

How Does Altered Phenology Affect Pollination and
Reproduction of *Delphinium nuttallianum*?

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Abstract

Due to climate change, wildflowers are blooming earlier and earlier and suffering lower reproductive success. There are two general, non-mutually-exclusive hypotheses about why this lower reproductive success may be occurring. One is that plants are blooming before their pollinators and therefore are experiencing a pollen deficiency. The other is that plants are coping with other environmental effects associated with earlier bloom time, such as drought and frost, which directly affect their survival and reproduction. Here I used a snow removal experiment to stimulate earlier flowering and measured pollen receipt, a proxy for pollinator visitation, and seed set. I also used pollen supplementation experiments to attempt to distinguish the effects of potential pollinator deficiency vs. other environmental factors on plant reproduction. I found that plants in the snow removal treatment received significantly less pollen than the plants in the controls; however, I failed to provide additional pollen in the pollen supplementation experiment and so I could not experimentally assess whether pollen limitation affected seed set. Nevertheless, these results suggest that early-blooming plants suffer harsh abiotic conditions, causing them to divert less energy to pollinator attraction in favor of seed creation and survival.

Introduction

Phenology is the timing of cyclical life history events, such as when flowers begin to blossom, birds migrate, and animals hibernate. Many organisms rely on abiotic cues, such as photoperiod, temperature, and snowmelt date, for their phenology. However, climate change has already shifted these abiotic cues and is expected to shift them even more in the future; atmospheric temperature alone will rise by an estimated 1.7-4.9°C from 1990-2100 (Wigley and Raper 2001). In response, many phenological events have shifted: amphibians in the United States now breed earlier in the season than they historically have, while many flowering species now bloom at an earlier date, both in the United States and Europe (Bradley et al 1999, Menzel et al 2006).

Changes in phenology can prove harmful for many organisms. If a bird migrates south too late in the season, it may encounter colder temperatures than it is adapted for. Additionally, wildflowers rely on date of snowmelt and ambient temperature to determine their flowering date. The melting of snow warms the temperature of the soil, initiating springtime flowering (Thompson 2010). According to Schmitt (1983), flowers that bloom too early in the season have lower reproductive success than those that bloom toward the middle of the season, because those individuals induced to bloom early might not be adapted to early-spring conditions. In short, shifts in phenology precipitated by climate change could pose a hazard to flowering plants.

There are at least two hypotheses, which are not mutually exclusive, about what causes the reduced reproductive success for early-blooming plants: phenological-mismatch and abiotic factors. The first theory posits that the plant-pollinator mutualism becomes disrupted by phenological changes. If plants bloom before their pollinators have the chance to emerge from their winter dormancy, a temporal dislocation may occur between plants and their pollinators, known as a phenological mismatch. Phenological mismatches could lead to pollen limitation and reduced plant reproduction. However, Forrest et al (2010) found that although plants may be flowering at a time when the insects that traditionally pollinate those plants have not yet emerged, other insect

pollinators emerge who are also effective pollinators, so that most plants are pollinated by at least some insects. The second hypothesis posits that plants with advanced flowering phenology experience dramatically different, and potentially deadly, abiotic conditions. For example, although global temperatures continue to rise, frost still strikes every year, causing damage to early-blooming plants (Inouye 2008). Additionally, earlier-blooming plants also face threats of differing photoperiod (around a 35 minute difference in daylength for a single month) than the plants that bloom later. Additionally, the lessening of snowpack that comes with climate change causes less water to melt out when spring arrives, causing a higher incidence of drought. Thus, plants could be pollen-limited, face environmental challenges that cause them to have lower reproductive success than plants that bloom later in the season, or face a combination of these two problems. Without a match with their pollinators, plants may struggle to reproduce; but with a harsh outside environment, plants may struggle to survive.

Though studies suggest that both mechanisms might act in concert, few studies have attempted to differentiate between them and show their relative strengths. This study attempted to determine if a wildflower in sub-alpine environments near the Rocky Mountain Biological Laboratory (Gothic, CO) is pollen-limited, or if the lowered reproductive success in these plants is due to other environmental factors.

Methods

Study system:

This study was conducted at the Rocky Mountain Biological Laboratory in the Elk Mountains of Colorado. We studied the focal plants species *Delphinium nuttallianum* (Ranunculaceae). *Delphinium nuttallianum* is a perennial, protandrous herb with a mixed mating system that is distributed across the Western United States. Protandrous plants have both male and female flowers. The flowers first bloom with male parts, and the flowers become female later in 1-3 days. At our study site, *D. nuttallianum* is mainly pollinated by queen bumblebees, solitary bees, and hummingbirds (Bosch and Waser 1998). Seed set of *D. nuttallianum* has been shown to be greater when pollinated by outcrossed pollen, heightening the importance of pollinators in this system (Waser and Price 1991).

Experimental procedure:

The study took place in a subalpine meadow on the 401 bike trail, roughly 200m north of the Judd Falls parking lot (Figure 1, 38°57' 58.612"N 106°59'25, 168"W). We established 20 2x2m plots haphazardly distributed within the meadow. The plots were randomly assigned to one of two phenology treatments: snow removal and control. Snow was shoveled from the snow removal plots on April 9, 2013, when average snow depth was approximately 90cm. Snow was removed until only 5cm remained to prevent disturbance of snow and plants within plots. The 10 control plots were allowed to melt out naturally.

Within each of the 20 plots, we marked up to 12 individual *D. nuttallianum* plants. Half of the marked plants were randomly assigned as "open pollination" plants and the rest as "hand pollination". Open pollination plants could be visited by pollinators as usual, with no intervention from the researchers. Female flowers of hand pollination plants were given supplemental pollen—in addition to the pollen received from

pollinators—every other day throughout their flowering. I performed hand pollination by picking individual male flowers that were growing 1-10 m away from the plot, exposing dehiscing anthers, and gently rubbing them against the stigmas of all female flowers of marked, hand pollination plants.

Each time we hand-pollinated, we also recorded the number of male and female flowers on each focal *D. nuttallianum*, which can be used as a measure of phenology. On each sample day, we also identified all flowering species within each plot and recorded their abundance and number of open flowers per plant.

When the *D. nuttallianum* petals fall and fruits begin to expand, we collected stigmas from up to 3 flowers per plant. Collecting stigmas at this stage should not affect fruit or seed set. Stigmas were pressed and stained using fuschin jelly (Kearns and Inouye 1993), and the number of conspecific and heterospecific pollen grains were counted as a metric of pollen receipt. For each plant, we calculated the mean number of pollen grains received per flower. We collected all fruits once mature, recorded any aborted fruit capsules from flowers that failed to set fruit, and counted all seeds, using proportion fruit set (number of seed-bearing fruits/(seed-bearing + aborted fruits)), average seeds per fruit, and total seeds per plant as metrics of female reproduction.

Statistical analysis:

We first compared the difference between the flowering phenology of the two treatments (snow removal vs. control) by calculating average flowers per plant (averaging the number of *D. nuttallianum* flowers from each plot that we recorded, and then averaging those numbers per treatment). I then graphed these measures of average flowers against date to create a graph of floral phenology over time.

To test whether snow removal affected pollen receipt per flower and female plant reproduction, we performed t-tests. As the unit of replication, we used the plot. Thus, for each plot, we calculated pollen receipt per flower per plant per plot and metrics of female plant reproduction (proportion fruit set, mean seeds per fruit, and total seeds per plant) per plant per plot.

Results:

Graphical description of *D. nuttallianum* flowering phenology showed that the snow removal plots exhibited peak flowering approximately 4 days earlier (12 June 2013) than control plots (16 June 2013; Figure 2). However, although the snow removal plots bloomed earlier by about 13 days, they ceased blooming at the same time as control plots, so the total length of *D. nuttallianum* flowering was longer in the snow removal than control plots.

Despite hand-pollinating flowers throughout the blooming season, we failed to provide additional pollen to stigmas in the pollen supplementation treatment (Figure 3, $t_{146} = 0.4516$, $p = 0.6522$). Thus, we did not analyze the pollen supplementation treatment further for seed production.

Control plots had a higher number of seeds than snow removal plots ($t_{11} = 2.068$, $p = 0.0320$). Plants in the snow removal plots had 38% fewer grains of pollen per flower than plants in the control plots (Figure 4). When one outlier outside of the upper quartile was removed from the analysis, estimates of female reproduction, measured by average number of seeds per fruit, were greater for plants in control plots than plants in removal

plots (Figure 5, $t_{15} = 2.042$, $p = 0.0296$). There was no significant difference between the two plot treatments in terms of proportion fruit set ($t_{73} = 0.4754$, $p = 0.6359$).

Discussion

The plants in our snow removal plots had less pollen and a smaller seed set than the plants in the control plots. However, the longer flowering time of the plants in the removal plots, and the similarity in the peak flowering dates of the flowers in the two plots (off by only four days) suggest that this difference in pollen and seed set is not attributable to a phenological mismatch alone.

There are two possibilities for why there is no difference in amount of pollen between supplemented and open-pollination plants: either there was an error in hand-pollination, or the stigmas were saturated with pollen. If the stigmas were saturated, this would indicate that seed set is less in the snow removal plots for reasons other than pollen limitation, like drought or frost.

If the stigmas were saturated—something perhaps suggested by the high level of pollen in each flower (R. Irwin, personal communication, August 1, 2013)—this begs the question of why control plants have a higher level of pollen than snow removal plants. There are a few papers that suggest that plants vary their threshold for stigma saturation under different conditions. Parrie and Lang (1992) found that stigma saturation levels change in response to self versus outcrossed pollen in blueberry plants. This may suggest that plants in the snow removal plots may have had a lower stigma saturation point than the plants in the control plots, in response to environmental factors. Unfortunately, it is impossible to distinguish between failed hand pollination and changed pollen saturation levels in this analysis. More investigation is needed.

In their 1988 paper, Haig and Westoby suggest that female reproduction is limited not only by pollen availability, but by environmental factors as well. Energy allocated to pollinator attraction also takes energy away from the task of reproduction, implying that female plants should reach an equilibrium between these two tasks. At this equilibrium, if pollen receipt increases, reproduction will not be affected because the plant is still limited by environmental factors. A phenological mismatch with pollinators may not have been the cause of the plants in the snow removal plots having a smaller amount of pollen than the plants in the control plots (suggested by the similar phenology between the two plot treatments). Instead, poorer environmental conditions experienced at the beginning of the blooming period may have caused the plants to lower the energy they put into pollinator attraction in favor of putting effort into reproduction and seed creation with the pollen that they were able to amass.

Inouye (2008) found that early-blooming flowers have frost damage on bud heads, lowering reproductive success in those flowers. Factors such as frost and drought, or even just cooler temperatures, could have had a large effect on the plants in snow removal plots in my study as well, which would be some of the abiotic factors that forced them to divert less energy into pollinator attraction and seed production. The fact that the flowers in the snow removal plots had a similar peak flowering date and flowering end as the control plots suggests that the abiotic factors that were affecting them occurred toward the beginning of their flowering, making things like cooler temperatures and frost likely contenders for causes of lowered reproductive success in the early blooming *D. nuttallianum*.

However, Kudo et al (2004) and Gross and Werner (1983) found that phenological mismatch was at least partially responsible for lowered seed set in early-blooming, but not late-blooming, flowers, especially those pollinated by bees. In actuality, phenological mismatch and abiotic factors most likely interact to a high degree to cause lowered reproductive success in early-blooming plants. Phenological mismatch may not have been as much of a factor in the case of this study because of the similarity in peak flowering date between the plants in the control and removal plots. However, plants in removal plots bloomed much earlier than those in control plots (a difference of ~13 days; see Figure 2), causing there to be a higher chance for them to be affected by abiotic factors early in the season, such as frost and drought.

This suggests that as the climate continues to warm, and snow begins to become less frequent and melt up to forty days earlier (Stewart et al 2004), flowers will continue to bloom early and encounter environmental conditions they are not suited for, leading them to focus less on pollinator attraction and more on reproduction. This will cause both a reduction in pollen receipt and seed set, leading to lower reproductive success for early-blooming plants.

The obvious problem with this study was the lack of difference in pollen count between open pollination and supplemented plants. This problem caused us to be unable to distinguish with finality between the effects of phenological mismatch and abiotic factors. There was some evidence in the data to suggest that there may have been a time effect of a hand pollination "learning curve" or multiple hand pollination helpers before I was able to take over the project. For stigmas collected later in the season, there seems to be a more distinct difference between open and supplemented plants, with supplemented plants having more pollen than open plants. However, the sample size for these stigmas collected later in the season is small and therefore the differences are not significant. In future studies, it would be important to have a constant hand pollinator throughout the season with previous experience with pollinating *D. nuttallinaum*.

Marriage Meadow Plots

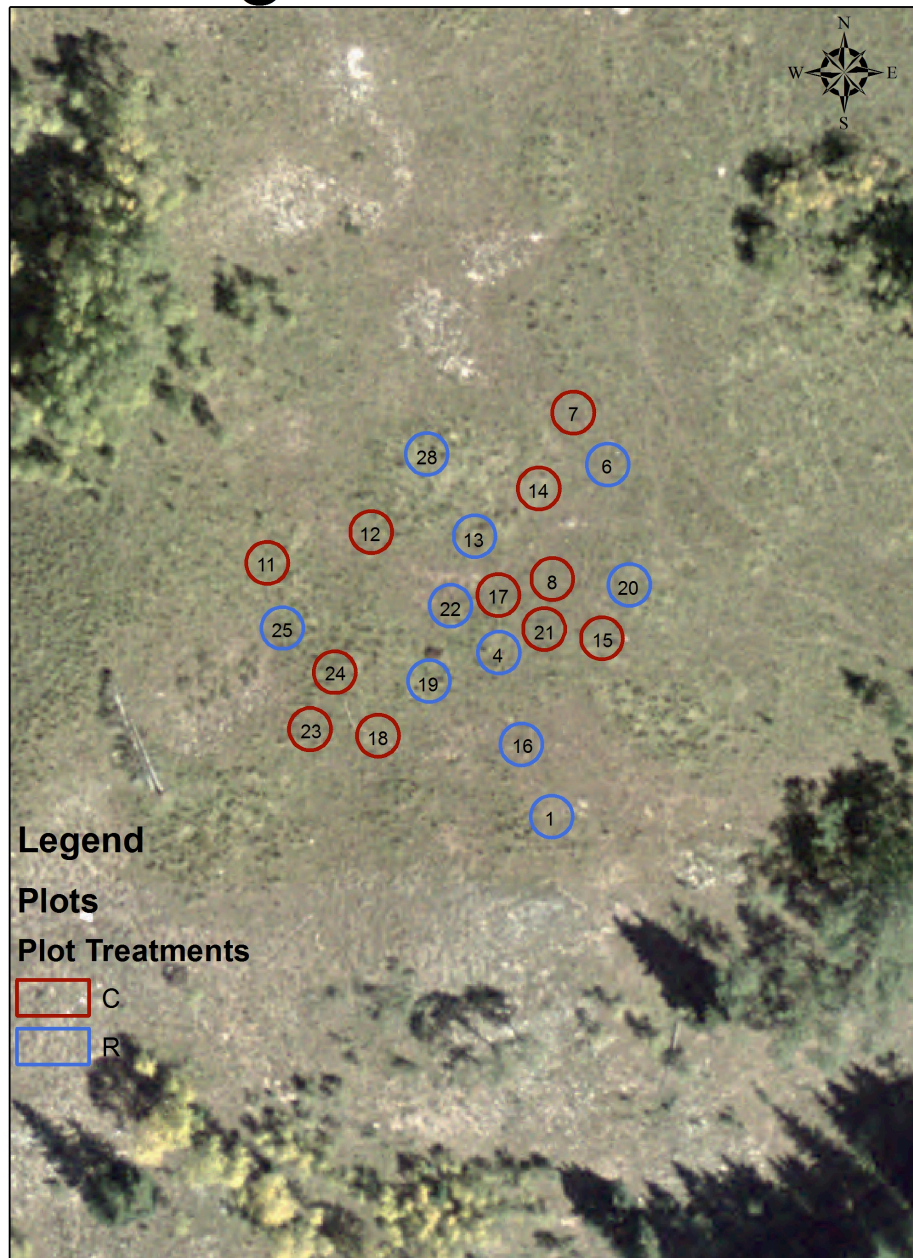


Figure 1: Map of focal study plots in Marriage Meadow

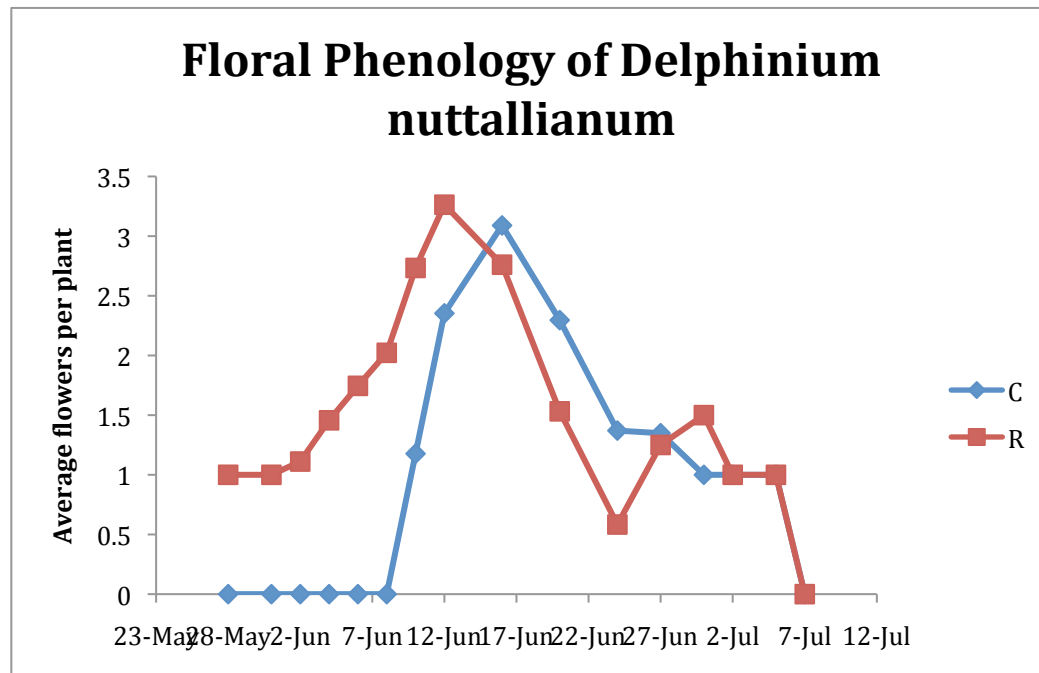


Figure 2: The average number of flowers per *D. nuttallianum* plant plotted over time for the control and removal plants. Although the removal plants begin to bloom earlier than the controls and have a slightly earlier floral phenology peak, both the control and removal plants finish blooming at the same time.

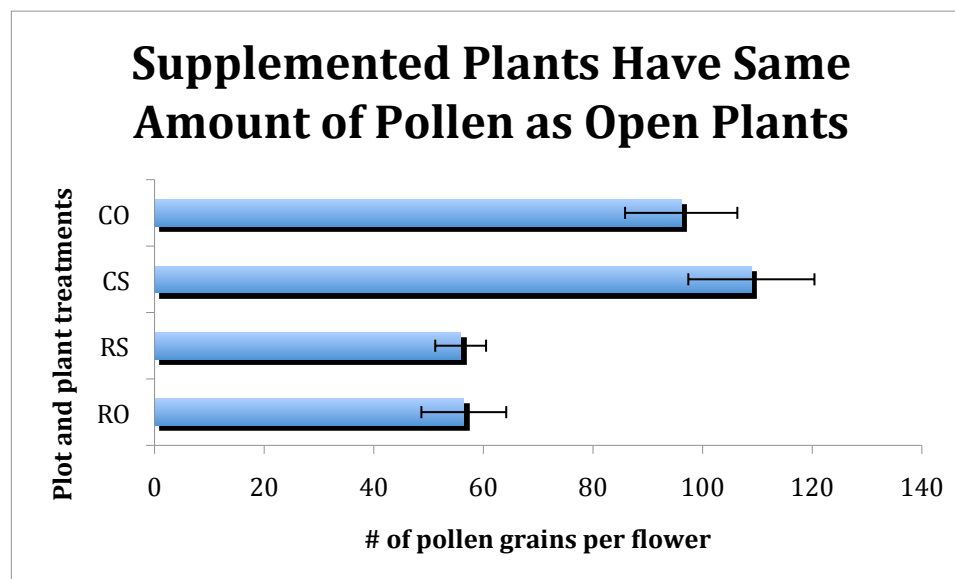


Figure 3: Average number of pollen grains per flower (number of pollen grains/number of flowers from which stigmas were collected). Although the removal plots (R) differ significantly from the control plots (C), open pollination plants (O) did not differ significantly from supplemented plants (S).

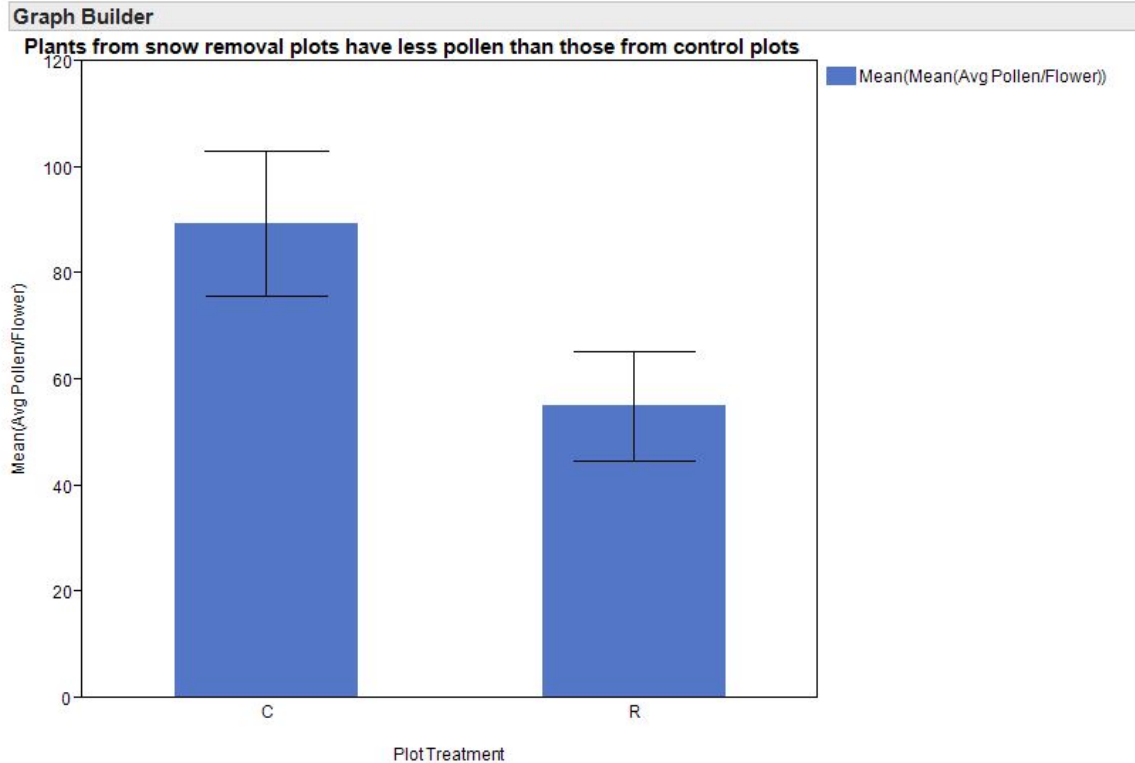


Figure 4: The average number of pollen grains per flower by plot for CO and RO plots only. There is a significant difference between the two treatments.

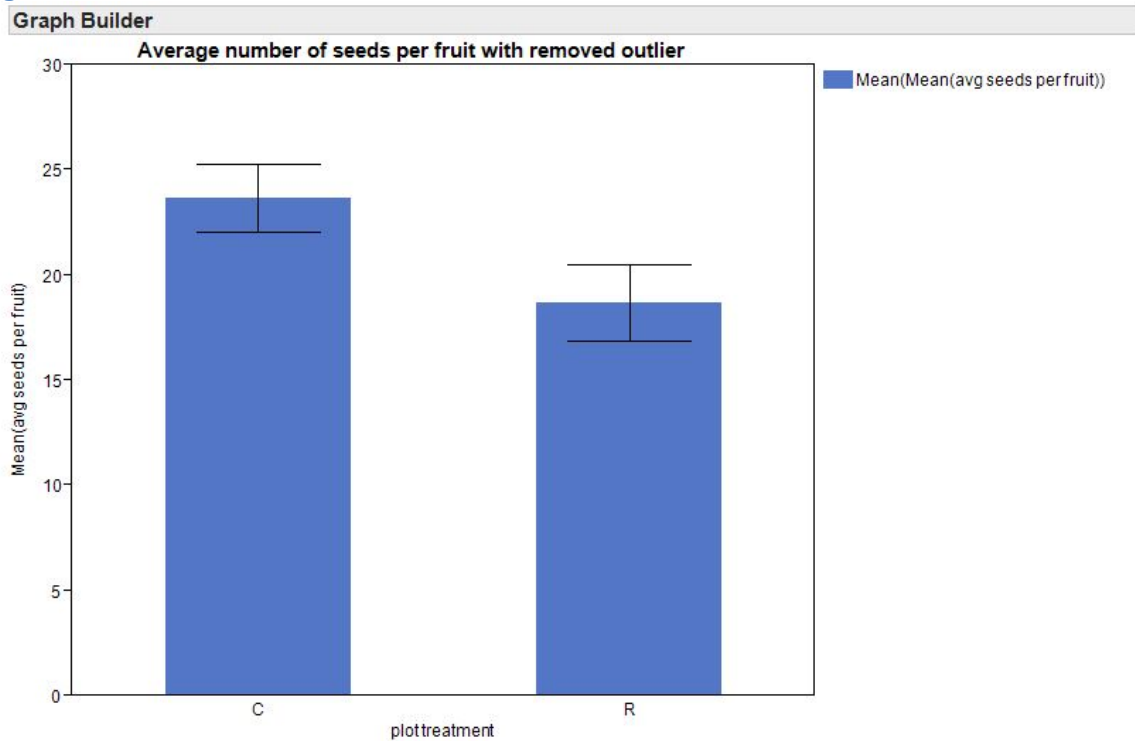


Figure 5: The average number of seeds per fruit for CO and RO plots only. When the single outlier is removed from the R treatment in the graph above, the difference between the two treatments becomes significant.

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