Temporal variation in pollen limitation in *Hydrophyllum fendleri* (Boraginaceae) individuals

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Abstract

Plant reproduction is highly dependent upon phenology, or biological timing, of flowering. However, little is known about how individuals vary in their flowering schedules relative to the population as a whole or how this variation could lead to differences in reproductive success of individuals. Here, it was shown that individuals of *Hydrophyllum fendleri* (Boraginaceae) were aggregated in time and that individuals that flowered later had a higher reproductive success than those that flowered earlier in the season. I tested the hypothesis that pollen limitation was responsible for the temporal variation in reproduction observed. Although it was shown that plants were indeed limited in fruit and seed production by pollen receipt, pollen limitation was consistent across the growing season, suggesting that other factors caused the variation in reproduction.

Introduction

Most ecological models of resource use and population dynamics treat individuals of the same species as equivalent entities. However, studies have suggested that individual specialization of resource use within a conspecific population is common across a wide variety of taxa, and in many cases, this intraspecific variation has a significant impact on ecological and evolutionary dynamics (Bolnick et al. 2003, Bolnick et al. 2011). Roughgarden (1972, 1974) provided a framework for quantifying intrapopulation niche variation of resource use. If the use of a resource follows a bell curve, then we can expect individuals within the population to behave in one of three ways: First, individuals may have a large range of resource use and a small variance in the means of resource use between individuals, indicating generalization. Conversely, individuals may have a small range of resource use and a large difference between the means of individuals, indicating a greater degree of specialization. Lastly, individual resource use may be randomly distributed within the population and the variance in the means of individuals (Poole & Rathke 1979).

In relation to flowering phenology, we can consider flowering date as the use of a resource. It is expected that fewer plants flower at the beginning and end of the flowering season, and the highest density of plants flower in the middle (or peak) of the season (Elzinga et al. 2007). If individuals are generalized, that is, there is a smaller difference between their first or peak flowering dates, this may be beneficial because pollinators tend to be more attracted to a given plant species only after a certain threshold density of individuals are in bloom (Heithaus et al. 1982). In addition, flowering synchronously may satiate seed predators, thus promoting stabilizing selection (Janzen 1971). However, other studies have found that flowering off-peak is associated with reduced seed predation and florivory (reviewed in Elzinga et al. 2007). In this case, plants would benefit from a greater degree of individual specialization. Off-peak flowering could also benefit individual plants if it results in a lesser degree of intraspecific competition for abiotic resources (shown to be true for interspecific competition, Veresoglou & Fitter 1984).

A previous study on two subalpine wildflower species found that individuals of both species were significantly aggregated (generalized) in time; however, reproductive success varied based on individual phenologies: in one species, individuals that ended their flowering earlier made more fruits, while in the other species individuals that ended

their flowering later made more fruits (Iler, unpublished data). Pollen limitation is a likely explanation for the temporal variation in fruit production in these two species. Pollen limitation is the concept that an inadequate quality or quantity of pollen restricts reproductive success (Ashman et al. 2004). Studies have shown that pollen supplementation often increases (Burd 1994) and rarely decreases (Young & Young 1992) seed and fruit production, indicating that pollen limitation is an important factor determining reproductive success. However, in general, little is known about how the amount of pollen limitation varies throughout the growing season. An earlier study in seasonal variation of pollen limitation on *Blandfordia grandiflora*, found that there was a significant difference in pollen limitation between early flowering and later flowering individuals due to variation in pollinator visitation across the season (Ramsey 1995). This shows that variation in pollination across the season can account for differences in reproductive success across the flowering period. In this study, I determined if there was variation in the amount of pollen limitation across the season for individuals of *Hydrophyllum fendleri.* In order to address this question, I also asked three other questions: (1) What is the flowering phenology pattern for *H. fendleri* individuals? (2) Does flowering phenology affect fruit and seed set? (3) Are fruit and seed set pollen limited?

Methods

Study design

Research was conducted on *Hydrophyllum fendleri* (Boraginaceae), a subalpine wildflower species, at the Rocky Mountain Biological Laboratory in Gothic, Colorado (Fig 5) in the summer of 2014. *Hydrophyllum fendleri* has a short flowering period from mid-June to early July (Inouye, unpublished data). This species requires insect pollination and is an obligate outcrosser (Beckmann 1979).

To confirm that *H. fendleri* requires insect pollination to successfully reproduce, the inflorescences on ten plants were covered with tulle baggies prior to blooming in order to prevent pollinators from accessing flowers (Kearns & Inouye 1993). Starting in mid-June when the first plants began to flower, approximately 5-10 pairs of newly flowering plants were marked for study each week, with an ending sample size of 25 total plant pairs (50 individuals). For each pair, one plant was left open to natural pollination as the control and 2-3 times per week, the other plant received supplemental pollen through hand pollination. Additionally, 2-3 times per week flowers were counted on all plants in order to determine the pattern of flowering phenology. All study plants were protected with chicken wire cages with holes large enough for pollinators to access flowers in order to prevent deer herbivory. In late July, fruits were collected, and fruit and seed set for each plant was determined. Fruits were dissected in the lab and the number of viable seeds counted. Viability was determined by gently squeezing the seed to check for sturdiness and by observation to check for roundness.

Data analysis

The flowering phenology pattern for *H. fendleri* was determined using the v-statistic described by Poole & Rathke (1979). A t-test was performed to determine if fruit and seed set were pollen limited (fruits per flower, seeds per flower, seeds per plant,

and seeds per fruit). The Welch correction was used when variances were unequal between treatments, and the number of seeds per flower and seeds per plant were both log transformed to meet normality assumptions. Lastly, an analysis of covariance (ANCOVA) test was performed to determine if there was temporal variation in reproductive success and in pollen limitation of fruit and seed set across individuals. Reproductive success (fruits per flower) was the response variable, and flowering date was a continuous predictor, while treatment was a categorical predictor. I tested for an interaction between flowering phenology and treatment to determine whether the amount of pollen limitation varied according to flowering time. This analysis was performed for each aspect of flowering phenology: first, peak, last, and duration of flowering.

Results

Using Poole & Rathke's v-statistic, it was found that the flowering pattern for both control and pollen supplemented plants was significantly aggregated (Table 1, Figs. 1 & 2). Individuals with a later first flowering and peak flowering date matured a higher proportion of fruits than those that flowered earlier (Table 3, Fig 4A, 4B respectively). Last day of flowering and duration of flowering did not have a significant effect on reproductive success (Table 3). Although it was shown that fruit set, seeds per flower, and seeds per plant were significantly pollen limited, seeds per fruit were not significantly pollen limited (Table 2, Fig. 3). Additionally, the amount of pollen limitation was consistent across the flowering season, as indicated by a non-significant interaction term between phenology and pollination treatment (Table 3, Fig 4).

Discussion

Individual phenologies of *H. fendleri* were significantly aggregated in time. This could suggest that there is either some benefit to flowering with conspecific individuals or some restriction on flowering time, such as abiotic constraints. Elzinga et al. (2007) reviewed a number of biotic effects that have been shown to promote selection for aggregated flowering, such as increased mate availability, seed predator satiation, and increased pollinator attraction, although here we find no evidence of pollinator attraction. Despite an aggregated individual flowering pattern, plant reproduction still varied with phenology.

It was also shown that reproduction in *H. fendleri* was significantly pollen limited. This was true for fruits per flower, seeds per flower, and seeds per plant. However, control and supplemental plants did not make a significantly different proportion of seeds per fruit. Therefore, although hand pollinated individuals made more seeds overall due to maturing a higher proportion of fruits, each individual fruit was not necessarily able to make more seeds. This suggests that the plants were limited in reproduction by the quantity of pollen receipt, not a limitation in the quality of pollen received. This could be because the sudden increase in pollen delivered through hand pollination overloads and clogs stigmas, thus reducing the benefits of receiving a higher quality of pollen (Ashman et al. 2004) An alternative explanation could be that because pollen supplementation has been shown to reduce floral longevity (Ashman 2004), the

hand pollinated flowers bloomed for a shorter period of time, causing a reduced seed set.

Additionally, it was shown that there was temporal variation in reproductive success in *H. fendleri.* Individuals that began to flower later and reached peak flowering later matured a greater proportion of fruits than those that flowered earlier and reached peak earlier. Since last flowering date and flowering duration did not have an effect on reproductive success, it seems that there is some benefit to beginning to flowering earlier, independent from the pattern that plants that begin to flower earlier have the ability to flower for a longer period of time. However, because pollen limitation was consistent across the flowering season, pollen limitation is not responsible for the temporal variation in reproduction. In contrast, Ramsey (1995) found that in the perennial herb *Blandfordia grandiflora* (Blandfordiaceae), seasonal variation in reproduction was pollen limited due to differing pollinator activity across the season. These results on *H. fendleri* indicate either pollinator activity is consistent across the season or that differences in pollinator activity has a negligible effect on the variation in reproduction observed.

Interspecific competition and plant size may both help to explain why plants that begin to flower later exhibited higher reproductive success. Interspecific competition may be reduced near the end of the flowering season for *H. fendleri.* This is consistent with the bimodal flowering pattern observed at the community level in this subalpine system (CaraDonna et al. 2014). Since *H. fendleri* is an early flowering species, individuals that flower later could have reduced interspecific competition as other early flowering species end their flowering. Further studies would have to be done at the study site to investigate whether other species do in fact end their flowering prior to *H. fendleri*, potentially providing a release from interspecific competition near the end of its flowering period. Size has also been shown to be an important factor affecting reproductive success in plants (Ollerton & Lack 1998; Iler, unpublished data), so perhaps the *H. fendleri* plants that began to flower later are larger than those that flowered earlier. However, one factor leading to higher reproductive success in Ollerton & Lack (1998) was that larger plants suffered a lower rate of seed predation, while seed and fruit predation was not observed in this study. This would indicate that instead, larger plants in this study could utilize resources more efficiently or have greater stored resources to increase reproductive success. Size, however seems unlikely to underlie all of the variation in fruit set with phenology observed in this study. Indeed, two other plant species at the study site have shown significant effects of phenology on fruit set independent of plant size (Iler, unpublished).

Although the causes of differential reproduction in this species are not yet clear, the individual variation based on phenology suggests that it is unwise (and in fact incorrect) to treat individuals as ecological equivalents. This is in contradiction to what might be expected based on the finding that *H. fendleri* individuals were aggregated in time. If individuals follow a more generalized pattern, it could be presumed that individual variation would have negligible effects on ecological dynamics. Yet despite the aggregated flowering pattern observed, individual variation in phenology still played an important role in the reproduction for this species. The extent and magnitude of individual phenological variation among plant species is largely unknown, but these results are evidence that intraspecific individual differences may have important impacts

on ecology and evolution, and further research into this area is needed in order to better understand phenology at the population level.

Table 1: V-statistic for *Hydrophyllum fendleri,* testing for generalized, random, or specialized flowering patterns ($p < 0.05$: specialized; $p > 0.95$: generalized).

Treatment	V-statistic	p-value
Control	0.0066	>0.999
Supplemental	0.0062	>0.999

Table 2: Pollen limitation of *Hydrophyllum fendleri* reproductive success. A significant p-value indicates that pollen supplemented plants had higher reproductive success than control plants

Response variable	t	p
Fruit set	-2.87	0.0065
Seeds/flower	-3.20	0.0025
Seeds/fruit	-1.58	0.12
Seeds/plant	-2.89	0.0062

n=24 plants/treatment

Table 3: ANCOVAs for pollen limitation of *Hydrophyllum fendleri* reproductive success across the flowering season (fruit set), FDF=first day of flowering, PDF=peak day of flowering, LDF=last day of flowering

Predictors	Df	F	p
FDF	1	4.52	0.039
Treatment	1	8.71	0.0051
FDF*Treatment	1	0.007	0.93
PDF	1	7.47	0.0093
Treatment	1	6.41	0.0015
PDF*Treatment	1	0.08	0.78
LDF	1	2.23	0.14
Treatment	1	7.05	0.011
LDF*Treatment	1	0.78	0.38
Duration	1	0.53	0.47
Treatment	1	9.05	0.0043

Duration*Treatment	1	0.25	0.62

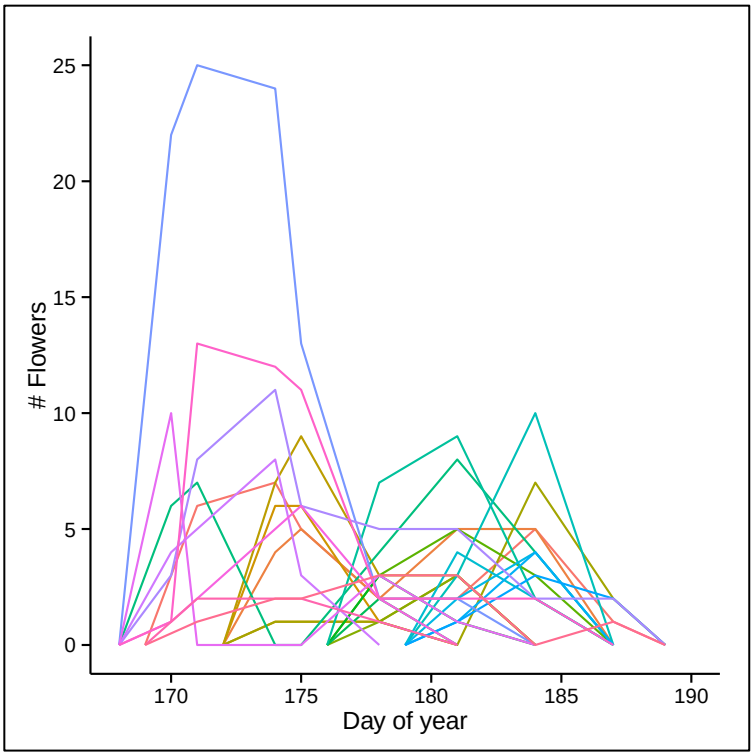


Figure 1. Flowering pattern for control individuals of *Hydrophyllum fendleri.* Each line corresponds to one individual plant.

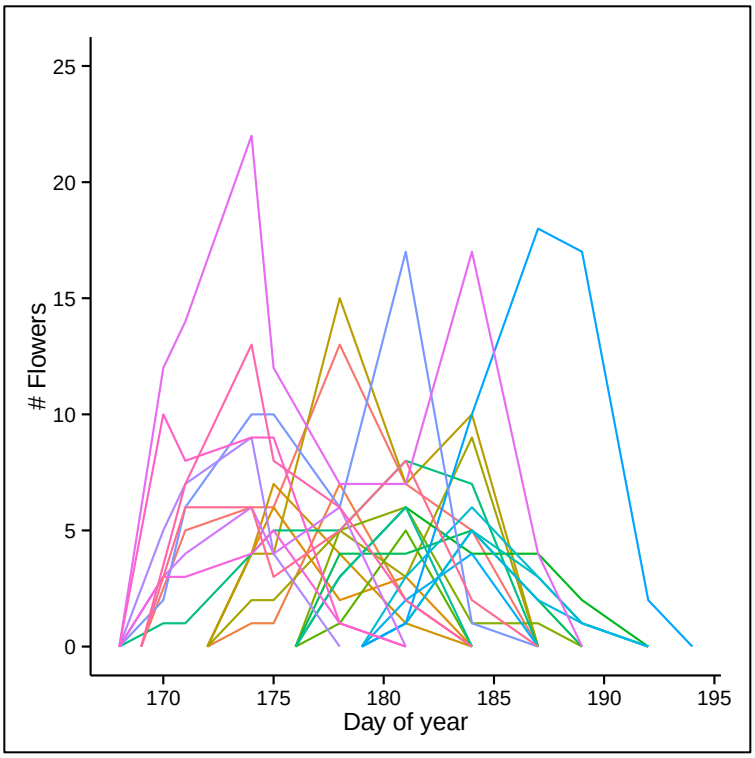
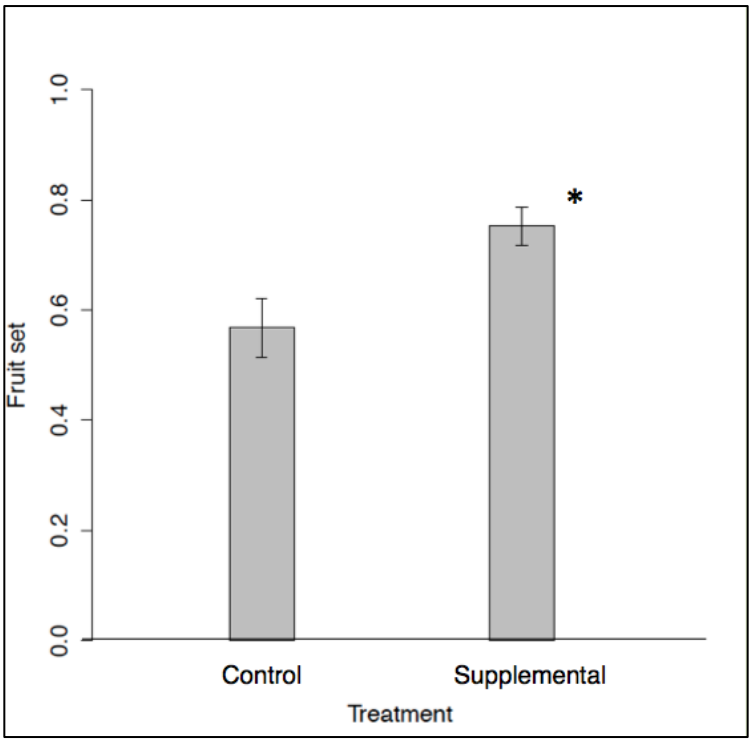
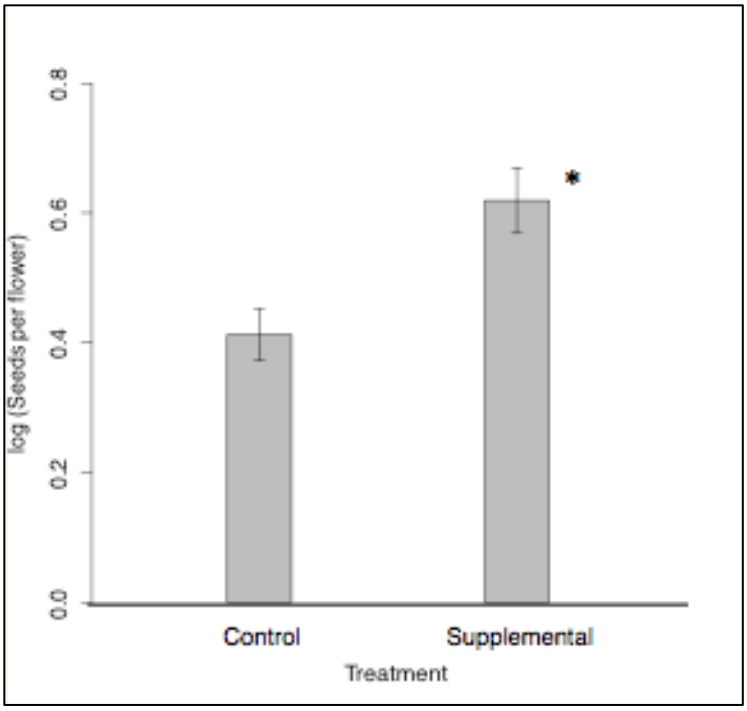


Figure 2. Flowering pattern for pollen supplemented individuals of *Hydrophyllum fendleri.* Each line corresponds to one individual plant.

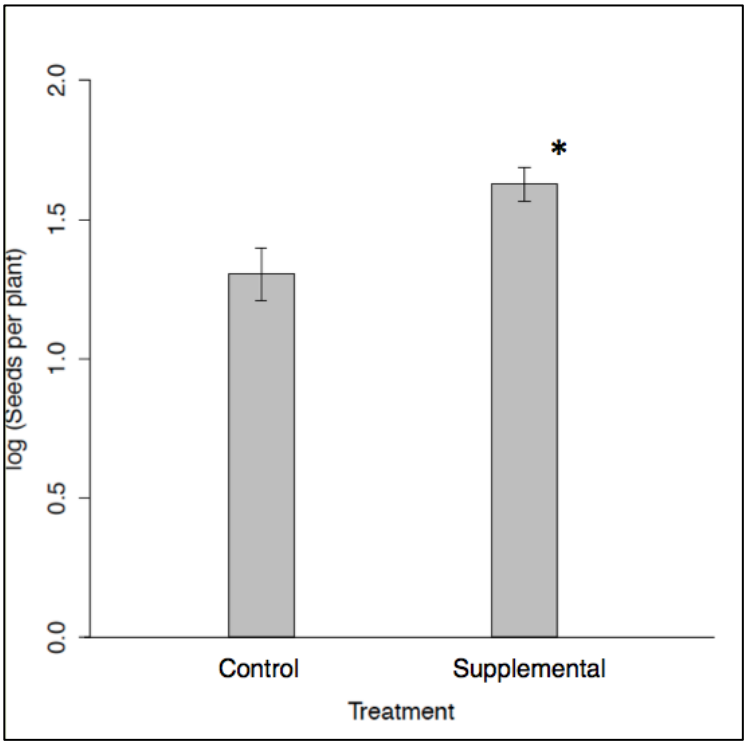
A



B


log (Seeds per flower)
0.0
0.2
0.4
0.6
0.8
*
Control
Supplemental
Treatment

C


log (Seeds per plant)
0.0
0.5
1.0
1.5
2.0
*
Control
Supplemental
Treatment

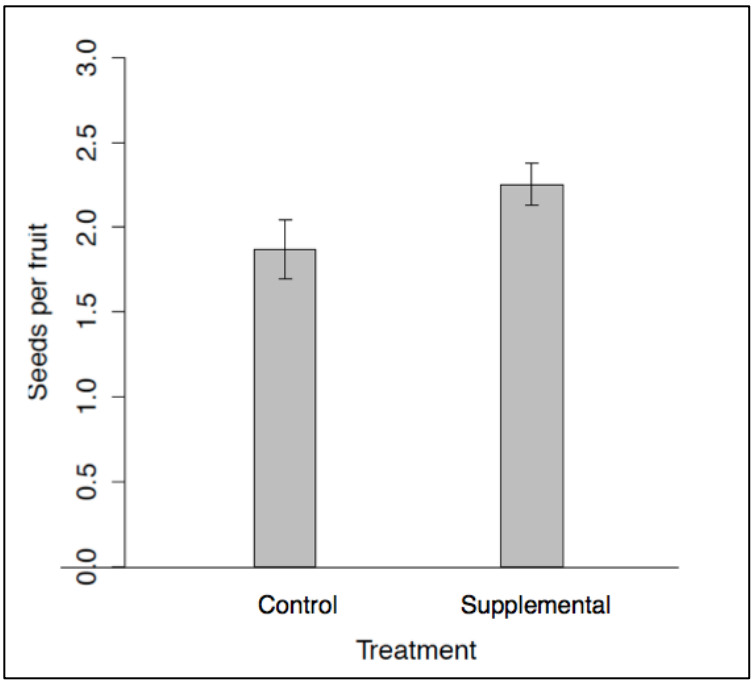
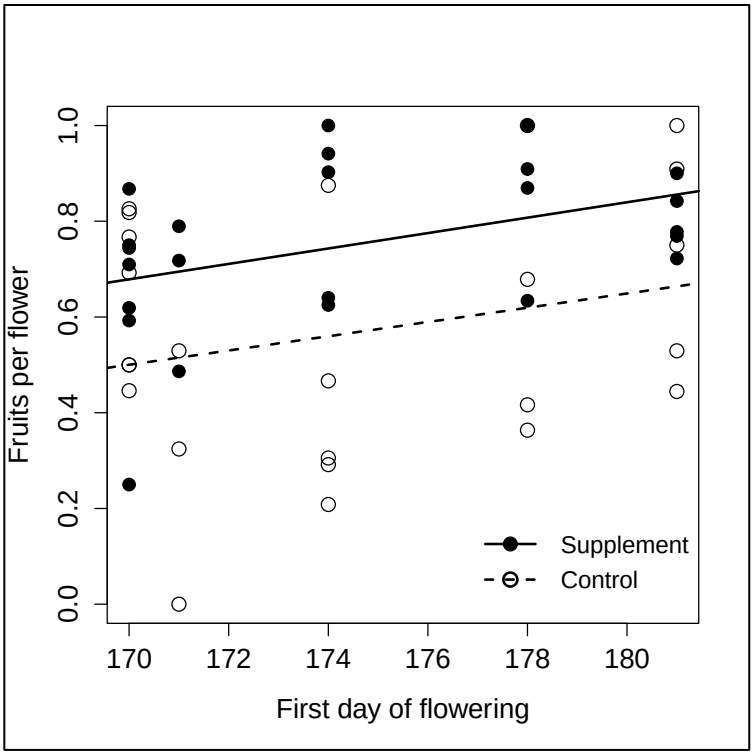


Figure 3. Pollen limitation of *Hydrophyllum fendleri* reproductive success (A) fruit set (fruits/flower), (B) seeds/flower, (C) seeds/plant, and (D) seeds/fruit.



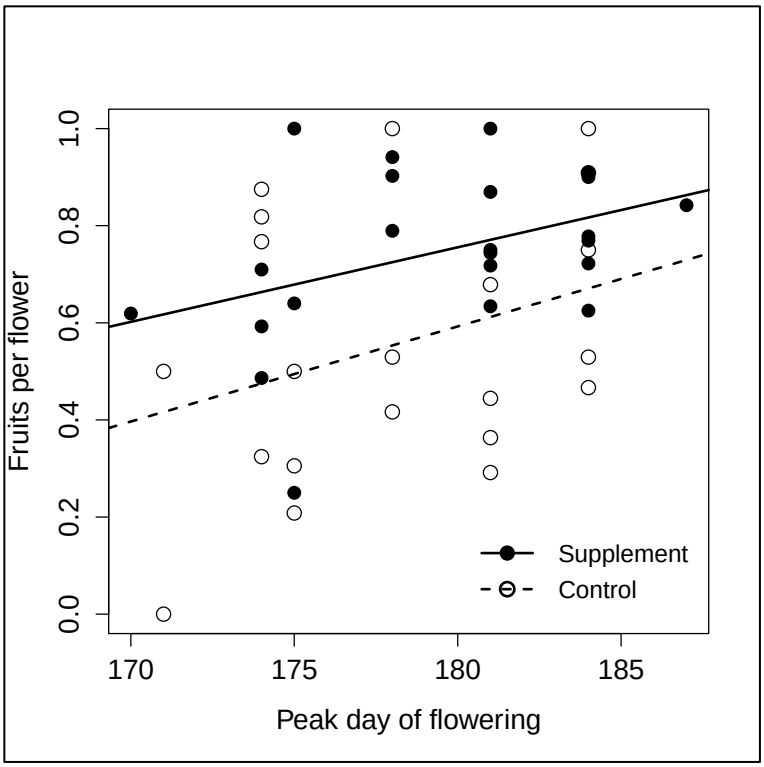


Figure 4. Degree of pollen limitation in *Hydrophyllum fendleri* based on (A) first day of flowering and (B) peak day of flowering. ANCOVAs indicated no difference in the slope of the control and supplemental lines

Figure 5. GIS map of research site near Gothic townsite at the Rocky Mountain Biological Laboratory, Colorado

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