ASSESSING THE RELATIONSHIP BETWEEN FLOWERING TIME AND FITNESS IN *LEAVENWORTHIA STYLOSA*

by

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ABSTRACT

Recent research has shown a trend towards early flowering time in a variety of species around the world. *Leavenworthia stylosa*, a cedar glade species endemic to the Central Basin of Tennessee, appeared to also follow this pattern. Herbarium specimens showed a trend towards earlier flowering in *L. stylosa* over the last century. In two years of research, there was no overall fitness cost or benefit to flowering earlier than the population average within years, but there was a fitness cost for individuals that flowered later than average. Between years, higher fruit set was found during a year with unusually early flowering compared to a year with more typical flowering time. Based on analyses of historical climate data, earlier flowering did not appear to be due to warmer spring temperatures. Early flowering was also not the result of earlier fall germination time.

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INTRODUCTION

Timing of life history events is crucial to the reproductive success and survival of individuals of all species. In plants, key life history events include germination time (Manzano-Piedras et al. 2014), the length of the growth period (Bolmgren & Cowan 2008), initiation of flowering (Manzano-Piedras et al. 2014), and the duration of the flowering period (Austen et al. 2017). A shift in the timing of any of these can result in changes in both the abiotic and biotic environments to which the plant would be exposed during these and subsequent life history phases (Rafferty et al. 2015). This could result in plants flowering when their usual pollinators are not present (Hegland et al. 2009), or when environmental conditions are unfavorable for pollination or seed production. Therefore, a small change in the timing of a single life history event of a plant can have a tremendous influence on plant fitness.

The timing of seed germination is among the most important life history events for a plant, as it can have a large influence on survival rate and reproductive success (Baskin & Baskin 1972; Philippi 1992; Baskin et al. 2003; Gremmer et al. 2016). Early seed germination may expose seedlings to unfavorable abiotic conditions such as freezes for spring germinating species, or hot and dry conditions for fall germinating species (Baskin & Baskin 1972). On the other hand, early germination may allow plants a longer growth period, which could result in having more resources available for flowering and seed production (Austen et al. 2017). Additionally, since the onset of flowering is often related to plant size, germination time could influence the onset and duration of flowering

(Pemadasa & Lovell 1974; Lacey 1986; Munguia-Rosas et al. 2011). Although it is typically assumed that there is a trade-off between plant growth and the onset of flowering (i.e. earlier flowering plants tend to be smaller than those that flower later) (Elzinga et al. 2007, Austen et al. 2017), in a study of annual and biennial dioecious plants, Forrest (2014) found that in 24 of 28 species, early-flowering plants were actually larger than plants that had not yet flowered. One factor that could explain the larger size of early-flowering plants is differences in plant age between early- and late-flowering plants (Austen et al. 2017) that would result if early-flowering plants had also germinated earlier. Early germination could be beneficial if it allows earlier onset of flowering and a longer flowering season (Austen et al. 2017), which could result in greater seed production. However, early germination may be costly if earlier-germinating seeds have reduced survival (Baskin & Baskin 1972).

The flowering time of a plant, both in terms of the initiation of flowering and the duration of flowering, are also key life history events. Early-flowering individuals can potentially have a longer duration of flowering (Hendry & Day 2005; Austen et al. 2017). However, if an individual flowers too early, it may not have had enough time to build up sufficient resources and will then have a limited capacity for seed production (Elzinga et al. 2007; Austen et al. 2017). Conversely, if an individual plant flowers too late, there may be less time available in the season for the individual to adequately produce flowers and fruits (Elzinga et al. 2007; Austen et al. 2017). Additionally, selection on the flowering time of individuals will depend on the flowering schedules of other individuals of the same species. Synchronous flowering among conspecific individuals allows them to increase the chances of successful outcrossing and reproduction within a short flowering season (Fleming 2006) while pollinators are available (Grant 1971; Waser & Real 1979).

Environmental conditions also play a crucial role in flowering time of all plants. It can be detrimental for a plant to flower too early due to the risk of frost (Anderson et al. 2012) or lack of pollinators (Elzinga et al. 2007). However, it can also be costly for a plant to flower late if environmental conditions will become too harsh for survival before the completion of the plant's reproductive cycle (Franke et al. 2006). Therefore, flowering when conditions are most favorable is key to reproductive success (Ream et al. 2014) and requires plants to continuously and accurately monitor environmental cues and conditions such as temperature, precipitation, and day length (Bernier and Perilleux 2005).

Recent studies have shown that there has been an overall trend towards earlier flowering in a large variety of plant species (Fitter & Fitter 2002; Primack 2004; Amano et al. 2010; Munguia-Rosas et al. 2011). Fitter and Fitter (2002) found that there has been a distinct shift towards earlier date of first flower since the 1980's for several British plant species, including two species with significantly extreme deviations from their previous first-flowering dates. In a meta-analysis, Munguia-Rosas et al. (2011) found that in two data sets of 87 and 18 plant species, selection favors early-flowering plants, particularly in temperate environmental conditions, potentially because temperate climates typically have a shorter duration of flowering. Primack et al. (2004) used herbarium specimens and current flowering time observations to determine that multiple species of plants in the Boston area were flowering an average of eight days earlier than they were a century ago. So why are plants flowering earlier? Most studies attribute earlier onset of flowering, either entirely or partially, to climate change and the warmer spring temperatures associated with it (Fitter & Fitter 2002; Primack et al. 2004 Anderson et al. 2012; Austen et al. 2017). However, these studies are often correlational, and it is not clear whether earlier flowering is due to a response to warmer temperatures in flowering time itself, or variations in temperature influencing other life history stages as well. For example, if seeds of annual plants germinate earlier, all other phenophases may also occur earlier as a consequence of early germination.

Plant populations may be evolving earlier flowering times, either in response to climate change or in response to other factors. An array of studies and a meta-analysis have provided evidence to suggest that early flowering time is being favored via natural selection (Gerber & Griffen 2003; Harder & Johnson 2009; Munguia-Rosas et al. 2011; Austen et al. 2017). Munguia-Rosas et al. (2011) found that early flowering is being favored particularly in plant species with shorter growing seasons with a smaller range of suitable environmental conditions for seedling survival. Austen et al. (2017) found a strong correlation between onset of flowering and duration of flowering, suggesting that there may be selection for earlier flowering because it extends the reproductive period, which then allows for greater reproductive output.

Leavenworthia stylosa, a winter annual in the Brassicaceae, is a cedar glade endemic with a limited distribution in the Central Basin of Tennessee (Rollins 1963; Baskin & Baskin 1972). *L. stylosa* is commonly found in the natural drainage areas of the cedar glade environment where soil cover is thin atop limestone bedrock (Baskin & Baskin 1972). These areas are often very wet in the fall and winter and then very dry by late spring and throughout the summer (Baskin & Baskin 1972). Seeds typically germinate in September and October; rosette growth continues throughout the fall and early winter; and flowering begins in late winter to early spring (Rollins 1963). The fruits mature and drop their seeds in late April and early May. Seeds lie dormant until cooler, wetter weather triggers germination in the fall (Zager et al. 1971; Baskin & Baskin 1972). With excessively hot, dry summers and cool, wet winters in its very specific habitat, it is easy to imagine that the timing of life history events in *L. stylosa* would be highly influenced by environmental conditions. Flowering time may be influenced by temperature and precipitation in the months leading up to and during flowering (January, February, March). Warmer temperatures earlier in the year could cue plants to initiate flowering earlier. Alternatively, if flowering time depends on plants reaching a certain size before initiating flowering, flowering time may be more heavily influenced by germination time, which may depend on precipitation and temperature leading up to and during germination time (August, September, October). Since *L. stylosa* seeds germinating before the hot and dry summer season is over have severely reduced survival (Baskin & Baskin 1972), cooler temperatures and/or increased precipitation in late summer and early autumn could result in earlier germination time, potentially leading to earlier flowering.

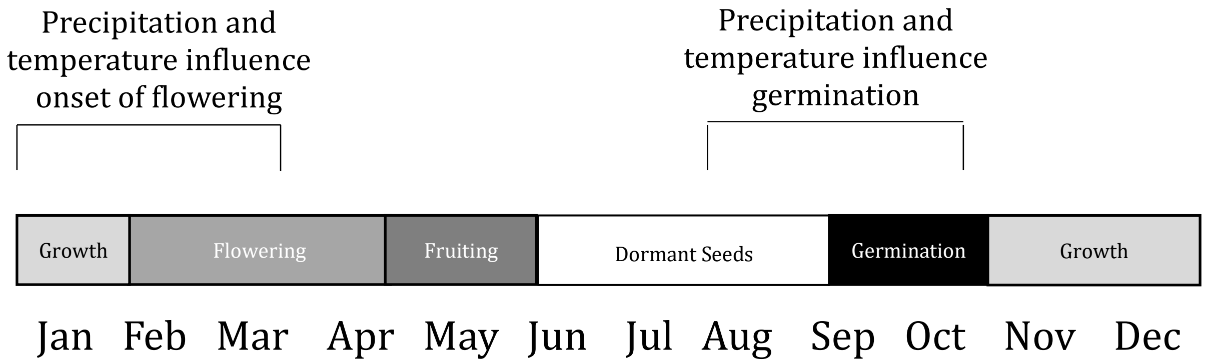

Figure 1: Schematic diagram of the life cycle of *Leavenworthia stylosa* showing the months where precipitation and temperature may have the greatest influence on germination and flowering times

In the winter of 2015-2016, *L. stylosa* was observed to be flowering at the Smith Springs field site along Smith Springs Road in Antioch, Tennessee (36.078619, -86.588787), as early as late December/early January, which is about 2 months earlier than usual (C.R. Herlihy, personal obs.). Baskin and Baskin (1972) studied the effects of germination time on plant survival and fitness in *L. stylosa* during a year of unusually early germination beginning at the end of July and found that germinating too early greatly decreased seedling survival rate to under 10% survival to flowering. Similarly, we now have a situation of a life history event occurring unusually early, but in this case the event is the onset of flowering. Therefore, I decided to use this opportunity of an abnormal flowering event to study the fitness effects of early flowering time in *L. stylosa.* Early flowering seems particularly risky in the specific case of *L. stylosa* since it is already one of the earliest plants to flower in cedar glades (Rollins 1963; Baskin & Baskin 1972).

The goal of this study was to quantify how flowering time affects reproductive success in *L. stylosa* and to investigate the factors that influence variation in flowering time. To do this, I asked the following questions: 1) How atypical was the early flowering seen in 2015/2016, and what is the long-term trend in flowering time in *L. stylosa*? 2) Are long-term patterns of temperature and precipitation change consistent with conditions that might favor either earlier flowering or earlier germination in *L. stylosa?* 3) How does flowering time influence survival and reproductive success of *L. stylosa* in a year of unusually early flowering and in a year of average flowering time? and 4) What is the relationship between germination and flowering time within and among generations of *L. stylosa*?

METHODS

Historical Variation in Flowering Time and Climate

Herbarium specimens were analyzed to investigate historical patterns in the timing of flowering and fruiting in *L. stylosa.* This allowed me to determine whether there is an overall trend towards earlier flowering, as well as to determine whether the early flowering in Late December 2015/January 2016 was an anomaly relative to historical flowering times. A total of 342 herbarium specimens were included in this survey. Of these, 238 were in a database from a previous herbarium survey conducted by Dr. James Beck (Wichita State University, unpublished), and 104 specimens were observed at the MTSU herbarium (MTSU). Beck's dataset included specimens from six herbaria: Missouri Botanical Garden (MO), Botanical Research Institute of Texas (BRIT/VDB/SMU), New York Botanical Garden (NY), United States National Herbarium (US), University of Texas at Austin (LL), University of Alabama Herbarium (UNA), and the Gray Herbarium at Harvard (G). Any specimens that had the same date of collection, location of collection, and collector(s) but were at different herbaria were considered duplicates. In cases of duplicate specimens, only one was included in analyses, which resulted in a final sample size of 312 specimens collected between 1915 and 2015.

Beck had previously classified the specimens in his database into four phenological categories: flowering, flowering/early fruiting, flowering/fruiting, and fruiting/late fruiting. After examining 10 specimens from each of Beck's phenological categories via BRIT VDB specimens 104000-104205, a list of phenological traits was created for each category: flowering plants were specimens that had open flowers but no fruit yet developed; flowering/early fruiting plants were specimens that had open flowers and very small, immature, new fruits developing; flowering/fruiting plants were specimens with open flowers but also larger, mature fruits; and fruiting/late fruiting plants were specimens with large, mature fruits only and no open flowers. The MTSU specimens were then assigned to the same phenological categories based on these criteria. For each phenological category, I plotted the collection date of specimens in Julian days by year over the last century in order to see if there is an overall trend towards earlier collection times as a proxy for earlier flowering.

Once it was determined if there was a trend towards earlier collection times, I quantified that trend by looking at the date of collection (Julian Day) of specimens collected at the beginning of the century and the date of collection of specimens collected at the end of the century. The range and average Julian Day of collection was calculated for all specimens collected between 1929 – 1954 to represent the beginning of the century and the same was calculated for all specimens collected between 1980 - 2005 to represent the end of the century. The difference was then calculated between the average Julian Day of collection at the end of the century and the beginning of the century in order to estimate change in onset of flowering time of *L. stylosa* over the last century.

Temperature and precipitation data spanning the past century (1915-2015) were downloaded for a weather station located near the center of the geographic range of *L. stylosa* in Murfreesboro, Tennessee

(GHCND:US1TNRD0091 [36.0153, -86.3718] from NOAA's National Climate Data Center – Climate Data Online; https://www.ncdc.noaa.gov/cdo-web/). Average monthly mean temperature and average total monthly precipitation were analyzed for any significant increasing or decreasing trends for three key months around germination time in the fall and three key months around flowering time in the spring. January, February, and March temperature and precipitation were assessed in order to determine if spring was becoming warmer and/or wetter, which might allow earlier onset of flowering. Similarly, August, September, and October temperature and precipitation were assessed in order to determine if cooler and/or wetter falls were occurring, which might allow for earlier germination, which could also lead to earlier flowering.

Costs and Benefits of Early Flowering

The goal of the field studies was to study the fitness costs and benefits associated with variation in flowering time. I was able to do this in a year of unusually early flowering (2016) and a year of average flowering time (2017).

Flowering Time in the Field – 2016 Early Flowering Event

To examine the fitness costs and benefits associated with the unusually early flowering seen over the winter of 2015/2016, a transect was set up to track the success of plants that flowered earlier than the population average, about the average time, and later than the population average in a single population in 2016. A 50 m transect was established at a cedar glade near Smith Springs

Road in Antioch, Tennessee (36.078619, -86.588787) on February 5, 2016. After establishing the transect, 50 flowering plants and 50 nonflowering plants were marked by walking down the transect and choosing one flowering and one nonflowering plant closest to the transect at approximately 1-m intervals. A uniquely numbered nail was put into the soil at the base of each plant, and the plant's position was mapped by recording both distance on the transect and distance from the transect to facilitate locating each plant during future visits. On March 10, 2016, when a majority of the previously marked nonflowering plants were now flowering, a third group of 50 additional plants was tagged and mapped in the same manner . None of the 50 plants in this new group were flowering at the time of tagging. This gave three treatment groups: early-flowering (the 50 flowering plants marked on February 5, 2016), average-flowering (the 50 plants marked on February 5, 2016 that were not yet flowering), and late-flowering (the 50 plants marked on March 10, 2016 that were not yet flowering). These flowering time descriptors (early, average, and late) are relative to the population flowering time in this particular year and site, however the site itself was flowering early as a whole based on long-term data. All flowering time categories were approximately 4 weeks earlier than the same categories used in a subsequent flowering season with average flowering time.

Each plant was observed weekly for 12 weeks, and the following data were recorded during each visit: number of open flowers, number of failed fruits, and the number of successful fruits. Since the flowers of *L. stylosa* are only open for 1-2 days, the open flowers during any visit were new flowers that were not open during the previous week. Flowers of *L. stylosa* are borne singly on individual pedicels, and these pedicels remain attached to the rosette even if the flower fails to produce a fruit. Therefore, I could categorize the success of every flower produced as either a successful fruit or a failed fruit (a pedicel lacking a fruit). The number of new successful fruits and new failed fruits produced each week were calculated by subtracting the total number of these counted during the previous weekly observation from the total number counted during the current weekly observation. The number of open flowers, new failed fruits and new successful fruits were compared among early-, average-, and late-flowering plants at their peak value via one-way analysis of variance (ANOVA). All statistical analyses were completed using JMP (Version 12, SAS Institute, Inc., Cary, NC).

In order to determine if flowering time was related to plant size, I tested whether earlier-flowering plants were larger than average-flowering or late-flowering plants. Rosette size was measured with digital calipers for all 150 plants on March 18, 2016 (just before the late-flowering category plants began to flower) and was compared among flowering time groups using one-way ANOVA.

On April 26, 2016, I conducted a final count of successful fruits and failed fruits. No plants in any treatment category were producing new flowers on this date. Fruits from all plants were harvested and stored in envelopes at room temperature until seeds were counted.

I compared fruit set (percentage of flowers that developed into a fruit), the mean number of flowers per plant, the mean number of fruits per plant, and rosette size between flowering time groups via one-way ANOVA. Any plants that did not survive were excluded from these analyses.

For a given plant, fruits were opened and seeds were collected, with all seeds being combined for counting purposes. The total number of viable seeds and inviable seeds was recorded. Seeds were considered viable if they appeared plump and were not transparent in the center when held up to a spotlight, indicating that there was a developed embryo within the seed. Seeds were considered inviable if they were transparent, indicating that there was no developed embryo within the seed. I weighed all viable seeds for each plant as a group, and then divided this total seed mass by the number of viable seeds to determine the average seed mass for each plant. I compared the average number of viable seeds per plant, the average number of inviable seeds per plant, the average single seed mass, the average number of viable seeds per fruit, and rosette size between the three flowering time groups with one-way ANOVA followed by Tukey-Kramer comparison of means to determine which means were statistically different from one another.

Flowering Time in the Field – 2017 Average Flowering

The 2016 field experiment was repeated for a second year at Smith Springs and at three additional field sites during a year with average flowering time. This allowed me to compare the success of plants that flowered early, normal, and late, both within years and between years. Two 25 m parallel transects were set up at four cedar glades located within the Central Basin of

Tennessee: Flat Rock State Natural Area (35.858214, -86.295611), Butler Cedar Glade (36.090215, -86.617095), Quarterman Cedar Glade (36.048858, -86.560430) and the same site off of Smith Springs Road from 2016 (36.078619, -86.588787). On March 9-12, 2017, 50 flowering plants and 50 nonflowering plants were marked in the same manner as in 2016. Once a majority of the 50 previously nonflowering plants had begun flowering at each of the sites, a third group of 50 late-flowering plants, which still had not flowered, were marked at each site on March 30-31 2017. In total, 600 plants were marked (150 plants at each site).

Each plant was censused approximately every 10 days for the duration of flowering and fruiting (4-6 weeks depending on their flowering time category). During each census, the phenophase of each plant was categorized from 0 to 7: 0 – nonflowering rosette, 1 – first open flower, 2 – open flower/s and spent flower/s, 3 – spent flower/s only, 4 – open flowers and early fruits, 5 – open flowers and fruits, 6 – open flowers and mature fruits, and 7 – mature fruits only. All plants were collected once they were reached category 7. This change in protocol from 2016 allowed for a more efficient weekly assessment of plants and therefore allowed us to collect more data. Collection dates ranged from April 21, 2017 to May 5, 2017. All fruits were brought back to MTSU and stored at room temperature in envelopes until the seeds could be counted. The seeds were counted and the same data were collected as described for the 2016 field season.

Mean number of viable seeds per plant, mean number of inviable seeds per plant, mean number of seeds per fruit, average single seed mass, and mean number of fruits per plant were analyzed within sites via one-way ANOVA followed by Tukey-Kramer multiple means comparison and via two-way ANOVA to analyze the relationships between flowering time and study sites. Differences between years regardless of flowering time and site were analyzed using t-tests. Survival rate was analyzed via chi-square.

Does Germination Time Influence Flowering Time?

Germination and Overwinter Survival in the Field

In order to determine if germination time has an effect on flowering time, I followed groups of plants from the week they germinated through to their first flower. Eight 30.5 cm by 30.5 cm plots were established at the same four sites that were used for the flowering time field experiment in 2017. Each site was monitored weekly starting in mid-August 2016 to identify germination as early as possible. Germination was first detected at Butler, Smith Springs, and Flat Rock in the last week of September 2016. The first four plots at each of those three sites were marked between September 29, 2016 and October 1, 2016 and were specifically placed in areas where there were at least three but no more than 100 germinated seeds in a 30.5 cm^2 area. These plots were visited weekly, and the number of germinated seeds within each plot was recorded. It should be noted that the seedlings were too small to be successfully marked for individual identification. Therefore, the total number of seedlings was recorded without definite knowledge of whether they were the exact same seedlings as the week before (i.e., if one seedling died and another seed germinated within a week, the data would not reflect this change). No plots were established at Quarterman during the first round because no germination was yet observed, but the site was still visited weekly in anticipation of the first signs of germination. Four additional plots were established at Butler, Smith Springs, and Flat Rock, and eight plots were established at Quarterman between December 8 and 14, 2016, for a total of eight plots per site and 32 plots across all four sites. All plots continued to be monitored weekly for continued germination and survival until March 31, 2017, when a majority or all of the plants in each plot had begun to flower. Data were only collected until date of first flowering for these plants. Data were analyzed for differences between median date of germination and date of first flower for each plot. Median date of germination was calculated for all seedlings that germinated and survived until flowering time. The first day of germination of a seedling at all four sites combined was considered Day 0, and all germination days and day of first flower were then counted as the number of days after the germination of that first seedling.

Germination in the Incubator

After the seeds from the 2016 field experiment at Smith Springs were counted, all parent plants with 10 or more viable seeds were included in a germination experiment to examine the relationship between flowering time of plants in the field and germination time of their offspring. In October 2016, seeds were placed on moistened filter paper in petri dishes and placed in an incubator set at optimal germination conditions, 20°C day/10°C night (Baskin & Baskin 1971) with 12-hour day length. Up to 20 seeds per parent plant were used in an attempt to ensure that at least 10 healthy seedlings survived to be transplanted and grown in the greenhouse. In total, 27 early-flowering plants were represented with 437 seeds (mean: 16.2 seeds per plant), 22 average-flowering plants were represented with 387 seeds (mean: 17.6 seeds per plant), and 22 late-flowering plants were represented with 382 seeds (mean: 17.4 seeds per plant).

Incubation of seeds started on October 14, 2016, and were observed daily. The number of days until germination (with October 14 being day 0) was recorded for each seed. Percentage of seeds germinating and the average number of days until germination were calculated for the offspring of plants in each of the three flowering time groups. Data were statistically analyzed via one-way ANOVA to determine if there was any significant difference in the average number of days until germination among flowering time categories. Differences in the percentage of seeds germinating from the three flowering time categories were analyzed using chi-square analysis. Once germinated, the seeds were transplanted into 10.2 cm by 10.2 cm individual pots filled with a 1:2 mixture of sand to Miracle Grow potting mix. The pots were then placed in a temperature-controlled greenhouse, with the temperature set at 23°C. Due to low survival of transplanted seedlings, I was unable to collect flowering time data from these plants.

Germination in the Greenhouse

This same germination experiment was then replicated with hopes of achieving a higher survival rate through flowering time than was achieved in the previous incubator germination experiment. The seeds were from the same plants from the 2016 Smith Springs Road field study. All plants with more than five viable seeds remaining after the first incubator germination experiment were included in the greenhouse germination time experiment with the same goal of assessing any relationship between the flowering time of a plant in the field and the germination time of its offspring. In this second attempt, all seeds were direct seeded into 10.2 cm by 10.2 cm pots filled with a 1:2 mixture of sand to Miracle Grow potting mix on March 24, 2017 and placed in the greenhouse set at 23°C. Supplemental lighting was used to extend natural daylight to a total of 12 hours of light per day. Pots were hand watered and monitored for germination once daily until there was no additional germination. They were then watered 2 times daily for 15 minutes each time with an automatic watering system. In total, 20 early-flowering parent plants were represented with 224 seeds (mean: 11.2 seeds per plant), 23 average-flowering parent plants were represented with 248 seeds (mean: 10.8 seeds per plant), and 24 late-flowering parent plants were represented with 247 seeds (mean: 10.3 seeds per plant). Germination data were collected and analyzed in the same manner as the incubator germination data.

RESULTS

Historical Variation in Flowering Time and Climate

The survey of herbarium specimens showed an overall trend towards earlier flowering for *L. stylosa* over the last century. Across all specimens (Figure 2A), there was a trend towards earlier collection times ($R^2 = 0.122$; $P < 0.0001$). The same pattern was present in each of the phenological categories. The flowering/early fruiting (Figure 2C) and flowering/fruiting (Figure 2D) categories both have a significant negative correlation with Julian Day ($R^2 = 0.037$, $P = 0.023$ and $R^2 = 0.281$, $P < 0.0001$, respectively). While the relationships for flowering (Figure 2B) and fruiting/late fruiting (Figure 2E) were not statistically significant ($R^2 = 0.076$, $P = 0.126$ and $R^2 = 0.035$, $P = 0.262$, respectively), they both showed a trend towards earlier collection time as well. It should be noted that the flowering and fruiting/late fruiting categories have smaller sample sizes ($n = 31$ and $n = 24$, respectively) than the other categories. For comparison, flowering/early fruiting had a sample size of $n = 135$ and the flowering/fruiting category had a sample size of $n = 89$.

In order to examine the magnitude of change in phenology over the period of the herbarium survey, the average day of collection (Julian Day) was calculated for 25 years at the beginning of the century (1929 – 1954) and 25 years at the end of the century (1980 – 2005 – years chosen for evenness of sample sizes and by data availability) for all herbarium specimens (Table 1). Overall, specimens were collected an average of 13.2 days earlier in our most recent 25 years of data compared to the earliest 25 years of data. Additionally, the earliest specimen collected at the end of the century was collected 21 days earlier than the earliest specimen collected at the beginning of the century.

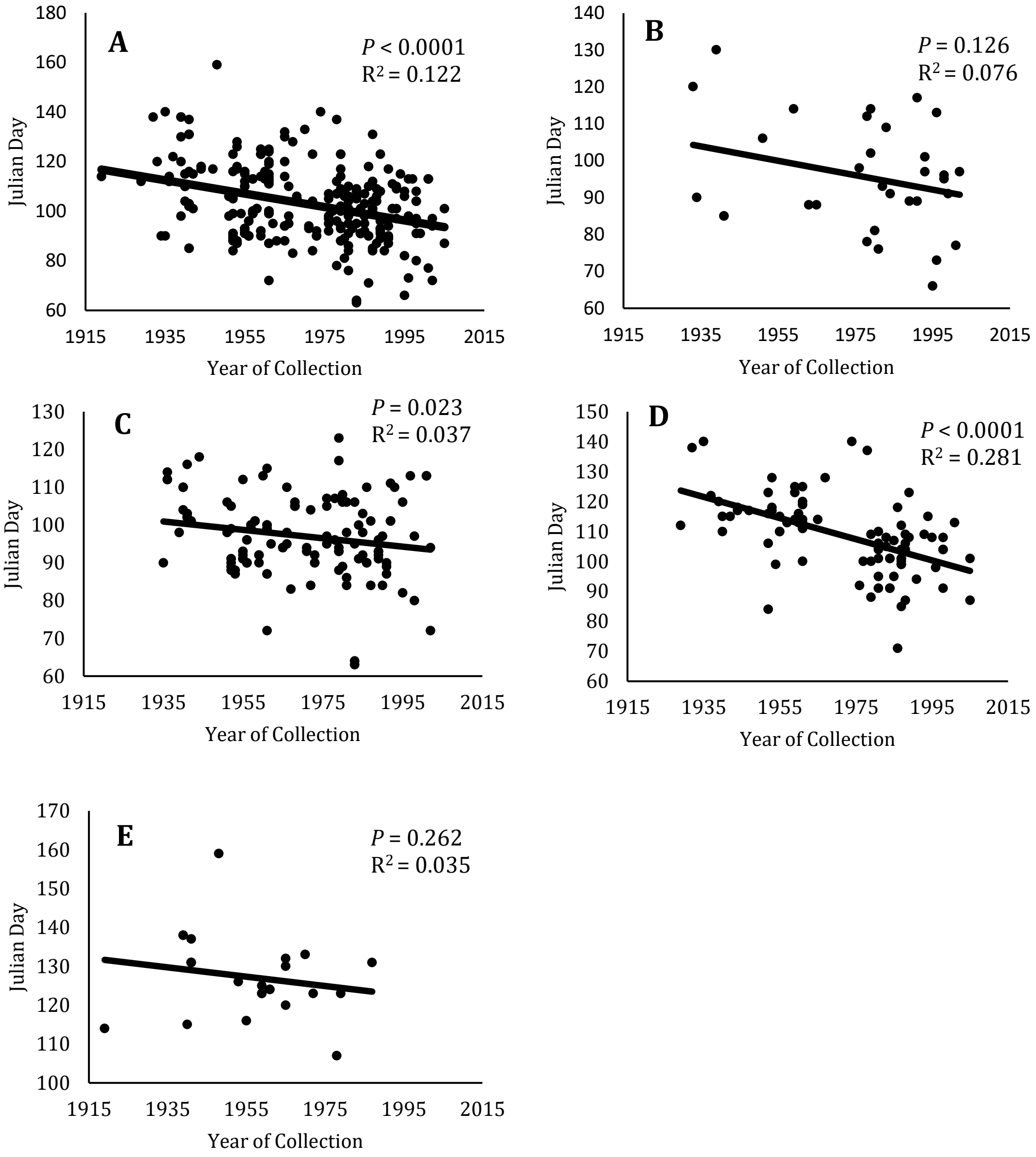

Figure 2: Date of collection of herbarium specimens indicates an overall trend towards earlier flowering and fruiting. (A.) all specimens together; (B.) flowering; (C.) flowering/early fruiting; (D.) flowering/fruiting; (E.) fruiting.

Table 1. Change in date of collection between herbarium specimens collected 1929 – 1954 and specimens collected 1980 - 2005.

Date range	Number of specimens observed	Range of Julian Day of collection	Average Julian Day of collection	Change in average Julian Day of collection
1929 - 1954	72	84 - 159	111.1	
1980 - 2005	110	63 - 131	97.9	-13.2

The early-flowering observed in the spring of 2016 was unprecedented, even within the overall trend towards earlier flowering and fruiting seen in the herbarium data. Prior to 2016, the earliest herbarium specimen with *L. stylosa* flowering was March 4, 1983 which is about two months later than what was observed in 2016. However, it should be noted it is unlikely that someone would have collected a plant with only its first flower or the earliest flowering individuals in the population such that herbarium records will likely not reflect date of first flowering.

In order to see if changes in climate during the fall were consistent with conditions that would favor earlier germination (decreased temperature and/or increased precipitation), I analyzed a century (1915-2015) of temperature and precipitation data for August, September, and October. I found a significant increase in total monthly precipitation for the month of September ($R^2 = 0.055$, $P = 0.019$) with a 31.8 mm increase in average monthly precipitation from the beginning of the century to the end of the century when compared with the same date ranges as the herbarium specimens (1929 – 1954 compared to 1980 – 2005). There was also a significant decrease in average mean temperature in the months of September ($R^2 = 0.061$, $P = 0.014$) and October ($R^2 = 0.084$, $P = 0.004$) with average mean temperatures decreasing by 1.21°C in September and 1.26°C in October when compared with the same date ranges as the herbarium specimens (1929 – 1954 compared to 1980 – 2005). While October also showed a slight trend towards increased total monthly precipitation over the last century, it was not statistically significant ($R^2 = 0.017$, $P = 0.196$; Figure 3).

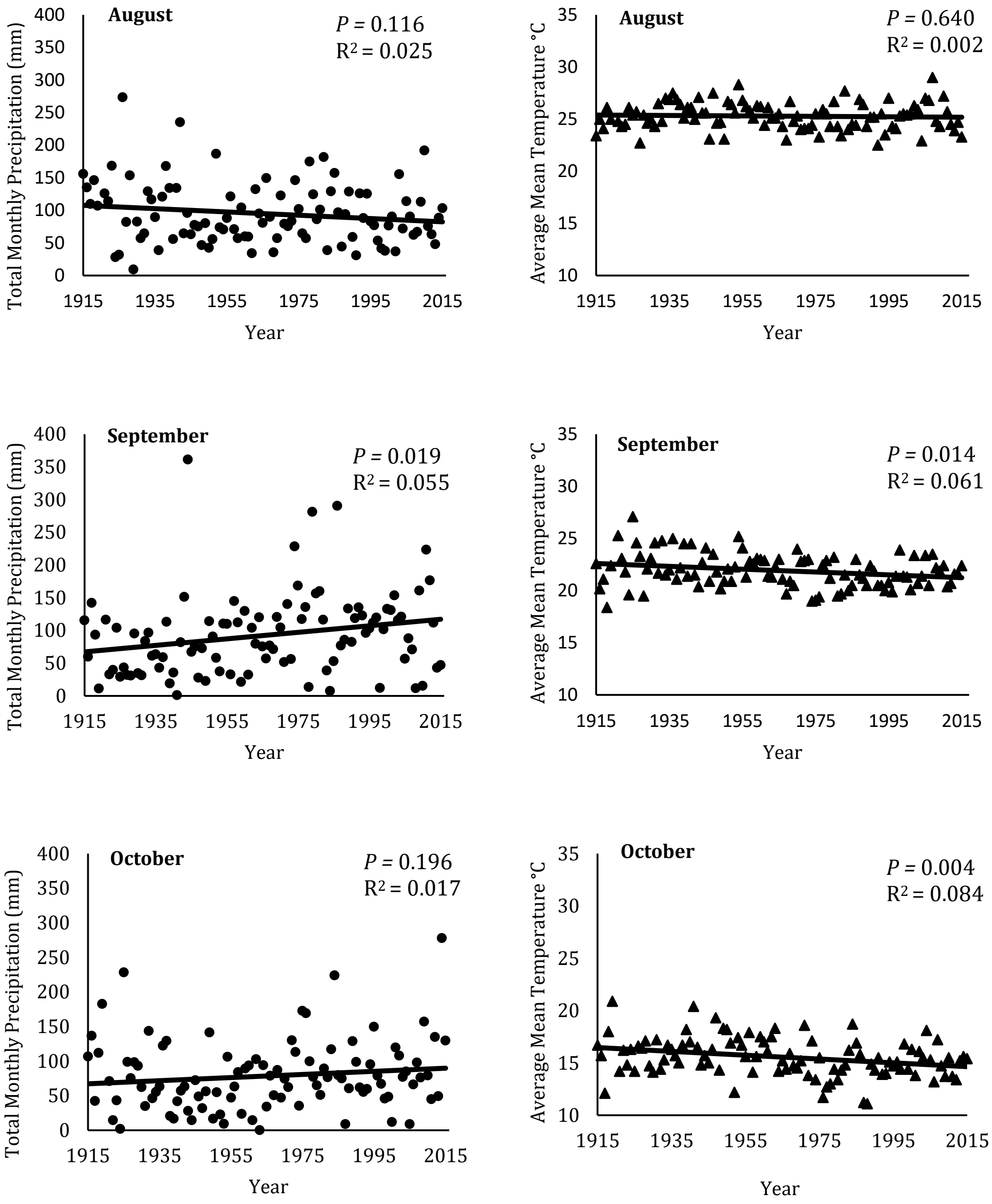

Figure 3: Total monthly precipitation and average mean monthly temperature over the last century (1915 – 2015) for the months most associated with germination and seedling survival in *Leavenworthia stylosa* – August, September, October.

Using the same climate data, I also examined if there were increases in temperature and precipitation in the months of January, February, and March, which could favor earlier flowering time. There were no significant changes in total monthly precipitation for the months of January ($R^2 = 0.013$, $P = 0.262$), February ($R^2 = 0.000$, $P = 0.949$) or March ($R^2 = 0.002$, $P = 0.680$) over the last century (Figure 4). However, there was a significant decrease in average mean temperature for the months of January ($R^2 = 0.087$, $P = 0.003$) and February ($R^2 = 0.098$, $P = 0.001$ over the last century (Figure 4). Average mean temperatures decreasing by 2.41°C in January and 1.51°C in February examining the same date ranges as the herbarium specimens (1929 – 1954 compared to 1980 – 2005). There was also a slight trend towards lower average mean temperatures for the month of March, but this was not statistically significant ($R^2 = 0.012$, $P = 0.267$). Overall, there was evidence of historical change in climate during fall that might favor earlier germination, but there was no evidence suggesting that climate change in spring would be associated with earlier initiation of flowering.

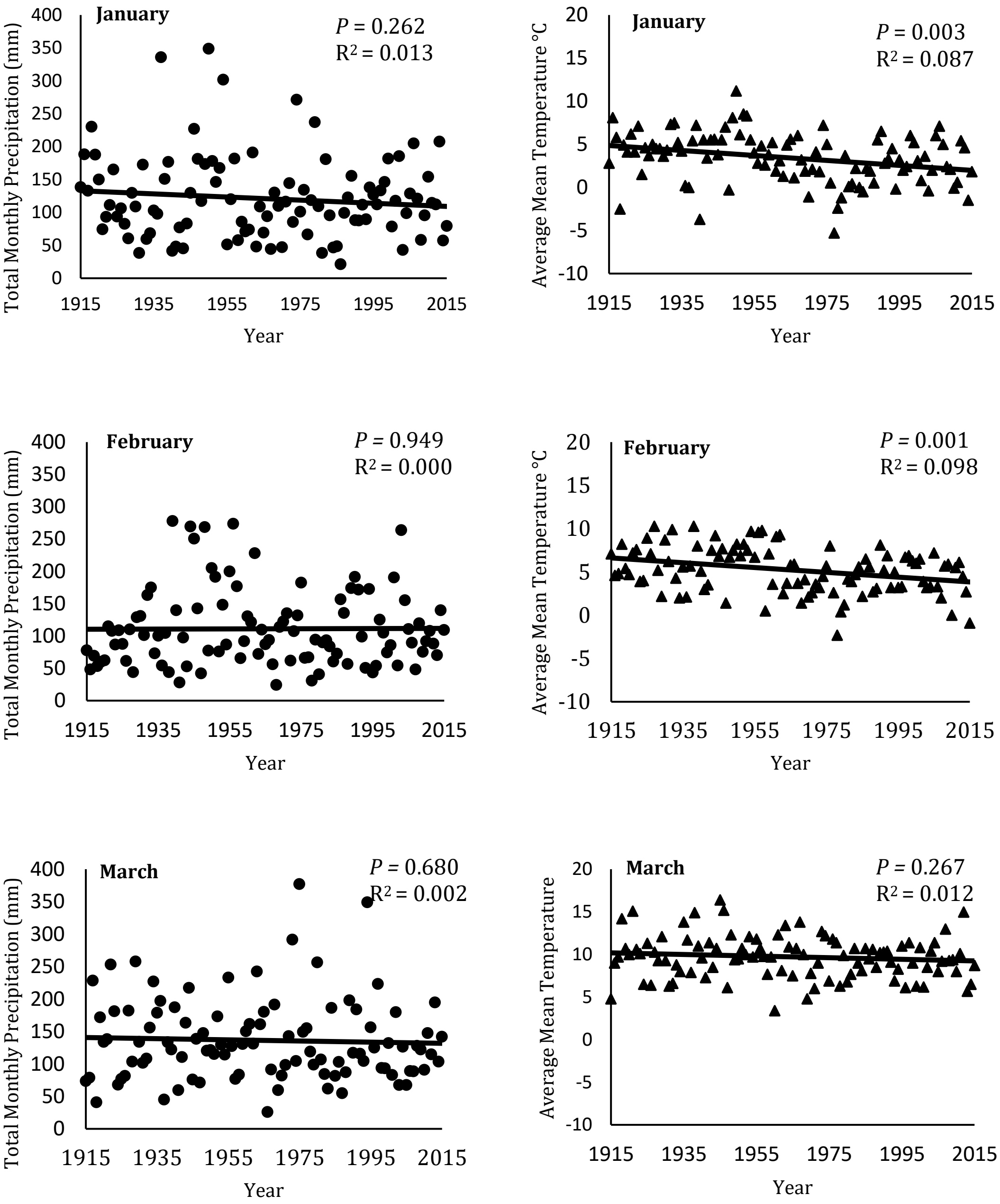

Figure 4: Total monthly precipitation and average mean monthly temperature over the last century (1915 – 2015) for the months associated with initiation of flowering time in *L. stylosa* – January, February, and March.

Costs and Benefits of Early Flowering

Flowering Time in the Field – 2016 Early Flowering Event

There was an initial spike in the mean number of open flowers per plant for the early-flowering group. However, flowering of plants in this group declined during the subsequent four census periods (Figure 5A). Early- and average-flowering plants produced their peak number of open flowers the same week while the late-flowering group produced their peak number of open flowers one week later (Figure 5A). All three flowering time groups had approximately the same number of open flowers at peak (F = 0.202, $P = 0.817$).

The early-flowering group had a small spike in failed fruits early on, but early- and average-flowering time groups still had their peak number of failed fruits the same week, while late-flowering plants had their peak number of failed fruits one week earlier (Figure 5B). Average-flowering plants produced the highest mean number of new failed fruits per plant at their highest weekly peak, followed closely by early-flowering plants, with late-flowering plants producing the lowest number of new failed fruits, however these differences in the mean number of new failed fruits per week was not significant between groups (F = 2.645, $P = 0.075$).

The early- and average-flowering time groups produced their peak mean number of new successful fruits per plant on the same week and, the late-flowering time group produced its peak mean number of new successful fruits per plant one week later (Figure 5C). The mean number of new successful fruits produced at their peak was significantly different among the flowering time groups (F = 3.741, *P* = 0.026). Early-flowering plants produced significantly more new successful fruits at their peak than did late-flowering plants (*P* = 0.019), while average-flowering plants did not differ significantly between early- (*P* = 0.363) and late-flowering (*P* = 0.347) plants.

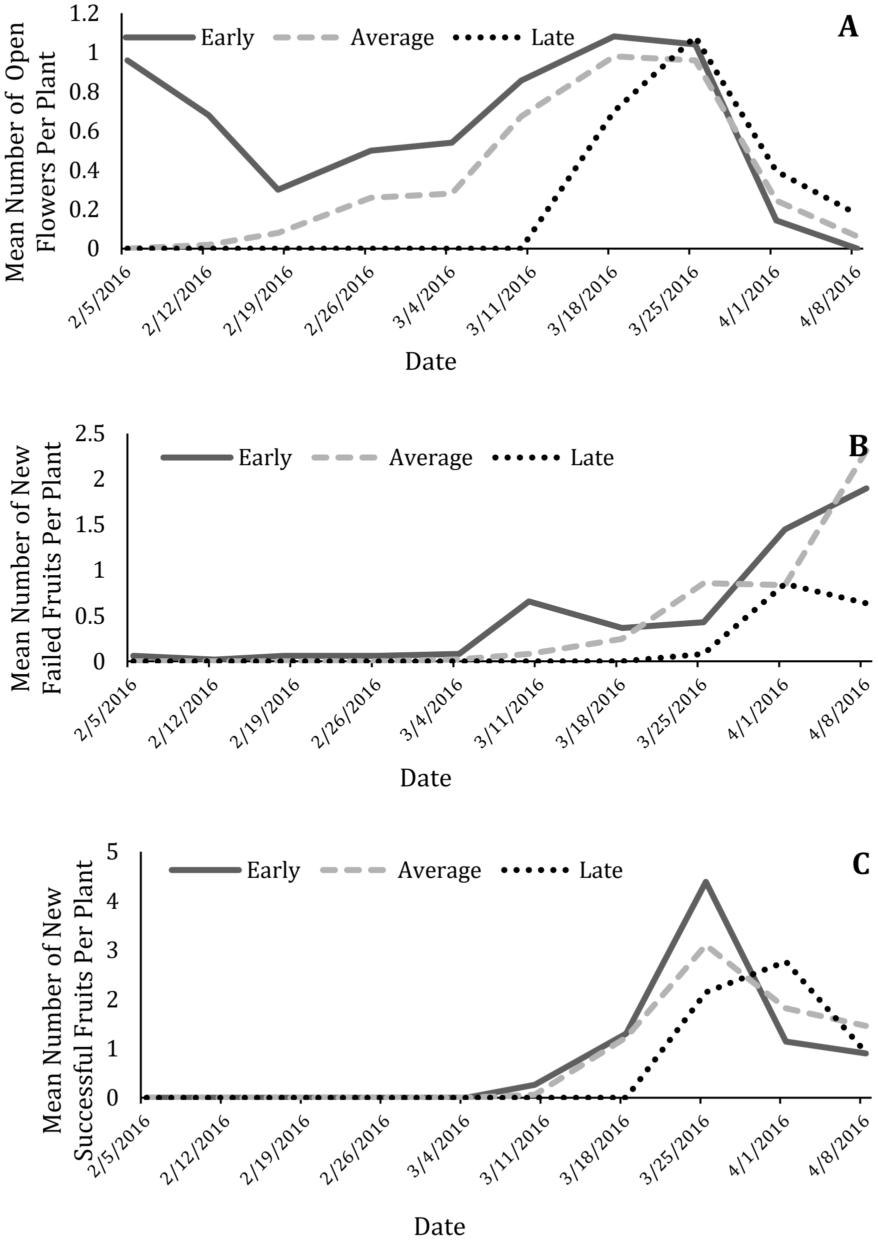

Figure 5. Peak flowering and fruiting times for Smith Springs 2016 early flowering event. (A.) Mean number of new open flowers per plant by week for early-, average-, and late-flowering plants; (B.) Mean number of new failed fruits per plant by week for early-, average-, and late-flowering plants; (C.) Mean number of new successful fruits per plant by week for early-, average- and late-flowering plants.

There were no significant differences in the success rate (% flowers that developed into fruits) (F = 0.001 *P* = 0.999), the mean number of fruits per plant (F = 0.257, *P* = 0.774), or the mean number of flowers per plant (F = 1.504, *P* = 0.226) among the flowering time categories in 2016 (Figure 6). There were decreasing trends in the mean number of flowers per plant and mean number of fruits per plant, indicating that late-flowering plants may be slightly less successful, but these trends were not statistically significant.

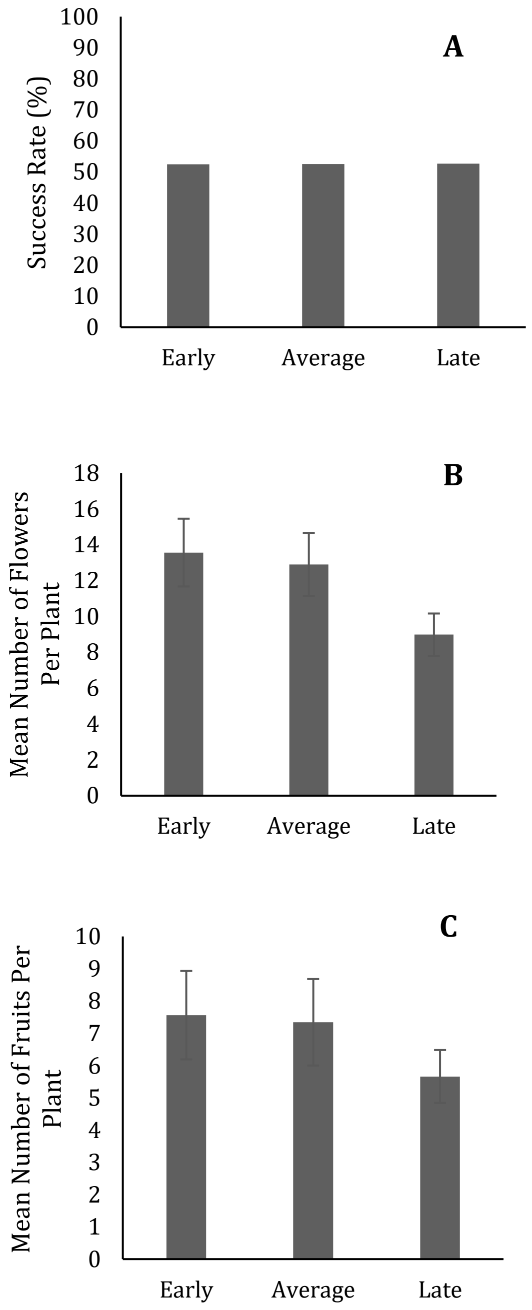

Figure 6. Results of field observations analyzed by flowering time for three fitness-related traits for Smith Springs 2016. (A.) success rate (percentage of flowers that developed into a fruit), (B.) mean number of flowers per plant, and (C.) mean number of fruits per plant. Each bar represents means ± 1 standard error.

There were no significant differences in any of the fitness categories among the flowering time treatments (Figure 7, Table 2): number of viable seeds per plant (F = 0.408, *P* = 0.666), number of inviable seeds per plant (F = 0.129, *P* = 0.879), mean seed mass (F = 0.032, *P* = 0.969), mean number of seeds per fruit (F = 1.442, *P* = 0.240), or survival (X^2 = 3.433, *P* = 0.1797). There was also no significant difference in rosette size among flowering time groups (F = 0.703, *P* = 0.497) (Figure 8), suggesting that earlier flowering plants were not flowering earlier simply because they were larger.

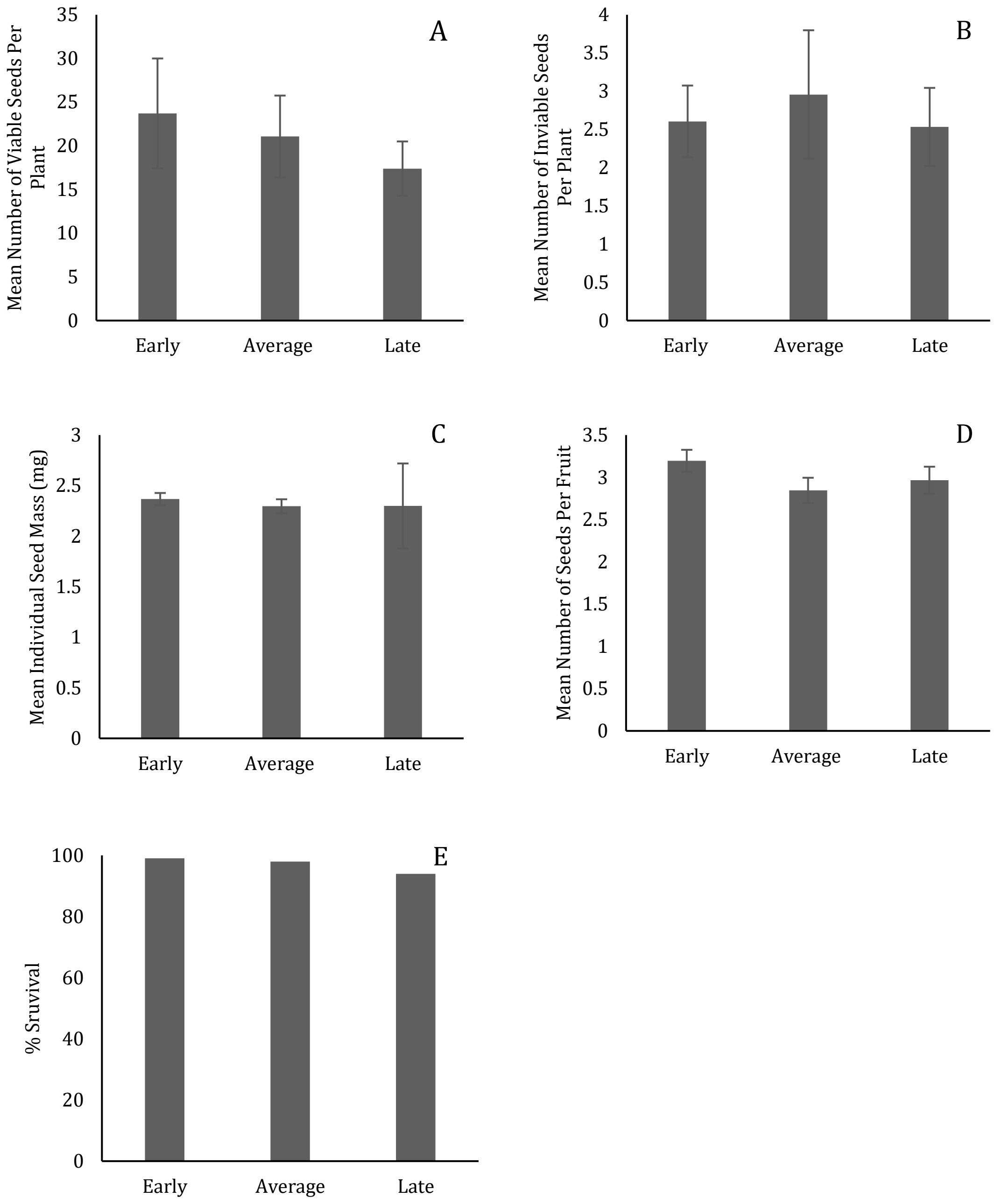

Figure 7: Fitness-related traits for Smith Springs seeds 2016. (A.) The mean number of viable seeds per fruit, (B.) mean number of inviable seeds per fruit, (C.) mean individual seed mass, (D.) mean number of seeds per fruit, and (E.) survival for each flowering time category for the Smith Springs 2016 seeds.

Table 2. Comparisons of fitness for Smith Springs 2016. *P* – values denote difference among all flowering time groups within a plot.

	Mean # viable seeds per fruit	Mean # fruits per plant	Mean individual seed mass (mg)	Mean # inviable seeds per plant
Early	3.2	8.3	2.37	2.6
Average	2.8	7.9	2.30	2.9
Late	2.9	5.9	2.30	2.5
P - value	0.240	0.344	0.969	0.879

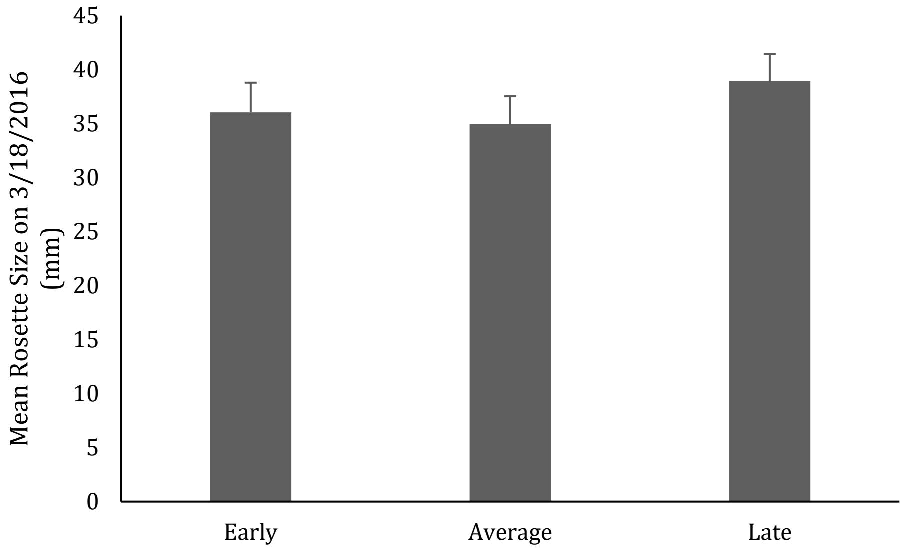

Figure 8. The mean rosette size for each flowering time group as measured on March 18, 2016 for Smith Springs. This measurement was taken when a majority of the early- and average-flowering plants had flowered but before any of the late-flowering plants began to flower. Bars represent ± 1 standard error.

Flowering Time in the Field – 2017 Average Flowering

There were significant differences among flowering time groups for the mean number of fruits per plant, the mean number of seeds per fruit, the mean number of viable seeds produced per plant, the average single seed mass and the mean number of inviable seeds per plant (Table 3). Significant differences among sites were seen in the mean number of fruits per plant, the mean number of seeds per fruit, the mean number of viable seeds produced per plant, the average single seed weight, and the mean number of inviable seeds produced per plant (Table 3). However, the site x flowering time interaction term was not significant for any of the measured traits, indicating that the difference among flowering time groups was consistent across sites.

Table 3. Effects of flowering time and site on mean number of seeds per fruit, mean number of viable seeds produced, average maximum number of fruits per plant, average single seed weight, and mean number of inviable seeds per plant in 2017.

	df	SS	*F*	*P*
Number of Viable Seeds Per Fruit				
Flowering Time	2	156.950	39.149	**<0.0001**
Site	3	50.656	8.424	**<0.0001**
Site x Flowering Time	6	21.664	1.801	0.097
Error	436	873.974		
Total	447	1127.693		
Number of Viable Seeds Produced				
Flowering Time	2	6208.791	24.442	**<0.0001**
Site	3	1793.455	4.707	**0.003**
Site x Flowering Time	6	1349.937	1.771	0.103
Error	436	55375.854		
Total	447	65934.748		
Mean Number of Fruits				
Flowering Time	2	468.273	43.091	**<0.0001**
Site	3	64.817	3.976	**0.008**
Site x Flowering Time	6	39.512	1.212	0.299
Error	459	2494.008		
Total	470	3116.357		
Single Seed Mass				
Flowering Time	2	0.014	25.590	**<0.0001**
Site	3	0.014	16.935	**<0.0001**
Site x Flowering Time	6	0.003	1.649	0.133
Error	385	0.107		
Total	396	0.146		
Number of Inviable Seeds Per Plant				
Flowering Time	2	137.758	5.592	**0.004**
Site	3	153.641	4.158	**0.006**
Site x Flowering Time	6	58.957	0.798	0.572
Error	437	5382.728		
Total	448	5792.802		

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At each site in 2017, late-flowering plants had significantly lower mean number of viable seeds per plant, survival rate (Figure 9), mean number of fruits per plant and mean number of seeds per fruit when compared to early- and average-flowering time groups (Table 4). At Butler and Smith Springs only, late-flowering plants also had lower average single seed mass, and at Butler only, late-flowering plants had significantly fewer inviable seeds per fruit (Table 4).

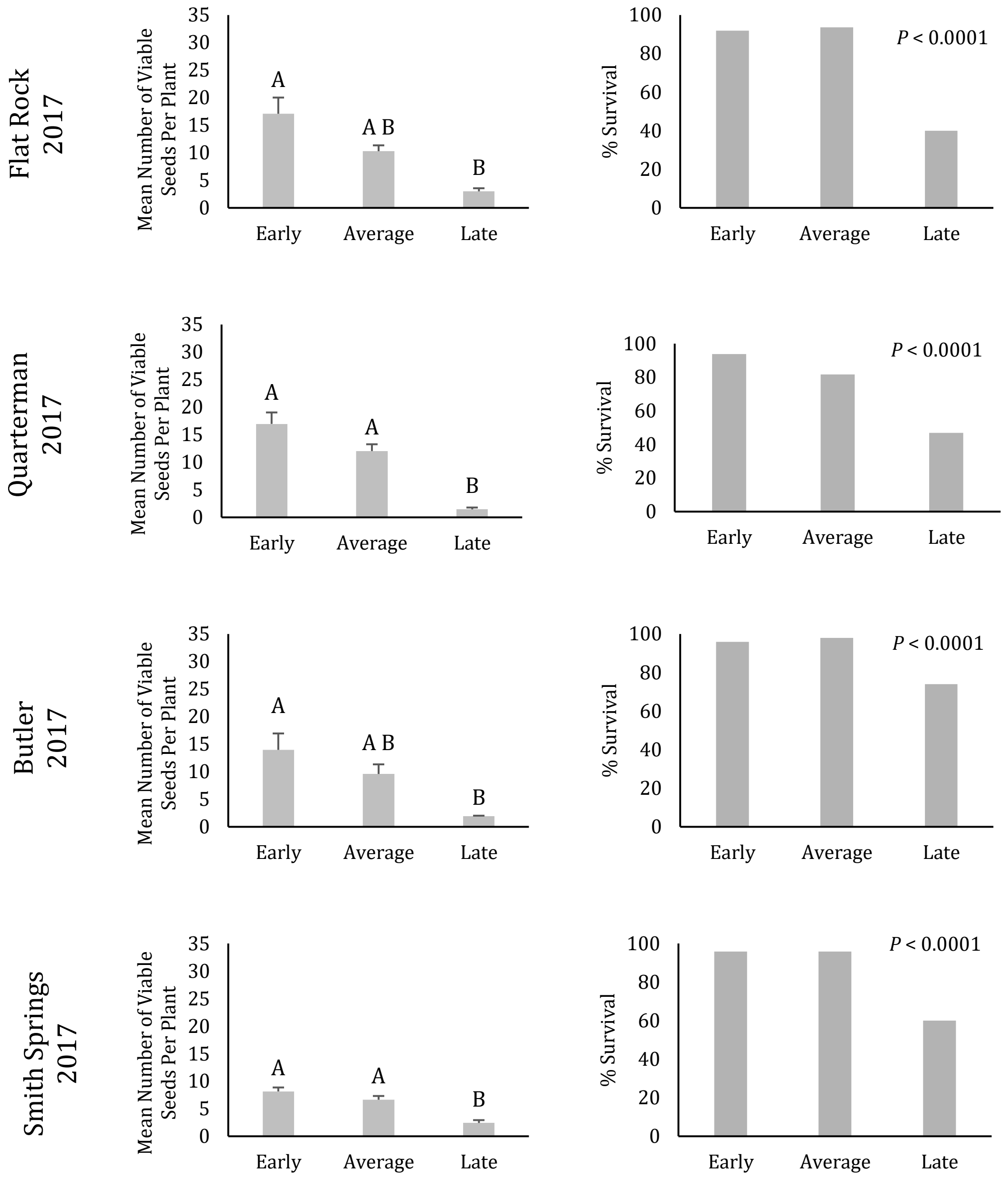

Figure 9: Quantifying fitness in terms of mean number of viable seeds per plant and survival rate for early-, average- and late-flowering time groups at four sites in 2017: Flat Rock, Quarterman, Butler, and Smith Springs. Letters above bars denote statistical difference among flowering time groups within a plot. Bars not sharing a letter are statistically different at *P* < 0.05.

Table 4. Additional comparisons of fitness for Flat Rock, Quarterman Glade, Butler Glade, and Smith Springs in 2017. *P* – values denote difference among all flowering time groups within a plot. Letters associated with means denote statistical difference among individual flowering time groups within a plot.

	Mean # Viable Seeds per Fruit	Mean # Fruits per Plant	Mean Individual Seed Mass (mg)	Mean # Inviable Seeds per Plant
FLAT ROCK				
Early	3.8^{A}	3.8^{A}	1.21	4.3
Average	3.6^{A}	2.8^{A}	1.11	3.3
Late	2.0^{B}	1.3^{B}	0.90	2.4
P - value	0.002	< 0.001	0.118	0.372
QUARTERMAN				
Early	3.4^{A}	4.6^{A}	1.73	3.5
Average	3.3^{A}	3.5^{A}	1.54	2.9
Late	1.5^{B}	1.2^{B}	1.40	1.7
P - value	<0.0001	<0.0001	0.141	0.169
BUTLER				
Early	2.4^{A}	4.4^{A}	1.98^{A}	3.6^{A}
Average	2.7^{A}	3.1^{A}	1.64^{B}	$2.0^{A\ B}$
Late	1.6^{B}	1.1^{B}	1.15^{C}	1.0^{B}
P – value	<0.0001	<0.0001	<0.0001	0.031
SMITH SPRINGS				
Early	2.8^{A}	2.7^{A}	1.85^{A}	1.5
Average	2.4^{A}	2.4^{A}	1.67^{A}	1.6
Late	1.5^{B}	1.1^{B}	1.11^{B}	1.2
P - value	0.0003	<0.0001	<0.0001	0.765

Differences Between Years

Across all flowering time groups and populations, plants in 2016 had a significantly higher number of seeds per fruit, number of viable seeds produced, number of fruits per plant, and average single seed mass compared to plants in 2017 (Table 5). Plants in 2016 also had a significantly higher survival rate than plants in 2017 ($X^2 = 16.064$, $P < 0.0001$).

The same results were found when I compared all plants from Smith Springs in 2016 to all plants from just Smith Springs in 2017. The plants from 2016 had a significantly higher number of seeds per fruit, number of viable seeds produced, number of fruits per plant, and average single seed mass (Table 5). The only difference found between this data and the data comparing all plants from 2016 to all plants at all four sites from 2017 was that Smith Springs 2017 plants had significantly fewer inviable seeds per plant compared to 2016. However, the 2017 plants also had significantly fewer viable seeds per fruit so it is most likely relative due to the fact that Smith Springs 2017 plants produced fewer seeds overall compared to 2016 plants. Plants in 2016 also had a significantly higher survival rate than plants from Smith Springs in 2017 ($X^2 = 16.382$, $P = 0.0013$).

Table 5. Effects of flowering time and year on mean number of seeds per fruit, mean number of viable seeds produced, average maximum number of fruits per plant, average single seed weight, and mean number of inviable seeds per plant. Asterisks indicate *P* < 0.05.

	Smith Springs 2016	Smith Springs 2017	All populations 2017	Smith Springs 2016 vs Smith Springs 2017 t-value	Smith Springs 2016 vs. all populations 2017 t-value
Mean number of Viable Seeds Per Fruit	3.0	2.3	2.1	-4.8*	-5.5*
Mean Number of Viable Seeds Per Plant	19.7	4.3	6.7	-5.6*	-7.8*
Mean Number of Fruits Per Plant	7.4	2.2	2.9	-7.3*	-10.1*
Mean Single Seed Weight (mg)	2.3	1.6	1.0	-4.8*	-13.6*
Mean Number of Inviable Seeds per Plant	2.7	1.4	2.5	-3.0*	-0.5

It is important to reiterate that the dates defining early, average, and late, flowering categories were different between 2016 and 2017. In 2016, early plants began flowering in January/Early February, average-flowering plants began flowering in late February/early March, and late-flowering plants began flowering in late March/early April. In 2017 early-flowering plants began flowering in early March, average-flowering plants began flowering in mid/late March, and late-flowering plants began flowering in early April. In 2016, early-flowering, average-flowering, and late-flowering plants flowered for approximately 8, 5, and 3 weeks, respectively. In 2017, early-flowering, average-flowering, and late-flowering plants flowered for approximately 4, 2, and 2 weeks, respectively.

Does Germination Influence Flowering Time?

Germination and Overwinter Survival in the Field

Seed germination time varied across the four sites (Table 6, Figure 10). At Flat Rock and Smith Springs, there were two distinct rounds of germination: one from late September to mid-October (September 29, 2017 – October 21, 2017; days 0 – 22), and another at the beginning of December (starting on day 65). However, the seeds that germinated in the early round in September and October did not survive, most likely due to lack of adequate precipitation. It should also be noted that the majority of the first and second rounds of germination were in different plots (i.e. there were not two bursts of germination in the same plot twice). At Flat Rock, the first flowering within plots was seen on March 10, 2017 (day 162) and the majority of plants within plots (51 out of 53 plants) were flowering by March 31, 2017 (day 183). At Smith Springs, the first flowering within plots was seen on March 22, 2017 (day 174) and the majority of plants within plots (150 out of 173 plants) were flowering by March 31, 2017 (day 183).

At Quarterman I did not see any germination until the beginning of December (day 63), the same time as the second round of germination for Flat Rock and Smith Springs. The first flowering within the Quarterman plots was seen March 11, 2017 (day 163), and a majority of plants in the plots (82 out of 103 plants) were flowering by March 31, 2017 (day 183). Germination and seedling survival at Butler was unique in that while I did see two peaks in germination, seedling survival for the first round of germination in September/October was much higher than at Flat Rock and Smith Springs with a 79% survival rate for seedlings that germinated between September 29, 2017 and October 21, 2017 (Days 0 – 22) compared to the 0% survival at Flat Rock and Smith Springs. At Butler I saw first flowering within our plots on March 22, 2017 (day 174) and a majority of the plants in the plots (155 out of 170 plants) were flowering by March 30, 2017 (day 182). Despite the different patterns of germination among sites, there was no correlation between germination time and flowering time across all 32 plots ($R^2 = 0.012$, $P = 0.5649$). Plants at all sites began flowering at approximately the same time, suggesting that germination time does not have a strong effect on the timing of flowering.

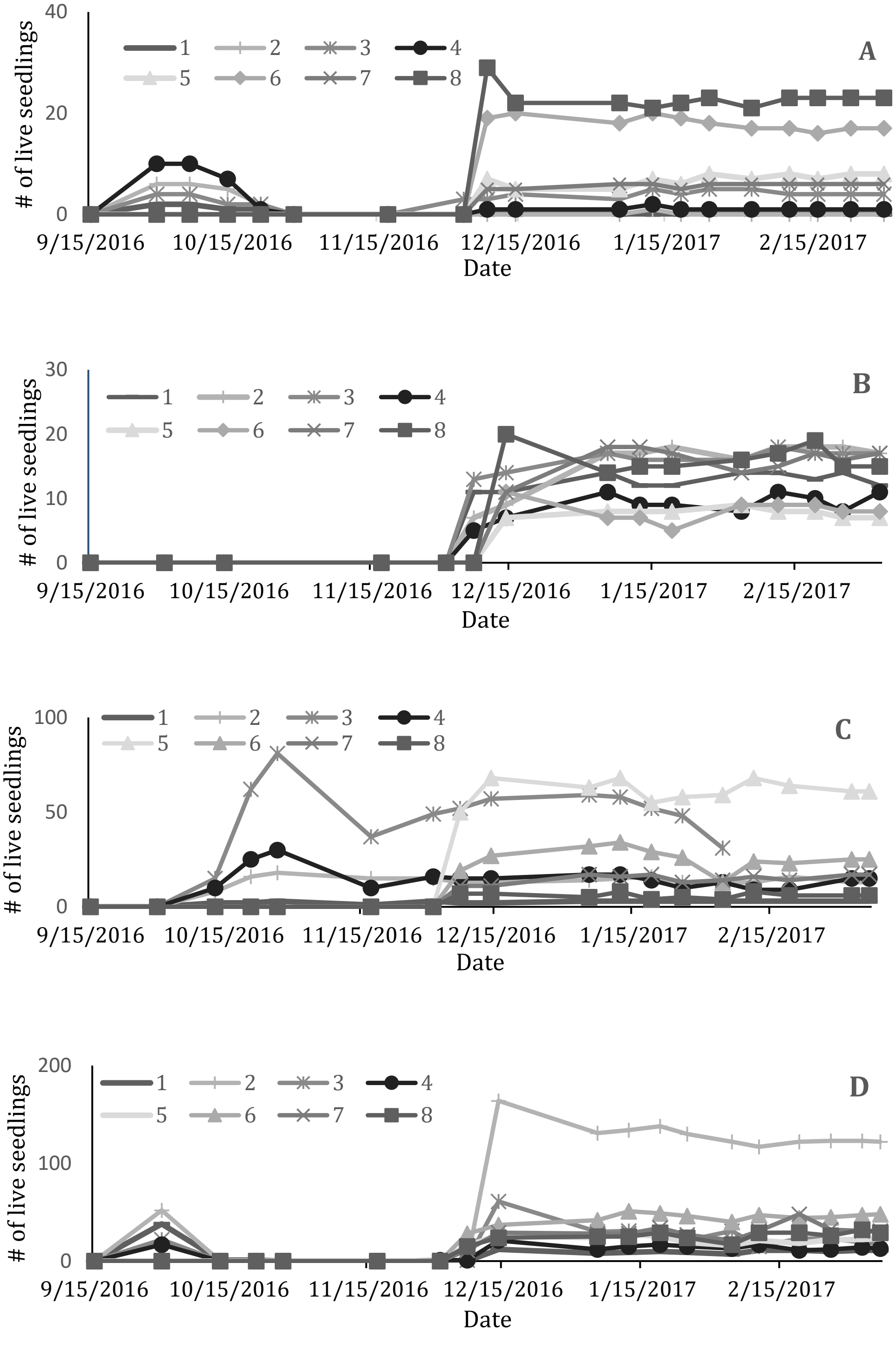

Figure 10: Number of live seedlings counted weekly within each of the eight plots established at the four field sites. (A.) Flat Rock (B.) Quarterman (C.) Butler (D.) Smith Springs.

Table 6. Germination and flowering observations for plants that survived until flowering, averaged by site. Day zero indicates the first day germination observed at any site and each day after that is the number of days from the date of first germination observed at any site. The median germination day is only using the seedlings that survived until flowering.

Site	Range of Germination Day	Median Germination Day	Range of Date of First Flower	Average Date of First Flower
Flat Rock	65 - 76	70.2	162 - 183	176.0
Smith Springs	70 - 76	74.5	174 - 183	176.3
Butler	22 - 76	52.8	174 - 183	179.6
Quarterman	70 - 76	74.5	163 - 183	179.3

Incubator Germination

While there was a trend for the seeds of the early-flowering plants from 2016 to germinate slightly earlier than the seeds of the late-flowering plants in the incubator (Figure 11), no significant differences were found in the average number of days until germination among flowering time groups (F = 0.484, P = 0.619). The percentage of seeds that germinated was significantly different among the three groups ($X^2 = 26.699$, $P < 0.0001$) with late-flowering plants having the lowest percentage of seeds that germinated and average-flowering plants having the highest percentage of seeds that germinated (Table 7).

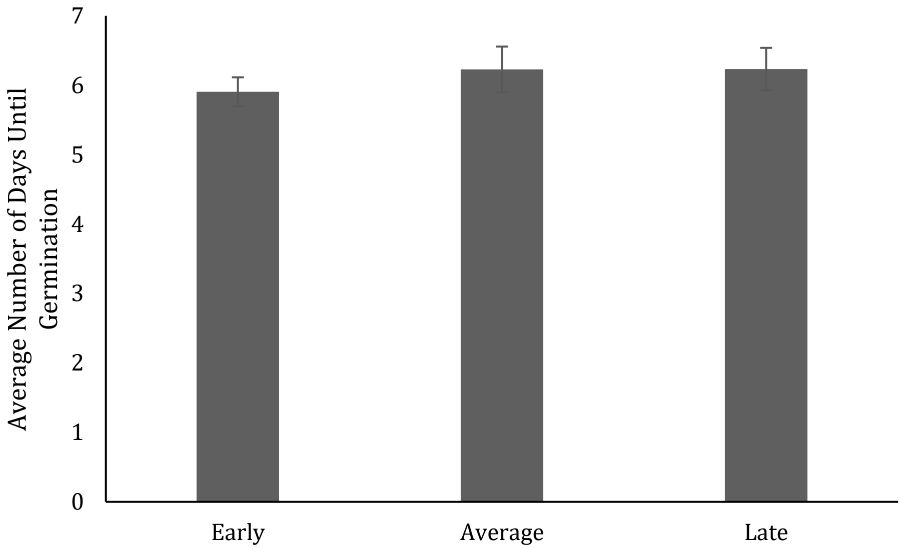

Figure 11: Average number of days until germination in an incubator for the seeds of the early-flowering plants, average-flowering plants, and late-flowering plants from Smith Springs 2016.

Table 7. Percentage of seeds from the offspring of early-flowering, average-flowering, and late-flowering plants that germinated in the incubator.

Group	Germinated	Did not Germinate	Total Started	% Germination
Early	255	182	437	58.35
Average	247	140	387	63.82
Late	175	207	382	45.81
P-value				**<0.0001**

Greenhouse Germination

The greenhouse germination experiment resulted in a similar pattern to that seen in the incubator germination experiment. While there was a trend towards the seeds of the early-flowering plants from 2016 germinating earlier than the seeds of the late-flowering plants (Figure 12), there was no significant difference in their average number of days until germination (F = 2.876, P = 0.064). However, the germination percentage among the groups was significantly different (X^2 = 20.316, P < 0.001), with late-flowering plants having the lowest germination percentage and average-flowering plants having the highest germination percentage (Table 8).

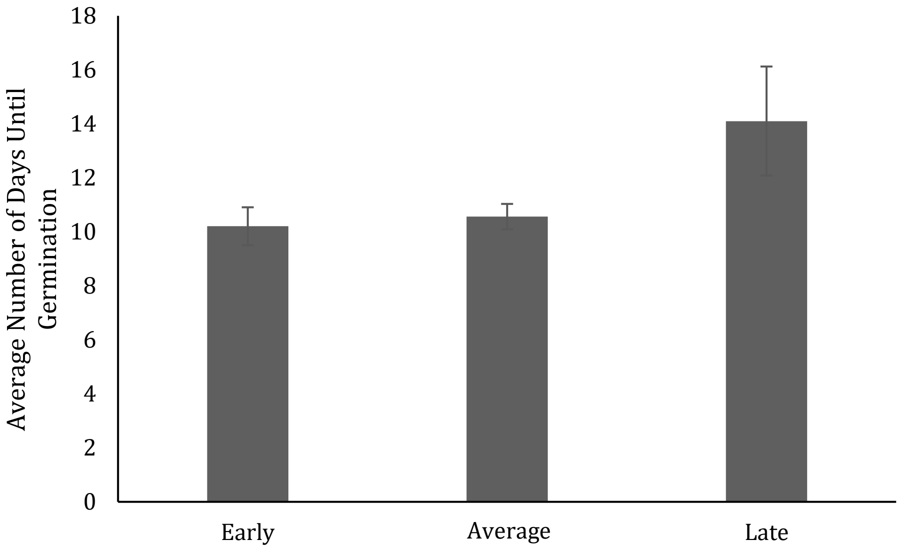

Figure 12: Average number of days until germination in the greenhouse for the seeds of early-flowering plants, average-flowering plants, and late-flowering plants from Smith Springs 2016.

Table 8. Percentage of seeds from the offspring of early-flowering, average-flowering, and late-flowering plants that germinated in the greenhouse.

Group	Germinated	Did not Germinate	Total Started	% Germination
Early	122	102	224	54.46
Average	139	109	248	56.05
Late	93	154	247	49.24
P-value				**<0.0001**

DISCUSSION

Historical Variation in Flowering Time and Climate

Leavenworthia stylosa seems to be following the same trend of earlier flowering that has been reported for numerous plant species across the globe (Fitter & Fitter 2002; Primack 2004; Amano et al. 2010; Munguia-Rosas et al. 2011). A century of herbarium specimens supported a trend towards earlier collection times, implying a trend towards earlier flowering times. In *L. stylosa*, I found that plants were flowering and fruiting approximately 13.2 days earlier, as compared to the 8-day flowering time advancement found by Primack et al. (2011) in Boston and 4.5-day advancement for 385 British plant species combined (Fitter & Fitter 2002). Furthermore, the earliest date of collection observed in our herbarium specimens for the end of the century was 21 days earlier than the earliest date of collection of specimens collected at the beginning of the century. The study conducted by Primack et al. (2011) focused on woody plants, which may be less susceptible to short-term, climate fluctuations, whereas the study conducted by Fitter and Fitter (2002) included both perennial and annual plants, with annuals showing more extreme advancement in onset of flowering than perennials. The early flowering event observed for *L. stylosa* in the winter of 2015-2016 was an anomaly compared to the species' overall trend towards early flowering, as there are no records of *L. stylosa* previously flowering as early as December. In the herbarium specimens I observed, the earliest date of collection is in early March.

Costs and Benefits of Early Flowering Time

I did not find that early flowering time decreased fitness in *L. stylosa* in the spring of 2016. When compared to average- and late-flowering time categories from the same year, early-flowering plants were not significantly more successful. In 2017, there was a clear disadvantage to flowering late in the season (first flowers not appearing until April). Late-flowering plants at all four field sites in 2017 had a significantly lower survival rate, mean number of viable seeds per plant, mean number of fruits per plant, mean number of seeds per fruit, and at Smith Springs and Butler only, a lower average single seed mass than early-flowering plants. At Flat Rock in particular, survival of late-flowering plants was less than half that of early and average-flowering plants. Therefore, within years a clear disadvantage to flowering late but no significant advantage or disadvantage to flowering earlier than the population average flowering time.

Between years, plants from the early flowering year of 2016 produced significantly more fruits, a higher number of seeds per fruit, more viable seeds per plant, had an increased average single seed mass and a higher survival rate compared to plants from the average flowering year of 2017. Previous research has attributed the increase in success during years when early flowering is observed in part to the longer flowering season associated with flowering earlier (Hendry & Day 2005; Kudo 2006; Elzinga et al. 2007; Munguia-Rosas et al. 2011). In an experimental manipulation of *Brassica rapa,* Austen and Weis (2015) found that while there was no significant relationship between the age of a plant at flowering time and seed production, there was a fitness advantage for early-flowering plants due to superior environmental conditions during earlier reproduction. In my study, while I did not find an advantage to early flowering in individual years, I did find a disadvantage to late flowering and an overall increase in fitness in plants regardless of flowering time in the early flowering event year. Consistent with the previously mentioned findings of selection favoring a longer duration of flowering season (Hendry & Day 2005; Kudo 2006; Elzinga et al. 2007; Munguia-Rosas et al. 2011), my study suggested that it is perhaps the longer reproductive season that is being favored and not early flowering time itself.

In 2016, all plants flowered 1 to 4 weeks longer than those in 2017. While my data showed that the duration of flowering time increased during the early-flowering event of 2016, I did not see an increase in successful fruits early on in the flowering season, particularly for early-flowering plants, indicating that those early flowers did not result in successful fruits. Overall, my data suggested that there was no benefit to flowering early, but that flowering late was costly due to shortened reproductive time, especially in the mid to late part of the season when most successful flowers were produced. However, even though early flowers were not successful in the two years of my study, it does not necessarily mean that there are not years in which early flowers are successful.

Based on these data, it is crucial to ask why a long-term pattern of early flowering time (as demonstrated by the herbarium specimen study) is occurring. *Leavenworthia stylosa* was found to be flowering earlier over time, but based on my data, I did not find a benefit to earlier flowering. Additionally, I did not find any climatic trends that would seem to result in earlier flowering nor was there evidence that alterations in germination time influenced flowering time. So why was a historical trend towards earlier flowering found?

What is Influencing Flowering Time?

Previous studies point to climate change as a factor influencing earlier flowering times (Anderson et al. 2012; Austen et al. 2017). Studies on wheat in eastern Australia found that an increase in temperature resulted in a delay in flowering dates (2.4 days) for winter wheat and earlier flowering dates (6.2 days) in spring wheat (Wang et al. 2015). Panchen (2016) also used herbarium specimens and temperature records to determine that flowering times in Arctic plants have advanced over the last 120 years with a corresponding increase in mean monthly temperatures during flowering times. More specifically, Panchen (2016) found that plants with traditionally later flowering times advanced at a faster rate than plants with traditionally earlier flowering times, suggesting that late growing season temperatures were rising faster than early growing season temperatures. Looking at the spring climate data, I did not see a warming trend in the Central Basin of Tennessee despite the long-term trend in earlier flowering in *L. stylosa,* indicating that the long-term early-flowering trend was probably not cued by warmer spring temperatures. However, in the two years of the field study, I did observe warmer than average winters, with the average temperature from December through March being 7.6°C in 2015/2016 and 8.5°C in 2016/2017, compared to the century average (1915 – 2015) of 5.9°C. Spring precipitation is also not likely to be a strong influence on the long-term trend in flowering time since records showed no significant change in total monthly precipitation for the months leading up to and during flowering over the last century. However, in the two years of the field study, I did see slightly higher than average monthly rainfall for the winter of 2015/2016 and even higher for the winter of 2016/2016. For the months of December to March, there was an average of 124.1 mm of rain per month in the winter of 2015/2016 and an average of 135.3 mm of rain per month for the same months in 2016/2017, compared to the century average of 122.8 mm. Wetter winters during the time of the field study could have influenced the flowering time data collected here, even though long-term precipitation data does not seem to be consistent with earlier flowering times.

I next examined whether there were long-term changes in temperature or precipitation in late summer and fall, when *L. stylosa* seeds germinate, since earlier germinating plants might also flower earlier. Plant maturity is a known factor that partially controls the onset of flowering in winter annuals with larger plants flowering earlier than smaller ones (Pemadasa & Lovell 1974; Lacey 1986; Munguia-Rosas et al. 2011). In a meta-analysis of 296 species with varying life histories conducted by Munguia-Rosas et al. (2011), a weak trend was observed for larger plants to flower earlier than small ones. For annuals specifically, Lacey (1986) asserts that, due to the imminent death within a year of germination and their unpredictable growing seasons, size and density at first flowering varies much more for annuals than for other plants. Since *L. stylosa* is a fall germinating winter annual, fall weather conditions are likely to influence germination time and seedling size as the reproductive season approaches making it possible that fall weather conditions could affect flowering time in *L. stylosa.* In fall 2016, when the spring 2017 flowering plants germinated, I saw that seeds that germinated in September or October at two of our sites (Flat Rock and Smith Springs) did not survive while at a third site (Quarterman) seeds did not germinate at all during these dates. This observed seedling death was most likely due to insufficient rainfall with just 5.6 mm of rain in the month of October 2016 (NOAA National Climate Data Center – Climate Data Online https://www.ncdc.noaa.gov/cdo-web/) compared to the average of 78.6 mm of rain for the month of October over the last century. Almost all of the seedlings that survived to flowering were those that germinated in mid-December (aside from Butler, which had a 79% survival rate for seeds that germinated in September/October). Looking back at the 2015 fall precipitation data, the Central Basin of Tennessee received approximately 129.5 mm of rain total in the month of October (NOAA National Climate Data Center – Climate Data Online https://www.ncdc.noaa.gov/cdo-web/). With the 2015 total October rainfall being higher than the average of 78.6 mm, it is possible that this rainfall provided enough moisture to keep the earlier germinating seeds alive through the fall. This would mean that in the winter of 2015/2016, the early-flowering plants we saw may have germinated earlier in the fall. In contrast, in the spring of 2017, the plants that were flowering had mostly germinated later in December. If cooler, wetter germination seasons cause earlier germination, plants would reach maturity faster, potentially explaining earlier flowering. However, in their controlled study manipulating germination times and environmental conditions of *Brassica rapa,* a rapid growth annual, Austen and Weis (2015) found the influence of plant age on overall fecundity to be far weaker than the influence of the environmental conditions that the plant was exposed to during seed maturation. Congruent with the findings of Austen and Weis (2015), my germination and overwinter to flowering data did not show any relationship between germination date and date of first flowering. I observed wa relatively wide range of germination dates both among sites and within sites, but there was no relationship between flowering time and germination time. The date of first flowering at each plot seemed to be relatively consistent across sites and plots regardless of germination time, as they all flowered within the same two weeks in mid- to late-March. At one site (Butler) there were seedlings that germinated in October that survived through flowering in March, but flowered at the same time as the other seeds that germinated in December. This does not necessarily tell us that seed germination time does not influence flowering time, but it does mean that there are at least more factors at play influencing flowering time.

According to the 2016 rosette-size data, there was no trend for larger plants to flower earlier, as rosette size did not differ among flowering time groups. This result is in contrast to the findings of a meta-analysis by Forrest (2014), who showed that in 24 of 28 studies of annuals and perennials, larger plants flowered earlier. However, my data did show that there was a positive relationship between plant size and total number of seeds produced, potentially supporting the idea of condition-dependence, or that larger plants were in better condition than smaller plants of the same age and have accumulated more resources (Forrest 2014; Austen et al. 2017).

In the germination experiments in the greenhouse and incubator, I did not observe any significant influence of parental flowering time on offspring germination time. In contrast with these findings, in a similarly conducted study of *Daucus carota*, Lacey and Pace (1983) found that seeds of early-flowering parents germinated earlier, possibly because the seeds matured earlier. Additionally, Elwell et al. (2011) found that parental environment in *Arabidopsis thaliana* significantly affected the germination time, formation of first flower, and seed weight of the offspring.

However, it should be noted that my ability to detect differences in germination time due to parental flowering time was limited by several factors. First, I did not know the exact flowering time of parental plants, but rather had plants grouped into three broad flowering time categories. Secondly, plants of all three flowering time groups produced flowers at the same time late in the season. This means that plants that flowered early could have had flowers that were pollinated by late-flowering plants and vice versa. Thus, my experimental results could be confounded by the fact that I only know the flowering time of the maternal plant.

Ideally plants in the greenhouse and incubator germination experiments would have survived to flowering, so that if there was a relationship between germination time and flowering time in the greenhouse it could have been discovered. Additionally, I would have been able to assess the relationship between parental flowering time in the field, and the flowering time of their offspring.

In *Arabidopsis thaliana,* Elwell et al. (2011) showed that parental flowering time can influence seed mass, and seed weight can influence flowering time. I observed a decline in average seed weight with later flowering times in 2017 but not in 2016, and a significantly lower seed weight for the seeds produced in 2017 vs. 2016.

For both the greenhouse and incubator germination experiments I observed that parental flowering time affected the percentage of seeds that germinated, with the late-flowering group having the lowest offspring germination percentage. Case et al. (1996) also found in controlled, hand-pollinated studies on *Plantago lanceolata* that parent flowering time significantly influenced offspring germination percentage, with extremely early and later flowering plants producing seeds that have a lower germination percentage. Overall, my germination experiments showed that although parental flowering time may not influence germination time, late flowering is costly because seeds produced by later flowering plants were smaller and had lower germination percentages than earlier flowering ones.

Overall, I found that over the past century, *L. stylosa* has been flowering earlier. However, within both years of my study, there was no apparent advantage to early flowering. There was, however, a cost to flowering late. Between years, plant fitness was higher during a year of extremely early flowering compared to a more average flowering time year, likely due to an extended flowering period. Based on the data, the long term trend in earlier flowering of *L. stylosa* could not be attributed to climate variables, fall germination time, or plant age, but the early-flowering event of 2016 may be linked to a wetter fall and warmer spring.

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