MEASURING CHANGES IN PHENOLOGY OF OKLAHOMA ASTERACEAE USING HERBARIUM SPECIMENS

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ABSTRACT

Analyzing shifts in plant flowering times (flowering phenology) in response to changing climate is crucial to understanding the impacts of climate change on plants. Herbaria contain the physical record of reproductive events from past seasons, making them an important source of long-term data for studies of phenology. We measured changes in flowering phenology of four Oklahoma native plants in the Asteraceae (sunflower) family: *Grindelia ciliata*, *Liatris punctata*, *Ratibida columnifera*, and *Vernonia baldwinii*. These species were selected to represent the morphological and phylogenetic diversity of the Asteraceae in Oklahoma and were represented in the Robert Bebb Herbarium (OKL) with over 100 specimens each. We created novel protocols for scoring the flowering phenology of these species into numeric categories, called phenophases. We looked for correlations between the collection date and both the year of collection and the temperature in that year. There was a significant relationship between collection date and year only in peak flowering specimens of *G. ciliata*. There was a significant relationship between statewide annual temperature and collection date only in peak flowering specimens of *V. baldwinii*. There was a significant relationship between the annual temperature of the climate division of the state where the plants were collected and collection date for peak flowering in *G. ciliata*, *R. columnifera*, and *V. baldwinii*, for first flowers in *V. baldwinii*, and for last flowers in *L. punctata*. More precise temperature data thus lead to an improvement of the model, but in all cases temperature or year explained relatively little of the total variation in flowering time.

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INTRODUCTION

Phenology is the study of the timing of recurring biological events. Analyzing shifts in flowering times in response to changing climatic conditions is crucial to comprehending and forecasting the impacts of climate change on the world's plants. Climate and phenology are physiologically linked and changes in the climate have the potential to alter phenological responses (Kooyers et al. 2019). In the Rocky Mountains, for example, many alpine plant species have shifted their flowering times earlier in the year in response to a 1°C rise in temperature since the mid-1990s (Munson and Sher 2015). The change in flowering phenology for these species has been steady since the late 1800s, leading them to flower over a month earlier than they once did, which has massive consequences for the ecosystem (Munson and Sher 2015). Other studies have found mixed results with increased temperature; some taxa in Oklahoma flowered later than in the past and others flowered earlier (Messick 2017). A study in the Netherlands found a similar result (Van Vliet et al. 2014). Pearson (2019) found that fall flowering taxa flowered later with increasing July temperatures, while spring flowering taxa flowered earlier in response to rising March temperatures. Species within the same genus in the same geographic range may have different responses, as was the case in a British study, with *Geranium rotundifolium* L. delaying its first flowering date and *Geranium dissectum* L. advancing its first flowering date in response to increasing temperature over time (Fitter and Fitter 2002). These different responses may be due to a delay in flowering with less winter cold by plants that require vernalization before flowering (Gremer et al. 2020; Messick 2017). The way that climate change links to phenology varies between taxa and environments, depending on the most important abiotic factors in each environment. For example, Matthews and Mazer (2016) found that with greater precipitation, the mean date of flowering moved later along the Pacific Coast of North America. In addition, plants in xeric environments tend to have greater phenological changes than plants in more mesic environments and are at a higher risk for changes in community composition (Park 2014).

These changes in flowering phenology will alter ecosystem functioning and productivity, as well as ecological interactions across trophic levels (Pearson 2019). A study on broad-tailed hummingbirds in Colorado and their preferred nectar sources revealed that changes in the flowering phenology of food sources in northern breeding grounds, if they continue at current rates, would lead to hummingbirds eventually arriving after flowering begins (McKinney et al. 2012). This projected mismatch in ecological timing may result in flowering of some important species ending their flowering before the hummingbirds raise their young, which would lower reproductive success (McKinney et al. 2012). In some cases, these phenological shifts may also increase competition for pollinators if the changes in phenology cause taxa that used to flower at different times to flower at the same time (Park and Mazer 2019). Differential changes in flowering phenology may also allow co-occurring species of *Viburnum* to hybridize, which was previously prevented by non-overlapping flowering periods (Spriggs et al. 2019).

Many studies of plant phenology are based on herbarium specimens. Herbaria contain longer consistent records of phenological events than are available from observational data from historical documents like newspapers or journals (e.g., Aono and Kazui 2008; Haggerty et al. 2013b) or detailed observations on flowering from individual observers (e.g., McKinney et al. 2012; Jánosi et al. 2020).

Herbarium specimens also allow us to examine changes in all stages of flowering, instead of being limited to the specific stage(s) previous observers chose to record (generally the date of first flowering; Amano et al. 2010). The advantage to dividing flowering specimens according to their phenological phase, or phenophase, is that it has the potential to uncover changes in phenology in more detail. Treating all flowering individuals as a single category is much less precise (e.g., Bowers 2007). Careful delineation of specific phenophases is a challenge for phenological research (Love et al. 2019). This must be done in a consistent manner, so that different researchers will score the phenology in the same way (Love et al. 2019; Yost et al. 2019).

We used records from the Robert Bebb Herbarium (OKL) at the University of Oklahoma to investigate the flowering periods of four members of the Asteraceae that are native to Oklahoma: *Grindelia ciliata* (Nutt.) Spreng., *Liatris punctata* Hook., *Ratibida columnifera* (Nutt.) Wooton & Standl., and *Vernonia baldwinii* Torr. We chose taxa that belonged to different tribes of the Asteraceae to capture more of the evolutionary variation in the family. In addition, we chose taxa with different inflorescence types. We were interested in answering the following questions: 1) Are there significant shifts in flowering periods? 2) If so, has flowering shifted earlier or later through time? 3) Which flowering stages show the largest shifts?

MATERIALS AND METHODS

Selected Species

Grindelia ciliata is in the tribe Astereae (Figure 1B). It is a fall-flowering annual with clusters of one to a few heads at the ends of each stem. While many individuals have only one stem and are 45 cm tall or less, plants can be up to 2 m tall. These larger plants have many side branches, which are often branched again, and each branch ends in a group of heads. Each individual head contains 100–200 disk florets and 25–40 ray florets (Strother and Wetter 2006), both of which produce fruit in this species.

Liatris punctata is in the tribe Eupatorieae (Figure 2B). It is a fall-flowering, long-lived perennial that comes from a corm or rhizome. It has many, small heads borne in an elongated cyme-like inflorescence that flowers from the top to the bottom. Each individual head contains 3–8 disk florets and no ray florets (Nesom 2006).

Ratibida columnifera is in the tribe Heliantheae (Figure 3B). It is a summer-flowering perennial from a rosette. Each plant bears 1–15 heads, with individual heads consisting of 4–12 ray florets and 200–400 disk florets borne on a columnar receptacle, with only the disk florets producing fruits in this species (Urbatsch and Cox 2006).

Vernonia baldwinii is in the tribe Vernonieae (Figure 4B). It is a late summer-flowering perennial, which forms rhizomatous clumps. Each individual stem has many heads in a corymbose arrangement, with each clump having many stems. Individual heads consist of 20–25 disk florets and no ray florets (Strother 2006).

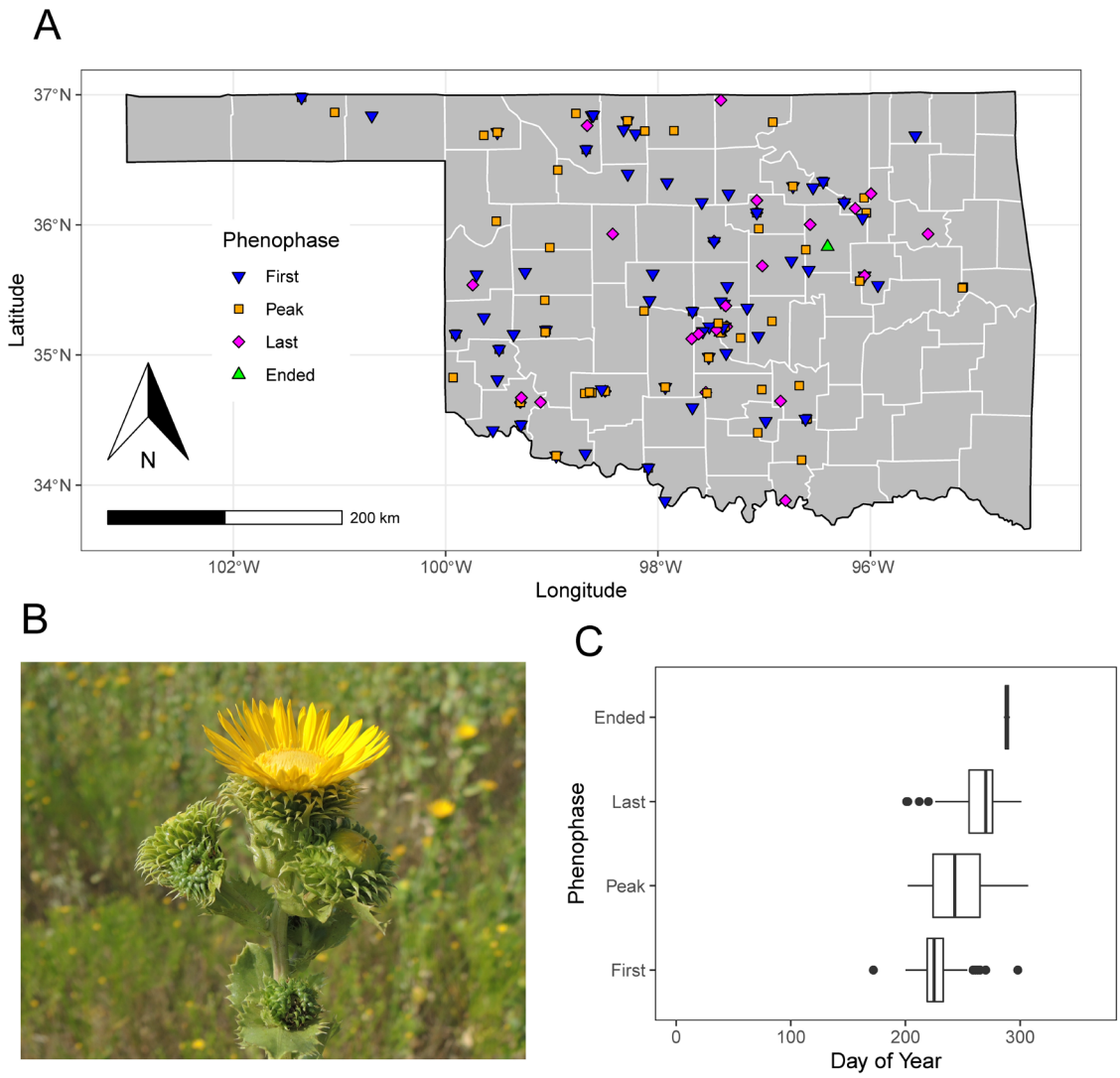

Figure 1 *Grindelia ciliata* (Astereae). A) Distribution map of specimens examined in this study, all from OKL. B) Plant from Sutton Urban Wilderness, Norman, Cleveland Co., Oklahoma. C) Boxplot showing the range of date of collection (DOY) for each of the four phenophases: First (First Flowers, 1), Peak (Peak Flowering, 2), Last (Last Flowers, 3), and Ended (Flowering Finished, 4).

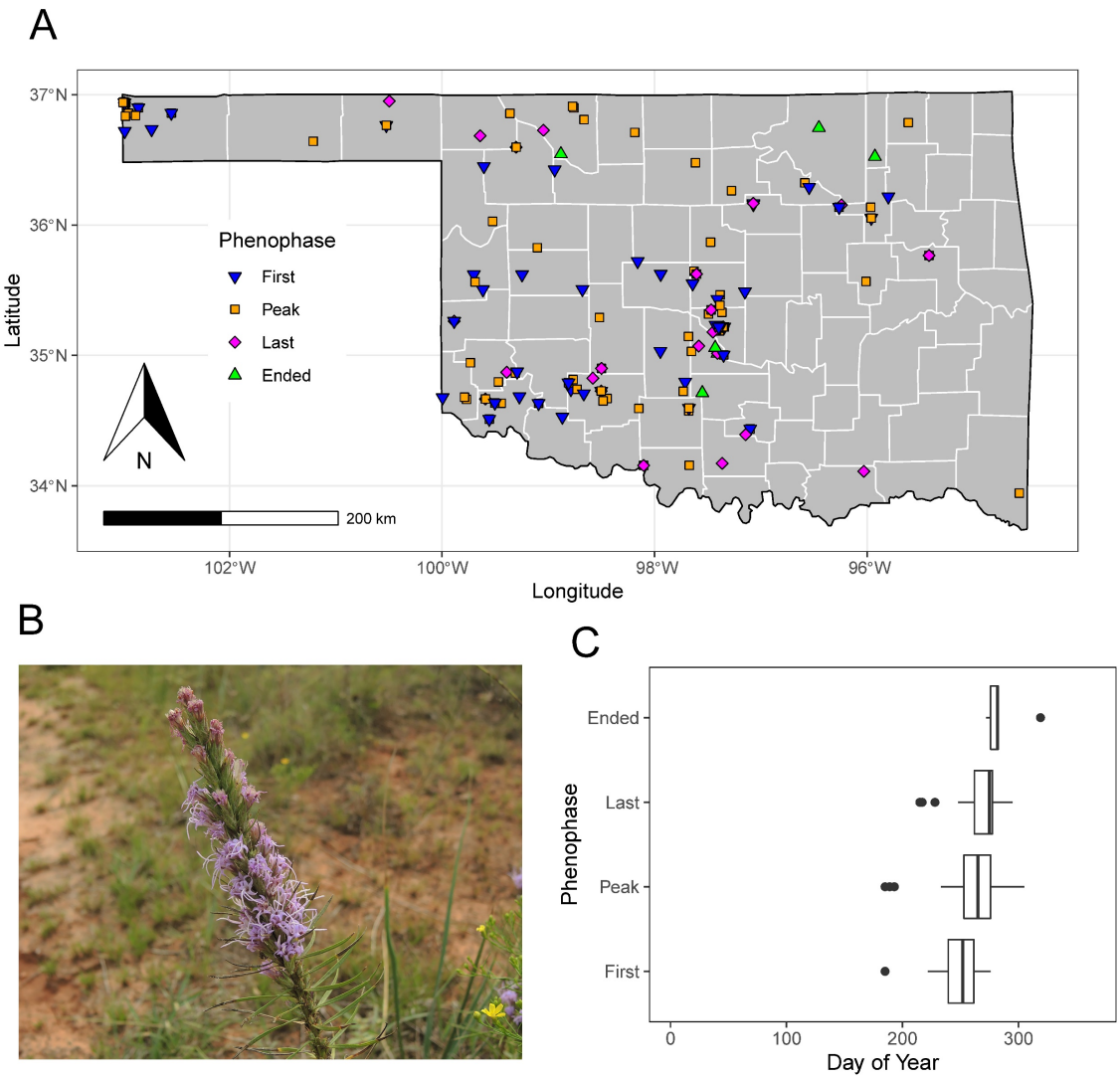

Figure 2 *Liatris punctata* (Eupatorieae). A) Distribution map of specimens examined in this study, all from OKL. B) Plant from Lake Thunderbird State Park, Cleveland Co., Oklahoma. C) Boxplot showing the range of date of collection (DOY) for each of the four phenophases. Labeling of phenophases following Figure 1.

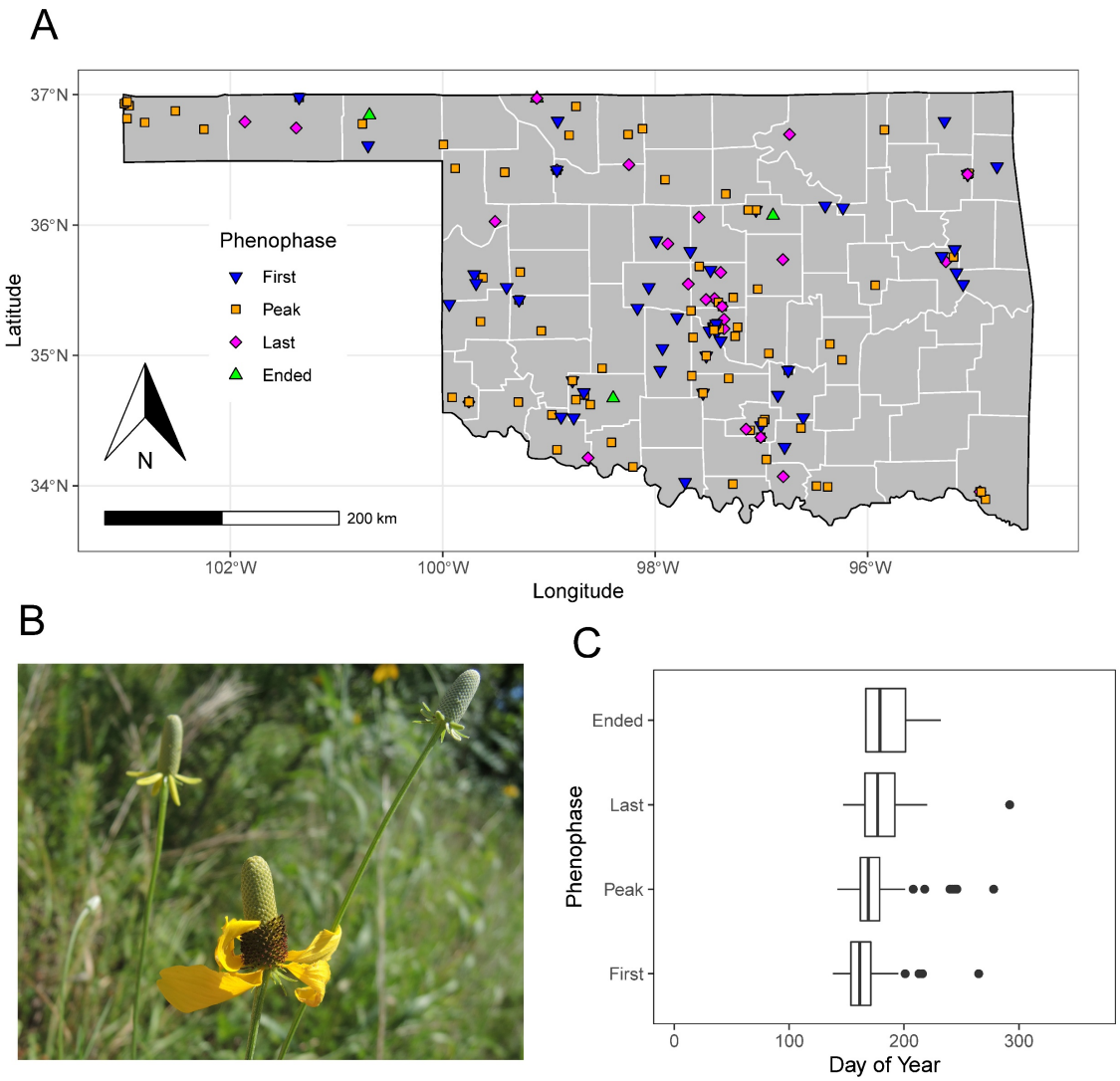

Figure 3 *Ratibida columnifera* (Heliantheae). A) Distribution map of specimens examined in this study, all from OKL. B) Plant from Sportsman Lake, Seminole Co., Oklahoma. C) Boxplot showing the range of date of collection (DOY) for each of the four phenophases. Labeling of phenophases following Figure 1.

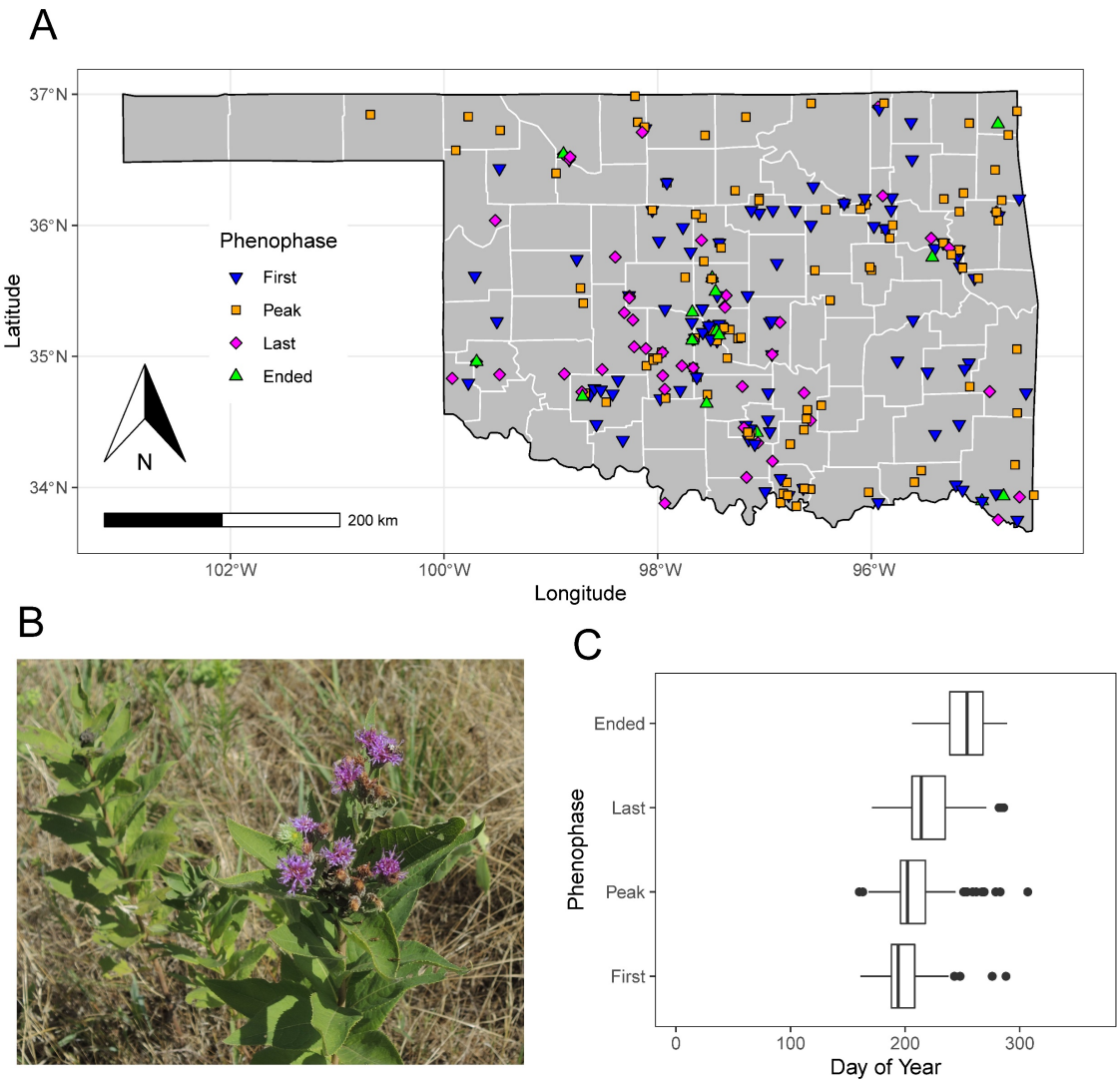

Figure 4 *Vernonia baldwinii* (Vernonieae). A) Distribution map of specimens examined in this study, all from OKL. B) Plant from US-412, Major Co., Oklahoma. C) Boxplot showing the range of date of collection (DOY) for each of the four phenophases. Labeling of phenophases following Figure 1.

Dataset Selection

We selected taxa that were present in the Bebb Herbarium (OKL) in high numbers, with at least 100 useable herbarium specimens per taxon. Specimens were only included in the analyses if their phenophase could be determined. Specimens were excluded if they did not include precise locality information or had no date. Multiple plants on the same herbarium sheet were treated as separate data points, because they often had different phenophases. In this case, each plant would receive a different phenophase score and would be included in the analysis as an independent data point from the same location with the same date.

We used the Oklahoma Vascular Plants Database (OVPD; Hoagland et al. 2019) to access dates and localities of specimens for georeferencing. This database includes each specimen's label information. Collection date was converted to day of year (DOY) with January 1 as day one and with DOY adjusted for leap years. We used Google Earth Pro (Google, Mountain View, California) to manually georeference the specimens. The accuracy of the georeferencing was checked by ensuring each specimen mapped to the county in which it was collected.

Determination of Phenophases

We based our strategy for determining phenophases on the primer by Haggerty et al. (2013a). There were four phenophases, based on how many open florets were present on the specimen and whether there were fruits present. Category 1 (first flowers) was assigned to specimens with at least one and up to 25% of the florets on the plant open. "Open" referred to visible stamens and pistil. Category 2 (peak flowering) applied to any specimen with 25%–75% open flowers, with more flowers than fruits. Category 3 (last flowers) corresponded to specimens with more fruits than flowers, but with at least some open flowers. Finally, category 4 (flowering finished) included specimens completely in fruit. For each taxon, the protocol for assigning a specific phenophase changed due to changes in morphological characters (Appendix).

Statistical Analyses

Data on the mean annual temperature for Oklahoma as a whole (henceforth statewide annual temperature) and for each of the nine climate divisions within Oklahoma (henceforth climate division annual temperature) were obtained from NOAA National Centers for Environmental Information (2021a, 2021b; procedure similar to that used by Calinger et al. 2013). In addition, both statewide and climate division temperature data were obtained for each of the four seasons separately from the same source (NOAA National Centers for Environmental Information 2022a, 2022b). Specimens were classified into climate divisions based on their counties (as each county was only in one climate division). This allowed us to investigate flowering responses on a broad statewide scale and on a finer scale which could be more informative in the potential flowering response to temperature changes.

All data analysis was performed in R, version 4.1.1 (R Core Team 2021). We performed simple linear regressions between DOY and various predictor variables: calendar year (year), statewide and climate division mean annual temperature, and statewide and climate division mean seasonal temperatures, with the specimens grouped by phenophase in all cases. Graphs and maps were plotted with ggplot2 (Wickham 2009) and sf (Pebesma 2018). The Bonferroni correction was applied to the p-values to account for multiple tests. (All seasons were tested for each species, as significance of the tests did not vary when only the season of flowering and the season prior to flowering were included.)

The R code, the datasets for each of the four species, and the table with the results from the analyses of all variables are available on ShareOK (https://hdl.handle.net/11244/336289).

RESULTS

The 203 examined specimens of *Grindelia ciliata* were found in approximately the western two-thirds of Oklahoma (Figure 1A). They were rather evenly scattered throughout the state, with clusters in Cleveland County (the location of the Bebb Herbarium) and in Comanche County (Wichita Mountains National Wildlife Refuge). They were collected from 1916 to 2020 (Figure 5). The median day of year (DOY) for peak flowering specimens of *G. ciliata* was 243 (31 August, Figures 1C, 5). There were no significant trends for first flowers or last flowers or for peak flowering with year (Figures 5, 6). There were only two specimens in the Flowering Finished category, so trends in this category could not be examined. The DOY for peak flowering was significantly correlated with statewide summer temperature ($p = 0.024$) and climate division summer temperature ($p = 0.050$; Figure 6). Both relationships were positive, showing that flowering became later by 4.81 and 3.75 days for each degree increase in temperature for statewide and climate division temperature, respectively, although temperature explained a relatively small amount of variation in flowering in both cases ($r^2 = 0.167$ for statewide summer temperature and $r^2 = 0.149$ for climate division summer temperature; Figure 6).

The 211 examined specimens of *Liatris punctata* were also found in approximately the western two-thirds of Oklahoma and were collected from 1913 to 2013 (Figures 2A, 7). They were not randomly distributed in the state, with clusters in Cleveland and McClain Counties, in southwestern Oklahoma, and in Cimarron County. The median DOY for peak flowering specimens of *L. punctata* was 265 (22 September, Figure 2C). There were no significant trends for the relationship of any flowering category with year or any of the temperature categories after correction for multiple tests (Figure 7).

The 191 examined specimens of *Ratibida columnifera* were found throughout the state, although there was a gap in collections in southeastern Oklahoma (Figure 3A), and a cluster of specimens in Cleveland County. They were collected from 1906 to 2015 (Figure 8). The median DOY for peak flowering specimens of *R. columnifera* was 169 (18 June, Figure 3C). There were no significant trends for any flowering category for year or any of the statewide temperature datasets (Figure 8). There were significant relationships between peak flowering DOY and three of the climate division datasets: annual ($p = 0.0057$), spring ($p = 0.0081$), and summer ($p = 0.0020$; Figure 9). In all cases, flowering advanced in response to an increase in temperature (by 3.6 days for annual temperature, 2.46 days for spring temperature, and 4.31 days for summer temperature), with temperature explaining a relatively small amount of the variation in the data ($r^2 = 0.132$ for annual, $r^2 = 0.126$ for spring, and $r^2 = 0.148$ for summer; Figure 9).

The 309 examined specimens of *Vernonia baldwinii* were spread throughout the body of the state, with clusters in Cleveland County, Comanche County, Marshall County, and Murray County (Figure 4A). They were collected from 1903 to 2013 (Figure 10). The median DOY for peak flowering specimens of *V. baldwinii* was 202 (21 July, Figure 4C). There were no significant trends in the relationship of any flowering category with year or any of the temperature categories after correction for multiple tests (Figure 10).

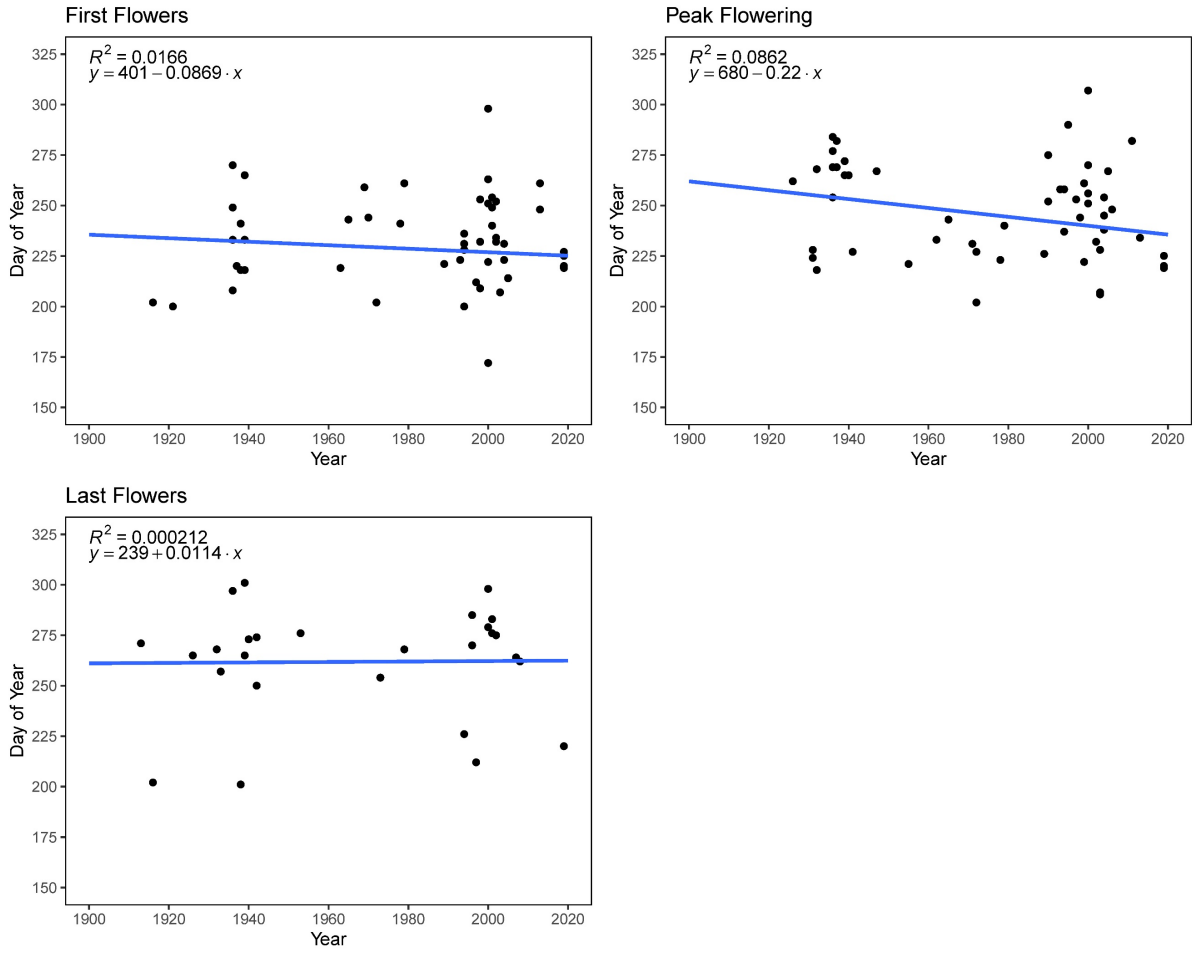

Figure 5 Scatterplot of day of year of collection for each of the three phenophases versus year of collection for *Grindelia ciliata*. (Too few specimens in the Ended category were present to analyze the relationship of day of year and year for that category.) Labeling of phenophases following Figure 1.

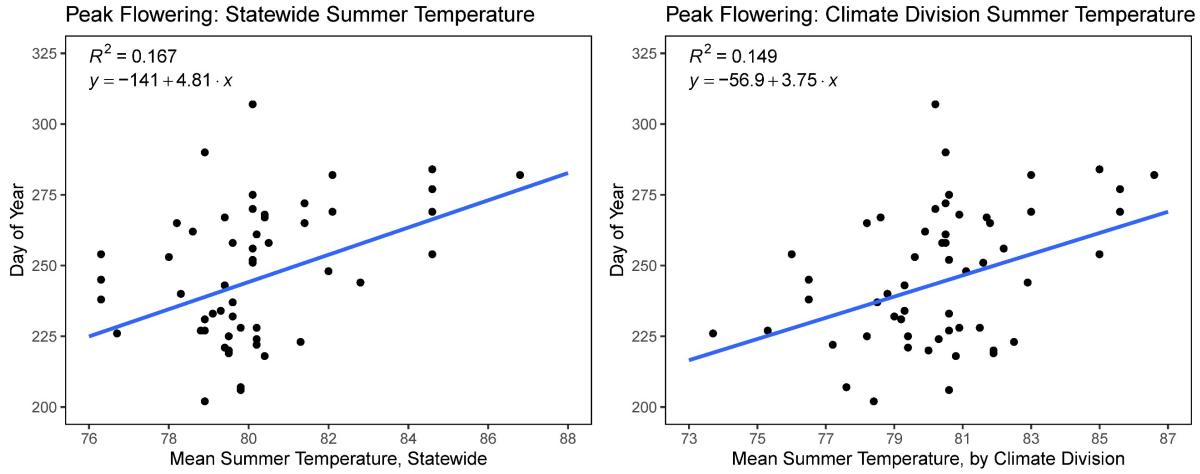

Figure 6 Scatterplot of the significant relationships between day of year of collection for *Grindelia ciliata*: Peak Flowering with yearly mean summer temperature statewide ($p = 0.024$) and yearly mean summer temperature in the climate division in which the specimen was collected ($p = 0.050$).

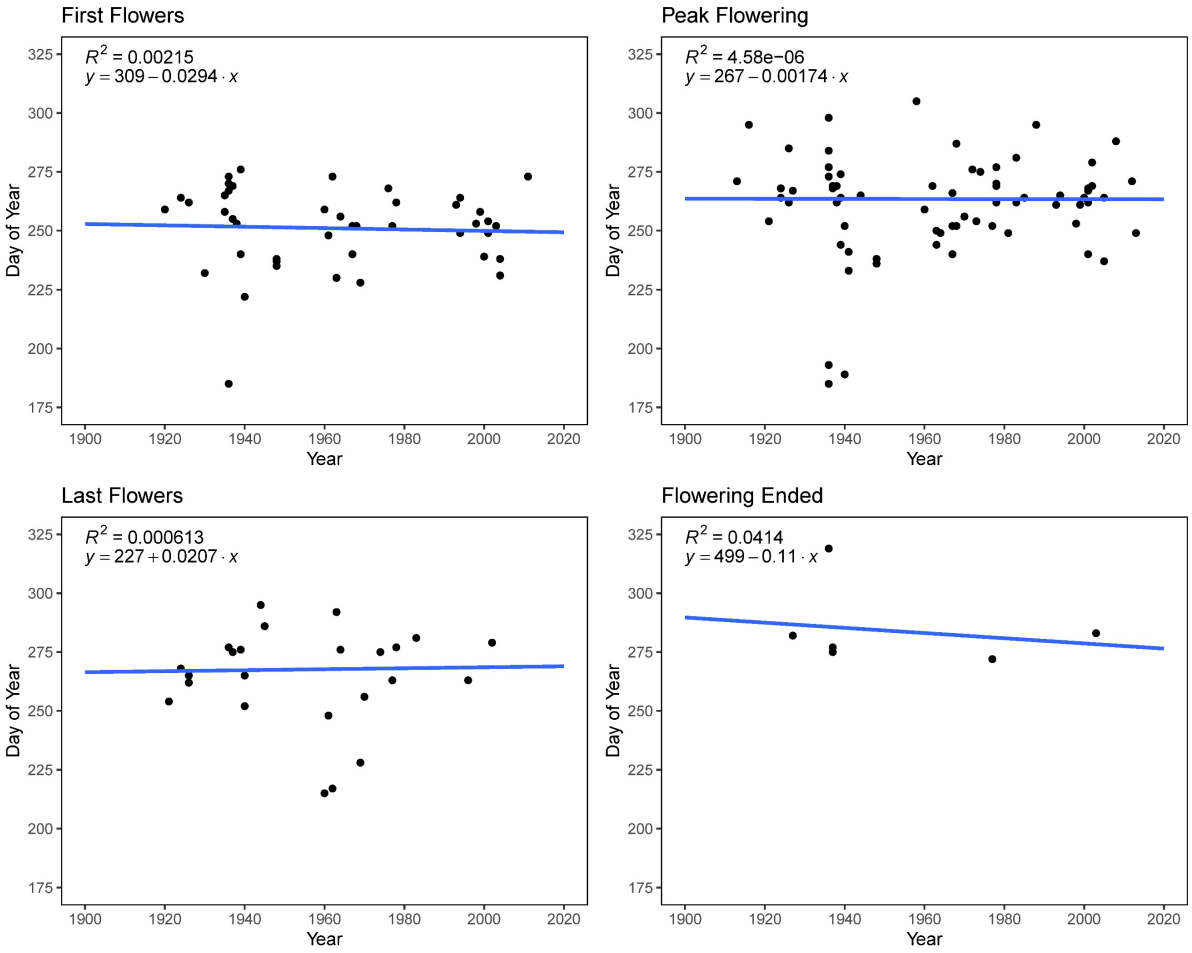

Figure 7 Scatterplot of day of year of collection for each of the four phenophases versus year of collection for *Liatris punctata*. Labeling of phenophases following Figure 1.

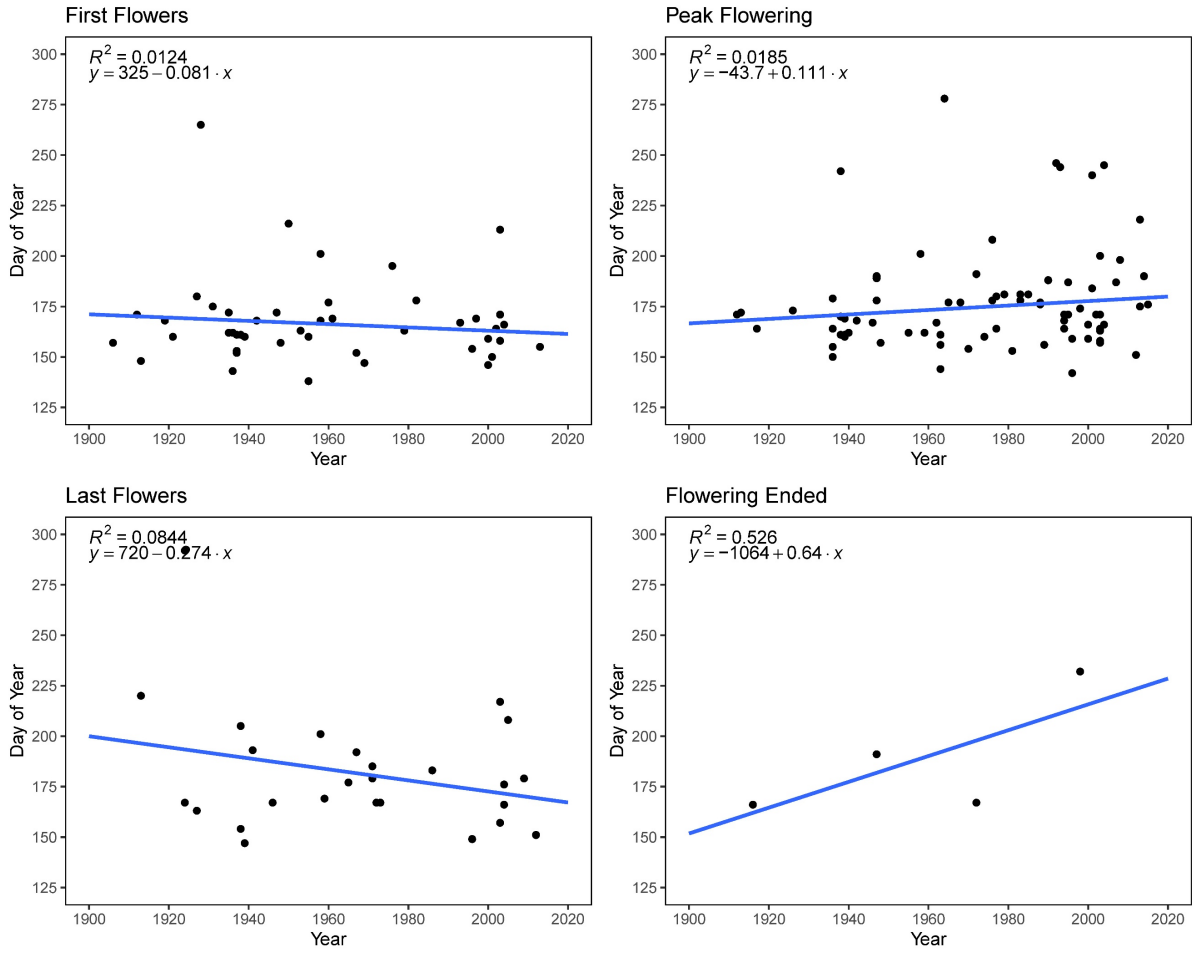

Figure 8 Scatterplot of day of year of collection for each of the four phenophases versus year of collection for *Ratibida columnifera*. Labeling of phenophases following Figure 1.

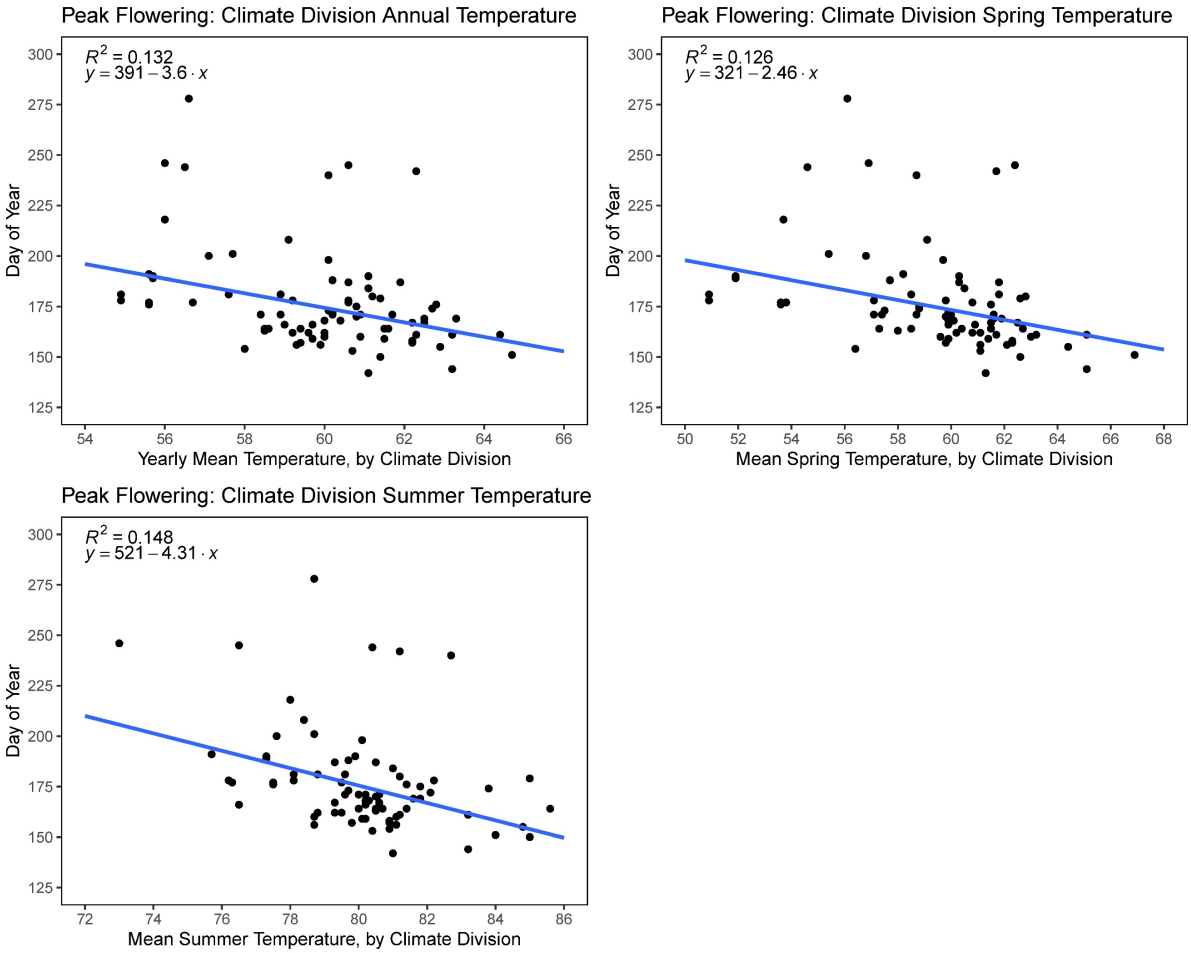

Figure 9 Scatterplot of the significant relationships between day of year of collection for *Ratibida columnifera*: Peak Flowering with the yearly mean annual temperature (p = 0.0057), yearly mean spring temperature (p = 0.0081), and yearly mean summer temperature (p = 0.0020), all for the climate division in which each plant was collected.

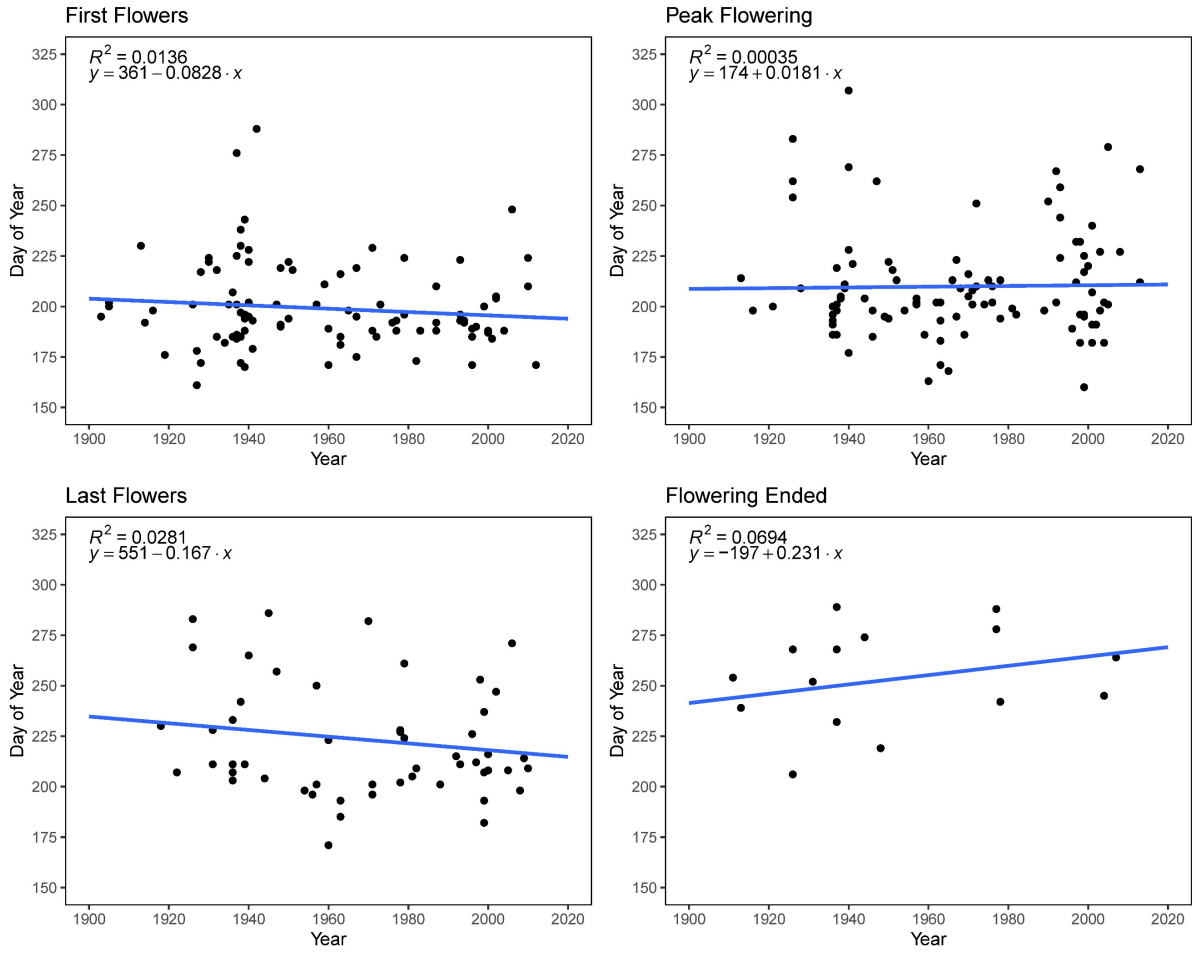

Figure 10 Scatterplot of day of year of collection for each of the four phenophases versus year of collection for *Vernonia baldwinii*. Labeling of phenophases following Figure 1.

DISCUSSION

We did not detect a significant relationship between collection date of year (DOY) and year in any of the species/phenophase combinations after correcting for multiple tests (Figures 5, 7, 8, and 10). In some cases, these species/phenophase combinations did show weak directional trends of earlier or later flowering through time. A lack of significant relationship between DOY and year of collection has been found in other multi-species phenology studies. Calinger et al. (2013) analyzed peak flowering of 141 species and found 66 species to show significant changes, either advances or delays, in flowering times. Messick (2017) found 10 of 20 species to show a significant change in flowering while the other species showed no trend or weak non-significant trends when year and DOY were regressed. Within this same study (Messick 2017), a mix of significant and non-significant trends were found when analyzing the same phenophases as in the present study. Three possible explanations for this lack of relationship found in the current study are that flowering is not strongly related to temperature, year and temperature are not strongly related, or that the selected species simply have not had enough time to show a significant response to temperature changes.

As noted in the results for each species, sampling is not evenly spread throughout the state, with sampling often concentrated in Cleveland County (where the Bebb Herbarium is located). This clumping of samples could have an effect on the results if the climate is different in different parts of the state or if plants are responding differently to climate in different parts of the state. While we corrected for differences in climate across the state by using the climate division datasets, the data to test how plants are responding to the climate across their ranges do not currently exist for these species.

Statewide annual temperature in Oklahoma does not show a steady warming trend over the period of this study (Oklahoma Climatological Survey 2021). The annual temperature has been warmer than the long-term average since the mid-1990s, but it was colder than the long-term average from the mid-1960s to the mid-1990s. The relationship between statewide annual temperature and DOY was not significant for any of the species. Two possible explanations for this lack of relationship are that flowering is not strongly related to temperature and that statewide annual temperature is not the most pertinent temperature variable for plant flowering.

When we looked at the data at a finer scale, either by dividing Oklahoma into its nine climate divisions or by dividing the temperature by season, more of the relationships were significant. Summer temperature, both statewide and divided by climate division, was significantly related to DOY for peak flowering in *G. ciliata* (Figure 6), while annual, spring, and summer temperature divided by climate division were significantly related to DOY for peak flowering in *Ratibida columnifera* (Figure 9). The remaining relationships did not show significant trends.

It is possible that flowering time may be governed strictly by temperature, but that annual or seasonal temperature is too coarse of a measure, even when it is for a specific climate division. For example, Jánosi et al. (2020) found that even monthly mean temperatures were too coarse to predict flowering accurately and that instead snowfall anomaly a certain number of days prior to flowering was the most pertinent variable governing the start of flowering for numerous cultivated bulb species. Messick (2017) found several species of Brassicaceae and Lamiaceae were responding to mean temperatures one to three months prior to date of collection with either an advance in

flowering times or contractions in flowering period length.

Even if annual or seasonal temperature for their climate division is what plants are responding to, it may be that they are responding to temperature in a complex way. There is often a relationship between flowering time and temperature for spring-flowering species or for budburst (e.g., Bowers 2007; Miller-Rushing and Primack 2008; Amano et al. 2010; Munson and Sher 2015), with strong trends in earlier flowering over the last hundred years for these species. However, the plants we examined are summer- or fall-flowering species, and flowering time in these species may not be governed, or may not be governed exclusively, by the start of spring. Pearson (2019) found that warming temperatures made spring flowering earlier but delayed fall flowering, with summer-flowering species showing intermediate responses. Except for *L. punctata*, which was fall-flowering with a median DOY for peak flowering of September 22, all of our species began flowering in mid- to late-summer, so they could be experiencing conflicting signals which would lead to no overall change in flowering time. In addition, for taxa that require vernalization (a period of cold before they are able to germinate, grow, or flower), warmer winters may delay sprouting or flowering, because the plants may wait to start to grow until they have experienced a certain number of cool or cold days (e.g., Hepworth et al. 2018; Gremer et al. 2020; Jánosi et al. 2020). However, while the only annual, *G. ciliata*, typically germinates in the fall (Kistenmacher and Gibson 2016), it does not require a cold period to flower after germination (A. J. Moore, pers. obs. of greenhouse plants). The other three species are perennials, and the vernalization requirements to induce flowering in adult plants do not appear to have been investigated.

Other factors besides temperature may be equally important for the timing of flowering in these plants. On its own, the influence of precipitation on flowering times has shown a mix of responses. Some species have not changed flowering times in response to increased precipitation (Abu-Asab et al. 2001; Matthews and Mazer 2016), while other species have delayed flowering with increased precipitation (Von Holle et al. 2010; Mazer et al. 2013), and yet other species advanced flowering with increased precipitation (Crimmins et al. 2010; Lambert et al. 2010). Precipitation has been shown to interact with temperature to determine the timing of various phenological events (e.g., Lesica and Kittelson 2010; Xie et al. 2015; Matthews and Mazer 2016). Messick and Hoagland (in prep.) found that budburst in *Quercus marilandica* Münchh. and *Q. stellata* Wangenh. responded to the interactions of temperature (chilling period followed by warming period), cumulative precipitation, and increasing photoperiod from February through April.

In Oklahoma, precipitation or available soil moisture is highly variable year to year. Drought may delay flowering, and abundant precipitation could prolong flowering. In the annual *G. ciliata*, plants in dry years or dry sites remain quite short and produce only a few flower heads, while plants growing in wetter years or more mesic sites can become tall and branched, producing many more heads (A. J. Moore, pers. obs.). These taller plants would also be classified as at peak flowering for longer, both because they would have a large number of open heads for a longer period and because botanists are likely to preferentially collect flowering branches of plants that are too large to collect and press in their entirety, thus further biasing the data to increase the length of time a plant is at peak flowering (Willis et al. 2017; Daru et al. 2018).

Even for species that do not have longer flowering with increased

precipitation, variability in flowering within a population would mean that some individuals were flowering while others were either pre-flowering or in fruit. These flowering specimens are more likely to be collected than pre-flowering or mainly fruiting specimens (Daru et al. 2018), in part because they better represent the ideal herbarium specimen, in part because they have the characters needed to identify the plant, and in part because plants that are still green can be pressed more easily than plants that have already completely dried out. Therefore, in the absence of notes on the phenological stage of the population as a whole, we assume that the specimen is representative of the population, when that might not be the case.

It is also possible that some of the species are not able to respond to climatic cues to change their flowering time, but instead respond to day length (e.g., Song et al. 2015). If this is the case, then these taxa may be at risk of declining, because they cannot track their optimal flowering period (Hulme 2011). In a prairie ecosystem, where one or more members of the Asteraceae are in flower throughout the summer, the selection for a particular flowering time may not be that strong. However, if some species track climate while ecologically similar species do not, then their formerly non-overlapping flowering times could begin to overlap, allowing for hybridization or increased competition for pollinators (Park and Mazer 2019; Visser and Gienapp 2019).

We found few significant results in our search for correlations between DOY and year, statewide annual temperature, and annual temperature of the climate division in which specimens were collected across four Oklahoma members of the Asteraceae. This result could be due to annual temperature being too coarse of a measure, to a lack of information on precipitation, or to a bias towards collecting specimens in full flower—artificially extending the time plants were considered to be in Peak Flower or Last Flowering stages. It is also possible that the flowering phenology of these taxa has not shifted with climate change, like it has for so many other taxa.

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APPENDIX

Scoring Protocols

General Protocol

This protocol is based on the protocol of Haggerty et al. (2013a), with the same four categories and metrics for splitting them up. The First Flowers phenophase (1) indicates specimens with up to 25% open flowers, the Peak Flowering phenophase (2) indicates specimens that have between 25% and 75% open flowers with few fruits present, the Last Flowers phenophase (3) is for specimens with fruits present and more than 50% closed flowers, and the Flowering Finished phenophase (4) is for specimens that are fully in fruit.

If the herbarium sheet includes multiple individual plants or multiple pieces of plants that are not currently connected (even though they may have come from the same plant originally), each plant or plant piece gets its own score and is a separate data point. To score the individual plants or parts, it is important to take all of the flowering heads on that plant into consideration. The entire plant should not be scored based on the state of just one part of the plant. Each head on a branch or each part of the inflorescence can be scored separately, and those scores can be averaged to get the score of the entire plant.

Grindelia ciliata Protocol

Grindelia ciliata has radiate heads with yellow ray florets and many disk florets. The ray florets produce seeds and thus factor into the higher scores that indicate fruits (3 or 4). The presence of relatively long ray florets (up to about 4 cm) makes the interior disk florets much harder to investigate and also indicates a mature inflorescence. Because the disk florets are so tiny, they are often pressed into the page and harder to view from the side in a dissection scope. When the disk florets are flowering, they will have prominent yellow anthers. The achene is a brown color with a pappus of long bristles, which can be seen without peeling back the ray florets.

Without developed ray florets, if the plant does have any open disk florets, it would be in the First Flowers (1) category. If a head has ray florets, they must be gently pulled back to look for the prominent anthers that each open disk floret will produce. The proportion of disk florets with visible anthers will allow the plant to be scored. If more than 25% of the florets have conspicuous anthers and there is not a lot of pappus sticking out of the receptacle, the plant is likely in the Peak Flowering (2) category. If more than 75% of the florets are open and there are fruits present (with prominent pappus bristles), the plant belongs in the Last Flowers (3) category. Last flowers specimens have lots of pappus, but they also have some open florets that must be confirmed by using a dissection probe to carefully sift through the pappus to search for conspicuous anthers. If no yellow anthers are seen, the specimen is in the Flowering Finished (4) category.

Liatris punctata Protocol

Liatris punctata has discoid heads, which only have purple disk florets. The inflorescence is a spike-like cyme with many heads. The heads at the top (distal) end of the inflorescence open first and those at the lower (proximal) end of the spike open last. When the disk florets are open, their light purple stigmas will be visible. If the florets do not have a visible stigma the dissection probe can be used to manually open a "closed" floret to see if it is a bud or a developing achene. The achenes appear slightly pinkish and are very hard to the touch with the dissection probe.

Scores are determined by starting at the top of the inflorescence in this species. If there are achenes in the proximal heads, then the specimen must be in either the Last Flowers (3) or

Flowering Finished (4) category. While, if the terminal heads are still developing and their florets are all not fully open, then the plant would be in the First Flowers (1) category, since the heads on the rest of the inflorescence will open later than the top heads. If the top heads have open florets or fruits, then the rest of the inflorescence must be examined to see how open the other florets are and keeping a rough count to estimate percentages. It is crucial to count the emerging heads that may only appear as buds to get accurate percentages to differentiate between First Flowers (1) and Peak Flowering (2) categories.

Ratibida columnifera Protocol

Ratibida columnifera has radiate heads, with the ray florets that are dark red with yellow edges or entirely yellow and the ray corollas that are dropped when the heads are in fruit. The disk florets are borne on an elongated, column-like receptacle, where they open from bottom to top of the receptacle, so florets on the top of the head can be assumed to be younger. The receptacles and florets are green when they are immature and, as the heads develop, they turn brown. Individual florets must sometimes be investigated with a dissection probe, because they are very small and hard to see inside of. Florets in fruit are much larger in diameter and harder to break open with a dissection probe than undeveloped florets. The achenes are dark brown to black in color.

Plants with many green immature heads will be in the First Flowers (1) category. Plants in this category must also have some open florets. Individual heads should be scored from bottom to top, noting the number of florets with pollen visible. In most plants in the Peak Flowering (2) category, the ray florets will be colorful, and the disk florets will also appear yellow because of their pollen. The fruits that will be present in the Last Flowers (3) category cannot be broken with the dissection probe, while undeveloped buds can be. Specimens that are in the Flowering Finished (4) category have all of their florets in fruit or will have some missing florets, because the fruits have already been dispersed.

Vernonia baldwinii Protocol

Vernonia baldwinii has discoid heads, with purple disk florets. The heads form a cyme in this species, with each section of the cyme flowering at roughly the same time. For example, if upon investigation of a specimen, a certain group of heads in the cyme contains an achene, the other heads in that branched group should be checked for achenes. The cyme has a more developed, longer, and thicker main branch, with shorter, younger branches coming from the bottom. Undeveloped florets are much smaller and green in color, while open florets are purple and have visible anthers and styles. The filaments are white, and the style extends beyond the corolla tube. When the plant is in fruit, the achenes produce a pappus with brown bristles. These florets developing into achenes are harder to the touch with a dissection probe.

The presence of many smaller, green, undeveloped florets on a specimen would indicate the First Flowers (1) category. When 25–75% of all florets on a plant, after considering each branched group, appear purple and the corolla containing stigma and anthers can be seen appearing out of the pappus, the plant is in the Peak Flowering (2) category. If many branched groups within the cyme inflorescence are found with maturing or mature achenes and there are only a few purple corolla tubes present, the plant would be in the Last Flowers (3) category. Plants with fully developed inflorescences with no open florets and all achenes with visible brown pappus would be in the Flowering Finished (4) category.