

The effects of water and phosphorus on floral traits and nectar production of
Ipomopsis aggregata

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Abstract:

Floral traits and nectar production of *Ipomopsis aggregata* were measured in response to water and phosphorus treatments applied to two morphotypes. Typical morphotypes underwent water and phosphorus treatments. Because stunted floral traits may be a response to drought stress, only water treatments were applied to stunted morphotypes. Observations were completed within three sites in Gothic, Colorado. In typical *I. aggregata*, corolla width was increased by phosphorus treatments and nectar production rate was increased with water additions. Water had no significant effect on plants containing stunted flowers; however, stunted plants produced typically sized flowers within the second week of the experiment.

Introduction:

Variation in floral traits can be genetic or it can reflect phenotypic plasticity, the ability of an organism to change its phenotype in response to environmental conditions. Floral traits may respond plastically to many abiotic factors, particularly to soil water and nutrient availability (Caruso 2006). Plasticity of inflorescence traits has been established as a response to drought and nutrient availability (Carroll et al 2001). The results of pollinator-mediated natural selection on these traits may be altered by the abiotic environment (Cheplick 1995).

Water is necessary throughout the lifetime of a flower and is required for floral bud expansion, flower opening, nectar production, floral display and attractiveness (Galen 1999; Mohan and Rao 1984). Under drought conditions, plants often produce fewer flowers with shorter corollas and reduced volumes of nectar (Caruso 2006; Carroll

et al 2001). Water availability and nectar secretion can be strongly correlated; however, the extent of stress experienced by a plant in dry conditions is still unclear (Carroll et al 2001).

Phosphorus aids in the production of healthy roots and flowers. Soil phosphorus availability can affect plant reproductive traits such as flower and pollen production (Lau and Stephenson 1994). In previous studies, plants which have undergone phosphorus treatments have produced more seeds and more flowers per plant (Lau and Stephenson 1994; Scagel and Schreiner 2006).

I tested the effects of water and phosphorus additions on floral traits and nectar production of wild *Ipomopsis aggregata* subsp. *aggregata* (Polemoniaceae). *Ipomopsis aggregata* is a monocarpic plant which spends 2-10 years in a vegetative state before producing a stalk which generates an average of 85 flowers during a five week summer growing season (Campbell and Halama 1993). The inflorescence is a panicle with several lateral cymes which produce long, red, trumpet-shaped flowers lasting about 3-5 days each (Campbell 1993). The flowers produce nectar at a constant rate and are mostly pollinated by Broad-tailed and Rufous Hummingbirds (*Selasphorus platycercus* and *Selasphorus rufus*; Price et al 2005).

Ipomopsis aggregata occasionally produces a morphotype with stunted corollas. Although there are no data on the frequency or distribution of the stunted plants, one local observer noticed more stunted plants this summer than in previous years (personal communication, George Aldridge). The persistence of less attractive flowers may be explained by effects of the abiotic environment on floral traits (Carroll et al 2001; Caruso 2006). The summer of 2007, as of early July, had been dry compared to previous

summers. Traits influencing floral attractiveness to pollinators, such as corolla length and width, may vary with plant water status; therefore, it may be possible that production of stunted flowers would be a response to the drought and interfere with pollination, given that pollinators of *I. aggregata* typically prefer plants with large flowers and high nectar production (Campbell et al. 1991). This study tested the effects of water and phosphorus additions on floral traits and nectar production of wild *Ipomopsis aggregata*, as well as the effects of water addition on plants containing stunted flowers.

Methods:

Three sites containing populations of *Ipomopsis aggregata* were examined within the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado.

Characterization of stunted vs. typical flowers:

Characterization of stunted morphotypes came from data on 32 stunted and 32 typical plants measured in three *I. aggregata* populations along Cement Creek Road, south of Crested Butte. In comparison to the Gothic sites, Cement Creek Road occurs at a lower elevation and is drier (Zach Turner, personal communication). Measurements of stunted and “typical” morphotypes along Cement Creek Road were taken during June 27-July 5, 2007. Hereafter, “typical “ refers to non-stunted flowers. For each measured plant, the number of flowers and plant height were recorded. Three flowers per plant were measured for: height from the ground (cm), corolla length (mm), corolla width (mm), style length (mm) and standing nectar crop (μ L).

Effects of water on stunted I. aggregata:

Two plots containing both stunted and typical *Ipomopsis aggregata*, located in Gothic, Colorado, were studied to determine the effect of water on plants containing stunted flowers. Plot one was located directly alongside Gothic Road at 2,898.5 m elevation at 38.961° N; 106.991° W. Plot two was located directly alongside Gothic Road at 2,875.8 m elevation and 38.959° N; 106.989° W. Every three days, 400 ml of water were applied to two stunted and two typical plants, while two stunted and typical plants were left untreated as controls. The eight *Ipomopsis aggregata* from each plot were measured once a week for: plant height (cm), number of flower types, flower height from the ground (cm), corolla length (mm), corolla width (mm), style length (mm), stamen length (mm) and nectar production rate (npr) (L). Nectar production was determined by covering the appropriate flowers with crimped drinking straws to prevent pollinator access and returning 24 hours to extract the nectar with microcapillary tubes. During the first two weeks, soil moisture was recorded by inserting a Hydrosense soil moisture probe near the base of each sampled plant 24 and 48 hours after treatments were applied. Rainfall measurements were recorded from the RMBL weather station and website: <http://rmb1.org/home/index.php?module=htmlpages&func=display&pid=91>.

Effects of water and phosphorus on typical I. aggregata:

The effects of water and phosphorus were tested in a plot with a larger sample size of plants. This plot was located near East River at 2,895.92 m elevation and 38.96179532° N; 106.99296205° W. Forty-five *Ipomopsis aggregata*, at least 0.5 meters apart, were assigned at random to three manipulations: control (no manipulation), water

addition and phosphorus plus water addition. Every three days, 400 ml of each treatment was applied directly to the appropriate plants. The water treatment consisted of East River water collected on site. A phosphorus aqueous solution consisted of one tablespoon of 0-44-0 Monosodium Phosphate per one gallon (3.8 liters) of water for the first three weeks, and 0-45-0 triple super-phosphate for the last week. Of the sampled flowers within each site, plant height and the number of flowers occurring on each plant were recorded. Three flowers per plant were measured once a week for: height of the flower from the ground (cm), corolla length (mm), corolla width (mm), style length (mm), stamen length (mm) and npr (μ L). Soil moisture was also recorded during the first two weeks of treatments. Soil moisture was significantly increased for plants which underwent water and phosphorus treatments (ANOVA for all measured days $P < 0.05$).

Statistical Analysis:

To compare stunted and typical morphs at Cement Creek Road, a one-way ANOVA was used. To test the effects of water on the two morphs along Gothic Road, a two-way ANOVA was used. The two factors in the latter ANOVA were morphotype and treatment. To determine the effects of water and phosphorus on typical flowers, traits were analyzed using one-way ANOVAS for each trait at the end of the study. To determine the effects of water or phosphorus independently, flower measurements were compared between phosphorus vs. watered plants and watered vs. control plants. Measurements were also compared between controls vs. treatment plants to determine an overall treatment effect. A multivariate analysis of variance (MANOVA) tested the effects of treatments on all measured traits, except for the height of the flower, as a

group. In addition, a repeated measures ANOVA was run to determine possible changes in each trait over time. All statistical tests were performed with the statistical analysis program SAS 9.1. All figures were constructed using EXCEL.

Results:

Characterization of stunted morphotype:

At the Cement Creek Road site, stunted *I. aggregata* had much smaller corolla lengths, corolla widths and style lengths than typical flowers ($P < .0001$) (table 1). The number of flowers, plant heights and flower heights did not differ significantly ($P > 0.05$) (table 1). Stunted flowers did not produce nectar.

Table 1: Mean measurements of 32 stunted and 32 typical *Ipomopsis aggregata* within three plots along Cement Creek Road.

Measurement	Stunted Means	Typical Means
Corolla Length (mm)	9.9 +/- 0.45	25.1 +/-0.43
Corolla Width (mm)	2.78 +/- 0.06	3.67 +/-0.05
Style Length (mm)	15 +/-0.51	23.9 +/-0.47
Stigma Exertion (mm)	4.8 +/-0.47	-1.32 +/-0.43
# Flowers	38 +/-3.69	34 +/-3.56
Plant Height (cm)	48.46 +/-1.18	47.5 +/-1.14
Flower Height (cm)	41.96 +/-1.13	40.22 +/-1.09

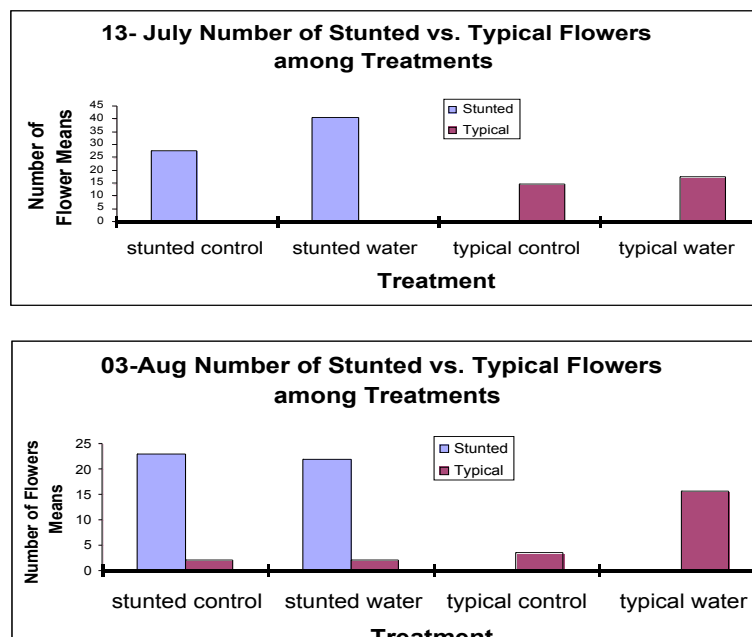
Effects of water on the stunted morphotype:

In the smaller experiment with both stunted and typical morphotypes, water had no significant effects on floral traits ($P>0.05$) (table 2). Nor did water have differential effects of the two morphs. Both control and watered stunted plants, however, began producing a few flowers with typically sized corollas by the end of the study (figure 1).

Table 2: August 3, 2007 measurements of stunted and typical flowers of both control and watered plants. ($P>0.05$)

Measurement	Stunted Control	Stunted Water	Typical Control	Typical Water
Corolla Length (mm)	8.29 +/- 1.07	8.47 +/- 1.07	25.54 +/- 0.95	23.5 +/- 1.54
Corolla Width (mm)	2.62 +/- 0.16	2.40 +/- 0.16	3.25 +/- 0.141	2.93 +/- 0.23
Style Length (mm)	9.65 +/- 1.11	1.31 +/- 1.11	20.45 +/- 1.60	17.53 +/- 2.61
Stamen Length (mm)	7.73 +/- 1.01	7.56 +/- 1.01	25.7 +/- 1.08	23.16 +/- 1.76

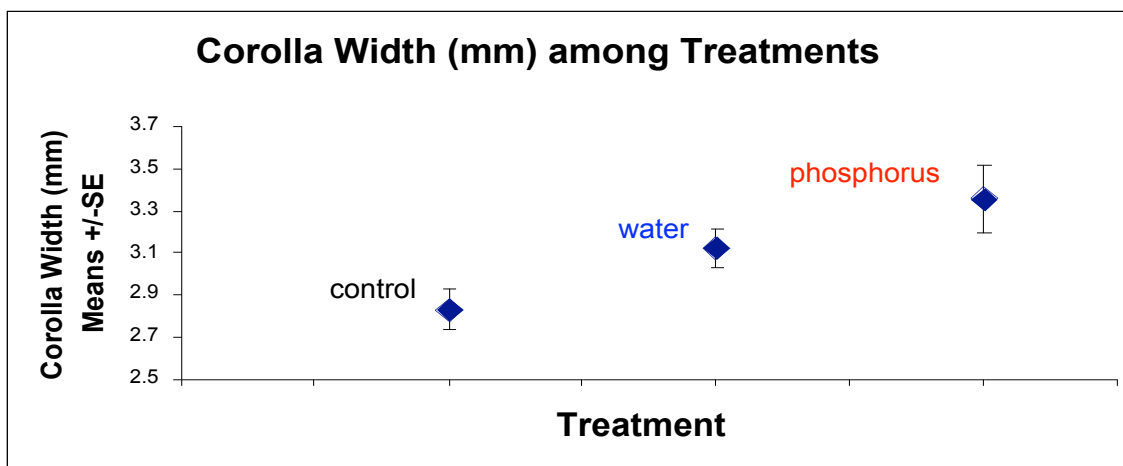
Figure 1: Numbers of stunted and typical flowers at the start of the manipulations (July 13, 2007) and at the end of the study (August 3, 2007) illustrating a growth in typical flowers on stunted plants.



Effects of Water and Phosphorus on typical morphotypes:

By August 3, 2007, plants in the various treatments differed significantly in corolla width ($F_{2,17}=4.60$, $P=0.0236$), with the control plants having the narrowest flowers (figure 2). When the analysis included plants for which all traits were measured on the last day, as in a MANOVA, plants receiving P had wider flowers than those only receiving water ($P=0.0142$). Both treatment groups differed from the controls ($P=0.0063$). The repeated measurement analysis showed that water significantly increased corolla width over the whole course of the study ($P=0.0132$). All other traits showed a similar trend of P treatments having increased measurements; however, they were not significant ($P>0.05$), with the exception that when the analysis included only the plants for which all traits were measured on the last day, water significantly increased npr compared to the average of the other treatments ($P=0.0487$). The MANOVA detected no overall affect of the treatments on the floral traits as a group (Wilk's Lambda $P=0.2124$).

Figure 2: Corolla Widths (mm) in the control, water and phosphorus treatments as of August 3, 2007 (ANOVA $P<0.05$)



Discussion:

Characterization of stunted morphotypes:

Stunted morphotypes along Cement Creek Road had significantly shorter corolla lengths, corolla widths and style lengths; furthermore, stigmas of stunted flowers were more exerted than typical flowers. A previous study showed that plants with strongly exerted stigmas receive more pollen per flower indicating selection favoring stigma exertion; however, narrower corollas don't allow as much pollen to be exported as wider corollas (Campbell 1994). Also, stunted corollas may discourage pollinators since hummingbirds, *I. aggregata*'s primary pollinators, typically prefer larger, more attractive flowers (Campbell et al. 1991). Such traits may effect pollination and consequently fitness of an *Ipomopsis aggregata* population containing stunted morphotypes.

The effects of water on stunted morphotypes:

Although there were no significant differences among treatments, plants previously containing only stunted flowers began growing a few typically sized corollas. Control and watered plants demonstrated this response showing there was no reaction to the water treatment. The first measurements were completed within the drought period, and there was considerable rainfall (>0.87 in) within the 3 weeks post-manipulation. Such growth could have been a response to rainfall or to other changes over the season. This may reflect the ability for plants with stunted morphotypes to grow typical flowers in the presence of a threshold amount of water or seasonal change.

The effects of water and phosphorus on typical morphotypes:

Plants which underwent phosphorus additions showed a significant increase in corolla width. A previous study established that *I. aggregata* plants with wider corollas export more pollen since hummingbirds can insert their bills more deeply into the tube, strongly contributing to efficient pollen export (Campbell et al 1991; 1996). In addition, most measured traits demonstrated a trend of increased variables compared to the watered and control plants. Hummingbirds typically prefer larger flowers which may aid in long distance attraction (Campbell et al 1991). Because such traits increase reproductive success, phosphorus may be an important nutrient in the overall fitness of *I. aggregata*.

A similar study by Campbell and Halama in 1990 was conducted within a much longer time-span of June 17- September 7. This study indicated a correlation between available soil phosphorus with the number of flowers and nectar volume of *Ipomopsis aggregata* (Campbell and Halama 1990). My study showed no significant effects on these traits which may indicate *Ipomopsis aggregata* is incapable of responding in the shorter time-span of July 13- August 3, 2007. Treatments may have to have been applied earlier in the season or during the plants' vegetative state.

The 1990 Campbell and Halama study found that water alone had no effect on any component of reproductive success. I found that water did increase corolla width and nectar volume within the plot consisting entirely of typical *I. aggregata*. The summer of 1990 experienced much more rainfall (4.4 in.) than the summer of 2007 (1.29 in.). Because rain was sparse, the flowers may have responded positively due to the previously limited moisture.

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