

UNIVERSITY OF CALIFORNIA,
IRVINE

Invasive plants and water availability mediate outcomes of plant-pollinator interactions

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Ecology and Evolutionary Biology

by

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DEDICATION

To

My mother, Wildelina,
for her perpetual and unconditional support and encouragement.

“Quienquiera que padece por la verdad y la justicia, ese es mi amigo”
Eugenio Maria de Hostos, Carta a Nicolás Salmerón

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ABSTRACT OF THE DISSERTATION

Invasive plants and water availability mediate outcomes of plant-pollinator interactions

By

Wilnelia Recart González

Doctor of Philosophy in Ecology and Evolutionary Biology

University of California, Irvine, 2019

Professor Diane R. Campbell, Chair

Animal pollination occurs in more than three quarters of all flowering plants. These plant-pollinator interactions can be greatly affected by the environment. The increasing presence of invasive species and changes to abiotic conditions could change the outcomes of plant-pollinator interactions. This dissertation focused on identifying how invasive plants and changes to water availability influence seed production and pollinator-mediated selection.

The first chapter demonstrated how water availability to pollen recipient plants (*Phacelia parryi*) as well as conspecific and heterospecific pollen (the invasive plant, *Brassica nigra*) donors interacted to influence seed production. In this experiment, water availability to recipient plants did not influence seed production. High-water availability to conspecific pollen donors led to higher seed production. Water availability to heterospecific pollen donors set the context for how water given to conspecific donors and

recipient plants impacted seed production. These results show the potential impacts that environmental heterogeneity has on post-pollination events that lead to seed production.

The second chapter explored how water availability influences the shape of the relationship between seeds and pollen received. Water availability influenced the pollen-to-seed relationship and the degree to which seed production is limited by pollen quantity. Data for chapters one and two were collected through a series of hand-pollination experiments done in a pollinator-free greenhouse.

The third chapter documented the impacts that the presence of an invasive plant (*Linaria vulgaris*) across years had on pollinator visitation and pollinator-mediated selection on floral traits in a native plant species (*Penstemon strictus*). This was done through a multi-year field experiment in Colorado, USA. The presence of *Linaria* increased pollinator visits to *Penstemon*. Both pollinator preferences and the relationship between pollinator visits and seed production changed across years.

Together, this work documents two previously-unknown mechanisms on how seed production can be influenced by water to pollen donors and to a change in the pollen-to-seed relationship. This work also illustrates how year-to-year variation in plant-pollinator interactions and the presence of a plant invader affects pollinator visitation and pollinator-mediated phenotypic selection.

INTRODUCTION

It is estimated that about thirty-percent of all crop species and up to eighty percent of all flowering plants rely on pollinators for reproduction (Ollerton et al., 2011). The environments where native plants and their pollinators coexist are changing through both modifications to abiotic conditions (e.g., CO₂, water availability, and temperature) and biotic context (e.g., the introduction of invasive species; (Walther et al., 2002; Tylianakis et al., 2008). Climate change can affect the pollination of native species through changes in plant distribution, phenology and pollinator visitation rate (Poorter and Navas, 2003; Cleland et al., 2007; Gornish and Tylianakis, 2013; CaraDonna et al., 2014; Gallagher and Campbell, 2017). Invasive plant species can affect native plants through changes to their reproductive success, pollinator visitation, structure of plant-pollinator networks, and pollinator-mediated selection (Bjerknes et al., 2007; Morales and Traveset, 2009; Albrecht et al., 2014; Beans and Roach, 2015). These ecological responses of native plants to environmental change tend to be both context dependent and species-specific, making it harder to establish generalizations and imperative to continue their study. Lastly, the presence of invasive species combined with changes to abiotic conditions could further thwart our ability to predict future reproduction of pollinator-dependent plant species (Schweiger et al., 2010). This dissertation aims to identify how invasive plants and changes to water availability influence seed production and pollinator-mediated selection.

Water availability is known to influence seed production (de Jong and Klinkhamer, 1989; Waser and Price, 2016). These effects of water availability could be directly driven by resource allocation to reproduction-related traits of maternal plants. Changes in seed production have also been related to the amount of pollen deposited and the quality of

pollen deposited (outcross versus self-pollen) (Ramsey and Vaughton, 2000; Waser and Price, 2016). The way pollen received is translated into seeds produced follows that of a saturating dose-response curve. This pollen-to-seed relationship can be divided into two main regions, a region in which an increase in pollen deposition leads to higher seed production and a region where additional pollen deposition leads to relatively fewer seeds being produced. In other systems, the pollen-to-pollen tube relationship can be affected by co-flowering species (Arceo-Gómez and Ashman, 2014). In a similar way, this pollen-to-seed relationship could also be influenced by changes in water availability. When this pollen-to-seed relationship is affected it could influence seed production, pollinator effectiveness and even pollinator-mediated selection.

Most plant species receive visits from pollinators that have visited a wide array of plant species. Thus, stigmas can receive not only conspecific but also heterospecific pollen. Such heterospecific pollen is also known to affect seed production in many plant species (Brown and Mitchell, 2001; Morales and Traveset, 2008; Bruckman and Campbell, 2016b). Yet we know little about the effects that changes to abiotic conditions could have on how heterospecific and conspecific pollen will impact seed production (Celaya et al., 2015). In an heterogeneous abiotic environment, quality and quantity of both conspecific and heterospecific pollen could vary (Delph et al., 1997). Under these conditions, abiotic conditions to pollen donors (both conspecific and heterospecific) could influence seed production, pollinator effectiveness and the strength of pollinator-mediated selection.

Selection on floral traits of a plant species can be affected by both the biotic and abiotic components of its surroundings (Caruso, 2000; Maad and Alexandersson, 2004; Kilkenny and Galloway, 2008; Sletvold et al., 2013; Campbell and Powers, 2015). Co-

flowering species can influence selection on floral traits of a focal plant species (Fishman and Wyatt, 1999; Caruso, 2000; Wassink and Caruso, 2013; Beans and Roach, 2015). Even though floral communities are shifting in composition due to anthropogenic disturbances (e.g., habitat fragmentation, invasive species) and climate change (e.g., flowering phenology, range shifts), we still know little about how selection is influenced by co-flowering species and how it will operate under novel ecosystems (Mitchell et al., 2009). In addition, temporal variation in plant-pollinator interactions could influence the strength and direction of pollinator-mediated phenotypic selection.

Understanding the mechanisms by which changes to abiotic conditions and the presence of invasive plants influence seed production and pollinator-mediated selection will serve as important tools to forecast how ongoing environmental change will influence the ecological and evolutionary outcomes of plant-pollinator interactions.

Chapter 1: Water influences how seed production responds to conspecific and heterospecific pollen. Outcrossing plant species depend on pollen from individuals that may not be exposed to the same abiotic conditions as maternal plants. Most studies focus on how maternal conditions influence seed production and leave unexplored the effect of abiotic conditions to pollen donors. This chapter tested when and how water availability to pollen donors, both conspecific and heterospecific, influenced the seed production of pollen recipient plants exposed to different water availability regimes. High-water availability to conspecific pollen donors led to higher seed production. Under low-water to heterospecific pollen donors, seed production was unaffected by recipient or conspecific pollen donor treatment. Under high-water to heterospecific pollen donors, seed production was highest

when conspecific pollen donors and pollen recipients also received high-water. These results illustrate potential impacts of environmental heterogeneity on post-pollination events that lead to seed production and thus impact a pollinator's contribution to plant fitness.

Chapter 2: Water availability influences the relationship of pollen received to seed production. The number of seeds produced by a flower is in part influenced by how much pollen is placed on the stigma and by the likelihood that pollen fertilizes an ovule. This relationship follows that of a saturating dose-response curve and characteristics of the dose-response (first slope, second slope, and breakpoint value) likely vary with changes in abiotic conditions influencing plant function. The breakpoint value between the first and second slope of the pollen-to-seed relationship was earlier on low-watered plants compared to high-watered plants. This change in the breakpoint value affected the duration in which seed production was limited by pollen quantity. These results demonstrate how changes to water availability could affect the relationship between pollen received and seeds produced.

Chapter 3: Pollination in a subalpine plant varies across years and with the presence of an invasive species. The third chapter examined whether the presence of the invasive plant *Linaria vulgaris* and temporal variation in plant-pollinator interactions influenced pollinator-mediated selection in the native *Penstemon strictus*. In the field, we conducted small spatial *Linaria* manipulations within a year, that were repeated through three years. The presence of *Linaria* at the small/neighboring scale facilitated pollinator visitation rates to *Penstemon*. The presence of *Linaria* did not influence pollinator-mediated selection on platform length of *Penstemon strictus*. There was significant variation in

pollinator visitation through time, on the relationship between seed production and pollinator visitation, and on the relationship between pollinator visitation and platform length. Although components of selection varied across years, no net selection on platform length was detected in any of the three years. These results show how the presence of an invasive plant and year-to-year variation in plant-pollinator interactions can affect the pollination and pollinator-mediated selection of native plant species.

CHAPTER 1

Water influences how seed production responds to conspecific and heterospecific pollen

RECART, W., B. OTTOSON, and D.R. CAMPBELL. 2019. Water influences how seed production responds to conspecific and heterospecific pollen. *American Journal of Botany* 106: 1–9.

INTRODUCTION

Abiotic conditions have well-documented impacts on the number of seeds produced by a plant. For example, under natural conditions, additional water or nutrients to maternal plants can lead to increases in seed production (e.g., Willson and Price, 1980; de Jong and Klinkhamer, 1989; Campbell and Halama, 1993; Burkle and Irwin, 2009). These studies document how seed production is affected by abiotic conditions of maternal plants but leave unexplored under what circumstances abiotic conditions experienced by pollen donors influence seed production. Outcrossing species depend on pollen from individuals that are not necessarily exposed to the same abiotic conditions as maternal plants (i.e., pollen recipient plants). Pollen vectors could further aid the movement of pollen between plants exposed to markedly different abiotic conditions. For example, common pollinators such as bumblebees and honey bees can forage over ranges from hundreds of meters up to several kilometers (Beekman and Ratnieks, 2000; Walther-Hellwig and Frankl, 2000), and some wind-pollinated species can receive pollen from plants up to 20 km away (Bittencourt and Sebbenn, 2007; Van De Water et al., 2007). Thus, the abiotic environment

where pollen donor plants grow could affect the seed production of individuals elsewhere. This potential effect is poorly understood and is the focus of this research.

Abiotic conditions experienced by pollen donors can influence pollen quantity and quality, although their impact on seeds sired has been less explored. For example, water availability to pollen donors can influence pollen production, pollen germination, and pollen tube growth (Turner, 1993; Pacini et al., 2006; Waser and Price, 2016), which in turn can influence whether pollination successfully leads to seed production (Wilcock and Neiland, 2002). Quantity and quality of pollen produced are incorporated in studies about pollen limitation (Ashman et al., 2004; Knight et al., 2005; Aizen and Harder, 2007). However, most studies focus on pollen quality through the lens of self and outcross pollen and not on variation in paternal environmental conditions (Burd, 1994; Ramsey and Vaughton, 2000; Aizen and Harder, 2007). Only a few studies have set out to manipulate abiotic conditions of donor plants and link pollen-related changes—such as pollen germination, pollen tube growth, and pollen viability—to the seed production of recipient plants (Young and Stanton, 1990; Turner, 1993; Jakobsen and Martens, 1994; Waser and Price, 2016). This is surprising given that the effects of the maternal and paternal environment can influence offspring seed and seedling characteristics, as well as seedling germination and survival (Schmid and Dolt, 1994; Galloway, 2001a; b).

In natural populations of plant species, flowers tend to receive heterospecific as well as conspecific pollen, and heterospecific pollen deposition, on average, has negative impacts on seed production (Ashman and Arceo-Gómez, 2013). Thus, it is important to understand how the abiotic environment also influences the impact of heterospecific pollen deposition on seed production. Heterospecific pollen deposition influences seed production

through stigma clogging, a mechanism in which heterospecific pollen reduces the available space for conspecific pollen to attach to the stigmatic surface (reviewed in Morales and Traveset, 2008). Pollen grain size and pollen quantity can change with abiotic conditions, and thus changes to the abiotic conditions of heterospecific individuals could also affect the degree of stigma clogging on pollen recipient plants. Heterospecific pollen on stigmas can also cause chemical pollen inhibition (pollen allelopathy), in which conspecific pollen germination and growth are reduced (Kanchan and Chandra, 1980; Galen and Gregory, 1989; Murphy, 2000; Morales and Traveset, 2008). It is likely that variation in abiotic conditions could alter the effects of chemical pollen inhibition through changes in the chemical composition or chemical concentration. If abiotic conditions do alter heterospecific pollen amount, size, and chemistry, then recipient plants—even under the same abiotic conditions and receiving equal amounts of conspecific pollen—could vary in seed production.

In contrast to conditions experienced by pollen donors, much more is known about how abiotic conditions of pollen recipients influence their seed production. Most of this work, however, results from manipulating single abiotic variables (e.g., nutrients, water, temperature) of recipient plants and leaving unchanged abiotic conditions of pollen donors (Willson and Price, 1980; de Jong and Klinkhamer, 1989; Campbell and Halama, 1993; Burkle and Irwin, 2009). In recent years, research has expanded to consider the impacts and importance of multi-stressors to reproduction (Schweiger et al., 2010; Celaya et al., 2015). For example, under low-water conditions for maternal plants, maternal plants exposed to high light had styles with more pollen tubes than plants exposed to low light (Celaya et al., 2015). In addition, the effects of changing both light and water also

influenced the impact that heterospecific pollen had on seed production and pollen tube number (Celaya et al., 2015). Experiments that manipulate abiotic conditions of pollen recipients provide information on how sensitive seed production is to maternal environmental factors but do not examine how environmental effects on pollen donors (conspecific and heterospecific) influence seed production.

To study the joint effects of water and heterospecific pollen on seed production, we selected a plant species, *Phacelia parryi*, whose seed production is reduced by the presence of another plant species, *Brassica nigra*, through heterospecific pollen transfer (Bruckman and Campbell, 2016a, b). With this species pair we conducted a series of hand pollinations, using conspecific and heterospecific pollen from donor plants exposed to high- or low-water treatments. Pollen recipient plants were also exposed to the high- or low-water treatments. With this experimental setup, we asked the following questions: (1) Does water availability to recipient and donor plants (both conspecific and heterospecific) affect seed production of pollen recipient plants? (2) Do donor-plant (conspecific and heterospecific) and recipient-plant water treatments interact to affect seed production of pollen recipient plants? (3) Can the effects on seed production be explained mechanistically by differences in pollen quantity or in pollen grain traits?

MATERIALS AND METHODS

Study System

Phacelia parryi (Hydrophyllaceae) is an annual herb native to southern California and Baja California, where it grows in coastal sage scrub and chaparral ecosystems (Bruckman and Campbell, 2014). A single plant of *P. parryi* produces from a few to hundreds of flowers.

Flowers are hermaphroditic and deep royal purple. This species is self-compatible, although it produces higher seed set per fruit from outcross pollen (Bruckman and Campbell, 2014). *Brassica nigra* (Brassicaceae), also known as black mustard, is a highly invasive, introduced, plant species in southern California. This annual hermaphroditic self-incompatible plant species (Conner and Neumeier, 1995) was used as our heterospecific pollen donor.

Both *B. nigra* and *P. parryi* bloom throughout the southern California rainy season (February–May). Black mustard invades various ecosystems including coastal sage scrub and chaparral where it co-occurs with *P. parryi* (Bruckman and Campbell, 2014). *Brassica nigra* has a strong negative impact on the seed production of *P. parryi*. In a greenhouse setting, pollen tube number and seed production of *P. parryi* were decreased by 30% when hand pollinations contained both conspecific pollen and *B. nigra* pollen when compared to hand pollinations with only conspecific pollen (Bruckman and Campbell, 2016b). The timing of the placement of *Brassica* pollen matters for pollen tube number and seed production of *Phacelia* (Bruckman and Campbell, 2016b). Stigmas that received *P. parryi* pollen before *B. nigra* pollen had more pollen tubes in comparison to treatments when the two types of pollen were placed simultaneously or when *Brassica* pollen was placed first (Bruckman and Campbell, 2016b). Seed production per flower was twice as low when *Brassica* and *Phacelia* pollen were placed simultaneously as when flowers were pollinated with only *P. parryi* pollen (Bruckman and Campbell, 2016b). Under low density of conspecific plants, stigmatic pollen loads on flowers of *P. parryi* in Orange County, California, typically contained from 10 to hundreds of conspecific pollen grains and zero to 10 grains of *B. nigra* pollen (Bruckman and Campbell, 2016a), depending on rates of

pollinator visitation and *B. nigra* densities (Bruckman and Campbell, 2016b). *Brassica nigra* pollen grains are larger than those of *P. parryi* (Bruckman and Campbell, 2016b).

Greenhouse conditions and water availability treatment

Plants were grown from seed during February 2016 inside a pollinator-free greenhouse at the University of California, Irvine. Individuals of both species were grown in 3 L pots with a soil mixture of 1:1:1 parts of peat moss, vermiculite, and perlite.

Two water availability treatments, low- and high-water, were used. Every two days, each low-water plant received 120 mL of fertilized water, and each high-water plant received 120 mL of fertilized water and 120 mL of water filtered through reverse osmosis. Fertilized water contained a mix with 95% of Peters Professional 20-20-20 complete water-soluble fertilizer and 5% of Best Ammonium Sulfate 21-0-0 at a concentration of 350–400 ppm in water. The low-water treatment simulated average February precipitation during 1906–2014, and the high-water treatment simulated twice the average precipitation value. Water volume for each of the water treatments was calculated from daily precipitation records for the area (Santa Ana Fire Station weather station from 1906 to 2014) and proportional to the area of the pots (precipitation records from the National Centers for Environmental Information of the National Oceanic and Atmospheric Administration, weather station ID: GHCND:USC00047888). Sixteen plants each of *P. parryi* and *B. nigra* were used as donor plants; eight plants of each species were given the low-water treatment and eight were given the high-water treatment. Twenty-eight other plants of *P. parryi* were used as recipient plants; 14 were given the low-water treatment and the other were 14 were given the high-water treatment. A separate experiment with *P. parryi* (W. Recart, unpublished data) shows that these water treatments produce significant

differences in both petal length and width, suggesting that the low-water treatment induced some water stress.

Hand pollination experiment

Once flower buds were evident, the largest purple buds of *P. parryi* recipient plants were emasculated to avoid deposition of self-pollen on stigmas. Hand pollinations were made 24 h after emasculation when stigmas were receptive. *Phacelia parryi* exhibits protandry, and receptivity of the stigma occurs when the stigma has grown past the stamens and is bifurcated. Six pollen mixes were generated using a full factorial design considering conspecific pollen treatment (low- and high-water) and heterospecific pollen treatment (no pollen, low- and high-water; Table 1.1). These six pollen mixes were applied to at least four receptive flowers on all recipient plants.

To generate these pollen mixes, one anther from three fresh flowers per plant from all eight donor individuals under a given water treatment and of the same species were collected and placed in a 1.5 mL microcentrifuge tube. In the case of the pollen treatments with both species of pollen, the microcentrifuge tube contained both *Brassica* and *Phacelia* pollen. A toothpick was used to transfer pollen from the microcentrifuge tube to a single stigma. Hand pollinations were done each day from April 19 to May 26, 2016. Flowers receiving pollen of both species typically received more grains of *P. parryi*, which is also the case in natural settings where the two species are intermixed (Bruckman and Campbell, 2016a). Pollen mixes were discarded after each day, and new mixes were prepared prior to hand pollinations. In total, we performed 702 hand pollinations.

Pollen transfer, pollen traits, and seed production

Pollen deposition and pollen traits (volume and eccentricity) were measured to examine the effect of water on the quantity and quality of transferred pollen, respectively. Stigmas were collected 48 h after hand pollination so that seed set of these flowers could be measured. Stigmas were collected in a 0.2 mL microcentrifuge tube and then squashed on a microscope slide with basic fuchsin gel (following methods in Kearns and Inouye, 1993). Pollen was counted using a compound microscope (Optiphot-2; Nikon, Tokyo, Japan). Pollen traits were measured using a microscope at 40× magnification (Leica DM750; Leica, Wetzlar, Germany) with an attached camera (Leica MC120HD). Because pollen grains of both species are ellipsoidal, two diameter measurements were taken, along the polar and equatorial axes of the pollen grain. The volume of the pollen grains was calculated using the volume equation for a spheroid, where the smallest diameter was used to determine the first two radii and the longest diameter determined our third radius. The eccentricity of each pollen grain was also calculated. Lower eccentricity values represent more spherical pollen grains. Pollen traits were measured on four randomly selected pollen grains from 30 microscope slides containing pollen from the different conspecific and heterospecific water treatments.

Pollinated flowers were harvested, and seed number for each flower was calculated. Seed number per flower and related reproductive measurements, the probability of a flower developing into a fruit (hereafter “fruit set”), and seed number per fruit were calculated for each recipient plant and pollen mix type.

Statistical analysis

Linear mixed models were used to analyze whether average number of seeds per flower or one of its multiplicative components—fruit set or average number of seeds per fruit—depended on the fixed crossed effects of recipient water treatment, conspecific-pollen water treatment, and heterospecific-pollen water treatment. Both fruit set and seeds per fruit can be limited by pollen (Knight et al., 2005). The identity of the recipient plant was nested within the recipient-plant water treatment and set as a random effect in these models. After the linear mixed model was generated, we assessed normality of the residuals by viewing a plot of the residuals and running a Shapiro-Wilks test on the model. From this process, only average number of seeds per fruit needed to be square root transformed to achieve normality of the residuals. To explore the three-way interaction, we ran linear mixed models controlling for heterospecific-pollen water treatment (low-water, high-water, or no-pollen), conspecific-pollen water treatment (low or high) and recipient water treatment (low or high).

Pollen count found on *Phacelia* stigmas (separately for *Phacelia* and *Brassica* pollen) was analyzed in the same way as seeds per flower and using a square root transformation to achieve normality of the residuals. For this analysis we used a linear mixed model, with fixed crossed effects of recipient water treatment, conspecific-pollen water treatment, heterospecific-pollen water treatment, and the random effect of the recipient-plant identity nested within recipient-plant water treatment. A post hoc Tukey HSD test was done to identify differences between groups if a significant heterospecific-pollen water treatment was detected.

A generalized linear mixed model was used to analyze pollen volume, and a linear model was used to analyze pollen eccentricity. For the pollen volume model, we used a negative binomial distribution because the data were not normally distributed, and this distribution yielded a low Akaike Information Criterion value and accounted for overdispersion of the data. For the pollen eccentricity model, we used a linear model because the residuals were normally distributed. For both models, we set as crossed factors the plant species (*B. nigra* and *P. parryi*) and the water treatments (low and high).

Statistical analyses were done using R version 3.4.4 (R Core Team, 2018). Linear mixed models were done using the “lmer” function in the “lme4” package (Bates et al., 2015). The results of the analysis of variance (ANOVA) from these models were obtained by running the “anova” function of the “lmerTest” package (Kuznetsova et al., 2017). Normality of the residuals was tested with the Shapiro-Wilks test using the “shapiro.test” function in the “stats” package (R Core Team, 2018). The generalized linear mixed model was created with the “glmmadmb” function of the “glmmADMB” package (Fournier et al., 2012; Skaug et al., 2016). For these models we used the “Anova” function in the “car” package, set to type 3 sum of squares to obtain the results for the ANOVA (Fox and Weisberg, 2011). The linear model for the pollen eccentricity was analyzed using the “lm” function in the “stats” package (R Core Team, 2018). The “Anova” function in the “car” package was set to type 2 sum of squares to obtain the results for the ANOVA for this model, for which sample sizes were equal.

RESULTS

Effects of water availability on seed production

Seed production per flower was influenced by conspecific-donor water treatment, in which pollination by high-water conspecific donors led to 31% more seeds per flower than low-water conspecific donors (Table 1.2). Main effects of recipient-plant and heterospecific-pollen water treatments on seed production per flower were not detected, but a three-way interactive effect did arise from recipient-plant, conspecific, and heterospecific water treatments (Table 1.2). These effects were driven more by effects on fruit set than seeds per fruit. Fruit set showed the same pattern of effects as did seeds per flower (Table 1.3), whereas we detected no impacts on seeds per fruit (Table S1.1).

We did further analysis, controlling for heterospecific pollen treatment (none, or exposed to high or low water availability), to understand whether water availability to conspecific donors and recipient plants influenced seed production per flower differently depending on heterospecific pollen treatment. When there was no heterospecific pollen present, flowers pollinated with high-water conspecific pollen produced, on average, 49% more seeds than flowers pollinated with low-water conspecific pollen, independent of the recipient-plant water treatment (Fig. 1.1A; Table 1.4). Under low-water heterospecific pollen, there were no detectable differences in seed production, regardless of recipient-plant water treatment or conspecific-pollen water treatment (Fig. 1.1B; Table 1.4). Under high-water heterospecific pollen, flowers on recipient plants exposed to high water availability and pollinated with pollen from high-water plants produced 58% more seeds compared to the other treatments (Fig. 1.1C; Table 1.4).

Effects of water availability on pollen transfer and pollen traits

Unlike seed production, number of pollen grains transferred to the stigmas of recipient *P. parryi* plants did not differ between water treatments to conspecific donors ($F_{1,130} = 1.35$, $P = 0.25$; Fig. 1.2A; Fig. S1.1). Although there was some contamination of heterospecific pollen in the no-pollen treatment (presumably due to accidental touching of flowers in the greenhouse), those stigmas received much less heterospecific pollen than stigmas pollinated with heterospecific pollen from high- or low-water treatments (Fig. 1.2B; Tukey-HSD test $P < 0.0001$ for both water treatments compared to pollen absence treatment). Mean heterospecific pollen deposition on the stigma did not differ detectably between the low- and high-water treatments ($F_{1,78} = 0.18$, $P = 0.67$; Fig. 1.2B). There was a significant three-way interaction when analyzing heterospecific pollen deposition in relation to recipient-plant water treatment, conspecific-donor water treatment, and heterospecific-pollen water treatment ($F_{2,130} = 3.69$, $P = 0.03$; Fig. S1.2). This three-way interaction was likely detected because when there was low-water heterospecific pollen there was an interactive effect between conspecific-pollen water treatment and recipient-plant water treatment, which was not seen in other heterospecific treatment combinations ($F_{1,26} = 6.36$, $P = 0.02$). On average, there were 4.8× more *P. parryi* pollen grains transferred (mean = 126 in pollinations with both species of pollen) than *B. nigra* pollen (mean = 26 in pollinations with both species of pollen). Pollen transferred with hand pollinations (considering all water treatments) ranged from one to 740 pollen grains for *P. parryi* and from zero to 214 pollen grains of *B. nigra* per stigma.

Pollen volume did not differ detectably between water treatments applied to the pollen donor ($F_{1,174} = 0.12$, $P = 0.73$; Fig. 1.3). Volume was 58% higher for *B. nigra* pollen

than for *P. parryi* pollen ($F_{1,174} = 54.51$, $P < 0.0001$; Fig. 1.3). There was a significant interactive effect of water availability and species identity on pollen eccentricity ($F_{1,175} = 5.95$, $P = 0.02$; Fig. 1.4). This interactive effect was likely a species-specific response, as seen by the result of extra water increasing *B. nigra* pollen eccentricity values while having no effect on *P. parryi* pollen eccentricity (Fig. 1.4). On average, eccentricity values between the two species did not differ ($F_{1,175} = 2.65$, $P = 0.10$; Fig. 1.4).

DISCUSSION

Environmental conditions experienced by conspecific pollen donors

Water availability to conspecific pollen donors was the main factor influencing seed production in this experimental study. If similar processes occur in natural populations, there are two potentially important consequences. First, environmental conditions experienced by plants in one place could influence the seed production of plants growing elsewhere—if there is pollen movement between these individuals. This effect could operate on a variety of spatial scales. For example, in environments that vary on a fine spatial scale, pollen movement even from relatively nearby plants could lead to differences in seed production. In coarse-grained environments, pollinators or abiotic vectors (e.g., wind, water) could move pollen long enough distances that environmental conditions for the recipient plants are expected to differ from that of donor plants. In our study system, the most frequent visitor to *P. parryi* is the nonnative *Apis mellifera* (Bruckman and Campbell, 2014); this pollinator can move pollen anywhere from a few meters to several kilometers (Beekman and Ratnieks, 2000). Bumblebees (*Bombus vosnesenskii* and *B. californica*) and halictid bees are native pollinators of *P. parryi* (Bruckman and Campbell,

2014). Bumblebees can move pollen from a few meters away to hundreds of meters away (Walther-Hellwig and Frankl, 2000), whereas some halictid bees forage at small scales (Waddington, 1979). Thus, these pollinators are capable of moving pollen from plants exposed to different environmental conditions on a variety of spatial scales. The second consequence is that environmental conditions could influence male fitness of plants. Although our study focused on seed production by the recipient rather than siring success by the donor, the results suggest that well-watered pollen donors would have higher male fitness. If a pollinator deposits pollen from multiple plants exposed to different environmental conditions in a single visit, a well-watered donor might also win out in pollen competition (Kalla and Ashman, 2002). For example, a well-watered pollen donor could exhibit higher pollen germination or pollen tube growth (Turner, 1993).

The mechanism behind the effect of the pollen donor's environment on seed production in *P. parryi* remains to be determined. There are two general ways in which water availability to pollen donors could have an impact, through pollen quantity or pollen quality. We can rule out pollen quantity as a mechanism for our observed results on seed production, as there were no differences between water treatments in the amount of conspecific pollen that we applied in the hand pollinations. Nevertheless, at least in other systems, water availability can influence pollen production, as seen in *Ipomopsis aggregata* (Waser and Price, 2016).

As a proxy for pollen quality, we measured pollen size, which can correlate with pollen viability (Kelly et al., 2002). Yet we did not detect any effects of water on conspecific pollen size. To our knowledge, the correlation between pollen size and pollen quality has rarely been established, with one demonstration in *Mimulus guttatus* (Kelly et al., 2002).

There was no difference between water treatments in the amount of conspecific pollen transferred; thus, variation in pollen quality, mediated through a trait other than size, remains a potential mechanism for the differences in seed production seen in this study.

One factor that may influence the impact of water supplied to conspecific pollen donors is the degree of pollen limitation on seed production. In a scenario of pollen limitation, the greatest difference in seed production should be driven by effects of water on pollen quality or quantity. By contrast, under resource limitation, when saturating amounts of pollen are transferred, the effect of water availability to conspecific pollen could be obscured by the impact of water availability to recipient plants. It would be interesting to test this prediction by comparing the impact of water supplied to conspecific donors for species in which seed production is not pollen limited with that in species like *P. parryi*, in which natural variation in seed set does relate to pollen receipt (Bruckman and Campbell, 2016a).

Environmental conditions experienced by recipient plants

Low water availability to recipient plants often decreases seed production, even when ample pollen is supplied, by reducing pollen tube growth, provisioning of seeds, or altering resource allocation (Turner, 1993; Galen, 2000; Celaya et al., 2015). Surprisingly, this main effect of recipient-plant water treatment was not seen in our study. The lack of a detectable effect is not the result of low sample size or low statistical power, given that we did detect interactions, for which larger sample sizes are generally required (Leon and Heo, 2009). Instead, one possible explanation is that recipient plants reallocated resources from unpollinated flowers to pollinated ones, such that sufficient resources were always available for provisioning seeds in the experimentally hand-pollinated flowers. This

resource reallocation phenomenon has been previously discussed in terms of overpredicting the degree to which seed production is pollen limited when not all flowers are pollinated (Knight et al., 2006). In greenhouse conditions, *P. parryi* can produce over a hundred flowers through its flowering season, and we pollinated only a small subset of these flowers; thus, it is feasible that resource reallocation occurred in this experiment. A second possibility is that plants received pollen doses low enough that seed production was pollen-limited. The average conspecific pollen deposition seen in this experiment (average = 127) is still within the region of pollen limitation based on other experiments (W. Recart, unpublished data). The amount of water supplied to recipient plants is likely to have more effect when there are high pollen loads to all flowers on the plant—when pollen limitation is attenuated and resource limitation is highest. Natural populations of *P. parryi* are likely sometimes pollen limited, as average amounts of conspecific pollen deposition show a considerable range, from ~10 up to hundreds of pollen grains (Bruckman and Campbell, 2016a). Thus, under some natural conditions we expect pollen limitation to cause seed production of this species to respond to environmental conditions experienced by pollen donors more than to those experienced by pollen recipients.

Environmental conditions experienced by heterospecific pollen donors

Heterospecific pollen deposition can negatively affect seed production of recipient plants (Brown and Mitchell, 2001; Morales and Traveset, 2008). This phenomenon has been documented previously in our study system (Bruckman and Campbell, 2016a, b). In Bruckman and Campbell (2016a) it is likely that all plants (e.g., recipient plants and pollen donors) were under well-watered conditions. Under these conditions and when stigmas received heterospecific and conspecific pollen simultaneously, average seed production

was 30 seeds per flower (Bruckman and Campbell, 2016b). When we match these conditions as best as possible to that of our experiment (i.e., high-water to all individuals), we see similar values in the seed production per flower (average = 28 seeds per flower). With high water to all individuals, we did not, however, see a significant increase in seed production per flower caused by the absence of heterospecific pollen (Table S1.2), as had Bruckman and Campbell (2016a). This difference could have been an effect of subtle differences in the watering treatments and conspecific and heterospecific pollen transferred. In the present study, the biggest impact of heterospecific pollen from well-watered plants appeared instead to occur when there was high water availability to conspecific donors but low water availability to the recipients (Fig. 1.1C). This context dependency on when heterospecific pollen had the greatest impact on seed production could explain why, in previous studies, there was great variability in the effect that heterospecific pollen had on seed production, especially in *in situ* experiments (Morales and Traveset, 2008). Our results suggest that abiotic conditions experienced by pollen donors and recipient plants could influence the impact of heterospecific pollen receipt.

More research needs to be done to elucidate the mechanism that led to a three-way interaction between heterospecific-pollen water treatment, conspecific-pollen water treatment, and recipient-plant water treatment. Heterospecific pollen grains became prolate (elongated in the polar axis in relation to the equatorial axis) when produced under the high-water rather than the low-water treatment. It is possible that this morphological change affected the impact that heterospecific pollen had on seed production by limiting the stigmatic surface area for conspecific pollen to successfully attach. Another possibility is that water availability altered the production of chemicals involved in chemical pollen

inhibition. *Brassica nigra* secretes allelochemical compounds, including glucosinolates, from its roots that inhibit seedling growth (Bell and Muller, 1973). Glucosinolates have also been found in the pollen of both *B. napus* and *B. juncea* (Dungey et al., 1988). There is evidence from other plant species suggesting that water availability can influence both chemical concentration and composition of root exudates associated with allelochemical inhibition (Yosef Friedjung et al., 2013). In *B. oleracea*, increases in water availability were correlated with increases in glucosinolate content in aboveground plant tissue (Khan et al., 2010). In *B. nigra*, it has yet to be determined whether allelochemical compounds vary with water availability or even if they are found in the pollen as they are in some other *Brassica* species. If allelochemicals found in the heterospecific pollen increase under wetter conditions, then conspecific pollen could exhibit lower pollen tube formation even when conspecific donors receive plenty of water. This could also be context dependent on the water condition of the recipient plant, because its water status could also dictate the severity of the interference of such heterospecific pollen.

In natural environments, water could also influence the impact of heterospecific pollen in yet another way. Pollen production could increase with more precipitation (Waser and Price, 2016), and if that effect occurs on heterospecific plants, there could be higher levels of heterospecific pollen deposition from these species to focal plant species—which could further increase the effects that heterospecific pollen has on seed production.

Interactive effects of changes in multiple abiotic conditions of recipient plants can lead to changes in seed production (Celaya et al., 2015). Here, we have also documented that even changes to one abiotic variable, water availability, for all plant participants (i.e., recipient plants, conspecific and heterospecific pollen donors) can influence seed

production in unexpected and intricate ways. It will be important to consider these impacts on all participants in predicting the repercussions of future climatic conditions for plant–pollinator interactions.

CONCLUSIONS

These results illustrate that environmental conditions in which male gametes develop can be just as important to seed production as conditions experienced by the seed parent. That phenomenon could also explain some of the variability seen in effectiveness of pollinators and in the degree of pollen limitation seen within and across plant species. For example, if abiotic conditions of pollen used for supplemental pollinations differ from that of natural pollinations, then estimates of pollinator effectiveness and pollen limitation could show significant variation in both space and time. In systems, where pollen is frequently moved between plant species, the environment in which heterospecific pollen develops can also influence seed production, and as illustrated here its effect could be context dependent on the environment experienced by conspecific pollen donors and pollen recipients. An important consequence of these results is that environmental conditions, such as water availability, can impact seed production at distances that extend well beyond the local root zones of plants where the conditions are altered.

TABLES

Table 1.1. Description of the six pollen-donor × water-treatment combinations used for the hand pollinations given to pollen-recipient plants (*Phacelia parryi*) under low- and high-water-availability treatments.

Conspecific pollen (<i>P. parryi</i>)	H ₂ O treatment	Heterospecific pollen (<i>B. nigra</i>)		Pollen absent
		Low	High	
	Low	Donors from same H ₂ O treatments	Donors from different H ₂ O treatments	Control
	High	Donors from different H ₂ O treatments	Donors from same H ₂ O treatments	Control

Table 1.2. Results for the ANOVA determining whether average number of seeds per flower was dependent on recipient-plant water treatment (low or high availability), conspecific-donor water treatment (low or high availability), and heterospecific-donor treatment (low, high, or no pollen). Significant *P* values are in bold.

Source	df	SS	F	P
Recipient-plant water treatment	1, 26	2.69	0.01	0.891
Conspecific-donor (<i>Phacelia parryi</i>) water treatment	1, 130	1645.54	11.70	0.001
Heterospecific-donor (<i>Brassica nigra</i>) water treatment	2, 130	399.89	1.42	0.245
Recipient × conspecific	1, 130	28.57	0.20	0.653
Recipient × heterospecific	2, 130	27.37	0.10	0.907
Conspecific × heterospecific	2, 130	246.05	0.87	0.419
Recipient × conspecific × heterospecific water treatment	2, 130	1182.12	4.20	0.017

Table 1.3. Results for the ANOVA determining whether fruit set is dependent on recipient-plant water treatment (low or high availability), conspecific-donor water treatment (low or high availability), and heterospecific donor treatment (low, high, or no pollen). Significant *P* values are in bold.

Source	df	SS	F	P
Recipient-plant water treatment	1, 26	0.083	2.074	0.162
Conspecific-donor (<i>Phacelia parryi</i>) water treatment	1, 130	0.222	5.540	0.020
Heterospecific-donor (<i>Brassica nigra</i>) water treatment	2, 130	0.027	0.335	0.716
Recipient × conspecific	1, 130	0.004	0.096	0.757
Recipient × heterospecific	2, 130	0.048	0.598	0.552
Conspecific × heterospecific	2, 130	0.017	0.218	0.804
Recipient × conspecific × heterospecific water treatment	2, 130	0.260	3.255	0.042

Table 1.4. Results from the ANOVA on average seed production per flower controlling for heterospecific pollen deposition (no pollen, low or high water availability). Significant *P* values are in bold.

Source	df	Heterospecific pollen treatment								
		No pollen			Low water			High water		
		SS	F	P	SS	F	P	SS	F	P
Recipient treatment	1, 26	0.20	0.001	0.972	0.28	0.002	0.967	18.61	0.13	0.722
Conspecific donor treatment	1, 26	1303.88	8.04	0.001	242.43	1.47	0.236	345.28	2.40	0.134
Recipient × conspecific treatment	1, 26	24.03	0.15	0.704	487.64	2.97	0.097	699.03	4.85	0.037

FIGURES

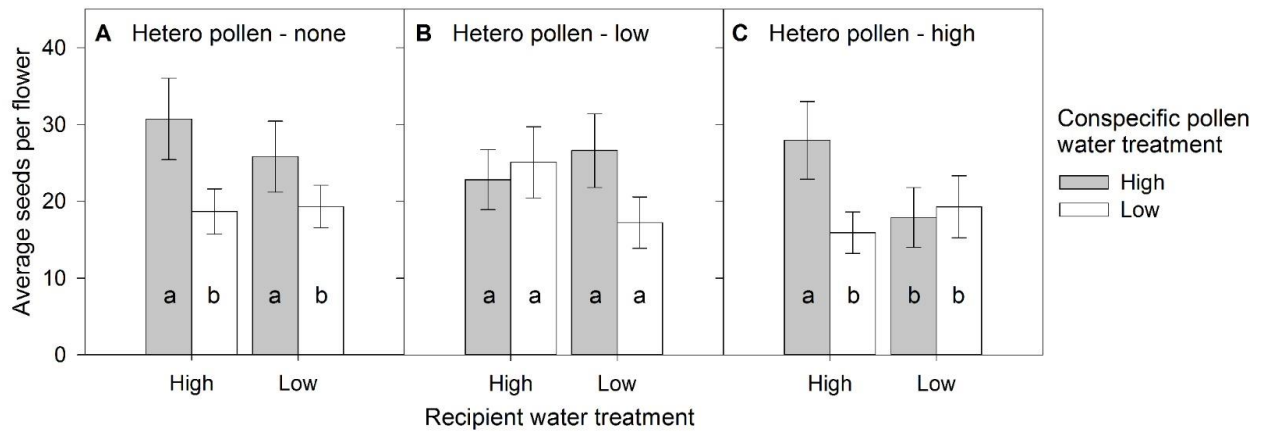


Figure 1.1. Effects of water availability to recipient plants and conspecific pollen donors on average seed production per flower under different heterospecific pollen treatments: (A) no pollen, (B) low-water pollen, or (C) high-water pollen. Means and standard errors are shown, based on the means of each pollen-mix $\times$ recipient-plant identity. Different lowercase letters represent statistically significant differences among treatments within each panel.

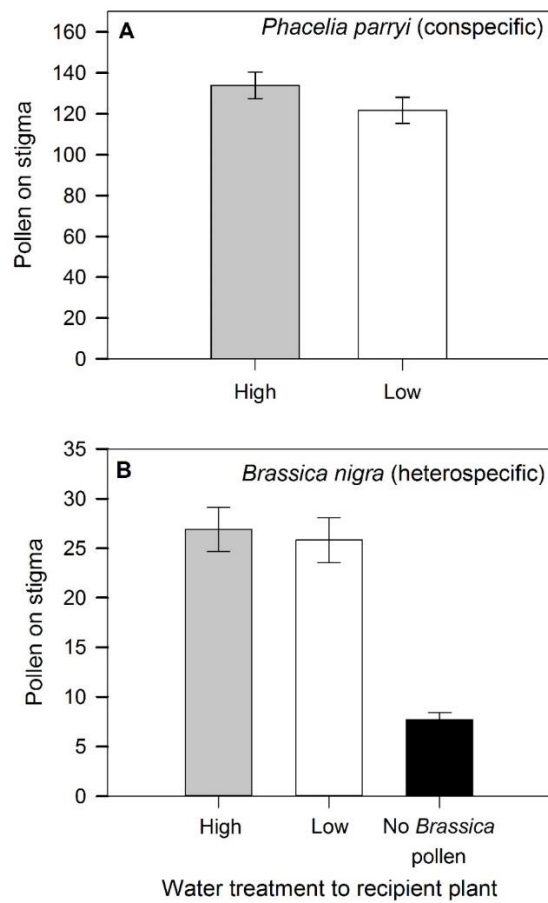


Figure 1.2. Effects of water availability treatment on (A) conspecific (*Phacelia parryi*) and (B) heterospecific (*Brassica nigra*) pollen deposition. Means and standard errors are shown, based of pollen count data for conspecific and heterospecific pollen deposition.

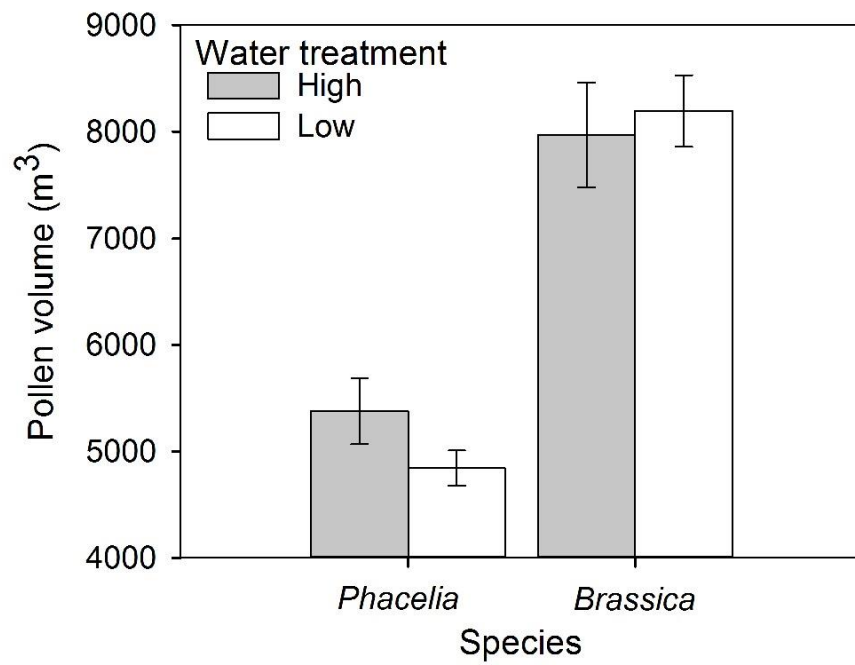


Figure 1.3. Effects of water availability to the average conspecific and heterospecific pollen volume. Means and standard errors are shown, based on the means of pollen measurements grouped by water treatment.

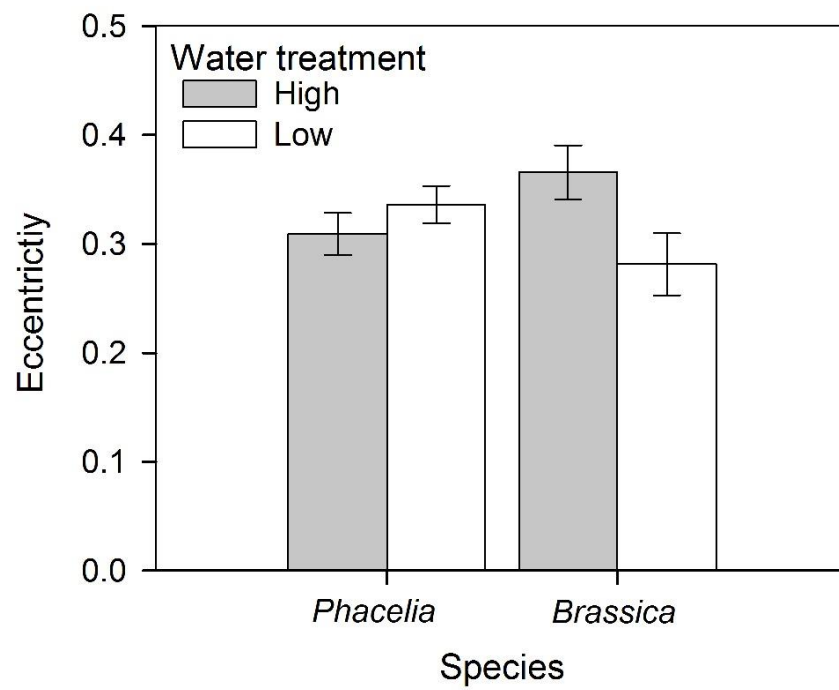


Figure 1.4. Effects of water availability to the average conspecific and heterospecific pollen eccentricity. Means and standard errors are shown, based on the means of pollen measurements grouped by water treatment.

SUPPORTING INFORMATION

Table S1.1. Results for the ANOVA done to determine if average seeds per fruit is dependent on recipient plant water treatment (low or high-water), conspecific donor water treatment (low or high-water) and heterospecific donor treatment (low, high, or no pollen).

Source	d.f.	Sum sq.	<i>F</i>	<i>P</i>
Recipient water treatment	1, 26	0.956	0.638	0.432
Conspecific (<i>Phacelia parryi</i>) donor water treatment	1, 126	4.870	3.250	0.074
Heterospecific (<i>Brassica nigra</i>) donor water treatment	2, 126	8.220	2.743	0.068
Recipient x conspecific	1, 126	1.074	0.717	0.399
Recipient x heterospecific	2, 126	0.415	0.139	0.871
Conspecific x heterospecific	2, 126	3.033	1.012	0.366
Recipient x conspecific x heterospecific water treatment	2, 126	7.696	2.568	0.081

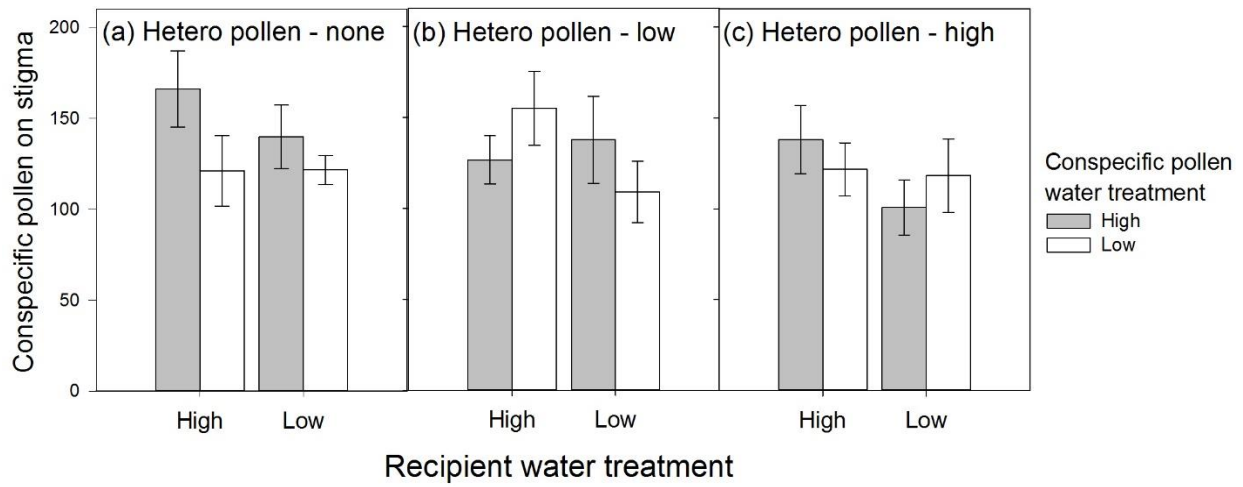


Figure S1.1. Average number conspecific pollen grains found on *Phacelia parryi* stigmas for each hand pollination treatment and by recipient plant water treatment. Figure shows means and standard errors based on the means of each pollen mix by recipient plant identity.

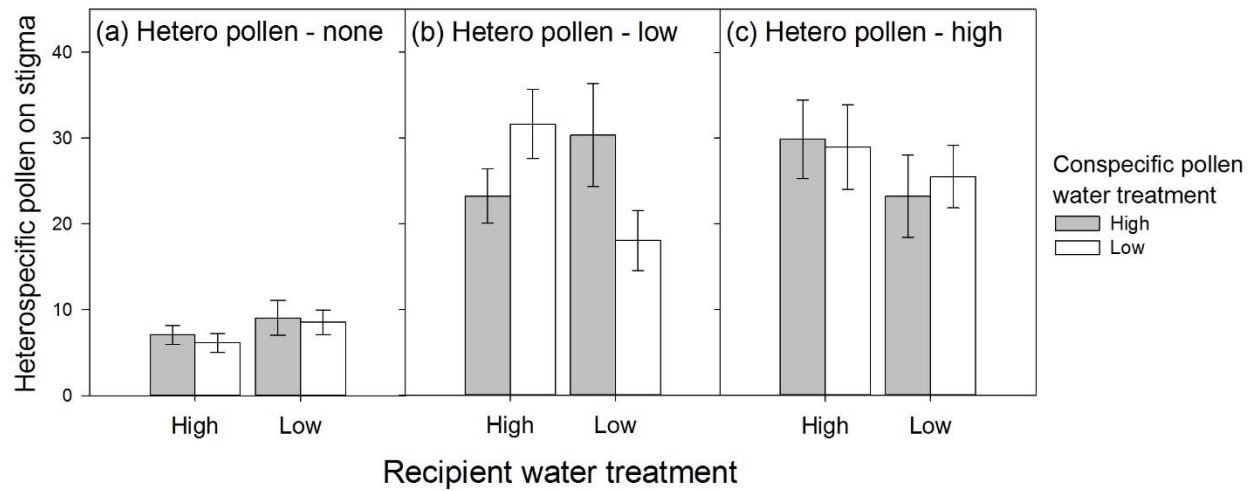


Figure S1.2. Average number heterospecific pollen grains found on *Phacelia parryi* stigmas for each hand pollination treatment and by recipient plant water treatment. Figure shows means and standard errors based on the means of each pollen mix by recipient plant identity.

Table S1.2. ANOVA results testing for the effect of the presence of high water heterospecific pollen when compared to no heterospecific pollen deposited on average seeds per flower when controlling for recipient plant and conspecific water treatments. Analysis done to test whether results are comparable to a previous study on this system (Bruckman and Campbell, 2016b a).

Square root of Average Seeds Per Flower	d.f.	Sum sq.	F value	P value
Low recipient plant / Low conspecific donor				
Presence of heterospecific pollen	1	2.4	0.015	0.904
Residuals	26	4218.7		
High recipient plant / High conspecific donor				
Presence of heterospecific pollen	1	34.9	0.091	0.765
Residuals	26	9936.7		
Low recipient plant / High conspecific donor				
Presence of heterospecific pollen	1	680.0	2.404	0.133
Residuals	26	7353.6		
High recipient plant / Low conspecific donor				
Presence of heterospecific pollen	1	76.46	0.641	0.431
Residuals	26	3102.5		

CHAPTER 2

Water availability influences the relationship of pollen received to seed production

INTRODUCTION

Pollen deposition is crucial for seed production and it varies both among plants and among flowers (Herrera, 2004; Arceo-Gomez et al., 2016). Even when pollen deposition on the stigma is held constant, seed production can vary due to differences in the environmental conditions experienced by maternal plants (e.g., herbivory, water availability, nutrients) (Willson and Price, 1980; de Jong and Klinkhamer, 1989; Campbell and Halama, 1993). Since pollen deposition and environmental conditions experienced by maternal plants can vary extensively, it raises the question, is the effect of environmental conditions on seed production the same under different amounts of pollen deposition?

The relationship between pollen received and seed production follows that of a dose-response relationship, where seed production increases greatly with initial increases in pollen deposited but eventually plateaus as the effect of pollen saturates (Bierzychudek, 1981; Haig and Westoby, 1988; Ashman et al., 2004). Using a piecewise regression model this pollen-to-seed relationship can be condensed to three attributes: the first slope (b_1 , see Fig. 2.1A), when seeds increase most rapidly with greater pollen deposition; the second slope (b_2), when seeds increase less rapidly with greater pollen deposition; and the breakpoint (c), defined as the amount of pollen at which the pollen-to-seed relationship switches from high efficiency (b_1) to low efficiency (b_2) (adapted from Alonso et al., 2012).

Little is known about the extent to which the pollen-to-seed relationship varies within and across species, what causes this variation to occur, and how environmental

conditions influence this relationship. In only one species it has been documented that the pollen-to-seed relationship shows great intraspecific variation (Mitchell, 1997). These relationships have also been used to study pollen limitation, to determine the degree to which selection on a floral trait is mediated by pollen deposition and to document how environmental conditions influence selection on floral traits (Knight et al., 2005; Burd, 2008; Campbell and Bischoff, 2013; Campbell and Powers, 2015). Recently, a piecewise regression approach has been implemented to document the pollen-to-pollen tube relationships (Alonso et al., 2012, 2013; Arceo-Gómez and Ashman, 2014). For example, piecewise regression models have been used to determine differences among species in the degree to which these species are limited by pollen quantity (region before the break point) or by pollen quality (region after the break point) (Alonso et al., 2013). These models have also been used to detect how pollen-to-pollen tube relationships drive the degree to which plants are limited by pollen quantity versus pollen quality in female and hermaphroditic flowering individuals found in marginal and central populations (Castilla et al., 2016).

Here we document how pollen addition influences seed number instead of pollen tube number. By studying the pollen-to-seed relationship we can also consider how resource allocation to pollen recipient plants influences seed production and the pollen-to-seed relationship. Within this framework we expect pollen quantity to be most important in the first slope and resource limitation by pollen recipient plants to be most important in the second slope. In this framework aspects of pollen quality or reproduction-related traits in recipient plants could influence seed production at any point in the pollen-to-seed

relationship. What we can determine is that after the breakpoint (c) seed production stops being governed by pollen quantity.

Changes to the pollen-to-seed relationship could influence the degree to which seed production is pollen limited. For example, if there are changes to the pollen-to-seed relationship due to changes in the maternal environment then plants under different environmental conditions but receiving the same amount of pollen could exhibit variation in the degree of pollen limitation. In particular, a plant with a shallow initial slope (dashed line, Fig. 2.1B), may be more likely to be pollen limited than a plant with a steep initial slope (solid line, Fig. 2.1B) when both are receiving natural low amounts of pollen, and theoretically exhibit the same maximum number of seeds. If environmental conditions decrease the maximum number of seeds, then there is a higher probability of seed production to be resource limited.

Changes to the pollen-to-seed relationships could also influence pollinator effectiveness, defined as the number of seeds produced from a single pollinator visit compared to that of unvisited flowers (Spears, 1983). For example, a shallow initial slope (b1) in a plant can make a visit by two pollinators, one less capable of depositing pollen than the other, to have almost the same contribution to seed production (Fig. 2.2A). In contrast, plants that exhibit differences in their b1 slope and are visited by the same pollinator species could display differences in the effectiveness of such a pollinator visit (Fig. 2.2A). In addition, changes to the efficiency of translating pollen received to seeds produced (at the population level) could influence the strength of pollinator-mediated selection (Campbell and Bischoff, 2013; Campbell and Powers, 2015). For example, the strength of pollinator-mediated selection could be weakened if b1 is shallow enough that

any pollinator preferences would not lead to substantial differences in seed production (Fig. 2.2B). Alternatively, pollinator-mediated selection could be strengthened if b_1 is steep enough that pollinator preferences lead to large differences in seed production (Fig. 2.2B).

Determining if abiotic conditions change the pollen-to-seed relationship could help explain some of the known impacts of abiotic conditions on pollination. The goal of this study was to determine if the efficiency of translating pollen received to seeds produced changes with changes to water availability, and 2) relate the pollen-to-seed relationships to pollen deposition under natural conditions. Water availability was chosen as the abiotic factor because of its high importance to plant reproductive success under natural conditions (Galen, 2000; Carroll et al., 2001; Waser and Price, 2016; Gallagher and Campbell, 2017).

MATERIALS AND METHODS

Study species and greenhouse conditions

Phacelia parryi (Hydrophyllaceae) is an annual herb native to Southern and Baja California, where it grows in coastal sage scrub and chaparral ecosystems (Bruckman and Campbell, 2014). A single plant of *P. parryi* produces from a few to hundreds of flowers. Flowers are hermaphroditic and self-compatible, although flowers produce higher seed production per fruit from outcross pollen (Bruckman and Campbell, 2014).

Phacelia parryi plants were grown from seed during Fall 2014 inside a pollinator-free greenhouse at the University of California, Irvine (UCI). Plants were grown in 3 L pots with a soil mixture of 1:1:1 parts of peat moss, vermiculite and perlite. Bulk seeds were obtained from the Irvine Ranch Conservancy seed farm. The greenhouse was used for the

main component of this study, to determine how water availability influences the pollen-to-seed relationship (detailed methods described below).

Collection of stigmas in natural conditions was done to calculate average pollen deposition on *Phacelia parryi* stigmas (detailed methods described below). Field work was done on April 17 and April 23, 2018 at the Crystal Cove State Park in Orange County, California, USA near the Lower Moro Campground (33.575694, -117.794115 WGS 84 Web Mercator). The field site was located on the side of the trail on a sandy and steep slope in coastal sage scrub habitat.

Greenhouse water manipulation treatment

Two water availability treatments, low- and high-water, were applied to potted plants germinated from seed in the greenhouse. Every two days each low-water plant received 120 mL of fertilized water, and each high-water plant received 120 mL of fertilized water and an additional 120 mL of water filtered through reverse osmosis. Fertilized water contained a mix with 95 % of Peters Professional 20-20-20 complete water-soluble fertilizer and 5 % of Best Ammonium Sulfate 21-0-0 at a concentration of 350–400 ppm in water. The low-water treatment simulated average February precipitation during 1906–2014 for Santa Ana, CA, and the high-water treatment simulated twice the average precipitation value (Recart et al., 2019). Thirty *P. parryi* individuals – randomly chosen from the germinated bulk seeds – were used as recipient plants, with fifteen exposed to a low-water treatment and the other fifteen exposed to a high-water treatment. Another ten plants of *P. parryi* were used as donor plants and received similar water amount as the pollen recipient plants under the high-water treatment. To document treatment effects, soil

volumetric water content was measured using a soil moisture probe (HydroSense II, Campbell Scientific) - right before watering and 24 hours after watering.

Greenhouse hand pollination treatments

The biggest purple buds of *Phacelia parryi* recipient plants were emasculated to avoid deposition of self-pollen on stigmas. Hand pollinations were made after 24 hours of emasculation, when the stigma was receptive (appeared bifurcated). Hand pollinations were divided into three treatments: low pollen load (a toothpick was swabbed from a randomly selected donor, then the toothpick was flicked four times before swabbing onto the stigma of a recipient flower), medium pollen load (same as low pollen load but toothpick was flicked twice), and high pollen load (same as others but toothpick was shook quickly and then swabbed onto the stigma of a recipient flower). These three treatments were done to be able to assure variation in pollen deposition. Each plant was exposed to all treatments and each treatment was replicated at least five times on each plant. A total of 703 hand pollinations were done. Each hand pollinated flower was given a unique flower number to be able to relate pollen received to seeds produced.

Greenhouse stigma collection, pollen count and fitness measurements

Stigmas were collected in a microcentrifuge vial after 48 hours of hand pollination and squashed with basic fuchsin gel in a microscope slide (methods detailed in Kearns and Inouye 1993). Pollen in the stigma slide was counted and related to its flower identification number. Fruits were harvested at the time of ripening, and seeds were counted and weighed to relate seed production to pollen deposition and to calculate average fitness in terms of average seeds per flower, fruit set, and average seeds per fruit.

In situ pollen deposition in relation to water availability

Average pollen deposition of naturally occurring *Phacelia parryi* plants was calculated to relate impacts of a change in the pollen-to-seed relationship to natural pollen deposition conditions. To do this pollen deposition of naturally occurring plants was documented in the field and related to soil moisture. For each plant a soil volumetric water content measurement was taken using a 12 cm long soil moisture probe (HydroSense II, Campbell Scientific). Stigmas were collected from thirty-one *Phacelia parryi* individuals flowering at Crystal Cove State Park. From each plant we collected one to three stigmas. Each stigma was placed on a microcentrifuge vial and squashed with basic fuchsin gel in a microscope slide to allow counting of pollen grains (methods detailed in Kearns and Inouye 1993). Collection date did not influence the average amount of pollen deposited per plant in an initial ANOVA ($F_{1,29} = 0.28$, $P = 0.60$).

Statistical analysis

All statistical analyses were done using the R statistical program version 3.5.2 (R Core Team, 2018).

Greenhouse data analysis

With the data collected from the greenhouse experiment we analyzed how water treatment influenced three fitness measures. We then analyzed differences in the pollen-to-seed relationship due to water treatment by using a piecewise regression analysis and a general linear mixed model.

We first analyzed the impact of water availability on three fitness measures averaged by plant identity: seed production per flower, proportion of fruits set, seed production per fruit. These overall means were analyzed using an analysis of variance with

water treatment set as a fixed effect. Normality of the residuals was tested with the Shapiro-Wilks test. The same analysis was done to analyze average pollen deposition per flower averaged by plant identity. The linear model was implemented using the 'lm' function in the 'stats' package (R Core Team, 2018). Normality of the residuals was tested with the Shapiro-Wilks test using the 'shapiro.test' function in the 'stats' package (R Core Team, 2018). The 'aov' function in the 'car' package was used to obtain the sum of squares of the analysis of variance.

The pollen-to-seeds relationships were analyzed in two ways: using piecewise regression and using a general linear mixed model with linear and quadratic terms for pollen. A piecewise regression was done to calculate slopes and breakpoint values of pollen recipient plants exposed to low- and high-water availability conditions (Fig. 2.1A). Instead of using the Michaelis Menten equation, commonly used to fit dose-response data (Ashman et al., 2004), we used a piecewise regression approach. As demonstrated by Alonso et al. 2012, both the Michaelis Menten equation (dose-response curve) and the piecewise regression approach (dose-response relationship) can produce similar outcomes in the pollen-to-pollen tube relationship. By using the piecewise regression approach, we were able to distil the curve into two slopes with a breakpoint value marking the pollen amount at which the first slope no longer fits the rest of the pollen-to-seed relationship (Alonso et al., 2012).

To generate the piecewise regression, we used a linear model where seed number was dependent on plant identity and the amount of pollen deposited. This model was used to estimate b1, b2 and c values (Fig. 2.1A). This model was run separately for the low-water treatment plants and the high-water treatment plants. For each model we used the Davies

test to determine whether the change in slope was significant (Davies, 2002). The 'segmented' function in the 'segmented' package was used to run the piecewise regression model. The 'davies.test' function in the 'segmented' package was used to run the Davies test. The 'slope' function was used to obtain the slope estimates and associated confidence intervals. The 'confint' function was used to obtain the breakpoint estimates and associated confidence intervals.

To test for a significant difference between water treatments in the b1 and b2 slope, for each water treatment, we divided the data into two datasets using the breakpoint value obtained for each water treatment (Arceo-Gómez and Ashman, 2014). To detect differences between water treatment in the b1 slope we used all the data points below the breakpoint value ($< c$) and to detect differences in the b2 slope we used all the data points above the breakpoint value ($> c$). We then used a general linear mixed model to determine if water treatment influenced the b1 and b2 slope (Arceo-Gómez and Ashman, 2014). For this model we used seed number as the response variable and pollen amount and water treatment as crossed fixed effects, with plant identity nested within water treatment and set as a random effect. A Gaussian distribution was used for this model.

A general linear mixed model was used to test for the second slope being significantly different from zero. Two models were run, one for low-water plants and one for high-water plants; for both models, seed count was set as the response variable, and pollen amount was set as a fixed effect with plant identity set as a random effect. A Gaussian distribution was used for these models. A t-test was done to compare the breakpoints between the low- and high-water treatments using their estimates of mean and standard error.

As an additional way of analyzing the data that fits a smooth function rather than a piecewise function, we used a general linear mixed model to determine whether there was a linear or quadratic relationship between pollen amount and seed number, and whether this relationship was influenced by water treatment. For this model we used a Gaussian distribution which yielded a much lower AIC value compared to using a Poisson distribution. In this model we set as crossed factors the linear pollen term with water treatment (low and high-water availability) and the quadratic term with water treatment, with plant identity nested within water treatment and set as a random effect.

All general linear mixed models were created with the 'glmmadmb' function of the 'glmmADMB' package (Fournier et al., 2012; Skaug et al., 2016). For these models we used the 'Anova' function in the 'car' package, set to type 3 sum of squares to obtain the results for the analysis of variance (Fox and Weisberg, 2011).

In situ pollen deposition

To determine whether there was a relationship between average pollen deposition per plant in the field and water availability experienced by these plants we used a linear model with a linear soil moisture term (to test for linear relationship) and quadratic soil moisture term (to test for a quadratic relationship), using type 2 sums of squares.

RESULTS

Seed production per fruit was 21% higher in the high-water availability than in the low-water availability treatment ($F_{1, 28} = 6.80$, $P = 0.02$). We did not detect an effect of water availability on fruit set ($F_{1, 28} = 0.26$, $P = 0.62$) or seed mass ($F_{1, 28} = 1.54$, $P = 0.23$). Average pollen transferred per flower to each pollen recipient plant did not differ significantly with

water treatment ($F_{1,28} = 3.69$, $P = 0.07$, mean = 79 pollen grains per flower). The average pollen transfer of flowers that set fruit was 18% higher in the high-water than in the low-water treatment ($F_{1,28} = 4.15$, $P = 0.05$).

Water availability influenced the shape of the relationship between pollen and seeds. Pollen deposition ranged from 1 to 652 pollen grains (Fig. 2.3). Seed production ranged from zero to 162 seeds per flower (Fig. 2.3). When looking at the results from the piecewise regression we found that the b1 slope did not change significantly with water treatment (interaction term: $F_{1,553} = 0.05$, $P = 0.81$), but the b2 did (interaction term: $F_{1,136} = 11.29$, $P = 0.001$) (Fig. 2.4 and Fig. 2.5A). The b2 slope for the low-water treatment was significantly higher than zero ($F_{1,107} = 8.17$, $P = 0.005$), whereas the b2 slope for the high-water treatment was not significantly different from zero ($F_{1,27} = 3.56$, $P = 0.07$). The breakpoint for the two regressions was also significantly different ($t_{1,699} = 93.77$, $P < 0.0001$), with the low-water treatment having an early breakpoint compared to the high-water treatment (Fig. 2.4 and Fig. 2.5B).

Using the general linear mixed model, we detected an interaction between water availability and the linear effect of pollen amount (Fig. 2.3; $F_{1,695} = 5.73$, $P = 0.02$), with pollen recipient plants under low-water having a shallower slope than high-water plants. We detected a negative quadratic effect of pollen amount on seed production (Fig. 2.3; $F_{1,695} = 117.51$, $P < 0.0001$). Water treatment and the quadratic effect of pollen amount did not interact to influence seed production ($F_{1,695} = 0.09$, $P = 0.77$).

A linear or quadratic relationship between pollen deposition per flower and soil moisture availability in the field was not detected (linear term: $F_{1,28} = 2.87$, $P = 0.10$; quadratic term: $F_{1,28} = 2.52$, $P = 0.12$). Conspecific pollen deposition ranged from 9 to 524

pollen grains and averaged 136 grains per stigma. This mean pollen deposition fell after the breakpoint value for the low-water plants and before the breakpoint value for the high-water plants (Fig. 2.6).

DISCUSSION

Water availability can influence the pollen-to-seed relationship. In the case of *Phacelia parryi* the slope breakpoint changed with water; low-water plants exhibited a smaller duration under pollen quantity limitation than high-water plants (Fig. 2.4). Other research has documented changes to the pollen-to-pollen tube relationship and the degree to which seed production is limited by pollen quantity (calculated from natural pollen deposition) due to changes in flowering community (Arceo-Gómez and Ashman, 2014). Additionally, in a gynodioecious species a change in the b1 slope (pollen-to-pollen tube relationship) was detected between female and hermaphroditic flowers, and this effect differed between central and marginal plant populations (Castilla et al., 2016). Our result shows how water availability can influence the impact that pollen deposition has on average seed production, through a change in the pollen-to-seed relationship.

Water availability did not influence the initial slope of the seeds to pollen relationship in *Phacelia parryi*. This lack of change seen in the initial slope could be due to low variation in pollen quality, since in this study we used the same pollen donor individuals for the hand-pollinations to low and high-watered plants. Research comparing pollen-to-pollen tube relationships have detected a change in the initial slope in relation to whether a species is native or not (Alonso et al., 2013), a plant is at the edge or center of a

population (Castilla et al., 2016), and the presences of co-flowering species (Arceo-Gómez and Ashman, 2014).

The average pollen deposition found in naturally occurring *Phacelia parryi* individuals falls within the b1 slope of high-watered plants and the b2 slope of low-watered plants (Fig. 2.4). Since the average pollen deposition falls after the breakpoint for the low-water plants but not for the high-water plants this could suggest that the same pollination intensity environment can have a different effect on seed production when plants are under low- or high-water treatments. In another study conducted on *Phacelia parryi*, the presence of the invasive plant *Brassica nigra* drove variation in pollen deposition to *Phacelia parryi* individuals (Bruckman and Campbell, 2016a). In this study, the lowest pollen deposition experienced by *Phacelia parryi* was seen when *Brassica* individuals were at least 5 to 7 meters away (average of 10 conspecific pollen grains) (Bruckman and Campbell, 2016a). The highest pollen deposition was detected when *Brassica* was nearby (< 3 meters away) with an average of a 100 conspecific pollen grains (Bruckman and Campbell, 2016a). In our experiment the breakpoint for low-water plants falls close to the pollen deposition seen in the near *Brassica* treatment, whereas it falls in the first slope for the high-water plants (Fig. 2.5B). These data suggest that during drought years there will be a smaller difference in seed production of *Phacelia* plants in nearby versus far away proximity to *Brassica* flowering individuals. During a wet year we will expect a greater difference between these two treatments (near and far). Thus, we expect the distance from a *Brassica* patch to have a greater impact on wet than on dry years. On a *Phacelia* population that only has *Brassica* individuals at faraway distances the amount of precipitation will not likely influence the impact that *Brassica* has on seed production.

Our data shows that pollen limitation estimates could differ between *Phacelia parryi* individuals under different water availability regimes. For example, if pollen deposition is the same under high and low-watered plants, then pollen limitation estimates could be high for plants under well-watered conditions and low for plants under water stress. In addition, pollinator effectiveness data (amount of pollen deposited by a single visit to a flower) collected on *Phacelia parryi* (Bruckman and Campbell, 2014), shows that water availability to pollen recipient plants would have had little to no impact on the seed production obtained from these single visits, since pollen deposition by these pollinators fell well within the b1 slope and this b1 slope was not influenced by water treatment (15 to 25 pollen grains). Since our data was collected using only outcross pollen, it will be important to determine whether differences in pollen quality (in terms of outcross versus self pollen) carried by different pollinators could influence our prediction. Water availability to *Phacelia parryi* pollen donors can influence the seed production of pollen recipient plants (Recart et al., 2019). Thus, it will be important to determine how water to conspecific pollen donors could also influence the pollen-to-seed relationship of pollen receiving plants.

Lastly, other environmental conditions could have the potential to influence the pollen-to-seed relationship or the pollen-to-pollen tube relationship, and in certain species this could lead to changes in the degree to which seed number or pollen tube number is limited by pollen quantity (Arceo-Gómez and Ashman, 2014; Castilla et al., 2016). More studies need to be conducted to identify whether environmental conditions are frequently influencing the pollen-to-seed relationship. Environmental factors that have been shown to directly influence seed production can be an ideal starting point to determine whether

changes in seed production are caused by changes in the pollen-to-seed relationship.

Nutrient availability can directly influence seed production (Campbell and Halama, 1993).

Low nutrient availability to pollen recipient plants can decrease pollen germination (Smith-Huerta et al., 2008) and could thereby reduce the initial b1 slope.

CONCLUSION

Water availability can alter the efficiency by which pollen received is translated into seeds produced. In the insect-pollinated *Phacelia parryi*, water availability influenced the breakpoint value at which an increase in pollen receipt no longer has much of an effect on seed production. This change in the breakpoint value caused a change in the duration in which seed production is mainly limited by pollen quantity. Taken together, our field and greenhouse data suggest that only under certain pollen intensity environments will water availability affect how pollen received is translated into seeds produced.

FIGURES

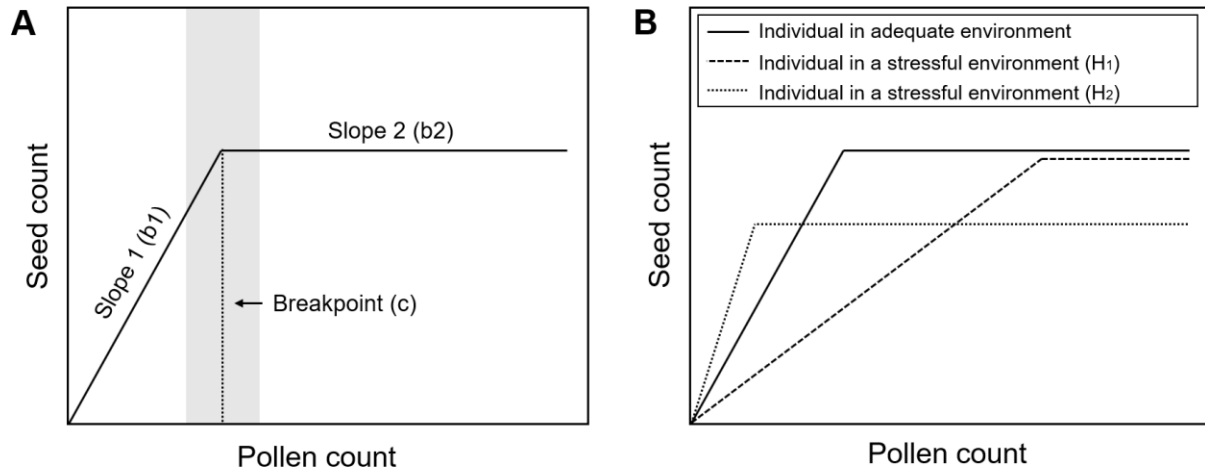


Figure 2.1. (A) Diagram of pollen-to-seed relationships using piecewise regression analysis. Slope 1 (b_1) depicts the most efficient part of the pollen-to-seed relationship and Slope 2 (b_2) depicts the least efficient part of the pollen-to-seed relationship. The breakpoint (c) shows the point at which the slope changes. The gray rectangle around the c value represents the c value confidence interval. Figure adapted from Alonso et al. 2012. (B) Potential outcomes of how pollen-to-seed relationships could be influenced by stressful environmental conditions. Solid line represents a pollen-to-seed relationship of plants under adequate environmental conditions. Dashed and dotted lines represent potential alternative outcomes of how a stressful environment could influence a pollen-to-seed relationship. Notice that the dashed line is from a pollen-to-seed relationship that exhibits a shallower b_1 , a later c value and maintains the same intercept for b_2 . In contrast, the dotted line is from a pollen-to-seed relationship that exhibits a steeper b_1 , an earlier c value and a decrease in the intercept for b_2 .

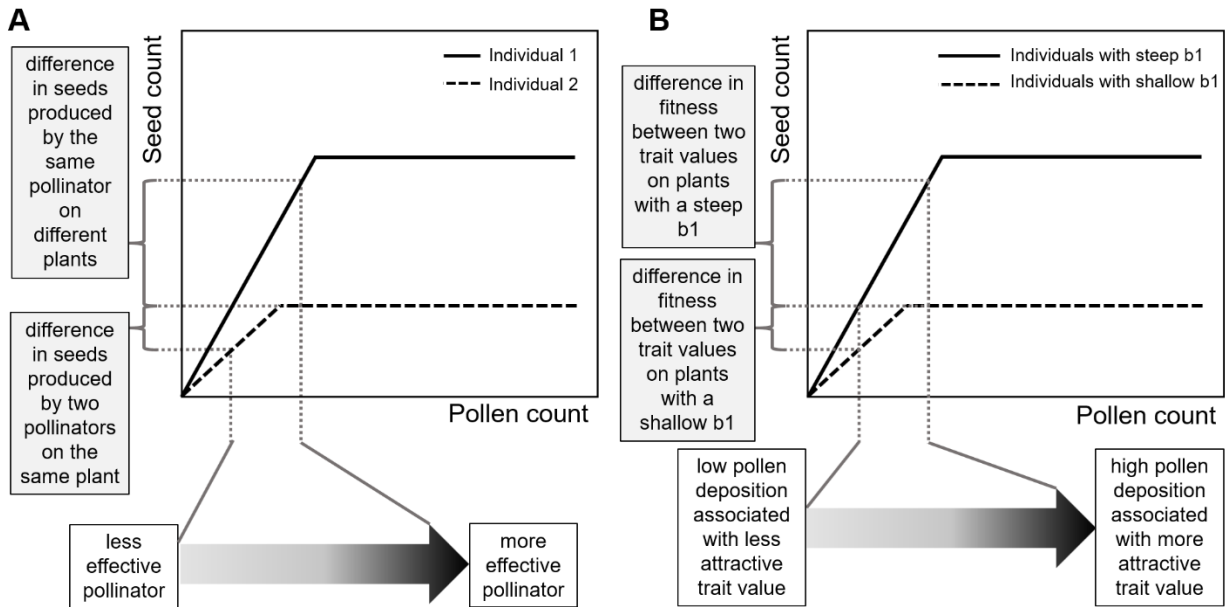


Figure 2.2. (A) Implications of changes to the pollen-to-seed relationship on pollinator effectiveness outcomes: a scenario when two different pollinators visit the same plant, and a scenario when the same pollinator species visits plants with different pollen-to-seed relationships. Solid and dashed lines represent different individuals. (B) Implications of changes to the pollen-to-seed relationships on pollinator-mediated selection. Solid and dashed lines represent different individuals.

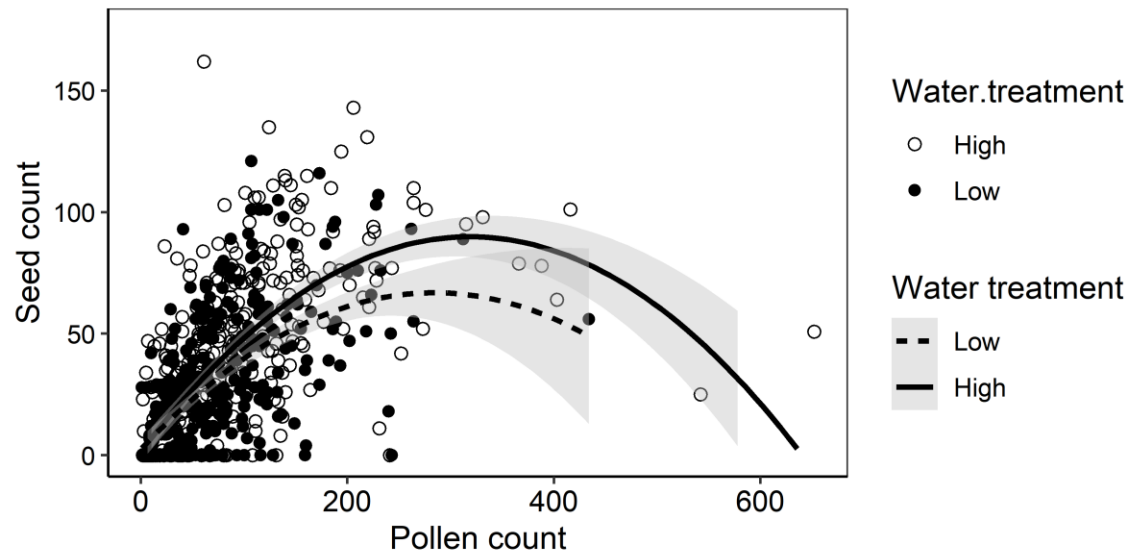


Figure 2.3. Scatterplot of pollen count and seed count data obtained from the 703 hand pollinations. Black circles represent the hand pollinations to low water treatment plants and white circles represent the hand pollinations to high water treatment plants. Quadratic relationship between pollen count and seed count for low-water treatment plants (dashed line) and high-water treatment plants (solid line). Gray shading around each regression line represent 95% confidence intervals.

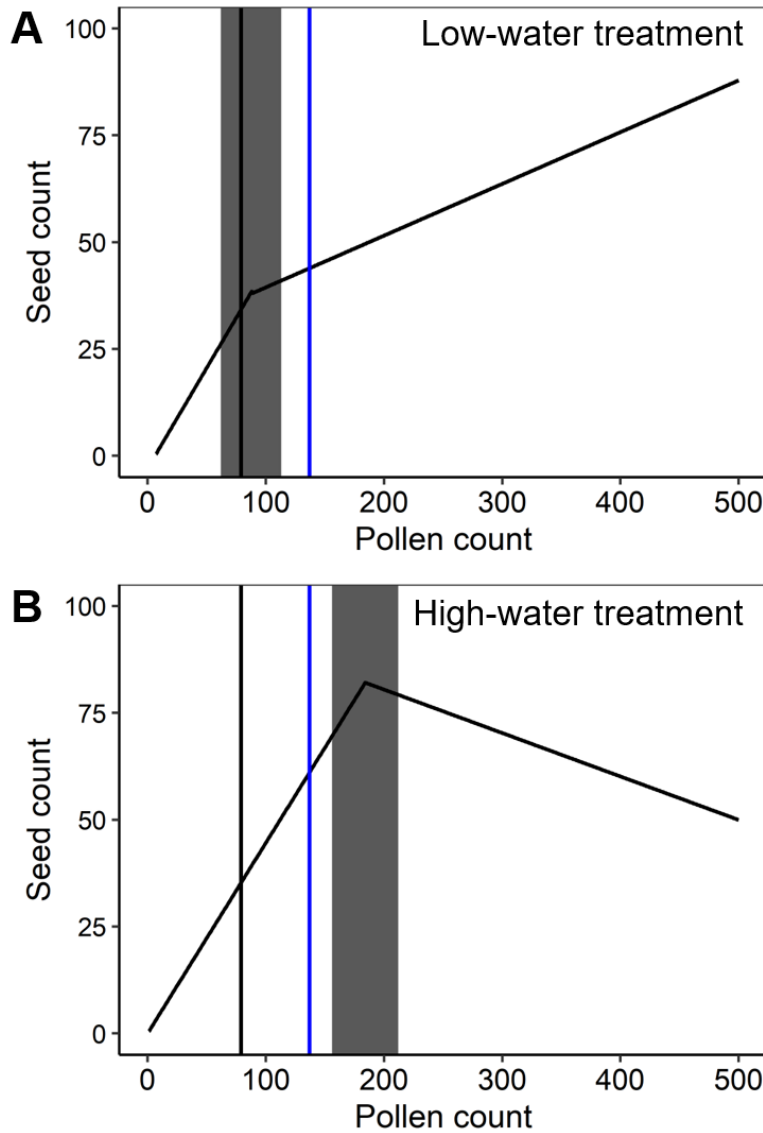


Figure 2.4. (A) Piecewise regression slopes of pollen recipient plants exposed to the low-water treatment. (B) Piecewise regression slopes of pollen recipient plants exposed to the high-water treatment. For both panels, gray rectangle represents confidence intervals around the breakpoint. Vertical black solid line represents average *Phacelia parryi* pollen deposition per flower found on stigmas from the hand pollination experiment. Vertical blue solid line represents average *Phacelia parryi* pollen deposition per flower found on stigmas of *P. parryi* individuals growing under natural conditions.

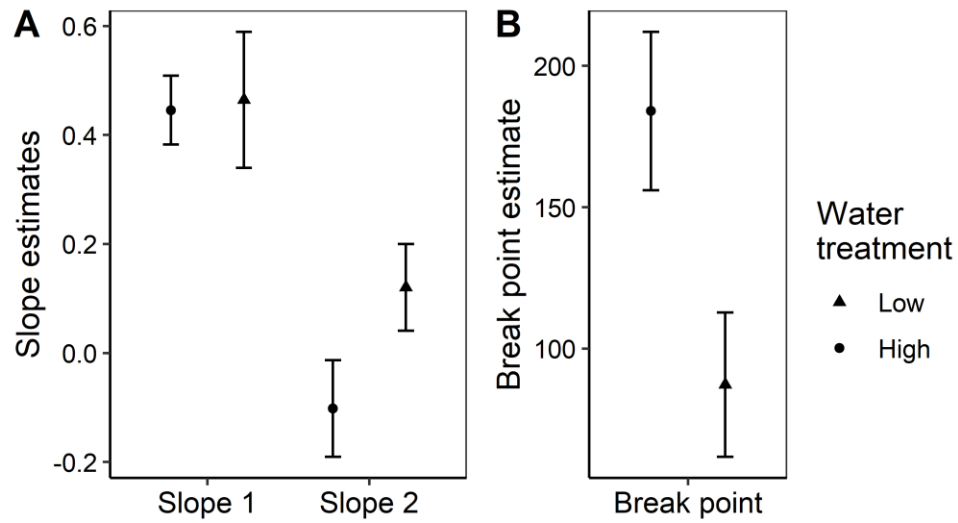


Figure 2.5. (A) Piecewise regression Slope 1 (b_1) and Slope 2 (b_2) estimates for pollen recipient plants under low- and high-water treatments. (B) Piecewise regression breakpoint (c) estimates for pollen recipient plants under low- and high-water treatments. Both figure panels show means and 95% confidence intervals.

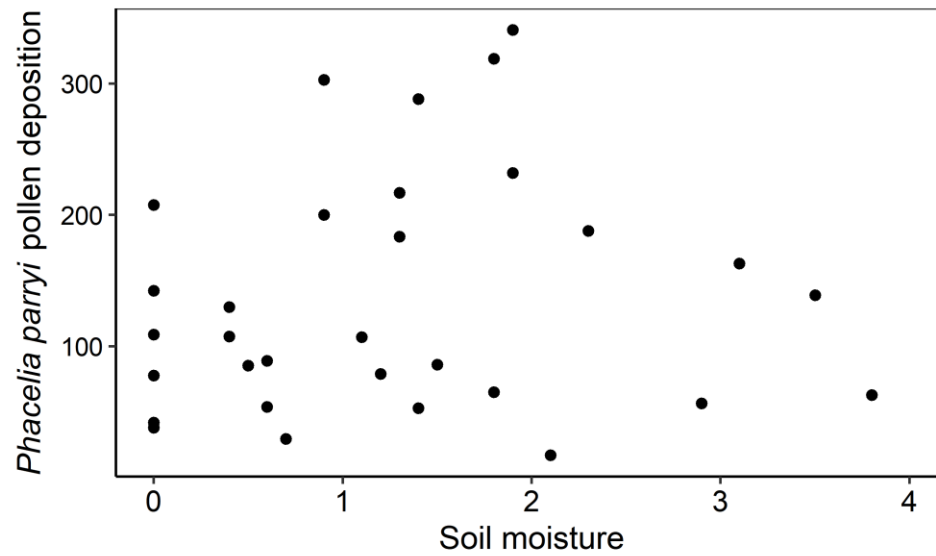


Figure 2.6. Scatterplot of soil moisture against average *Phacelia parryi* pollen deposition per plant on the stigmas of naturally occurring *P. parryi* individuals.

CHAPTER 3

Pollination in a subalpine plant varies across years and with the presence of an invasive species

INTRODUCTION

Invasive plants can have contrasting effects on the pollination of native plant species (Morales and Traveset, 2009; Charlebois and Sargent, 2017). For example, non-native plant species can negatively influence pollination through decreases in pollinator visitation rates (Brown et al., 2002; Bjerknes et al., 2007; Albrecht et al., 2016). Furthermore, if pollinators exhibit low floral constancy (i.e., they visit many different plant species in a single foraging bout), they could increase pollen wastage, or lead to deposition of heterospecific pollen onto stigmas (Brown and Mitchell, 2001; Bruckman and Campbell, 2016a), in some cases decreasing seed production of the native plant (Flanagan et al., 2009). In other instances, non-natives could facilitate the pollination of native species. For example, invasive plants can generate a larger floral display that attracts more pollinators to a patch, thus increasing the chances that a co-flowering species is visited by a pollinator (Jakobsson and Padrón, 2014). Invasive plants can also change the composition of pollinators that are visiting native plants; for example, when only a subset of the pollinator species that visit a plant species change their visitation frequency in the presence of the invader (Albrecht et al., 2016). Additionally, changes in pollinator community composition can lead to differences in seed production if pollinators vary in effectiveness (Sahli and Conner, 2007; Rafferty and Ives, 2012). Thus the impact of invasive plants on the pollination of native plant species can be quite varied and, as recent research suggests, these effects can be highly context

dependent on the spatial scale and relative floral densities of the interacting species (Albrecht et al., 2016; Bruckman and Campbell, 2016a).

Plant community composition can not only influence the average level of pollination in a population, it can also change natural selection exerted by pollinators on the floral traits of plants. For pollinator-mediated selection to occur the pollinator (e.g., selective agent) needs to show a preference (e.g., higher visitation rate) for certain trait values, or be more effective at transferring pollen to plants with certain trait values, such that a trait influences pollination success. Here we focus on the impact of a trait on pollinator visitation, as one component of fitness (Arnold and Wade, 1984; Campbell et al., 1991) and refer to that relationship as “selection based on visitation” (Campbell et al., 1996). In addition, the higher visitation must translate into higher fitness for the trait to experience net selection. We focus on female fitness as estimated by seed production and use the shorthand “net selection” to refer to the impact of a trait on seed production, recognizing that selection can also occur through male fitness (Stanton et al., 1986) or be influenced by events after seed formation (Campbell et al., 2017). If either of these relationships breaks down, between the impact of a trait on pollinator visitation (Fig. 3.1A) or between visitation and fitness (Fig. 3.1B), net selection will be weakened. (Fig. 3.1C).

In communities of native plants, the presence of a competing plant species has been shown to influence the advantageous trait value for flowering phenology (Campbell, 1985), floral daily display size (Wassink and Caruso, 2013), selfing rate (Fishman and Wyatt, 1999), and flower size (Caruso, 2000). In a similar way invasive plant species could affect the evolution of native plants, but so far only one study has described the effects of an invasive plant on pollinator-mediated selection of a native plant species (Beans and Roach

2015). In this study, the native *Impatiens capensis* experienced selection for shorter corollas in the presence of the non-native *Impatiens glandulifera*. More research is necessary to understand the effects that plant invasions will have on pollinator-mediated selection of native plants, particularly the consistency of such effects through time.

Temporal variation in pollination, across flowering seasons is an often assumed yet not widely studied pattern (Price et al., 2005). Spatiotemporal variation in pollinator community composition, effectiveness and visitation by pollinators and pollen eaters can directly influence pollination (Herrera, 1988; Horvitz and Schemske, 1990; Fishbein and Venable, 1996; Fenster and Dudash, 2001; Price et al., 2005). Such temporal variation in pollination success could in turn influence the strength and direction of pollinator-mediated selection. Environmental factors, including the timing of flowering for sympatric species (Waser and Real, 1979; Rafferty and Ives, 2012), could drive the observed temporal variation in pollination. The effects of variation in floral neighbors on pollination have mostly been tested by examining the level of within-season variation rather than year-to-year variation.

Here we assess how an invasive plant influences pollinator visitation rate and net selection through female function in a native plant species and determine if these effects are consistent across three years. *Penstemon strictus* is a plant species native to the Colorado Rocky Mountains that is visited by a wide array of pollinators (Williams and Thomson, 1998; Castellanos et al., 2004). In dry subalpine meadows *P. strictus* and the invasive plant *Linaria vulgaris* can flower simultaneously and share the same species of bumblebee visitors (Castellanos et al., 2004; Burkle et al., 2007). We experimentally removed flowers of *Linaria vulgaris* to test the impacts of the presence of *Linaria* on *P.*

strictus visitation and seed production during a growing season, and we looked at whether these effects were consistent over years. We answered the following questions; 1) Does the nearby presence of the invasive *Linaria vulgaris* influence the mean rate of pollinator visitation or seed production in *Penstemon strictus*? 2) Does the invasive influence the relationship of a floral trait to pollinator visitation? 3) Does the invasive influence the relationship between pollinator visitation and seed production? 4) What are the resultant effects of the invasive on net selection through female function? 5) How consistent are all these effects over three years?

MATERIALS AND METHODS

Species and study site description

Penstemon strictus Benth. (Scrophulariaceae), also known as the Rocky Mountain Penstemon, is an herbaceous perennial plant native to the southern Rockies, present in Colorado, eastern Utah, eastern Arizona, southern Wyoming and northern New Mexico (Ogle et al., 2013). This plant has a basal rosette from which one to a few inflorescences can arise (Ogle et al., 2013). Each inflorescence can have up to 50 purple flowers open at any given time (Thomson, 1996). Our study site was at the Lupine Trail near Nicholson Lake, Crested Butte, CO (GPS coordinates: 38.903260, -107.011476 - WGS 84 Web Mercator). In this area, *P. strictus* is visited by many insects, including wasps, solitary bees and several species of bumblebees including *Bombus bifarius*, *B. flavifrons*, *B. appositus*, and *B. californicus* (Thomson 1996, Wilson et al. 2004, W. Recart personal observation). In the region of our study site *Penstemon strictus* co-occurs with the invasive *Linaria vulgaris* Mill. (Scrophulariaceae). *Linaria vulgaris*, also known as butter-and-eggs and yellow toadflax, is

an herbaceous perennial plant that is native to Europe and Asia (Saner et al., 1995). It was introduced to North America in the 19th century and has spread widely throughout the continental United States and Colorado (Saner et al., 1995). This plant spreads through rhizomes and seeds. *Linaria vulgaris* can produce an average of 27 pale yellow flowers per flowering shoot (Burkle et al., 2007). Near Crested Butte, CO it is visited by several bumblebee species, *B. flavifrons*, *B. appositus*, and *B. californicus* (Burkle et al., 2007). For the experiment described below we used potted or cut inflorescences of *Penstemon strictus* collected from the Lupine Trail.

The degree of flowering overlap between the two species varied across the three years. In 2015 and 2017 both *Penstemon strictus* and *Linaria vulgaris* flowered at the same time, from early July (around July 10th) through late August (around August 20th). In 2016 *Penstemon strictus* flowered from early July through late August, and *Linaria vulgaris* flowered during early August and less abundantly.

***Linaria* removal, pollinator observation, seed production and floral traits**

We examined whether the nearby presence of the invasive *Linaria vulgaris* influenced mean visitation rate by floral-visitors (as a proxy for pollinator visitation), pollinator community composition, selection based on visitation, or net selection in the native *Penstemon strictus*. We tested these effects from 2015, 2016, and 2017. In each year, we established experimental blocks (2016 and 2017 – five blocks; 2015 seven blocks), each with two 3 by 3 m plots that were 3 meters apart, one with *Linaria* flowers present and the other with *Linaria* flowers removed by clipping inflorescences (Fig. 3.2A). Five potted or cut inflorescences of *Penstemon* were placed into each plot, arranged in the form of an X (Fig. 3.2A). Each block was at least 10 meters away from other blocks.

In 2015 we used cut inflorescences only, allowing measurements of pollinator visitation but not of seed production. Cut inflorescences were placed in floral picks with water, and floral traits were measured immediately after pollinator observations were complete. In 2016 we used potted *P. strictus* individuals. In 2017 two of the blocks contained cut inflorescences, and the other three contained potted *P. strictus* individuals. In all three years, pollinators were observed for three hours at both plots in each block to determine plot level visitation rate (visits per open flower per hour) and species composition of the flower visitors. Plots were observed simultaneously when two observers were in the field. When there was only one observer in the field, the observer alternated every hour between the two plots. The total experiment included 102 plot-hours of observation. All individuals (potted and cut inflorescences) were observed only once for three hours during the experiment. Seed production per flower and per fruit were calculated from all the flowers open during the pollinator observations from the potted *Penstemon* individuals (data only available in 2016 and 2017). When potted *Penstemon* individuals were not used for pollinator observations they were inside a pollinator-free enclosure.

We measured pollinator-mediated selection on platform length (Fig. 3.2B). We used platform length because a landing platform is present in bee-pollinated *Penstemon* species and absent in hummingbird-pollinated *Penstemon* (Castellanos et al., 2003; Wilson et al., 2004), suggesting it could be a trait under selection by bees. Platform length was measured with a digital caliper and averaged across three measured flowers for each *Penstemon strictus* individual. To estimate selection, we examined the effects of platform length on

both pollinator visitation rate (i.e., selection based on visitation) and seed production (i.e., net selection).

Statistical analysis

Supplemental Table S3.1 contains a summary of the collected data for each year this experiment was conducted. The models described below are summarized in Supplemental Table S3.2 and Table S3.3. All data analysis was done using the R statistical program version 3.4.4 (R Core Team, 2018).

Effects of *Linaria vulgaris*

Mean plot pollinator visitation rate and mean plot seed production were analyzed using a linear mixed effects model (lme4 package, lmer function) with *Linaria* treatment and year as fixed crossed effects and block identity nested within year as a random effect (Bates et al., 2015). Normality of the residuals was tested using the Shapiro-Wilks test (shapiro.test function). A square root transformation was done on mean pollinator visitation rate to achieve normality of the residuals. Mean pollinator visitation rate for a plot was analyzed for all pollinator visitors, only bumblebee pollinators and only non-bumblebee pollinators. Mean seed production for a plot was analyzed as seeds per flower (total seeds divided by the number of open flowers) and seed per fruit (total seeds divided by the number of fruits). During 2016, only a few individuals of *Linaria vulgaris* bloomed in this area and they did so later in the summer (early August through late August) than *P. strictus* (see Table S3.1). Thus, we were not able to implement the *Linaria* inflorescence removal in 2016. We still collected data during this year and used the same experimental design (without conducting the *Linaria* removal but keeping two observation plots within each block) and data collection protocol (Fig. 3.2A).

The relationship between pollinator visitation rate and seed production was analyzed for the 2017 experiment, as that was the only year when we had both pollinator visitation data and seed production data in the absence and presence of *Linaria* (see Table S3.1). This data analysis was done using the three blocks that had both pollinator visitation and seed production data from 26 *Penstemon strictus* individuals. Due to the small sample size we could not analyze the full model with block nested within year. Instead we first ran a linear model determining whether block identity influenced seed production. Since we detected no variation due to block, we then ran a linear model to determine whether pollinator visitation rate (analyzed using visit rate by all pollinators, only bumblebees, only non-bumblebees) and *Linaria* treatment interacted to influence seed production. For residuals to be normally distributed, pollinator visitation rate was square root transformed.

We used a linear mixed effects model to determine whether *Linaria* treatment influenced the relationship between platform length and pollinator visitation rate. For this model each data point consisted of a *Penstemon* individual for which we had a mean platform length value and a pollinator visitation rate value. We set the platform length, the *Linaria* treatment and the year as fixed crossed effects and block identity was nested within year. For residuals to be normally distributed, pollinator visitation rate was square root transformed. Analysis was done considering visitation rates by all pollinators, only bumblebees or only non-bumblebee pollinators.

Effects across years

To analyze effects of year, we took out the *Linaria* treatment factor from the statistical analysis and analyzed the effect that year had on pollinator visitation, relationship between

pollinator visitation and seed production and the relationship between platform length and pollinator visitation. The effect of year (data from 2015 to 2017) on mean plot pollinator visitation rate was analyzed using a linear mixed effects model with year as a fixed effect and plot identity nested within year as a random effect. Normality of the residuals was tested using the Shapiro-Wilks test. A square root transformation was done on mean pollinator visitation rate to achieve normality of the residuals. Mean pollinator visitation rate for a plot was analyzed for all pollinator visitors, only bumblebee pollinators and only non-bumblebee pollinators.

The relationship between pollinator visitation rate and seed production was analyzed in 2016 and 2017 – when we had both pollinator visitation data and seed production data. A linear mixed effects model tested if seed production per flower was related to the continuous variable of pollinator visitation rate (analyzed using visit rate by all pollinators, only bumblebees, only non-bumblebees) and to year. Pollinator visitation rate and year were set as fixed crossed effects and plot identity nested within year was set as a random effect. For residuals to be normally distributed, pollinator visitation rate was square root transformed.

We used a linear mixed effects model to determine whether the year (data from 2015 to 2017) influenced the relationship between platform length and pollinator visitation rate. For this model each data point consisted of a *Penstemon* individual for which we had a mean platform length value and a pollinator visitation rate value. We set the platform length and the year as fixed crossed effects and plot identity was nested within year. For residuals to be normally distributed, pollinator visitation rate was square root

transformed. Analysis was done considering visitation rates by all pollinators, only bumblebees or only non-bumblebee pollinators.

We used a linear model to determine whether the year (using 2016 and 2017 data) influenced the relationship between platform length and seed production. Due to the small sample size we could not analyze the full model with block nested within year. Instead we first ran a linear model determining whether block identity influenced seed production. Since we detected no variation due to block, we then ran a linear model to determine whether platform length and year interacted to influence seed production.

RESULTS

***Penstemon strictus* visitors and effectiveness**

Penstemon strictus was visited by several species of hymenopterans including: *Anthophora terminalis*, *Bombus appositus*, *Bombus bifarius*, *Bombus californicus*, *Bombus flavifrons*, *Pseudomasaris vespoides* and a wide variety of unidentified small solitary bees. We measured the effectiveness of several of these visitors, in terms of seeds produced from a single visit. A visit by *Bombus* (regardless of species) yielded three times more seeds than the seeds produced by a visit of a non-*Bombus* insect, making the bumblebees a more effective pollinator group (see Supplemental Methods S3.1, Table S3.4 and Fig. S3.1).

***Effects of Linaria vulgaris* on pollinator visitation and seed production**

In the removal experiments, the presence of *Linaria vulgaris* increased by 50% the mean visitation rate by all flower visitors to *Penstemon strictus* ($F_{1,10} = 6.87$, $P = 0.03$, Fig. 3.3). This increase in visitation was likely due to both a 76% increase in visitation by *Bombus bifarius* ($F_{1,10} = 3.94$, $P = 0.07$) and a 57% increase in visitation by other visitors ($F_{1,10} =$

3.73, $P = 0.08$). Even with only 3 blocks in 2017 available for testing the impact on seed production, there was a marginally significant effect of the presence of *Linaria* on the seed production per flower of *Penstemon strictus* ($F_{1,2} = 13.34$, $P = 0.07$), with seed production 80% higher in the presence of the invasive species.

In 2017, we detected a significant positive effect between visitation rate by all visitors and seed production per flower ($F_{1,22} = 13.43$, $P = 0.001$, Fig. 3.4). This relationship was not affected by the presence of *Linaria* (interaction term: $F_{1,22} = 0.10$, $P = 0.75$, Fig. 3.4). Seed production also increased with higher bumblebee visitation ($F_{1,22} = 4.94$, $P = 0.04$). This pattern was seen for visitation only by *Bombus bifarius* ($F_{1,22} = 5.59$, $P = 0.03$), but not for other bumblebee visitors ($F_{1,22} = 0.05$, $P = 0.82$). The relationship between seed production and bumblebee visitation was not influenced by the presence of *Linaria vulgaris* (interaction term $F_{1,22} = 0.01$, $P = 0.92$). The presence of *Linaria* also did not influence the relationship between visitation by *Bombus bifarius* and seed production ($F_{1,22} = 0.11$, $P = 0.74$).

Effects of Linaria vulgaris on pollinator-mediated selection

Plants with longer platforms had higher visitation rate by all flower visitors combined ($F_{1,95} = 4.41$, $P = 0.04$, Fig. 3.5). The presence of *Linaria vulgaris* did not alter the slope of this relationship ($F_{1,90} = 0.83$, $P = 0.37$, Fig. 3.5). This relationship between platform length and visitation rate was lost when looking at any given visitor group (*Bombus*: $F_{1,93} = 1.58$, $P = 0.21$; non-*Bombus*: $F_{1,92} = 2.67$, $P = 0.11$; *B. bifarius*: $F_{1,90} = 1.39$, $P = 0.24$; other *Bombus* sp.: $F_{1,92} = 0.35$, $P = 0.56$).

Yearly variation in pollinator visitation and seed production

When grouping all the data by year independently of the *Linaria* treatment we found that mean visitation rate by all flower visitors (averaged by plot identity) to *Penstemon strictus* varied across years ($F_{2,14} = 9.71$, $P = 0.002$, Fig. 3.6). Visitation rate by all visitors was 195% higher in 2016, the year of non-overlap, than in 2015 and 2017 (Tukey-HSD test $P \leq 0.001$). This increase in visitation rate was not due to grouping together visitation rate independently of *Linaria* treatment (for 2015 and 2017 data); when we analyzed visitation rate using data only from one treatment at a time (either *Linaria* present or *Linaria* absent) along with the 2016 visitation data we saw no difference in the patterns reported above. Visitation rate by bumblebees also varied across years ($F_{2,14} = 13.30$, $P \leq 0.001$, Fig. 3.6), with bumblebee visitation being 310% higher in 2016 than in 2015 and 2017 (Tukey-HSD test $P \leq 0.001$). Visitation by *Bombus bifarius* (the most frequent visitor to *P. strictus*) did not vary detectably across years ($F_{2,14} = 0.98$, $P = 0.40$). In contrast visitation by other species of *Bombus* changed across years ($F_{2,14} = 17.94$, $P \leq 0.001$) and was highest in 2016 when compared to 2015 and 2017 (Tukey-HSD test $P \leq 0.001$). We did not detect a significant effect of year on visitation by other non-bumblebee visitors ($F_{2,14} = 0.66$, $P = 0.53$, Fig. 3.6). Seed production per flower did not significantly differ between 2016 and 2017 ($F_{1,6} = 1.64$, $P = 0.25$).

The relationship between visitation rate by all flower visitors and seed production per flower was significantly different between 2016 and 2017; the relationship was positive in 2017 and absent in 2016 (interaction term $F_{1,70} = 9.84$, $P = 0.002$, Fig. 3.7). This was also the case when using visitation by bumblebees only (interaction term $F_{1,50} = 11.27$, $P = 0.001$). The same was true when looking at *Bombus bifarius* visitors (interaction term

$F_{1,70} = 16.69$, $P = 0.0001$) but not for other bumblebee visitors (interaction term $F_{1,70} = 1.09$, $P = 0.30$) or other kinds of insects (interaction term $F_{1,70} = 0.32$, $P = 0.57$). There was no relationship between visitation rate and seed production per flower when looking at non-*bifarius* bumblebee species ($F_{1,70} = 2.01$, $P = 0.16$) and non *Bombus* sp. ($F_{1,70} = 3.37$, $P = 0.07$).

Yearly variation in pollinator-mediated selection

We detected a positive relationship between *Penstemon strictus* platform length and visitation by all flower visitors, meaning that selection based on visitation was present during the three years of the experiment ($F_{1,140} = 5.66$, $P = 0.02$, Fig. 3.8). There was no significant interactive effect between platform length and year, meaning that selection on *Penstemon strictus* platform length based on visitation by all pollinators did not change in direction or magnitude across the three years of the experiment ($F_{2,140} = 0.46$, $P = 0.63$, Fig. 3.8). A similar pattern was seen when only looking at the bumblebee visitors – where there was no significant interactive effect between year and visitation rate ($F_{2,137} = 1.75$, $P = 0.18$) and there was a significant positive effect of platform length on visitation rate by bumblebees ($F_{1,137} = 3.86$, $P = 0.05$). Interestingly, there was no detectable effect of platform length on *Bombus bifarius* visitation ($F_{1,32} = 0.33$, $P = 0.56$) nor a significant interaction between platform length and year ($F_{2,132} = 1.37$, $P = 0.26$). We found a positive relationship between platform length and visitation by other bumblebees ($F_{1,137} = 6.13$, $P = 0.01$). We also detected a significant interaction between platform length and year on visitation by other bumblebees ($F_{2,137} = 5.51$, $P = 0.005$) where in 2016 there was a stronger relationship between platform length and visitation. Visitation by non-bumblebees had a positive relationship with platform length ($F_{1,139} = 3.99$, $P = 0.05$), but

this relationship was not influenced by year ($F_{2,139} = 0.24$, $P = 0.79$). There was no detectable relationship between platform length and seed production within or across years ($F_{1,56} = 3.02$, $P = 0.09$; Fig. 3.9), meaning that net selection was not detected throughout this experiment.

DISCUSSION

*Effects of *Linaria vulgaris* on pollinator visitation and seed production*

The nearby presence of *Linaria vulgaris* can positively influence the pollinator visitation rate and seed production of *Penstemon strictus*. Invasive plants can have varied effects on the pollination of native plant species, although research tends to be biased to document negative effects of plant invasions (Charlebois and Sargent, 2017). A small but growing body of research suggests that the degree of spatial and temporal overlap, as well as the density of flowering individuals can influence the magnitude and direction of the effect that non-native plants have on the pollination of native plant species (Albrecht et al., 2016; Bruckman and Campbell, 2016a; Charlebois and Sargent, 2017). These findings align with literature on competition and facilitation for pollination between native plant species, in which facilitation and competition for pollination are viewed as extremes of a continuum that is dependent on various aspects of floral neighborhood composition (e.g., species diversity, species richness, floral trait diversity, relative species flower densities) (Rathcke, 1983; Ghazoul, 2006). Facilitation for pollination can occur between native and invasive plant species, independently of the amount of pollinator sharing, floral trait similarity or distance and density of the invader (Charlebois and Sargent, 2017). It is plausible that *Linaria* serves as a magnet species, attracting pollinators to patches where *Linaria* is present

which then leads to an increase in pollinator visitation to *Penstemon*. Experiments aimed to manipulate all these factors could provide us with a better picture of when an invasive plant could influence the pollination of neighboring plant species.

The relative densities and distance of a non-native plant relative to native co-flowering individuals can influence pollinator visitation and interspecific pollen transfer (Bruckman and Campbell, 2016a). In a similar way, the scale of the invasive plant removal could have affected the pollination outcome (facilitation, or competition for pollination) that *Linaria* had on *Penstemon*. It is plausible that under different *Linaria* removals or *Linaria* flowering densities we could have seen a different impact of the invasive plant on the native plant species. The scale of our removal was small compared to the area that *Linaria vulgaris* flowering individuals can occupy, although there is variation in the patch sizes of this invader. While the small-scale manipulation of the presence of *Linaria vulgaris* led to a facultative scenario for the pollination of *Penstemon* there is the possibility that *Linaria vulgaris* can successfully compete for pollination. For example, our yearly pollinator visitation data to *Penstemon strictus* suggested that in the absence of *Linaria* (2016 the year of non-overlap between *Linaria* and *Penstemon*), *Penstemon strictus* exhibited higher visitation rates. This finding suggests that *Linaria* could be competing at a scale larger than our experimental manipulation.

We expected the *Linaria* treatment to strongly influence the visitation rates by shared pollinators (e.g., *Bombus* sp.) when compared to non-shared pollinators, yet this was not the case. This is an interesting result since in other systems the shared pollinators were the group that exhibited variation in visitation rate with the invasive treatment (Albrecht et al., 2016; Bruckman and Campbell, 2016a). One possibility is that pollinators

cue on *Linaria* flowers to identify flowering patches and once near these patches they forage for suitable flowers (Hegland et al., 2009). If this is the case, we should expect a relationship between *Linaria* flowering density and pollinator visitation rate to *Penstemon strictus*.

The invasive plant did not influence the relationship between pollinator visitation and seed production per flower. Thus, it is plausible that the increase in seed production in the presence of *Linaria* was not due to changes in pollinator effectiveness. This result suggests that the mechanism by which *Linaria* facilitated the pollination of the native plant was through a sheer increase in pollinator visitation. This result aligns with other research studying ‘magnet species’ where the presence of another species increases both pollinator visitation and seed production (Molina-Montenegro et al., 2008).

In our system visitation by *Bombus* sp. was more tightly and positively correlated with seed production, when compared to that by other pollinator guilds – even though this was the pollinator guild that was shared between the two species. This pattern has also been detected in another system, where a native co-flowering species increased visitation rates and seed production of another native co-flowering species (Yang et al., 2013). In this system the authors hypothesized that the lack of interspecific pollen transfer was due to differences in flower morphology between the two species and high pollinator flower constancy (Yang et al., 2013). Both differences in floral morphology and pollinator flower constancy could be factors that in our system maintained the effectiveness of the shared pollinator group, *Bombus* sp. (Fig. S3.1).

Effects of Linaria vulgaris on pollinator-mediated selection

Interestingly the removal of *Linaria* did not influence pollinator-mediated selection on the platform length of *Penstemon strictus*. This effect could be in part due to a lack of change in the pollinator community composition with *Linaria* treatment (MANOVA: $F_{1,21} = 0.57$, $P = 0.64$). For example, if pollinator community composition changed and pollinators were tracking different floral traits or floral trait values, then a change in pollinator community composition should have led to a change in pollinator-mediated selection in the absence or presence of *Linaria*. Alternatively, pollinators could change their floral preferences due to changes in floral neighborhood and thus influence pollinator-mediated selection on the focal species (Caruso, 2000; Beans and Roach, 2015). Our data suggest that the pollinators retain their trait preferences independently of the presence or absence of the invader. Previously it has been documented that an invasive plant (*Impatiens glandulifera*) can influence the pollinator-mediated selection experienced by the native plant *Impatiens capensis* (Beans and Roach, 2015). The two species of *Impatiens* had more similar flower morphology compared to the species in our study. Flower shape similarity could increase interspecific pollen transfer which could further reinforce floral trait divergence. In this system, *Impatiens capensis* experienced selection for shorter corollas in the presence of the non-native *Impatiens*. More research could address whether the presence of pollinator sharing, and shared pollinator-attractant trait values are necessary characteristics for pollinator-mediated selection to be altered. Additionally, it would be important to determine when pollinator-mediated selection is expected to change due to neighboring species. This question is ever more relevant as climatic change alters the species composition of flowering communities (CaraDonna et al., 2014).

Yearly variation in pollinator visitation and seed production

Our results show that pollinator visitation could change considerably across years.

Although pollinator abundances can vary significantly from year to year (Horvitz and Schemske, 1990), over the timeframe of our experiments the years with low bumblebee pollinator abundance were not correlated with the years of low pollinator visitation to *Penstemon strictus* (Ogilvie et al., 2017). Since 2016 was a year without *Linaria* overlap it begs the question of whether *Linaria* competes for pollinators at a scale larger than our *Linaria* removal. In addition, we detected an increase by bumblebees other than *B. bifarius* in the year when *Linaria* was not co-flowering with *Penstemon*. These pollinators did not exhibit a relationship between visitation and seed production, and thus the temporal absence of *Linaria* could potentially influence seed production of *Penstemon strictus*.

The relationship between pollinator visitation and seed production varied across years, suggesting that visitation data alone is not a reliable measure of impacts on seed production. Other studies have documented variation in pollinator effectiveness within and across flowering seasons (Fishbein and Venable, 1996; Ivey et al., 2003). For example, individuals of *Asclepias incarnata* can exhibit yearly variation in seed production that is in part driven by variation in pollinator effectiveness across years (Ivey et al., 2003). This temporal variation in pollinator effectiveness could attenuate or accentuate the impacts of the plant invader through time.

The relationship between visitation by *Bombus bifarius* and seed production per flower changed considerably between 2016 and 2017. Changes in water availability throughout the flowering season between the two years could not explain this change since for both years we were using potted plants and watering regimes were the same. Since all

potted plants were overwintering in the ground it is possible that any differences in snowmelt timing between the two years could have influenced soil moisture (Blankinship et al., 2014) and altered resource allocation in these plants. One possibility is that these pollinators were foraging for different floral resources during the two years (foraging for nectar or pollen), which could have led to differences in their effectiveness (Wilson et al., 2004). Another possibility is that pollen quantity and quality was different across the two years, which could have generated differences in the contribution that pollinator visitation made to seed production. This mechanism has been documented in *Ipomopsis aggregata* to explain differences seen across years in the relationship between pollinator visitation and seed production (Waser and Price, 2016). It has also been documented in *Phacelia parryi*, where variation in water availability to conspecific pollen donors influenced seed production independently of the water conditions experienced by the pollen recipient plants (Recart et al., 2019). An important avenue of research is to study how temporal variation in environmental conditions influences pollinator effectiveness.

Yearly variation in pollinator-mediated selection

Pollinator preferences varied greatly through time, with stronger preferences for long platforms in 2016. These pollinator preferences did not translate to net selection, as seen by a lack of a relationship between platform length and seed production in both 2016 (Fig 3.1 – solid line) and 2017 (Fig 3.1 – dashed line). This lack of net selection in the year of strong pollinator preferences (2016) was most likely due to a lack of a positive relationship between pollinator visitation rate and seed production. Even when pollinator preferences are detected, there still needs to be a correlation between visitation rate and seed

production to generate net selection, and that latter relationship varied across years in out system.

CONCLUSION

The nearby presence of the non-native invasive *Linaria vulgaris* facilitated the pollination of *Penstemon strictus* and did not influence the relationship between visits and seed production, or pollinator-mediated selection based on visitation. In one year, we detected a strong relationship between visitation and seed production but no pollinator preferences. In another, we detected pollinator preferences on platform length but no relationship between visitation and seed production. Thus, in both years we were not able to detect net selection. This study emphasizes the importance of temporal variation in shaping the impacts that invasive plants have on plant-pollinator interactions.

FIGURES

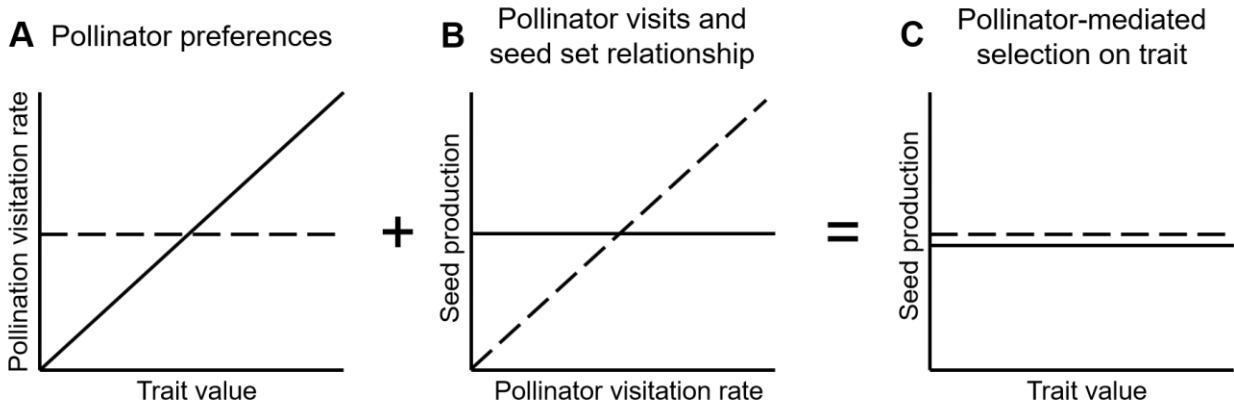


Figure 3.1. Contributions of (A) pollinator trait preferences and (B) the relationship between pollinator visits and seed production (C) to net selection through female function. Different line types (solid and dashed) represent different years or invasive species treatments.

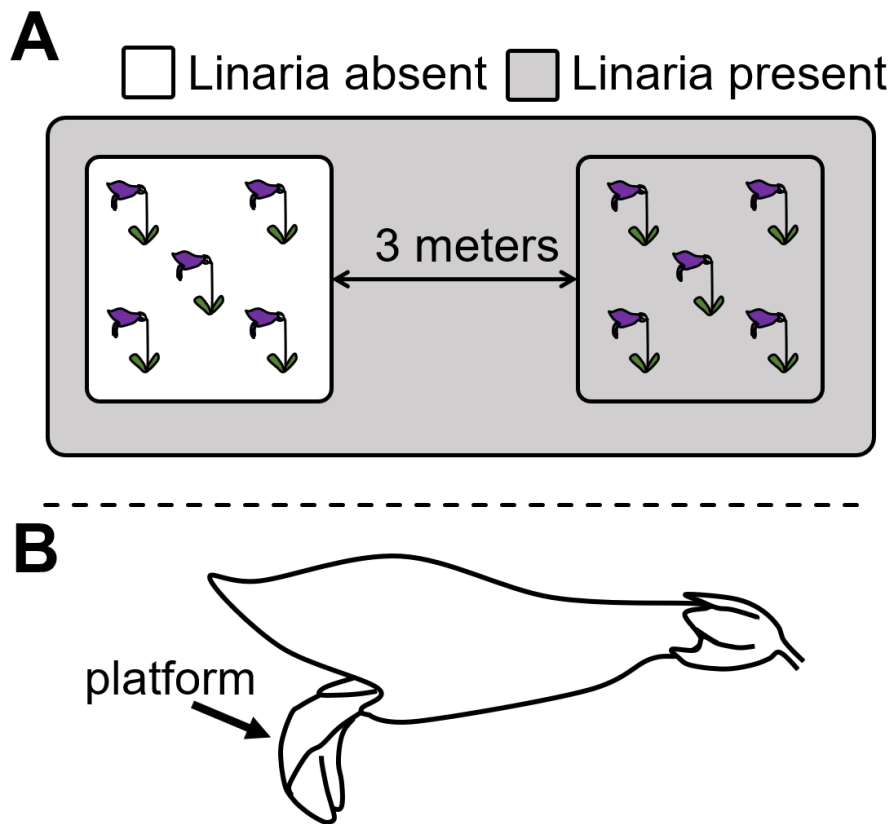


Figure 3.2. (A) Diagram of the experimental design applied and replicated during the three years of the experiment. Gray space represents areas where *Linaria vulgaris* is present. The bigger outer square represents the block and the inner two squares represent the plots for the *Linaria* absent treatment (white square) and the *Linaria* present treatment (gray square). The plots are three meters by three meters and are three meters apart. Inside the plots there are five *Penstemon* individuals – represented by each green rosette with a purple flower. (B) Flower of *Penstemon strictus* showing the landing platform – the floral trait of interest in this experiment.

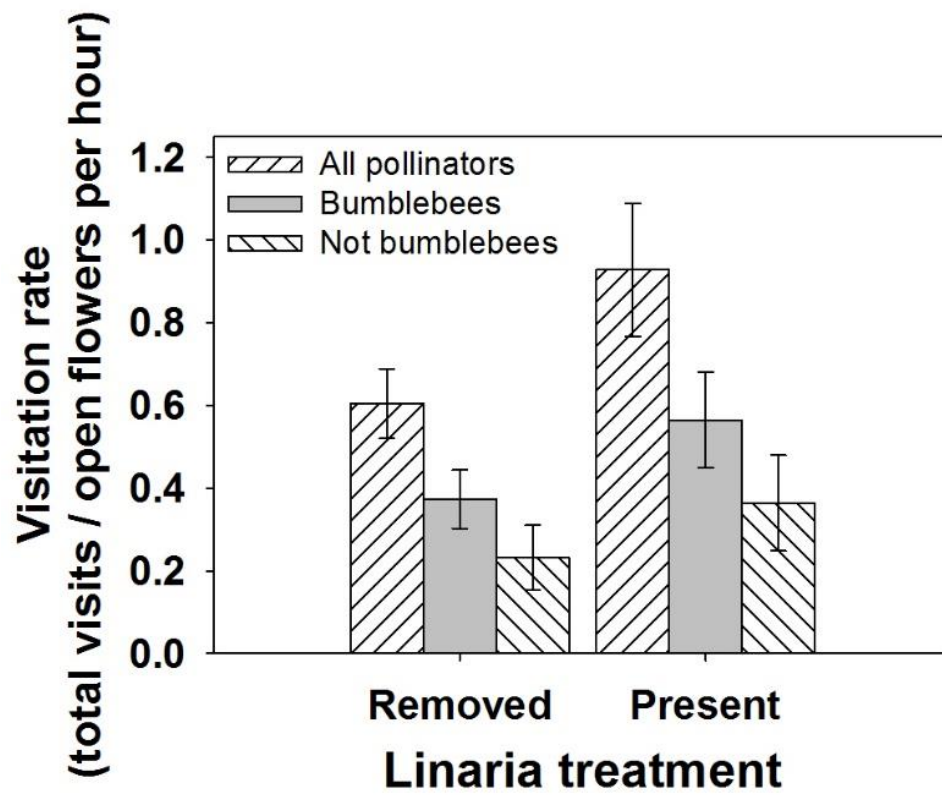


Figure. 3.3. Pollinator visitation rates to *Penstemon strictus* in the absence or presence of *Linaria vulgaris* sorted by different pollinator groups. Means and standard errors are shown, based on the means of visitation rate grouped by *Linaria* treatment and pollinator group.

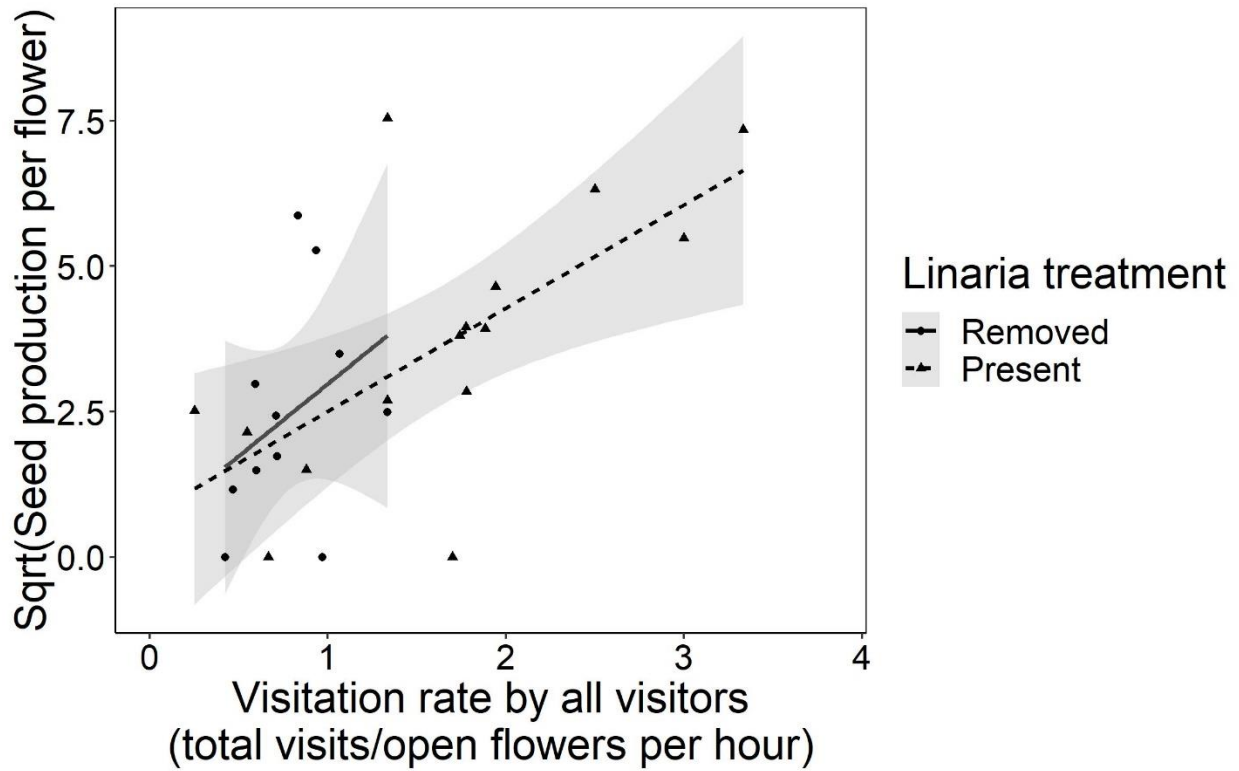


Figure 3.4. Relationship between pollinator visitation rate and seed production per flower in *Penstemon strictus* in either the presence or absence of *Linaria vulgaris*. Gray shading around each regression line represent 95% confidence intervals.

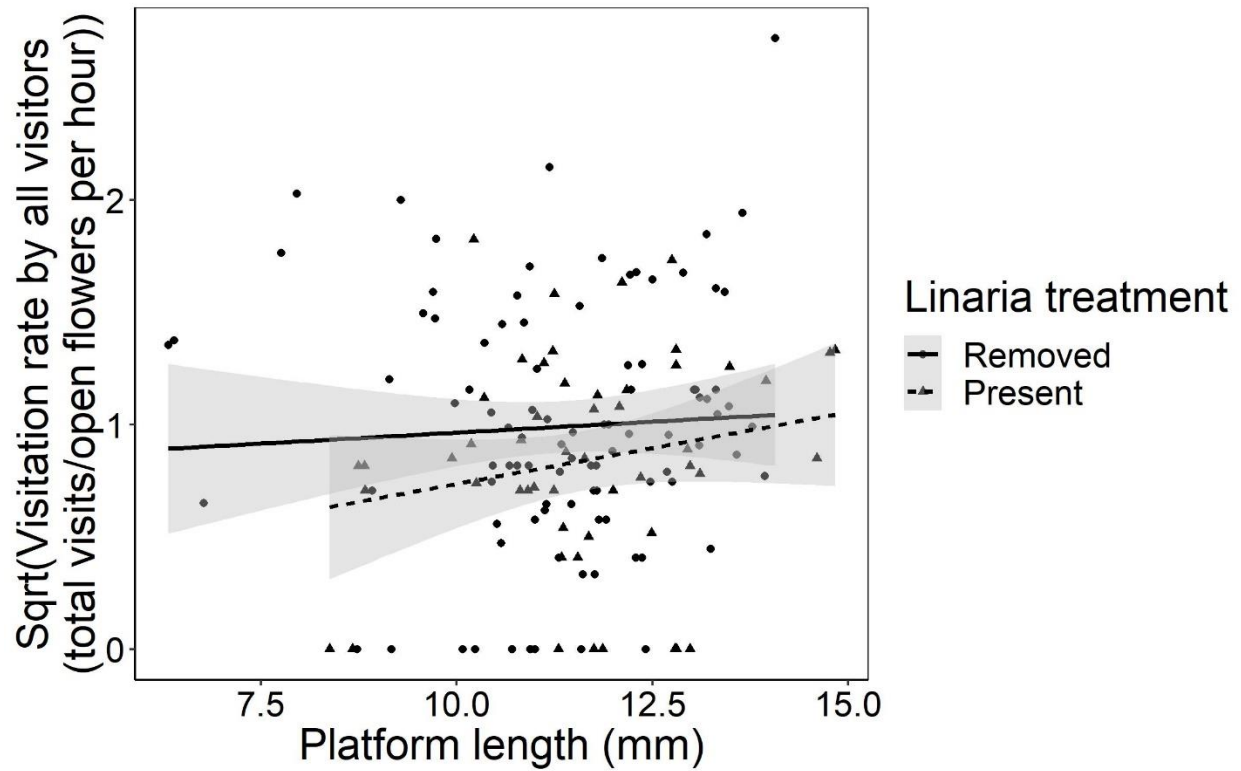


Figure 3.5. *Penstemon strictus* platform length in relationship to visitation rate by all pollinators in the presence and absence of *Linaria vulgaris*. Gray shading around each regression line represent 95% confidence intervals.

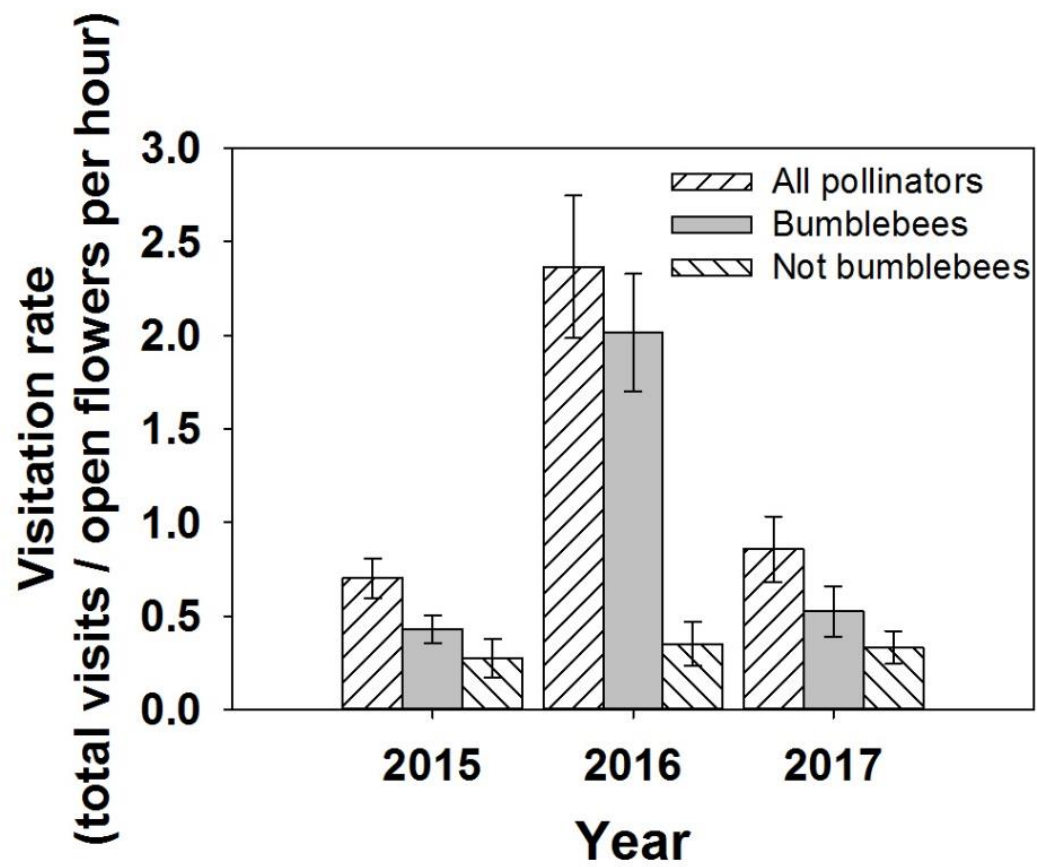


Figure 3.6. *Penstemon strictus* pollinator visitation rate from 2015 to 2017. Means and standard errors are shown, based on the means of visitation rate grouped by year and pollinator group.

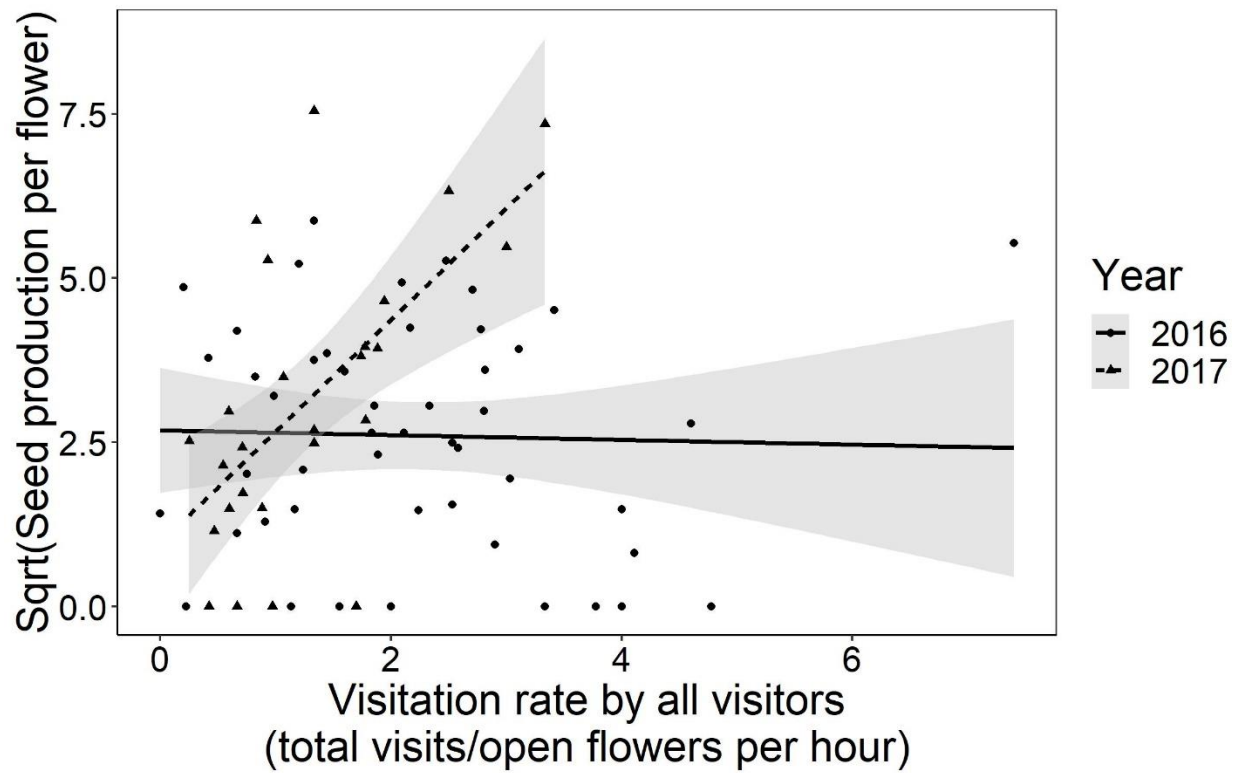


Figure 3.7. Relationship between visitation rate by all visitors to *Penstemon strictus* and seed production per flower in 2016 and 2017. Gray shading around each regression line represent 95% confidence intervals.

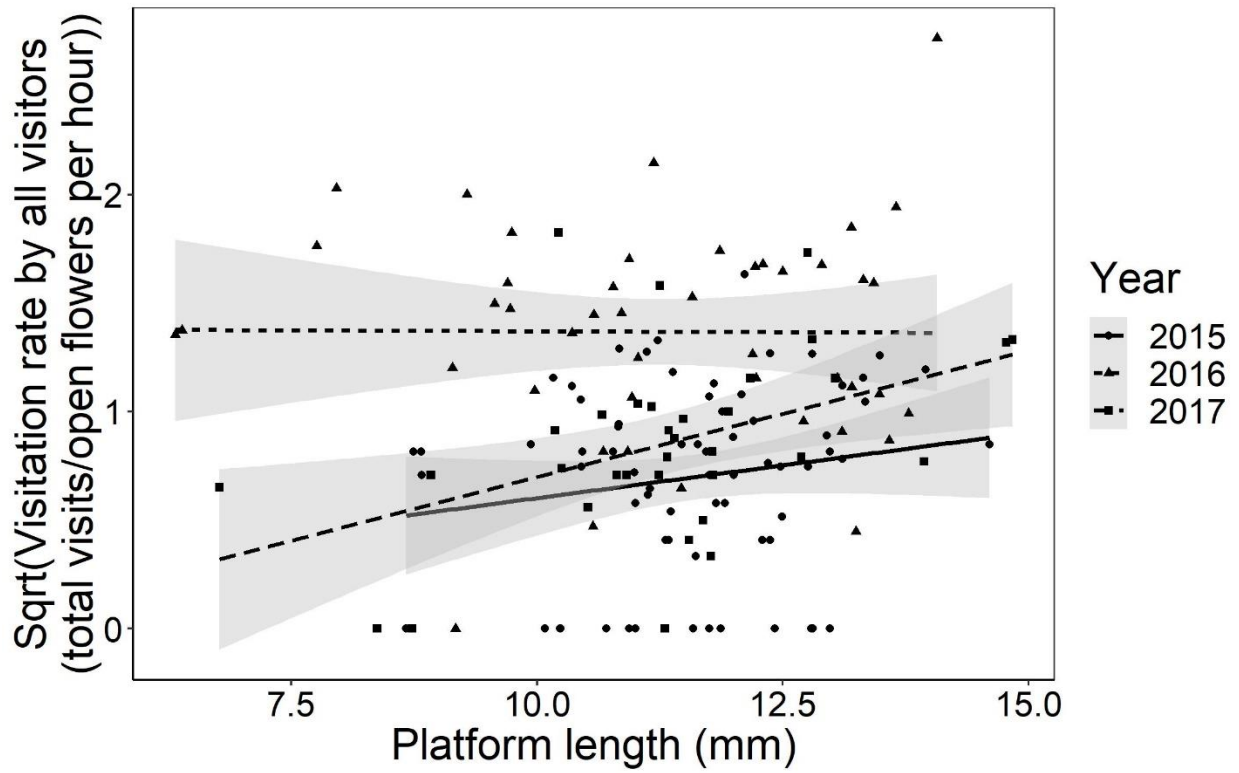


Figure 3.8. Relationship between *Penstemon strictus* platform length and visitation rate by *Bombus* sp. from 2015 to 2017. Gray shading around each regression line represent 95% confidence intervals.

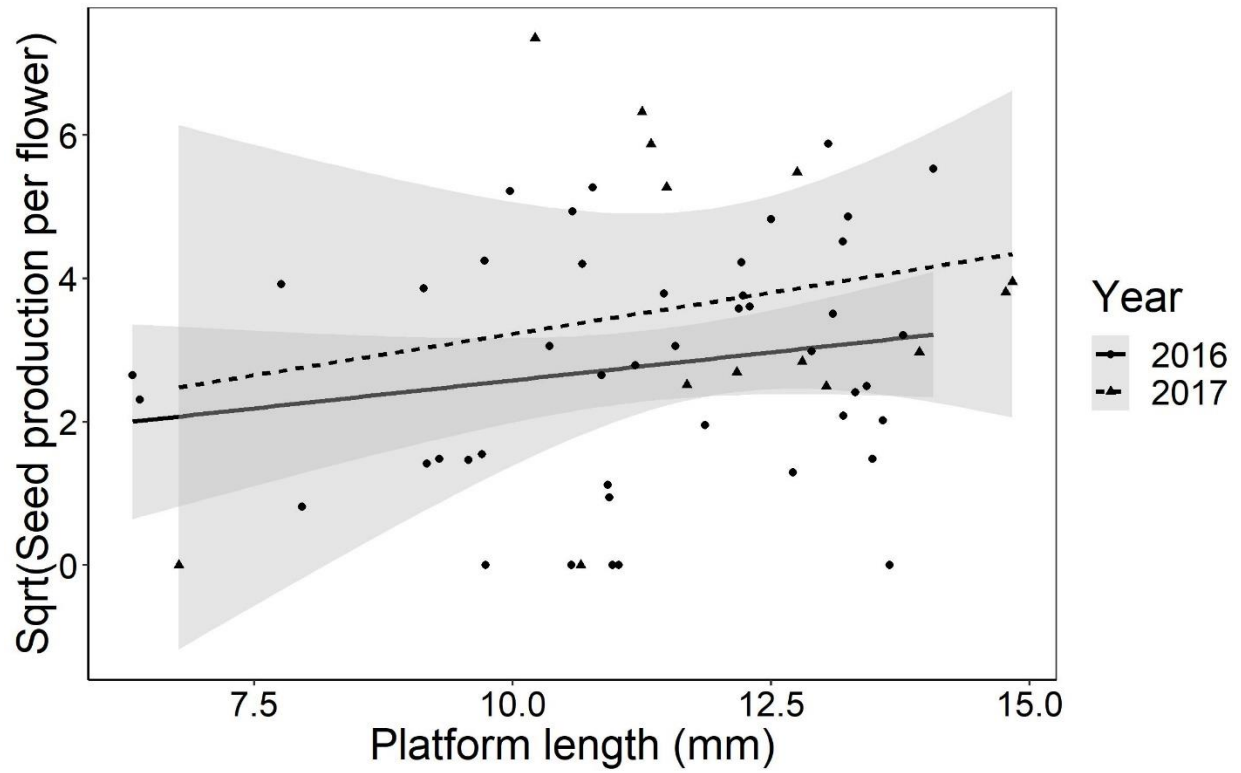


Figure 3.9. Relationship between *Penstemon strictus* platform length and seed production per flower in 2016 and 2017. Gray shading around each regression line represent 95% confidence intervals.

SUPPLEMENTAL MATERIALS

Table S3.1. Data collected during the three years of the experiment.

Variables of interest	2015	2016	2017
Linaria treatment	Yes	No	Yes
Platform length data	Yes	Yes	Yes
Visitation data	Yes	Yes	Yes
Seed production data	No	Yes	Yes
Pollinator effectiveness (details in Methods S3.1)	No	No	Yes

Table S3.2. Models generated to evaluate the ecological and evolutionary effects of the presence of *Linaria vulgaris*. Visitation rate was evaluated in terms of all pollinators, *Bombus* sp., not *Bombus* sp., *Bombus bifarius*, and *Bombus* sp. not *B. bifarius*.

Response variable	Year included in analysis	Dependent variables
Visitation by plot ID	2015 and 2017	Linaria treatment Year Linaria treatment x Year Block identity nested within Year
Seed production by plot ID	2017	Linaria treatment Block identity
Seed production by plant ID	2017	Visitation Linaria treatment Visitation x Linaria treatment
Visitation by plant ID	2015 and 2017	Platform length Linaria treatment Year Platform length x Linaria treatment Platform length x Linaria treatment x Year Block identity nested within Year

Table S3.3. Models generated to evaluate the ecological and evolutionary effects of year-to-year variation in plant-pollinator interactions. Visitation rate was evaluated in terms of all pollinators, *Bombus* sp., not *Bombus* sp., *Bombus bifarius*, and *Bombus* sp. not *B. bifarius*.

Response variable	Year included in analysis	Dependent variable
Visitation by plot ID	2015, 2016 and 2017	Year Block identity nested within Year
Seed production by plant ID	2016 and 2017	Visitation Year Visitation x Year Block identity nested within Year
Visitation by plant ID	2015, 2016 and 2017	Platform length Year Platform length x Year Block identity nested within Year
Seed production by plant ID	2016 and 2017	Platform length Year Platform length x Year

Methods S3.1. Methods for calculating pollinator effectiveness to *Penstemon strictus*.

In 2017 thirty-six *Penstemon strictus* flowering individuals (same site where main experiment was conducted) were bagged to conduct pollinator effectiveness observations. We observed flowers and after a flower was visited once by a flower visitor we documented the visitor group identity (*Bombus bifarius*, bumblebees not *B. bifarius* and non-*Bombus* sp. visitors) and, tagged and bagged (using an organza bag) the flower to prevent further visitation. Control flowers were used to determine how seed production made by a given visitor group compared to unvisited flowers. Seed production was calculated in all tagged flowers. Samples sizes for each visitor group are as follows: *Bombus bifarius* (n = 21), bumblebees not *B. bifarius* (n = 29), non-*Bombus* sp. visitors (n = 97), control flowers (n = 38). Residuals were not normally distributed in analysis of variance and we used non-parametric statistics to detect differences among visitor groups. We use the Dunns' test for stochastic dominance (dunn.test function in the dunn.test R package) with a Bonferroni correction for multiple group comparisons to identify differences in seed production per flower between visitor groups.

Table S3.4. Sample size of single visits observed on *Penstemon strictus* by *Bombus* species to calculate effectiveness measurements.

<i>Bombus</i> species names	Sample size
<i>Bombus appositus</i>	19
<i>Bombus bifarius</i>	21
<i>Bombus californicus</i>	6
<i>Bombus flavifrons</i>	4

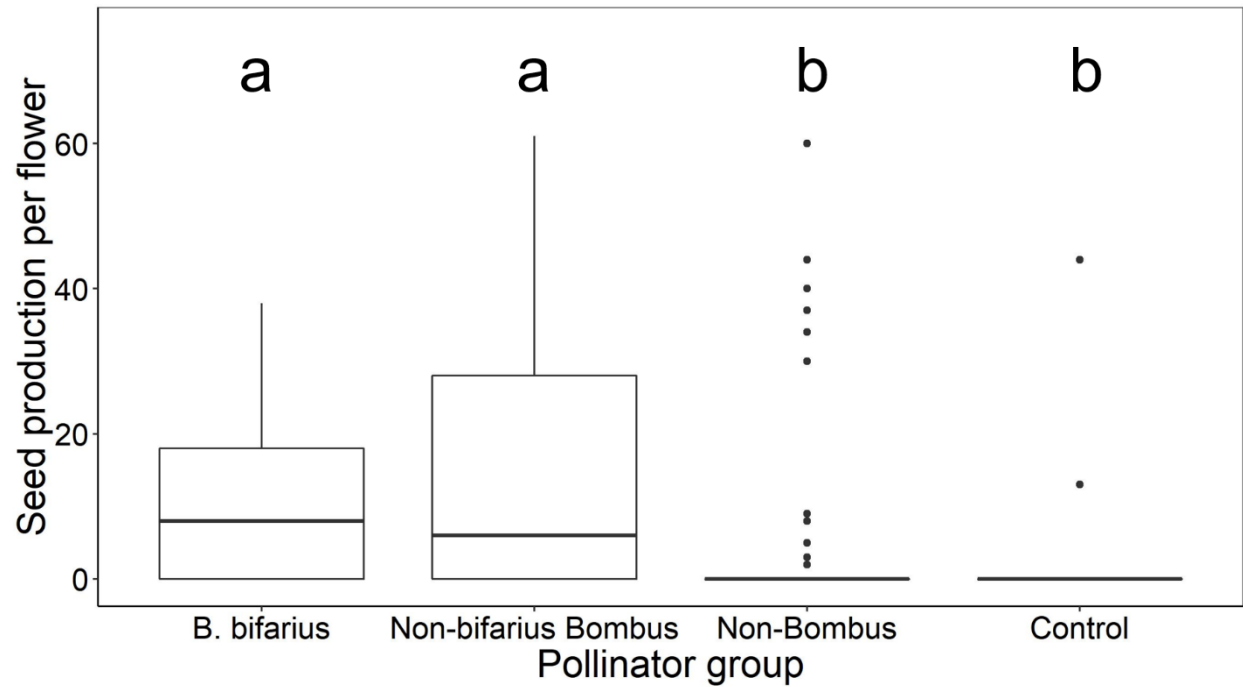


Figure S3.1. Effectiveness of a single visit by different pollinator groups to the seed production per flower of *Penstemon strictus*. Seed production of the control group was obtained from flowers not exposed to pollinators. Boxplots represent the median, the lower and upper quartile, and dots represent outlier points. Lower case letters represent significant differences ($P \leq 0.001$) detected among pollinator groups using the non-parametric Dunn's test.

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