EXECUTIVE SUMMARY

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

SERDP Project RC-2432

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ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of variance
BOLD	Barcode of Life Database
CO1	Mitochondrial gene cytochrome oxidase 1
DNA	Deoxyribonucleic acid
DoD	U.S. Department of Defense
ha	Hectare
ISI	Index of Self-Incompatibility
m	Meter
NCBI	National Center for Biotechnology Information
NIP	Non-native invasive predator
OTU	Operational Taxonomic Units
PLI	Pollen Limitation Index
PTA	U.S. Army Pōhakuloa Training Area
SERDP	Strategic Environmental Research and Development Program
SHELOB	SERDP Hawai'i Ecosystem-Level Observation-Based model
USDA	U.S. Department of Agriculture

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1.0 INTRODUCTION

This project examined the impacts of non-native invasive predators (NIPs) on pollination and native plant reproduction in a tropical dryland ecosystem in Hawaiʻi. Investigators combined field observation, experimental manipulation, and laboratory analyses to examine interactions between focal native plants, insect pollinators, and NIPs. Pollination is a critical ecosystem process, and non-native species invasions can disrupt pollination services and result in negative impacts on native plant reproduction and genetic diversity. Such impacts are particularly acute for oceanic islands, which are well known for high endemism and unique biological diversity, but are also particularly susceptible to invasions.

Field work was conducted on the U.S. Army Pōhakuloa Training Area (PTA) on Hawaiʻi Island. PTA contains part of a remnant sub-alpine tropical dryland ecosystem and supports 20 federally designated threatened and endangered plant species. Project results provide basic and applied scientific information for the management of tropical dryland ecosystems on military lands in the Pacific. These lands are home to numerous threatened and endangered species, some of which are found only on military holdings. Results can be used by the U.S. Department of Defense (DoD) to effectively steward the land base while simultaneously supporting the military's operational, training, and testing missions.

2.0 OBJECTIVES

The principal objective of the research was to determine impacts of NIPs on insect pollinators and pollinator services in an invaded tropical dryland ecosystem in Hawaiʻi. NIPs consume animal pollinators and, by doing so, reduce pollinator populations and possibly eliminate entire pollinator guilds. Loss of pollination services due to NIPs likely is an important, although poorly understood, factor in both native plant conservation and management for long-term sustainability of native island ecosystems. The study focused on both endangered and common Hawaiian plants that are pollinated by insects.

3.0 TECHNICAL APPROACH

Investigators examined interactions between eight native plants (endangered and common), pollinators (native and non-native), and NIPs. They used various approaches to: (1) identify current insect pollinators and pollination effectiveness for focal native plant species; (2) examine diets of study site NIPs to identify direct interactions with insect pollinators; (3) apply common NIP control methods to experimentally determine their effectiveness at both reducing NIP populations and NIP impacts on pollinator-plant interactions; and (4) analyze the data using Bayesian hierarchical models to identify the direct and indirect relationships between NIPs, insect pollinators, and native plants.

Field data collection included one year of baseline assessment prior to experimental treatments (March 2015-February 2016), approximately 18 months during treatment implementation (August 2016-January 2018), and three months after the end of experimental treatments (February 2018-April 2018).

3.1 STUDY AREA AND FOCAL SPECIES

PTA is the largest U.S. Army holding in the State of Hawai‘i and encompasses approximately 53,750 hectares (ha) in the saddle region between Mauna Kea, Mauna Loa, and Hualālai volcanoes on the island of Hawai‘i. Field work occurred in Kīpuka Kālawamauna East on PTA, a 794-ha mix of grassland-shrubland and *Metrosideros polymorpha* (‘ōhi‘a) dominated woodland located at approximately 1,675 meters (m) in elevation. This unit is fenced to exclude non-native invasive ungulates (primarily goat and sheep) and contains many threatened and endangered plant species.

Focal plant species for the study included four endangered species (*Haplostachys haplostachya* (*H. haplostachya*), *Silene lanceolata (S. lanceolata)*, *Stenogyne angustifolia* (*S. angustifolia*), and *Tetramolopium arenarium* (*T. arenarium*)) and four common native species (*Argemone glauca* (*A. glauca*), *Bidens menziesii* (*B. menziesii*), *Dubautia linearis* (*D. linearis*), and *Sida fallax* (*S. fallax*)). The focal NIPs in the study site included rodents (*Mus musculus* (*M. musculus*), *Rattus rattus* (*R. rattus*)), ants (*Linepithema humile* (*L. humile*), *Tapinoma melanocephalum* (*T. melanocephalum*)), and yellowjacket wasp (*Vespula pensylvanica* (*V. pensylvanica*)).

3.2 EXPERIMENTAL PLOTS

Investigators established 20 experimental plots to assess the effects of removal of NIPs (rodents, ants, yellowjackets) on the insect pollinator community and pollination of focal plants (**Figure 1**). Sixteen plots received predator control treatments (four Rodent treatments; four Ant treatments; four Yellowjacket treatments; and four All combined rodent/ant/yellowjacket treatments) and four 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) treatment area. Potted plants of the focal plant species were placed at the center of the central core area during the experimental treatments. 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 were regularly monitored at a subset of these stations within the plots.

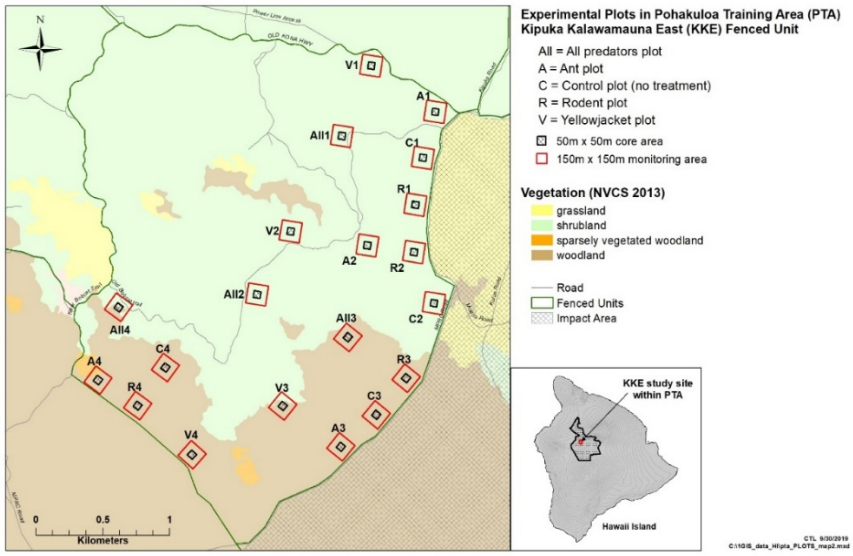

Figure 1. Experimental Plots within Kīpuka Kālawamauna East Fenced Unit at PTA, Hawai‘i Island

Plots were arranged in four blocks of five plots in order to account for habitat differences. 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. Plot buffers were included in the arrangement, with larger buffers for the plots receiving yellowjacket treatment.

3.3 FLOWER VISITATION OBSERVATIONS AND POLLINATION TREATMENTS

Investigators conducted flower visitation observations to determine the identity of current flower visitors (all of which were insects) for each of the focal native plant species, as well as the rate of visitation exhibited by each insect taxon. This enabled construction of partial pollination networks for the site (limited to the eight focal plant species but comprehensive for those plants). In the year prior to implementing experimental treatments, observations were made on naturally occurring wild plants to assess the baseline pollination regime. After implementation of treatments, observations were made on the potted plants within experimental plots.

Investigators also performed manual pollination treatments to evaluate the dependence of each plant species on outcrossing via pollinators. Fruit set and seed set from treated flowers were analyzed separately for each plant species, using parametric analysis of variance (ANOVA) or non-parametric Kruskal-Wallis tests. Results from the treatments were used to calculate the Index of Self-Incompatibility (ISI) and Pollen Limitation Index (PLI) for each plant species.

3.4 PREDATOR MONITORING AND TREATMENTS

Abundances of focal NIPs were monitored in all plots throughout the full duration of the field study. In each plot, NIPs were monitored at all or a sub-set of the 25 monitoring stations situated along transects radiating outward from the center of the plot. Rodents were monitored by tracking tunnels, ants were monitored by baited index cards, and yellowjackets were monitored by baited traps.

In the NIP treatment plots (four Rodent, four Ant, four Yellowjacket, and four All), suppression methods included continuous snap-trapping for rodents, periodic applications of granular formicide for ants, and periodic applications of insecticide-laced bait for yellowjackets throughout the experimental treatment period. NIP monitoring was performed immediately before and after treatments in treatment and control plots to assess the efficacy of treatments. Effectiveness of NIP treatments were assessed by comparing data between these plots, with the expectation that treatment effects would decrease with greater distance from the center of each plot. Data were analyzed with ANOVA tests.

3.5 DIET ANALYSES

NIPs were collected in the field for analysis of whole body, mouth, or fecal contents, which were then screened for insect prey deoxyribonucleic acid (DNA) to determine the types of insect pollinators consumed. Ants were collected in pooled samples, rats and mice were collected in traps and then dissected, and yellowjackets were captured as they returned to their nest carrying prey items in “prey balls.” For each prey ball, DNA was extracted, the “barcoding” region of the mitochondrial gene cytochrome oxidase 1 (CO1) was amplified, and sequences were obtained by Sanger sequencing. For pooled ant samples and fecal contents of individual rodents, the contents 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, Colorado).

Sequences for all sample types were then queried against the National Center for Biotechnology Information's (NCBI) GenBank and the Barcode of Life Database (BOLD) to assign species or Operational Taxonomic Units (OTU). The OTUs then enabled matching of the NIP-consumed prey with the flower visitors that were observed on target plant species in the field, or with other arthropod groups.

3.6 DATA SYNTHESIS

To capture seasonal and treatment-induced variation in predators and pollinators across the experimental time period, as well as to account for the timing of all monitoring, treatments, and pollinator observations, the experimental analysis period was divided into three approximately half-year periods: August 2016-January 2017, February 2017-June 2017, and July 2017-November 2017.

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

While treatments were effective, stochasticity of NIPs across the landscape made it so that abundances of predators in treated plots were at times higher than in untreated plots. Instead of using plot treatment as a categorical variable, the investigators modeled predator frequencies as continuous variables across all plot/block combinations to account for this natural variability.

Investigators performed binomial regression with a logit link on each pollinator species group for each plant species, using mean frequencies of NIPs as independent variables. For each combination of pollinator species group and plant species separately, the model estimated the relationships with NIP frequencies for that combination. The number of trials in the binomial distribution for each pollinator species was the number of 180-minute observation periods for each plot within each half year period. No flower visitors belonging to the honey bee, non-syrphid fly, or non-Vespula wasp groups were observed on *S. lanceolata* so these interactions were left out of the analysis. Investigators also ran the same analysis but combined all pollinator observations for all plant species across a given pollinator species for the three half-year periods, to determine an average effect of NIP on pollinator species.

4.0 RESULTS AND DISCUSSION

4.1 FLOWER VISITATION OBSERVATIONS

Across all observations, the large majority of flower visitors were either known non-natives or of insufficient resolution to determine native or non-native origin (i.e., recorded at order or family level where the taxon is known to include both natives and non-natives in the study area).

4.1.1 Baseline year

Figure 2 shows the visitation network for observations made during the pre-experiment, baseline year. Endangered plant species had fewer flower visitor species than common plant species. The only known native flower visitors observed in systematic observations for all plant species were the moth *Orthomecyna* sp. (Lepidoptera: Crambidae) and butterfly *Udara blackburni* (Lepidoptera: Lycaenidae). Taxa with uncertain origin included unidentified micromoths (Lepidoptera), unidentified wasps (Hymenoptera), *Hylaeus* sp. bees, and unidentified beetles (Coleoptera). All other taxa were known non-natives.

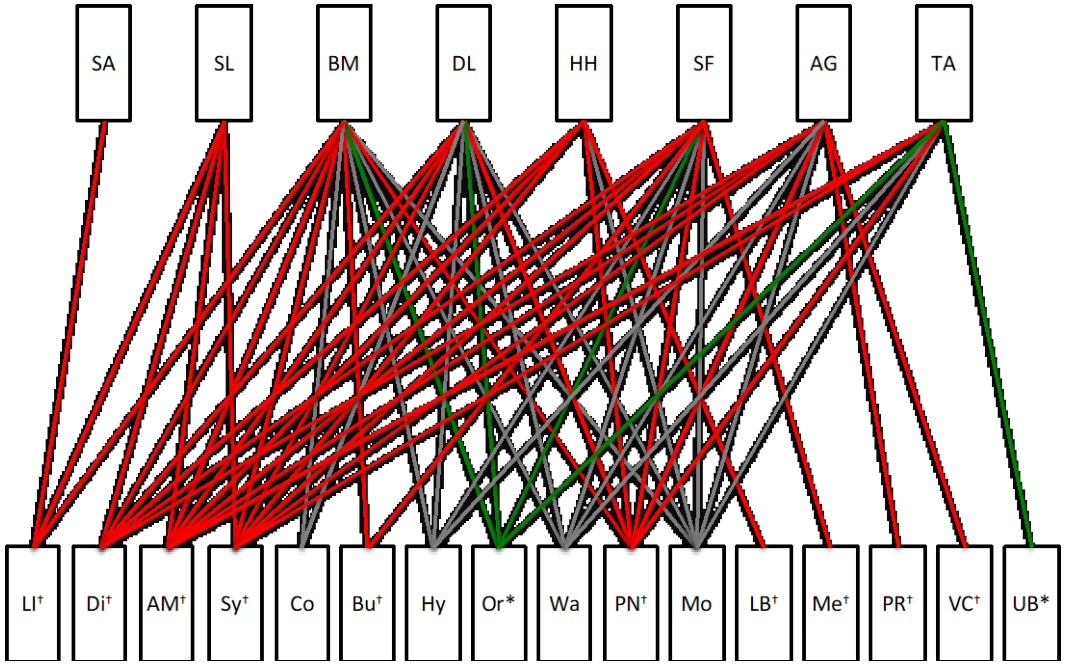

Figure 2. Networks Displaying Observed Interactions between Flower Visitors and the Eight Focal Native Plant Species in this Study

Green connectors = native flower visitors. Gray connectors = flower visitors of indeterminate nativity. Red connectors† = non-native flower visitors.*
Plants appear in the top row: SA = S. angustifolia (endangered), SL = Silene lanceolate (endangered), BM = B. menziesii, DL = D. linearis, HH = H. haplostachya (endangered), SF = S. fallax, AG = A. glauca, and TA = Tetramolopium arenarium (endangered). Flower visitors appear in the bottom row: LI = Lasioglossum impavidum, Di = Diptera (unspecified), AM = Apis mellifera, Sy = Syrphidae, Co = Coleoptera (unspecified), Bu = Butterfly (unspecified), Hy = Hylaeus sp. (unspecified), Or = Orthomecyna sp., Wa = Wasp (unspecified), PN = Pachodynerus nasidens, Mo = Moth (unspecified), LB = Lampides boeticus, Me = Megachilidae (unspecified), PR = Pieris rapae, VC = Vanessa cardui, and UB = Udara blackburni.

4.1.2 Treatment period

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

4.2 POLLINATION TREATMENTS

All plant species produced some seed when bagged to exclude outcrossing, indicating some degree of self-compatibility for each species based on ISI values. This implies that the species are capable of producing fruits and seeds even in the absence of all pollination. Self-compatibility may be critical for the survival of a population when pollinators are rare or individual plants are rare across a landscape. Island endemics are known to exhibit a high rate of self-compatibility, thought to be a result of the importance of self-compatibility to the earliest colonizers of a particular island, since it removes the need to arrive along with a mate. The self-compatibility observed for the focal species limits their dependence on pollinators in general. At the same time, bagging decreased fruit or seed set for all of the focal plants, indicating a likely cost to self-fertilization and emphasizing the importance of outcrossing in this system.

PLI values indicate that most of the focal plants are producing similar amounts of seed from ongoing, ambient pollination as from hand-supplementation, suggesting low pollen limitation in this system. This suggests that many of the flower visitors observed are likely successfully transferring pollen, and that these plant species are experiencing active pollination in this ecological community in spite of the heavy dominance of non-native pollinators. However, more of the endangered plant species are pollen limited than common species.

4.3 NIP SPECIES COMPOSITION AND SEASONALITY

Rats (*R. rattus*) and mice (*M. musculus*) were present in all 20 study plots on a year-round basis. The highest rat tracking during the year occurred in July through November, and the lowest periods of rat activity occurred during March and April. The lowest mouse activity tended to be in May and June, with a steady increase in subsequent months to reach peak activity during December through March. Plots in grassland-shrubland habitat tended to have the most mouse activity, whereas rats tended to dominate the woodland plots. Although the ratio of rats to mice differed seasonally and between habitat types, mice generally outnumbered rats at the study site by a ratio of roughly 2:1.

Abundance of Argentine ants (*L. humile*) and ghost ants (*T. melanocephalum*) varied by sampling event, but did not show a strong seasonal pattern. Argentine ants were found in Blocks 1-3, and ghost ants were only found in Block 4. Abundance of yellowjacket wasps (*V. pensylvanica*) varied both temporally and spatially across study sites. Wasps were most abundant in woodland plots and least abundant in grassland-shrubland plots. In all plots, wasp abundance peaked during the summer months (June to August) and was lowest in the spring.

4.4 EFFICACY OF NIP SUPPRESSION

Both rat and mouse trapping significantly reduced rodent activity in treatment plots compared to untreated control plots (**Figure 4**). However, although mouse activity was reduced, the target suppression level of ≤ 20% activity was unable to be maintained aside from the first four months following suppression. The most likely reasons for this are habituation to traps by mice, non-target trap interference, and the naturally high abundance of mice at the study site.

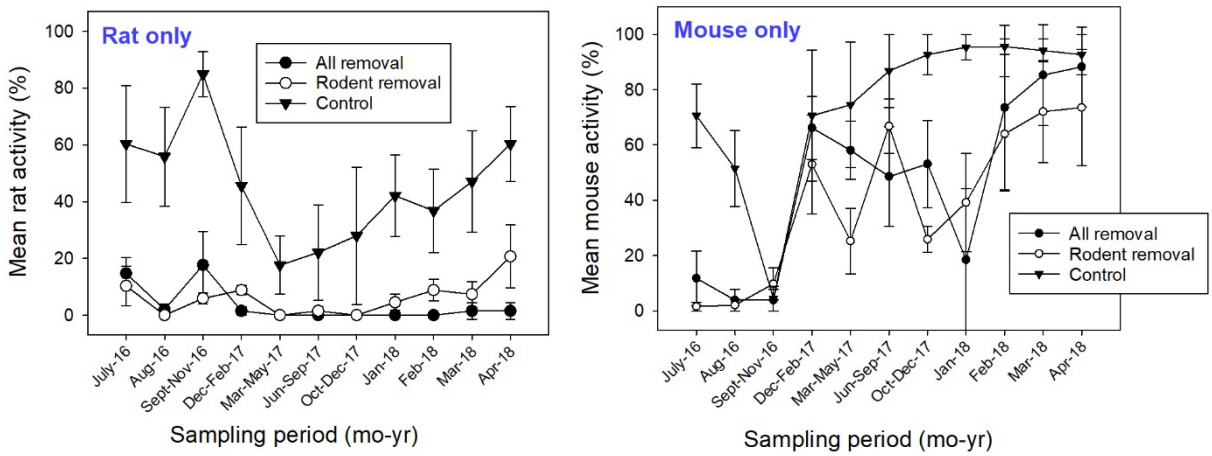

Figure 3. Rodent Activity in Experimental Plots

Ant treatments were effective at significantly reducing Argentine ants in treatment plots compared to untreated control plots, and effective at significantly reducing ghost ants in some but not all treatment plots (**Figure 5**). Ant populations rebounded soon after treatment, so frequent bait application was necessary to maintain suppression.

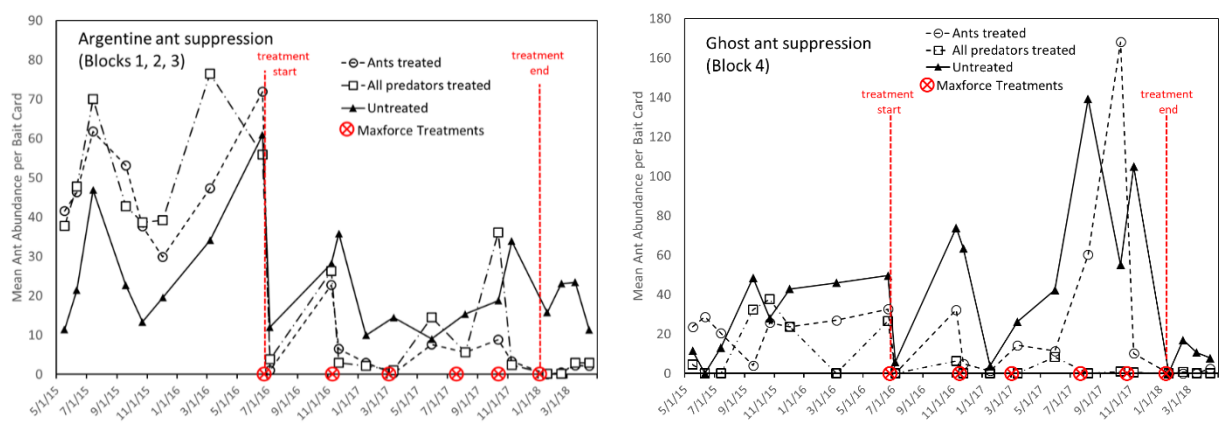

Figure 4. Suppression of Argentine Ants and Ghost Ants with Maxforce Granular Insect Bait

Yellowjacket treatments were effective at reducing wasps in treatment plots compared to untreated control plots (**Figure 6**). Suppression was not long-lasting, however, nor were target suppression levels met. 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.

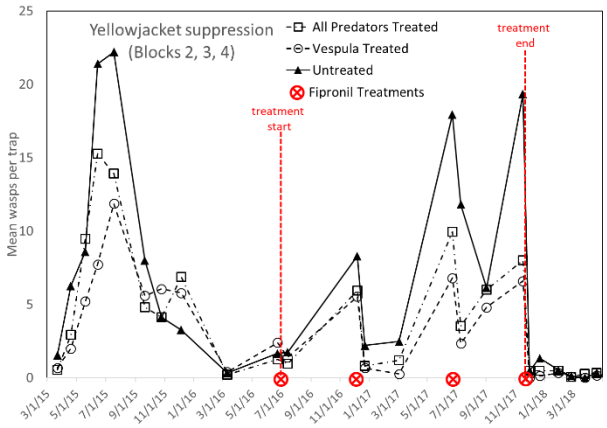

Figure 5. Yellowjacket Abundance in Traps Baited with Heptyl Butyrate

4.5 DIET ANALYSIS

Figure 3 shows the results for the insect DNA sequences which were extracted from NIPs. These analyses show that NIPs consumed insect taxa that were observed to visit flowers of focal plant species.

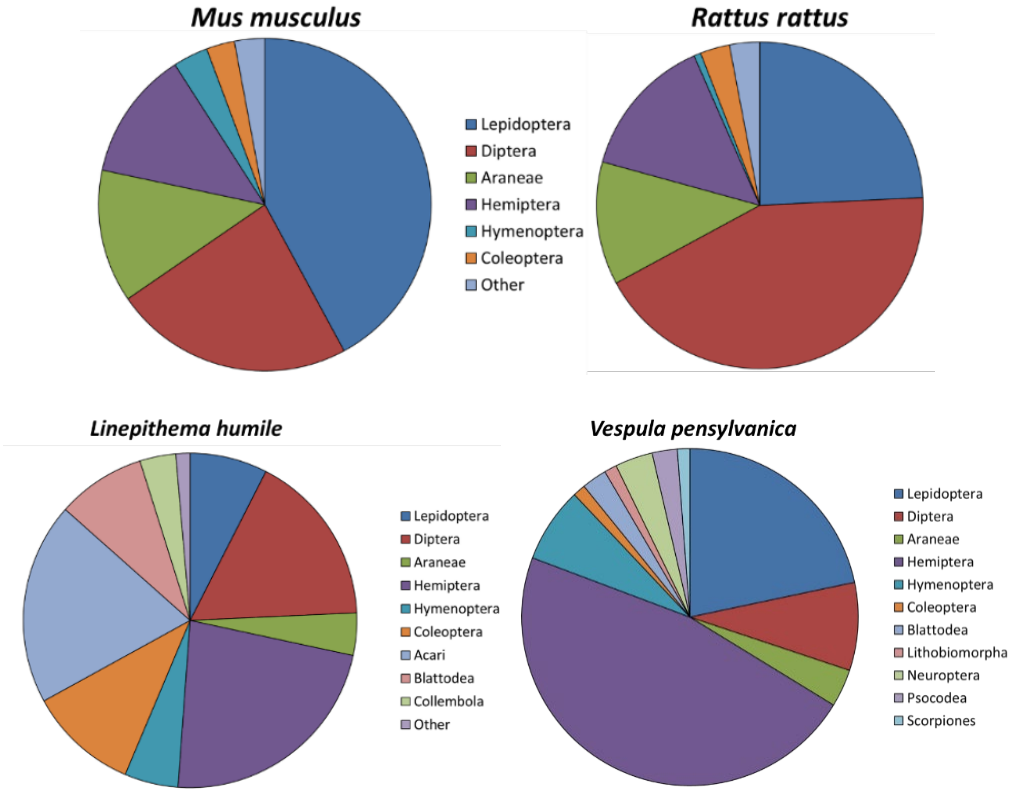

Figure 6. Proportion (by Arthropod Order) of CO1 Sequence Reads Detected in NIPs

4.6 DATA SYNTHESIS AND SHELOB MODEL

For the SHELOB model, the investigators classified six pollinator species groups known to perform frequent, legitimate pollen transfer: solitary bees, honey bee, moths and butterflies, non-syrphid flies, syrphid flies, and wasps excluding *V. pensylvanica* (hereinafter, "non-Vespula wasps"). During the entire experimental treatment, the most important pollinator species groups were syrphid flies (for *D. linearis*, *H. haplostachya*, *S. lanceolata*, and *S. angustifolia*), honey bees (for *S. fallax*), and non-Vespula wasps (for *B. menziesii*).

The SHELOB model analyzed relationships between NIP frequencies and pollinator frequencies for six of the eight focal plant species. *A. glauca* and *S. angustifolia* were excluded from analysis due to no presence in the treatment plots and very low flower visitation, respectively. Frequencies of NIPs at PTA varied by experimental block. Yellowjacket frequencies in particular generally displayed higher frequencies in woodland blocks than in grassland/shrubland blocks for all treatment types. Ant frequencies demonstrated the opposite trend in that they generally displayed higher frequencies in grassland/shrubland blocks than in woodland blocks. Both rats and ants demonstrated a clear treatment effect.

Investigators found significant negative relationships between the frequency of NIPs and the frequency of flower visitation by at least one of the six pollinator species groups for four of the six plant species (**Figure 7**). There were primarily negative relationships between NIPs and nearly every pollinator species group for *H. haplostachya, S. fallax,* and *T. arenarium*.

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

Figure 7. Relationships between NIPs and Flower Visitation by Pollinator Species Groups

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

Investigators calculated effect sizes for the relationships between pollination interactions and NIP. Effect sizes in this case quantify how much the pollinator visitation frequency would be expected to increase (assuming a negative relationship between NIP and pollinator group) if the NIP frequency were decreased from 0.6 to 0. NIP frequency of 0.6 was the untreated average frequency of NIPs in the experimental plots, and NIP frequency of zero would represent eradication of the predator species. Increases in pollinator visitation frequency when lowering NIP frequency to zero were positive for many plant species-pollinator interactions. Eradication of mice, rats, and ants could produce up to a > 90% increase in pollinator visitation for specific pollinator-plant combinations. Mean increases in non-syrphid flies and honey bee pollination of *S. fallax* with rat and ant eradication, respectively, were predicted to have the largest positive effect sizes.

4.6.1 NIP-Pollinator Frequency Relationships Across Plant Species Combined

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

5.0 IMPLICATIONS FOR FUTURE RESEARCH AND BENEFITS

Investigators identified relationships between NIPs, insect pollinators, and native plants (both endangered and common species) at PTA. This research has several management relevant results and implications.

First, current pollination differs for endangered versus common plant species in the study system. Endangered species have fewer flower visitor species than common species, and more of the endangered species are pollen limited. For most of the endangered species, additional pollen and outcrossing improves seed set. Taken together, this indicates that pollinator management and conservation are important for endangered plant species.

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

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

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