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Drought, pollen and nectar availability, and pollination success

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Abstract. Pollination success of animal-pollinated flowers depends on rate of pollinator visits and on pollen deposition per visit, both of which should vary with the pollen and nectar "neighborhoods" of a plant, i.e., with pollen and nectar availability in nearby plants. One determinant of these neighborhoods is per-flower production of pollen and nectar, which is likely to respond to environmental influences. In this study, we explored environmental effects on pollen and nectar production and on pollination success in order to follow up a surprising result from a previous study: flowers of *Ipomopsis aggregata* received less pollen in years of high visitation by their hummingbird pollinators. A new analysis of the earlier data indicated that high bird visitation corresponded to drought years. We hypothesized that drought might contribute to the enigmatic prior result if it decreases both nectar and pollen production: in dry years, low nectar availability could cause hummingbirds to visit flowers at a higher rate, and low pollen availability could cause them to deposit less pollen per visit. A greenhouse experiment demonstrated that drought does reduce both pollen and nectar production by *I. aggregata* flowers. This result was corroborated across 6 yr of variable precipitation and soil moisture in four unmanipulated field populations. In addition, experimental removal of pollen from flowers reduced the pollen received by nearby flowers. We conclude that there is much to learn about how abiotic and biotic environmental drivers jointly affect pollen and nectar production and availability, and how this contributes to pollen and nectar neighborhoods and thus influences pollination success.

Key words: drought; experiment; hummingbird visitation; nectar neighborhood; nectar production; pollen limitation; pollen neighborhood; pollen production; pollen receipt; pollination success.

Introduction

J. G. Kölreuter (1761) first demonstrated experimentally that the production of fruits and seeds depends on how much pollen insects deliver to flowers. Since his time, it has become clear that various ecological factors influence pollen receipt (reviewed in Knight et al. 2005) and that receipt depends jointly on the rate at which pollinators visit flowers and on the amount of pollen deposited per visit, the two multiplicative components of "pollinator effectiveness" (Ne'eman et al. 2010).

Per-visit deposition often is treated as a fixed attribute of a pollinator species, so that pollination success is equated implicitly or explicitly with limitation in numbers or activity of pollinator taxa (e.g., Bierzychudek 1981, Parker 1997, Knight et al. 2005). There is increasing appreciation, however, that both the rate at which a given pollinator species visits a flower (hereafter "visit rate") and the per-visit receipt of pollen by the flower will be sensitive to the availability of pollen and nectar in and around the flower—to its *pollen neighborhood* and *nectar neighborhood*. The conspecific pollen neighborhood should directly influence conspecific pollen receipt via the amount of pollen that pollinators pick up and carry as they move from flower to flower (Harder 1990, Campbell et al. 1996). Similarly, the conspecific and heterospecific nectar neighborhood should influence pollinator attraction to an area and hence visit rate (Pleasants 1981, Dreisig 1995). Per-flower nectar volume also may be positively related to per-flower visit duration, which in turn can affect conspecific pollen removal and deposition (e.g., Thomson 1986).

Pollen and nectar neighborhoods will vary through space and time. Conspecific flower density fluctuates across years and sites, and the seasonal phenology of flowering affects not only flower density but also effective ratios of conspecific plant genders or mating types (the "mating environment" of Brunet and Charlesworth [1995] or "pollination context" of García-Camacho et al. [2009]). These variables, along with species composition of the surrounding plant community, are obvious determinants of pollen and nectar neighborhoods, but they are not the only determinants. Pollen and nectar neighborhoods also depend on the amounts of pollen and nectar produced by individual flowers.

Per-flower pollen and nectar production, in turn, are likely to respond to abiotic and biotic environmental context. For example, pollen production is sensitive to temperature, water availability, nutrients, and herbivory of leaves (Vasek et al. 1987, Stephenson et al. 1992, Turner 1993, Lau and Stephenson 1994, Quesada et al.

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1995). Some herbivores directly reduce pollen availability by collecting or consuming it without moving it to recipient stigmas (Hargreaves et al. 2009). Nectar production is affected by similar abiotic and biotic factors (Kenoyer 1917, Huber 1956, Shuel 1967, Pleasants and Chaplin 1983, Devlin 1988, Petanidou et al. 1999).

Ecologists have mostly overlooked variation in per-flower availability of pollen and nectar as a feature of the floral neighborhood that contributes to pollination success. We include ourselves here: we did not consider abiotic influences on pollen and nectar production and availability until we were confronted by a surprising result. In a previous study (Price et al. 2005) we found that variation in stigma pollen loads across years in the montane wildflower *Ipomopsis aggregata* was negatively, rather than positively, correlated with the rate of visitation by hummingbirds, the primary pollinators of this species at our sites.

Here we report on further studies of *I. aggregata* that were partially motivated by that earlier result. We explore three hypotheses: (1) that water availability, a major environmental driver in many ecosystems, is correlated with hummingbird visit rate; (2) that both pollen and nectar production respond to water availability in the form of soil moisture; and (3) that a plant's pollen neighborhood affects pollen receipt. Reanalysis of data from the prior study suggests that years of high hummingbird visits were drought years. In a greenhouse experiment, per-flower pollen and nectar production both decreased with decreasing soil moisture, a relationship confirmed by variation in four unmanipulated populations sampled across six summers. Finally, brushing dehiscing anthers to reduce pollen availability in natural populations resulted in lower stigmatic pollen loads, demonstrating that variation in the pollen neighborhood does influence pollen receipt. We discuss how coupled variation in pollen and nectar production within flowers might contribute to the negative cross-year correlation between hummingbird visit rate and pollen receipt in *I. aggregata* and, more generally, how attention to pollen and nectar neighborhoods can shed light on temporal and spatial variation in pollination success.

Methods

Study system

Scarlet gilia (*Ipomopsis aggregata* [Pursh] V. Grant [Polemoniaceae]) is a common wildflower of open meadows in mountains of the western United States. It has been studied extensively at the Rocky Mountain Biological Laboratory (RMBL) at 2900 m elevation in west-central Colorado. Plants are semelparous, growing as vegetative rosettes from seed for several years before flowering during a single summer season and then dying (Price et al. 2008). Reproductive individuals vary greatly in size; one sample of >1000 plants yielded a range from 3 to 703 flowers per plant, with a mean of 84 (Campbell 1989). Flowering around the RMBL lasts for about one month beginning in late June (Waser 1978). Plants are distributed in a patchy manner with higher densities in dry soils where there is little vegetative cover to shade rosettes; average density within patches is about five flowering plants (and many vegetative rosettes) per 3 × 3 m area (Price et al. 2008). The flowers are protandrous, typically opening and beginning to dehisce pollen in mid-afternoon. After about 2 d in male phase, the styles have completely elongated and the stigma lobes open. Female phase lasts an additional 1–3 d before corollas fall off (Campbell et al. 1994). The main pollinators near the RMBL are Broad-tailed (*Selasphorus platycercus* Swainson) and Rufous Hummingbirds (*S. rufus* Gmelin), but bumble bees, solitary bees, and lepidopterans also visit and carry pollen (Mayfield et al. 2001, Price et al. 2005). Reproductive plants face various antagonists, including nectar-robbing bumble bees, pollen-eating flies and other insects, pre-dispersal seed predators, and herbivores (Brody 1997, Irwin and Brody 1998, Price et al. 2005).

Hummingbird visitation, precipitation, and soil moisture (1996–2002; 2009–2014)

To explore drought as a possible driver of variation in hummingbird visit rates, we undertook new analyses of the data of Price et al. (2005) on average visit rates in three natural populations around the RMBL from 1996 through 2002, using long-term precipitation records maintained by the RMBL (W. Barr, *unpublished data*). Multiple regression allowed us to characterize relationships between visit rate and season-specific precipitation from 1996 to 2002 and between soil moisture and precipitation from 2009 to 2014. In these and all other analyses, we used JMP version 5 (SAS Institute, Cary, North Carolina, USA) and transformed variables as needed to normalize model residuals.

Greenhouse manipulation of soil moisture (2009)

To explore whether water availability influences pollen and nectar production, we chose four representative natural populations near the RMBL in July 2008 that were used throughout this entire study (Table 1). In June 2009, we returned to transplant three pairs of bolting plants in native soil from each population (24 plants total) into 12.5 cm diameter fiber flower pots. Plants were paired as closely as possible by inflorescence height, number of flower buds, and anticipated date of flowering onset. Pots were placed in a greenhouse at the RMBL and their positions were rotated regularly. We chose one plant of each pair at random to receive 50 mL of water (~4.5% of pot volume) every 2–3 d; the other received 100 mL. These treatments were an attempt to bracket preliminary measurements of soil moisture in the field. We periodically took between two and four replicate measurements of moisture in each pot with a Campbell Scientific HydroSense probe (Campbell Scientific, Inc., Logan, Utah 84321, USA)

Table 1. The four natural populations of *Ipomopsis aggregata* near the Rocky Mountain Biological Laboratory (RMBL) used throughout this study.

Population name and abbreviation	Location	Description
Maxfield Meadow (MM)	38°57′00.5″ N, 106°59′23.7″ W	gently east-facing meadow
Ore House (OH)	38°57′35.7″ N, 106°59′27.6″ W	gently south-facing meadow
Vera Falls (VF)	38°57′41.9″ N, 106°59′35.1″ W	gently west-facing meadow
Beyond Billy's (BB)	38°57′50.5″ N, 106°59′33.7″ W	non-sloping meadow

inserted 3–5 cm into the soil (always before applying water) to characterize achieved soil moistures.

After plants started to flower, we identified elongated flower buds each morning that would open that afternoon. To measure pollen production, we collected some of these buds (between 1 and 7 per plant, mean = 3.3), transferred all the anthers from a given bud into a single microcentrifuge tube, allowed the anthers to dehisce, and added 0.5 mL of 70% ethanol that had been lightly stained with basic fuchsin. Using a standard Neubauer-style hemacytometer, we counted four replicate samples drawn from each microcentrifuge tube with a Pasteur pipette after the contents had been mixed in standard fashion. Counts were made at 250× with a compound microscope. Each sample comprised a volume of 0.9 mm^3; the mean count across replicate samples was scaled up to a volume of 0.5 mL to estimate total pollen produced by the flower. To measure nectar production, we placed a length of plastic drinking straw over other elongated buds (between 1 and 9 per plant, mean = 4.3) to exclude any insects that might have entered the greenhouse. After 24 h we removed straws and measured the volume and concentration of accumulated nectar in the open flowers. This method yields values that are highly correlated with those obtained by the more delicate process of first draining flowers of nectar and then bagging them (Campbell et al. 1991).

Our original intent was to generate two distinct soil moisture treatments, but flower pots dried down quickly, so that within-pot variation across days comprised 71% of total variance in moisture readings compared to 27% due to treatment and 2% due to pot (thus, plant) nested within treatment. We therefore included the average of moisture readings on the day a bud was collected or enclosed in a straw as a covariate in general linear models of pollen and nectar production. Because we were not interested in estimating the repeatable plant-to-plant variation in production exhibited by *I. aggregata* (Campbell et al. 1991, Mitchell 1993), we removed plant as a fixed effect and analyzed the relationship between day-to-day variation in soil moisture and deviations in pollen or nectar production from each plant's mean value.

Field measurements of soil moisture, pollen production, and nectar production (2009–2014)

To confirm that greenhouse patterns were repeated in nature, we marked 10 flowering plants in each of the four field populations (Table 1) in each summer from 2009 through 2014. We took two replicate measurements of soil moisture within 20 cm of each plant 10 and 20 d after the first flower appeared in each population, i.e., about one-third and two-thirds of the way through the flowering season of *I. aggregata*. One day after soil measurements we collected anthers from up to two elongated buds from each marked plant for pollen counts, and placed lengths of drinking straw on up to two additional buds for 24-h nectar production measurements. If a marked plant was browsed by deer after the first collection date or lacked enough elongated buds on a collection date, we took one or more elongated buds as needed from an adjacent (within 1 m) unbrowsed plant, if available. Methods for pollen counts and nectar measurements were as described in *Greenhouse manipulation of soil moisture*. We calculated means of individual plant means for soil moisture and pollen and nectar production for each population, collection date, and year. Quadratic regression allowed us to evaluate population-level relationships between pollen and nectar production and soil moisture.

Field manipulation of the pollen neighborhood (2008)

To determine whether the local pollen neighborhood affects pollen receipt we manipulated pollen availability independent of soil moisture. In June 2008, we marked between 16 and 25 individuals in each of two areas (hereafter "patches") within each of the four field populations (Table 1). The two patches in each population were <10 m in diameter and were >5 m apart at their closest points. We chose these values because *Ipomopsis* pollen rarely travels >5 m (Campbell and Waser 1989). One patch in each population was assigned at random to receive a pollen removal treatment; the other served as control.

Approximately 1 week after first flowers opened in each population, we returned in the early afternoon, when newly opened flowers dehisce pollen. In pollen-removal patches, we inserted a round wooden toothpick into the corolla of each newly opened flower to remove some of the pollen. In control patches, we touched plants and flowers similarly but did not insert toothpicks into the corollas. These treatments were applied once per day for 3 d. One day later we collected up to two stigmas from senescent flowers of each of the marked plants in each population. One day after that we reversed the

treatment each patch received and repeated treatments as just described. This design, in which treatments were blocked within populations and each patch (and plant) received treatments in one of two orders (removal first, control second, or vice versa), allowed us to control as much as possible for temporal and spatial variation in pollen production and pollinator activity. By collecting after 4 d within each of these two consecutive treatment periods, we also ensured that stigmas had been exposed to the local pollen-neighborhood treatment, since flowers spend at most 3 d in female phase. Stigmas were mounted on microscope slides in basic fuchsin gel (Kearns and Inouye 1993) and pollen loads counted at 100×. Replicate stigmas were averaged to obtain a single value for each plant in each treatment period. In all, we obtained samples from 13 to 32 plants for each patch and treatment period, for a total of 317 plants sampled. We also marked calyces of flowers whose stigmas we collected, to measure seed sets. On 109 plants, we relocated at least one fruit that had retained its mark and was undamaged by predispersal seed predators, for a total of 161 fruits. Only six plants in two populations had undamaged fruits from both treatment periods.

To analyze this experiment, we first explored patterns in the data by fitting a full factorial model with population, treatment period (first vs. second), and treatment as explanatory variables and per-plant mean pollen loads for each population, period, and treatment combination as the dependent variable. Pollen loads were square-root transformed to normalize model residuals. We then simplified the model by removing population, whose main and interaction effects were not significant, and reran the analysis. This approach is a split-split plot design, where population is the blocking factor, treatment period and treatment are fixed treatments applied to each population, and plants are nested within population-period-treatment combinations.

Results

Hummingbird visitation, precipitation, and soil moisture (1996–2002; 2009–2014)

New analysis of our earlier data (Price et al. 2005) showed that hummingbird visitation in the years 1996–2002 was a decreasing function of precipitation at the RMBL (multiple regression of square-root-transformed visitation rate on mm of water input during the previous winter and current summer: $F_{2,18} = 6.15$, $P = 0.01$, $r^2_{adj} = 0.34$). Precipitation effects were entirely due to snowmelt input ($F_{1,18} = 11.26$; $P = 0.003$; Fig. 1); summer rainfall through the blooming period of *I. aggregata* (late June-early August) had no detectable effect ($F_{1,18} = 0.112$, $P = 0.74$), and eliminating it from the model actually increased r^2_{adj} to 0.37.

Soil moisture values taken in four natural populations 10 and 20 d after the onset of flowering from 2009 through 2014 allowed us to relate precipitation to soil moisture (Fig. 2). As with hummingbird visitation, percent soil moisture was explained entirely by snowmelt water (multiple regression of square-root-transformed percent soil moisture on millimeters of water input during the previous winter and current summer: overall model $F_{2,45} = 118.89$, $P < 0.0001$, $r^2_{adj} = 0.83$; snowmelt input $F_{1,45} = 202.33$, $P < 0.0001$); cumulative summer rainfall to the time when we measured soil moisture in our populations had no detectable effect ($F_{1,45} = 1.329$, $P = 0.225$).

If we assume that the quantitative relationship between winter precipitation and soil moisture observed during 2009–2014 held during earlier periods, the years of particularly high hummingbird visit rate from 1996 through 2002 were drought years (~400–500 mm winter snowmelt water input) with soil moistures of 2–4% by volume (Fig. 1).

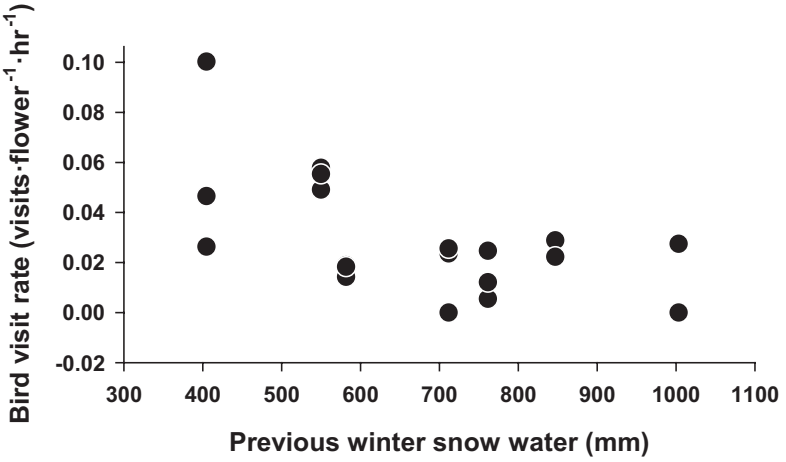

Fig. 1. Rate of hummingbird visitation to *Ipomopsis aggregata* at three sites over 7 yr decreased with the previous winter's snow-water input (visitation data from Price et al. 2005).

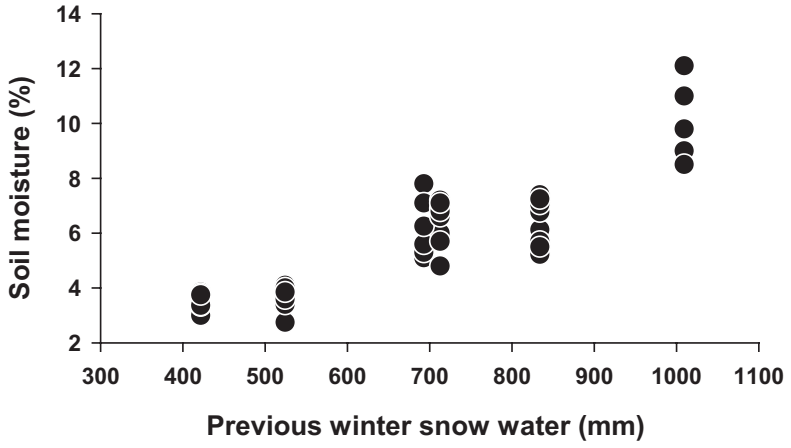

FIG. 2. Relationship between winter precipitation and soil moisture (mean percent by volume) from 2009 to 2014 in four natural populations. Precipitation values come from a single sampling location at the Rocky Mountain Biological Laboratory (RMBL); soil moisture values are averages across samples taken near *I. aggregata* plants in each population at each of two collection times (10 and 20 d after onset of flowering).

Greenhouse manipulation of soil moisture (2009)

Pots that received the 50 mL watering treatment in 2009 had drier soil on average (8.5% water by volume) than those that received 100 mL (12.4%). Both treatments, however, exhibited considerable and equivalent variation (range 4–27%) around these means on days when we collected elongated buds for pollen counts or covered buds with drinking straws for nectar measurements. This range tended toward higher values than the 2.75–12.1% range in soil moisture in the field between 2009 and 2014 (Fig. 2), but the two ranges overlap substantially.

ANCOVA indicates that pollen production, nectar volume, and nectar sugar content varied among plants, as expected, and increased with soil moisture in a decelerating fashion. Both positive first-order and negative second-order terms were significant in all cases, but the latter were far smaller, especially for nectar, indicating a nearly linear response for nectar variables (Table 2).

Ninety-five percent confidence intervals for pollen and nectar production in potted plants overlapped those of plants in the field in 2009 (Table 3), indicating that there was no transplant shock and that greenhouse results are commensurate with those from the field. With 713 mm of winter precipitation and mean soil moisture of 6.4%, 2009 was a "middling" year for the 2009–2014 study period (Figs. 2 and 3).

TABLE 2. ANCOVA tables summarizing effects of soil moisture (volume %) on pollen and nectar production by potted plants in the 2009 greenhouse experiment.

Source	Coefficient	df_{num},df_{den}	*F*	*P*	r^2_{adj}
Pollen production (grains/flower)†					
Model		22,47	3.373	0.0002	0.43
Plant		20,47	2.921	0.0013	
Moisture	1.926	1,47	17.520	<0.0001	
Moisture^2	−0.395	1,47	16.580	0.0002	
Nectar production (μL·flower^{-1}·24 h^{-1})					
Model		22,26	5.430	<0.0001	0.67
Plant		20,26	5.952	<0.0001	
Moisture	0.400	1,26	16.444	0.0004	
Moisture^2	−0.018	1,26	14.223	0.0003	
Nectar production (mg sucrose equilvalents·flower^{-1}·24 h^{-1})					
Model		22,26	3.727	0.0008	0.56
Plant		20,26	4.005	0.0006	
Moisture	0.215	1,26	15.655	0.0005	
Moisture^2	−0.009	1,26	11.525	0.0022	

† Square-root transformed.

TABLE 3. Comparison of pollen and nectar production of potted vs. intact field plants in 2009. Values are based on means of plant means.

Variable and sample	No. plants	Mean	95% CI
Pollen production (grains/flower)			
2009 potted	21	1,0001	8,933–1,1070
2009 field	32	1,0994	9,710–1,2278
Nectar production ($\mu L \cdot flower^{-1} \cdot 24\ h^{-1}$)			
2009 potted	21	3.19	2.51–3.87
2009 field†	13	3.96	3.21–4.71
Nectar production (mg sucrose equilvalents·$flower^{-1} \cdot 24\ h^{-1}$)			
2009 potted	21	0.86	0.70–1.01
2009 field†	13	0.91	0.73–1.09

†In 2009, nectar measurements were only taken from one population (OH).

Field measurements of soil moisture, pollen production, and nectar production (2009–2014)

Mean soil moisture varied between 2.7% and 12.1% by volume in the four natural populations across the 2009–2014 study period (Figs 2 and 3). Year contributed the most (81.1%) to variance in moisture, followed by collection date (10 vs. 20 d after onset of flowering) nested within population and year (5.4%; soil moisture was lower 20 d after flowering onset) and population nested within year (2.2%); residual variance constituted the rest (11.4%).

Pollen production per flower increased in a decelerating fashion with soil moisture across populations, years, and collection times (Fig. 3A). The relationship may be asymptotic or may be truly hump-shaped, with an intermediate peak, although sample size at high moisture values is limited. The overall second-order model was significant ($F_{2,45} = 12.57$, $P < 0.0001$, $r^2_{adj} = 0.33$), as were positive first-order ($F_{1,45} = 21.63$, $P < 0.0001$) and negative second-order terms ($F_{1,45} = 17.30$, $P = 0.0001$). Nectar volume and sucrose equivalents produced per flower per 24 h increased linearly with soil moisture across populations, years, and collection times (Fig. 3B,C; overall model fits for both nectar variables were significant, $F_{2,38} > 53.0$, $P < 0.0001$, $r^2_{adj} > 0.55$; first-order coefficients were significantly positive for both variables, $F_{1,38} > 5.95$, $P < 0.0001$; second-order coefficients were insignificantly positive for nectar volume and insignificantly negative for mg sucrose, $F_{1,38} < 1.33$, $P > 0.26$).

Field manipulation of the pollen neighborhood (2008)

A simplified model of pollen receipt containing only treatment period, treatment, and their interaction was significant ($F_{3,313} = 9.13$, $P < 0.0001$, $r^2_{adj} = 0.072$; Fig. 4). Stigma pollen loads were lower in the second treatment period (period effect: $F_{1,313} = 14.28$, $P = 0.0002$), and after pollen removal (treatment effect: $F_{1,313} = 8.57$, $P = 0.004$), particularly in the first treatment period (period × treatment interaction: $F_{1,313} = 3.21$, $P = 0.074$). Pollen removal reduced average pollen grains per stigma of nearby flowers by 19.4%, from 181.05 ± 8.62 (mean ± SE; $n = 141$ plant means) for the control to 148.97 ± 6.73 ($n = 134$ plant means) for the removal treatment. Based on a nonlinear relationship between pollen load and seed set reported previously for *I. aggregata* (Waser and Fugate 1986), this predicts an average reduction of 0.88 seeds per fruit or about 10%. The observed mean seed sets per fruit for control and pollen removal treatments (based on plant means) were 4.91 and 4.03, respectively, a difference of 0.88 seeds or about 18%. Our seed set data were too sparse and unbalanced for a statistical analysis of treatment effects to be unconfounded by other sources of variation.

DISCUSSION

The starting expectation for this study was that variation in environmental factors can affect pollen and nectar production by flowers and thereby local pollen and nectar neighborhoods, which in turn will influence how much pollen is delivered to the stigmas of flowers by pollinators. Greenhouse and field studies supported the first part of this expectation, showing that pollen and nectar production are positively related to soil moisture. This agrees with past reports that drought can affect the output of both pollen (Stephenson et al. 1992, Turner 1993) and nectar (Huber 1956, Zimmerman 1983, Petanidou et al. 1999, Carroll et al. 2001).

In both greenhouse and field, nectar production increased with soil moisture in a nearly linear fashion, whereas pollen production approached an asymptote, or perhaps even declined, at higher moisture levels. Such an asymptotic or humped response is unsurprising because the number of pollen mother cells is determined early in flower development (Goldberg et al. 1993). The result is a ceiling on the number of pollen grains produced per flower, which might be reached once the plant has enough moisture to mature all pollen mother cells (see Devlin 1988), but not so much moisture that roots are waterlogged. Nectar production was not similarly constrained at the soil moisture levels attained in this study.

The pollen removal experiment confirmed the third part of our starting expectation, that pollen availability in the neighborhood of a plant can affect its receipt of pollen—not a surprising result given that most hummingbird movements from *I. aggregata* plants are to neighboring conspecifics (Waser 1982) and, as a consequence, that most pollen travels <5 m (Campbell and Waser 1989). We expect a similar effect in other species at a spatial scale of meters to tens of meters, since pollen dispersal for most plant species is concentrated at short distances (Levin and Kerster 1974). Thus we predict that differences in pollination success will occur whenever there is local spatial variation in the abiotic or biotic environment (such as in

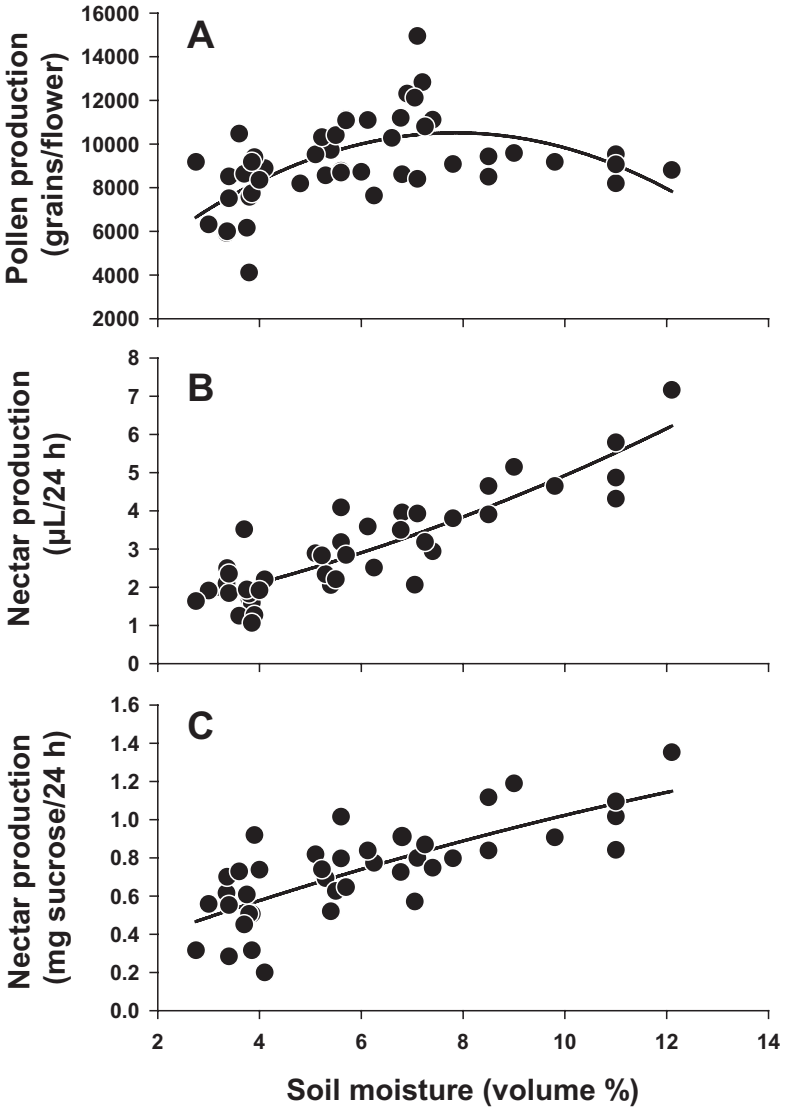

FIG. 3. Effects of soil moisture (A) on pollen production in flowers and (B, C) on two measures of nectar production in the field. Data represent means of plant means from each of two collection times per year in each of four populations from 2009 through 2014. Lines indicate best-fit quadratic equations.

conspecific or heterospecific flower densities, the genetic makeup of plants, or the gender ratios of populations) that leads to spatial variation in the availability of compatible pollen. Temporal variation in the abiotic or biotic environment ought to have an analogous effect. Further study of such patterns should improve our understanding of pollen limitation in nature.

The pollen removal experiment also yielded evidence of a response of seed set to reduction in pollen loads per stigma, as opposed to the augmentation of pollen loads that has yielded almost all evidence of pollen limitation to date (see Knight et al. 2005). It is encouraging to see an interpretable response to both lower (this study) and higher (Campbell and Halama 1993) pollen loads in *I. aggregata*, and to find as we did that the response to lower loads matches reasonably well what we expect from the nonlinear dose-response function relating pollen load to seed set.

The effects of the pollen neighborhood on pollen receipt, in combination with the joint response of pollen and nectar production to soil moisture, shed light on the surprising result from our earlier work (Price et al. 2005) that years of high average hummingbird visit rate around the RMBL were years of low pollen receipt by *I. aggregata* flowers. New analysis of data from the earlier study indicates that hummingbird visit rates were high in drought years. Reduced nectar production in dry soils, such as we have documented, might force hummingbirds to visit flowers more frequently to support their extraordinary energy demands (e.g., Pearson 1954), and to spend less time per flower (thereby visiting more flowers per unit

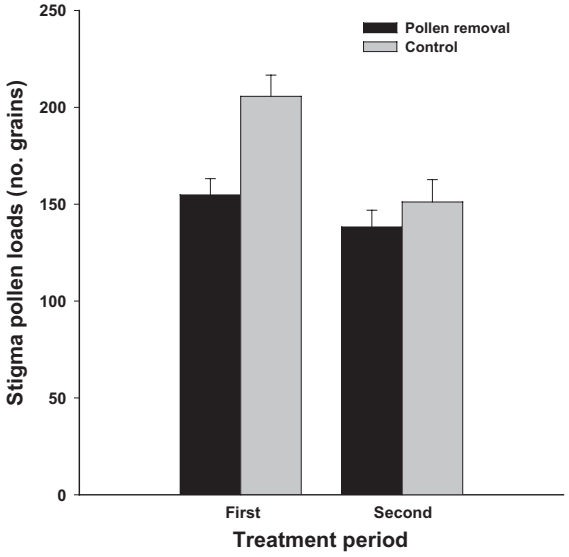

FIG. 4. Stigma pollen loads (number of *I. aggregata* pollen grains per stigma) in field populations as a function of pollen removal treatment and treatment period. Values are least-squared means of plant means + 1 standard error of the mean, pooled across populations.

time), because it takes less time to drain flowers with little nectar. Mitchell and Waser (1992) reported a slight decline in pollen removed and deposited during shorter visits to *I. aggregata* flowers with lower nectar volumes, an effect that may have contributed, along with the lower per-flower pollen production under drought conditions documented here, to the negative correlation between hummingbird visit rates and stigma pollen loads. These effects taken together might cause hummingbirds to deliver less pollen even as they visit flowers more frequently (Fig. 5). In this scenario it also is important to point out that whereas hummingbirds visit flowers of many other species around the RMBL (e.g., Table 1 in Waser 1983), the meadows used in our earlier study and in this study contain few of these other flowers when *I. aggregata* is in bloom, so that nectar neighborhoods are effectively conspecific.

The evidence presented here only allows us to argue that the scenario just outlined is plausible. We lack the information needed to assess whether direct and indirect effects of soil moisture on pollen and nectar neighborhoods were strong enough to completely explain the previously observed negative relationship between hummingbird visit rate and stigma pollen loads. Other factors, such as correlated variation in abundance of insects that rob flowers of nectar or pollen, may have contributed as well. Taking a larger view, however, our results illustrate the value of recognizing that pollination success depends both on the number of visits a flower receives and on the amount of compatible conspecific pollen delivered per visit; that both of these components of success are related to pollen and nectar neighborhoods; and that these neighborhoods in turn are influenced by abiotic or biotic factors such as soil moisture, insolation, nutrients, and herbivory.

Our focus on soil moisture, rather than other potential drivers of pollen and nectar production, reflects the facts that water is a limiting resource in meadows around the RMBL and that precipitation is highly variable across years (Price and Waser 2000, Loik et al. 2004). There is a larger context as well. Water availability is a major environmental driver in many ecosystems worldwide (Loik et al. 2004), and anthropogenic climate change is anticipated to increase aridity of already-dry regions at both temperate (Cayan et al. 2010) and tropical (Cook et al. 2012) latitudes. To date, research that relates climate change to pollination success has focused on phenological and numerical responses of plants and pollinators (e.g., Hegland et al. 2009). The patterns described here for *I. aggregata* suggest that it will be valuable to expand the focus to include links between environmental

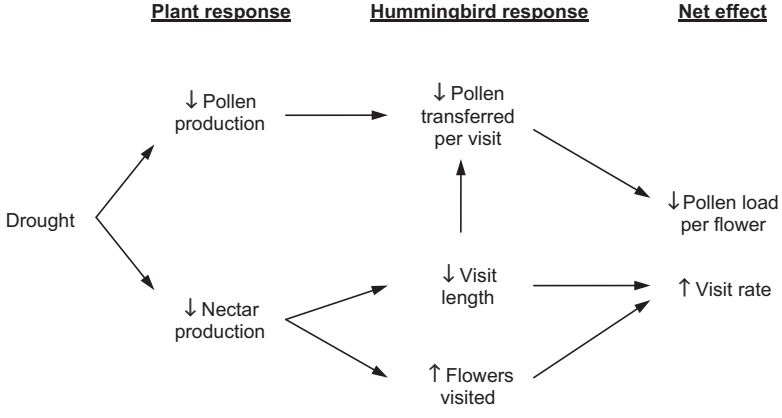

FIG. 5. A scenario for links between drought, nectar and pollen availability, hummingbird behavior, and per-visit pollen deposition that could contribute to an unexpected negative correlation across years between rates of hummingbird visit rate and stigma pollen loads of *I. aggregata* flowers.

factors, pollen and nectar neighborhoods, and pollen movement by animals.

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