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RESEARCH ARTICLE

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Chilling consequences: Herbarium records reveal earlier reproductive phenology of winter annual gladecress in a wetter, cooler climate

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Societal Impact Statement

Networks of digitized herbarium records are rich resources for understanding plant responses to climate change. While the climate is warming globally, some localities are experiencing climate cooling, the effects of which are poorly understood. Our herbarium-based study of a geographically restricted species shows that the timing of reproduction can shift earlier as the climate becomes cooler and wetter. Local variation in climate change may be a key factor driving the high variability of changes observed in plant reproduction and climate cooling should be considered along with other global change drivers. This will help enable accurate predictions for the successful management of climate change effects.

Summary

- Plant phenological responses to global warming are well studied. However, while many locations are experiencing increased temperatures, some locations are experiencing climate cooling. Little work has been conducted to understand plant phenological responses to cooling trends, much less the combined effects of cooling and other factors, such as changing precipitation. Furthermore, studies based on herbarium specimens have been instrumental in demonstrating plant responses to global warming; but to our knowledge, herbarium records have not been used to investigate responses to cooling.
- We collected data from 98 years of herbarium records to evaluate whether the reproductive phenology (flowering/fruiting) of an annual mustard, cedar gladecress (*Leavenworthia stylosa*), has changed as the climate has become cooler and wetter in central Tennessee, USA. Additionally, we conducted two field experiments to assess reproductive consequences of different flowering times.
- Over the last century, gladecress reproductive phenology has shifted 2.1 days earlier per decade, concurrent with wetter conditions during germination and cooler conditions during reproduction. Field experiments showed that plants with extremely early and moderately early flowering had equivalent reproduction,

Caitlin Banaszak and Joshua B. Grinath contributed equally to this study.

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but these plants had greater reproduction than intermediate- and late-flowering plants.

- Counter to expectations from global warming studies, our work demonstrates that climate cooling and greater rainfall can result in earlier plant reproductive phenology, potentially due to asymmetric selection for early flowering. Future studies may need to consider climate cooling along with other global change factors to fully explain changes in plant phenology. Our understanding of plant responses to climate cooling can be enhanced through additional herbarium-based research.

KEYWORDS

climate cooling, flowering phenology, global change, herbarium, *Leavenworthia stylosa* (cedar gladecress), plant reproduction, precipitation, winter annual life history

1 | INTRODUCTION

Numerous studies now show that plant phenology has changed, and will continue to change, as a result of climate warming (Amano, Smithers, Sparks, & Sutherland, 2010; Cleland et al., 2012; Ibanez et al., 2010; Miller-Rushing & Primack, 2008; Parmesan & Yohe, 2003). For many spring-flowering plants, research indicates that the timing of reproductive life-history stages (flowering, fruiting) is occurring earlier as the climate warms (Beaubien & Hamann, 2011; Fitter & Fitter, 2002; Wadgymar, Ogilvie, Inouye, Weis, & Anderson, 2018; Wolkovich et al., 2012). However, these phenological responses are highly variable across species (Cleland et al., 2012; Dunnell & Travers, 2011; Fitter & Fitter, 2002; Ibanez et al., 2010; Miller-Rushing & Inouye, 2009; Miller-Rushing & Primack, 2008) and may be complicated by other climatic factors, such as changes in precipitation in addition to changes in temperature (Crimmins, Crimmins, & Bertelsen, 2010; Inouye, Saavedra, & Lee-Yang, 2003; Konig et al., 2018; Moore & Lauenroth, 2017). For instance, flowering phenology in water-limited systems can be driven more by changes in precipitation than by temperature (Crimmins et al., 2010). Moreover, though the climate is warming globally, there is great variation in trends at local and regional scales, with some areas experiencing climate cooling (Doran et al., 2002; Schwartz & Reiter, 2000). Few studies have considered how plant phenology responds to cooling climate trends (Crimmins et al., 2010; Meng et al., 2017), but evidence suggests that phenological responses will not simply be the inverse of responses to climate warming (Li et al., 2016). By focusing research on global warming, ecologists have assembled an incomplete picture of phenological responses to climate change (Cleland, Chiariello, Loarie, Mooney, & Field, 2006; Pau et al., 2011). At present, it is unclear how plant reproductive phenology may be altered by local climate cooling and simultaneous changes in precipitation, which may inhibit our ability to accurately predict responses to climate change.

Herbarium records have proven to be reliable sources of phenological data, revealing changes in reproductive phenology in response to climate warming and altered precipitation (Davis, Willis, Connolly, Kelly, & Ellison, 2015; Hufft, DePrenger-Levin, Levy, & Islam, 2018; Matthews & Mazer, 2016; Park et al., 2018). Though much effort has been devoted to digitizing herbarium records to expand their use in research, they remain a largely untapped resource in the study of global change (Meineke, Davis, & Davies, 2018). Presently, no study to our knowledge has collected data from herbarium specimens to evaluate plant phenological responses to climate cooling.

Here, we conducted a herbarium-based study to investigate whether there has been change in the reproductive phenology of a spring-flowering plant as the local climate has become wetter and cooler. Cedar gladecress (*Leavenworthia stylosa* Gray) is a species of early-flowering mustard (Brassicaceae) that is endemic to the Central Basin of Tennessee (Baskin & Baskin, 2003). This species grows in cedar glade habitats (Harper, 1926), which are characterized by shallow, rocky soils that are hot and dry in summer and waterlogged during the cool, wet winter (Cartwright & Hui, 2015). To cope with these conditions, gladecress has a winter annual life history: Seeds are dormant during the summer before germinating in the fall, then the plants overwinter as rosettes before flowering, fruiting, and senescing in the early spring (Baskin & Baskin, 1971, 1972). This life-history strategy is highly responsive to environmental cues (Baskin & Baskin, 1971; Pemadasa & Lovell, 1974), and we expected gladecress reproductive phenology would be sensitive to changes in climate. Specifically, changes in temperature or precipitation in the summer and fall could influence the timing of germination, while changes occurring in the winter and spring could influence the initiation and/or duration of flowering. Such shifts in phenology in response to climate have been observed in the well-studied annual mustards *Arabidopsis thaliana* (Fournier-Level et al., 2016; Springate & Kover, 2014; Wilczek et al., 2010) and *Brassica rapa* (Austen & Weis, 2015; Franks, Sim, & Weis, 2007; Hamann, Weis, & Franks, 2018). Over the past century, annual precipitation has increased while the average temperature has decreased in the home range of cedar gladecress (Figure S1), as elsewhere in Tennessee (Lesser & Fridley, 2016; Trepanier, Roberts, & Keim, 2015).

We anticipated that cedar gladecress reproductive phenology could either occur later or earlier with a wetter, cooler climate. Research on other annual mustards found that the onset of flowering

becomes earlier with warmer temperatures and/or drought conditions and is delayed by greater precipitation (Fournier-Level et al., 2016; Franks et al., 2007; Hamann et al., 2018; Manzano-Piedras, Marcer, Alonso-Blanco, & Pico, 2014; Springate & Kover, 2014). These studies suggest that the timing of gladecress reproductive stages might be later in a wet and cool climate. However, previous work with gladecress has found earlier germination during wet and cool weather conditions (Baskin & Baskin, 1972), and reproductive phenology could potentially become earlier in such conditions if early-germinating plants also initiate reproduction earlier (Post, Pedersen, Wilmers, & Forchhammer, 2008; Wilczek et al., 2010).

In either case, shifts in plant phenology are expected to impact reproductive success. For example, drought selects for early flowering in *B. rapa* (Franks et al., 2007; Hamann et al., 2018; Weis, Wadgymar, Sekor, & Franks, 2014), and early-flowering individuals exhibit greater seed production (Austen & Weis, 2015). Alternatively, changes in flowering phenology could cause mismatches with the availability of pollinators (Hegland, Nielsen, Lazaro, Bjerknes, & Totland, 2009; Rafferty, CaraDonna, & Bronstein, 2015) or expose reproductive structures to damaging conditions, such as frost (Gu et al., 2008; Inouye, 2008), that could decrease plant reproduction.

To assess changes in gladecress phenology, we collected data from 98 years of herbarium specimens (years 1919–2016). We measured the day of year corresponding to three phases of reproductive phenology (flowering only, flowering and fruiting, fruiting only) represented in the herbarium records and then evaluated whether the timing of gladecress reproduction changed along with the climate. To help understand the climatic drivers influencing reproductive phenology, we compared the dates with the temperature and precipitation conditions corresponding to the 6 months leading up to and including the reproductive season (winter/spring), and the 6 months leading up to and including the preceding germination season (summer/fall). We also conducted two field experiments to evaluate plant reproductive performance in relation to flowering time. We measured fruit and seed production for early-flowering versus later flowering plants in each of 2 years: one with very early flowering and another with typical flowering phenology. Additionally, we tested seed germinability for early-flowering versus later flowering plants to assess whether phenology impacted seed quality. Unlike most previous studies, our work shows that earlier reproductive phenology can result from trends in climate cooling and increased precipitation that are advantageous for plant reproduction.

2 | MATERIALS AND METHODS

2.1 | Herbarium Study

We matched data from herbarium records with the longest available temperature and precipitation time series measured within the range of cedar gladecress. During October and November 2018, we collected herbarium record data from digitized images available via the Southeast Regional Network of Expertise and Collections (http://sernecportal.org). Data collected from the digital MTSU records were validated by directly viewing the specimens at the herbarium. Additional herbarium record data were provided by James Beck (Wichita State University), who performed a previous survey of *Leavenworthia* sp. specimens from seven herbaria (BRIT, G, LL, MO, NY, UNA, US; J. B. Beck, unpubl. data). For each herbarium record, we categorized the phenological stages of the plants as "flowering" (without fruits present), "flowering and fruiting," or "fruiting" (without flowers present), and we recorded the date, location, and collector. Specimens were considered to be fruiting if they had at least one fruit with developing seeds. Phenophases were classified using a binary method (presence/absence of flowers or fruits) because this provides similar results to methods based on fine-scale phenological data, but is much less time-consuming (Ellwood, Primack, Willis, & HilleRisLambers, 2019; Pearson, 2019). Many records contained more than one plant; for these, we categorized the phenological stage exhibited by all plants combined. Although there were specimens collected by Gattinger from 1879 to 1885, collection dates were frequently missing from these records and they occurred outside the range of available climate data. The next oldest herbarium record was collected in 1919, and we focused our data collection on 546 herbarium specimens collected between 1919 and 2016. Because many records were duplicates that were collected concurrently, we synthesized data from records occurring on the same day to express gladecress phenology at the level of the entire population. Thus, if two records from the same day had been scored as "flowering" and "fruiting," respectively, then their combined phenology was recorded as "flowering and fruiting." This procedure resulted in a total of 268 observations, each a unique record of reproductive phenology on a specific day. Month and day were then translated into day of year (Julian date), correcting for leap years.

Monthly environmental data were downloaded from the National Oceanic and Atmospheric Administration's online climate data (accessed October 2018: https://www.ncdc.noaa.gov/cdo-web/datatools/findstation). Cedar gladecress has primarily been documented from three counties in central Tennessee (Davidson, Rutherford, and Wilson), with occasional records from surrounding counties (Bedford, Marshall, Maury, Smith, Sumner, and Williamson). The longest and most complete climate record within this range is for the Murfreesboro station (USC00406371; latitude: 35.9204, longitude: −86.3727, elevation: 163.1 m). We used climate data starting in 1918 to describe the environmental conditions leading up to the first herbarium data point in 1919. There were several small gaps in this climate record, and to fill these, we input data from the next longest climate record within the gladecress range in Lebanon, TN (station: USC00405108); a few points were input from the nearby station in Franklin, TN (USC00403280). Data from these stations covaried with those of Murfreesboro; thus, these inputs were appropriate because they reflected the climate in Murfreesboro. Altogether, we amassed a dataset of total monthly precipitation and average monthly temperature from 1918 to 2017, covering the range of the herbarium data and our field experiments in 2016 and 2017 (Figure S1). These data were combined to describe the total precipitation and average temperature in two 6-month

periods, from June through November and from December through May. Germination usually occurs from August through October, but can occur as early as July and span multiple months in the same year (Baskin & Baskin, 1971, 1972). Therefore, the summer/fall climate variables describe the conditions during seed maturation, germination, and early seedling survival, hereafter referred to as the "germination season." February was the earliest flowering month in the herbarium records, but we have personally observed cedar gladecress flowering as early as December in 2015 (C. R. Herlihy, pers. obs.). Thus, the winter/spring climate variables characterize the conditions leading up to and including flowering, fruiting, and senescence, hereafter called the "reproduction season." While there are many ways to relate climate and plant phenological data, such as considering climate records for each month separately, in various combinations, or using finer temporal scale events, results are often hard to interpret due to collinearity among a large number of predictor variables and because life-history periods potentially span multiple months. Our method dissects the gladecress annual life cycle into two, broad seasons (germination vs. reproduction) with minimal covariation among climate variables to enhance our ability to interpret drivers of phenology in the herbarium data at a coarse scale.

2.2 | 2016 Field Experiment

To examine the fitness costs and benefits associated with earlier flowering, we conducted a field experiment during the extremely early-flowering year of 2016 to compare plants that initiated flowering at different times. This study was conducted at a cedar glade near Smith Springs Road in Antioch, Tennessee (36.078619, −86.588787). On February 5, we established a 50 m transect through the glade, along which we marked 50 flowering plants and 50 nonflowering plants with numbered nails at the base of each plant. Experimental plants were selected by marking one flowering and one nonflowering plant closest to the transect at approximately 1 m intervals. By March 10, a majority of the previously marked nonflowering plants were now flowering, and we marked a third group of 50 nonflowering plants. This experimental design resulted in three treatment groups for the onset of flowering: extremely early flowering (prior to February 5), early flowering (between February 5 and March 10), and intermediate flowering (after March 10). See Figure 1f for the dates corresponding with these groups. Treatment group designations are in reference to flowering phenology in a typical year. A late-flowering group was not present in 2016; thus, the intermediate-flowering group were the last plants to flower in this year.

Plant maturation was tracked via weekly observations from February 5 to April 26. To determine whether flowering time was related to plant size, we measured rosette size with digital calipers for all plants on March 18. On April 26, when the experimental plants had ceased flower production, we counted the number of fruits and failed flowers. Cedar gladecress flowers are borne singly on individual pedicels, and these pedicels remain attached to the rosette even if the flower fails to produce a fruit. Therefore, we could measure total flower production as the number of fruits plus the number of failed flowers (a pedicel lacking a fruit). Fruits from all plants were harvested at this time and stored in envelopes at room temperature in the laboratory.

After allowing to air dry, we opened the fruits to count the total number of seeds produced by each plant. Seeds were only counted if they had developed a mature embryo. Seed number per fruit was then measured as total seed number divided by the number of fruits. If a plant had already dispersed a portion of their seeds, then the number of uncounted seeds produced was estimated by multiplying the number of seeds per fruit (calculated from the intact remaining fruits) by the number of fruit that had opened. This estimated number of seeds was then added to the observed seed count to obtain total seed number. Average single seed mass was measured for each plant by weighing all seeds together and dividing by total seed number.

Next, we assessed seed germination to evaluate differences in viability across treatment groups. All plants with 10 or more seeds were included in an incubator experiment. 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-hr day length. Up to 20 seeds per parent plant were used. In total, 27 extremely early-flowering plants were represented with 437 seeds (mean: 16.2 seeds per plant), 22 early-flowering plants were represented with 387 seeds (mean: 17.6 seeds per plant), and 22 intermediate-flowering plants were represented with 382 seeds (mean: 17.4 seeds per plant). Incubation of seeds started on October 14 and the presence of germination was observed daily over the next 20 days. For each seed, we recorded whether the seed germinated and the number of days until germination.

Following the incubator trial, a second germination trial was conducted in a greenhouse to determine whether the same results were obtained when the seeds were sown in soil. All plants with more than five viable seeds remaining after the incubator trial were included in the greenhouse trial. On March 24, 2017, all seeds were directly sown into 10.2 × 10.2 cm pots filled with a 1:2 mixture of sand to Miracle Grow potting mix and placed in the greenhouse set at 23°C. Supplemental lighting was used to extend natural daylight to a total of 12 hr of light per day. Pots were hand watered and monitored for germination daily over the next 20 days. In total, 20 extremely early-flowering plants were represented with 224 seeds (mean: 11.2 seeds per plant), 23 early-flowering plants were represented with 248 seeds (mean: 10.8 seeds per plant), and 24 intermediate-flowering plants were represented with 247 seeds (mean: 10.3 seeds per plant). Germination data were collected in the same manner as the incubator trial.

2.3 | 2017 Field Experiment

Flowering began at a typical time in 2017, and we used this opportunity to conduct a field experiment assessing the fitness

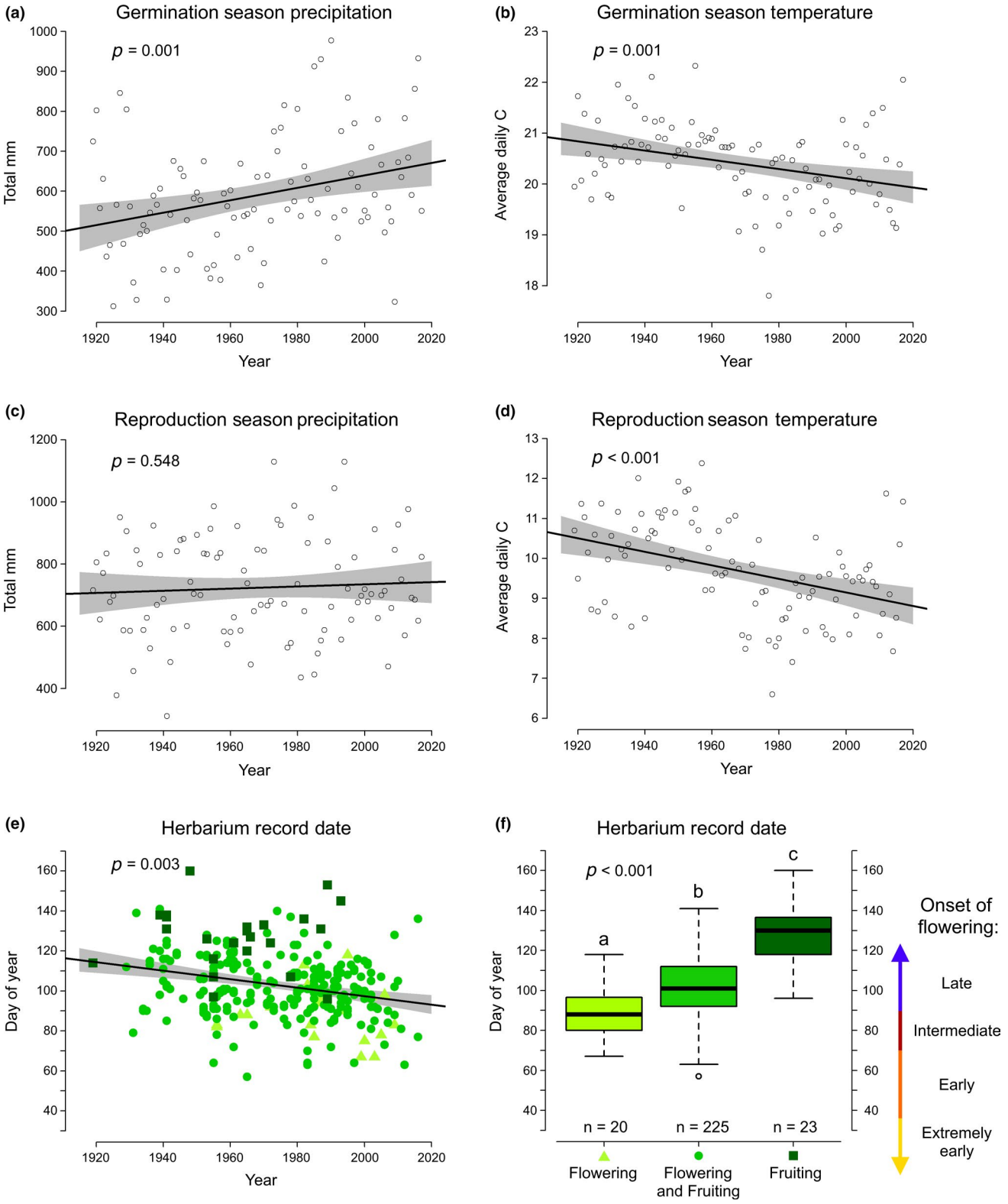


FIGURE 1 Changes in climate and gladecress reproductive phenology through time, and the categorization of treatment groups corresponding to the onset of flowering. (a) Precipitation and (b) average temperature during the germination season from June to November. (c) Precipitation and (d) average temperature during the reproduction season from December to May. (e) Day of year of herbarium records through time and (f) across phenological phases. Regressions are shown with 95% confidence intervals and corresponding *p*-values for the slope (generalized least square models). Points in (e) indicate phenological phases (flowering: light green triangles, flowering and fruiting: green circles, fruiting: dark green squares). Significant ($p \leq .05$) post hoc Tukey contrasts are indicated by lowercase letters above boxplots in (f). Dates for treatment groups for the onset of flowering in the field experiments are provided in (f). Colors for flowering-onset treatment groups correspond to later figures

costs and benefits associated with differences in flowering phenology in a normal year. This experiment was conducted at three field sites in addition to the site used in 2016 at Smith Springs: Flat Rock State Natural Area (35.858214, −86.295611), Butler Cedar Glade (36.090215, −86.617095), and Quarterman Cedar Glade (36.048858, −86.560430). At each site, two parallel 25 m transects were established on March 9–12, and 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 plants, which still had not flowered, were marked at each site on March 30–31. Thus, the three treatment groups for the onset of flowering (Figure 1) included in this experiment were: early flowering (prior to March 9–12), intermediate flowering (between March 9 and March 30–31), and late flowering (after March 30–31). An extremely early-flowering group was not present in 2017; thus, the early-flowering group were the first plants to flower in this year. In total, 600 plants were marked (150 plants at each site).

We monitored plant maturation approximately every 10 days through the end of the reproductive season. From April 21 to May 5, we counted fruit production for each plant and collected fruits in envelopes. Fruits air-dried in the laboratory; then for each plant, we measured the total number of seeds, seed number per fruit, and average single seed mass as described above.

2.4 | Statistical Analyses

We conducted all data analyses in R v3.5.2 (R Core Team, 2018). First, we used generalized least squares (GLS) regression to evaluate whether climate variables changed through time. Year was rescaled as a time series from 1 to 99 for analysis, and was included in the models as a continuous fixed effect. The regressions were run with the "gls" function (package "nlme"), and for each analysis, we considered models with different variance structures to meet the assumption of homoscedasticity. We used Akaike information criterion (AIC)-based model selection to determine the optimal variance structure (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). The AIC score from the regression without variance structure was compared with those from regressions with three structures (fixed, power, exponential), and the inclusion of variance structure was supported if the AIC score was > 2 units lower than that of the model without structure. Herbarium record date was analyzed as a function of time in the same way, except that phenological phase was included as a fixed three-level factor and the model included a term for the interaction between time and phenology. In addition to the variance structures described above, for this ANCOVA we also considered a model assuming unequal variances across phenological stages and compared AIC scores using the same procedure.

Next, we used GLS to analyze herbarium record date as a function of precipitation and temperature during the germination and reproduction seasons (four continuous variables), phenological stage, and all interactions among these five factors. To determine the optimal variance structure for this ANCOVA, we compared the AIC of the model without structure to those for models with three structures for each continuous variable (12 models) and for the model assuming unequal variances across phenological phases. Prior to analysis, we assessed correlations between each pair of climate variables using the "cor.test" function to determine whether there was collinearity among predictor variables in the ANCOVA. The climate variables were mostly non-correlated, but germination season temperature was moderately correlated with germination season precipitation and reproduction season temperature (Table S1). To assess the impact of this correlated variable on the results, we first ran the ANCOVA including all climate variables and their interactive effects, then we re-ran the model with germination season temperature included as a main effect without considering its interactive effects. There were no differences in conclusions drawn from these models; therefore, we proceeded with the simpler model. To avoid overfitting, we next conducted a selection procedure to further simplify the model. Following the method of Zuur et al. (2009), we used likelihood ratio tests ("ANOVA" function) to remove non-significant interaction terms from the model. The final model included any significant interaction terms and their lower-order dependent terms, and all main effects.

Data from the 2016 field experiment were analyzed using GLS for variables with Gaussian-distributed residuals and generalized linear models (GLMs) for variables with other error distributions. For these analyses, only plants that flowered were considered. The GLS models were conducted with flowering phenology included as the sole fixed factor (a three-level nominal variable). As above, for each analysis we compared the AIC score for the model without variance structure with the AIC for the model assuming unequal variances. These models were performed for total seed number, seed number per fruit, average single seed mass, and rosette size; total seed number and rosette size were natural log (LN) transformed (plus one) to meet the assumption of normality. Flower and fruit production were analyzed with GLMs appropriate for count data. Similar to above, we compared the AIC scores for models with Poisson and Negative Binomial distributions, using the functions "glm" and "glm.nb" (package "MASS"), respectively. The initial flower production analysis indicated that treatment groups may differ, but there were extreme outlying data values that could have influenced this result. To obtain a model with appropriate fit, we removed the two highest outlying points from each treatment group, which were all over three times the interquartile range higher than the upper quartile for each treatment level. To aid comparison, these same data were used to evaluate fruit production. Germination data were evaluated with similar methods, but we used generalized linear mixed-effects models (GLMMs; package "lme4") with parent plant identity included as a nested random effect (random intercepts) because multiple seeds derived from the same plant were not independent of each other. Days to germination was analyzed with GLMMs assuming Poisson and Negative Binomial error structures ("glmer" and "glmer.nb"

functions with a "bobyqa" optimizer) and AIC scores were compared as above. Germination presence was analyzed using a GLMM with a Binomial error structure.

We analyzed data from the 2017 field experiment with GLMMs according to the appropriate error distribution for each response variable. Field site identity was included as a nested random effect (random intercepts) in these models to account for the non-independence of data collected within each site, and flowering phenology was included as a fixed three-level variable. Fruit production was analyzed with the same method described above for days to germination. Total seed number (LN transformed), seed number per fruit, and average single seed mass were analyzed with models assuming Gaussian-distributed residuals using the "lme" function (package "nlme"). We used this R function because it allowed us to consider models assuming unequal variances across the phenology factor, using the same AIC procedure as for the GLS models.

Because sample sizes were unbalanced across treatment levels (some plants died or never flowered), we used the "ANOVA" function ("car" package) to conduct all models with Type II SS (Wald tests). Results were considered significant if $p \leq .05$. Tukey post hoc contrasts were implemented to discern differences among phenological stages following significant results ("glht" function from "multcomp" package). Interactive effects of continuous variables were visualized with response surface graphs ("persp" function, "rsm" package).

3 | RESULTS

From 1918 to 2017, the climate changed during both the germination (summer/fall) and reproduction (winter/spring) seasons for cedar gladecress, but in different ways (Figure 1; Table S2). Germination season precipitation increased through time (15.5 mm/decade), concurrent to a decrease in temperature (Figure 1a,b). During the reproduction season, precipitation did not significantly change, but temperature decreased across years (Figure 1c,d). The trend in climate cooling was stronger for the reproduction season (slope = −0.17 °C/decade) than the germination season (slope = −0.09 °C/decade). The herbarium records showed that from 1919 to 2016 the mean date for gladecress reproductive phenology (across all three phases) decreased by 2.1 days per decade (20.6 days over the 98 year dataset), indicating that the timing of reproduction became earlier (Figure 1e). While the dates for the phenological phases all differed from each other (Figure 1f), there was not a statistical interaction with time, indicating that the trend for earlier phenology was not different across the three phases (Table S2).

Herbarium record date was significantly related with climate variables, but there were no significant interactions between phenological phase and climate variables. The simplified model following the selection procedure is provided in Table S3. Rather, we found that date responded to a three-way interaction ($\chi^2 = 6.641$, $p = .010$) between germination season precipitation and reproduction season temperature and precipitation (Figure 2). This result indicates that the changes in germination season precipitation and reproduction season temperature through time (Figure 1a,d) jointly influenced gladecress reproductive phenology, but that this effect was dependent on reproduction season precipitation. When there was low precipitation during the reproduction season (Figure 2a), the mean date was early if there was high precipitation during germination and high temperatures during reproduction, and when there was low germination season precipitation and low reproduction season temperature. The mean date was late when high germination season precipitation co-occurred with low reproduction season temperatures

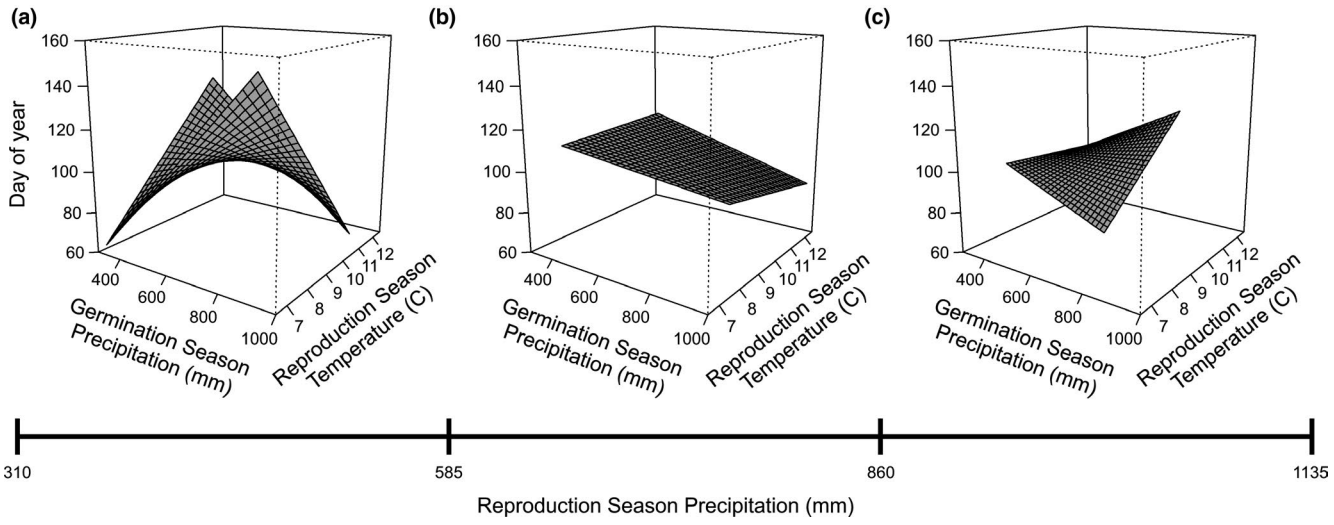


FIGURE 2 Response of the mean herbarium date to the three-way interaction between germination season precipitation and reproduction season temperature and precipitation. Response surfaces are shown for day of year as a function of total precipitation during the germination season (June–May) and average daily temperature during the reproduction season (December–May) at (a) low, (b) average, and (c) high reproduction season total precipitation. Reproduction season precipitation was divided into equal bins of 275 mm for visualization. The corner in the foreground of the plots corresponds with high germination season rainfall and low reproduction season temperature

and vice versa. When precipitation was average during reproduction (Figure 2b), there was little variation in mean date across levels of germination season precipitation and reproduction season temperature. At high reproduction season precipitation (Figure 2c), the mean date was relatively early under high rainfall during germination and cool temperatures during reproduction, and was late when germination season rainfall and reproduction season temperature were both high. Altogether, this result indicates that gladecress phenology has shifted earlier as the climate has become wetter during the germination season and cooler during the reproduction season, primarily in years with high reproduction season precipitation.

During the atypically early-flowering year (2016), the field experiment showed that there was little difference in reproductive performance between extremely early and early-flowering plants, but that these plants tended to have higher performance than those that flowered at a later, intermediate time period (Figure 3; Table S4). Rosette size was not different across flowering phenology periods; however, total flower production tended to be lower for intermediate-flowering plants compared to plants flowering at earlier time periods (Figure 3a,b). There was no difference in fruit production across flowering phenologies, nor were there differences in total seed number or seed number per fruit (Figure 3c–e). Yet, seed mass was lower for plants with an intermediate-flowering time compared to those flowering earlier (Figure 3f). In addition, seed germinability in the incubator was lowest for intermediate-flowering plants and highest for early-flowering plants, while the germinability of plants that flowered at an extremely early time period fell between these phenological groups (Figure 3g). The number of days to germination in the incubator was not different across groups (Figure 3h). The germination results from the greenhouse are qualitatively similar to those from the incubator (Figure S2; Table S4).

In a year of typical flowering time (2017), the field experiment found consistent differences in reproductive performance between early, intermediate, and late-flowering plants (Figure 4; Table S5). Fruit production was highest for early-flowering plants and progressively declined for intermediate-flowering and late-flowering plants (Figure 4a). These relative differences were also seen for total seed number (Figure 4b). The number of seeds per fruit did not differ between early- and intermediate-flowering plants, but both of these treatment groups had higher seed numbers per fruit than late-flowering plants (Figure 4c). Early-flowering plants had the highest single seed mass, followed by intermediate-flowering plants and late-flowering plants (Figure 4d).

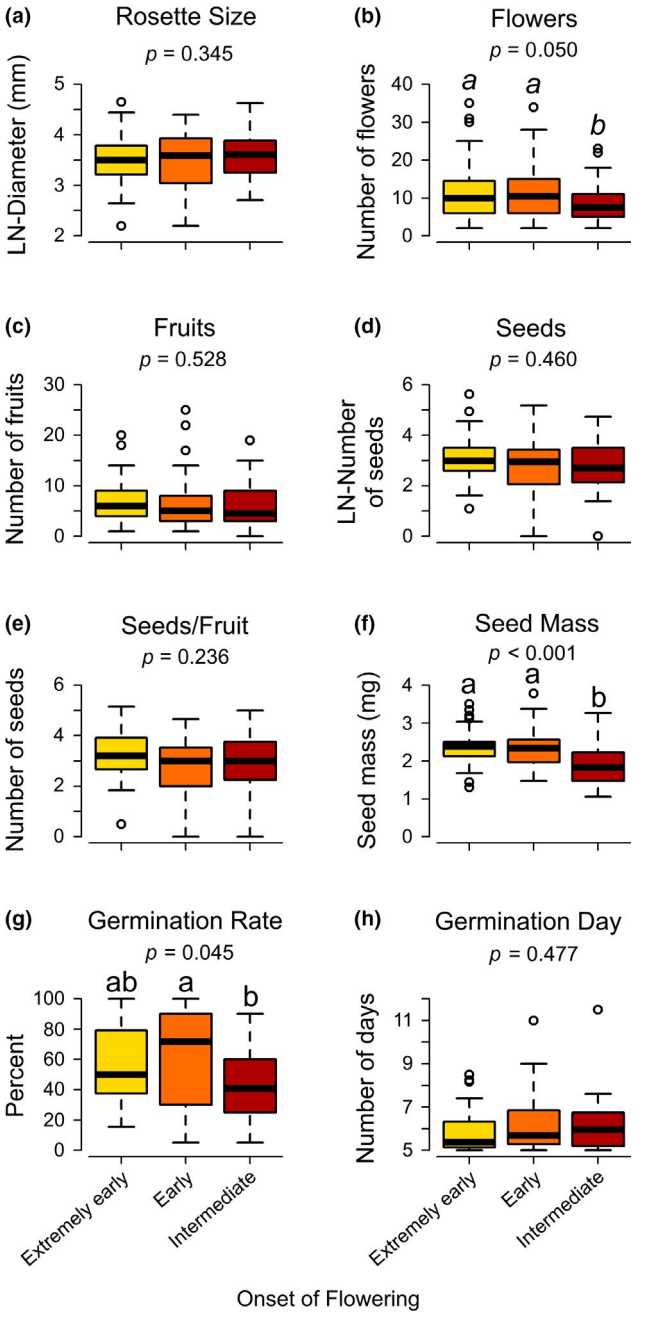


FIGURE 3 Gladecress performance across flowering phenology treatment groups in the extremely early-flowering year of 2016. (a–f) Results from plants in the field experiment. (g,h) Results from the incubator germination trial. The mass of the average individual seed per plant is shown in (f). To aid visualization, (g) germination rate, and (h) average days to germination are shown at the level of parental plants. *p*-values from corresponding generalized linear models, generalized linear mixed-effects models, or generalized least square models are provided for each response variable and Tukey post hoc contrasts are indicated by lowercase letters above boxplots for significant results ($p \leq .05$). Within each panel, letters for post hoc tests indicate differences using $\alpha = .05$, or when italicized, $\alpha = .10$

4 | DISCUSSION

Our research demonstrates that the timing of reproduction of a spring-flowering plant is arriving earlier as the climate becomes wetter and cooler. This finding is counter to expectations drawn from previous work with other annual mustard species (Fournier-Level et al., 2016; Franks et al., 2007; Hamann et al., 2018; Manzano-Piedras et al., 2014; Springate & Kover, 2014), as well as plant species in other families (Gordo & Sanz, 2010; Konig et al., 2018; but see Crimmins et al., 2010; Dorji et al., 2013). Phenological responses to local climate cooling and greater precipitation may

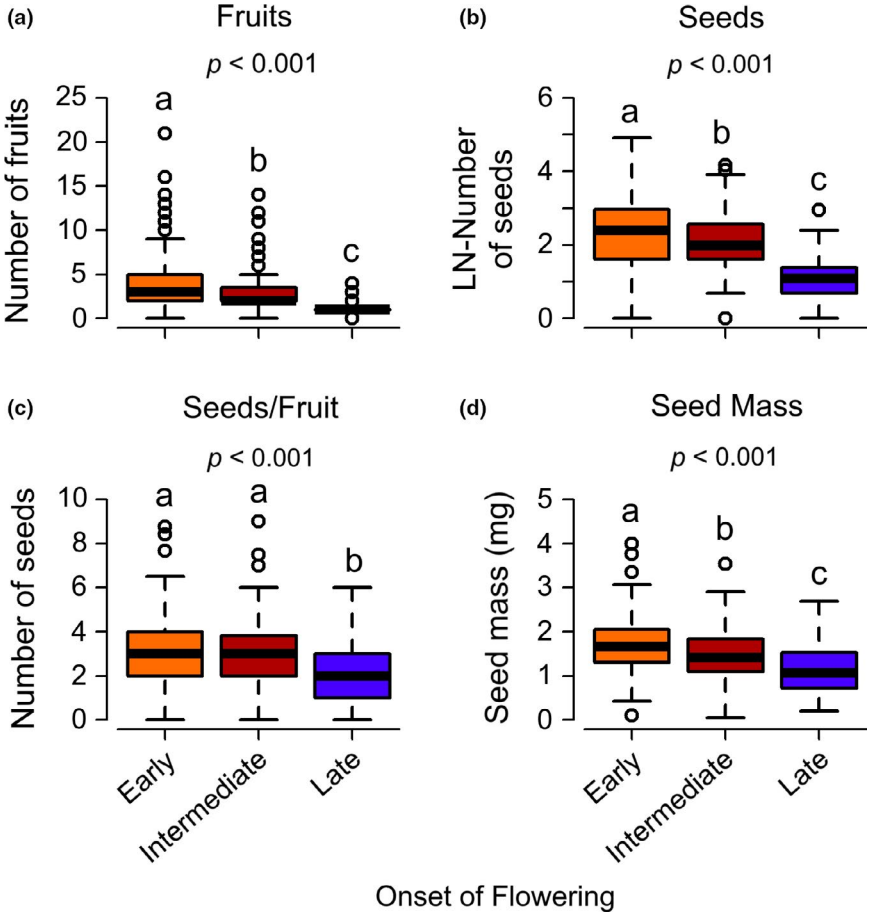


FIGURE 4 Gladecress performance across flowering phenology treatment groups in the typical flowering year of 2017. (a–d) Results from plants in the field experiment. The mass of the average individual seed per plant is shown in (d). *p*-values from generalized linear mixed-effects models are provided for each response variable and Tukey post hoc contrasts are indicated by lowercase letters above boxplots for significant results ($p \leq .05$). Within each panel, letters for post hoc tests indicate differences using $\alpha = .05$

frequently not be the inverse of responses to warming and drying (Li et al., 2016). These local-scale responses likely add to the variation in reproductive phenological responses to climate change observed among species (Cleland et al., 2012; Dunnell & Travers, 2011; Fitter & Fitter, 2002; Ibanez et al., 2010; Miller-Rushing & Inouye, 2009; Miller-Rushing & Primack, 2008) and across populations within species (Park et al., 2018). The entire range of cedar gladecress is restricted to the Central Basin of Tennessee (Baskin & Baskin, 2003), and low variation in climate conditions across this small range likely helped us to detect a relationship between climate and phenology. Studies of species with large ranges and of areas with high environmental variability may need to account for climate trends at a fine-grain, local scale to discern larger-scale patterns in plant phenology.

Our results indicate that the reproductive phenology of cedar gladecress depends on precipitation levels during the germination season, in combination with the precipitation and temperature conditions during the season of reproduction. This complex interaction suggests that anticipating reproductive phenology responses to climate change will also involve consideration of climate effects on earlier life-history stages, including how the timing of early life-history stages influences the timing of later life-history stages (Post et al., 2008; Wilczek et al., 2010). Germination in cedar gladecress is cued by cool temperatures and increased soil moisture, typically from late summer to early fall (Baskin & Baskin, 1971). Previous work with this species has shown that plants which germinated extremely early had greater seed production than later germinants (Baskin & Baskin, 1972). The local trend toward wetter, cooler conditions during the summer/fall may be causing gladecress plants to germinate earlier, which could contribute to earlier reproductive phenology if there is a correlation between germination time and initiation of flowering. Research with *B. rapa* has found that plants with earlier germination have greater reproductive output (Weis, Turner, Petro, Austen, & Wadgymar, 2015), indicating that germination phenology may be particularly important for later life history in annual mustards. However, gladecress that germinate too early can have lower survival compared with later germinants (Baskin & Baskin, 1972). The degree to which survival may have influenced our results is unclear.

Trade-offs between growth, survival, and reproduction can impose constraints on aggregate life-history responses to climate change (Post et al., 2008). Interestingly, developmental constraints on flowering phenology in annual mustards may be weak, where there is little or no fitness cost for plants that flower early at a smaller size compared to later-flowering, large plants (Franks & Weis, 2008; Gnan, Marsh, & Kover, 2017). We found no difference in rosette size between extremely early-flowering and subsequent flowering groups, suggesting that a trade-off between growth and reproduction was not present during our study. Future work documenting phenological sequences of life-history events will be necessary to

evaluate how reproductive phenology is dependent on earlier developmental stages.

Herbarium records were a useful resource for measuring changes in gladecress reproductive phenology and are an important potential resource for understanding climate cooling effects on other plant species. A growing number of studies are using herbarium records to document phenological change (Davis et al., 2015; Hufft et al., 2018; Matthews & Mazer, 2016; Park et al., 2018), and much potential exists for herbarium-based research to inform our understanding of ecological responses to global change (Meineke et al., 2018). Our study suggests that herbarium records of species with small geographic ranges may be especially informative for evaluating responses to climate change. However, studies using herbarium records do have important limitations to consider (Daru et al., 2018; Meyer, Weigelt, & Kreft, 2016). In particular, herbarium records are frequently only collected for mature plants in a reproductive stage and records for earlier life-history stages are lacking (Meineke et al., 2018), which limits their use in studies of aggregate life history. While herbarium specimens are often great resources for studying reproductive phenology, they may also be of limited use for investigating the onset of flowering because the earliest-flowering individuals in a population are not likely to be collected, and collectors frequently select plants with both flowers and fruits present (Pearse, Davis, Inouye, Primack, & Davies, 2017). Moreover, the environmental data that are available to compare with herbaria-derived data is often coarse, and specific cues for life-history events may be difficult to decipher. Herbarium-based research may frequently need to be paired with additional studies to understand fine-scale relationships between plant phenology and the environment, inter-dependencies among life-history events, and the reproductive consequences of changes in phenology.

Using field experiments to augment our herbarium-based research, we found that early-flowering cedar gladecress had a reproductive advantage over those that flowered late in the season. In a year with typical-flowering time, fruit and seed production and single seed mass progressively declined the later that plants initiated flowering, similar to the reproductive cost to late-flowering exhibited by *B. rapa* (Austen & Weis, 2015). Yet in a year with atypically early flowering, there were no differences in fruit and seed production between plants flowering with extremely early, early, and later intermediate phenologies, though seed mass and germinability were lower for this last group. These results suggest that the earlier reproductive phenology of gladecress may be an evolved response to asymmetric selection, where there is weak selection against flowering too early but strong selection against flowering too late (Austen, Rowe, Stinchcombe, & Forrest, 2017; Weis et al., 2014). Cedar gladecress is a self-incompatible insect-pollinated plant (Norton, Fernando, Herlihy, & Busch, 2015), and weak selection against early flowering could be more related to a mismatch with pollinators (Hegland et al., 2009; Rafferty et al., 2015) than to adverse environmental conditions in this system (Gu et al., 2008; Inouye, 2008). Previous research has shown that selection frequently favors earlier flowering (Munguia-Rosas, Ollerton, Parra-Tabla, & De-Nova, 2011), and the flowering phenology of other annual plants has been shown to rapidly evolve in response to climate variability (Franks et al., 2007; Lustenhouwer, Wilschut, Williams, Putten, & Levine, 2018). Alternatively, climate-driven shifts in flowering time could be due to phenotypic plasticity without evolutionary change, or some combination of plasticity and adaptive evolution (Anderson, Inouye, McKinney, Colautti, & Mitchell-Olds, 2012; Fournier-Level et al., 2016; Nicotra et al., 2010; Richardson, Chaney, Shaw, & Still, 2017). Cooler winters could potentially result in earlier flowering if gladecress requires chilling as a reproductive cue via vernalization (Kim, Doyle, Sung, & Amasino, 2009), which has helped to explain unexpected responses to climate change for other plant species (Cook, Wokovich, & Parmesan, 2012; Yu, Luedeling, & Xu, 2010). Additional research is required to understand to what degree changes in gladecress phenology are influenced by plasticity versus evolution. Either way, it seems clear that early flowering in gladecress is a strategy to escape the stressful hot and dry conditions in cedar glade habitats brought by the onset of summer (Kazan & Lyons, 2016).

Experimental approaches based on species' functional traits, such as traits related to resource acquisition, may provide a direction forward for mechanistically understanding changes in plant phenology (Dorji et al., 2013; Konig et al., 2018; Rollinson & Kaye, 2012). For instance, rooting depth is a key trait influencing the delayed reproductive phenology of the sedge *Kobresia pygmaea* in response to warming, likely because this shallow-rooted species is reliant on moisture availability in the upper soil, which is impacted by the effect of warming on evapotranspiration (Dorji et al., 2013). Traits related to water acquisition may be particularly influential in determining the phenology of plants that experience water limitation, such as those in cedar glades and arid/semi-arid ecosystems (Kimball, Angert, Huxman, & Venable, 2010). Cedar gladecress grows in shallow soils that fluctuate from mesic to xeric conditions as the reproductive season transitions to the season influencing germination (Baskin & Baskin, 1971, 1972; Cartwright & Hui, 2015). Climate change effects on the timing of reproduction in gladecress will largely be governed by the influence of both precipitation and temperature on soil water availability. Perhaps theoretical frameworks based on species' traits can be developed to explain the complex phenological responses to climate change observed in water-limited systems (Crimmins et al., 2010).

This study has shown that gladecress phenology has shifted earlier as the climate has become cooler and wetter, yet other drivers of global change, such as elevated atmospheric carbon dioxide and nitrogen deposition, may have influenced this shift (Cleland et al., 2006; Jagadish et al., 2016; Walker & Ward, 2018) but were unaccounted for here. More detailed environmental records are necessary to determine how plant phenology responds to the separate and combined effects of these various global change drivers. Manipulative experiments also provide a powerful approach to determine the individual and combined effects of these factors on plant phenology (e.g., Cleland et al., 2006). Local climate cooling should be considered as an additional variable in manipulative experiments

to obtain a fuller understanding of the ecological repercussions of global change (Li et al., 2016; Meng et al., 2017).

While the focus of this study is on a single plant species, other species are likely responding to local climate cooling as well. Plants with winter annual life histories, including wild and domesticated species, may be especially prone to respond to cooling in similar ways to those described here; and species with other life histories may also have non-intuitive responses that are not predicted by warming studies. Further study is needed to evaluate the prevalence of climate cooling effects on plants and the repercussions of these changes for ecological communities and ecosystems, including both natural and agricultural systems. Climate cooling must be considered along with other global change factors to develop accurate predictions for plant responses to climate change, which are essential for developing mitigation policies and enacting effective management.

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AUTHOR CONTRIBUTIONS

CB and CRH conceived the study; all authors contributed to data collection; JBG analyzed the data and wrote the first draft of the manuscript; all authors contributed to manuscript revisions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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