in the blocks to encourage bee nesting) at each plot and periodically returned to them to assess bee colonization for several months before abandoning this sampling technique on account of an absence of bee colonization. We have not reported results of the light or malaise traps in this User's Guide on account of the low sampling frequency. Furthermore, we have focused our results for this section of the User's Guide on the seasonal and habitat differences of arthropods on the understory vegetation prior to treatment at KKE.

Results

Understory vegetation arthropods

From year-long understory vegetation sampling at KKE, we identified arthropods in 12 orders (or subclasses, in the cases of Acari and Collembola) and 57 families overall. A total of 40 different families were identified from samples in the grassland-shrubland habitat and 39 in the woodland; a total of 3289 and 3071 individuals were collected in the grassland-shrubland and woodland habitats, respectively, over the course of the study. We found significant relationships between the abundance of some arthropod groups and weather and habitat type, as well as significant correlations between certain arthropod families.

Influence of weather on understory arthropod diversity at PTA

Although there were no strong seasonal patterns for number of arthropod individuals overall collected throughout one year in the Hawaiian dry forest, we found that weather variables were significantly related to other arthropod metrics including the abundances of some taxonomic orders. We tested for relationships between local rainfall and temperature and the abundance of the most commonly collected taxonomic orders: Araneae, Homoptera, Coleoptera, Hymenoptera (including Formicidae), Heteroptera, Diptera, and Lepidoptera.

Average daily temperature was a significant positive predictor of Heteroptera, Homoptera, Hymenoptera, and Lepidoptera abundances (all $p < 0.05$). Sum of daily precipitation was a significant positie predictor of Araneae and Coleoptera abundances (all $p < 0.05$). There were no predictive relationships between precipitation or temperature and total arthropod abundance, family diversity, or family richness.

Precipitation varied widely throughout our year-long study; January, February, and April tended to be the driest months, whereas June, August, and October were the wettest months. This pattern of wetter summer than winter months is typical of an El Niño year in Hawai‘i, and was experienced in 2015–2016.

Temperature fluctuations can often trigger arthropod reproduction or changes in activity levels (Hartley and Lester 2003, Hartley et al. 2010), and temperature was a significant predictor of abundances of Hemiptera, Homoptera, Hymenoptera, and Lepidoptera in our study. Average daily temperature varied slightly and followed expected seasonal patterns of warmest temperatures (~16° C) in July–September, and the coolest temperature in January (~10° C). Hartley and Lester (2003) used modeling to demonstrate the number of days at the threshold temperature of 15.9 (+/- 0.8)° C that were needed for Argentine ants (*Linepithema humile*) to develop from egg to adult, indicating the importance of warmer temperature months and microsites at our study site for Argentine ant activity and success.

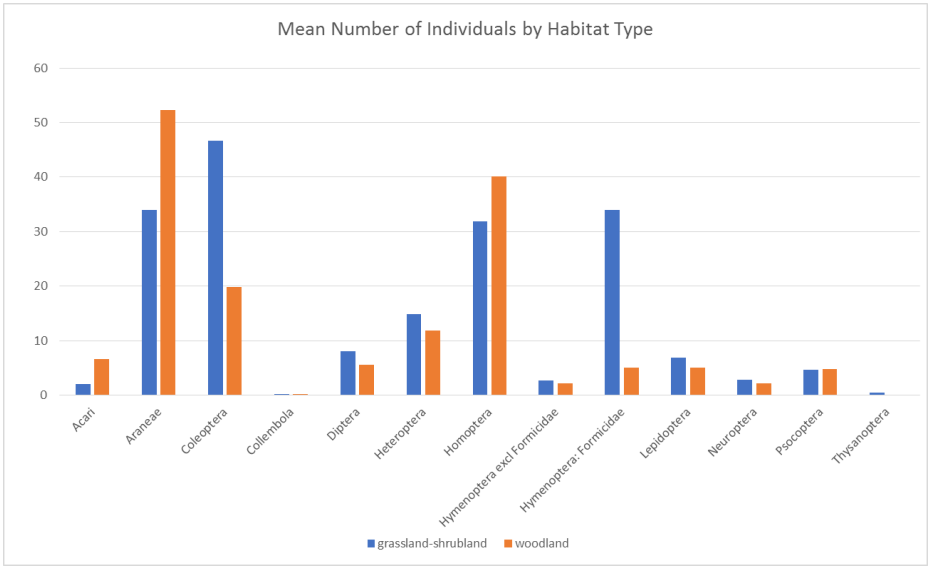

Figure 7. Mean number of understory arthropod individuals in each Order that were collected per ten sampling events during the year-long study, from 20 plots in the two habitat types (woodland and grassland) at our PTA study site.

Influence of habitat type on understory arthropod diversity at PTA

The mean number of individuals in each order by habitat type is shown in Figure 7. Habitat type was a significant predictor of the abundance of certain groups and also a

significant predictor of family-level Shannon diversity. Coleoptera (beetles) and ants (Formicidae) were more abundant in grassland-shrubland, and Araneae (spiders) and Homoptera (leafhoppers, aphids, and relatives) were more abundant in woodland.

Grassland-shrubland and woodland habitat types tend to have differences in vegetation density, habitat quality for different ecological guilds, and soil abundance. While we collected slightly more individuals and families from the grassland-shrubland habitat type, differences by habitat type were pronounced in certain families, perhaps indicating preferences. Coleoptera and Formicidae, for example, were more abundant in the grassland-shrubland habitat type (blocks 1 and 2), while Homoptera and Araneae were more abundant in the woodland habitat type (blocks 3 and 4). We speculate that the woodland provides a better habitat for both web-building spiders, due to the presence of more woody vegetation and taller trees, and for hunting spiders such as *Lycosa*, due to lower vegetation density, providing more bare ground as foraging space. Our spider findings were consistent with those of Vandergast and Gillespie (2004), who found that spiders (including web-builders) were more abundant in the forest interior and edge habitats relative to the more open lava flow habitat on Hawai‘i Island. Cole et al. (1992) found that *Lycosa* spider abundance was significantly reduced by the Argentine ant in high-elevation Maui. Our grassland-shrubland habitat contained more ants, and this may be expected since ants are predatory and predators tend to be early colonizers of lava flows (Ashmole et al. 1992). The woodland habitat exhibited more Homoptera, which are entirely herbivorous; plant-eating guilds like Homoptera tend to be more numerous on older lava substrate, such as demonstrated in the Azores Island chronosequence (Borges and Brown 1999).

Potential interactions among arthropod groups

To explore potential interactions among different groups, for instance predator-prey relationships or competition among taxa for similar resources, we tested for pairwise correlations in abundance between the most commonly collected arthropod families. We found significant relationships between several pairs of arthropod groups, both across the entire sampling area (Figure 8A) and within the two habitat types (Figures 8B, 8C). Some positive correlations among groups were significant and consistent between both habitat types.

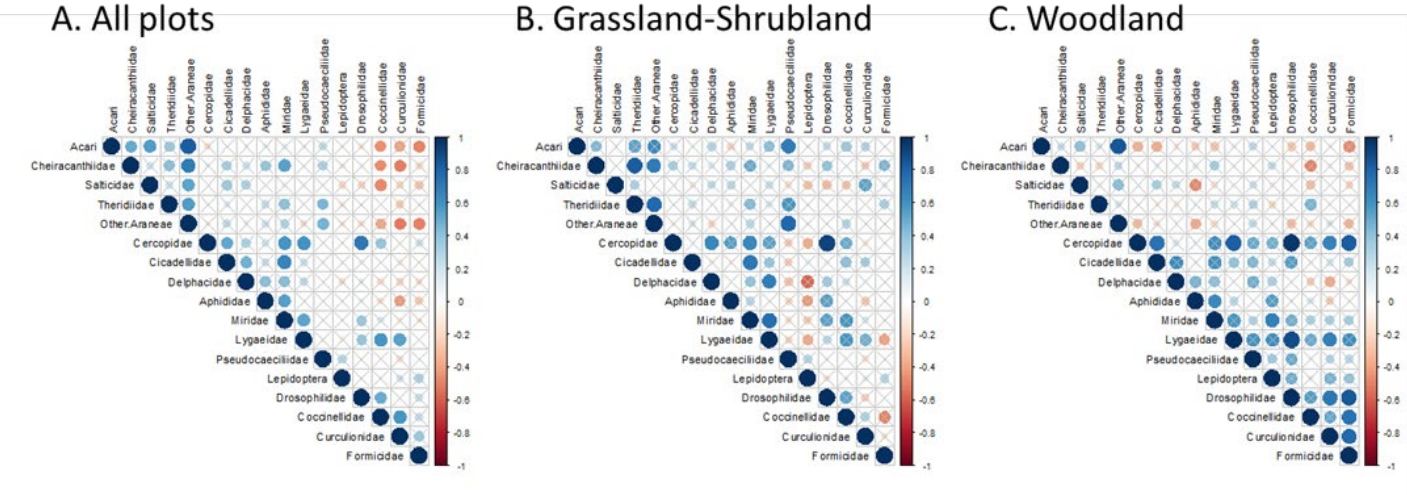

Figure 8. Correlations among arthropod taxa on the plot level (A) across all plots, (B) within the grassland-shrubland habitat in blocks 1 and 2, and (C) within the woodland habitat in blocks 3 and 4. Positive correlations are shown in blue, and negative correlations are shown in red. Circle size and color intensity represents the magnitude of the relationship. Cells with an "X" indicate a non-significant correlation ($p > 0.05$).

Several families of Hemiptera showed consistent positive correlations, particularly Lygaeidae, Miridae, Cercopidae, and Cicadellidae (Figure 9). Although these families often feed on different plant tissues (seeds, leaves, phloem, etc.) they all use sucking mouth parts to tap into their food source. The positive association among these families may be due to common host plant preferences; if certain host plants are more favorable for sucking insects, perhaps due to more accessible vascular tissues or lower levels of chemical defenses, plots with higher numbers of these host plants might be expected to have higher numbers of hemipterans. Top down factors, such as the prevalence of generalist predators or parasitoids in some plots, could also conceivably drive correlations among hemipteran families, but we found no evidence of significant negative correlations between any of the hemipteran families and ladybird beetles (Coccinellidae), which often attack hemipteran insects.

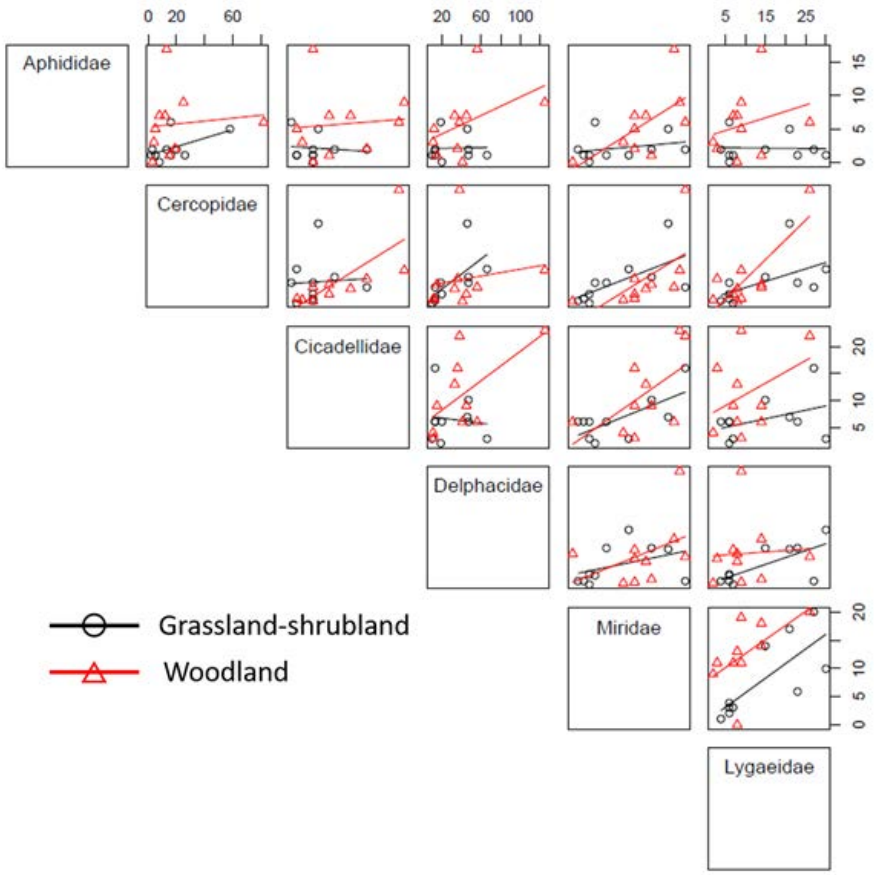

Figure 9. Pairwise relationships between Hemiptera families in grassland-shrubland and woodland plots.

Surprisingly, we found little evidence for negative correlations among ants (Formicidae) and other arthropod taxa. Although there were some significant negative correlations between ants and Acari (mites) and "other Araneae" (spiders) when data from all plots were included (Figure 8A), neither of these negative correlations remained significant when examined separately within each habitat type (Figures 8B and 8C).

Prominence of invasive ants

The highly invasive Argentine ant was abundant in blocks 1, 2, and 3, and occurred in both of the habitat types sampled in our study. This ant is well known to directly threaten native invertebrates and vertebrates, and indirectly affect plant reproduction in Hawai'i (Cole et al. 1992, Krushelnycky et al. 2005, Hartley et al. 2010). Ants were, on average, seven times more abundant in the grassland-shrubland habitat than in the woodlands of our study site, and the Argentine ant was the dominant ant species in both habitat types. Through predictive modeling, Hartley et al. (2010) demonstrated several factors were important for Argentine ant success and spread, including that the number of days at the threshold temperature of 15.9^{o} C, bare-ground microsites for nesting, and lack of thick woody cover. Both our woodland and

grassland-shrubland have an abundance of bare-ground microsites, and the woodland has greater woody cover than the grassland-shrubland, but it is unclear at our site if the nesting temperatures are greater and thus more favorable at the grassland-shrubland habitat or at the woodland habitat. Cole et al. (1992) proposed that Argentine ants may be negatively impacting pollination of native and endangered plants after documenting that these ants reduced abundances of native pollinators, such as larvae of *Hylaeus* bees and *Agrotis* moths, on Maui. With the ubiquity and high abundance of these ant at our study site, changes in ecosystem properties including pollination of native plants, is probable. Efforts to control or eradicate Argentine ants in Hawai'i have resulted in variable, but generally little, success (Krushelnycky et al. 2005). The Argentine ant thrives in higher elevation climates in Hawai'i, like those of our study site, and it has been outcompeted by other invasive ant species in lowland ecosystems in Hawai'i (Krushelnycky et al. 2005).

Study conclusions

At the end of our study, we had gathered species from 57 families, of which 40 families were from the grassland-shrubland and 39 families were from the woodland. While climatic variables influenced individual arthropod groups within our study, the strongest overall predictor of the invertebrate community in each plot was habitat type (grassland-shrubland vs. woodland). Our study provided a year-long overview snapshot of the invertebrate diversity within this high elevation, dryland ecosystem, and highlights the importance of diversity in substrate and cover type. Because the site is home to endangered plants and animals in a number of guilds, this assessment of the invertebrate community and its dynamics provides important baseline information to support the development of understanding and appropriate management decision-making in the future.

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VII. Rodent Populations, Dynamics, and Suppression

In a Nutshell:

- Trapping rather than toxic baits/rodenticides is the best method to control invasive rodents in areas such as PTA where toxicants are unwelcome.
- Trap interference by non-target species, habituation toward baits and traps, and high labor are the main barriers to successful rodent control using traps.
- Tracking tunnels provide an independent assessment of effectiveness of rodent trapping.
- Trapping was effective at suppressing invasive rats (*Rattus rattus*) for an 18-month period, but only the first 3 months for invasive mice (*Mus musculus*).
- Labor for rodent trapping and monitoring (not including transportation time or costs) in our 8 treatment plots, and 12 total monitoring plots, totaled: 192 hours (setup), 373 hours (first 3 months of intense trapping for knockdown), and 984 hours (1 year of trapping and monitoring). On top of labor, the estimated equipment cost for traps, trap boxes, and monitoring equipment was $9,500.

General background

Traps and toxicants to suppress invasive rodents

The two most common methods to suppress invasive rodents on islands and in natural areas include the use of traps and/or toxicants. There are two types of rodent traps that receive the most use in Hawai‘i and other natural areas worldwide: classic snap-traps (e.g., Victor brand, U.S.A.) and A24 self-resetting rat + stoat traps (Goodnature brand, N.Z.) (Figure 10). The benefits of using snap-traps are that they are inexpensive (~$2 per trap) and they are well-tested and used for >100 years (Victor brand since 1898, rat and mouse traps); however, they require frequent re-setting and they often require placement inside a cover to decrease non-target interference. A24s can be triggered and fire ~24 times before the CO_2 cartridge must be replenished and the bait/lure is now automated out of a reservoir such that servicing A24s may not be necessary for 3-6 months in the field; however, they are ~$160-200 for each trap and are relatively new on the market (~6 years) and have not had nearly as much testing and use as snap-traps.

Figure 10. Images of commonly used rat traps in Hawaii. Left image is a Victor snap-trap, and right image is a Goodnature A24 automatic self-resetting trap. Bait lure is placed on the yellow treadle on the snap-trap, and within the black reservoir on the upper right side of the A24 trap.

There is currently only one type of toxicant—diphacinone (an anticoagulant rodenticide)—that is registered for conservation use as a rodenticide in Hawaii. The two formulations of diphacinone bait approved for conservation in Hawai‘i include Ramik bait blocks and Diphacinone-50 pellets (see EPA labels for these products). Both can be applied in bait stations (Figure 11), and Diphacinone-50 can be applied by hand- or aerial-broadcast. Even though these rodenticide bait products are legal to use in Hawaii, they must be used according to label and they must have the land owner’s permission prior to use them—in some cases this may require completion and approval of an Environmental Assessment (EA) or an Environmental Impact Statement (EIS) prior to rodenticide use. The EA process generally takes a minimum of 3-6 months to gain approval, whereas an EIS generally takes longer (e.g., a minimum of ~1 year+).


Figure 11. Images of diphacinone rodenticides used in a bait station in block formulation (Ramik minibars; left picture), and in pelleted formulation (Diphacione-50; right picture) that can be used in bait stations or through hand- or aerial-broadcast. Both formulations contain 50 ppm active ingredient (diphacinone) that is mixed in a cereal bait matrix. Diphacinone is the only rodenticide approved for conservation use in the State of Hawaii.

Traps (snap- and A24s) and diphacinone toxicants are by far the two most commonly used and effective methods of invasive rodent control on landscapes in Hawaii. A summary of the pros and cons of each of these three methods in Hawai‘i are as follows:
-Snap-traps: Inexpensive for equipment (including placement in boxes to limit non-target interference), but very labor intensive and thus very expensive for longer-term use. Effective at suppressing and maintaining both rat and mouse populations (but see difficulties in maintaining target levels for mice below). Best used for very localized rat and mouse suppression to protect confined resources, but not economically sustainable on landscape scales for long-term use due to the need for frequent trap maintenance (i.e., resetting, rebaiting).
-A24 self-resetting traps: Very expensive for equipment, but large saving of labor over the long-term if the trap visits/maintenance is at least occurring at 2-month intervals (3-4 months has been the maximum typical interval for mesic forest on Oahu). Effective at maintaining rat populations, but not mice, in most natural areas in Hawai‘i (but ineffective at obtaining target levels in some habitats, possibly due to bait/lure attractiveness and/or rat densities).
-Diphacinone toxicant: Relatively inexpensive and relatively low labor (refill bait stations approx. monthly). Major downside of this method is that it is not a species-specific toxicant and it gets into the food web and persists for several months at low levels. It is also not well accepted by the majority of the public and some land owners, even though cases of non-target mortality due to exposure to the toxicant are rare. Effective at both rat and mouse control in localized and landscape-level use.

Additional methods that have been practiced elsewhere to manage invasive rodent populations and their associated damage include: physical (e.g., barriers, frightening devices), chemical (e.g., glues, tracking powder, repellents, attractants, sterilants, fumigation), and biological (sniffer dogs, habitat modifications, predators) methods (for more detail see Witmer and Shiels 2018).

How to determine success of large-scale rodent suppression

Determining success in rodent suppression requires the use of an independent monitoring device to 1) assess if your rodent suppression methods are indeed reducing the rodent population (e.g., using tracking tunnels, chew blocks, or trail cameras), and/or 2) assess whether the natural resource that you are trying to protect is benefitting from your rodent suppression (e.g., % seed/fruit destroyed, % pollinating insect present, % birds fledge from nest). Unfortunately, there is no known rodent suppression level or tipping point that is universal for protecting all native species of conservation concern. There has been a single New Zealand study involving an endangered bird, as well as a single Oahu study involving an endangered plant, that provide guidelines for the level of tracking tunnel activity that must be

achieved in order to protect the reproductive rate of the focal species of concern. These levels were <10% rat tracking in the New Zealand study, and <20% rat tracking in the Oahu study (Pender et al. 2013). Thus, for our PTA study, our target level of rodent tracking (for both rats and mice) to continuously maintain within the rodent treatment and combined treatment plots was <20%; we hypothesize that maintaining rat and mice populations below this level will benefit target native insects and plant pollination. Further results from our PTA study may help us establish a minimum rodent tracking percentage that must be maintained to benefit target pollinators or native and endangered plant pollination at PTA.

During our study at PTA, we used tracking tunnels to monitor rodent populations. By simultaneously measuring rodent activity in treatment and untreated plots, we could be sure that we were interpreting fluctuations in the tracking tunnel activity due to our intentional suppression or that of natural seasonal fluctuations. As described above, we adjusted our suppression in accordance with our tracking tunnel results (e.g., switching bait, identifying nontarget interfering species using trail cameras, and reinforcing mouse trap boxes when rodent tracking became elevated [especially >20% tracking]; and reducing the number of traps armed so that we could improve efficient use of our labor when suppression was below our target level of <20%). This adaptive management strategy is necessary to reach management goals.

Methods

Rodent trapping and monitoring at PTA

The objective of our invasive rodent portion of our PTA study was to experimentally test whether snap-traps, placed in plastic boxes to limit non-target interference, were effective at suppressing invasive rodents. Our design had a total of 12 plots, each 2.25 ha, that included three treatments (n = 4 per treatment): rodent treatment (where rodents were suppressed), combined treatment (where rodents+ants+yellowjackets were suppressed simultaneously), and untreated control plots. We utilized and monitored the core 150 m x 150 m are of each of these 12 plots. In each rodent and combined treatment plot, a grid of 169 mouse traps (each 12.5 m apart) and 49 rat traps (each 25 m apart) was installed and armed continuously for 1.5 years, with bait refreshed each 1-2 weeks. All snap-traps were placed in corrugated plastic boxes to reduce non-target interference, such as trapping non-target vertebrates, especially birds (Figure 12). The two types of snap-traps accounted for the differences in rodent sizes (mice are ~8 times smaller than rats), and the distances between each trap type reflected the daily movement differences between rats and mice (i.e., mice have about half as small of daily ranges than rats). Due to the high intensity of labor required to visit, clean, reset, and rebait each trap (and record data), we could not service all traps in a day so we completed trap servicing by block such that all rodent suppression plots (i.e., all rodent and combined treatment plots) could be serviced each week with our crew of 4-5 staff members. All activities involving rodents were approved under the USDA Wildlife Services IACUC Study Protocol number QA-2452.

Figure 12. Images of rodent snap traps at PTA that were placed in plastic boxes to limit non-target inference. Top picture shows a mouse snap-trap attached to a plastic sleeve that will be placed into the box. Wooden lath (paint-stir-sticks) were attached to some boxes to reduce large vertebrates (game birds, cats) from tipping them over. Bottom picture shows a trapping station where the large box on the left has a rat snap-trap in it and the small box on the right has a mouse snap-trap in it (both traps on plastic sleeves to ease the safety and efficiency of checking, resetting, and rebaiting traps).

We monitored rodent populations in all 12 plots using tracking tunnels, which are baited ink cards placed in tunnels so that foot prints of animal visitors can be identified (Figure 13). The foot tracks of rats and mice can easily be distinguished, as can be the foot tracks of non-target species such as cats, mongoose, insects, and birds. For each monitoring session with tracking tunnels, 17 tracking tunnels per plot (spaced ~25 m apart) were set by placing an inked and baited tracking cards inside each tunnel, and tracking cards were left for three consecutive days before removing the cards and identifying and recording the foot tracks to species. We did not attempt to quantify the number of tracks on a given card, but instead used the tracks to assign presence or absence of each rodent species targeted. Tracking tunnels were used to monitor rodent populations for one year prior to experimental treatments, during eight periods during the 1.5 years of experimental treatment (July 2016–January 2018), and for three periods

(February, March, and April 2018) post-experiment. For each tracking tunnel monitoring event, we determined rodent tracking in the rodent treatment, combined treatment, and untreated control plots. Based on tracking tunnel results, we often adjusted our levels of rodent trapping to improve rodent suppression efficiency by: reducing the number of armed traps when suppression was effective, occasionally changing the lure/bait from peanut butter to Goodnature chocolate lure for a short period, adding a wooden lath to the trap boxes to reduce occurrence of the mouse trap boxes from getting knocked over by game birds and cats, and adding monitoring cameras aimed at trap boxes to better evaluate non-target trap interference.

Figure 13. Images of inked foot tracks and tracking tunnel equipment used at PTA for monitoring invasive rodent populations. The center picture shows the black plastic tracking tunnel with a baited and inked white tracking card ready to be inserted. The left picture shows rat tracks only, and the right picture shows rat tracks (larger foot prints) and mouse tracks (smaller foot prints).

Results

Barriers and short-comings to general rodent suppression within our PTA project

There is a wide range of barriers to effectively manage and suppress invasive rodent populations, and many of these are site specific and cannot be easily predicted without frequent and diverse monitoring. As outlined above, rodent traps and rodenticide baits are not species-specific control devices, and therefore other animals in the area may interfere or be harmed by using such devices. However, precautions can be taken, such as using trap-boxes, and this prevented most non-targets from being trapped (i.e., two mongoose, one feral cat, and

four Erckel's francolin game birds—all non-native and unwanted species in the conservation area—were lethally trapped by the rat traps as bycatch during the 1.5-year study). An important barrier to effective rodent trapping is that target rodents can get habituated to the traps in place, and otherwise may not go into the trap box or trigger the trap. We noticed this at PTA once we aimed trail cameras at the trap boxes. Effective trapping takes skill—setting the traps so they are 'hair-trigger' is important in trap boxes, changing the type of bait/lure on occasion to help revive interest in a long-term device, and preventing non-target interference with the traps and trap boxes are all important considerations and items that required adjustment (adaptive management) in our PTA study, as well as in other studies.

Trap interference at PTA was considerable. The francolins (particularly Erckel's and black francolins) either knocked the mouse trap boxes over before springing the trap and eating the bait, or simply sprang the trap and eat the bait. Cats and mongoose also interfered with our trapping by pulling out the traps from the boxes and consuming the dead mice that were trapped. Without the use of game cameras and some personal observations in the field, we would not have known which non-target species were responsible for traps pulled out of the boxes, boxes tipped over, and traps sprung. Non-target invertebrates are also known to consume the peanut butter bait, thereby rendering the traps unattractive to target rodents. The most common non-target invertebrates that were found spoiling or consuming the bait on traps at PTA were cockroaches and ants. Within mesic and wet sites in Hawaii, slugs commonly interfere with rodent trapping by sliming over bait and consuming it, but there are very few slugs that were found in the arid study site of PTA, and the one confirmed species occasionally present was *Limax maximus*.

Interference from non-target species during rodent trapping can be managed in a number of ways, including adjusting trapping methods, installing barriers through which vertebrates larger than rodents cannot pass, and suppressing the populations of non-target vertebrates (especially if they are non-native pest species, like all of those that we identified in our PTA study). In our PTA study, we had minimal success at local suppression of mongoose that were visiting our plots. We had used DOC-250 traps (New Zealand design and product), which are powerful snap-traps. The DOC-250 traps have worked exceptionally well in other locations in Hawai'i (e.g., Oahu), and we used the same traps and methods as those used by Oahu Army Natural Resources. We began to use the DOC-250s to target mongoose before we had determined that non-native game birds were in fact the more substantial vertebrate pests that were interfering with our rodent trapping. SEML/NRO has found success in keeping out large vertebrates (including game birds) from accessing rodent traps by covering the area around each trap with chicken wire—the chicken wire has small enough openings to keep large vertebrates out but allows small vertebrates (rats and mice) access to the traps. However, it is likely that rats would have to be quite motivated to press through the small holes in the chicken wire, and a slightly larger-sized opening, such as that in common chain-linked fencing, would provide less of a barrier for invasive rats to pass through. Mice are expected to freely pass through both chicken wire and chain-linked fencing without any deterrence.

Overall rodent trapping at PTA

In nearly 3 years of rodent trapping at our plots at PTA, we only detected two species of rodent: black rats (*Rattus rattus*) and house mice (*Mus musculus*) (Figure 14). Given our extensive trapping at KKE, it is unlikely that the other two invasive rodent species that are established in Hawai‘i (*R. exulans* and *R. norvegicus*) are present at PTA in woodland-grassland habitats. We determined that both rats and mice could be effectively suppressed (<20% detection in tracking tunnels) for ~4 months after trapping initiated; yet only rat, and not mouse, suppression was sustainable thereafter. Trail camera evidence revealed that mice became habituated to traps in some cases, leading to trap avoidance, and that some non-target animals interfered with mouse traps. Therefore, 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. In contrast, if field labor can support it, grids of snap-traps appear to be a sustainable method to maintain suppressed rat populations at <20% tracking at PTA.

Figure 14. Black rats (*Rattus rattus*; upper photograph, and from http://aepma.com.au) and house mice (*Mus musculus*; lower photograph, and from Ed Freytag) were the only two rodent species found in our study plots at KKE. An adult black rat is approximately 10 times larger (by mass) than an adult house mouse in Hawaii.

Rat suppression at PTA

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 15). 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 15).

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-experiment levels within three months (Figure 15).

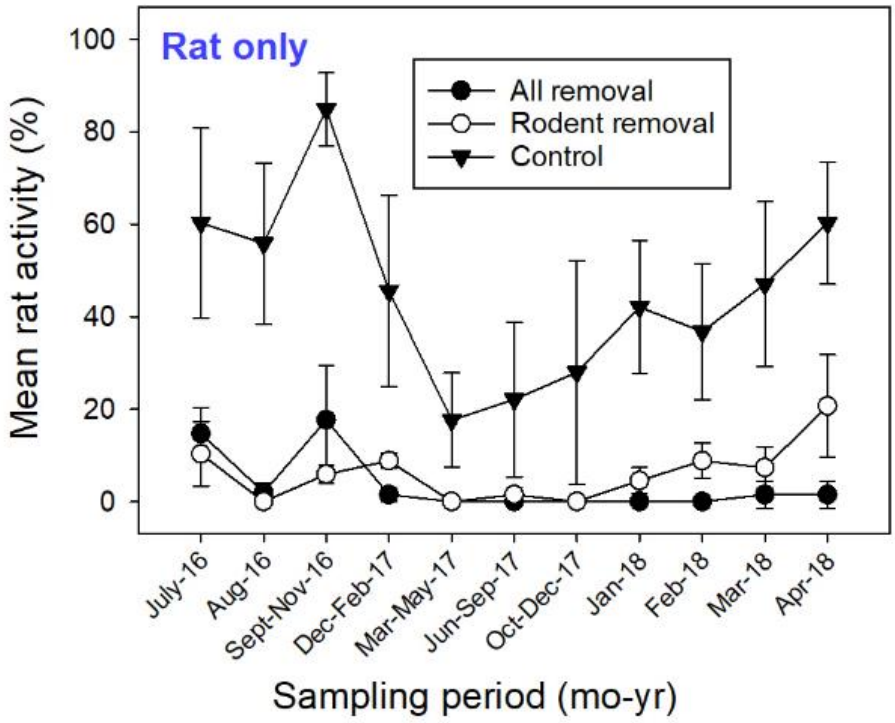

Figure 15. PTA rat tracking tunnel results in the rodent treatment (Rodent removal) and combined treatment (All [rodents+ants+yellowjackets] removal), and the untreated Control or reference plots where rodents were not suppressed. Each data point represents an average of n = 3 or 4 treatment plots that 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-experiment period that occurred prior to July 2016; the post-experiment period was February–April 2018.

Mouse suppression at PTA

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%), aside from the first four months following suppression (Figure 16). As a mirror opposite to the spike in rat abundance in September–November 2016, house mouse abundance simultaneously plummeted naturally during this period, which may be a result of competition between these two rodent species.

Once mouse trapping ceased in January 2018, we monitored mouse incursion into the plots for the subsequent three months. Mouse tracking had recovered to pre-experiment levels within three months in some plots, but not in others (Figure 16).

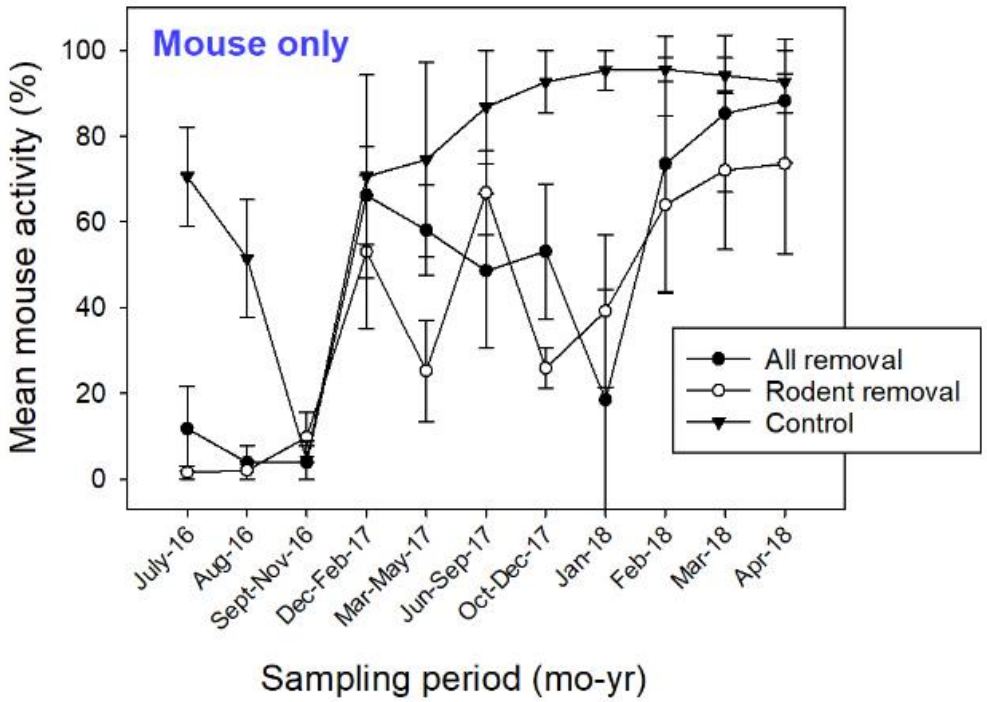

Figure 16. Results for house mouse tracking tunnels at PTA in the rodent treatment (Rodent removal) and combined treatment (All [rodents+ants+yellowjackets] removal), and the untreated Control or reference plots where rodents were not suppressed. Each data point represents an average of n = 3 or 4 treatment 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-experiment period that occurred prior to July 2016; the post-experiment period was February–April 2018.

Best Management Practices: General to all predator control projects

For any predator control project, it is of the upmost importance to follow the listed five general principles:

1. Identify the problem, objectives, and questions (e.g., which predator(s) are targeted, and which native species are you trying to protect?).
2. Identify control tools and field design considering best management practices (BMPs), nontarget risks, and available staff and funding (e.g., which is most realistic given your constraints?).
3. Identify the monitoring method, evaluation procedures, and timeline (e.g., how will predator and native species be monitored?).
4. If feasible, proceed with project implementation.
5. Use adaptive management (e.g., using frequent monitoring, determine if goals are being met over the long periods and identify and correct parts that may be failing).

Best Management Practices: Specific to PTA project

Rodent control requires a great deal of attention, labor, and equipment; and importantly, such rodent control projects are indefinite. Therefore, it is critical to have a well-vetted plan and to be realistic about the resources needed to fulfill the plan and meet the conservation goals. This is why the five general principles of predator control MUST be followed. It is unclear the level of rodent damage that is occurring at PTA and which species are experiencing elevated rodent damage. Clearly there are rats and mice in all plots and habitats that we sampled at PTA, yet the level of damage that they are causing will certainly depend on the species of interest combined with local site conditions. In general, rats are much more damaging to native plants and birds than mice (see Shiels et al. 2014); and both mice and rats consume invertebrates so the arthropod pollinators and decomposers at PTA would be at risk from these rodent species. It is advised to make sure you have demonstrated the need to suppress invasive rodents prior to doing so, and that you monitor the rodent populations and the target natural resource that you are trying to protect during any rodent management project—without this you are unlikely to meet your management goals while wasting time and money, and possibly doing more harm than good (e.g., causing rodents to be habituated to traps so future control efforts using traps may be unsuccessful using traps; putting unnecessary toxicants into the environment when using rodenticide baits, and risking rodents developing resistance to these toxicants). Furthermore, monitoring the rodent population is among the least expensive actions during a rodent suppression project (e.g., $6 hours/plot/year in our study; see below and Table 2).

The scale of rodent suppression is particularly important to establish for best results in meeting management goals. Is a rat trapping grid of 150 m x 150 m necessary to protect a small population of a target natural resource in the center of such a plot? Should there be a greater density of mouse traps (tighter than 12.5 m spacing) or more frequent re-baiting to maintain mouse densities to target levels? Unfortunately, our study did not attempt to address these questions, but plot size (and buffer zones), trap spacing, and trap-check frequency must be adjusted for the given site and will likely influence project success. If such factors cannot be determined at your site(s), start by using parameters that have been tested elsewhere and then be open to adaptive management based on your monitoring results.

Cost breakdown for PTA project

As a final (and important) area to assist in the establishment of best management practices for rodent suppression projects like ours, we have provided a cost breakdown for the various field activities that accompanied our SERDP PTA rodent suppression study. Please keep in mind that these values apply to just this one project (i.e., one dryland site at PTA, one type of rodent suppression methodology).

The size of plots that we aimed to protect, and the methodology used, required 3-5 people working full-time; we employed a group of five because of the many duties beyond rodent trapping and monitoring that were a part of our larger project. Once our trap grids were established, there was a necessary knockdown period where traps were checked frequently

(daily or multiple times a week), lasting 3 months and costing 373 hours of labor for completing all 8 plots; then to maintain the rodent trapping grids for all eight plots each year, it would cost about 912 hours of labor, but will vary depending on how many traps are set upon each check (i.e., see 'potential scenarios' for 'trap maintenance' in Table 2). Some evidence pointed to scaling back the trapping frequency to servicing traps each 2 weeks instead of each week, and this is how past rat control projects maintained their snap-trapping grids on Oahu with mediocre success (Shiels et al. in press). At PTA, we implemented trap checks each 2 weeks for the last 4 months of our study (i.e., September 2017–January 2018). Although not statistically analyzed, it appears that the 2 week trap checks were similar to the 1 week trap checks in their effectiveness in maintaining suppressed rat and mouse populations (see Figures 15 and 16); thus, altering the duration between trap checks to determine the most efficient level is a recommended area for future investigation. Even under a 2-week frequency, this is a serious labor investment (e.g., 54-108 hours/plot/year depending on the rodent trap maintenance strategy; Table 2).

Duron et al. (2017) conducted an assessment of invasive rat control (not eradication) on islands worldwide, and part of their analysis revealed that rat control projects cost a median of $17,262 ($252/ha), or mean of $39,766 ($843/ha), per year. If we paid all labor at the State of Hawai'i minimum wage of $9.25 in 2017, our project cost was approximately $25,000 for all equipment, setup and knockdown, 1 year of maintenance, and monitoring for rats and mice. Furthermore, and using the $9.25 hour rate, each year cost for trapping (trap maintenance) and monitoring would be about $9,102, which is $337/ha if the control plots are included ($506/ha if only the eight rodent suppression plots were included). One major difference between Duron et al. (2017) estimates for rat control and ours was that our PTA project included both rat and mouse control (e.g., there were >3 times the number of mouse traps per plot as rat traps). A more likely scenario that we practiced was to pay our field staff on average $16-$20 per hour, making total costs (using $20/hour) estimated at $29,180, instead of ~$25,000 (using $9.25/hour). As a reminder, these cost estimates do not include any transportation costs (e.g., from Hilo to PTA by vehicle, vehicle costs, and hiking out to each plot).

Table 2. Labor hours and associated costs for rodent suppression and monitoring at PTA, Hawaii. Costs for labor were calculated based on the 2017 hourly wage of $9.25 (note that in 2018 it was $10.10; and $8.50 in 2016), once out at the plots (i.e., travel time was unaccounted for; KKE is approximately 1.5-2 hour travel by vehicle from Hilo, where staff held their duty stations; once at KKE, staff walked to field plots, which could take up to 30 minutes one-way). Our project had 8 rodent suppression plots (each 150 x 150 m or 2.25 ha), and monitoring occurred at all 8 rodent suppression plots and an additional 4 plots that were the untreated control plots. Treatment knockdown was April–June 2016, and trap maintenance occurred from July 2016 through January 2018, which was 18 months; but the first 12 months were used for estimates below).

Activity	# traps per plot: R = Rat,	Effort rate (hr/plot)	Frequency	Effort rate (hr/plot/year)	Our estimated

	M = Mouse				total project hours[¥]
Treatments in place	49 (R), 169 (M)	24	Once per plot	24 hr/plot one-time	72
Initial knockdown (2-3 months)	Variable; Full then Reduced/ Perimeter	42-53 hr/plot over 2-3 months	Variable; daily to weekly	42-53 hr/plot over 2-3 months	373
Potential Scenarios:					
Trap maintenance: Full	49 (R), 169 (M)	4	Weekly	208	1664
Trap maintenance: Elevated	49 (R), 103 (M)	3	Weekly	156	1248
Trap maintenance: Reduced	49 (R), 73 (M)	1.5	Weekly	78	624
Trap maintenance: Perimeter	24 (R), 48 (M)	0.75	Weekly	39	312
Our project's actual trap maintenance	Variable: all 4 levels used	0.75-4	Weekly	109-117	912
Rodent monitoring (ea. 3 month; 1 yr pre-experiment; 1 yr post-experiment)	17	1.5	Quarterly	6	72[€]
Take down	392 rat, 1352 mouse trap+box+ tracking tunnels	24	Once per plot	24 hr/plot one-time	192

Rodent trapping equipment	392 rat, 1352 mouse trap+box+ Spares	NA	NA	NA	$6,500
Rodent monitoring equipment	12 plots x 17 per plot = 204 tunnels plus spares & supplies	NA	NA	NA	$3,000

[¥]=based on rodent traps in all 8 plots (each 150 x 150 m or 2.25 ha), and for monitoring an additional 4 plots (the untreated Control plots). For "Potential Scenarios" Activity, the annual estimated costs are shown.

[€]=12 plots used to calculate because tracking tunnels were run on the 8 rodent suppression plots and the 4 untreated Control plots where no trapping occurred.

Study conclusions

In our 150 m x 150 m (2.25 ha) plots at PTA, we determined that both rats and mice could be effectively suppressed (<20% detection in tracking tunnels) for ~4 months after trapping initiated; yet only rat, and not mouse, suppression was sustainable thereafter. Trail camera evidence revealed that mice became habituated to traps in some cases, leading to trap avoidance, and that some non-target animals interfered with mouse traps. Therefore, it is our suggestion that in areas with high mouse populations, grids of snap traps are unlikely to be a sustainable management technique for long-term house mouse control. Instead, use of diphacinone bait and repeater mouse traps, such as are currently implemented at Ka'ena Point on Oahu, could be considered for sustaining mouse populations at low levels. In contrast, if field staff and labor can support it, rat suppression using grids of snap-traps appears to be sustainable to maintain suppressed rat populations at <20% tracking at PTA; based on past studies in Hawai'i (e.g., Pender et al. 2013; Shiels et al. 2019), we feel that this level of rat suppression is likely to be sufficient to protect target natural resources that are vulnerable to rat predation.

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VIII. Ant Populations, Dynamics, and Suppression

In a Nutshell:

- Ants are not native to Hawai‘i and are an important threat to native arthropods.
- Ants can be surveyed and monitored using baits and visual surveys.
- Formicidal baits are the most effective options for controlling ants, though there are relatively few options registered for use in natural areas in Hawaii.
- Ant populations at PTA are patchy, and different ant species occur in different areas.
- Maxforce Complete Granular Insect Bait suppressed Argentine ants and ghost ants at PTA, but populations often rebounded quickly.
- It was necessary to apply Maxforce repeatedly every two to three months to maintain suppression of ant populations.

General background

Ants in Hawai‘i

Due to the extreme isolation of the Hawaiian Islands, its native biota is disharmonic, since organisms vary in their abilities to disperse over long distances and colonize new habitats. Many taxonomic groups are underrepresented compared to continental biota, or are completely absent. Social Hymenoptera (bees, wasps, and ants) are one of the groups absent from Hawaii's native fauna. Although there are many endemic solitary wasps and bees, there are no native colony-forming wasps, bees, or ants. Unfortunately, there is a long (and ever-growing) list of nonnative ants that have established in Hawaii, and many of these are highly invasive. At the time of preparing this guide, at least 57 ant species have established in the islands (Krushelnycky et al. 2005, https://www.antweb.org/island.do?name=Hawaii).

In continental ecosystems where they are native, ants play major ecological roles through predation of other invertebrates, competition with other ants and predators, and mutualisms with plants. Because Hawai‘i's native flora and fauna have not evolved with social insects, ants are highly destructive in Hawaiian ecosystems. Not only do they prey directly on native arthropods, but they often farm pest insects such as scales, mealybugs, and aphids, which can severely impact the health of native plants.

Methods

Surveying and monitoring ants using baits

Ant management can consist of several stages, including initial detection and mapping, species identification, and control. Surveying and monitoring are critical parts of ant management during all of these stages. Baits are commonly used during surveys for ants, since they can give a relative measure of abundance. Because forager ants recruit additional foragers when they find a food source, baiting can amplify the chances that ants will be detected and can be a reliable and efficient way to sample for ants over large areas.

Different ant species are attracted to different types of foods; some are attracted to sweet sugary foods, others to greasy lipid-heavy baits, and others to high-protein foods. It is

important to take this into account when selecting baits for monitoring and surveying. For monitoring a single species, a single type of bait can be used. When surveying broadly to document which ant species are in an area, it is best to use a variety of baits, typically a carbohydrate (e.g. corn syrup, fruit jelly, or honey), a lipid (e.g. peanut butter), and a protein (e.g. canned tuna or chicken). These can either be deployed separately (which may reduce competition among multiple species) or together on the same bait card or vial (as in Figure 17).

There are several methods for deploying baits. Baits can be placed in snap-cap plastic vials so that they can be quickly capped upon retrieval and brought back to the lab for sorting, counting, and identification. Bait is smeared on the inside of the vial, near the entrance, and the vial is placed open on its side, with the bottom of the vial slightly lower than the top, so that bait does not drip out of the vial. This method is useful when conducting broad surveys for multiple species that are difficult to identify in the field. Alternatively, baits may be deployed on paper index cards. A small amount of bait (about 1 cm diameter) is placed in the center of the card. Bait cards must be weighed down with small pebbles and placed somewhere sheltered from wind so that they don't blow away (Figure 17). Ants will typically gather around the perimeter of the bait, where they can be counted without disturbing them. When ants are very abundant, there may be more than a hundred ants recruited to a single bait, making counting difficult and tedious. In these cases, a digital camera may be useful for monitoring; baits can be photographed so that more precise counts can be made from the photographs in the laboratory.

Figure 17. An example of an ant bait card baited with honey, canned tuna, and peanut butter.

Bait placement is important. Bait cards or vials should ideally be placed in locations sheltered from the direct sun and wind (e.g., at the base of a shrub or beneath the shelf of a rock). Baits are typically left in place for 45 to 90 min before counting to allow enough time for

forager ants to detect and recruit to the bait. If baits are left for too long, they may dry out or degrade, or be consumed by non-target animals such as wasps or rodents.

There are some limitations to sampling for ants using baits. Ant foraging activity is highly dependent on weather conditions, and most ants will not be active in rainy or excessively cold or hot weather. This can be particularly problematic when using baits to compare ant abundance in different sampling events, for instance, when examining seasonal fluctuations of ant populations, or comparing ant abundance before and after pesticide treatments. There are several ways to account for the variation in weather and increase consistency among sampling events. First, it is important to establish weather requirements that dictate whether or not sampling can occur. For instance, do not sample for ants during rainy or misty weather, and try to sample during the same time of day (e.g. late morning to early afternoon) during each sampling event to better control for the effect of temperature or solar radiation. Second, when the goal is to compare ant abundance in multiple areas (e.g., in a treated plot and an untreated plot), conduct sampling simultaneously in all plots that will be compared directly to one another.

Another limitation to baits as a sampling method is that they vary in attractiveness across species. Ants that form small colonies, or ants that are primarily predaceous rather than scavengers, may not recruit to baits at all. If multiple ant species co-occur, a more dominant species may competitively exclude another species from baits, so bait counts should generally not be used to compare densities of two different species in the same area. Additionally, when ant populations are very low, baits may not reliably detect them. For instance, when attempting complete eradication of ants from an area, baits should not be relied on exclusively to assess success. Small colonies may persist in an area, but at such low densities that foragers do not find baits or recruit other workers. In these situations, a combination of baits and visual searches for foragers and nests, perhaps in combination with passive sampling methods such as pitfall traps or sticky traps, should be used over many months to assess eradication.
That said, baits are highly useful for mapping and monitoring ant populations, and are particularly appropriate when dealing with a single species whose biology is well known.

Ant identification

Proper identification of ants to the species level is essential for ant management, as the species identification will determine the best baits to use for monitoring and the options available for chemical control. There are several online resources for ant identification in Hawai‘i. Reliable identification is based on traits such as the number of nodes in the petiole (the “waist” between the thorax and abdomen) or the number of antennal segments. It generally requires a microscope and some knowledge of insect morphology. Before taking any actions to control ants, confirm species identifications with an expert such as the staff of the Hawai‘i Ant Lab in Hilo or Dr. Paul Krushelnycky at the University of Hawai‘i at Manoa.
Ant web:
https://www.antweb.org/island.do?name=Hawaii
Includes a species list for Hawai‘i and high quality images of ant specimens for most species.

Pacific Invasive Ant Key:
http://idtools.org/id/ants/pia/
An interactive key that includes most of the invasive ants likely to be encountered in Hawai‘i or other Pacific Islands.
Hawai‘i Ant Key at Discover Life:
https://www.discoverlife.org/mp/20q?guide=Ants_Hawaii
An interactive key that includes only species known to occur in Hawai‘i. It does not separate some closely related species but can help narrow down the list depending on which traits you are able to see.

Controlling ants using insecticides

Spray applications of contact insecticides, though they may kill foraging ants, are unlikely to provide any lasting control, since they do not affect the whole colony and do not target reproductive individuals. Formicidal (ant-killing) baits, on the other hand, have the potential to control or even eradicate colonies if the bait is brought back to the nest and fed to other workers, larvae, and the queen. However, there are many types of bait formulations, using a variety of bait carriers and active ingredients, and not all baits are effective against all ants, so correct species identification is crucial. As mentioned above, some ants are more attracted to sugary baits, others are attracted to oily baits, and still others prefer protein-based baits.

Ant baits, like all pesticides, *must* be applied according to the directions listed on the product label. Among other things, the product label specifies the permitted methods of application, the kinds of areas in which it can be used, the rate at which it can be applied, the types of personal protective equipment (PPE) required, and the type of pests it can be used to control. Many insecticides are not labeled for use in natural areas, so there are limited options available for pest control in conservation areas.

Formicidal baits are available as liquids, granules, pastes, and gels, and methods of application are also diverse. Depending on the product and its label restrictions, baits can either be broadcast into the environment or placed in bait stations. Bait stations reduce the likelihood of nontarget effects, and also protect the bait itself from the environment, as many baits and active ingredients degrade when in contact with sunlight or water. However, bait stations can also limit access by the target ants and to be effective they need to be applied at high densities, perhaps one or two meters apart. When using a granular bait, it is easier to achieve complete coverage if the bait is broadcast, rather than placed in bait stations, and most granular baits are designed for this application method.

Suppressing Argentine ants and ghost ants at PTA

We used Maxforce Complete Granular Insect Bait (1.0% hydramethylnon) to suppress Argentine ant and ghost ant populations in our experimental plots. This product delivers the slow-acting active ingredient hydramethylnon in a granular protein/carbohydrate bait matrix. The product is labeled for use against both species of ant in a diversity of non-crop areas.

Although the product label does not specifically list natural areas such as forests and shrubland, we consulted with the EPA Office of Pesticide Programs, and they agreed that application to our plots would be consistent with the product label. Maxforce bait has been used to control Argentine ants in Hawai'i since the 1990s, including in natural areas at Haleakala National Park (Krushelnycky et al. 2011) and at PTA (Oboyski et al. 2001). Although it has not been successfully used to eradicate Argentine ants in Hawaii, it is effective at dramatically reducing numbers, and presumably slowing rates of spread.

We applied Maxforce bait to our 2.25-ha experimental plots using hand-cranked seed spreaders at the labeled rate of 1.5 lb per acre (3.78 kg per plot). We assured even coverage by dividing the plots into 36 smaller squares (25 m by 25 m) and applying 105 g of bait to each square. Plots were treated multiple times annually, when monitoring indicated that ant numbers had increased in our treated plots. In all, each treated plot was treated six times: in late June 2016, mid-November 2016, early March 2017, late July 2017, late October 2017, and early January 2018.

Monitoring using bait cards was conducted roughly monthly for 15 months prior to predator treatments, then periodically over the course of the study. A mixture of canned tuna blended with corn syrup (4:6 ratio) was used as a bait, with an approximately 1 cm drop of the bait placed in the center of a paper index card (7.6 cm x 6.4 cm). We placed cards at 13 monitoring stations along two transects running diagonally through each plot, and left them in place for 45-90 min before returning to count ants.

Results

Ants present at PTA

At least six ant species have been recorded at PTA: the Argentine ant (*Linepithema humile*), the big-headed ant (*Pheidole megacephala*), the ghost ant (*Tapinoma melanocephalum*), the pharaoh ant (*Monomorium pharaonis*), *Cardiocondyla kagutsuchi* (no common name, often identified as *C. venustula* in earlier literature), and *Hypoponera opaciceps* (no common name) (Oboyski et al. 2001, W. Haines personal observation). The first four of these species are considered highly invasive, while *C. kagutsuchi* and *H. opaciceps* form small colonies and are not considered to be as destructive. The Argentine ant and ghost ant (Figure 18) were both present in our experimental plots, and these were the two species we attempted to control using formicidal baits. *C. kagutsuchi* was also present at low levels in some plots, but was not targeted for control.

Figure 18. Three ant species were present in our study area at PTA: (A) the Argentine ant *Linepithema humile*, (B) the ghost ant *Tapinoma melanocephalum*, and (C) *Cardiocondyla kagutsuchi*, which was only found in low numbers in some plots. Photos: April Nobil, from www.AntWeb.org

Ant populations at PTA

Prior to treatments, ant populations were heterogeneous across our study area. Argentine ants were most abundant in blocks 1 and 2 (grassland habitat), patchily distributed in block 3, and virtually absent from block 4 (woodland habitat). Ghost ants, on the other hand, were abundant in block 4, but absent from the other three blocks. Within each of the four blocks, one plot was treated for ants using Maxforce, one plot was treated for ants as well as yellowjackets and rodents, and a third plot was untreated for any of the predators and served as a control. Maxforce was applied to treated plots 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.

During the pretreatment 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 posttreatment 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 19). However, Argentine ants were not always suppressed to near-zero levels, sometimes rebounding soon after treatments. Frequent treatments were needed to maintain suppression.

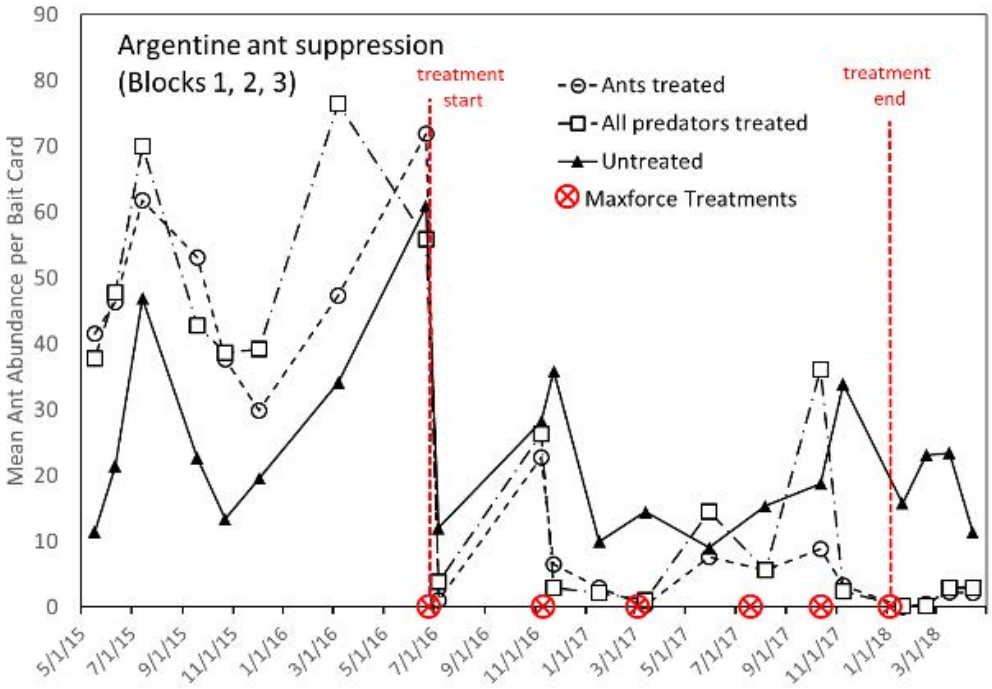

Figure 19. Suppression of Argentine ants (*Linepithema humile*) with Maxforce Granular Insect Bait. During the pretreatment period, ant numbers were significantly higher in plots designated as treatment plots compared to untreated plots. Following treatment, ant numbers were significantly lower in “Ant” and “All” plots compared to untreated plots, indicating that suppression was effective.

Maxforce treatments were not as effective against ghost ants, though they did achieve some suppression. During the pretreatment 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 posttreatment period, there was a significant effect of treatment overall (ANOVA: $F_{2, 26} = 5.463$, $p = 0.010$) (Figure 20). Post-hoc Tukey tests revealed that the “All” plot had fewer ants than the untreated plot ($p = 0.008$), but the “Ant” plot did not differ significantly from the untreated plot ($p = 0.282$). As with Argentine ants, ghost ant populations rebounded soon after treatment, so frequent bait application was necessary to maintain suppression.

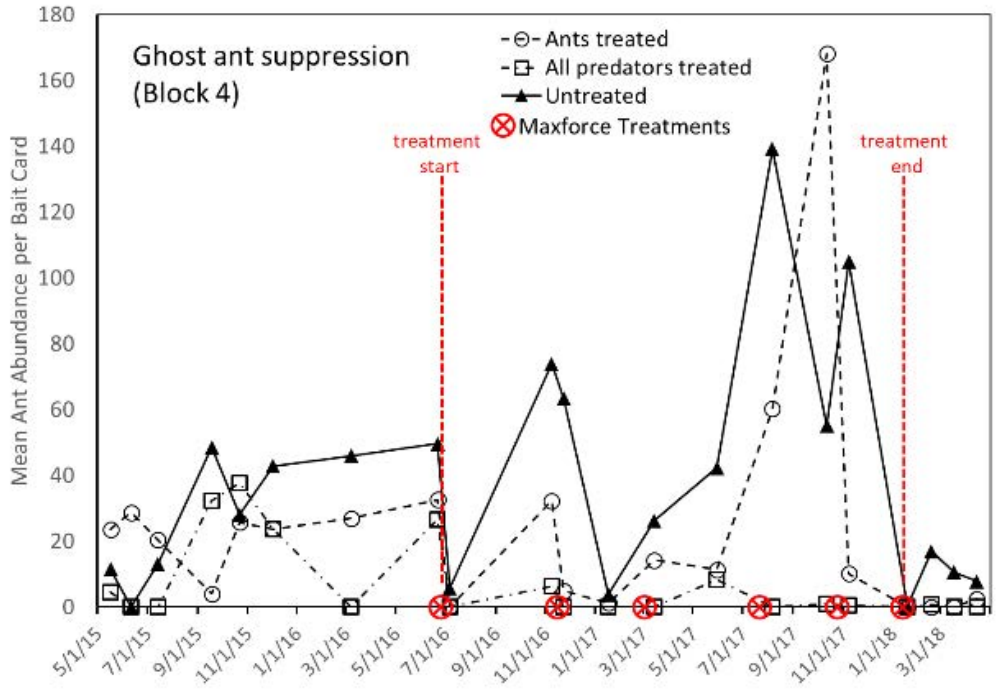

Figure 20. Suppression of ghost ants (*Tapinoma melanocephalum*) with Maxforce Granular Insect Bait. During the pretreatment period, there were no significant differences among the three different plots. Following treatment, ant numbers were significantly lower in the "All" plot compared to the untreated plot, but were not significantly lower in the "Ant" plot.

Cost breakdown for ant suppression

Here, we provide a cost breakdown for the various field activities associated with ant suppression at PTA. Please keep in mind that these values apply to just this one project (i.e., one dryland site at PTA, one type of ant suppression methodology).

The size of plots that we aimed to protect, and the methodology used, required 3-5 people working full-time; we employed a group of five because of the many duties beyond ant suppression and monitoring that were a part of our larger project.

Labor and materials to treat one 150 m x 150 m plot once:

- Maxforce Complete Granular Insect Bait (1.0% hydramethylnon).
  - purchased in 4 gallon jugs from BEI in Hilo
  - 3.8 kg = approx. $152.55
- 2 hours to apply bait throughout plot.

Labor and materials for monitoring one 150 m x 150 m plot at 13 monitoring stations once:

- Canned tuna, corn syrup, index cards, applicator bottles
  - less than $20.00
- 1.5 hours to set out and retrieve baited index cards.

Note that ideally, plots should be monitored multiple times before and after treatments.

Study conclusions

Ants can be suppressed using available registered formicidal baits that are labeled for outdoor use. However, ant populations can rebound quickly, so repeated treatments every two to three months are likely necessary to keep levels low.

When treatments must be applied frequently to maintain suppression, impacts on nontarget species are important to consider. Granular formicidal baits, such as the Maxforce Granular Insect Bait used in this study, are most likely to impact ground-dwelling scavengers that may consume the bait. Nontarget arthropod groups likely to be impacted by granular baits include isopods, springtails, earwigs, roaches, and crickets. Indeed, earwigs, roaches, and crickets are listed on the Maxforce label as pests controlled by this product. 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.

References and further reading:

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IX. Yellowjacket Population, Dynamics, and Suppression

In a Nutshell:

- Hawai‘i lacks native yellowjackets or social insects of any kind, and yellowjackets are important predators of native arthropods.
- Yellowjacket populations at PTA are heterogeneous across the landscape, very abundant in some areas
- Few registered products exist for control of yellowjacket wasps on a landscape level.
- Under an experimental use permit, we tested fipronil-laced bait, applied at a very low rate, as a control method for yellowjackets at PTA.
- Evaluation of yellowjacket suppression was difficult due to extreme spatial and temporal variation in yellowjacket populations across the study area.

General background

Yellowjackets in Hawai‘i

The western yellowjacket (*Vespula pensylvanica*), native to North America, is a generalist predator, forming large colonies in subterranean nests. It has been established in Hawai‘i since the early 1900s, and occurs in many native habitats at high elevations, becoming highly invasive in many areas. It is not only a nuisance pest, but heavily impacts native Hawaiian insects (Gambino 1992, Wilson and Holway 2010), which have evolved in the absence of social wasps and ants. Yellowjacket wasps are known to prey on important pollinator groups, including moths and butterflies (both larval and adult life stages) and native yellow-faced bees (Wilson and Holway 2010). In natural areas of Hawaii, the primary motivation for controlling yellowjackets is to improve ecosystem integrity and conserve native invertebrates (Gambino and Loope 1992, Hanna et al. 2012). Previous studies have assessed the efficacy of various control methods for yellowjacket wasps (Harris and Etheridge 2001, Hanna et al. 2012), but the non-target impacts of these methods have been little studied.

Methods

Monitoring yellowjacket wasps

Yellowjacket wasps in Hawai‘i are highly attracted to heptyl butyrate, a nontoxic, food-grade compound found abundantly in some fruits. In this study, wasp abundance was monitored in treated and untreated plots using plastic yellow jacket traps (Seabright) baited with heptyl butyrate. Wasp traps were arranged in diagonal transects extending outward from the corners of each plot, and were baited for three days during each monitoring period. Wasp abundance was monitored approximately monthly in all plots for a full year prior to initiating experimental treatments, and continued to be monitored on roughly a quarterly basis while treatments were in place, including pre-treatment and post-treatment monitoring.

Options for controlling yellowjackets

Limited chemical products are available for yellowjacket control in natural areas. Most registered products are dusts or sprays that must be applied directly to the entrance of yellowjacket nests. For landscape-level control, this is generally impractical because yellowjacket nests can be widely dispersed and extremely difficult to locate, while yellowjacket workers can forage over long distances (Gambino and Loope 1992). Non-toxic traps (e.g., water traps) baited with attractants can be used to control nuisance wasps on a small scale (e.g., around buildings and picnic areas), but generally have little impact on yellowjacket colonies or populations on the landscape level.

Baits, though none are currently registered for use in natural areas in Hawaii, show great promise for control of yellowjackets. Fipronil, when applied using a meat-based bait carrier, has been shown to provide excellent control of *V. pensylvanica* in native forests in Hawai‘i (Hanna et al. 2012) and New Zealand (Harris and Etheridge 2001). In Hawaii, fipronil mixed with canned chicken has been demonstrated to be effective in wet and mesic forests of Hawai‘i Island within Hawai‘i Volcanoes National Park (Hanna et al. 2012), but has not been tested in dry forests at higher elevations, where yellowjacket wasps are very problematic and may have larger foraging distances. Previous studies have demonstrated that fipronil baits can be effective at low application rates, and data on visitation rates suggests that bait deployment periods may be reduced considerably without reducing efficacy (Hanna et al. 2012).

Although there is evidence that yellowjacket suppression using fipronil improves pollination of the native tree ‘ōhi‘a, *Metrosideros polymorpha* (Hanna et al. 2013), the overall impacts of this method on pollinators and other non-target insects have not been evaluated. Although fipronil is very safe for vertebrate animals, it is a persistent insecticide, and has been documented to have negative effects on non-target insects, especially pollinators such as honeybees, when broadly applied (Bonmatin et al. 2015, Pisa et al. 2015).

Suppression of yellowjackets with fipronil baits at PTA

In this study, we tested the efficacy and impacts of fipronil baits at PTA. Baits were applied in a small area for a reduced exposure time in order to minimize any impact on non-target invertebrates in treatment areas. Baits were applied under an experimental use permit (EUP-16-01) issued by the Hawai‘i Department of Agriculture.

Fipronil was applied by mixing a flea and tick control product with canned chicken to achieve a final concentration of 0.1% fipronil, using methods similar to previous studies (Hanna et al. 2012). The product used was Sentry Fiproguard for Dogs (89-132 lbs.) (9.7% fipronil; EPA Registration Number: 2517-136; HI Product Number: 9227.300), a product used to control fleas and ticks through direct application to a dog’s skin. Canned chicken was drained, manually mixed to break into small pieces, and weighed in quantities of 89.07 g, the amount to be applied to each plot after mixing with Fiproguard. While mixing toxicant, the handler wore a long-sleeved shirt, long pants, covered shoes, an apron, face shield, and elbow-length gloves, greatly exceeding the PPE requirements listed on the Fiproguard label. Fiproguard was mixed with canned chicken at a rate of 0.93 g product per 89.07 g chicken to achieve a final

concentration of 0.1% fipronil. Green food coloring was mixed with the Fiproguard prior to mixing with chicken, allowing us to assess evenness of mixing of Fiproguard into the bait, and serving as a warning coloration to distinguish treated chicken from untreated chicken.

Bait was applied in the morning (around 0900) to maximize availability to foraging yellowjackets, and removed the same day before sundown (1700), because wasps are most active from mid-morning to late afternoon, and previous studies have found that the majority of bait was taken within the first 6 h of deployment (Hanna et al. 2012). Applicators wore a long-sleeved shirt, pants, covered shoes, latex gloves and a face shield while dispensing bait, to prevent accidental contact with the skin and eyes. Bait was applied in bait stations hanging from shrubs. Bait stations consisted of plastic 2 oz. portion cups with four holes (17 mm diameter) evenly spaced around the top of the cup (Figure 21). Bait stations were hung at a height of 1.0-1.5 m from a wire punched through the lid of the cup. Hanging wires were coated in a narrow (1-2 cm) band with Tree Tanglefoot, a sticky barrier which prevented access by ants and other crawling insects. Bait stations were spaced 25 m apart in a grid formation in the 50 m by 50 m square at the center of each treatment plot, for a total of 9 bait stations per plot (3 rows of 3). Each bait station was filled with 10 g treated bait, for a total of 90 g treated bait in each plot. This corresponds to 0.93 g Fiproguard product (0.090 g fipronil) in each plot. This rate of application is extremely low, considering that the labeled single dose of this product for a large dog is 4.02 mL (approximately 4 g) Fiproguard applied directly to the dog's skin. The dose that is applied to one dog was sufficient product to treat four plots. Any remaining bait was removed at the end of the day, placing bait stations in a zippered plastic bag.

Figure 21. Yellowjacket bait station containing canned chicken laced with fipronil. Bait stations were constructed from 2-oz cups and hung from shrubs at a height of 1-1.5 m.

To increase attractiveness of bait stations to yellow jackets, a wick of the non-toxic attractant heptyl butyrate was hung from each bait station. This consisted of a rolled paper towel "wick" saturated with heptyl butyrate and placed in a 1.5 mL microcentrifuge tube, which was affixed to the wire holder of each bait station.

Wasp traffic at each bait station (the number of wasps visiting each station over a three-minute period) was counted at regular intervals throughout the deployment period. Any non-target insects observed on the bait were also counted.

Treatment schedule at PTA

Yellowjacket treatments were applied in the treated plots on four occasions during our study: on 6/30/2016–7/1/2016, 12/15–12/16/2016, 6/26–27/2017, and 11/20–21/2017. Wasp abundance was monitored using heptyl butyrate traps one week prior and two weeks after each treatment. Block 1 did not receive yellowjacket treatment after the first application, due to very low numbers of yellowjackets during the pretreatment monitoring period. Block 1 was also excluded from our analysis of treatment efficacy.

Results

Yellowjackets at PTA

There was some evidence that our treatments reduced yellowjacket numbers, but suppression was weak and did not keep yellowjacket levels near zero as was desired (Figure 22). During the year prior to initiation of treatments, yellowjacket abundance did not differ significantly among plot treatments (ANOVA: $F_{2, 76} = 2.962$, $p = 0.058$), but during the treatment period, yellowjacket numbers were significantly lower in treated plots after application of fipronil (ANOVA: $F_{2, 108} = 5.905$, $p = 0.004$). Pairwise comparisons of plots revealed that wasp numbers in untreated control plots were significantly higher than those in both "All" plots ($p = 0.031$) and "V" plots ($p = 0.004$).

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 22).

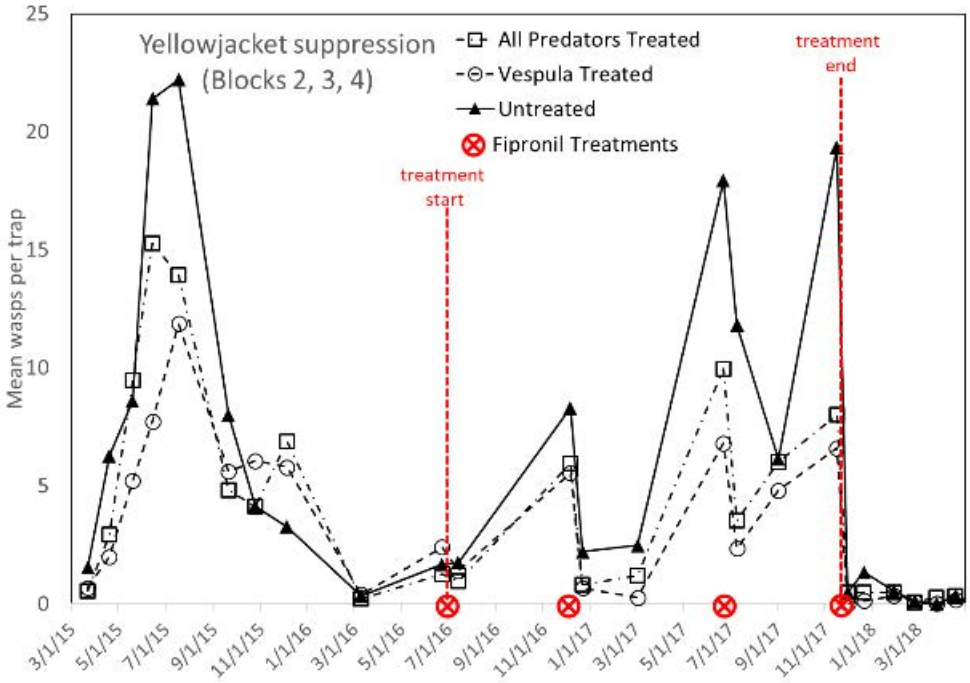

Figure 22. Yellowjacket abundance in traps baited with heptyl butyrate throughout the study period. Although wasp abundance was significantly lower in treated plots than in untreated plots after treatments were initiated, any suppression was slight and was not sustained for long periods.

Cost breakdown for yellowjacket suppression

Here, we provide a cost breakdown for the various field activities associated with yellowjacket suppression at PTA. Please keep in mind that these values apply to just this one project (i.e., one dryland site at PTA, one type of ant suppression methodology), and this use was allowed only under an experimental use permit. This product is not registered for use, and the costs are provided here for informational purposes only.

The size of plots that we aimed to protect, and the methodology used, required 3-5 people working full-time; we employed a group of five because of the many duties beyond yellowjacket suppression and monitoring that were a part of our larger project.

Labor and materials to treat one 150 m x 150 m plot at 9 bait stations:

- Sentry Fiproguard for Dogs (89-132 lbs).
  - purchased from Amazon
  - approx. $19.00
- Canned chicken, food coloring dye, bait stations (plastic cup, wire, Tanglefoot)
  - less than $20.00
- 8 hours to set out bait stations and monitor throughout the day.

Study conclusions

It is unclear why yellowjacket treatments were not very effective. During treatments, foraging wasps quickly found the toxic baits, and in many cases, wasps collected virtually all of the available bait in the plot, presumably taking it back to share with nestmates. 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).

The natural seasonal fluctuations of yellowjackets also made the timing of yellowjacket treatments difficult, and also complicated evaluation of suppression. Our goal was to treat yellowjackets in the early summer, after overwintering queens had established new nests, but before wasp populations had grown to very high levels. Our hope was that new nests would be eradicated in the vicinity of our treated plots, and that wasp levels would remain low for the rest of the year. However, these summer treatments did not lead to long-lasting suppression of foraging wasps in our plots, and populations soon increased. The summer treatments were followed up with a second treatment each year (in December 2016 and November 2017), and wasp numbers decreased immediately after these winter treatments, but they decreased in the untreated control plots as well as the treated plots. There are two possible explanations for this. Firstly, treatments may have coincided with natural seasonal decreases in populations due to colder weather. Alternatively, our treatments may have impacted wasp populations in the untreated plots as well as treated plots, due to long-distance foraging of yellowjackets in dry forest habitats.

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X. Invasive Predator Diets

In a Nutshell:

- Diets can be measured for predators, including wasps, ants, and rodents using various methods for sampling and analysis.
- Different analysis methods (stomach/fecal content IDs, stable isotopes, DNA, field trials) have different pros and cons, with some methods being more suited for certain species than others and fine- vs. coarse-scale interpretations.
- Our diet analyses at PTA showed that wasps, ants, and rodents are consuming a broad variety of arthropods, including many pollinator groups.

General background

General diet assessment for predators

Determining predator diets is the single most straightforward method of assessing which prey species may be at risk to predators. Furthermore, diet assessments reveal the feeding habits and impacts of the predator on the environment. Methods of diet assessment generally require animal capture and euthanasia to obtain gut or fecal contents. Collections of such target predators can be relatively easy in areas like Hawai‘i where trapping invasive animals occurs frequently as a means of controlling these invasive populations. Capture methods are well established for such invasive animals and these are detailed later in this section for our target species at PTA. The four most common methods of diet assessment, along with the pros and cons of each, are detailed below.

Types of predator diet assessments

There are four major types of predator diet assessments. No single diet assessment is perfect, and all have limitations. We suggest that the investigator clearly define their questions of pursuit, and then choose the appropriate methods that will best answer those questions. In most cases, and if resources allow, a better understanding of the target species' diet will be uncovered if multiple techniques are simultaneously used. In all cases, there must be sufficient replication (i.e., multiple individuals from each target population must be assessed to make any meaningful inference about the species' diet at the particular site). Below, each of the four methods are listed with their associated pros and cons.

1-Morphological assessment of stomach and fecal contents: This is the classic method of diet assessment, and it is also known as the microhistological method, as stomach and fecal contents are extracted from the animal carcass and visually scanned and identified using a microscope. This method is commonly practiced on vertebrates, but less on invertebrates (especially those of the sizes of common insects). Because it is a common method, it allows for comparisons across taxa, habitats, and islands. Obtaining carcasses can be relatively easy if trapping is occurring in an area of interest; fresh roadkills are another way of obtaining target carcasses. Once carcasses are obtained, they are necropsied and the stomachs and/or fecal

contents are removed and placed in ethanol and/or frozen for preservation. Typically, the stomach contents are rinsed and sieved to remove gastric juices and other liquidated and unidentifiable material. Contents can be quantitatively assessed under the microscope by using gridded petri dishes where the contents are spread out (see Shiels et al. 2013). This can give an estimate of the relative abundance of life forms (plant, animal, fungus), and the phenological stage of the organism consumed can give more information on the impact of the predator on the prey population (e.g., seed destruction or dispersal vs. vegetative material; eggs and chicks vs. adult birds).

Pros:

- Inexpensive for materials (but need access to microscope and ethanol).
- Moderate labor required.
- Very common method so it is easily comparable across species, sites, and continents/islands.
- Enables a quantification of the relative abundance of life forms consumed (i.e., X% plant and Y% animal in diet).
- Storage in ethanol allows possibility of future DNA analyses.

Cons:

- Moderate labor intensity that requires training to be able to distinguish the types of food items.
- Items are generally highly fragmented (especially when animals have teeth) and difficult to identify to lower taxonomy than Phylum or Order.
- Requires animal capture and euthanasia (often requiring special permitting, even if it is a non-native predator species).
- Requires personal protective equipment (PPE) such as mask and gloves.
- Gives a snap-shot of what the animal was eating within the last several hours (e.g., 24-48 hours for most rodents).
- May be biased towards hard-bodied prey items, while soft-bodied prey may be less recognizable in the gut.
- Generally impossible to use for small arthropod predators such as ants which generally liquify prey items prior to consumption.

2-Stable isotope analysis of target animal tissue: Stable isotope analysis ($\delta^{15}N$ and $\delta^{13}C$) of an individual's tissues can be an indicator of longer-term (e.g., weeks to years) average diet. This type of analysis helps determine the trophic positions of the target species. Unlike stomach or crop/gizzard contents, which do not persist long in the digestive tract, stable isotopes reflect the assimilation of diet contents over time. Interpretations of diet using stable isotopes are complicated by variation in tissue turnover rates among organs. For example, liver tissue has a higher turnover rate than blood cells or muscle, whereas bone collagen is deposited and modified throughout life, so its isotopic values represent a long-term average of an animal's diet. The difference in isotopic composition between a consumer and its food (discrimination

values) is presumed to average ca. 3% for $\delta^{15}N$ and 1 % for $\delta^{13}C$; however, discrimination values can differ widely among food sources (Shiels et al. 2013; Shiels et al. 2018).

Pros:

- Ability to just send the animal tissue (with minimal processing) to a chemistry analysis lab and pay for relatively inexpensive analysis (~$10-$15 per sample for isotopic $\delta^{15}N$ and $\delta^{13}C$).
- Gives a longer-term estimate of diet and trophic position of the target organism.
- Helpful for augmenting short-term diet analysis like gut contents.

Cons:

- Very difficult to determine the genera or species that are consumed and does not estimate quantity well, despite fancy 'mixing models' and researchers claiming this ability.
- Requires gathering and determining isotopic ratios of potential prey items from the site of interest (and where the predator was obtained).
- Generally a coarse estimate of long-term diet.

3-DNA assessment of gut contents: Obviously, this is a more modern technique that is getting cheaper and better with each year of use. It is also called meta-barcoding or high-throughput sequencing. Like those methods listed above, the internal gut or fecal contents are needed to complete DNA screening. Once obtained, DNA is extracted using standard extraction kits (e.g., Qiagen DNeasy Blood and Tissue Kit for animal prey), then the "barcoding" region of the target genes (e.g., CO1 gene for animals; other genes for plants) are amplified using specific (universal) PCR primers, and sequenced on an expensive machine. The sequences are then compared to reference sample sequences, and there are 'libraries' that are publicly available, such as NCBI Genbank and the Barcode of Life Database (BOLD). Once queried through such databases, the sequences are assigned species/genera/Family/Order or Operational Taxonomic Units (OTU).

Pros:

- Ability to just send the whole insect or gut content to a lab and pay them to produce a spreadsheet listing all likely organisms (to at least Order/Family) contained in each sample.
- Theoretically possible to identify specific taxa without requiring expertise in the morphology of a particular group.
- Allows identification of prey items that do not physically hold up well during consumption and are not physically recognizable.
- Theoretically can be used on arthropod predators such as ants and spiders that liquify their prey prior to consumption.

Cons:

- Moderate cost ($25-$60 per sample for extraction, sequencing, and bioinformatics, at time of writing of this Guide).

- Rarely can species be determined unequivocally, as taxonomic identification to Order or Family is common and Genus is less common.
- Unable to give a reliable quantitative estimation of diet due to primer biases.
- DNA from some taxonomic groups may not be amplified by primers, and therefore will not be detected.
- Unable to determine the part of the organism present (e.g., for plants, the likely taxa is given but whether it is a seed, fruit, or vegetative part ingested by the predator is unknown).

4-Field trials offering potential prey items and observing prey take and/or consumption:
Understanding predator diets can be accomplished by conducting food offering trials of potential prey items. This involves gathering the fresh prey items and setting them out in areas where the target predator is known to forage. It is important to make sure that the target predator has access to the food item and it does not get consumed or spoiled by non-target species—trail cameras are useful for determining if the predator is indeed the culprit or a non-target is responsible for prey removal and consumption. Fruits and seeds are often trialed as singly by species, but groupings of multiple species (i.e., 'cafeteria trials') is also a common method. Animals may be more difficult to assess as prey of a target predator because animals are mobile. Therefore, eggs are obvious choices for birds and reptiles, trail cameras can help depict predation on nests, and otherwise carcasses can be used to determine incidences of scavenging.

<u>Pros:</u>
- Inexpensive and does not require any fancy equipment and can occur 'in house,' without having to rely on anything from the outside.
- Can target food items suspected of being vulnerable to the predator or otherwise particular prey species (e.g., plants, arthropods, vertebrates) of conservation concern.

<u>Cons:</u>
- Much of the diet is missed.
- Takes a lot of time to properly test (leaving prey items out long enough in 'palatable state' and ensuring that target and not non-target species truly get access), especially with multiple species.
- Can be difficult to ensure it was the target predator that interacted or consumed the target prey (though monitoring cameras are helpful for such surveillance).
- May still need special permitting (e.g., IACUC approval) as animal behavior is arguably altered by the actions of the investigator.
- Difficult or impossible to offer mobile prey items in a way that truly reflects the prey's behavior and ability to evade predation.

Additionally, it should be noted that using methods 1-3 above, it is impossible to determine if animals in a predator's diet were killed and consumed, or if they were scavenged. This can be very important if diet contents are being used to infer the impacts of predators on populations.

If predators are consuming individuals of a species only after they are dead, that species might make up a considerable portion of their diet, yet the predators may not have any impact on the population of that species.

Methods

PTA diet assessments of NIP

The objective of the diet assessment portion of our PTA study was to determine the arthropod prey species that our target non-native invasive predator species (NIP; including yellowjackets, ants, mice, and rats) were consuming. Although previous studies have characterized the diets of some of our NIP in some ecosystems in Hawaii, we sought to empirically determine the arthropod groups and species that NIP are consuming at PTA, with particular focus on those that are likely to be pollinators.

In our PTA study, gut and fecal contents of NIP were screened for insect prey DNA to determine the types of insect pollinators consumed by NIP at our site. Field collection of NIP 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 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 (but not our field staff) 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 23). 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 yellowjackets were found foraging 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 23. 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 CO1 gene was amplified using the universal PCR primers LCO1490 and HCO2198, 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 samples 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 flower visitors (potential pollinators) that we observed on our target plant species in the field, or with other arthropod groups.

Results

Rodent prey items at PTA

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 operational taxonomic units (OTUs), which were matched to the most similar sequences in reference libraries. The results are summarized by arthropod order in Figure 24. 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. 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 >25% of the total black rat diet and >50% of the total house mouse diet (Shiels et al. 2013).

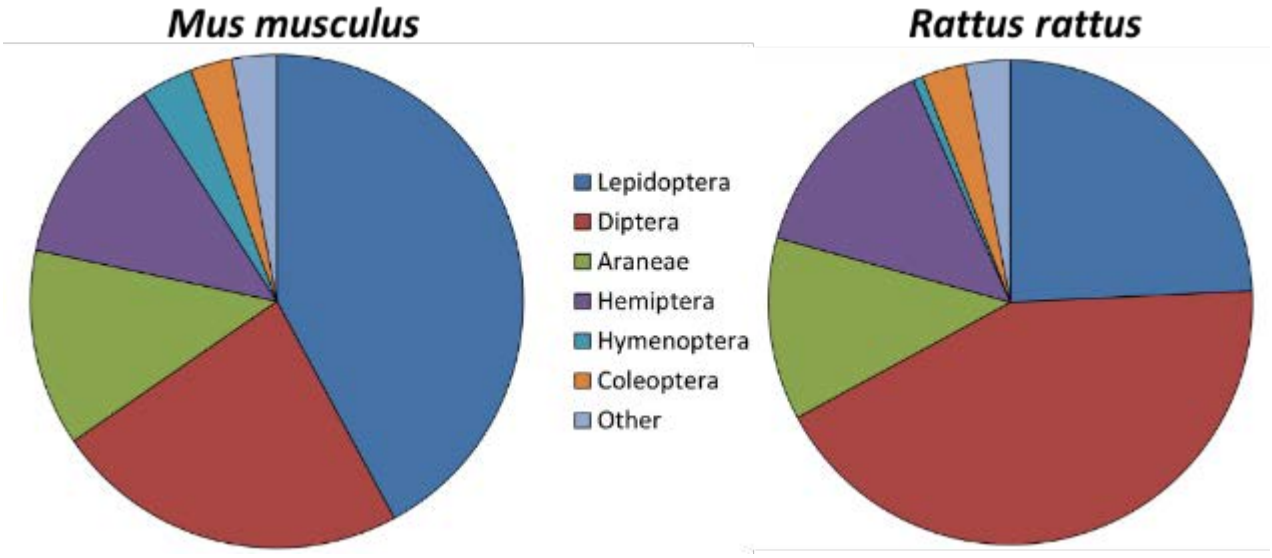

Figure 24. Proportion (by arthropod order) of CO1-gene sequence reads detected in mouse and rat fecal pellets by high throughput DNA sequencing.

Ant prey items at PTA

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 25. 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.

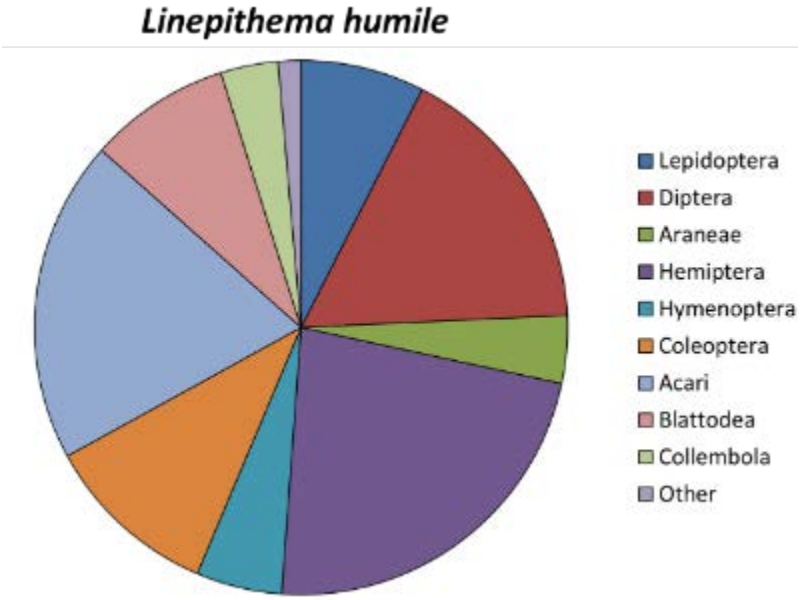

Figure 25. Proportion (by arthropod order) of CO1-gene sequence reads detected in Argentine ant samples (N=30) by high-throughput DNA sequencing. Sequence reads identified as Argentine ant DNA were excluded from the total.

Yellowjacket prey items at PTA

In all samples, 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 26. 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, prey balls consisted of *Vespula pensylvanica* itself, indicating intraspecies predation or scavenging.

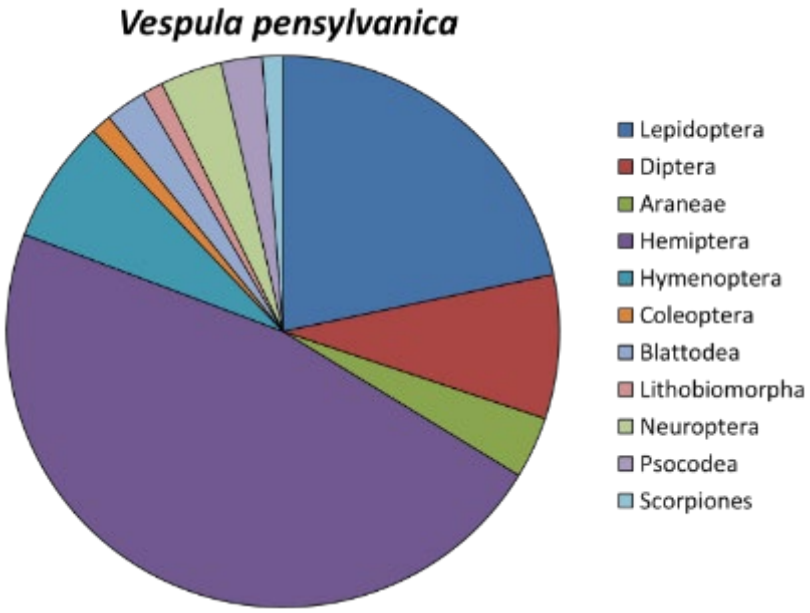

Figure 26. Proportion (by arthropod order) of prey fragments (N=82) collected from foraging yellowjacket wasps as they returned to their nest. Fragments were identified by Sanger sequencing the CO1-gene region.

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 Hawaii. 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.

Study conclusions

Knowing the life forms, and lower taxonomic classifications, that are preyed upon by the target NIP at PTA helps estimate the native species or groups that are likely to be most vulnerable to population-level changes and conservation needs. All of our NIP species consumed arthropod orders that are known to be frequent flower visitors and pollinators. Lepidoptera are one such group that appears in the diets of all NIP, and the frequency of this group was ~20-45% for rodents and nearly 50% for yellowjackets. These findings imply that

suppression of NIP could release some groups of insects from their suppression of NIP feeding. An understanding of NIP diets at our sites at PTA should help guide management and native insect conservation.

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XI. Greenhouse Propagation for Eight Native Plant Species

(notes courtesy of greenhouse manager, Kim Dillman)

In a Nutshell:

- The two endangered mint species (*Haplostachys haplostachya* and *Stenogyne angustifolia)* grew from cuttings.
- The other two endangered species (*Silene lanceolat*a and *Tetramolopium arenarium*) grew from seeds, but it took a long time for the seeds to sprout in our greenhouse.
- The common species (*Argemone glauca, Bidens. menziesii, Dubautia linearis, and Sida fallax*) grew from seeds.
- The fastest-growing and healthiest plants were the first set of cuttings and seeds, which grew when the soil medium was 50-75% cinders and the greenhouse was freshly cleaned and dry.

Project background

We propagated our eight focal plant species in a greenhouse at the University of Hawai‘i Experimental Station in Volcano, HI. Seeds were collected from wild plants at PTA for *Argemone glauca*, *Bidens*. *menziesii*, *Dubautia linearis*, and *Sida fallax*. Seeds were obtained directly from PTA NRO for *Silene lanceolat*a and *Tetramolopium arenarium*. Additionally, we planted cuttings of *Haplostachys haplostachya* and *Stenogyne angustifolia* (all obtained from PTA Natural Resources Office). 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 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.

Greenhouse conditions

Elevation at the greenhouse in Volcano is 4,000 ft. The greenhouse was 100 ft long by 20 ft wide, containing 16 benches 4 ft x 16 ft each. The front half (Ka‘u side) had 8 benches under UV-protected plastic cover and (about) ~27-36% shade cloth. The back half (windward or Hilo end towards the lawn) had UV-plastic cover only (Figure 27).

Some of the species might have preferred a little more shade to sprout, but most benches were fairly bright, and temperatures on sunny days got warm, 80F or higher during southwest winds or lack of tradewinds. The shaded times usually came in winter, which also brought cool nights that may have encouraged some species to start. Hot and sunny weather may have delayed sprouting on *D. linearis* and *A. glauca*. All of the endangered species seemed to green-up in cool weather with partial shade, although they tolerated hot sunny weather as long as the soil medium did not dry out completely.


Figure 27. Propagation of plants in greenhouse.

***Argemone glauca* (pua kala, Hawaiian prickly poppy); common species**

Fresh seeds were sown in June of 2014, and sprouted in October. This first batch grew fast and well to a meter tall, then flowered and ultimately died back. They did not flower a second time and did not produce new basal growth that survived for more than a few weeks. They did produce a lot of seeds. Some were spilled onto existing flats, and others were used to test pre-treatments.

Pre-treatments consisted of soaking seeds in different materials overnight; tap water, hot water or gibberellic acid. Treatment did not seem to make a difference, as seeds from all pre-treated sets sprouted around the same time, and had similar vigor, once the weather cooled down. Most of the pre-treated plants grew to a few inches (4-8) and then died. A handful produced a single flower bud before dying. The reason that nearly all sprouts died was not definitively determined.

In hindsight, perhaps pre-treatments were not good for this species. However, other factors may also have had some influence, such as volcanic smog and unusually hot daytime temperatures for weeks at a time. Additionally, it might be that poppies need deeper containers, and/or they just needed less moisture while indoors, or more shade and cooler temperatures from sprouting until roots are strong. Various mediums were tried, including

cinders with perlite, cinders with peat, and a mix of all materials (cinder, peat, perlite, vermiculite). None of these stood out as working better or worse.

***Bidens menziesii* (koʻokoʻolau); common species**

Fresh seeds were sown in June 2014. The first sprouts followed within a few weeks. Pretreatment on the seeds was soaking overnight in tap water. Medium was perlite:peat at a ratio of 3:1 at first, and a standard mix (cinder, perlite, peat) or any other mix thereafter. Any mix seemed to be okay, as long as moisture was closely monitored to keep pots on the dry side, but not too dry. Small plants in flats were usually okay, while large plants in smaller containers dried quickly. Most droopy plants bounced back after watering, as long as the dry period was just a day. Plants began flowering in under a year, and then seasonally (1x/year) thereafter. Older plants in larger pots attracted pests, specifically ant, aphids, and whitefly.

***Dubautia linearis* (kūpaoa); common species**

Dubautia linearis were grown from cuttings and from fresh seeds. Cutting mortality was high, so the majority of plants were from seeds. Seeds sprouted well in perlite:peat 3:1 mix, in a cool, damp environment (the windward end of the greenhouse). Fresh seeds sprouted fast in cool weather. Growing the plants to flowering took patience. These plants attracted ants and aphids. In addition to ants and aphids, the plants that stayed in the greenhouse the longest also contracted mealy bugs. Some of the mealy bugs went into the shallow root layer and were destroyed when plants were dipped for ants. Plants produced flowers once per year but it took more than 12 months for the first flowers, even with extra fertilizing. Plants flowered seasonally, 1x per year.

***Sida fallax* (ʻilima); common species**

ʻIlima were started from seeds and cuttings at first. Seedlings grew larger, quicker, and more upright than cuttings that tended to grow horizontally along the ground. Though cuttings had flowers from the start, seedlings soon also produced flowers. Once flowering began, it was fairly continuous. Seeds were sown in a perlite:peat 3:1 mix, pretreated by soaking in tap water overnight. The first batch was started in late June 2014, sprouted in about 4 weeks and flowered by December. Any medium worked for sowing seeds and transplanting this common species, the hardiest of all species grown for the project. Stems are flexible and transport well, and plants can tolerate water or dryness for extended periods. Plants were somewhat attractive to whitefly in the greenhouse, but damage was minimal, easily controlled by leaf trimming and cleaning with soapy water + alcohol.

***Haplostachys haplostachya* (honohono); endangered species**

This species was propagated by cuttings from mature plants. Materials were collected from the greenhouse specimen at PTA, with the first batch in mid-July 2014. The plant was flowering from nearly every stem, so cuttings were selected by trying to find those that had not started budding. Even those cuttings continued producing buds while trying to root, so buds

were pinched off as they emerged. The cut stems were transported in plastic bags, with no water or refrigeration, to the Volcano greenhouse on the same day. They were then trimmed of excess foliage, dipped in powdered rooting hormone and placed into individual containers using a growing medium of perlite:vermiculite at a ratio of 3:1. They were watered with a light solution of Miracle-Gro all-purpose plant fertilizer crystals in water. This first attempt worked the best (that is, it had the lowest mortality rate of all attempts).

When plants were rooted they were transplanted to 4-inch containers, using a medium of 3:1 cinder:perlite. This very dry mix helped prevent the plant from getting too much moisture. When plants were too wet, the first sign was powdery mold. Trimming off older foliage got rid of most of the mold, but then the plants required even less watering. They were sprayed with potassium bicarbonate mixed with water after trimming, to prevent new spores from taking hold. This treatment worked best in sunny or breezy weather when the solution dries quickly. Any moisture on foliage overnight or for several hours on overcast days resulted in more powdery mold. *Haplostachys haplostachya* seems to need daily attention to survive in a greenhouse. The plants will indicate when they need water by drooping. Watering before drooping might cause some root rot. Letting them droop or dry out for too long will also cause mortality.

Of the failed attempts, it is possible:

- They were collected during a dormant growth period.
- They had too much moisture during transport or prior to placing into containers with soil.
- They were left as cuttings too long before being placed into containers (sometimes overnight, sometimes also in water).
- Subsequent mediums may have been too wet (largely weather dependent).
- Rooting powder might have made a difference although it is also possible the rooting powder worked better when plants were not waterlogged. Plants grew with and without the powder (though formal tests were not conducted and results were not quantified).
- Vog did not seem to be a factor in mortality, although it did certainly burn foliage at times.
- Excessive moisture was probably the cause for most of the mortality.

Seeds: The greenhouse plants produced many seeds that were collected and stored. No germination attempts were made with these seeds. Upon inquiring with the nursery managers at three known *H. haplostachya*-permitted sites on the island, none were found to have had success growing this species from seeds.

Mound layering as opposed to cuttings: One plant that had an extra-long branch was experimentally assessed to see if an air-layer-type action could produce roots. The result was positive. An 8-inch shallow dish was filled with cinder rocks, with larger material at the base and smaller at the top. Over that was a thin (about a half-inch) layer of 3:1 perlite:vermiculite. The stem was placed on the medium, held down with a rock, and covered with more of the perlite:vermiculite mix and watered. The buried part of the stem was a curvy section, mid-way between the roots and the tip, including one or two nodes where roots could emerge. The tip extended upward to be the future new plant. It worked, and after a few months the stem was noticeably rooting and then cut from the mother plant.

***Silene lanceolata*; endangered species**

It took longer than expected for the seeds to start, and there was some concern that they might not germinate. It was first thought that the seeds had been watered through the medium and were resting at the bottom of the flats. To test this, the materials from each of the seeded flats were spread over the top of three other flats containing a standard mix bottom (with cinders, perlite and peat). This might have helped, as those flats did eventually germinate. They germinated in concert with other species during the colder winter months, about one year after first being sown.

Once the seeds started germinating, *S. lanceolata* was the easiest and least problematic of the endangered species propagated for this project. The plants enjoy being watered weekly with a light mix of Miracle-gro all-purpose in water. The foliage exhibits burns in vog, and this is more likely if this foliage is wet when vog rolls through. Pests did not bother the plants much, but they did catch flies from time to time.

Root systems on *S. lanceolata* are small compared with tree species like *B. menziesii* or *D. linearis*. The species preferred a drier mix (50-75% cinders) that will never be soggy, but plants were okay in a wet mix (perlite/vermiculite) as long as the containers were small and dried up on occasion. Larger containers stayed wet longer and roots began to rot in some cases.

***Stenogyne angustifolia*; endangered species**

The first set of plants was started from cuttings taken from the PTA greenhouse specimen in July 2014. Cut ends were dipped in powdered rooting hormone then placed in 2-inch containers with growing medium 3:1 perlite:vermiculite and watered with a light solution of Miracle-gro all-purpose plant fertilizer and water. Most of the cuttings rooted and were transplanted to 4-inch containers in September. By December, some of them were in gallon containers.

Stenogyne angustifolia is easy to root from cuttings. After the first set grew long runners, they were cut and new plants were made. Various soil mediums were used, and all of them worked. Well-drained to very soggy, plants continued to grow.

When plants were wet, they collected powdery mold spores that matured and affected foliage at first. Left uncontrolled, the mold would settle in on the stems and flowers. Affected stems and foliage were trimmed regularly to keep the mold at a minimum. Plants were also treated with a spray solution of potassium bicarbonate and water to kill the mold.

The plants grew so well and required trimming so often, a shortcut to rooting new cuttings took effect. Using 3 or 2 gallon sized containers filled with 50:50 Perlite:Vermiculite, cuttings were placed into the medium, as they were trimmed from the plants. Those that rooted were fine with the crowded conditions of the pots. The pots were only half-filled with the medium, creating a humid environment within the container, above the soil. This created a void that was eventually filled with pest insects, whitefly and and/or aphids. The pests were gone after a few treatments, first with the soap-alcohol solution, followed by Talstar (bifenthrin), which seemed to work longer (better).

There were flowers on most of the rooted plants, most of the time. Seeds were found and collected, usually during a cleaning and trimming process, but never sown.

***Tetramolopium arenarium*; endangered species**

Seeds were provided by the PTA greenhouse manager in July 2014, and were sown within days, using a mix of 50:50 finely sifted cinder and perlite. After about eight weeks, only a few seeds had sprouted. Those first plants were transplanted to small containers in September. By November, not many more had sprouted and the reasons were unclear. Since it took longer than expected for the seeds to start, PTA greenhouse provided some trays of germinating seeds to get started. By the end of 2014 there were a dozen or so seedlings.

One possible reason that very few seeds sprouted from the first batch was that they could have blown out of the flats by strong winds. Another consideration was that the seeds were not fresh off a plant; rather they were previously in storage and possibly dormant. In order to mitigate the possibility that they may have blown out, fiberglass windowscreen material was used to cover some of the flats, but still nothing sprouted.

Ultimately, the flats were re-worked, just in case seeds had been washed to the bottom from months of watering. Materials in the flats were spilled into a tub, and vermiculite was mixed in to hold more moisture. A layer of the mixture was then spread out over flats that were filled with standard mix. Whether any of the original seeds sprouted is unknown. Flats did produce plants after some time, but the seeds that germinated were likely fresh seeds from flowering plants on the bench. There was hot and dry weather for many months, but when the weather changed to cool temperatures, sprouts emerged from most of the flats, late in 2015.

Plants attracted caterpillars (or moths; caterpillars were found in leaf terminals). They also attracted whitefly. Plants were treated by hand-cleaning with a soap-alcohol solution. Caterpillars were instantly found and removed after spraying, but damage was usually already done. The branch tip would typically die, then new branches would sprout from below. During treatments, whitefly were seen flying away or getting caught in the solution. It was hoped that the alcohol would penetrate eggs left behind. Small plants of 1 or 2 inches tall would die if whitefly went unnoticed. Taller, more woody plants could survive, and new branching often resulted.

General tips

The fastest-growing and healthiest plants were the first batches, when the soil medium was 50-75% cinders and the greenhouse was freshly cleaned and dry. The cinder mix was perfect for plants in the greenhouse. After the first year, cinders were rarely used in the mix because it dries out too fast in the field and is heavy, and these pots needed to be transported to the field once the pollinator visitation experiment began. It was difficult to find a (literal) "happy medium," or a soil mix the plants could handle in the greenhouse without getting soggy and could also survive in out in the field, where plants dry quickly. Ultimately, the wettest mix was 50/50 Perlite/Vermiculite. This mix was used for plants in the field, as well as for all transplants. Some plants never dried out in the greenhouse, but those in smaller containers did. Therefore, plants were kept in the smallest possible containers until going out to the field, and then put into the same medium in a larger container, in the hopes that it would hold water longer.

Before using any chemical or other treatment, all dead and dying foliage below the growing tips were trimmed off. Plants were turned upside down and all loose materials were removed from the top of the soil. A majority of the above-ground pests (and weed seeds) were removed in the process. Avoiding the use of systemic chemicals (due to research needs) made it more difficult to keep the plants clean and healthy. A variety of other products were used with limited success on whiteflies, aphids, and mealy bugs.

Greenhouse pests and pathogens

Ants (likely Argentine ant) moved into some of the older plants on the benches, in gallon or larger sized pots. This happened even though a regular ant-bait routine was in place. Maxforce Complete granules work well in dry weather, but they have very little effect once they are old (no shelf life in a damp environment). When ants were found in the plants, dipping was the next alternative. Carbaryl-4L (sevin) was mixed in water and root balls were dipped and briefly (5-10 minutes) soaked in the solution. Low-hanging foliage that was dipped showed damage the following day. Pests appeared to be dead or gone post-treatment. This was done for most of the second- and third-round *D. linearis* and *B. menziesii* (those that were in the greenhouse longer) just before moving to the field. Later on, Talstar (bifenthrin) was sprayed along the path the ants traveled along stored PVC pipes and bench legs. Those treatments worked for the ants, and also seemed to have gotten rid of spiders on the benches and plants. In hindsight, using Talstar (as liquid, applied with hand-pump pressurized gallon sprayer) around the perimeter of the house and on bench legs seems to be the best prevention option if ants are present on site before the house is full of plants. Read product labels and (M)SDS sheets before mixing or using pesticides.

Aphids, apparently a product of the ants, infested large plants with lots of foliage. They were found on *D. linearis* and *B. menziesii* and some of the infested plants also had ants in the roots. While chemicals such as Safer brand or M-pede insecticidal soaps in combination with setting out sticky card traps helped keep aphid populations down, the goal was zero aphids. To accomplish this, all old foliage, dried and yellowing were trimmed off. Next a mild washing of each leaf was done by hand, with a solution of soapy water and Isopropyl (70 or 90%) as 1 part, mixed with 4 parts water. A quarter tsp (one or two drops) of Dawn liquid dish soap was mixed in and the solution applied with a hand-held trigger squirt bottle to all plant parts above the soil. Additionally, ant-infested plants were isolated and treated separately. Read product labels and (M)SDS sheets before mixing or using pesticides.

Whiteflies and caterpillars were harder to control and did most damage to certain species, including *T. arenarium*. Regularly spraying the plants with the isopropyl/soap/water solution (see Aphids, above) worked on most of the whiteflies, for a short period of time. Plants did not always look good, but they survived. The alcohol/soap solution also disclosed the location of certain little caterpillars, possibly of the diamond moth variety, which were also fairly destructive as they ate the tender shoots from the tip of the plants. M-Pede and/or Talstar may have helped reduce the number of pests overall, including the moths/caterpillars. Read product labels and (M)SDS sheets before mixing or using pesticides.

Powdery mold on mints was remedied by leaf-trimming, then treating remaining foliage and stems by spraying potassium bicarbonate (Kaligreen), rubbing out white spots under or on top of the remaining leaves.

Chemical fertilizers

Miracle-Gro all-purpose plant food water soluble crystals was used to water plants 1 or 2 times per month, or more in certain sets of plants. The best response to this fertilizer was demonstrated by *S. lanceolata*; apparently this species enjoy being watered with fertilizer all the time. If this was not done, the plants eventually start turning more yellow than green. They probably produced more flowers, too, although all plants got equal treatment so there was no untreated control with which to compare.

Gaviota 14-14-14 slow-release, water-activated fertilizer beads were used in most species, 1 or 2 times per year, in addition to regular Miracle-Gro watering.

Water soluble chips of 8-8-8 were given to some species to get them growing better (greener) than naturally occurring plants in the wild. Reportedly, this product is temperature activated (heat); it was not applied during sunny, hot periods.

XII. Plant-Pollinator-Predator Dynamics

In a Nutshell:

- We used Bayesian hierarchical models to determine tri-trophic relationships, i.e., interactions between primary producers (native plant species), primary consumers (native and non-native insect pollinators), and secondary consumers (NIP species).
- Rats had the most negative relationships with the frequency of pollination interactions and ants had the second most negative relationships.
- Reduction of rats could increase pollination of *Haplostachys haplostachya* (honohono), *Sida fallax* (‘ilima), and *Tetramolopium arenarium*.
- Reduction of ants, in addition to rats, could increase pollination of *Sida fallax* (‘ilima) and *Tetramolopium arenarium*.

General background

Statistical term definitions

Regression: Any linear regression, also called a general linear model (GLM), that takes the form mu = a0 + a1*x1 + a2*x2 … where mu is the mean of the specified distribution (e.g. in a normal linear regression, mu would be the mean of the normal distribution that describes the response variable), a0 is the intercept of the linear model, and a1 and a2 (and beyond) are the slope relationships with the independent variables (x1, x2, …).
Bernoulli distribution: The distribution that results when only two outcomes of a trial are possible, e.g. a coin flip. When paired with a GLM, it is referred to as Bernoulli regression, or more frequently, logistic regression.
Binomial distribution: The distribution that results from the sum of multiple, independent, and identically-distributed Bernoulli trials. When paired with a GLM, it is referred to as binomial regression.
Lognormal distribution: Log-transformed normal distribution. When paired with a GLM, it is referred to as lognormal regression.
Logit link: In Bernoulli and Binomial regression, the linear model needs to be logit-transformed to fit the shape of the response variable data. The logit link performs this task.
Hierarchical model: A GLM with multiple levels (each of which is generally an average or regression of some kind), where the results of one level of the model feed into the next level of the model. An advantage of this kind of model–as opposed to running each average or regression separately–is that uncertainty in the mean or intercept and slope parameter estimation is transferred from one level of the model to the next.
Uncertainty shrinkage: Using an average of related parameters at one level of the hierarchical model to reduce the uncertainty of the parameter estimation at another level.
MCMC: Acronym for Markov chain Monte Carlo. A type of simulation through distribution sampling used to estimate the likelihood of any regression. Most often paired with Bayesian methods, but some recent frequentist statistics have started to use MCMC.

MCMC chain: One simulation of MCMC. It is standard in Bayesian regression to run three chains in an analysis, using different starting points (initial values) for each chain. The three chains are then used to assess convergence.
Gelman-Rubin statistic: Standard method of assessing convergence by calculating the similarity between the three (or more) MCMC chains run for a given analysis. Similarity values are expected to be between 1 and 1.1 (Gelman and Rubin 1992).

Modeling multi-trophic interactions using SEMs or Bayesian hierarchical models

Simple or even more complex linear mixed-effect models do not sufficiently represent whole-system or multi-trophic interactions, and understanding these types of interactions would require running multiple models. Instead, what is needed is some way to model a directed acyclic graph, which is a graphical representation of the relationships between levels of a multi-level model that often includes both direct and indirect effects (see Figure 28 for an example of a directed acyclic graph). The best options for directed acyclic graph analysis (also referred to as path analysis) in ecological statistics are structural equation models (SEMs) or Bayesian hierarchical models (Ogle 2009, Grace et al. 2010). Here we briefly review the advantages and disadvantages of each approach. This is not intended to be an exhaustive list and as such does not thoroughly review the vast statistical literature on SEMs and Bayesian hierarchical models.

SEMs can generally incorporate and estimate latent variables (unobserved variables), which can be intermediate levels of the multi-level model (Grace et al. 2010). They can be used with either maximum likelihood estimation (MLE) or MCMC, although most use is with MLE (Grace et al. 2010, Fan et al. 2016). Several R packages exist for SEM, (e.g., *Lavaan* (Rosseel 2012) and *piecewiseSEM* (Lefcheck 2015)), which allows for a 'plug-and-play' type analysis, and the output in these packages can include a p-value (Fan et al. 2016). *Lavaan* and other packages use MLE (Rosseel 2012), which requires certain assumptions to be met within the data, i.e., normal distributions, continuous predictor variables, and few missing data (Fan et al. 2016). The package *piecewiseSEM* instead estimates each relationship separately, but this approach has other drawbacks, e.g., it cannot incorporate latent variables (Lefcheck 2015). Models become unreliable with smaller data sets, and while many recommended minimum sample sizes for data exist in the literature, agreement does not (Fan et al. 2016). Finally, SEMs supposedly test causal relationships, but much debate about the veracity of that claim exists within the literature (e.g., Lindquist and Sobel 2013).

Bayesian hierarchical models, like SEMs, can incorporate and estimate latent variables (Ogle 2009). Because Bayesian analysis outputs include uncertainty and because parameters are estimated as part of a whole model, uncertainty is carried through multiple levels of the model. Uncertainty at any level can be reduced through averaging related parameters, also referred to as shrinkage (O'Hara and Sillanpaa 2009). Manager or researcher knowledge can be incorporated into the model via priors, which give a starting (or educated guess) value for parameters. Model outputs, posterior distributions, give a direct measure of belief in the models (the probability of the hypothesis, given the data) instead of *p*-values (the probability of the data, given the hypothesis; Ellison 2004). Model outputs can also be used to calculate effect

sizes, allowing for comparison between effects of different independent variables within the model. This comparison is similar, but not the same, as the comparison possible between independent variables with the results of SEM analysis. The biggest disadvantage, however, to Bayesian hierarchical modeling is that it requires the manager or researcher to code their own analysis in full (except for the MCMC evaluation) instead of using an R package like for SEMs. While this makes the analysis options more flexible, it does require a higher level of expertise.

Tri-trophic interactions at PTA

Non-native invasive predators (NIP) pose a potential threat to native and endangered plant species through consumption of pollinators. Our objective was to quantify the relationship between our NIP, native predators, native and non-native pollinators, and focal plant species to determine the indirect effects of NIP on native and endangered plant species at U.S. Army Pōhakuloa Training Area (PTA) on Hawai‘i island (Figure 28).

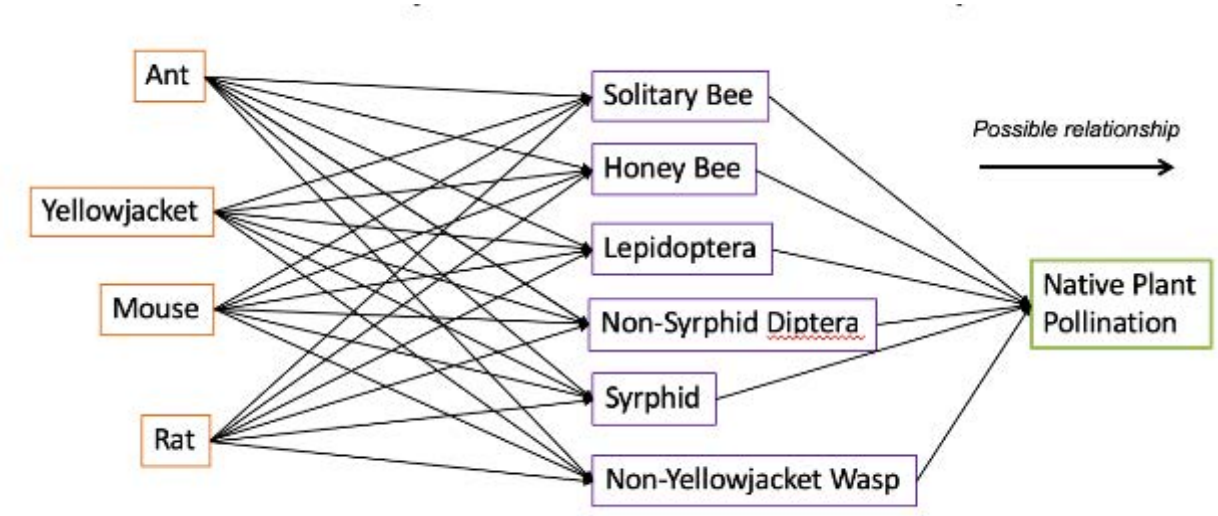

Figure 28. Conceptual model of all tri-trophic interactions between NIP, other predators, pollinators, and native plant species. NIP directly and indirectly affect pollinators, which in turn affect the pollination interactions with native plant species, and as a consequence, the seed set of these native plant species.

Methods

Experimental setup, predator treatments, and monitoring

Our experimental setup consisted of 20 plots that were grouped into four blocks. We located blocks 1 and 2 in grassland-shrubland habitat with largely a’a lava. We located blocks 3 and 4 in ‘ōhi‘a woodland habitat with pahoehoe lava. We placed pots of our focal plant species in the center of each plot. Plant species consisted of four federally endangered plant species (*Haplostachys haplostachya,* honohono, Lamiaceae; *Silene lanceolata,* Caryophyllaceae; *Stenogyne angustifolia,* Lamiaceae; and *Tetramolopium arenarium,* Asteraceae) and four

common species (*Argemone glauca,* pua kala, Hawaiian prickly poppy, Papaveraceae; *Bidens menziesii*, koʻokoʻolau, Asteraceae; *Dubautia linearis*, kūpaoa, Asteraceae; and *Sida fallax,* ʻilima, Malvaceae). Within each block, we randomly designated one treatment type to each of the 5 plots, to experimentally reduce populations of NIP: ant treatment plot, rodent treatment plot, yellowjacket treatment plot, combined treatment plot, and untreated control plot. These NIP (ants: primarily *Linepithema humile* but also *Tapinoma melanocephalum*; rodents: *Mus musculus* and *Rattus rattus*; and yellowjacket wasp: *Vespula pensylvanica*) are known to consume arthropods, including some known pollinators of our focal plant species.

We subjected predators to a treatment regime in corresponding plots. In the rodent treatment and combined treatment plots, we placed rat and mouse snap traps in 49 and 169 locations, respectively. We trapped rats and mice continually throughout the experimental treatment period, and we checked traps once every one or two weeks, dependent on the level of rodent activity (Section VII. Rodent populations, dynamics, and suppression). In the ant treatment and combined treatment plots, we applied granular formicide bait (Maxforce) three times per calendar year. We spread bait throughout the entire plot for each of the ant treatment and combined treatment plots (Section VIII. Ant populations, dynamics, and suppression). In the yellowjacket treatment and combined treatment plots, we left out canned chicken laced with fipronil insecticide for wasps to consume and take back to nests twice per calendar year. We placed yellowjacket bait stations at nine locations within a plot (Section IX. Yellowjacket population, dynamics, and suppression). In the untreated control plots, no predator reduction was undertaken.

We monitored rodent activity using plastic tunnels, lined with an inked card and baited with peanut butter (Section VII. Rodent populations, dynamics, and suppression). We monitored ant activity within the plots using index cards baited with 40% tuna-60% corn syrup mixture. We left cards out for 1 hour, after which we photographed the cards and collected and identified foraging ants. We monitored ants approximately one week prior to and one to two weeks after ant treatments (Section VIII. Ant populations, dynamics, and suppression). We monitored yellowjackets using heptyl butyrate traps that we left out for four days. Like ants, we monitored yellowjackets approximately one week prior to and one to two weeks after yellowjacket treatments (Section IX. Yellowjacket population, dynamics, and suppression).

Pollinator observations

All potted plant species were propagated and grown in a greenhouse (Section XI Greenhouse propagation for eight native species) before being placed in the plots. We observed pollination using a combined scan sampling and focal individual observation method wherein observers scanned all open flowers for the first minute of a 10-minute observation block and recorded all pollinators observed, before they observed pollinator individuals for the other 9 minutes of the observation block, following the focal individual and recording the number of flowers and plants visited. Observers recorded the number of all flowering individuals, the number of open flowers, and the plant species for each during each observation period (Section III. Flower visitation observations). Observers tracked individuals that landed on three species of plants during each observation block. *Argemone glauca* did not propagate well

in the greenhouse, leading to few flowering individuals and flowers (Section XI. Greenhouse propagation for eight native species). *Stenogyne angustifolia* had few pollinators and pollination events over the course of both the pre-experimental period of wild plant observations (Section III. Flower visitation observations) and the experimental treatment period. Both *A. glauca* and *S. angustifolia* had too few pollinator interactions to be included in the analyses.

We divided all pollinators into six major groups, including only observed species known to perform frequent, legitimate pollen transfer: solitary bees (primarily *Hylaeus spp.* and *Lasioglossum spp.*), honey bees (*Apis mellifera*), lepidopterans (primarily *Crambidae spp.*, *Lampides boeticus*, *Orthomecyna spp.*, *Pieris rapae*, and *Udara blackburni*), non-syrphid dipterans (primarily *Dioxyna sororcula* and *Muscidae spp.*), syrphids (primarily *Allograpta spp.*), and non-*Vespula* wasps (primarily *Pachodynerus nasidens* and *Polistes aurifer*). To standardize pollinator observations, we calculated pollinator visitation importance (PVI), which is an index of average flower visitations per pollinator group per plant species (Aslan 2015; modified from Renne et al. 2000)

Analysis of 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 treatment 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

Our 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). While this multi-trophic 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 with 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 (Figure 29).

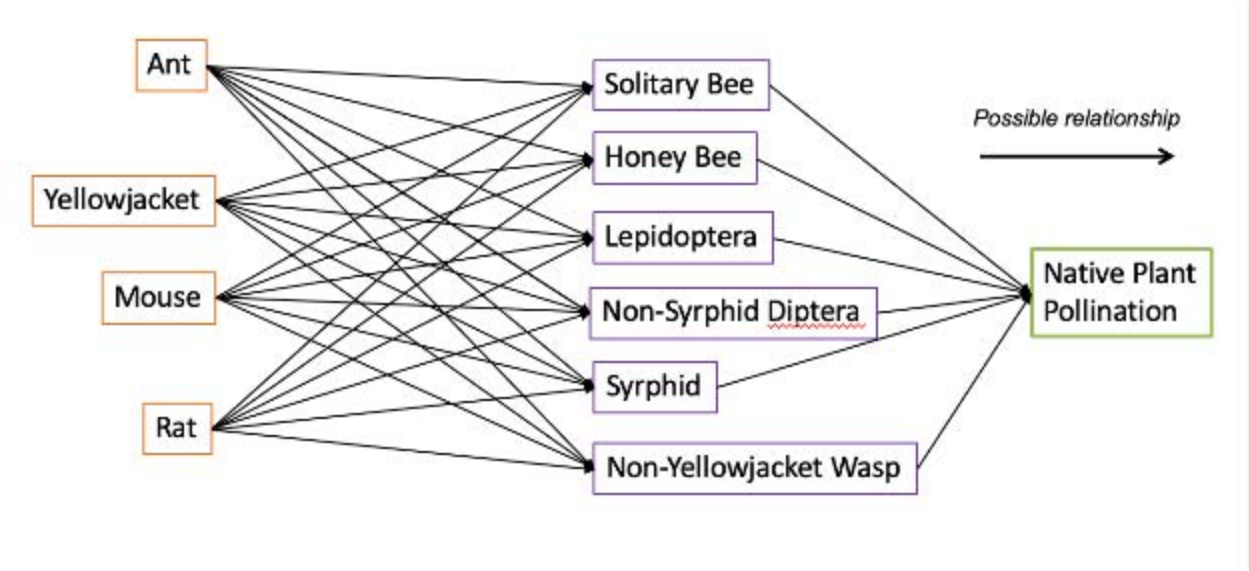

Figure 29. All possible interactions between NIP (boxes outlined in orange) and pollinators (boxes outlined in purple) modeled in SHELOB. For the presence/absence model, each pollinator is modeled in the context of its interactions with each of the six focal plant species. For the PVI model, each pollinator is modeled in the context of its interactions with all of the six focal plant species together.

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. To match the rat and mouse data, we converted ant and yellowjacket data to a frequency measure based on proportion out of maximum value recorded for the species. We modeled frequency of NIP within each plot at 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, rats and mice were only monitored in Rodent, All, and Control plots during the experimental phase after suppression began. During the pre-experiment phase of the project, however, rodents were monitored in all plot types. We compared pre-experiment 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 could still be higher than control or 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 as an experimental unit, we therefore used predator frequency as a continuous variable across all plot/block combinations to account for this 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 3 hour observation periods so data were transformed for every 3 hour 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 wasp frequencies for that combination. The number of trials in the Binomial distribution for each pollinator species was the number of 3 hour observation periods for each plot within each half year period. No pollinators of 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 lumped the pollinator observations for all plant species across a given pollinator species.

We fit both 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 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 ≥ 1 and <1.1 (Gelman and Rubin 1992).

Results

Pollinator species group PVI for each plant species

Pollinator interactions varied by time of year during the experimental treatment period (Figure 30). 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*) (Table 3). The honey bee group consisted of only *Apis mellifera*, which are non-native in Hawai'i, and the syrphid flies are all non-native as well. The other groups are composed of both native and non-native species. *Dubautia linearis* flowers seasonally and so did not flower during the February–June 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).

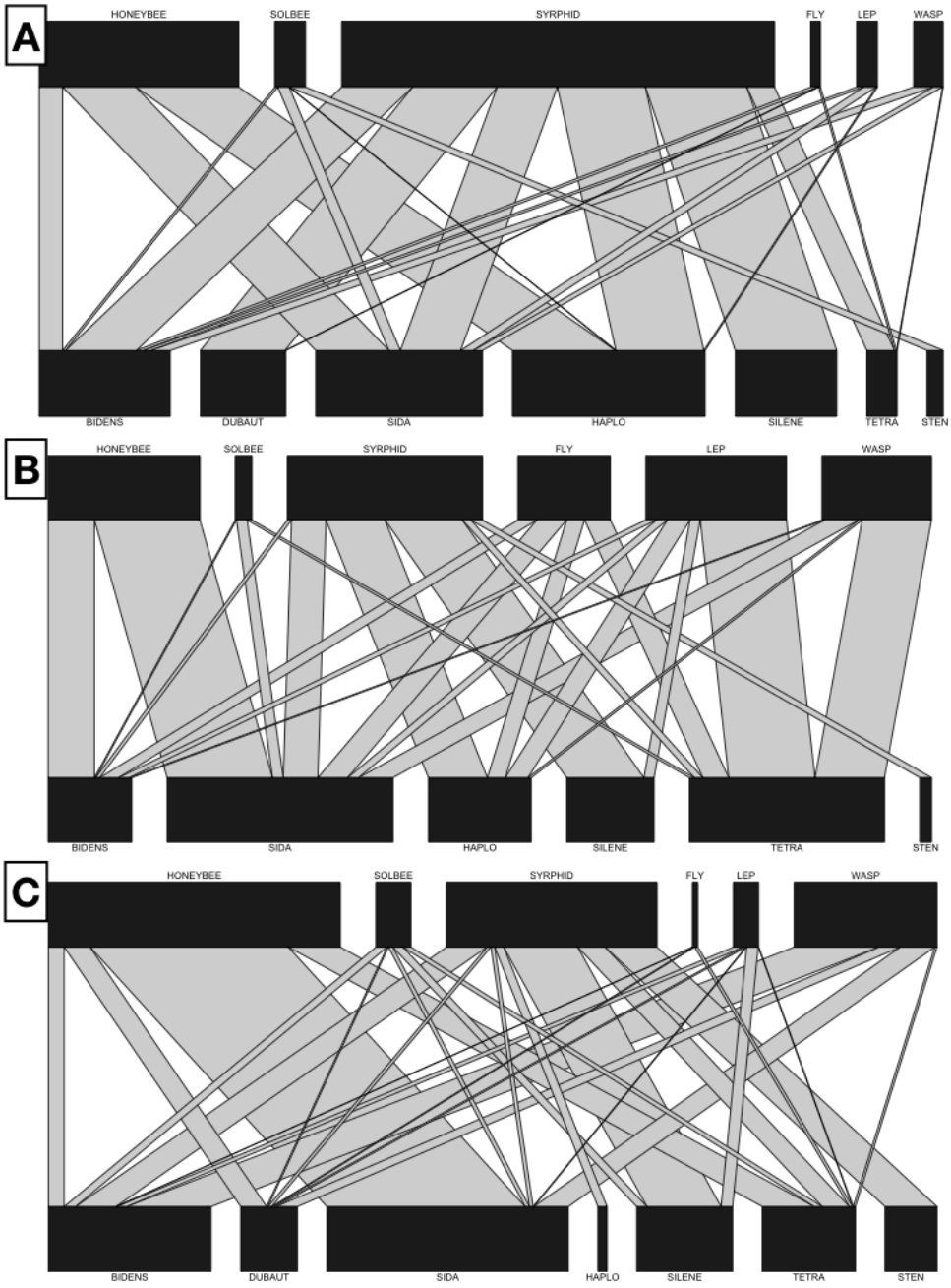

Figure 30. Plant-pollinator networks averaged across all plots and blocks for A) August 2016–January 2017, B) February–June 2017, and C) July–November 2017. Upper level: FLY = non-syrphid dipterans, LEP = butterflies and moths (lepidopterans), HONEYBEE = honey (social) bees, SOLBEE = solitary bees, SYRPHID = 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.*

Table 3. Most important pollinator species group for the pre-experiment and experimental treatment periods for all plant species except *Argemone glauca.* Importance values are based on observations performed on wild plants occurring across the study area during the pre-experiment period, but on potted plants located within treatment plots during the experimental treatment period.

Plant species	**Most important (pre-experiment period)**	**Most important (experimental treatment period)**
Bidens menziesii	Honey bees	Non-*Vespula* wasps
Dubautia linearis	Syrphid flies	Syrphid flies
Haplostachys haplostachya	Non-*Vespula* wasps	Syrphid flies
Sida fallax	Moths & butterflies	Honey bees
Silene lanceolata	Honey bees	Syrphid flies
Stenogyne angustifolia	Solitary bees	Syrphid flies
Tetramolopium arenarium	Moths & butterflies	Moths & butterflies

Predator frequencies at PTA

Frequencies of our target NIPs at PTA varied by experimental block (Figure 31). 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 31).

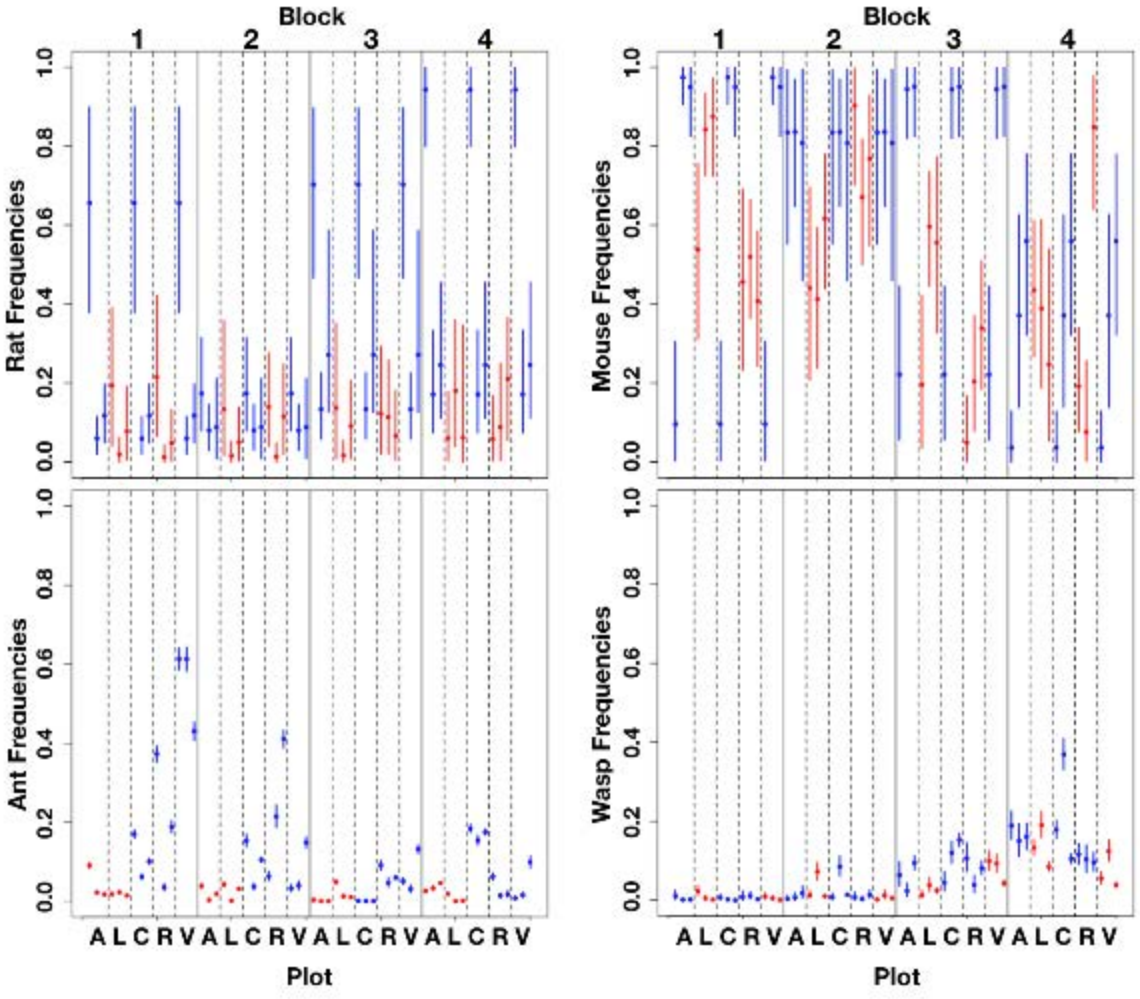

Figure 31. NIP frequencies across all blocks (labeled at the top), plots (labeled at the bottom), and time periods (within each block/plot combination: left = August 2016–January 2017, middle = February–June 2017, right = July–November 2017). Red represents treatment plots (Rodent and All plots for rats, 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 plant species at PTA

We found significant negative relationships between the frequency of NIP and the frequency of presumed pollination by at least one of the six pollinator species groups for four of the six plant species (Table 4). We found negative relationships between NIP and nearly every pollinator species groups 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*. Across all 36 possible interactions between pollinator groups and plant species, rats had a negative relationship with 7 of them, 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).

Table 4. Relationships between NIP and presumed pollination. Significant relationships between NIP and presumed pollination 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 highlighted in red, and positive relationships are highlighted in turquoise. NS = No significant relationship between NIP and presumed pollination on a given plant species by a given pollinator species group. NPE = No pollinator events recorded between the pollinator species group and plant species during the experimental treatment period. NPE are highlighted in grey. BID = *B. menziesii*, DUB = *D. linearis*, HAP = *H. haplostachya*, SID = *S. fallax*, SIL = *S. lanceolata*, and TET = *T. arenarium*.

	Bidens	**Dubautia**	**Haplostachys**	**Sida**	**Silene**	**Tetramolopium**
Solitary Bees	ns	ns	Mice (-)	Wasps (-)	ns	Ants (-)
Honey Bees	ns	ns	ns	Ants (-) Wasps (+)	no pollination events	Mice (+)
Moths & Butterflies	ns	ns	Rats (-)	ns	ns	Rats (-)

Non-syrphid flies	ns	ns	Rats (-)	Rats (-)	no pollination events	Rats (-) Ants (-)
Syrphid flies	Mice (-)	ns	ns	Mice (-) Wasps (-)	ns	ns
Non-*Vespula* wasps	Wasps (-	Mice (+)	Rats (-)	Rats (-)	no pollination events	Ants (-)

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 for many plant species-pollinator interactions were positive but uncertain (95% CI: <0.01, >0.90 for all pollinator groups): 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.

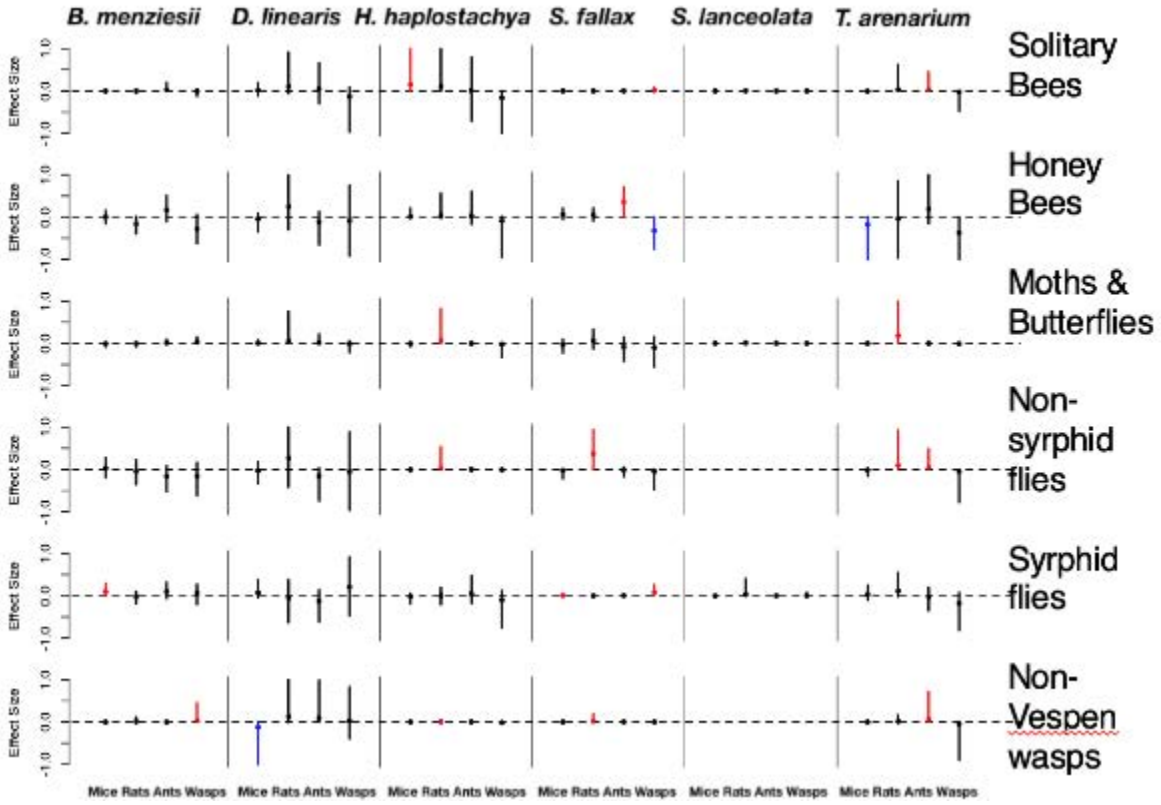

Figure 32. Effect sizes for the relationships between NIPs and pollination interactions between pollinator (row) and plant (column) species. Effect sizes were calculated as the change in pollinator frequency on a given plant species when a NIP frequency was reduced from 0.6 to 0. Positive effect sizes 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.

Study conclusions

Pollinator interactions varied by time of year during the experimental treatment period. 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 visiting potted plants in the experimental treatment period than during the wild plant observations (Table 3). One explanation for this finding is that pollinator importance

may be extremely localized in time or space. Pollinator observations are time- and labor-intensive, but to capture these spatial and temporal fluctuations, particularly for endangered species that are nearly or entirely self-incompatible, it may be necessary to perform them over repeated seasons and across multiple locations.

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 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 5). 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 5. 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 (Section IV. Pollination treatments). Therefore, reducing ant populations, if it increases pollination, would increase the reproductive success of this endangered species.

Management recommendations specifically for increasing pollinator frequencies at each of the focal plant species are shown in Table 6. Recommendations are given if managing for pollinators with the highest PVI for a given plant, and if managing for all pollinators for a given plant.

Table 6. NIP management recommendations for the purpose of increasing the likelihood of pollination on each of the focal plant species. The "Most important pollinator & NIP relationships" is the management recommendation for the most important pollinator species (highest PVI) for the given plant species during the experimental treatment period. "All pollinator species & NIP relationships" is the management recommendation across all observed pollinator species for the given plant species. If an NIP was found to have both positive and negative relationships across pollinator species for a given plant species, it was not included in this column.

Plant Species	**Most important pollinator & NIP relationships**	**All pollinator species & NIP relationships**
Bidens menziesii	Non-*Vespula* wasps: reduce yellowjackets	Reduce yellowjackets, reduce mice
Dubautia linearis	Syrphid flies: N/A	N/A
Haplostachys haplostachya (endangered)	Syrphid flies: N/A	Reduce rats, reduce mice
Sida fallax	Honey bees: Reduce ants	Reduce rats, reduce ants, reduce mice
Silene lanceolata (endangered)	Syrphid flies: N/A	N/A
Stenogyne angustifolia (endangered)	Syrphid flies: Possibly reduce ants	Possibly reduce ants
Tetramolopium arenarium (endangered)	Moths & butterflies: Reduce rats	Reduce rats, reduce ants

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XIII. Managing for Resilience

In a Nutshell:

- Identifying the appropriate *scale*, *state*, *stressor*, and *success* are critical to managing for resilience.
- A priori identification of the ecological and social characteristics of a particular system is essential to ensure that stakeholders are in agreement about focal system state, scale, stressors, and success.
- A proactive approach to defining resilience in a particular system should occur prior to management implementation so that success can be understood and recognized.

General background

Framework for managing for resilience

The highly invaded nature of the plant/pollinator system at PTA is evidence that this ecological community has been substantially disturbed by species introductions. This raises the question of whether managing for resilience should be a priority in this system, and how it might be accomplished. Resilient systems will retain or regain their characteristics following disturbance. As a result, resilience management is a priority of US resource management agencies. Yet understanding how resilience can be predicted and measured remains a matter of debate. High biodiversity, adaptive capacity, human prosperity, and sustainable development have all been linked to resilience (Folke et al. 2016), yet the drivers and characteristics of these elements are themselves matters of debate. Resilience by definition describes the response of a system to a specific disturbance or stress (Speranza et al. 2014), but in conversation the term is often used to mean simply "healthy" (e.g., Halpern et al. 2012; Speranza et al. 2014).

Resilience has very specific definitions and properties in conceptual literature. A system's defining characteristics are considered its "state," and most systems settle into stable states via domains of attraction that result at least temporarily in stability. Disturbances, or perturbations, can push a system out of its stable state. Under its classic definition, the term "resilience" encompassed the modern concepts of both resistance and resilience (Holling 1973). Resistant systems are those that will remain in their current state (i.e., retain their defining characteristics) in the face of perturbation, and resilient systems are those that will regain their defining characteristics after a perturbation that shifted them into a new state, for a time (Lake 2012).

Several key challenges make it difficult to measure and model resilience. Identifying the most effective spatial ***scale*** of management is essential but problematic when resilience mechanisms remain abstract and undefined (Timpane-Padgham et al. 2017). Similarly, description of a full system ***state*** can be difficult and subject to interpretation (Siedl et al. 2016). Solutions cannot be evaluated unless the ***stressor*** of concern is pinpointed; the interaction of stressors can by contrast impede responses (Müller et al. 2016). As management responses are employed, it becomes necessary to define ***success***, and unless indicators of success are

predetermined, a given manager can claim success from any activity (Kharrazi et al. 2016; Steelman & DuMond 2009).

In light of these challenges, we suggest that, within any given management effort, *a priori* definitions of the four key elements of resilience is a critical step. **Effective resilience management must (a) pinpoint the specific stressors to which resilience is sought, (b) identify the desired and current states of a system, as informed by management values, (c) define meaningful temporal and spatial scales of resilience management actions and targets, and (d) select clear social and ecological metrics of success**. We have termed these essential elements *the 4 S's*.

Results

Resilience at PTA

At PTA, we have found that the majority of pollination is now carried out by non-native insects. In order to plan effective resilience management in light of this wholesale ecosystem change, the 4 S's must be considered:

If the ecological *state* here is defined by functions, it may be that no change in stable state has occurred here. As long as pollination is occurring, the system would be considered resilient; loss of native pollinators has occurred, but the system recovered from this disturbance. If pollination declines, management may include support or introduction of pollinators (even non-natives). Because continued extinctions are unlikely to remove all pollination from the system, the system may be considered resilient. If state is defined by species assemblage, the system has entered a new stable state, with most pollinators being non-native. Managers may focus on preventing future state change. This may require tracking populations of individual species and restoring them through cultivation and rearing of native species. Depending on how managers define resilience components, they may arrive at very different management activities.

Meanwhile, the remaining S's are also at play. Because there is spatial heterogeneity in pollination, *scale* is important. Do managers restore native pollinators across the whole region, or is persistence in certain patches sufficient? Should they work on bolstering pollinator habitats at a broad scale or introducing and monitoring individual pollinator populations at fine scales? Does *success* signify that native plants are receiving pollination, that historic native plant/native pollinator relationships are restored across the landscape, that each species persists somewhere, or that native biodiversity is bolstered as much as possible in any form (a value for land managers and the public). Finally, in addition to the *stressor* of species extinctions, other stressors will dictate which management activities can be adopted. For example, invasive species have introduced novel predation and fire regimes to the region (D'Antonio & Vitousek 1992; Hanna et al. 2013), complicating future pollination management by impacting native pollinator populations as well as native, fire-susceptible plants; it may be necessary to remove some of these non-natives before attempting pollinator restoration. The four S's steer decision-making in this system by illuminating value systems.

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Natural History Notes

This section documents some natural history observations from our field work at U.S. Army Pōhakuloa Training Area on Hawai‘i island.

Rodent trapping

- Non-target catch was commonly found in rodent traps, e.g., game birds, cats, mongoose.
- Feral cats were consistently caught on trail cameras patrolling the trap lines at night (looking for dead rodents in the traps, particularly mice). The field crew observed mongoose during the day doing similar behavior.
- Some of the game birds (particularly Erckel's francolin) seemed to 'follow' the field crew as they reset and rebaited snap traps, to take the peanut butter bait from the traps.

Rodents

- There were relatively more rats in the ‘ōhi‘a woodland, and more mice in the grassland-shrubland.
- A mouse was found stuck by its tail on *Silene lanceolata*.

Ants

- Argentine ants were much more abundant in the grassland-shrubland than in the ‘ōhi‘a woodland.
- Argentine ants were observed to swarm and completely cover an ‘a‘ali‘i i (*Dodonaea viscos*a) shrub in the grassland-shrubland.
- Ants appeared to be distributed quite patchily in KKE; flipping over large stones was the best way to find their nests.
- At least six ant species have been recorded at PTA: the Argentine ant (*Linepithema humile*), the big-headed ant (*Pheidole megacephala*), the ghost ant (*Tapinoma melanocephalum*), the pharaoh ant (*Monomorium pharaonis*), *Cardiocondyla kagutsuchi* (no common name, often identified as *C. venustula* in earlier literature), and *Hypoponera opaciceps* (no common name).

Yellowjacket wasp

- There were very few yellowjackets in the grassland-shrubland, lots more in the ‘ōhi‘a woodland.
- Only one yellowjacket nest was ever found (in block 4 in the ‘ōhi‘a woodland), although honey bee nests were frequently encountered in KKE. This was despite our crew spending a lot of time walking around in and around our plots. Although yellowjackets were abundant in some areas, the nest density appears to be low compared to other parts of Hawaii Island, such as Hawai‘i Volcanoes National Park.
- Yellowjacket populations fluctuate seasonally.
- Dead goat in KKE was eaten by yellowjackets.
- Yellowjackets transport prey items in their mouths.

Endangered plants

- *Stenogyne angustifolia* received almost no flower visitors. In the first year of observations on wild plants, we observed only one visitation (by non-native *Lasioglossum* sp.) in over 120 hours of observation.
- Flowers of *Silene lanceolata* closed up during the day (closing in mid-morning, opening again in late afternoon). Nighttime video footage showed some noctuid moth visitation to flowers.

Acronyms

ANOVA	analysis of variance
CO1	cytochrome oxidase 1 mitochondrial gene
GLM	general linear model
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 (used in statistical analyses)
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
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, Argentine ant, *Apis mellifera*, *Bidens menziesii*, black rat, conservation, DNA barcoding, dryland tropical ecosystem, *Dubautia linearis*, endangered species, *Haplostachys haplostachya*, Hawai'i, honey bee, house mouse, Hymenoptera, insect, invasive species management, island endemics, Lepidoptera, *Linepithema humile*, *Mus musculus*, plant-animal interactions, plant reproduction, Pōhakuloa Training Area, pollination, *Rattus rattus*, SHELOB, *Sida fallax*, *Silene lanceolata*, *Stenogyne angustifolia*, *Tetramolopium arenarium*, *Vespula pensylvanica,* yellowjacket wasps