Plant-arthropod interactions of an endangered California lupine

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Abstract

The reintroduction of endangered plant species is an essential conservation tool. Reintroductions can fail to create resilient, self-sustaining populations due to a poor understanding of environmental factors that limit or promote plant success. Biotic factors, specifically plant-arthropod interactions, have been shown to affect the establishment of endangered plant populations. *Lupinus nipomensis* (Nipomo Mesa lupine) is a state of California (California Rare Plant Rank: 1B.1) and federally (65 FR 14888) endangered endemic plant with only one extant population located along the central California coast. How arthropods positively or negatively interact with *L. nipomensis* is not well known and more information could aid conservation efforts. We conducted arthropod surveys of the entire *L. nipomensis* extant population in spring 2017. Observed arthropods present on *L. nipomensis* included 17 families, with a majority of individuals belonging to Thripidae. We did not detect any obvious pollinators of *L. nipomensis*, providing support for previous studies suggesting this lupine is capable of self-pollinating, and observed several arthropod genera that could potentially impact the reproductive success of *L. nipomensis* via incidental pollination or plant predation.

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Abstract

The reintroduction of endangered plant species is an essential conservation tool. Reintroductions can fail to create resilient, self-sustaining populations due to a poor understanding of environmental factors that limit or promote plant success. Biotic factors, specifically plant-arthropod interactions, have been shown to affect the establishment of endangered plant populations. *Lupinus nipomensis*(Nipomo Mesa lupine) is

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Keywords

Coastal dune, endemic**,** Fabaceae, *Lupinus nipomensis* , pollination, restoration

Introduction

Plant-arthropod interactions, through processes such as pollination, herbivory, and frugivory, play an important role in the reproductive success of plant species (Strong et al. 1995, Nemec and Bragg, 2008; Schweizer et al. 2013). Low pollinator visitation diversity or abundance can explain reduced distribution, low reproductive output, or failure to establish novel plant populations in habitats that are otherwise abiotically suitable (Karron, 1987; Kearns, Inouye and Waser, 1998). Herbivorous arthropods meanwhile have been shown to impact establishment and seed production of plants (Bevill, Louda and Stanforth, 1999; Münzbergová and Herben, 2005). Both the mutualistic and antagonistic interactions between plants and arthropods are important drivers in determining the survival of a plant (Stahl, Hilfiker and Reymond, 2018).

Despite the importance of arthropod interactions to plant species, current conservation efforts to restore endangered plants often prioritize the presence of appropriate abiotic conditions to select reintroduction sites (Falk, Millar and Olwell, 1996; Guerrant and Kaye, 2007; Godefroid *et al.* , 2011). Knowledge of pollination and herbivory has been shown to be important in maximizing reproduction and increasing establishment success of rare plant species (Archer and Pyke, 1991; Kay, 2008; Ancheta and Heard, 2011). Pollination is especially important to endangered species with small populations, not only to maintain the population size, but also to increase outcrossing of individuals (Steffan-Dewenter, Mïnzenberg and Tscharntke, 2001; Reiter*et al.* , 2017; Horth, 2019). Maintaining genetic diversity and reducing the likelihood of inbreeding depression is essential to the conservation of rare plant species (Falk, 1990; Lee *et al.* , 2018).

Lupinus nipomensis Eastw. (Fabaceae, Nipomo Mesa lupine) is a state and federally endangered annual forb endemic to Nipomo, California (USFWS 2009; Fig. 1). Historically, *L. nipomensis* has occurred at low densities in back dunes and inter-dune habitat. The loss of coastal back dune habitat due to land conversion, fragmentation, and competition with the invasive perennial veldt grass (*Ehrharta calycina* Sm., Poaceae) limit the range and potential for natural regeneration of the*L. nipomensis* populations (Skinner and Pavlik, 1994). The entire extant population is geographically isolated within a 5 km^2 area along the central California coast in the Guadalupe-Nipomo Dune Complex and is comprised of seven dispersed colonies. Total population size is dependent on winter and spring climatic conditions and ranges between 139 and 771 individuals per year (USFWS, 2019).

The reintroduction of Nipomo Mesa lupine, and other rare plants with a limited initial population size, calls for abiotic requirements to maximize reproductive output from reintroduction efforts. Luong, Nolan, and Stratton (2019) provides an overview of abiotic microhabitat characteristics (i.e., landscape slope and aspect) and seed treatment relevant to *L. nipomensis* fecundity. A foundational study of*L. nipomensis* by Walters and Walters (1989) primarily focused on abiotic drivers of reproduction, specifically changes observed in flowering and fruit set as a factor of rainfall received; this publication also includes a record of herbivorous arthropod interactions, but did not specifically include pollinator observations. Although other annual lupines have had interactions with known pollinators, the absence of pollinator observations has led researchers to hypothesize that *L. nipomensis* is capable of both self-pollinating as well as outcrossing (USFWS 2019).

In this study, we sought to establish a baseline of plant-arthropod interactions of *in situ L. nipomensis* to inform future restoration efforts and especially targeted pollinator observations, which have not previously been studied. We surveyed arthropod use of*L. nipomensis* and classified plant visitors as potential pollinators when observed on or in the flowers. The primary goal of this study was to create an inventory of observed arthropod interactions with*L. nipomensis* to inform future research and conservation efforts.

Methods

Study Area

This study was conducted in the spring of 2017 at the Phillips 66 Oil Refinery (35.0388889, -120.5894444) in San Luis Obispo County, California (Fig. 2). The region is characterized by a Mediterranean-type climate with cool, wet winters and hot, dry summers, while also receiving occasional inputs of water from coastal fog (Baguskas *et al.* , 2016). San Luis Obispo County receives an average precipitation of 33.1 cm annually and an average wind speed of 10.83 km/hr (November – May) with average low and high temperatures range from 5.92˚C - 18.9˚C during the growing season (Western Regional Climate Center 2020).

In our study area, Phillips 66 was required to mitigate for their oil development by establishing protected areas where extant populations are regularly monitored and restrict management actions that could negatively affect *L. nipomensis* populations (USFWS, 2009).The protected area in which we conducted our study is a coastal back dune ecosystem and the oldest part of a dune complex. These less disturbed areas often have later successional plants with increased soil stability as well as higher plant and insect diversity (Buckler, 1979; Miller, Gornish and Buckley, 2010; Ferrier *et al.* , 2012). The study site is dominated by non-native, invasive perennial veldt grass (*Ehrharta calycina*) with scattered native annual forbs and perennial shrubs. The area is actively grazed by cattle during *L. nipomensis* dormant season (June-November) to suppress the invasive veldt grass.

The entire population of *L. nipomensis* is restricted to seven colonies within the Phillips 66 protected area. Two of the colonies were excluded due to their wide and sparse distribution, making these areas ineffective for targeted sampling. One colony was newly rediscovered from historic occurrence data not found until partway through the course of the study. A fourth population was located along a roadside with different ambient disturbance characteristics compared to other areas. We conducted plant visitor observations and vegetation monitoring at the remaining three colonies within the protected area in accordance to our California Fish and Wildlife permit (Permit No. 2081(a)-16-010) (Fig. 2).

Plant Visitor Observations

One monitoring plot was established per colony ($n = 3$). Each plot was 8m × 8m and contained 22 - 96 *L. nipomensis* individuals. Arthropod visitor surveys were conducted by two observers every other week from March 2017 to May 2017 for 40 minutes per observer per plot (48 hours total across all plots). Short observation times and single field season were due to limited accessibility, small population size, and the permitting protocol to access private property. It is important to note that not all flower visitors are pollinators, but they are considered potential "incidental pollinators"; incidental pollinators move pollen from flower to flower while foraging for other resources (Kearns, Inouye and Waser, 1998; Anandhan, Kazmi and Dey, 2020). Diurnal insects are most active during the warmest time of the day, which corresponds with peaks in flower nectar resources (Willimer, 1983) observations occurred between 12:00 – 15:00 to observe the greatest potential suite of insects visiting *L. nipomensis* (Herrera, 1990). One set of early and late sampling (beginning at 9:00 and 16:00, respectively) were conducted to increase the likelihood of sampling temporal niche visitors. During each collection we also classified cloud cover on a 4-point scale and ambient air temperature.

Insect samples were collected using aspirations, beat samples, hand or net collections, and flower collections for floral dissections. Nets were only used when necessary to minimize damage as dictated by the California Department of Fish and Wildlife collection permit (Scientific Collecting Permit SC-13574). Beat sampling was conducted on every individual in the plot during each observation period. Field flower samples were stored in air-tight bags with an ethyl acetate cotton ball and kept cool until returned to the lab. Flowers were then dissected and any arthropods found were placed in 75% ethanol vials. Specimen collection complied with California State and Federal laws and samples were vouchered at the Invertebrate Zoology Collection at the University of California, Santa Barbara (*Appendix 1*).

Insects were identified to family and received lower classifications if possible (Carvalho, 1955; Herring, 1976; Slater and Baranowski, 1978; Schuh and Slater, 1995; Gibson, Huber and Wooley, 1997; Ross, Thomas and Skelley, 2002; Daniel and Franz, 2012; Hoddle, Mound and Paris, 2012; Marshall, 2012; Iowa State University Department of Entomology, 2017). Specimens identified to family were sorted into putative species based on morphology, or morphospecies (Samways, McGeoch and New, 2010). New host records were determined and recorded via the Global Biotic Interactions (GloBI) database and through literature searches (Poelen, Simons and Mungall, 2014). Families classified as flower visitors are considered potential pollinators and were observed on or in flower parts of *L. nipomensis* .

Data Visualization

Interaction data between *L. nipomensis* and flower visiting individuals was visualized in R Studio (version 1.4.1106). An interaction web was created using the *bipartite* package (Dormann 2021). Arthropod icons were created or retrieved from http://phylopic.org/. Icons for Asilidae, Coccinellidae, Curculionidae, and Miridae are under a creative common license (http://creativecommons.org/licenses/by/3.0/) by Gareth Monger, Melissa Broussard, JCGiron, and Karina Garcia, respectively.

Results

A total of 351 arthropod individuals were observed interacting with*L. nipomensis* during our surveys (*Table 1*). Records of the 157 vouchered specimens are available on the UCSB Invertebrate Zoology Collection, Global Biodiversity Information Facility (GBIF) and Global Biotic Interactions (GloBI) databases (*Appendix 1*). Twenty-two unique morphospecies from 8 orders and 17 families were classified. At least one individual from 11 unique families were found in or on a*L. nipomensis* flower (*Fig. 3*).

Individuals observed that were identified to genus and species include members of orders Diptera, Coleoptera, Hemiptera, Hymenoptera, and Lepidoptera (Appendix 1). The one Dipteran observed was *Delia lupini* Coquillett (Anthomyiidae). The Coleoptera species were*Diabrotica undecimpuncata* Mannerheim (Chyrsomelidae),*Apleurus* sp., *Scaphomorphus* sp., *Trigonoscuta* sp., and *Rhigopsis effracta* LeConte (Curculionidae). Hemipterans identified to lower classifications include *Orius* sp. (Anthocordidae), *Closterocoris amoenus* Provancher (Miridiae),*Lygus* sp. (Miridae), and *Apiomerus californicus* Berniker & Szerlip (Reduviidae). Collected hymenopterans were all Formicidae, including *Crematogaster* sp. and *Linepithema humile* Mayr. A lepidoptera individual, *Plebejus lupini* Boisduval (Lycaenidae), was observed landing on a vegetative part of *L. nipomensis* . Thysanoptera individuals were identified to genus (*Thrips* sp.) and accounted for 234 individuals out of the total 320 observed (*Fig. 3*). A complete list of individuals is available in*Appendix 1* .

During our plant visitor observations, arthropods including *Apis mellifera* Linnaeus (Apidae), *Bombus vosnesenskii* Radoszkowski (Apidae), Syrphidae, and Lepidopterans were observed visiting neighboring flowers (*Acmispon glaber* (Vogel) Brouillet (Deerweed, Fabaceae), *Amsinckia spectabilis* Fisch. & C.A. Mey. (seaside fiddlehead, Boraginaceae), *Collinsia heterophylla* Graham (purple Chinese houses, Plantaginaceae), *Ehrharta calycina* Sm. (perennial veldt grass), *Lupinus chamissonis* Eschsch. (dune bush lupine, Fabaceae), *Nemophila menziesii* Hook. & Arn. (baby blue eyes, Boraginaceae) within 0 – 5 meters of *L. nipomensis* plots, but never visited *L. nipomensis* itself.

Discussion

Plant arthropod interaction studies associated with restoration efforts are becoming more common due to greater appreciation of the role arthropods play in the survival and reproductive output of plants through positive (pollination) and antagonistic (herbivory) interactions (Bucharova *et al.* , 2021; Cariveau, Bruningasocolar and Pardee, 2021; Sabatino, Rovere and Meli, 2021). While our study did not detect obvious pollinators, we recorded a high number of arthropod individuals that could be incidental pollinators and inadvertently pollinate*L. nipomensis* while feeding on pollen or other plant resources (Gill, 1991). We observed over 200 Thysanoptera individuals present in*L. nipomensis* flowers (Table 1). Thysanoptera have been known to pollinate members of several angiosperm families, including fabaceous plants (Velayudhan and Annadurai, 1986; Varatharajan *et al.* , 2016). However, Thysanoptera are well known flower pests that consume pollen, potentially causing withering of flowers and lowering plant reproductivity (Reitz, 2009). We also observed arthropods known to be important pollinators (i.e., *Apis mellifera* Linnaeus (Apidae)) visiting neighboring plants, including another species of lupine, but never interacting with *L. nipomensis* (Aslan, Galindo and Service, 2016; Hung *et al.* , 2018). The record of these arthropods visiting other, nearby plants indicates that while pollinators such as *Apis mellifera* were present, these arthropods were not visiting *L. nipomensis* . If cross-pollination is occurring between *L. nipomensis* individuals, this service is being performed by incidental pollinators, such as Thysanoptera, and not being performed by arthropods such as Apidae, known to pollinate other lupine species (Williams, 1987).

Arthropod genera were observed that could affect the reproductive success of *L. nipomensis* due to herbivory. The Dipteran found,*Delia lupini* (Anthomyiidae), was collected from a gall present on a *L. nipomensis* individual and has been observationally implicated to reduce fecundity in a previous study of *L. nipomensis* (Walters and Walters, 1988). While some galls aid in pollination and nutritional services, most are detrimental to plant health and in some cases, have been shown to threaten endangered plant species (Kolesik *et al.* , 2019; Harris and Pitzschke, 2020). The impact of *D. lupini* gall presence on *L. nipomensis*reproductivity was not quantified in this study; however, it is possible there were additional individuals we did not observe that are affecting the fecundity of this lupine. Formicidae species we observed included*Linepithena humile* , the invasive Argentine ant (Holway, 1999). Argentine ants have been shown to impact floral visitation patterns and nesting success of other arthropods with the potential to create cascading, negative effects and reduce pollinator visitation (Underwood and Fisher, 2006; Sahli *et al.* , 2016; Plentovich *et al.* , 2021). The presence of a gall-inducing and invasive arthropods may further inhibit recruitment within this singular extant population of*L. nipomensis* .

Plants face both biotic and abiotic barriers to reproductive success, and for rare, endangered species, these barriers can ultimately result in extirpation or extinction (Rejmánek, 2018). Small populations, like that of *L. nipomensis* , can face pollination limitation, as small plant populations often do not attract pollinators due to low pollen rewards (Shi, Michaels and Mitchell, 2005). A reduction in pollination leads to reduced outcrossing, ultimately resulting in a lower genetic diversity that further threatens already small, rare plant populations (Gray, 2019). Simultaneously, herbivorous arthropods can impact seed production and recruitment of plant species (Lucas-Barbosa, 2016). Active intervention may be necessary to promote outcrossing via hand pollination, as well as protect *L. nipomensis* from herbivorous arthropods to ensure the genetic diversity and successful establishment of new individuals (Walsh *et al.* , 2019; Serrano *et al.* , 2021).

Our results suggest that if cross-pollination is occurring at all among*L. nipomensis* individuals, it is only being performed by incidental pollinators, and that herbivorous arthropods are present that may threaten fecundity of this lupine. Additional work is necessary determine the frequency of cross-pollination and whether the potential threats this lupine faces from herbivorous arthropods will affect the establishment of novel populations during restoration efforts.

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Data Accessibility

The complete dataset has been uploaded to the PANGEA data repository; corresponding DOI will be provided pending acceptance and publication.

Competing Interests

Any findings or recommendations expressed in this material do not necessarily reflect the views of funding agencies. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

CIM led data presentation with input from JCL and KCS. CIM and JCL led writing of the paper, with input from KCS. CIM and JCL created the figures. JCL and KCS designed the study. JCL collected the data and JCL and KCS identified the arthropods.

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Figure & Table Captions

Fig. 1 Flower peduncle of *Lupinus nipomensis* Eastw. (Nipomo mesa lupine)

Fig. 2 Lupinus nipomensis populations and geographic placement of study site in California

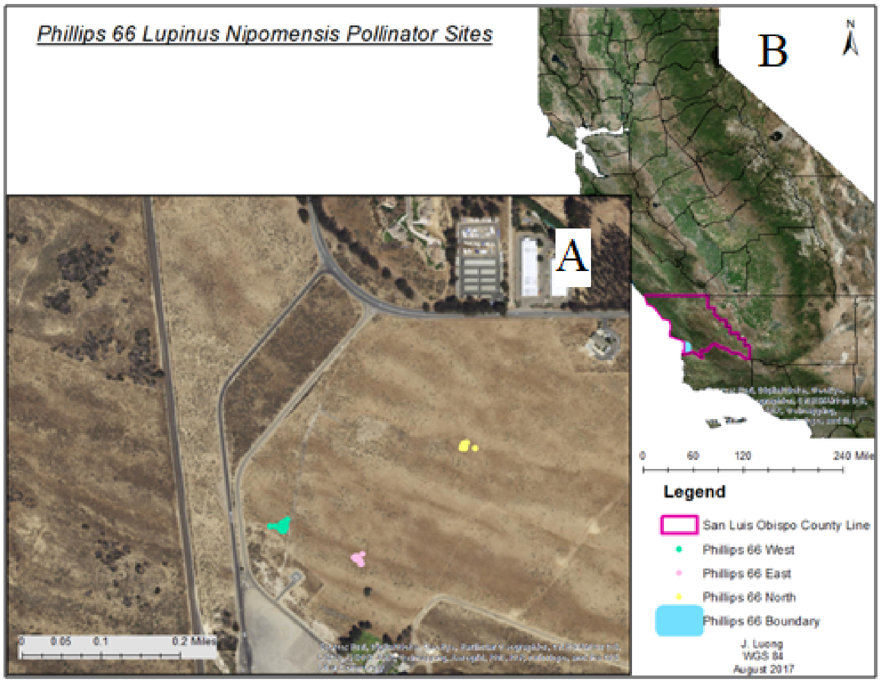
Table 1 Order, Family, flower visitation, number of morphospecies, and number of individuals of arthropods that interacted with *Lupinus nipomensis* .

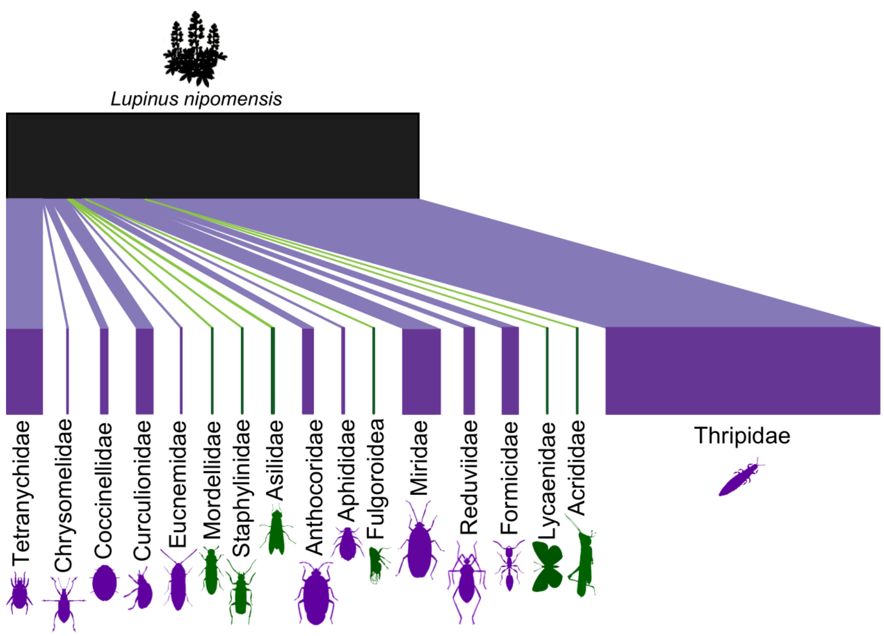
Fig. 3 Interaction web of arthropod families containing individuals found on *Lupinus nipomensis* . Families containing at least one individual found in or on flowers are colored in purple while families only containing individuals found on other, vegetative parts of the plant are colored in green.

Table 1

Order	Family	Flower Visitor	No. Morphospecies	No. Individuals
Trombidiformes				
	Tetranychidae	yes	1	31
Coleoptera				
	Chrysomelidae	yes	1	1
	Coccinellidae	yes	1	2
	Curculionidae	yes	4	14
	Eucnemidae	yes	1	1
	Mordellidae	no	1	1

Order	Family	Flower Visitor	No. Morphospecies	No. Individuals
	Staphylinidae	no	1	1
Diptera				
	Anthomyiidae	no	1	1
Hemiptera				
	Anthocoridae	yes	1	9
	Aphididae	yes	1	2
	Fulgoroidea	no	1	1
	Miridae	yes	1	28
	Reduviidae	yes	2	10
Hymenoptera				
	Formicidae	yes	2	13
Lepidoptera				
	Lycaenidae	no	1	1
Orthoptera				
	Acrididae	no	1	1
Thysanoptera				
	Thripidae	yes	1	234
Total			22	351


Phillips 66 Lupinus Nipomensis Pollinator Sites
B
N
A
0 0.05 0.1 0.2 Miles
0 60 120 240 Miles
Legend
San Luis Obispo County Line
Phillips 66 West
Phillips 66 East
Phillips 66 North
Phillips 66 Boundary
J. Luong
WGS 84
August 2017


Lupinus nipomensis
Tetranychidae
Chrysomelidae
Coccinellidae
Curculionidae
Eucnemidae
Mordellidae
Staphylinidae
Asilidae
Anthocoridae
Aphididae
Fulgoroidea
Miridae
Reduviidae
Formicidae
Lycaenidae
Acrididae
Thripidae