

Impacts of drought gradient on pollen limitation in flowers in dry subalpine meadows

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I. Abstract

Several theoretical frameworks suggest pollen limitation (when plants' seed set is limited by pollen) should be rare, but empirical studies show that it is common at equilibrium conditions in nature. One proposed mechanism for this incongruity is that widespread pollen limitation is the result of pervasive anthropogenic disruption of pollinator species. Alternatively, abiotic resource availability also poses a strong limit on seed production and can even negate any benefits of animal pollination. As such, an alternative theory to widespread pollen limitation may be 'bet-hedging' of individual plants due the uncertainty of whether pollen or resources will limit seed set at any given year. In this theory, plants cope with seasonally inconsistent resources (such as precipitation) by investing moderately in the number of flowers produced. In stressful years, when resources limit seed set more strongly than pollen availability, a moderate investment in flowers allows plants enough resources for survival and to develop as many seeds as it can support. In mild years, when resources are abundant, the number of flowers that form seeds is then more strongly limited by the availability of pollinators and pollen. A direct prediction of this theory is that pollen limitation should be greatest when abiotic conditions are less stressful. We used alpine meadows near Gothic, CO, USA, to test this theory under stressful abiotic conditions. We measured soil water content at each of seven meadows to quantify abiotic stress using a soil water content meter, and measured pollen limitation by comparing fruit production of pollen-supplemented plants to naturally pollinated controls. We found that under stressful conditions of the 2018 drought year, water availability significantly impacted seed set of asexually-reproducing plants, indicating that water stress was a limiting factor in plant reproduction. We also found that none of three pollination dependent species were pollen limited during this growing season, offering support for a bet-hedging hypothesis of pollen limitation.

II. Introduction

A. Project Narrative

Pollen limitation occurs when plants produce fewer seeds under natural conditions than they do with supplemental pollen addition (Johnston, 1991). Several conceptual frameworks suggest that pollen limitation should be rare in nature. For example, sexual selection theory posits that female reproductive success should be most strongly limited by access to resources, rather than access to male gametes (pollen in plants) (Janzen, 1977). In addition, Haig & Westoby (1988) suggested that in a constant environment, evolution should favor plants that invest in attracting pollinators just enough to provide as much pollen as the plant can produce seeds. Essentially, if any single factor (in this case pollen) limits reproduction, evolution should favor mutations that help alleviate that factor (in this case by attracting more pollinators), to the point that some other factor becomes limiting for reproduction.

Despite the predicted rareness of pollen limitation, meta-analyses of pollen limitation suggest that 63 - 73% of terrestrial, animal-pollinated plants are pollen limited (Knight et al., 2005; Ashman et al., 2004; Burd, 1994). Fewer studies exist on pollen limitation in wind pollinated plants, although initial experiments suggest that it does exist and can even shape species distributions (Davis et al., 2004). Ecologists have proposed several explanations for the prevalence of pollen limitation in animal pollinated plants. One possibility is that the current state of widespread pollen limitation is a non-equilibrium state resulting from worldwide disturbance caused by climate change, habitat conversion, invasive species, and other human activities (Ashman et al., 2004). Studies conducted in highly disturbed systems (eg. Chittka & Schurkens 2001; Buchman & Nabham, 1996) have provided some evidence for this human disturbance theory. Chittka & Schurkens (2001) found that the introduction of *Impatiens glandulifera*, a highly attractive invasive species, caused lower pollen deposition and greater pollen limitation in

native species in invaded areas. Similarly, habitat conversion can lead to losses of native pollinator species, leading to increased pollen limitation in disturbed areas (Buchman & Nabham, 1996). While human disturbance is associated with pollen limitation in heavily disturbed environments, pollen limitation has also been observed in many natural, relatively undisturbed ecosystems (Ashman et al., 2004; Garcia-Camacho & Totland, 2018). Proponents of the human disturbance theory may interpret this fact as evidence that human disturbance has extended even to relatively intact ecosystems, especially via wide-reaching changes in climate and nutrient cycling.

An intriguing alternative explanation is that pollen limitation in undisturbed systems may be an equilibrium state resulting from plants “bet-hedging” over years with stochastic resource availability (Burd, 1995). Plants allocate resources towards attracting pollinators and producing ovules early in the season without “knowing” what abiotic resources will be available when the plant must access them to mature seeds. Given unpredictable availability of abiotic resources, selection may favor a strategy of intermediate investment in attracting pollinators early in the season, which would result in pollen limitation in years when abiotic resources are plentiful (Haig & Westoby, 1998). If plants are investing an intermediate amount in attracting pollinators and creating flowers and ovules, to hedge bets on abiotic resource conditions later in the season, *pollen limitation should be far less pronounced when plants experience stressful abiotic conditions*. In stressful abiotic years, reproduction will be limited by access to abiotic resources, and left-over resources from the beginning of the season will help the plant survive to the next year. In favorable abiotic years, pollen will be the limiting factor for reproduction.

Dry meadows surrounding the Rocky Mountain Biological Station (RMBL, Colorado, USA) present a unique opportunity to test this hypothesis. These meadows have been the subject of intensive study of plant-pollinator interactions over the past 40 years (Price & Waser, 1998; Inouye, 1978, Brosi & Briggs, 2013). This depth of past knowledge about both individual plant species and species interactions between plants and pollinators makes these meadows a good study system to use to understand the interaction between abiotic stress and pollen limitation.

One common abiotic stressor in these dry meadow ecosystems is drought, which generally decreases both the number of flowers per plant and the volume of nectar in individual flowers (Phillips et al., 2018). Low snowfall over winter 2017-2018, and low rainfall in spring 2018 caused drought conditions over the 2018 growing season in the RMBL area. Drought conditions have had negative consequences in the past on flower communities, including reducing the total number of flowers of each type blooming (Aldridge et al., 2011). Using the stressful abiotic conditions available in the 2018 growing season gave us a unique opportunity to test the impact of abiotic stress on pollen limitation.

B. We Hypothesized: *Under stressful abiotic conditions of the 2018 drought, pollinator-dependent plants will not display pollen limitation.*

C. Research Questions: Do drought-stressed plants show similar pollen limitation to less drought stressed plants? If so, pollen limitation in this ecosystem may be a result of catastrophic human disturbance of pollinators. Alternatively, are drought-stressed plants significantly less pollen limited than less drought-stressed plants? In this case, pollen limitation may be an evolutionary result of bet-hedging between years with varying abiotic conditions. Answering this question in the relatively pristine dry meadow ecosystem surrounding RMBL will provide key evidence to explain the prevalence of pollen limitation in non-disturbed environments. At a broader scale, the results of this study will help answer an unresolved

question about the origin of pollen limitation, and the extent of human impact on pollination services worldwide.

III. Methods

We conducted this project at seven meadow sites (Stupid Falls, Avery Picnic, Rustler's Gulch, Virginia Basin, Bellview Bench, 403 Bench, and Gothic Townsite, Fig. 1) distributed throughout the East River Valley (Gothic, CO, USA). We assessed soil water availability at the site level using a soil water content (SWC) meter (Fieldsout TDR 100 Soil Moisture Meter, Spectrum Technologies, Aurora IL, USA). We measured SWC at multiple points throughout each plot twice, once on July 10th and once on July 24th, averaging these values to create an average plot-level soil moisture value.

Flower communities were largely similar at each site, and all focal flower species occurred at least three sites. I conducted experiments on three sexually reproducing species: *Fragaria virginiana*, *Lathyrus leucanthus* var. *lewansii*, and *Viola praemorsa*. I also assessed fruit set on three self-pollinating species: *Boechera stricta*, *Ranunculus inamoenus*, and *Viola praemorsa* (asexual fruits). For sexually reproducing species, we randomly selected 10 individual plants of each species to receive supplemental hand pollination. We marked hand pollinated plants with flags and wire ties for identification during seed set later in the season. I visited each site 1-2 times per week to pollinate each flower on the selected plant individuals. At the end of the flowering season, we harvested all hand-pollinated flowers, and all flowers from 10 randomly-chosen individuals from the immediate area that received only natural pollination. We calculated fitness as the proportion of flowers from each plant that successfully produced fruit. For asexually-reproducing species, we assessed total fruit set for 10 individuals at each site where they occurred.

We analyzed data for each flower species (*Boechera stricta*, *Fragaria virginiana*, *Lathyrus leucanthus lanzwerthii*, *Ranunculus inamoenus*, *Viola praemorsa*) separately using generalized linear mixed models in the package *glmmTMB* (R Development Core Team, 2011). For all species, we used zero-inflated models to account for an abundance of flowers that did not successfully reproduce (Brooks et al., 2017). For outcrossing species (*Fragaria virginiana*, *Lathyrus leucanthus lanzwerthii*, and outcrossed fruits of *Viola praemorsa*), we first calculated a value for fitness of each plant as the proportion of flowers that successfully produced fruit (i.e. were pollinated and had adequate resources to develop). We then examined the effect of pollination treatment and soil water content on fitness, with plot as a random effect. For some species that only produced fruit successfully at one site, we separated data by site to examine the effect of pollination treatment as well. We ran ANOVA's on single-site data and used a Tukey's HSD test to determine differences between pollination treatments.

For selfing species (*Boechera stricta*, *Ranunculus inamoenus*, and asexual fruits of *Viola praemorsa*) we ran models testing the effect of soil water content on number of fruits produced with site as a random effect. We used number of fruits rather than number of seeds because time did not permit for full analysis of seed heads during summer 2018.

IV. Results

Pollen limitation in sexually-reproducing plants

Pollination treatment (exclusion, open, or hand pollination) did not significantly impact fruit set of *Fragaria virginiana* ($p > 0.5$, Fig. 2A). We were not able to test for an impact of soil water content on fitness in *Fragaria* because all plants that successfully set fruit occurred at one site,

Avery Picnic. We analyzed data from Avery Picnic *Fragaria* separately and found a significant effect of pollination treatment at this site (ANOVA, $p < 0.0001$, Fig. 2D). Both open plants and hand pollinated plants set significantly more fruit than exclusion plants (TukeyHSD, $p < 0.0001$), but were not significantly different from each other ($p > 0.1$).

Treatment did not significantly impact fitness in *Lathyrus leucanthus lanzwerthii* ($p > 0.05$, Fig. 2B). Soil water content also did not significantly impact *Lathyrus* fitness ($p > 0.1$). *Lathyrus* set fruit most successfully at one site (Virginia Basin). Analysis of *Lathyrus* from Virginia Basin only revealed no significant impact of treatment on fitness (ANOVA, $p > 0.1$, Fig. 3E).

Treatment did not significantly impact *Viola praemorsa* fitness ($p > 0.10$, Fig. 3D). Soil water content also did not significantly impact fitness in *Viola praemorsa* ($p > 0.05$).

Soil water content impact on asexual plant fitness

Soil water content was not a significant predictor of fruit set in *Boechera stricta* ($p > 0.1$, Fig. 3A). *Ranunculus inamoenus* seed set was significantly correlated with soil water content ($p < 0.05$, Fig. 3B). Soil water content was not a significant predictor of asexual fruit set in *Viola praemorsa* ($p > 0.5$, Fig. 3C).

V. Discussion

Our results provide mixed evidence for pollen limitation in three sexually-reproducing flower species (*Fragaria virginiana*, *Lathyrus leucanthus*, and *Viola praemorsa*). Although hand pollination did not significantly impact *Fragaria* among all our sites, this pattern was driven by sites where no *Fragaria* was able to reproduce, likely due to extreme drought in the 2018 growing season. In one site where *Fragaria* were able to successfully reproduce (Avery Picnic), treatment was highly significant. At this site, hand pollinated flowers were significantly more fit than naturally pollinated flowers, indicating pollen limitation. Because *Fragaria* only reproduced at one site, we were unable to observe an interaction between pollen limitation and soil water content.

Fitness in *Lathyrus leucanthus* was not significantly impacted by treatment, and this pattern persisted even among Virginia Basin *Lathyrus* which were able to reproduce the most successfully. This result suggests that in a drought year, *Lathyrus* seed set is more strongly limited by resource limitation than pollen limitation. In average snowfall years and under less stressful conditions (potentially the 2019 growing season) *Lathyrus* may be more strongly pollen limited.

Pollination treatment did not significantly impact fitness in *Viola praemorsa*, indicating that this species was not pollen limited during the 2018 growing season.

Because the 2018 growing season represented highly stressful growing conditions for most species observed, our observation that pollen limitation was not widespread supports the bet-hedging hypothesis for pollen limitation. If plants always invest a moderate amount in attracting pollinators, this moderate investment would attract enough pollen to develop the small amount of seeds plants could support in such stressful conditions.

Observations from asexually-reproducing plants confirmed that soil water content was an important predictor of reproductive success for some plants under drought conditions. Soil water content significantly predicted total fruit set in one of three plants tested (*Ranunculus inamoenus*). We were unable to detect an impact of soil water content on fruit set in either *Boechera stricta* or *Viola praemorsa*, likely because these species only occurred at a subset of

sites with similar soil water content values, which may have impacted our ability to detect an effect.

This study provides preliminary support for a “bet-hedging” theory of pollen limitation for flowers in alpine Colorado meadows. Soil water content, a measure of abiotic stress, significantly affected reproductive success in some asexually-reproducing plants, indicating that this metric is meaningful to study in the context of flower reproduction. None of three sexually-reproducing species, however, showed strong signatures of pollen limitation, suggesting that the extreme abiotic limitations of the 2018 growing season outweighed any impacts of pollen limitation. Unfortunately, extremely low precipitation throughout the study area made it impossible to examine an extensive gradient of soil moisture availabilities and their impact on mediating the effects of possible pollen limitation. Data from future growing seasons with average precipitation will provide an invaluable opportunity to compare pollen limitation across a wider abiotic stress gradient.

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VI. References

- Aldridge G, Inouye DW, Forrest JRK, Barr WA, Miller-Rushing AJ. 2011. Emergence of a mid-season period of low floral resources in a montane meadow ecosystem associated with climate change. *Ecology* **99**(4): 905-913.
- Ashman T, Knight TM, Steets J, Amarasekare P, Burd M, et al. 2004. Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology* **85**(9): 2408-21.
- Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg, CW, Nielsen A, Skaug HJ, Maechler M, Bolker BM (2017). "glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling." *The R Journal*, 9(2), 378–400. <https://journal.r-project.org/archive/2017/RJ-2017-066/index.html>.
- Brosi BJ, & Briggs HM. 2013 Single pollinator species losses reduce floral fidelity and plant reproductive function. *PNAS* **110**:32: 13044-13048
- Buchman SL, & Nabham GP. 1996. The forgotten pollinators. Island Press, Washington D.C., USA.
- Burd M. 1994. Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. *Bot. Rev.* **60**: 83-139.
- Burd M. 1995. Ovule packaging in stochastic pollination and fertilization environments. *Evolution* **49**(1): 100-09.
- Chittka L, & Schurkens S. 2001. Successful invasion of a floral market. *Nature* **411**:653.
- Davis HG, Taylor CM, Lambrinos JG & Strong DR. 2004. Pollen limitation causes an Allee effect in a wind-pollinated invasive grass (*Spartina alterniflora*). *PNAS* **101**(38): 13804-07.
- Haig D, & Westoby M. 1988. On limits to seed production. *Am. Nat.* **131**: 757-59.
- Garcia-Camacho R & Totland O. 2018. Pollen limitation in the alpine: a meta-analysis. *Arctic, Antarctic, and Alpine Research*. **41**:103-111.
- Inouye, D. 1978. Resource partitioning in bumblebees: Experimental studies of foraging behavior. *Ecology* **59**(4): 672-678.
- Janzen DH. 1977. A note on optimal mate selection in plants. *Am. Nat.* **111**: 365-71.
- Johnston MO. 1991. Pollen limitation of female reproduction in *Lobelia cardinalis* and *L. siphilitica*. *Ecology* **72**: 1500-3.
- Knight TM, Steets JA, Vamosi JC, Mazer SJ, Burd M, et al. 2005. Pollen limitation of plant reproduction: Pattern and process. *Annu. Rev. Ecol. Evol. Syst.* **36**: 467-97.
- Price, M. V. and N. M. Waser. 1998. Effects of experimental warming on plant reproductive phenology in a subalpine meadow. *Ecology* **79**:1261-1271.

R Development Core Team. 2011. R: A Language and Environment for Statistical Computing. Vienna, Austria : the R Foundation for Statistical Computing. ISBN: 3-900051-07-0. Available online at <http://www.R-project.org/>.

VII. Figure Captions

Fig. 1: Map of study sites in the East River Valley, Gunnison County, Colorado, USA. Gothic Townsite is marked in blue for reference; all study sites are marked in pink.

Fig. 2: Fitness by treatment for three different sexually-reproducing plant species: *Fragaria virginiana*, *Lathyrus leucanthus*, and *Viola praemorsa*. Two of these species (*Fragaria virginiana* and *Lathyrus leucanthus* only reproduced successfully at one site; data for sites with successful reproduction only are shown in the second row (D & E). Widths of violin curves indicate the proportion of points under the graph at that fitness.

Fig. 3: Asexual fruit set and soil water content for three asexually-reproducing species, *Boechera stricta*, *Ranunculus inamoenus*, and *Viola praemorsa*.

VIII: Figures
Figure 1

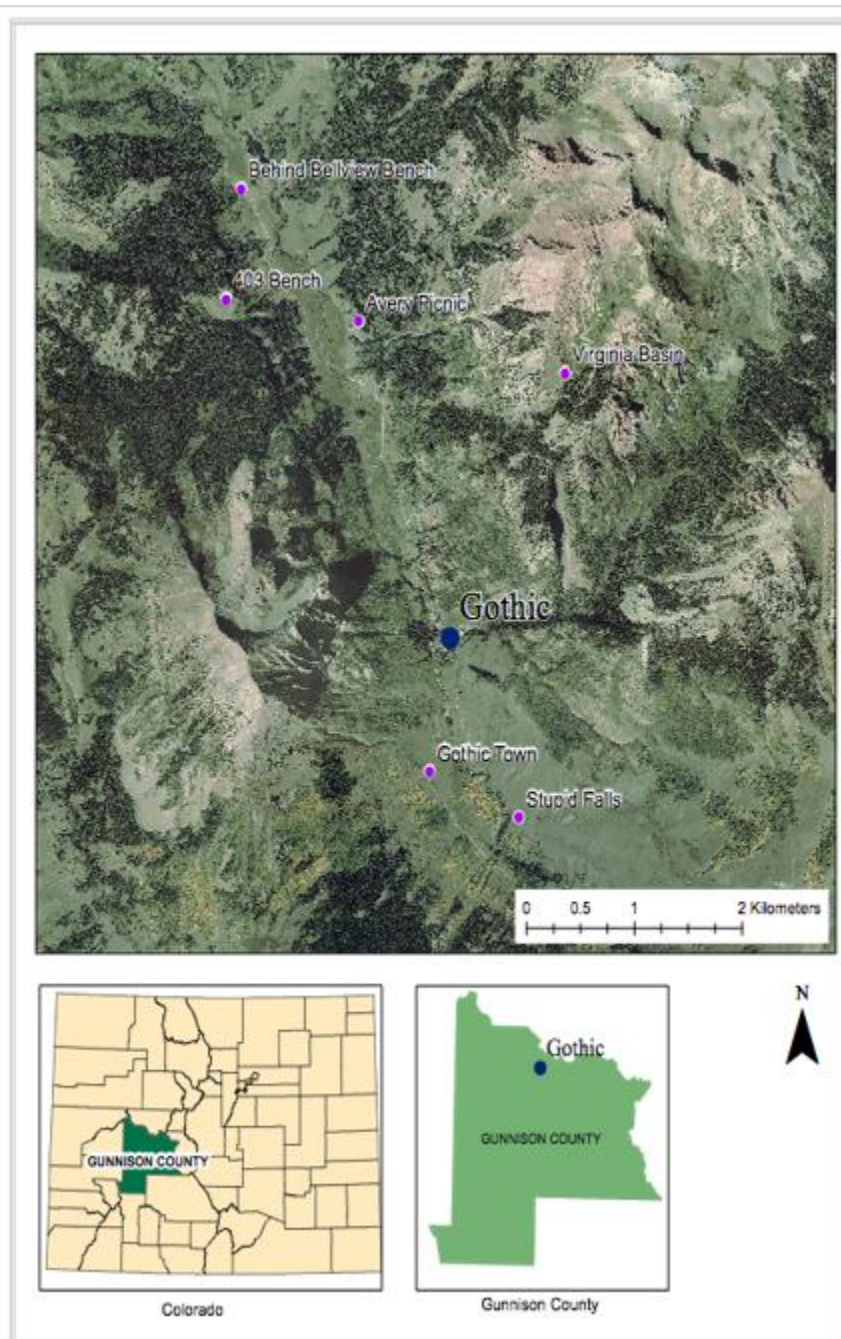


Figure 2

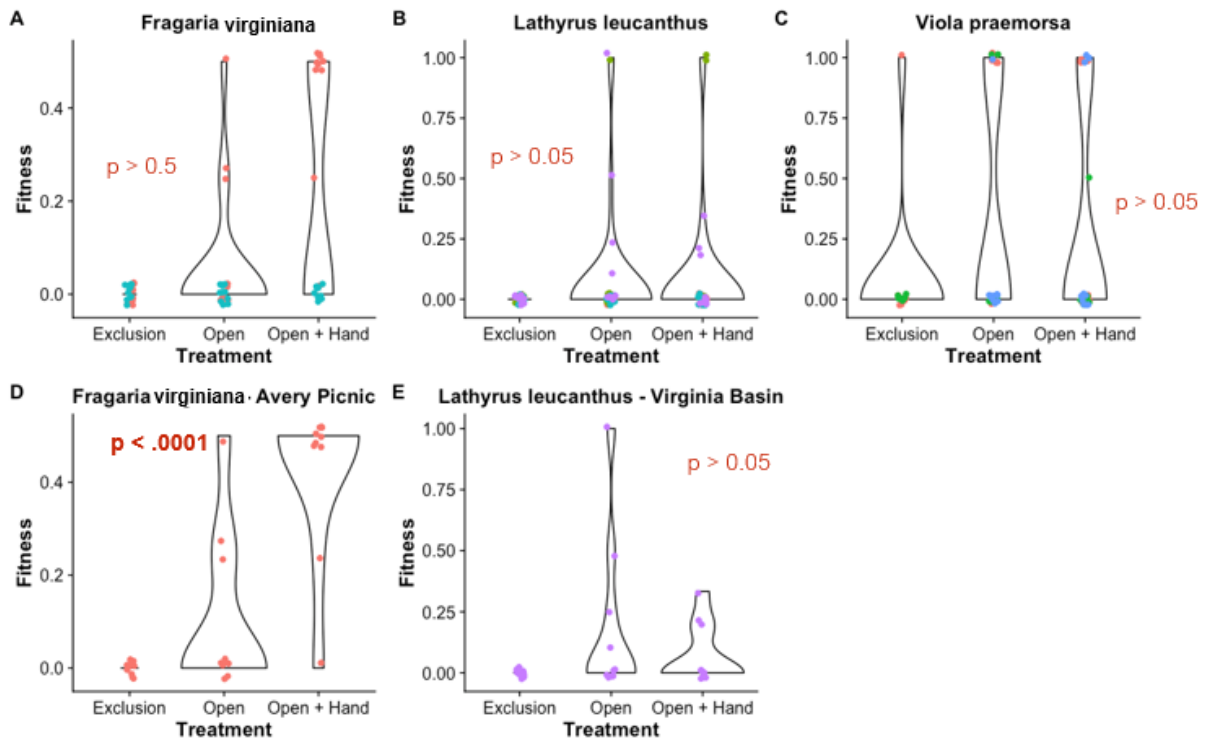


Figure 3

