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Experimental Removal of an Introduced Pollinator Reduces Reproductive Success of
California Native Clustered Tarweed

A Thesis submitted in partial satisfaction of the requirements
for the degree Master of Science

in

Biology

by

Annika Joy Nabors

Committee in charge:

      Professor David Holway, Chair
      Professor Joshua Kohn
      Professor James Nieh

2015

The Thesis of Annika Joy Nabors is approved and it is acceptable in

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ABSTRACT OF THE THESIS

Experimental Removal of an Introduced Pollinator Reduces Reproductive Success of

California Native Clustered Tarweed

by

Annika Joy Nabors

Master of Science

University of California, San Diego, 2015

Professor David Holway, Chair

Honey bees (*Apis mellifera*), introduced worldwide by humans, are often a

numerically dominant pollinator in non-managed ecosystems, but surprisingly few

experimental studies have examined the effect of honey bee visitation on wild plant

reproduction. I experimentally removed honey bees from plots of clustered tarweed

(*Deinandra fasciculata*: Madiinae), a native annual forb, to measure the contribution

of *Apis* visitation to tarweed seed set. While removal of *Apis* did reduce seed set, the

much higher rate of honey bee visitation suggests that honey bees contribute modestly

to seed set compared to non-*Apis* pollinators. Honey bees visit more than three times as many capitula per visitor, yet their removal results in only a 15 percent decrease in seed set. Visits by non-*Apis* visitors significantly increased when *Apis* was removed, indicating possible competition between *Apis* and other insects. In ecosystems where honey bees become numerically dominant, they can contribute a significant proportion of visits to native plants. *Apis* removal may negatively affect plant reproduction, especially during years in which native pollinators are relatively uncommon because of lack of floral resources, but it may also release native pollinators from competitive displacement.

Introduction

Western Honey Bees (*Apis mellifera* L.) were introduced to California from Europe less than 200 years ago for the purpose of honey and beeswax production (Crane 2013). Their ability to thrive on pollen and nectar from many different plant species and to survive in a wide range of climatic conditions has allowed them to spread throughout California (Crane 2013), where feral colonies survive and reproduce without human management (Gambino et al. 1990). In some ecosystems, non-native honey bees are the numerically dominant pollinator (Hermansen et al. 2014; Aizen and Feinsinger 1994). This dominance holds true in Southern California as well, where Africanization is common in feral honey bees. Africanized bees are a hybrid between the African subspecies *A. m. scutellata* and various European subspecies, originally bred in Brazil. Most managed hives in California are of European descent, but about 65 percent of foraging workers and 70 percent of feral hives sampled in San Diego carry the African mitotype (Kono and Kohn 2015).

An extensive literature exists on the biology of honey bees and their importance in agricultural pollination (Artz et al. 2011; Brittain et al. 2013; Smith et al. 2013), because the honey bee is the most economically important invertebrate (Crane 2013; vanEngelsdorp and Meixner 2010). In spite of what is known about *Apis* pollination in agricultural systems, surprisingly little is known about how introduced honey bees affect natural ecosystems or the extent to which they contribute to the reproduction of native plants. Because fragmented and degraded natural habitats may not provide sufficient resources to sustain intact assemblages of native pollinators (Cunningham 2000; Steffan-Dewenter and Tscharntke 1999; Kearns et al. 1998),

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honey bees may exert an increasingly strong influence over the quality of pollination services in human-modified habitats.

One native plant community in California where honey bees appear numerically dominant is coastal sage scrub (CSS), which is composed mainly of aromatic, drought-deciduous shrubs and annual forbs. As a result of urbanization, agriculture, and other human development, less than 15 percent of historic CSS remains (Jensen et al. 1990). Although native CSS plants in San Diego County may receive up to 90 percent of floral visits from honey bees (Hung and Holway, *unpublished data*), the ramifications of this phenomenon remain unknown. The quality of pollination services available to facilitate plant reproduction ultimately determines long-term ecosystem viability (Ashman et al. 2004), which is essential for the management and conservation of remaining CSS.

In order to measure the relative contribution of honey bees to the reproductive success of clustered tarweed (*Deinandra fasciculata*: Asteraceae) reproductive success, I removed *Apis* from observed plots of *D. fasciculata* in a paired-replicates experiment. I then compared the visitation of insect pollinators with the seed set of *D. fasciculata* capitula to determine what effect, if any, *Apis* removal had on seed production. I also performed a self-compatibility assay and sampled visitors to *D. fasciculata* to quantify some aspects of the plant's reproductive biology. By measuring the effect that removal of *Apis* has on the reproductive fitness of a California coastal sage scrub plant, this study examines the impact of visitation by a numerically dominant introduced pollinator on the plants of a threatened native ecosystem.

Methods

Study system and focal plant species

The study took place at the Elliott Chaparral Reserve (32º 53' 30" N, 117º 5' 15" W), a 74-hectare strip of native chaparral and coastal sage scrub habitat managed by the UC Natural Reserve System, and at the adjacent UC San Diego Elliott Field Station. These reserves abut more than 500 hectares of largely undeveloped scrub habitat owned by the Marine Corps Air Station (MCAS) Miramar. Collectively, these areas support a high diversity of native bees (Hung and Holway, *in preparation*), both feral and managed colonies of honey bees (*Apis mellifera*), and a representative assemblage of California native chaparral and coastal sage scrub plants, including the small annual forb clustered tarweed (*Deinandra fasciculata*), which is abundant at this site. Clustered tarweed, a species in the sunflower family (Asteraceae: Heliantheae: Madiinae), is common in CSS communities. This tarweed has a high tolerance of drought and an extended blooming phenology, usually flowering from May through August. Its composite flowers, hereafter referred to as capitula, cluster together at the apex of its thin stems in sticky, clumped inflorescences. Very little is known about its reproductive biology, but members of the tarweed tribe are frequently self-incompatible.

Experimental procedure

The experiment took place from April to June 2015. Each experimental unit, or replicate, consisted of a 50 x 50 cm area of ground that contained at least 20 mature *D. fasciculata* individuals, all of which were < 50 cm in height. I chose sites for replicates based on the local density of *D. fasciculata*. A minimum density of 20 tarweed

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individuals was deemed necessary based on observations to attract both honey bees and native bees. On replicates in the "*Apis*-removal" treatment (n = 16), I experimentally prevented honey bees from visiting plots of *D. fasciculata*. This removal permitted unrestricted access by native pollinators to tarweed flowers. Replicates in the "*Apis*-present" treatment (n = 16) allowed open access by all floral visitors to tarweed. Pairs of replicates, with each pair consisting of a replicate in each experimental group, were spatially and temporally interspersed throughout the study area. Individual replicates within each pair were > 5 m away from one another; replicates within each pair were assigned to experimental group at random. Coordinates for all replicates are listed in Appendix 1.

I delineated a 50 x 50 cm footprint that represented the border of each replicate with red yarn and enclosed the plants in a pollinator-exclusion cage except during the period when the experimental trials took place. Exclusion cages had an interior volume of 50 x 50 x 50 cm and were constructed of PVC piping and sturdy translucent fabric with a mesh size of 1 mm. The cage mesh effectively prevented visitation by flying insects, which make up the vast majority of visits to tarweed flowers, while allowing in moisture and sunlight. Within the same study area as the experimental procedures, I also established "uncaged control" replicates (n = 16) to measure the effect of the pollinator exclusion cage on experimental plants. I found no significant difference in seed set between plants from uncaged control plots (3.95 ± 0.18 SE seeds/capitulum/plot) and those from *Apis*-present plots (3.51 ± 0.16 SE seeds/capitulum/plot) (paired *t*-test: $p = 0.073$).

The day before observations began in *Apis*-present and *Apis*-removal replicates, I selected 20 unopened capitula within each plot. It was necessary to focus on unopened capitula to control pollinator access throughout the life span of the flowers therein. To identify the 20 "focal capitula," I tied 5 cm of red thread around the base of the sepals. I chose red thread because most insects cannot see red wavelengths of light well. The red thread therefore should not disproportionately attract or repel potential visitors. To ensure an overall similarity of plant architecture, I chose capitula only from plants ≤ 15 cm in height and with at least one node (i.e., only plants that had at least two "branches").

As focal capitula in each replicate opened to reveal their stigmas, I simultaneously uncaged and observed a pair of *Apis*-removal and *Apis*-present replicates for three to four hours per day between 0900 and 1500 hours. Simultaneous observation of experimental and control replicates ensured that any variation in visitor number or behavior was due to treatments and not to time of day, weather, replicate location, or season. During observations, I collected three types of data on insect visitors: the identity of the visitor, the length of its visit, and the number of capitula visited. Visitation to plants in the *Apis*-removal treatment was documented by direct observation. Visitation to plants in the *Apis*-present treatment was documented using a GoPro™ Hero 3 camera, set on a 45-cm tripod 45 to 60 cm away from the replicate and angled downward to capture simultaneous images of the entire plot and visitors to all flowers. Videos of control replicates shot using the GoPro were transcribed using a 1:1 temporal observation period identical to the transcription of field observations.

To perform the experimental *Apis* removal, I selectively removed individual honey bees from the vicinity of experimental replicates and documented all non-*Apis* floral visitors. I removed *Apis* by blowing exhaled air onto individual *Apis* through a modified insect aspirator until the individual honey bee flew away. I also preemptively drove away *Apis* individuals that were visiting plants adjacent to the replicate in order to eliminate any chance of visitation to plants within the replicate.

I observed replicates daily for the duration of blooming for focal capitula; capitula completed blooming within two to four days. When every focal capitulum within a given replicate had senesced, I enclosed plants bearing individual focal capitula inside 7 x 7 cm square mesh bags. These bags restricted additional pollinator visits and prevented seeds from blowing away.

Once tarweed was completely dry and senescent, I used scissors to harvest focal capitula from *D. fasciculata* individuals. In the lab, I then dissected each capitulum and weighed and counted the developed seed set from the ray flowers only. I chose to examine only ray seeds when quantifying seed set because the disc flowers of *D. fasciculata* produce pollen but rarely set fully developed seed (see Appendix 2). Seeds were assessed for development based on their fullness, color, and mass. Thick, black seeds > 0.2 mg were considered to be developed, while thin, straw-colored, and light brown seeds < 0.2 mg were considered to be undeveloped. For the focal capitula in each replicate, I divided the mass and the number of developed ray seeds by the number of focal capitula to obtain values of the average mass per seed and the average number of developed seeds set per capitulum.

To identify the common visitors to *D. fasciculata*, I collected voucher specimens three times during the study period. I collected between 1000 and 1300 hours only on warm, sunny days with light wind, after the experimental observations for the day were completed and in areas separated from the immediate vicinity of replicates. I used a short-handled insect net to catch floral visitors as they alighted on *D. fasciculata* flowers growing within 10 m of replicates, then trapped them for later pinning. All specimens were identified with as much specificity as possible.

Self-compatibility assay

I also tested the self-compatibility of *D. fasciculata* by hand-pollinating 20 capitula with pollen from a non-self plant and 20 capitula with self pollen. To collect pollen, I snipped capitula with mature anthers and carried them in a Petri dish to the focal capitulum for hand pollination using tweezers. For non-self pollen, I chose only plants growing > 5 m away from focal plants to reduce the chance of choosing a close relative of the focal plant. Donor capitula for self pollen were chosen from the same plant and stem as focal capitula.

To assess the degree of self-compatibility, I dissected hand-pollinated capitula and weighed and counted all ray seeds, developed and undeveloped. I divided the mass and number of all seeds by the number of capitula in each pollination group (i.e., outcrossed or selfed) to give an average mass per individual seed and average number of developed seeds per capitulum. I divided the average number of developed ray seeds produced by capitula hand-pollinated with self pollen by the average number produced by capitula hand-pollinated with outcrossed pollen to calculate the index of

self-incompatibility, or ISI. ISI is measured by the ratio of self:outcross seeds (Vogler

et al. 1998).

Results

Apis-removal experiment

The exclusion method used to prevent *Apis* from visiting flowers successfully reduced numbers of *Apis* visiting tarweed (Fig. 1a). *Apis*-present replicates received an average of 14.6 *Apis* visitors per hour, while *Apis*-removal replicates received an average of 0.2 *Apis* visitors per hour (two-tailed Wilcoxon signed-rank test: $V = 136$, $p < 0.0001$). Removal of *Apis* increased by 55 percent the number of native visitors per hour (Fig. 1b; two-tailed Wilcoxon signed-rank test: $V = 5$, $p = 0.00123$). All visitors to *Apis*-present replicates visited 71 percent fewer capitula per visitor on average than did all visitors to *Apis*-removal replicates (Fig. 2; pairwise *t*-test: $p < 0.0001$).

The selective removal of *Apis* led to a nearly 15 percent reduction in tarweed seed set (Fig. 3; pairwise *t*-test: $p = 0.027$). No relationship existed, however, between the number of insect visitors per hour and the subsequent seed set (adjusted $R^2 = 0.015$, $p = 0.234$). More specifically, seed set was unrelated to the number of non-*Apis* visitors in *Apis*-removal replicates, even though non-*Apis* visitors made up 95 percent of pollinators visiting these replicates (adjusted $R^2 = 0.0089$, $p = 0.305$). Examining only *Apis* visitors on *Apis*-present replicates revealed a similar pattern; honey bees made up over 85 percent of the visitors to these replicates, but seed set was independent of the number of *Apis* visitors (adjusted $R^2 < 0.0001$, $p = 0.543$). The mean number of capitula visited per visitor (adjusted $R^2 = 0.0598$, $p = 0.126$) and the mean duration of visits (adjusted $R^2 < 0.0001$, $p = 0.563$) also had no significant effect on seed set.

Time of year had a slight but significant effect on individual ray seed mass: as the season progressed from April to June, average seed mass decreased by about 6E-5 grams (adjusted $R^2 = 0.0775$, $p = 0.0311$). However, season had no effect on the average number of developed seeds per capitulum (adjusted $R^2 < 0.0001$, $p = 0.938$).

I collected 132 insects visiting tarweed between April 7 and June 23 and identified at least 21 genera and 31 species in 3 orders (Table 1).

Self-compatibility assay

The average number of developed ray seeds per capitulum produced by capitula pollinated with self pollen was significantly lower than the number of seeds produced in open-pollinated, uncaged controls (Fig. 4a; one-sample *t*-test: $t_{15} = 7.31$, $p < 0.0001$). Average individual seed mass was also lower for capitula hand-pollinated with self pollen than for open-pollinated capitula (Fig. 4b; one-sample *t*-test: $t_{15} = 16.5$, $p < 0.0001$).

Conversely, capitula pollinated with outcrossed pollen produced larger seeds compared to those of open-pollinated capitula (Fig. 4b; one-sample *t*-test: $t_{15} = -4.06$, $p = 0.00103$), although there was no significant difference in the number of developed seeds produced per capitulum (Fig. 4a; one-sample *t*-test: $t_{15} = 0.277$, $p = 0.786$). I calculated tarweed's index of self-incompatibility (ISI) to be 0.68.

Discussion

Observational studies of interactions between *Apis* and native pollinators are the common perspective on *Apis* and non-*Apis* impacts in the literature on plant-pollinator interactions (Aizen and Feinsinger 1994; Roubik and Wolda 2001; Santos et al. 2012; Steffan-Dewenter and Tscharntke 2000). Direct comparisons have also been made between *Apis* and non-*Apis* insects of pollination effectiveness in wild and agricultural systems (Carmo, Franceschinelli, and Silveira 2004; Garibaldi et al. 2013; Gross 2001; Freitas and Paxton 1998), but the approach that we took to experimentally remove *Apis* removal from a non-managed ecosystem is new. The impact on coastal sage scrub ecosystems of removing *Apis* can be examined through two lenses: the effect that honey bees have on other floral visitors, and the effect they have on plants.

Increases in visitation by non-*Apis* insects when honey bees are removed suggest release from competition by *Apis*. Native bees can be competitively suppressed by *Apis*, via both indirect competition for floral resources and through direct interactions at foraging sites (Gross 2001; Roubik 1978; Roubik 1980; Thomson 2004; Schaffer et al. 1983). Data collected in a pilot study by Hung and Holway (*in preparation*) support this hypothesis on California native plants in coastal sage scrub. The urban-surburban matrix increasingly prevalent in Southern California land use offers native pollinators few resources, and in drought years feral honey bees foraging in non-managed systems can have a large impact on the ability of native pollinators to forage successfully (Paini 2004). Native pollinators often lose species richness, diversity of community composition (Winfree et al. 2011), and reproductive success (Jha and Kremen 2013) as habitats become more fragmented and developed, unlike

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honey bees that are largely unaffected by habitat fragmentation due to their large foraging range and ability to exploit non-native floral resources (Steffan-Dewenter and Tscharntke 1999; Barthell et al. 2001). The inability of many native pollinators to exploit non-native suburban floral resources, and their reliance on native plant species, may be a reason for the significant increase in native visitation when *Apis* is removed. Regardless of the support in this study for direct or indirect competition, neither the presence of humans nor the method used to remove *Apis* from experimental replicates appeared to deter native floral visitors.

Because I found a significant reduction in seed set when *Apis* was removed, the conclusion could be drawn that *Apis* visitors contribute significant pollination services toward the reproductive success of *D. fasciculata*. However, despite the fact that honey bees were the numerically dominant pollinator, their removal resulted in a relatively small (albeit significant) decrease in seed set. There also did not appear to be synergistic pollination effectiveness as described by Brittain et al. 2013, such that the presence of native pollinators induces *Apis* to engage in more functionally effective pollination behavior. If such a relationship existed, one would expect seed set for *Apis*-present replicates to be proportionally higher for each additional visitor than for visitors to *Apis*-removal replicates. What was most likely to have induced this relationship between experimental treatment and seed set was the sheer number of *Apis* visitors. The threefold decrease of average capitula visited per visitor described in Figure 2 supports this interpretation; although native visitors foraged on 75 percent fewer capitula per average visit, their removal only resulted in a 15 percent drop in seed set. An interaction model by Vázquez, Morris, and Jordano (2005) argues that

animal mutualists that most frequently interact with plants usually contribute the most to reproductive success, even though per-interaction pollination effectiveness may not be related directly to plant reproduction. Even if native pollinators are more effective per visit, as shown in several pollination comparison studies (Celebrezze and Paton 2004; Freitas and Paxton 1998; Wilson and Thomson 1991), the prevalence of *Apis* in systems where they are numerically dominant might result in adequate pollination services for successful plant reproduction.

Another factor determining the relative pollination contribution of *Apis* may be its foraging profile: non-native honey bees foraging for pollen on a native annual flower in the balsam family removed more and deposited less pollen than did native bumble bees (Wilson and Thomson 1991), but when honey bees were foraging for nectar on the same plant, they delivered a quality of pollination services similar to that of natives (Young et al. 2007). However, *Apis* may also reduce pollination success regardless of competitive exclusion of natives; in one study, honey bee visitors removed more than 99 percent of pollen grains from floral male reproductive organs (Carmo, Franceschinelli, and Silveira 2004). As a more concrete measure of relative pollinator effectiveness, I anticipate an analysis of the relative pollen loads carried by *D. fasciculata* visitors to be published in the journal article adapted from this thesis. The relative paucity of visitor diversity seen for the majority of the season may also in part explain these data. Even single-species losses in native pollinator diversity can reduce the amount of conspecific pollen delivered to certain plant species (Brosi and Briggs 2013), and honey bees contribute relatively little toward stemming this lack of pollination services (Garibaldi et al. 2013).

I observed an increase in non-*Apis* species richness, especially native bee richness, over the course of the season, as measured by the types of pollinators documented in my focal *D. fasciculata* observations and my *D. fasciculata* floral visitor surveys. This increase over the course of *D. fasciculata*'s blooming period may be due to a mismatch in floral phenology and pollinator emergence times. The unseasonably hot spring of 2015 changed the phenology of *D. fasciculata* such that peak blooms were as many as 4 weeks earlier than normal (Hung, *pers. obs.*). In the last week of the experiment, when most *D. fasciculata* individuals had senesced, I observed the highest visitation rates and species richness of any during the experiment. Additionally, the seasonally linked reduction in *D. fasciculata* seed weight was probably due to increasing water stress rather than pollen limitation, because the seed set per capitula was not affected and asters set fewer and smaller seeds when under water stress (Cheptou et al. 2000).

The self-compatibility assessment illustrated in Figure 4 was unable to determine true self-incompatibility. The calculated index of self-incompatibility (ISI) of 0.68 indicates probable self-compatibility but better performance when provided with outcrossed pollen, which means that most developed seeds were probably set by the visitation of insects. However, contrary to the literature's tentative predictions of self-incompatibility in *D. fasciculata* and congeners (Sawyer and Keeler-Wolf 2009; Tanowitz 1985; Stevens, O'Brien, and Anderson 2006), selfed capitula produced some developed seeds rather than none. The production of developed seeds suggests that although pollination with outcrossed pollen is beneficial to the reproductive success of *D. fasciculata*, the plant is only partially self-incompatible. However, the significantly

lower average mass of seeds from self pollen indicates that seeds apparently developed to the naked eye may not actually have the robustness needed to germinate successfully. In an obligate outcrossing species of alpine shrub, hand pollination increased seed set by two- to threefold but reduced seed weight by 15 percent, perhaps because the intense climatic conditions penalize seed production additional to the seeds set by open pollination (Muñoz and Arroyo 2006). In *D. fasciculata*, however, seed weight in outcrossed capitula was increased by 12 percent. Additionally, a comparative study by Larson and Barrett (2000) found that pollen limitation is less intense in species that are self-compatible or autogamous. This suggests that *D. fasciculata* is prone to inbreeding depression.

Major aspects of the experimental design included the use of pollinator exclusion cages to restrict access by flying insects, and the use of videocameras to record visitation to *Apis*-present replicates. As the use of exclusion cages will always have some effect on natural conditions, the non-significant reduction in seed set between uncaged controls and experimental controls is not detrimental to the conclusions drawn from this study, especially since both experimental groups were caged in the same way and for the same amount of time. The use of a video camera to record visitation to almost every *Apis*-present replicate and the resulting significant increase in native visitors when *Apis* is removed may raise concerns, due to the possibility of missing some native visitors on video as opposed to in-person observation. The fidelity of the video transcription technique was not directly measured for every replicate, since human observers were needed to remove *Apis* from the paired experimental *Apis*-removal replicate. However, for the single replicate pair

in which both *Apis*-present and *Apis*-removal replicates were observed in person rather than on camera, native visitors increased by 48 percent when *Apis* was removed, an increase similar to that seen in the overall experimental treatments. After performing an additional simulation analysis, I would need to have missed transcribing 1 out of every 3 visitors to show no significant effect of *Apis* removal on native visitation, and 2 out of every 3 visitors to achieve a significant effect in the other direction.

Native bee populations in fragmented Southern California habitats are declining in diversity (Hung et al. 2015), although the broad-scale reasons for this decline are unclear. One potential contributor to this diversity loss was the prolonged drought in California between 2012 and 2015. 2014 was the third driest year on record in California (Howitt et al. 2014). With the lack of floral resources, bees and other pollinators possibly experienced a population crash, as has been seen in other drought-affected systems (Villanueva-Gutiérrez, Roubik, and Porter-Bolland 2015; Mitchell 2014). In a typical year, the increase observed in native visitation when *Apis* are removed may be even starker due to larger standing populations of native pollinators. Change in floral resource phenology and lack of water in an increasingly hot and dry California landscape likely both contribute to a loss of native pollinator diversity, which threatens the reproductive success of insect-pollinated plants in Southern California ecosystems. In their review article, Kearns et al. (Kearns, Inouye, and Waser 1998) argued that fragmented habitats would lead to pollen limitation in plants due to a loss of pollination services. But honey bees may serve as a partial replacement for the services that are being lost as native bee diversity dwindles.

As habitat fragmentation worsens in Southern California (Bolger et al. 2000), honey bees may not be affected to the same extent as native bees. *Apis* can exploit highly fragmented systems (Aizen and Feinsinger 1994) and, unlike many native pollinators, can forage on flowers regardless of their distance from native-quality habitat (Steffan-Dewenter and Tscharntke 1999). Honey bees may even provide a significant amount of pollination services: in one study, numerically dominant honey bees were the only visitors to carry large amounts of pollen and facilitate pollen transfer, despite not being native (Hermansen et al. 2014). However, as mentioned earlier, honey bees themselves may apply competitive pressure to native pollinators. In another example, specialist pollinator *Peponapis pruinosa* declined in visits to squash flowers when fields were supplemented with managed hives of honey bees (Artz, Hsu, and Nault 2011). Generalist native pollinators may therefore be better able than specialists like *P. pruinosa* to handle increasing competition from honey bees. Widespread *Apis* visitation therefore has both positive and negative effects. Although *Apis* may provide a certain quantity of needed pollination services to insect-pollinated native plants in drought and post-drought years, honey bee foragers may increase competition and limit food access for native pollinators.

This thesis, in full, will be prepared for submission for publication of the material. Nabors, Annika J.; Cen, Henry; Hung, Keng-Lou James; Holway, David. The thesis author was the primary investigator and author of this material.

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Tables

Table 1. Genus and species richness of visitors to *Deinandra fasciculata* sampled between April 9 and June 23. All specimens not identified to species were identified with as much specificity as possible.

| HYMENOPTERA: BEES	COUNT	DIPTERA: FLIES	COUNT
Andrenidae		Unknown	
Calliopsis pugionis	1	DIPTERA spp.	6
Apidae		**COLEOPTERA: BEETLES**	
Anthophora curta	4	Mordellidae	
Anthophorula nitens	2	*Mordella* sp. 1	2
Apis mellifera	37		
Melissodes sp. 1	2	Unknown	
Tetraloniella pomonae	7	COLEOPTERA sp. 1	1
Triepeolus sp. 1	1	COLEOPTERA sp. 2	1
		COLEOPTERA sp. 3	2
Halictidae		COLEOPTERA spp.	2
Halictus tripartitus	3		
Lasioglossum incompletum	14		
Lasioglossum microlepoides	4		
Lasioglossum sp. 1	1		
Megachilidae			
Ashmeadiella bucconis	2		
Ashmeadiella californica	3		
Dianthidium dubium	1		
Megachile fidelis	2		
Megachile frugalis	2		
Megachile sp. 1	1		
HYMENOPTERA: WASPS			
PARASITICA sp. 1	1		
VESPOIDEA sp. 1	1		
DIPTERA: FLIES			
Bombyllidae			
Pantarbes sp. 1	2		
BOMBYLLIDAE sp. 1	4		
BOMBYLLIDAE sp. 2	6		
Syrphidae			
SYRPHIDAE sp. 1	1		
SYRPHIDAE sp. 2	2		
SYRPHIDAE sp. 3	1		

Figures

(a)
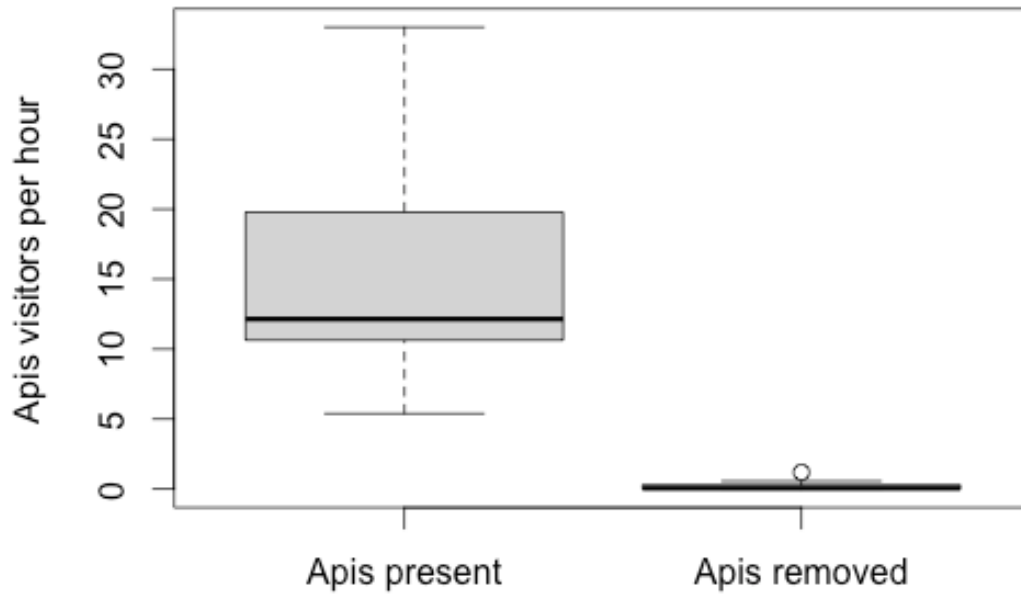
(b)
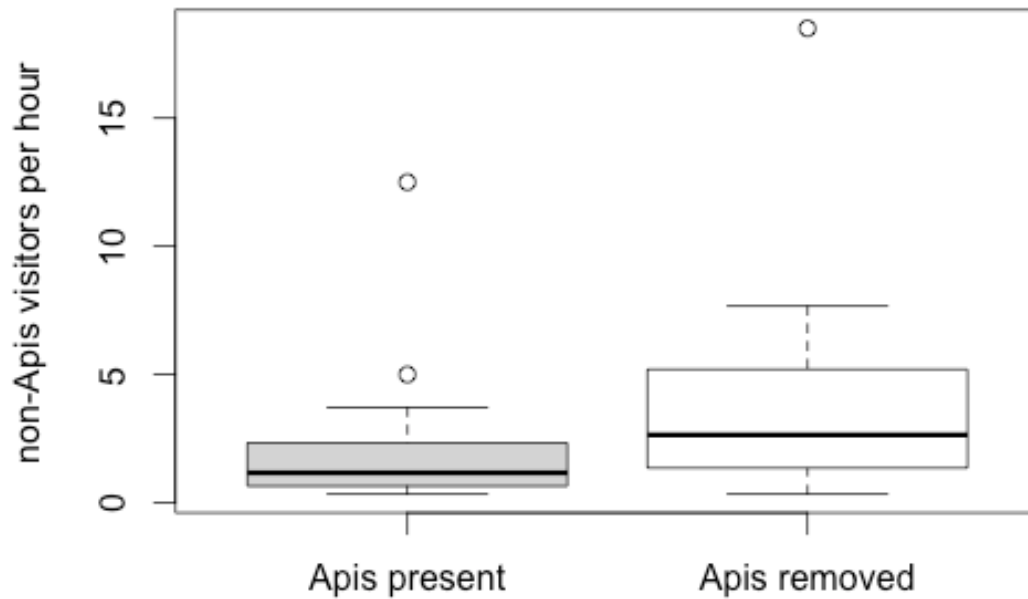
Figure 1. Number of (a) *Apis* and (b) non-*Apis* visitors to plots in *Apis*-present and *Apis*-removal experimental groups. Visitor number equals the sum of all visitors observed during the total hours of observation on each plot and divided by the number of hours of observation for that plot.
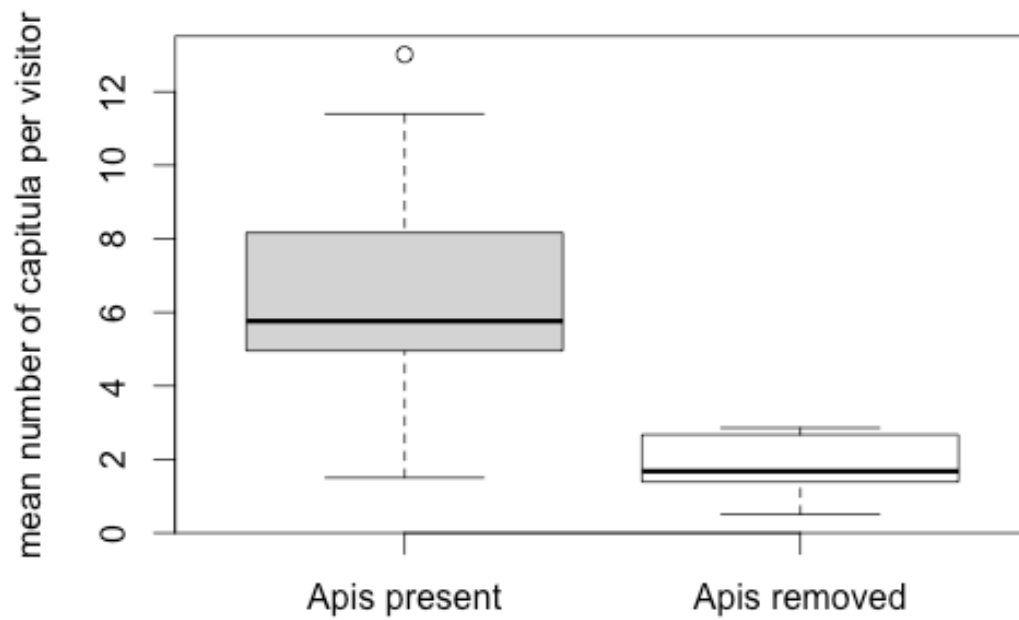
Figure 2. Mean number of capitula per visitor in *Apis*-present and *Apis*-removal experimental groups. All capitula visited by every visitor, *Apis* and non-*Apis*, were summed across each replicate and divided by the number of visitors.
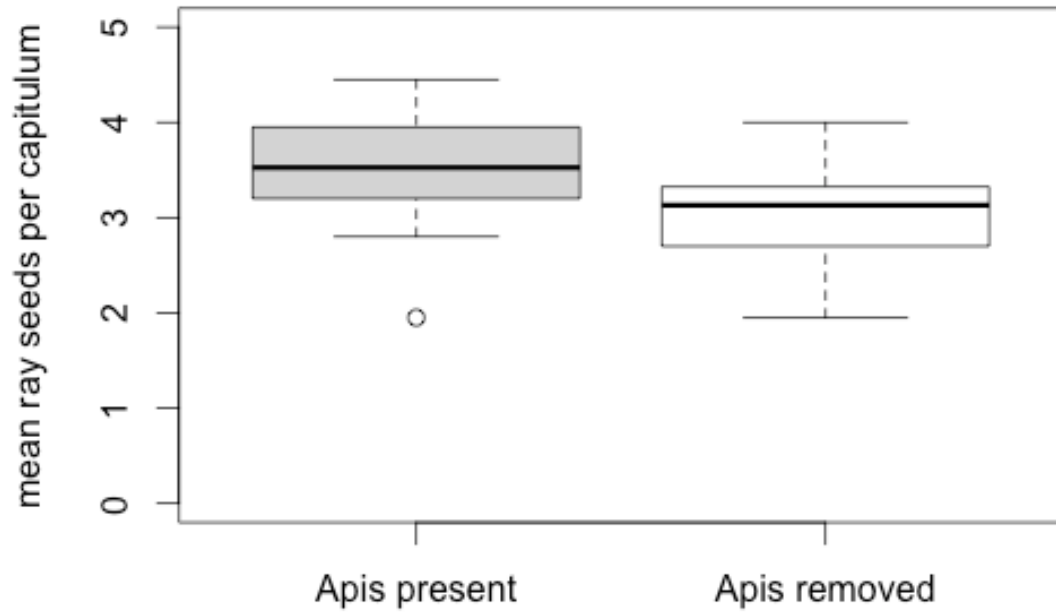
Figure 3. Seed production by *D. fasciculata* in the *Apis*-present and *Apis*-removal experimental groups, measured by mean number of developed ray seeds per capitulum per plot.

(a)

(b)
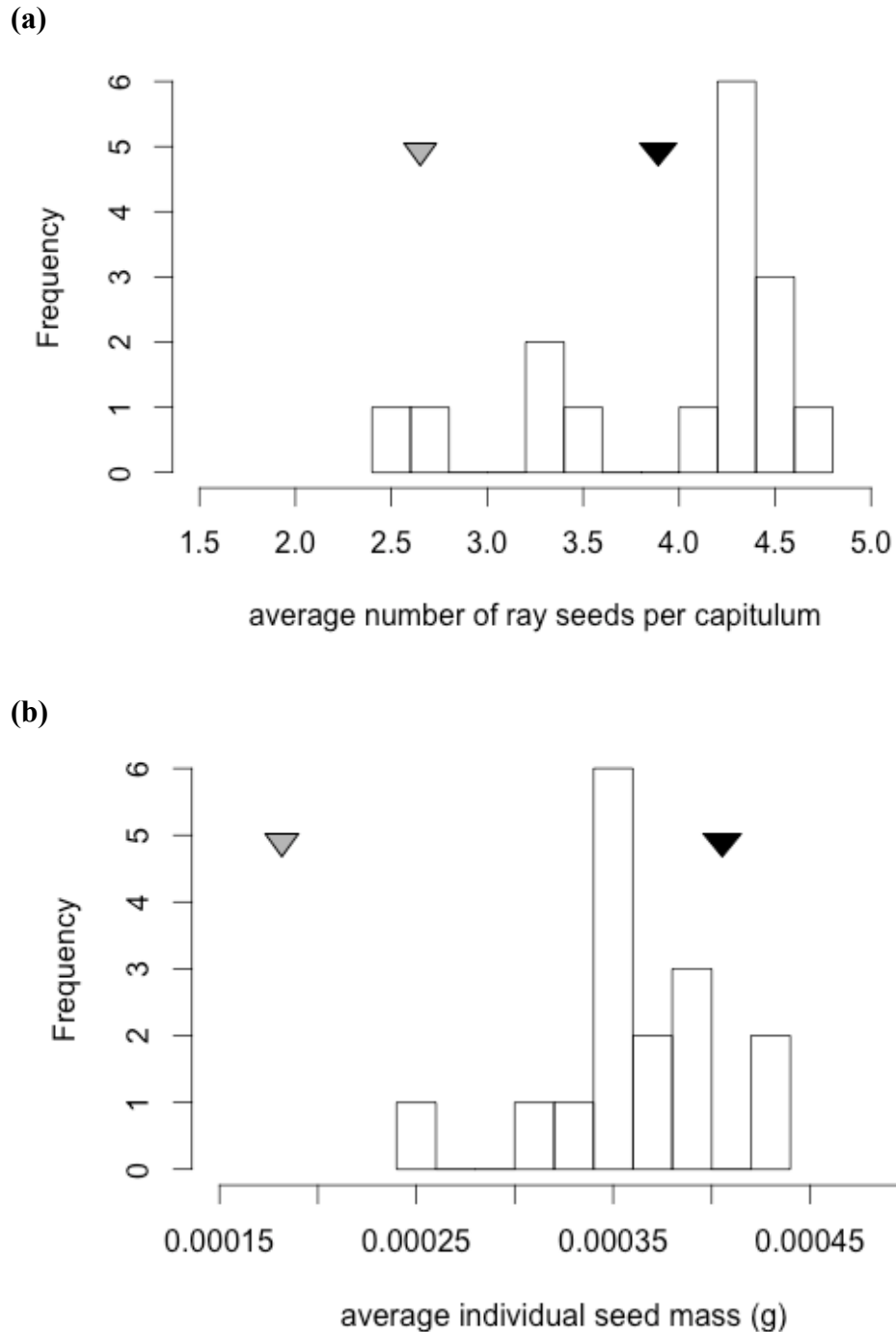
Figure 4. Seed set, measured by (a) average number of developed ray seeds set per capitulum and (b) average individual seed mass, in *D. fasciculata* capitula pollinated by hand with either self or outcrossed pollen. The gray arrow indicates seeds produced from self pollen and the black arrow indicates seeds from outcrossed pollen. The distributions depict average seed masses and counts from the uncaged control replicates used in this paper's primary experimental exclusion.

Appendices

Appendix 1. GPS coordinates of all experimental replicates. "Site" lists abbreviations for the greater sites at which replicates were placed, and "treatment" refers to the experimental treatment. Sites are listed in order of their use during the field season.

SITE	TREATMENT	LAT	LONG
SS2	*Apis* present	32.89547	-117.08919
SOL1	*Apis* present	32.8916	-117.09582
RS1	*Apis* present	32.89395	-117.09731
RS1	*Apis* present	32.89424	-117.09756
PEL1	*Apis* present	32.8867	-117.09601
PEL1	*Apis* present	32.88674	-117.09576
WR1	*Apis* present	32.89069	-117.09621
WR1	*Apis* present	32.89065	-117.09627
ELS1	*Apis* present	32.89192	-117.10174
ELS1	*Apis* present	32.89166	-117.10209
SOL2	*Apis* present	32.89381	-117.09591
SOL2	*Apis* present	32.89374	-117.09608
JAH1	*Apis* present	32.89094	-117.09214
JAH1	*Apis* present	32.89108	-117.09299
SOL3	*Apis* present	32.89382	-117.09576
JAH2	*Apis* present	32.89064	-117.0924
SS2	*Apis* removed	32.89554	-117.08717
SOL1	*Apis* removed	32.89162	-117.09598
RS1	*Apis* removed	32.89389	-117.09736
RS1	*Apis* removed	32.89425	-117.0974
PEL1	*Apis* removed	32.88681	-117.09573
PEL1	*Apis* removed	32.88668	-117.09565
WR1	*Apis* removed	32.89079	-117.09622
WR1	*Apis* removed	32.89004	-117.09623
ELS1	*Apis* removed	32.89202	-117.10161
ELS1	*Apis* removed	32.89157	-117.1022
SOL2	*Apis* removed	32.89379	-117.09612
SOL2	*Apis* removed	32.89379	-117.09583
JAH1	*Apis* removed	32.89095	-117.09246
JAH1	*Apis* removed	32.89099	-117.09284
SOL3	*Apis* removed	32.89394	-117.09577
JAH2	*Apis* removed	32.89043	-117.09196
SS2	Uncaged control	32.89547	-117.08719
SOL1	Uncaged control	32.89152	-117.0958
RS1	Uncaged control	32.89371	-117.09734
RS1	Uncaged control	32.8942	-117.09756
PEL1	Uncaged control	32.88685	-117.09611
PEL1	Uncaged control	32.88664	-117.09576

WR1	Uncaged control	32.89093	-117.09628
WR1	Uncaged control	32.8905	-117.0962
ELS1	Uncaged control	32.89196	-117.10169
ELS1	Uncaged control	32.89172	-117.10204
SOL2	Uncaged control	32.89382	-117.09588
SOL2	Uncaged control	32.89376	-117.09592
JAH1	Uncaged control	32.891	-117.09262
JAH1	Uncaged control	32.89116	-117.09281
SOL3	Uncaged control	32.89388	-117.09575
JAH2	Uncaged control	32.89081	-117.09233

Appendix 2. Histogram of masses of developed ray and disc seeds produced by plants in uncaged control replicates.