Does Altering Local Water Availability for an Invasive Plant (*Raphanus raphanistrum*) Affect Floral Morphology and Reproductive Potential?

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ABSTRACT

Abiotic environmental variation can have dramatic effects on plant floral morphology and nectar or pollen rewards. In response, pollinators may change their foraging behavior and distribution and if pollinators change their foraging behavior or distribution, this could have dramatic effects on the reproductive success of plant populations. To start tackling this problem, we measured the response of floral morphology (corolla diameter, stamen length, and ovule number) of *Raphanus raphanistrum* to experimental manipulations of field soil moisture. As soil moisture increased, corolla diameter and anther length grew. We expect these changes to provide more visitation rewards for insects in moist conditions. Therefore, water availability influences growth and development of flowers, and may have dramatic effects on insect community dynamics.

KEYWORDS
Floral Rewards, Climate, Rain-out Shelters, Flower Morphology, *Raphanus raphanistrum*, Brassicaceae

INTRODUCTION

Recently, plant and animal populations have experienced dramatic changes in the availability of water due to climate change; the prevalence of very dry areas (land areas with a Palmer drought severity index < −3.0) and the frequency of great inland flood catastrophes have doubled in the last 15 years relative to less recent history (1950's – 1980's)1, 2. With increasing infrared-absorbing 'greenhouse' gases, air temperature has risen by 0.89°C over the past century and rainfall during storms has intensified3. Obviously, climate can dramatically and directly influence the morphology and reproductive success of organisms, thereby altering population persistence and species geographic distributions4, 5. A large body of evidence now shows that changing climates have influenced the geographic distribution of many taxa6, moving species boundaries poleward an average 6.1 km per decade or up elevational gradients 6.1 m per decade5. Further, the timing of reproduction and development of biota has advanced an average of 2.3 days per decade (e.g., flowering, migration, etc.)5. Through anthropogenic climate change, changing water availability may change plant phenology and distributions and therefore altering the persistence and visitation patterns of pollinator populations. Altered water availability may affect pollinator visitation patterns by a) directly altering insect abundance and diversity or b) changing the features of flowers, including floral attractiveness and floral rewards.

Indirect environmental effects may negatively impact the survival and reproduction of some plant species7. Alternatively, some plants are capable of immediate and plastic phenotypic responses that may affect the attractiveness of their flowers to floral visitors. In response to environmental
cues, individual plants may change floral phenotypes via phenotypic plasticity⁸⁻¹¹. For example, under wet conditions, *Nicotiana quadrivalvis* produces larger flowers and greater nectar volume than under control conditions¹⁰. When dry conditions prevail, flowers tend to be smaller, to reduce water loss via transpiration¹⁰,¹². These changes may increase or decrease floral attractiveness. Larger flowers are more attractive to pollinators¹³⁻¹⁵. However, producing more nectar may decrease nectar quality, as the additional volume increases water quantity rather than sugar concentration, as observed in *N. quadrivalvis*¹⁰. Other floral attractants include corolla size, shape, and stamen length¹⁶,¹⁷.

In this study, we imposed a watering treatment on plants growing in experimental populations of the weedy annual *Raphanus raphanistrum* to investigate the effects of soil moisture variation on floral morphology and pollinator visitation patterns. *Raphanus raphanistrum* is a well-established model system in studies of plant evolution and ecology as well as insect foraging behavior¹⁸⁻²¹. Specifically, we asked does water availability influence the floral morphology and reproductive capacity of *Raphanus raphanistrum*? We predicted that flowers would get larger as water increases and therefore increase their reproductive potential both via floral attractiveness to insect pollinators and allocation to male and female function.

**METHODS**

**Study Species**

*Raphanus raphanistrum* is a self-incompatible, hermaphroditic, weed species that is closely related to the cultivated radish²²,²³. It is native to northern Europe and very abundant throughout the northeastern United States, among other places²⁴. Since *R. raphanistrum* is an invasive weed and has been noted growing in a variety of field crops, it is important to know the factors that influence its reproductive success. Cabbage butterflies, honey bees, small sweat bees, bumble bees and syrphid flies are the most frequent pollinators associated with this species²¹,²².

**Study Site**

The experiment was performed at Koffler Scientific Reserve (KSR) at Jokers Hill, King City, ON (44° 03’ N, 79° 29’ W) nestled in the Oak Ridges Moraine. The land offers about 350 hectares of diverse forests, pastures, and wetland communities. We carried out the experiment over the summer of 2011; the average monthly precipitation for that summer was 89.97mm²⁵.

**Watering Treatments**

We experimentally manipulated the amount of rainfall received by natural density plots (planted at a maximum density of 119 plants/11.15 m² plot or 10.67 plants per m²) of *Raphanus raphanistrum*. Each natural density plot was assigned a specific watering treatment: No Rain, Control Unsheltered, Control Sheltered, or Double Rain. To control the amount of rainfall received, rain exclusion structures (minimum 1.6 m above ground) were built to cover No Rain, Control Sheltered, and Double Rain plots (Figure 1). The No Rain plots received no rainfall. In contrast, Control Unsheltered plots received all natural rainfall and were used to represent conditions without manipulation of water availability or shading by the rain exclusion shelters. Control Sheltered plots received all the rainfall collected at their site, which we applied (sprinkling water on plots with a hose fitted with a spray nozzle) within 48 hours of any rainfall event. Control Sheltered plots acted as a control that accounted for the effect of the rain exclusion shelter. Finally, Double Rain plots received double the volume of rainfall of the control plots. We measured soil moisture at the center of each plot, using a TDR (Field Scout, TDR 100/200 Spectrum Technologies, Inc., Plainfield, IL, USA), at 10 cm depths after each redistribution of rainfall, a total of eight events during the growing season (between July 26, 2011 and September 28, 2011).
The 40 plots were organized in a completely randomized block design; each block consisted of eight plots, totaling in five blocks, or replicas, of the experiment. Plots within each block were located close together to increase the likelihood of sharing environmental conditions (i.e. soil quality, geographical landscape). There were 40 plots; each was $2.44 \text{ m} \times 3.05\text{m}$ in size and were separated by at least 200 m of distance between plots and scattered across the reserve, so that treatments imposed on one plot would have little effect on responses in another plot. The shelters were built with wooden frames held up approximately 2m above the ground by four fencing posts and metal rods, with an angled roof to let the rainwater run down the roof. Clear plastic sheets prevented rain from falling but allowed sunlight to reach plants. A gutter was attached to wooden frame and rainwater was diverted to a 208L barrel (note that 208L is approximately the amount of rainwater collected during a heavy rainfall at our site).

To determine the effect of the experimental rainfall treatment on soil moisture in each treatment type (No Rain, Control Shelter, Control Unsheltered, Double Rain), we used a repeated measures ANOVA that included type of watering treatment, date of moisture treatment, and their interaction. Block was treated as a random effect. Post-hoc comparisons among treatments were established using Tukey's HSD test.

**Floral Morphology**

In order to determine if plant floral morphology responded to variation in water availability, we measured three floral traits: corolla diameter, stamen length, and number of ovules for plants grown in Double Rain, No Rain, or Control Sheltered conditions.

Floral morphology was measured on a second group of 40 controlled density plots (72 plants per 11.15 m² or 6.46 plants per m²) where plant density within plots was held constant (another factor that may affect floral traits). Here, only three plots per block were sheltered and thus were the only plots to receive an experimental watering treatment (i.e. Double Rain, No Rain, or Control Sheltered); the other five plots per block were Control Unsheltered. For these Controlled Density plots, the rain-exclusion shelter design differed only in that the roofs were shorter than the shelters over the natural density plots (one meter above ground level) and the intercepted rain was diverted away from the plot, and plants in plots were watered with water from a local well instead. The Controlled Density plots were located within a meter of each other. *Raphanus raphanistrum* seeds were germinated in seedling trays. We thoroughly tilled the soil of a common garden. On June 20th – 23rd, 20 focal seedlings were transplanted into each prepared plot. In addition to the 20 focal seedlings, two border rows were added to maintain constant competition within the plots, resulting in a total of 72 plants per plot; all seedlings were planted within 30cm of each neighbor and plots were weeded to maintain constant levels of competition.
To measure the effects of soil moisture on three floral attributes (corolla diameter, stamen length, and number of ovules), two flowers per plant were collected randomly from three randomly selected plants per Controlled Density plot in late August 2011; a total of 240 flowers were measured. Corolla diameter was measured at the widest part of the corolla with digital calipers. Similarly, the longest stamen of a flower (measured from the point of insertion at the ovary) was measured with a caliper. In order to count the number of ovules, the pistil of a flower was carefully dissected and each ovule was counted.

To test for differences in floral morphology among plants grown in the four watering treatments, we ran a linear mixed model ANOVA for each floral trait. Watering treatment was considered to be a fixed effect and block was a random effect. Variance of random effects was estimated using restricted maximum likelihood in SPSS (v. 20, IBM, Armonk, New York). When differences were detected, a pairwise comparison was run to compare the mean values across the four watering treatments. Corolla diameter was natural log transformed to meet normality assumptions of the ANOVA.

RESULTS

The Effect of Watering Treatments on Soil Moisture

Average volumetric moisture content (VMC) of the soil differed significantly among watering treatments ($F_{3,240} = 720.2, P < 0.0001$, Figure 2). Control Unsheltered plots were 2.7% drier than Control Sheltered plots ($t_{212,240} = -3.80, P = 0.0002$), whereas the soil moisture in No Rain plots was 16.8% lower than the Control Sheltered plot ($t_{212,240} = 18.52, P < 0.0001$) and the soil moisture in the Double Rain plot was 6.3% higher than the Control Sheltered plot ($t_{212,240} = -6.94, P = 0.0001$). Soil moisture declined significantly over the season ($F_{5,240} = 35.2, P < 0.0001$) and there was a significant interaction between watering treatment and date of sampling ($F_{15,240} = 16.44, P = < 0.0001$).

![Figure 2. Average soil volumetric moisture content (% ± SE) across watering treatments.](image-url)
Responses of Floral Morphology to Water Availability

Changes in water availability may change the size and pollen reward provided by flowers, thus potentially changing the attractiveness of these flowers to insects. A multivariate ANOVA revealed that watering treatments significantly affected floral morphology ($F_{9,209} = 12.51, P < 0.001$).

Average corolla diameter differed significantly among the four watering treatments (Figure 3, Univariate ANOVA: $F_{3,88} = 20.96, P < 0.001$). There was no significant difference in corolla diameter between plants grown under No Rain and Control Unsheltered conditions (Mean difference = 2.64 mm ± 0.03, $P = 0.86$). Further, corolla diameter of flowers grown under Control Sheltered and Double Rain conditions was not significantly different from each other (Mean difference = −0.041 mm, $P = 0.487$). However, plants grown under No Rain and Control Unsheltered conditions tended to produce flowers with smaller corollas than plants grown under Control Sheltered and Double Rain conditions (e.g., Mean difference between the Control Unsheltered and Sheltered = 2.76 mm ± 0.03, $P < 0.001$; other comparisons had greater mean differences).

![Figure 3. Comparison of corolla diameter (mm) between treatments (log transformed mean ± SE, n = 240).](image)

Similarly, stamen length differed significantly among the four watering treatments (Figure 4, Univariate ANOVA: $F_{3,88} = 22.42, P < 0.001$). The stamen lengths of the Control Unsheltered and Sheltered treatments were not significantly different (Mean difference = 0.049 mm, $P = 1.000$). Stamen length was significantly shorter on plants grown under No Rain conditions than Control Unsheltered (Mean difference = 11.53 mm ± 0.22, $P < 0.001$) and stamen length was significantly longer on plants grown under Double Rain than Control Sheltered conditions (Mean difference = 12.45 mm ± 0.30, $P < 0.05$).
Finally, the number of ovules differed significantly between watering treatments (Figure 5, $F_{3, 88} = 7.44, P < 0.001$). Plants in No Rain plots tended to produce fewer ovules per flower than plants grown under other watering treatments (Mean difference of No Rain plants to: Unsheltered Control: 7.50 ovules ± 0.33, $P = 0.035$; Sheltered Control: 8.13 ovules ± 0.04, $P < 0.001$; Double Rain (7.87 ovules ± 0.45, $P = 0.003$). However, there was no significant difference in the number of ovules produced by plants grown in Unsheltered Control, Sheltered Control or Double rain ($P > 0.05$).
DISCUSSION

By imposing experimental watering treatments, we were able to successfully manipulate soil moisture (Figure 2). This led to predictable and significant differences in floral morphology between plants grown under the four watering treatments. We predict that floral attractiveness could change with consequent changes in floral morphology, and below we outline a research mandate for this question.

We predicted that floral traits should become smaller under dry conditions and larger under wet conditions compared to plants grown under control conditions\(^{10, 27}\). If a plant can produce larger flowers with more rewards (e.g., pollen), then it becomes more attractive to potential pollinators. Successful pollination will likely have a positive effect on fertilization and seed production.

Differences in corolla size can change the probability that an insect will notice and visit the flower; therefore environmental conditions that can affect the probability of insect visitation may have dramatic consequences for plant fecundity. Across the literature, corolla size is repeatedly correlated with water availability\(^{10-12}\). For instance, Frazee & Marquis\(^{12}\) found that, compared to controls, corolla diameter of *Chamaecrista fasciculata* decreased when plants experienced a low water treatment. Although larger corollas can attract more pollinators\(^{27}\), higher pollinator visitation may be costly. They found that *Mimulus ringens* plants with larger flowers were more prone to geitonogamy (i.e., pollination of one flower with pollen of another flower of the same plant) than plants with smaller flowers. Increased geitonogamy increases selfing rates and, in plants that experience strong inbreeding depression, this can lead to reductions in plant fitness\(^{27}\).

Surprisingly, plants grown in the Control Unsheltered plots had significantly smaller flowers than plants grown in the Control Sheltered plots. In fact, plants in Control Unsheltered plots produced small flowers, similar to those in the No Rain plots. In contrast, corolla diameter in the Control Sheltered plots produced relatively large flowers, similar to those in the Double Rain plots. We suspect that although the plants in the Control Unsheltered control plots had plenty of water from natural rainfall, plants in the Control Unsheltered plots experienced more evapotranspiration than those in sheltered plots and therefore the moisture environment of the Control Unsheltered plots was more similar to that of the No Rain treatment.

As expected, moisture had a significant and positive effect on stamen length. Similar results were obtained by Frazee & Marquis\(^{12}\) who found anther length to be significantly shorter in plants grown in a low water treatment as compared to a high water treatment. In *Raphanus*, differences in stamen length can attract different flower visitors\(^{28}\). Larger pollinators, such as honey bees or bumble bees, prefer flowers with a longer stamen such that the anthers are located higher above petals. In contrast, as smaller bees land on a petal, they can have more difficulty collecting pollen from a tall anther\(^{28}\). Therefore, the change in stamen length can lead to a more specialized pollinator requirement, thus reducing the total number of potential pollinators that can transfer pollen.

Finally, the number of ovules is directly related to how many offspring a plant can produce when there is successful and complete pollination. Thus, it stands to reason that a flower will produce the most ovules it can support, given the resources available. We predicted wetter environments would support more ovules per flower, whereas drier environments would support fewer ovules per flower. In our experiment, flowers grown in the drier environment did produce fewer ovules per flower than control populations. Similar results were obtained by Frazee & Marquis\(^{12}\); in drier conditions, *C. fasciculata* produced fewer ovules compared to plants grown with plenty of water\(^{29}\). While fewer ovules means reduced reproductive potential, it can also mean that the plant will spend more energy on producing more viable seeds. On the other hand, a flower with more ovules can afford to produce one or two seeds per fruit that are less viable than the rest\(^{12}\). However, we found that plants grown in the wetter environment produced similar number of ovules per flower compared to the controls. Therefore, increased water availability did not increase floral fertility via ovules. Other plants show this insensitivity to variation in soil moisture as well. For instance, *Trigonella coerulesa* revealed no significant difference between ovule production in either Double Rain or No
Rain conditions as compared to controls. Our results suggest that ovule production on plants grown under control conditions was not limited by water resources, but may have been limited by other nutrients. Intriguingly, water availability seems to have a greater impact on those floral traits that affect male fitness (corolla diameter, stamen length) more than floral traits that directly affect female fitness (ovule production).

Plants grown under dry conditions had decreased flower size. Moreover, the scarcity of water also lead to decreased ovule production and we hypothesize may reduce pollen production via the concurrent reduction in anther size. Thus, we predict that dry conditions may have reduced the attractiveness and fitness of *Raphanus* plants. On the other hand, increased water availability increased flowers size and stamen length (but not the number of ovules), thereby increasing traits normally associated with floral attractiveness but not their potential fecundity. Consequently, we predict that the plants grown under dry conditions spent less resources on petal and stamen growth in order to produce more viable (although fewer) seeds.

**Conclusion**

Pollinator and insect communities are essential to the reproductive success of many plants. The effects of environmental change can influence this intricate interactive web, influencing both the plants and the insects. In this experiment, watering treatments changed the environmental conditions of the plots, which in turn influenced the floral morphology of *R. raphanistrum*. As hypothesized, we saw the changes in traits normally associated with floral attractiveness as drier conditions lead to the development of smaller flowers. These changes can affect the reproductive success of the *Raphanus*, as pollinators rely on these floral cues when searching for flowers with high rewards (i.e. pollen and nectar). With the changes in floral morphology, we predict insect diversity and foraging behavior will also be significantly impacted.

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PRESS SUMMARY
As has been widely discussed recently, climatic conditions and specifically rainfall patterns, of our planet are changing dramatically. Changes in rainfall may alter the attractiveness of flowers to insect pollinators. We manipulated water availability (both increasing and decreasing soil moisture) and measured the consequences of water availability on flower size in an agricultural weed, wild radish. We concluded that increasing water availability may make flowers more attractive to insect visitors.

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