

THESIS

THE EFFECT OF TIMING OF GROWING SEASON DROUGHT ON FLOWERING OF
ANDROPOGON GERARDII

Submitted by

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ABSTRACT

THE EFFECT OF TIMING OF GROWING SEASON DROUGHT ON FLOWERING OF *ANDROPOGON GERARDII*

Timing of precipitation is equally important as amount for determining ecosystem function, especially aboveground net primary productivity (ANPP), in a variety of ecosystems. The particular precipitation period(s) of greatest importance varies between ecosystems. In tallgrass prairie of the central US, the relative importance of different precipitation periods is dictated by the phenology of the dominant C₄ grasses, in particular *Andropogon gerardii* which can contribute >80% to ANPP in this ecosystem. It is predicted that precipitation periods with the greatest impact on the highly variable flowering rates of *A. gerardii* are likely to be particularly important for determining ANPP, as flowering individuals are much larger (>2-fold) than non-flowering individuals. The potential for flowering may be affected by precipitation at different times via different mechanisms (e.g. carbon gain via rapid growth early in the growing season vs. direct effects on stalk elongation later in the growing season). In order to test the differential effects of precipitation timing, rainfall deficits (100% exclusion) at different periods of the growing season were imposed on native tallgrass prairie in Kansas, USA. Contrary to expectations, the most sensitive period in terms of flowering for *A. gerardii* did not coincide with the highest potential photosynthetic rates early in the growing season. Rather the most sensitive period was mid to late summer immediately preceding, and concurrent with, the initiation of flowering stalks. Growth rate, leaf water potential and carbon assimilation of *A. gerardii* were all most sensitive to drought late in the growing season, suggesting that growth regulation in

response to plant water status, not current year's carbon accumulation is the critical factor determining flowering responses to precipitation or lack thereof. Flowering, in addition to influencing ANPP, controls rates of sexual reproduction which in turn limit adaptation and migration, and thus understanding how flowering will be influenced by a changing climate is critical for predicting plant community and ecosystem responses in tallgrass prairie. My study suggests that increased frequency of growing season droughts forecast with climate change could result in reduced ANPP and reproductive success of the dominant grasses in the tallgrass prairie ecosystem.

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CHAPTER 1- THE EFFECT OF TIMING OF GROWING SEASON DROUGHT ON FLOWERING OF *ANDROPOGON GERARDII*

Introduction

It is becoming increasingly apparent that in addition to precipitation quantity, timing of rainfall is important for determining aboveground net primary productivity (ANPP) in a variety of ecosystems (Robinson et al. 2013), including the tallgrass prairie ecosystem of the US Central Plains (Knapp et al. 2002, La Pierre et al. 2011, Craine et al. 2012, Craine 2013). For example, La Pierre and colleagues (2011) showed that the two most important precipitation periods for describing inter-annual variation in ANPP were the “vegetative growth” period (April 15 to July 14), and to a lesser extent the “flowering stem elongation” period (July 15 to August 15). This precipitation timing effect may in large part be determined by the phenology of the dominant plants in tallgrass prairie - perennial C₄ grasses - which are responsible for the majority of energy flow in the system, often contributing more than 80% of ANPP (Smith and Knapp 2003). Tallgrass prairie in the Central US is dominated primarily by the C₄ grass, *Andropogon gerardii* (big bluestem). Flowering rates of this grass are highly variable (La Pierre et al. 2011), with flowering typically quite low (La Pierre et al. 2011). In peak flowering years biomass of flowering stalks can increase as much as 10-fold, and during these high flowering years, flowering stalks can contribute >70% to ANPP (Knapp and Hulbert 1986). Thus, understanding what controls flowering in *A. gerardii* is important for predicting inter-annual variation in ANPP of tallgrass prairie.

Multiple resources including light, nitrogen, and water may contribute to inter-annual variability in both ANPP and flowering of *A. gerardii* (Owensby et al. 1970, Knapp and Seastedt 1986, Hulbert 1988, Knapp et al. 2001). Periodic release from limitations of light and N, as a result of infrequent fires (e.g., every 4 years), has been shown to result in a transient pulse in

ANPP (Seastedt and Knapp 1993) and in flowering (LaPierre et al. 2011; Supplementary Fig. 1). In the absence of fire, a thick layer of detritus accumulates resulting in light limitation, as well as increased soil nitrogen availability (Knapp and Seastedt 1986). Both resources are then more abundant when a fire removes the thick detritus layer. With increased soil nitrogen and light availability, the stage is set for a productive year. Productivity peaks observed following infrequent fire are not just a result of high vegetative production, but also high flowering events (Knapp and Hulbert 1986), and it has long been noted that high flowering years tend to follow infrequent fires (Curtis and Partch 1950, Kucera and Ehrenreich 1962). However, potential evaporation is also increased post-fire, as the detritus provides insulation against soil water loss (Knapp and Seastedt 1986). Thus, post-fire years also can result in higher water stress and reduced ANPP, both due to decreased vegetative growth and decreased flowering of *A. gerardii* and other C₄ grasses. Sufficient precipitation is needed during the growing season to ensure both increased production and flowering post-fire (Briggs and Knapp 1995).

While precipitation amount during periods broadly encompassing vegetative growth and flowering stalk elongation has been shown to be important for determining ANPP and flowering of *A. gerardii* in tallgrass prairie (La Pierre et al. 2011, Craine et al. 2012, Craine 2013), even shorter periods during the growing season are likely to have impacts disproportionate to their length, especially if they affect *A. gerardii* flowering stalk initiation, elongation, and/or density and biomass. However, experiments imposing precipitation deficits at these critical times during the growing season are lacking. Yet, this understanding is particularly important with future climate change, as precipitation is expected to become more variable and drought periods more extensive during the growing season in the Central Plains region of the US (Sheffield and Wood 2008).

In order to experimentally assess the effect of timing of precipitation deficits on flowering of *A. gerardii*, droughts of equal magnitude were imposed for relatively short periods of time during the growing season. These periods included late spring (LS = April to May), early summer (ES = May to June) and mid-summer (MS = June to July), with the three representing critical periods of rapid early season growth, carbon accumulation, and flowering stalk initiation and elongation, respectively (Selbo and Snow 2005, Sherry et al. 2007). Flowering stalks of *A. gerardii* are large in comparison to their vegetative organs (>2-fold difference in mass (J. Dietrich, pers.obs.)), and thus flowering stalk production is likely to be sensitive to stored carbon and/or carbon gain during the growing season. Indeed, given that flowering is highest when light and nitrogen limitations are most reduced due to infrequent fire, the expectation is that carbon gain during the growing season may be most important for determining flowering post-fire. As carbon assimilation rates are highest early in the growing season (Knapp 1985), it was predicted that LS or ES drought would have the largest effect on flowering of *A. gerardii*. However, given the evidence that precipitation during the period of flowering stalk elongation can be important for flowering (La Pierre et al. 2011), MS drought also could influence density of flowering stalks, but this effect is likely of lesser importance than a drought during rapid carbon gain and accumulation. To test these hypotheses, we conducted a drought timing experiment over a two-year period on adjacent sites that had not been burned for three years prior to the experiment, but that were burned early in the growing season prior to initiation of the drought treatments. Selection of these sites was strategic in that we hoped to capture a potentially high flowering event by minimizing limitations by all other resources except for water.

Methods

Study Site

The study was conducted during the 2013 and 2014 growing seasons at the Konza Prairie Biological Station (KBPS), which is a NSF Long-term Ecological Research (LTER) site. The Konza Prairie is a 3,487 ha native, unplowed tallgrass prairie located in the Flint Hills region of northeastern KS. Two sites were selected in ungrazed, upland areas of two adjacent watersheds (<200 m apart) that were burned in the spring of the experimental year, but which had not been burned during the three preceding years (an approximate 4-year burn interval has been imposed in these watersheds for the last 30 years). Both sites are dominated by *A. gerardii* with a few other subdominant C₄ grasses and many minor graminoid and forb species. Both sites have soils of the Benfield-Florence complex, consisting of gravelly silt loam in the upper 15 cm, with increasing gravel and cobbles until bedrock is encountered generally within 140 cm of the surface (NRCS 2014). As a consequence of the shallow soils, drought during different periods of the growing season was expected to have a significant effect on growth and flowering of *A. gerardii* (Briggs and Knapp 1995).

Drought treatments

To assess the impacts of timing of growing season drought on flowering of *A. gerardii*, we conducted a two-year experiment consisting of a control treatment (CON, average growing season precipitation) and three drought timing treatments: late spring drought (LS, starting mid-April), early summer drought (ES, starting mid-May), and mid-summer drought (MS, starting mid-June). For each year of the experiment, the control and drought treatments were arranged in a randomized block design (n = 10 blocks), with 10 replicates per treatment. Drought treatments were imposed by excluding rainfall with 2.4 x 2.4 m clear polycarbonate roofs mounted on fence t-posts at heights ranging from approximately 60 cm to 130 cm above the ground (shelter height

increased with increasing canopy height during the growing season). The roof size provided a substantial buffer (~0.7 m) around each 1 x 1 m sampling plot. Control plots were not covered.

In 2013, each drought treatment was designed to exclude a target of ~150 mm or approximately 25% of the average growing season precipitation. For each year of the study, the first exclusion period began shortly after the prairie was burned, the second a month after that, and the third a month after that, and each continued until the target was reached (Table 1). Control plots (CON) without exclusion had water added by hand during dry periods to match the long term average precipitation. Additions occurred weekly (as required) from June 1 through July 16 and totaled 76 mm.

For 2014, exclusions began at approximately the same dates as in 2013 (Table 1). As more rain fell in May 2014 than in May 2013, the exclusion target was increased to ~170 mm, and this target was easily met with the LS and ES drought treatments. However, little rain fell during the MS exclusion period; rather than extend this exclusion to the end of August, four artificial rain events (each 21-24 mm in size) were simulated by adding a total of 93 mm to the LS, ES, and CON treatments in July and August. Aided by these events, the mid-summer exclusion target was met by mid-August.

Soil moisture of the top 20 cm was measured with (ECH₂O probes, Decagon Devices Inc., Pullman, WA, USA) in half of the plots (n = 5 per treatment). Soil moisture was measured every hour, and the probes were calibrated with soil samples collected throughout the summer for laboratory soil moisture analysis following Wilcox et al. (2015).

Flowering measurements

At the end of each growing season, *A. gerardii* flowering stem density (stems m⁻²) and dry mass of both reproductive and vegetative tissues (g m⁻²) were assessed. All flowering stems

were counted in each 1 x 1 m plot, including stems that elongated, but did not produce flowers. All *A. gerardii* plant material was harvested from two randomly selected 0.1 m² (20 x 50 cm) subplots in each plot. Flowering stems, inflorescences, and cauline leaves were considered “reproductive” tissues; basal leaves of flowering individuals and all parts of non-flowering individuals were considered “vegetative”. Biomass was dried for 48 hrs at 60 °C and weighed. From this data, the ratio of reproductive to vegetative (R/V) biomass was calculated.

Individual growth measurements

In 2013, ten individual *A. gerardii* tillers were tagged in each experimental plot at the beginning of the growing season and monitored from May 11 to Sept 7. Developmental stage (i.e., vegetative, elongating, flowering, seed set) and height (from base of plant to tip of longest leaf or flowering stalk) were assessed on an approximately biweekly basis for a total of eight measurements. Relative growth rate (RGR) was calculated for individuals in each of the treatments as follows.

$$\text{RGR} = \frac{\ln(\text{height}_2 - \text{height}_1)}{\ln(t_2 - t_1)}$$

Where t_1 is the day of the earliest assessment point within a given exclusion period, t_2 is the day of the latest assessment point within that exclusion period, and height_1 and height_2 are the heights measured at those times.

Ecophysiological measurements

During the 2014 growing season, plant water status and CO₂ assimilation were assessed for selected individuals of *A. gerardii* on a biweekly basis (starting May 27 and ending Aug 23) for all treatments in six of ten blocks. Leaf water potential (ψ_{leaf}) at pre-dawn and mid-day was measured with a pressure bomb (PMS model 1000). The youngest fully emerged leaf was collected from 2 or 3 canopy individuals (different plants each sampling period) before dawn and

again in the middle of the day (between 11:00 am and 3:00 pm). Net CO₂ assimilation (A_{net}) was also measured at mid-day using a LI-COR 6400 infra-red gas analyzer (IRGA) equipped with a fluorometer. A_{net} measurements were taken on the youngest fully emerged leaf of the same marked individuals each sampling period. Leaves were provided with full light ($2000 \mu\text{mol m}^{-2} \text{s}^{-1}$) and 400 ppm CO₂ and the assimilation rate was allowed to stabilize before being recorded. Time constraints required that both mid-day (ψ_{leaf}) and A_{net} measurements be split into two, generally consecutive days (unless there were suboptimal conditions) for sampling.

Statistical Analyses

Mixed model ANOVA was used to assess the effects of the drought treatments on soil moisture, flowering stem densities and R/V biomass. Drought treatments were treated as fixed effects and block as a random effect. Each year of the experiment was analyzed separately. Soil moisture for each treatment plot was averaged over each of the three exclusion periods and ANOVA was performed on the period means. A large number of plots without any flowering stalks resulted in a non-normal distribution for both flowering stem density and R/V biomass, and therefore these data were transformed before they were analyzed. The flowering stem densities were transformed with a natural log transformation and R/V biomass was transformed with a modified arcsin transformation following (McCune and Grace 2002) prior to analysis. Repeated measures ANOVA was performed on the growth, water potential and CO₂ assimilation data, with drought treatment as the fixed effect, block as the random effect, and date as the repeated measure. Several common covariance structures were used, and results from the analysis with the lowest AIC were selected. Additionally mean growth rates for each period were compared with ANOVA using R statistical software. All other analyses were performed

with SAS 9.3 (SAS Institute Inc., Cary, NC, USA). Significance was set at $\alpha = 0.05$ for all analyses.

Results

Drought timing effects on soil moisture

Total growing season precipitation was similar in both years of the study, but timing of rainfall events was different, with more rain falling early in the growing season in 2014 compared to 2013 (Table 1, Supplementary Fig. 2). This produced exclusion periods of different lengths and amount of overlap each year, and different reductions in total growing season precipitation (Table 1). Overall, each drought treatment excluded approximately 24% of the total growing season precipitation in 2013, while in 2014 each drought treatment excluded approximately 30% of the total growing season precipitation.

There were significant effects of drought on soil moisture in both 2013 and 2014 (Supplementary Table 1, Fig. 1). As expected, each drought timing treatment had lower soil moisture values during its focal period, although the difference between the drought and control treatments was not always significant. For the 2013 LS exclusion period, soil moisture for the LS treatment was significantly less than that of the ES and MS treatments, but not the CON treatment. For the ES exclusion period, which overlapped with both the LS exclusion period and the MS exclusion period, all three drought treatments LS, ES and MS had significantly lower soil moisture than the CON treatment. For the MS exclusion period, soil moisture of the MS treatment was significantly below the CON and LS treatments, but not the ES treatment which also had depressed soil moisture during this period due to overlap between the ES and MS exclusion periods. The magnitude of soil drying relative to the CON treatment was similar for each of the drought treatments, with reductions of 29% for the LS treatment during the late

spring drought, 25% for the ES treatment during the ES drought, and 35% for the MS treatment during the MS drought.

Soil moisture differences between treatments were generally less pronounced in 2014 (Fig. 1b). For the LS exclusion period, soil moisture was lowest for the LS and ES treatments. However, the difference in soil moisture between the LS treatment and, the thus far “undroughted”, MS and CON treatments was not significant. For the ES exclusion period, soil moisture was significantly different between the ES and both the MS and CON treatments. For the MS exclusion period, soil moisture was lowest for the MS treatment, but the soil moisture difference between the MS and CON treatments was not significant, although it was marginally significant between the MS and ES treatments (Supplementary Table 1).

Effects of drought timing on flowering

In 2013, the ambient level of flowering was as great as any year in the past 30 years for the study site (Supplementary Fig. 1a). While more modest in 2014, ambient flowering for that study site was still above-average (Supplementary Fig. 1b). Control plots with modest water additions had even higher levels of flowering both years of the experiment when compared to long-term data for the study sites. In 2013, CON treatment flowering stalk densities averaged 26.6 (21.1 SD) vs the long term mean of $5.1 \pm (8.2 \text{ SD})$ stems m^{-2} for the study site, and flowering stalk biomass was on average $65.7 \pm (60.7 \text{ SD})$ vs the long term site mean of $10.5 \pm (14.5 \text{ SD})$ g m^{-2} . In 2014, flowering stalk density for the CON treatment was on average $17.3 \pm (11.5 \text{ SD})$ vs the long term mean of $6.1 \pm (13.0 \text{ SD})$ stems m^{-2} , and flowering stalk biomass was $26.4 \pm (26.8 \text{ SD})$ vs the long-term site mean of $12.0 \pm (30.7 \text{ SD})$ g m^{-2} .

There were significant effects of growing season drought timing on flowering stalk density and R/V biomass (Supplementary Table 2, Fig. 2). In 2013, flowering stalk densities

were lowest for the ES and MS treatments, with the LS treatment intermediate in number of flowering stalks and the CON treatment with the highest flowering stalk density (Fig. 2a). Both the ES and MS treatments showed significant reductions in R/V biomass relative to the CON treatment, with the LS treatment intermediate (Figs. 2c). In 2014, flowering stalk densities were lowest for the MS treatment which was significantly below all other treatments (Fig. 2b). A similar pattern was observed for R/V biomass, the MS treatment had the lowest R/V biomass which was significantly less than that of the LS treatment, while the ES treatment was not significantly different from the LS or CON treatments (Fig. 2d).

Effect of drought timing on growth and ecophysiology

There was a significant effect of treatment, date and the interaction of treatment by date on mean tiller height (Supplementary Table 4). Early in the growing season of 2013 all treatments had a similar growth trajectory based on height (Fig. 3a), but later in the year the CON and then LS treatments increased in height much more rapidly than the ES and MS treatments. There was a significant treatment effect on growth rates for each of the three exclusion periods (Supplementary Table 3). During the LS exclusion period, the LS and ES treatments grew significantly slower than the CON and MS treatments (Fig. 3b). During the ES exclusion period, the growth rate for the ES treatment was significantly lower than the LS or MS drought treatments, which had intermediate growth rates when compared to the CON treatment (Supplementary Table 3, Fig. 3c). During the MS exclusion period, the growth rate for the MS and ES treatments was much less than for the LS or CON treatments (Supplementary Table 3, Fig. 3d). A pulse in precipitation following the end of the MS exclusion (Supplementary Fig. 2) provided an opportunity for recovery and all treatments showed increased growth. As a consequence, the mean height for the LS treatment caught up to the CON treatment by the end

the growing season, at which time individuals of both the LS and CON treatments were significantly taller than those of the ES and MS treatments (Fig. 3a).

There were significant effects of treatment, date and the interaction of treatment by date for all ecophysiological variables (Supplementary Table 5). Pre-dawn and midday leaf water potential (ψ_{leaf}) remained relatively constant throughout the growing season for the LS, ES, and CON treatments (Fig. 4a,b). The MS drought treatment on the other hand, showed significant reductions in pre-dawn and midday ψ_{leaf} relative to all other treatments by the second half of the MS exclusion (Fig 4a,b). All treatments had large reductions in A_{net} with time over the course of the growing season (Fig. 4c). Similar to ψ_{leaf} , the only significant differences between treatments occurred after mid-July. By July 29th, MS treatment had the lowest A_{net} and it remained significantly lower than the other treatments through August 23 (Fig. 4c).

Flowering Phenology

The first individuals to commit to flowering were first observed to elongate in early July. Out of approximately 600 individuals monitored, two were committed to flowering on July 5 with this number increasing to a total of 130 by Sept 7 (Supplementary Fig. 3).

Discussion

In each of the two years of the experiment, a similar pattern of drought timing effects on flowering of the dominant C₄ grass, *A. gerardii*, was observed even though the overall rate of flowering, timing of precipitation, and the length of the exclusion periods differed. Contrary to expectations, the effect of drought timing on flowering (both number of flowering stalks and the ratio of reproductive to vegetative biomass) of *A. gerardii* was greater for drought occurring mid-summer (MS treatment = ~June 21-July/Aug) than the drought in late spring (LS treatment = Apr 20- early/mid-June). Likewise the MS treatment had the greatest ecophysiological

responses (reductions in leaf water potential and photosynthetic rates) and, along with the ES treatment, the greatest reductions in growth. However, in 2013 the drought during early summer (ES treatment = May 21-July 2) also significantly reduced individual growth, flowering stalk densities and R/V biomass of *A. gerardii* when compared to the LS or CON treatments (no ecophysiological measurements were collected this year). On the other hand, in 2014 no ES treatment effects on flowering or ecophysiology were observed (no growth measurements were collected this year). This variation in the impact of drought in early summer, which began on approximately the same date each year of the study, was likely related to differences in its duration in 2013 vs. 2014. The ES treatment exclusion ended mid-June in 2014, with 29 mm of rain the next day. In contrast, the ES treatment exclusion ended in early July in 2013, with no rain events > 13mm until July 23 (Suppl. Figure 2).

These findings suggest that plant water stress, which typically peaks in July or August (Knapp 1984, Owensby et al. 1993), drove the decrease in photosynthetic rates, growth, and flowering observed with both the ES and MS droughts in 2013 and the MS drought in 2014. Collectively, the large impact on flowering for the ES drought in 2013, which effectively extended to late July, the lack of effect for the ES drought in 2014, which ended in mid-June, and the large effects of the MS drought in both years indicate that the most sensitive time for water stress effects on flowering is mid to late July. This timing also coincides with flowering stalk initiation, which began in early July and continued through August, with a in peak flowering occurring in late July to early August in 2013 (with similar timing of both observed in 2014; J. Dietrich, pers. Obs.). Sixty percent of individuals that would eventually produce flowering stalks were first observed to elongate between July 25 and August 8 (Supplementary Fig. 3).

This timing coincides with large reductions in water potential observed with the MS drought treatment.

Contrary to expectations, drought timing effects on flowering of *A. gerardii* did not appear to be due to impacts on accumulation of carbon stores during the growing season. Indeed, the LS treatment did not reduce photosynthesis in the late spring drought nor did the ES treatment reduce photosynthesis during the early summer drought. Moreover, individuals were already beginning to elongate flowering stalks by the time differences in photosynthesis between the MS treatment and other drought treatments emerged. On the other hand, if carbon utilized for flowering stalk initiation and growth is derived from assimilation during the MS drought timing period, depression of photosynthesis at this time could be important. However, relatively low assimilation rates for the CON treatment during the mid-summer exclusion period (Fig. 4) suggest that reserves likely provide at least a portion of flowering stalk carbon.

Without large negative effects on growing season carbon gain, it is likely that the differences in the impacts of the drought timing on flowering were due to the degree of water stress experienced by individuals. Although there was no difference in degree of drying for the top 20cm of soil between the drought treatments, the MS treatment experienced large reductions in leaf water potential that the other treatments did not. This was likely related to drying of soils to a greater depth (as supported by lower pre-dawn water potentials for the MS treatment) and/or, higher temperatures and greater leaf area later in the growing season. It is unclear to what extent MS treatment effects reflect seasonal variation in the sensitivity of *A. gerardii* flowering to water stress and to what extent they reflect seasonal variation in potential water stress. In other words, do the MS treatment effects occur because the MS treatment produces stress during a critical developmental stage, or do they occur because mid-summer is the only time when drought will

produce sufficient water stress? Regardless of the relative importance of these two aspects of timing it appears that only drought later in the summer is likely to have a large impact on flowering. Some combination of cooler temperatures/lower leaf area and higher availability of deep soil moisture prevent severe water stress early in the growing season, as indicated by the lack of significant difference between LS and CON treatment water potential at the end of the late spring drought, despite 100% exclusion for 45 days. On the other hand, the observed stress levels in the MS treatment, mid-day ψ_{leaf} around -3 or -4 MPa, are not unusual for *A. gerardii* in the middle of summer. Other studies on *A. gerardii* at Konza have shown comparable values for mid-day ψ_{leaf} in July, without artificial rain exclusion.

The production of a flowering stalk involves both a transition to production of reproductive tissues, and a dramatic increase in individual production (growth). Thus there are two potential avenues for stress effects on flowering; a generalized growth response, and the transition in the nature of a meristem from vegetative to reproductive. The effect of water stress leads to reduced growth even before reduced photosynthesis, and thus it is usually not carbon starvation that limits growth (Muller et al. 2011). It has been suggested that, in a region prone to summer drought, reduced flowering in response to water stress is a stress avoidance mechanism for *A. gerardii*, and that this response gives it a competitive advantage over similar, but less abundant tallgrass species (Swemmer et al. 2006). On the other hand the hormonal response to water stress may influence floral induction directly (Wang et al. 2013).

Craine et al. (2010) evaluated the long term flowering record at Konza and selected the precipitation period from May 25 to August 8 as being the most predictive of flowering of *A. gerardii*. This is generally consistent with our results. This period lines up well with the 2013 ES treatment exclusion which had a large effect on flowering and began on May 21 and

effectively continued until July 23, though the MS treatments had effects of similar magnitude while beginning a month later and of shorter duration. It is likely that a complete rainfall exclusion produces a strong effect over a shorter time interval than most natural droughts, but if the end result of a longer drought is water stress in mid to late-July it is likely to have a strong impact on flowering. Craine et al. (2010) also found that deep soil moisture was predictive of flowering, which suggests that the effects of the later exclusions we observed may have been caused by drying below the depth of our probes which did not show significant soil moisture effects between the drought timing treatments.

The impact of mid-summer drought on flowering of *A. gerardii* helps to explain why this period is important for ANPP in tallgrass prairie (La Pierre et al. 2011, Craine 2013). Drought related reductions in flowering are likely to be more common in the future as extended periods of anomalously low soil moisture during the growing season are predicted to increase in frequency central North America (Sheffield and Wood 2008). Specifically, decreased frequency or magnitude of high flowering years could have large ramifications for the reproductive success of this important species which already has low rates of sexual reproduction (Benson and Hartnett 2006). It is likely that fertilization rates increase with increasing flowering stalk density (Smith et al. 1990, Kelly 2001) and that large flowering years contribute disproportionately to seed production. Sexual reproduction is important for determining the response of this important species to changing climate; it results in genetic recombination, promoting adaptation, and the resulting seeds are potentially dispersed large distances increasing migration rates. Ultimately, drought effects on flowering have implications for, carbon sequestration and biofuel production.

Table 1. Rainfall exclusion periods in 2013 and 2014 for the late spring (LS), early summer (ES), and mid-summer (MS) drought timing treatments. Included are the duration of exclusion, amount of excluded rainfall, and amount of precipitation received for each treatment. Note that total precipitation includes watering events in addition to ambient precipitation (2013: 568 mm, 2014: 474 mm). In 2013, 76 mm was added to the CON treatment plots. In 2014, 93 mm of water was added to the LS, ES, and CON treatment plots, but not the MS treatment.

Year	Treatment	Exclusion period	Exclusion duration (days)	Excluded rainfall (mm)	Total precipitation (mm)	Mean period temperature (°C)
2013	LS	Apr 20- Jun 22	63	151	416	18.4
	ES	May 22- Jul 2	41	159	409	22.6
	MS	Jun 22- Jul 26	34	154	413	25.4
	CON	-	-	0	644	
2014	LS	Apr 20- Jun 4	45	166	401	18.8
	ES	May 21- Jun 14	24	171	396	21.9
	MS	Jun 19- Aug 12	54	175	391	24.7
	CON	-	-	0	568	

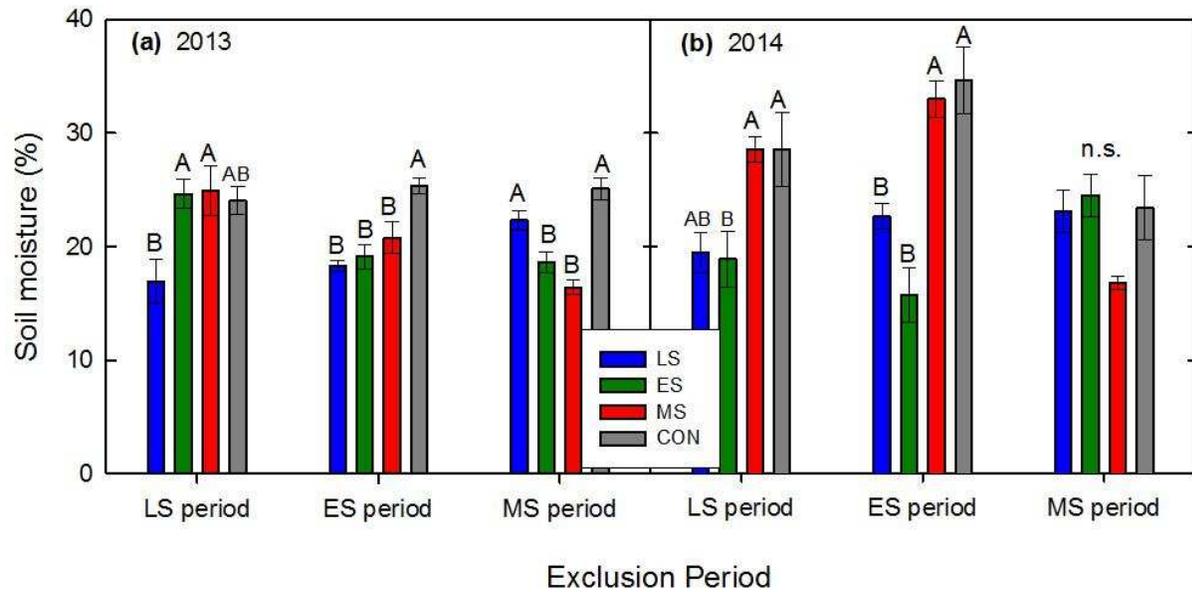


Figure 1. Soil moisture (%) for the control (CON) and drought timing treatments (LS = late spring drought, ES = early summer drought, and MS = mid-summer drought) averaged over the three exclusion periods in a) 2013 and b) 2014 (see text for details). Different letters indicate significant differences ($P \leq 0.05$) between treatments; n.s. indicates that there were no significant differences between treatments.

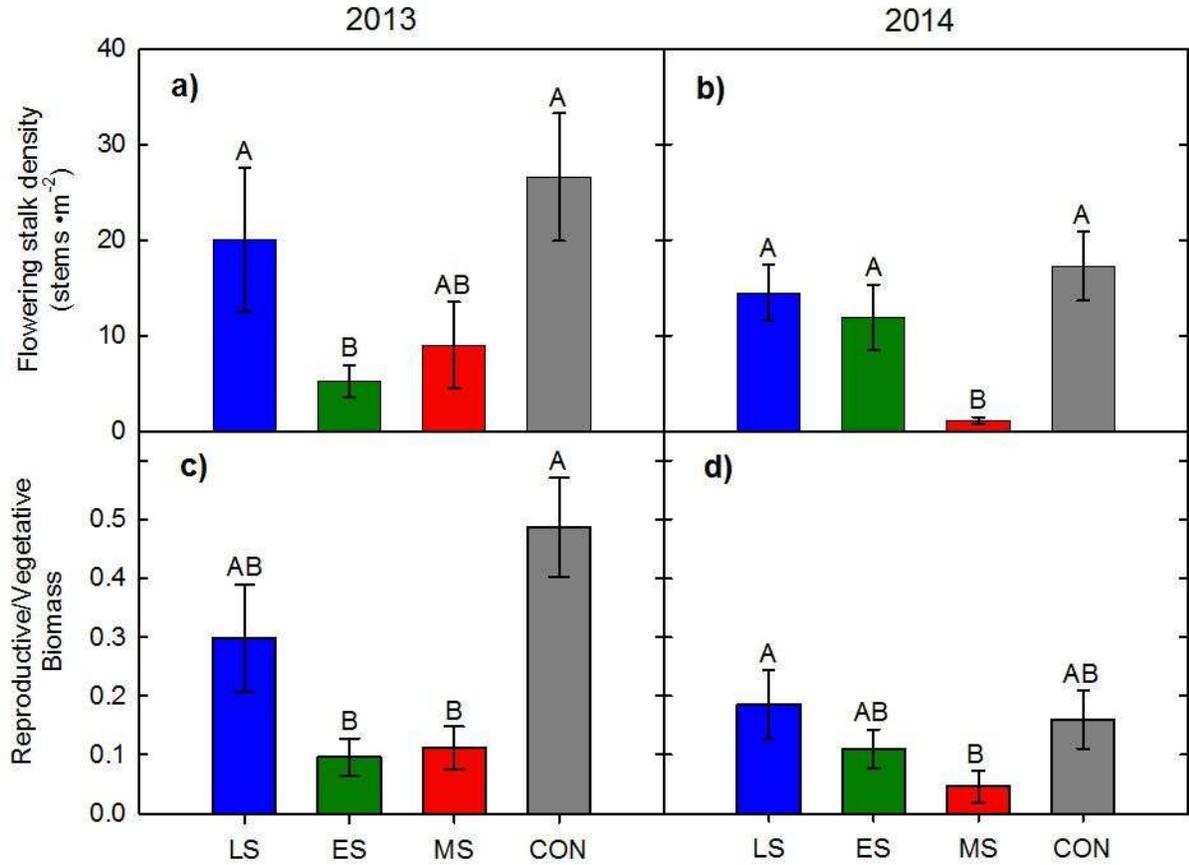


Figure 2. Effects of drought timing treatments (CON = control, LS = late spring drought, ES = early summer drought, MS = mid-summer drought; see text for details) on (a, b) Flowering stalk densities (m⁻²), and (c, d) the ratio of reproductive to vegetative biomass (R/V) in 2013 (left) and 2014 (right). Different letters indicate significant differences ($P \leq 0.05$) between treatments.

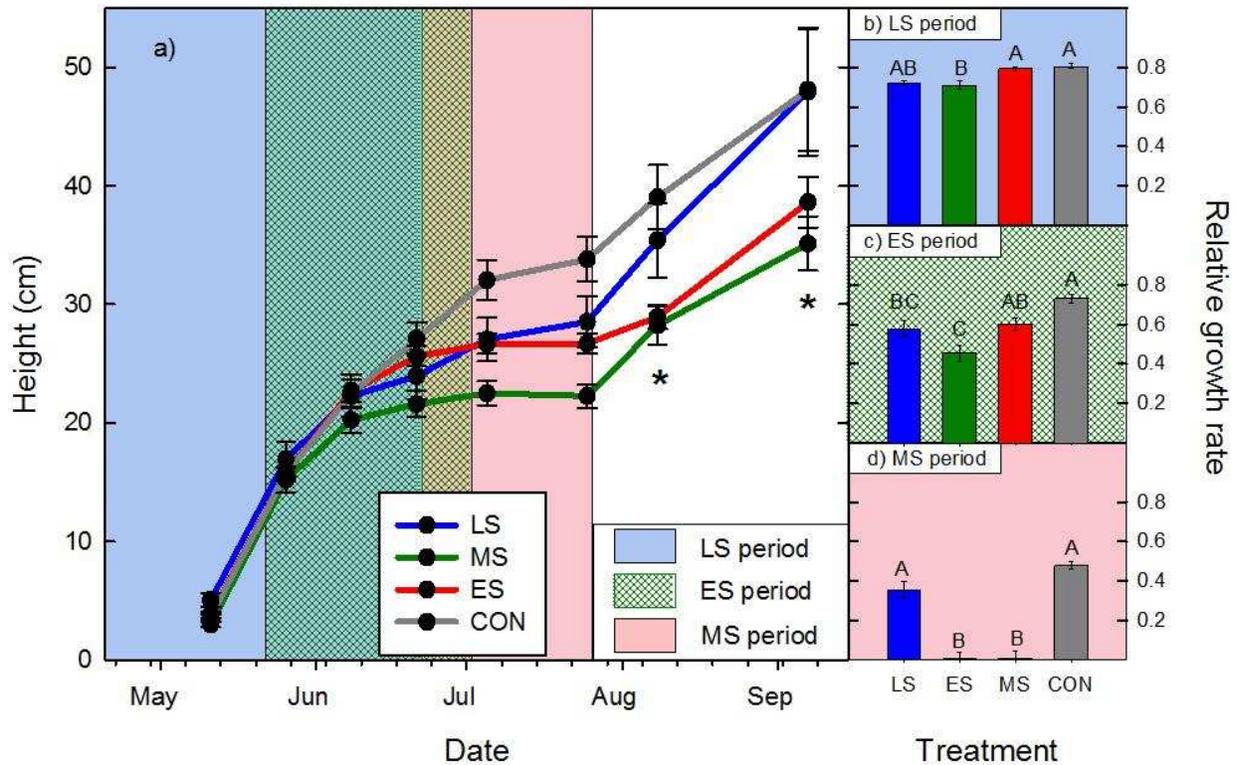


Figure 3. (a) Mean tiller height of *A. gerardii* over time during the 2013 growing season in response to the drought timing treatments (CON = control, LS = late spring drought, ES = early summer drought, MS = mid-summer drought; see text for details). Relative growth rates for drought timing treatments during the (b) late spring exclusion period, (c) the early summer exclusion period, and (d) the mid-summer exclusion period. Rainfall exclusion periods are indicated by background color. Asterisk indicates at least one significant difference in pairwise comparisons between treatments.

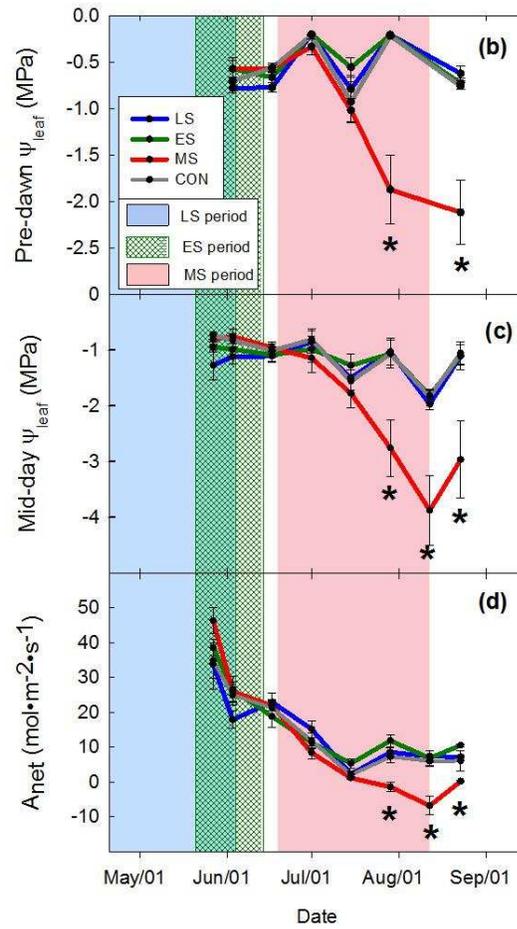


Figure 4: Ecophysiology of *A. gerardii* over time during the 2014 growing season in response to the drought timing treatments (CON = control, LS = late spring drought, ES = early summer drought, MS = mid-summer drought; see text for details). Ecophysiological measurements include (a) mean predawn water leaf water potential (ψ_{leaf}), (b) mean midday leaf water potential (ψ_{leaf}), and (c) mean net CO₂ assimilation rate (A_{net}). Rainfall exclusion periods are indicated by background color. Asterisk indicates at least one significant difference in pairwise comparisons between treatments.

CHAPTER 2- FUTURE RESEARCH ON CAUSES AND CONSEQUENCES OF DROUGHT EFFECTS ON FLOWERING OF *ANDROPOGON GERARDII*

Mechanisms

Drought occurring in mid-summer (late June through July) strongly reduces the rate of flowering of *Andropogon gerardii*, yet drought of similar duration occurring earlier does not affect flowering despite equal reductions in total precipitation (Chapter 1). The timing of impactful drought is coincident with both high temperatures, and the initiation of flowering stalks. So it is not clear whether this time is critical because of the intrinsic phenology of *A. gerardii* or because drought during this time tends to produce severe water stress. Would severe water stress earlier in the year effect flowering similarly? It is much less likely that severe stress would occur in May/June, but it may be possible with winter/spring drought and unusually warm temperatures in early summer. In such a circumstance June conditions may come to approximate those more expected with drought later in the year. For example in 1988 only 223 mm of precipitation fell between December 1 and June 30mm about half of the average 437mm for this period; this was combined with especially high temperatures in May (6 days with highs above 30 °C) and June (8 days with highs above 38 °C) (Konza data set AWE012). The cumulative pan water evaporation potential for April through June 1988 was 686mm which is nearly equal to the average for April through July— 736 mm (Konza data set APT025). Perhaps water stress in late June 1988 approached levels equal to those achieved with experimental exclusion in late July (Chapter 1). Unfortunately, we lack physiological data for that time, and no 4-year burn occurred in a watershed with flowering data, so it is hard to assess effects on flowering. In order to evaluate the effect of drought/water stress timing independently of magnitude a greenhouse experiment would be very useful.

An alternative approach to understanding the mechanism of drought effects on *A. gerardii* would be to perform hormone or RNA assays during the time of flowering stalk initiation. Within a drought timing experiment this data could be used to evaluate treatment effects on the signal to flower. Outside the context of such an experiment, one could look in a watershed likely to have high flowering, assay tagged individuals, and perhaps find differences between individuals that eventually flower and those that do not.

Reproduction

The density of *A. gerardii* flowering stalks varies dramatically from year to year; for example in the 4B watershed, the standard deviation, 11.5, is greater than the mean, 7.2 stalks m⁻² (Konza data set PRE022). Seed production may be even more variable if fertilization rates increase with flowering stalk density, as one might expect (Smith et al. 1990, Kelly 2001, Bjornstad et al. 2002) (Smith et al. 1990, Kelly 2001, Bjornstad et al. 2002). In 2013 there was a higher rate of flowering across the 4B watershed than there had been in ten years— mean 22 stems m⁻² (Konza data set PRE011). Yet even in this “peak” year only about half of sessile florets contained seeds (Dietrich, unpublished data) suggesting that pollen abundance may limit seed production even in a “peak” flowering year. In 1981 flowering stalk densities of *A. gerardii* were as high as 68 stalks m⁻²; this is nearly triple the flowering rate of a normal “peak” year. These super peak years may have disproportionate importance for sexual reproduction and population dynamics of *A. gerardii* especially if they are associated with increased fertilization rates.

Understanding the relationship between flowering stalk density and seed production is critical; for explaining the population dynamics of this important species. This relationship could be evaluated by looking at rates of fertilization or seed filling along a gradient of flowering

stalk densities that often exists within Konza related to different burning treatments. In addition to comparing fertilization/seed filling rates between watersheds with high and low flowering, the spatial pattern of pollen movement could be evaluated by looking at fertilization/seed filling rates across a single watershed, comparing plants from peripheral and central parts of a single high flowering watershed, as well as those with different orientations to the prevailing wind. Seed longevity is a potentially important factor as well; if seeds are only produced in significant number once or twice a decade their ability to survive several years until favorable conditions arrive could play a major role in reproductive success. Perhaps seed longevity could be evaluated in the lab or the soil seed bank could be compared between watersheds with different time intervals since the last big flowering year.

REFERENCES

- Benson, E. and D. Hartnett. 2006. The Role of Seed and Vegetative Reproduction in Plant Recruitment and Demography in Tallgrass Prairie. *Plant ecology* **187**:163-178.
- Bjornstad, O. N., M. Rees, D. Kelly, and O. Bjørnstad. 2002. Snow Tussocks, Chaos, and the Evolution of Mast Seeding. *American Naturalist*, The **160**:44-59.
- Briggs, J. and A. Knapp. 1995. Interannual Variability in Primary Production in Tallgrass Prairie: Climate, Soil Moisture, Topographic Position, and Fire as Determinants of Aboveground Biomass. *Am J Bot* **82**:1024-1030.
- Craine, J. 2013. The importance of precipitation timing for grassland productivity. *Plant ecology* **214**:1085-1089.
- Craine, J. M., J. B. Nippert, A. J. Elmore, A. M. Skibbe, S. L. Hutchinson, and N. A. Brunsell. 2012. Timing of climate variability and grassland productivity. *Proceedings of the National Academy of Sciences* **109**:3401-3405.
- Craine, J. M., E. G. Towne, and J. B. Nippert. 2010. Climate controls on grass culm production over a quarter century in a tallgrass prairie. *Ecology* **91**:2132-2140.
- Curtis, J. and M. A. X. Partch. 1950. Some Factors Affecting Flower Production in *Andropogon Gerardi*. *Ecology* **31**:488-489.
- Hulbert, L. 1988. Causes of Fire Effects in Tallgrass Prairie. *Ecology* **69**:46-58.
- Kelly, D. 2001. Evaluating the wind pollination benefits of mast seeding. *Ecology* **82**:117-126.
- Knapp, A. 1985. Effect of Fire and Drought on the Ecophysiology of *Andropogon gerardii* and *Panicum virgatum* in a Tallgrass Prairie. *Ecology* **66**:1309-1320.
- Knapp, A., P. Fay, J. Blair, S. Collins, M. Smith, J. Carlisle, C. Harper, B. Danner, M. Lett, and J. McCarron. 2002. Rainfall Variability, Carbon Cycling, and Plant Species Diversity in a Mesic Grassland. *Science* **298**:2202-2205.
- Knapp, A. and L. Hulbert. 1986. Production, Density and Height of Flower Stalks of Three Grasses in Annually Burned and Unburned Eastern Kansas Tallgrass Prairie: A Four Year Record. *The Southwestern naturalist* **31**:235-241.
- Knapp, A. K. 1984. Water relations and growth of three grasses during wet and drought years in a tallgrass prairie. *Oecologia* **65**:35-43.
- Knapp, A. K., J. M. Briggs, and J. K. Koelliker. 2001. Frequency and Extent of Water Limitation to Primary Production in a Mesic Temperate Grassland. *Ecosystems* **4**:19-28.
- Knapp, A. K. and T. R. Seastedt. 1986. Detritus Accumulation Limits Productivity of Tallgrass Prairie. *BioScience* **36**:662-668.
- Kucera, C. L. and J. Ehrenreich. 1962. Some Effects on Annual Burning on Central Missouri Prairie. *Ecology* **43**:334-&.
- La Pierre, K. J., S. Yuan, C. C. Chang, M. L. Avolio, L. M. Hallett, T. Schreck, and M. D. Smith. 2011. Explaining temporal variation in above-ground productivity in a mesic grassland: the role of climate and flowering. *Journal of Ecology* **99**:1250-1262.
- McCune, B. and J. Grace. 2002. *Analysis of ecological communities*. MjM Software, Bleneden Beach, Oregon, USA.
- Muller, B., F. Pantin, M. Genard, O. Turc, S. Freixes, M. Génard, M. Piques, and Y. Gibon. 2011. Water deficits uncouple growth from photosynthesis, increase C content, and modify the relationships between C and growth in sink organs. *Journal of Experimental Botany* **62**:1715-1729.
- NRCS. 2014. NRCS Web Soil Survey. USDA National Resources Conservation Service.

- Owensby, C., R. Hyde, and K. Anderson. 1970. Effects of Clipping and Supplemental Nitrogen and Water on Loamy Upland Bluestem Range. *Journal of range management* **23**:341-&.
- Owensby, C. E., P. I. Coyne, J. M. Ham, L. M. Auen, and A. K. Knapp. 1993. Biomass Production in a Tallgrass Prairie Ecosystem Exposed to Ambient and Elevated CO². *Ecological applications* **3**:644-653.
- Robinson, T. M. P., K. J. La Pierre, M. A. Vadeboncoeur, K. M. Byrne, M. L. Thomey, and S. E. Colby. 2013. Seasonal, not annual precipitation drives community productivity across ecosystems. *Oikos* **122**:727-738.
- Seastedt, T. R. and A. K. Knapp. 1993. Consequences of Nonequilibrium Resource Availability Across Multiple Time Scales: The Transient Maxima Hypothesis. *American Naturalist, The* **141**:621-633.
- Selbo, S. M. and A. A. Snow. 2005. Flowering Phenology and Genetic Similarity among Local and Recently Introduced Populations of *Andropogon gerardii* in Ohio. *Restoration Ecology* **13**:441-447.
- Sheffield, J. and E. F. Wood. 2008. Projected changes in drought occurrence under future global warming from multi-model, multi-scenario, IPCC AR4 simulations. *Climate dynamics* **31**:79-105.
- Sherry, R. A., X. Zhou, S. Gu, J. A. Arnone, D. S. Schimel, P. S. Verburg, L. L. Wallace, and Y. Luo. 2007. Divergence of reproductive phenology under climate warming. *Proceedings of the National Academy of Sciences* **104**:198-202.
- Smith, C., J. L. Hamrick, and C. Kramer. 1990. The Advantage of Mast Years for Wind Pollination. *American Naturalist, The* **136**:154-166.
- Smith, M. and A. Knapp. 2003. Dominant species maintain ecosystem function with non-random species loss. *Ecology Letters* **6**:509-517.
- Swemmer, A. M., A. K. Knapp, and M. D. Smith. 2006. Growth Responses of Two Dominant C4 Grass Species to Altered Water Availability. *International Journal of Plant Sciences* **167**:1001-1010.
- Wang, Y., L. Li, T. Ye, Y. Lu, X. Chen, and Y. Wu. 2013. The inhibitory effect of ABA on floral transition is mediated by ABI5 in *Arabidopsis*. *Journal of Experimental Botany* **64**:675-684.
- Wilcox, K., J. von Fischer, J. Muscha, M. Petersen, and A. Knapp. 2015. Contrasting above- and belowground sensitivity of three Great Plains grasslands to altered rainfall regimes. *Global Change Biology* **21**:335-344.

SUPPLEMENTARY TABLES

Supplementary Table 1. ANOVA results describing the effect of the drought timing treatments on soil moisture of the top 20cm for 2013 and 2014. Each exclusion period analyzed separately. d.f. = numerator degrees of freedom, denominator degrees of freedom.

Soil moisture				
	Exclusion period	d.f.	F-value	P-value
2013	LS	3, 14	4.72	0.018
	ES	3, 9.81	9.16	0.003
	MS	3, 14	19.28	<.001
2014	LS	3, 13	5.61	0.011
	ES	3, 15	19.22	<.001
	MS	3, 16	3.19	0.052

Supplementary Table 2. ANOVA results describing the effects of the drought timing treatments on the density of flowering stems and the ratio of reproductive to vegetative biomass in 2013 and 2014. d.f. =numerator degrees of freedom, denominator degrees of freedom.

Flowering				
	Effect	d.f.	F-value	P-value
2013	Stem Densities	3, 24.1	6.94	0.002
	R/V Biomass	3, 34	5.36	0.004
2014	Stem Densities	3, 22.4	16.65	<.001
	R/V Biomass	3, 27	4.47	0.011

Supplementary Table 3. ANOVA results describing the effects of the drought timing treatments on relative growth rate with each exclusion period analyzed separately. d.f. = numerator degrees of freedom, denominator degrees of freedom.

Relative growth rate			
Exclusion period	d.f.	F-value	P-value
LS	3, 16	5.417	0.009
ES	3, 36	10.7	<0.001
MS	3, 36	50.65	<0.001

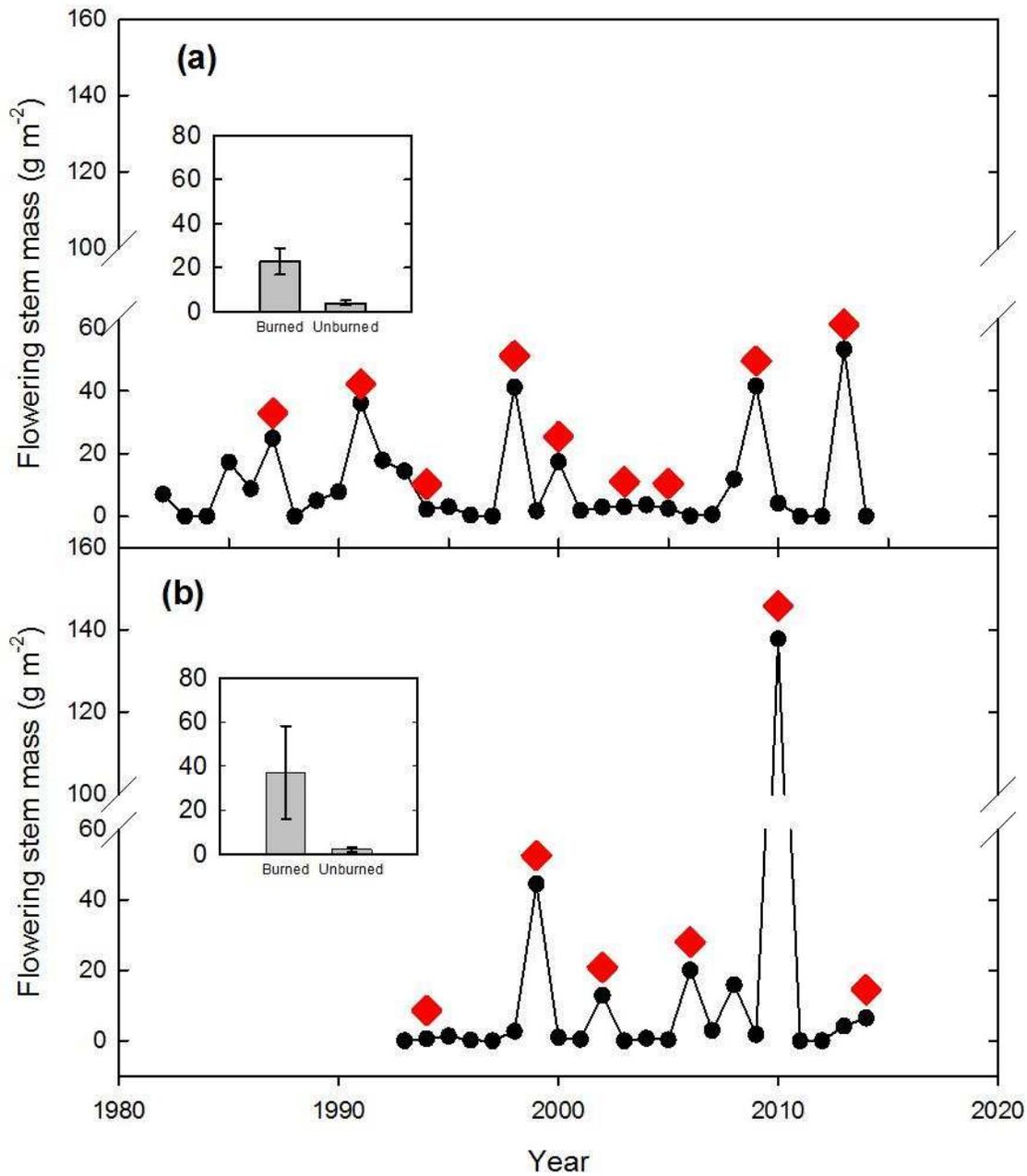
Supplementary Table 4. Repeated measures ANOVA results describing the effects the drought timing treatments and date on mean tiller height. Analysis with drought timing treatment (Trt) as the fixed effect and Date as the repeated effect. df. =numerator degrees of freedom, denominator degrees of freedom.

Tiller height			
Effect	d.f.	F-value	P-value
Trt	3, 259	3.92	0.009
Date	7, 259	316.16	<.001
Trt*Date	21, 259	7.66	<.001

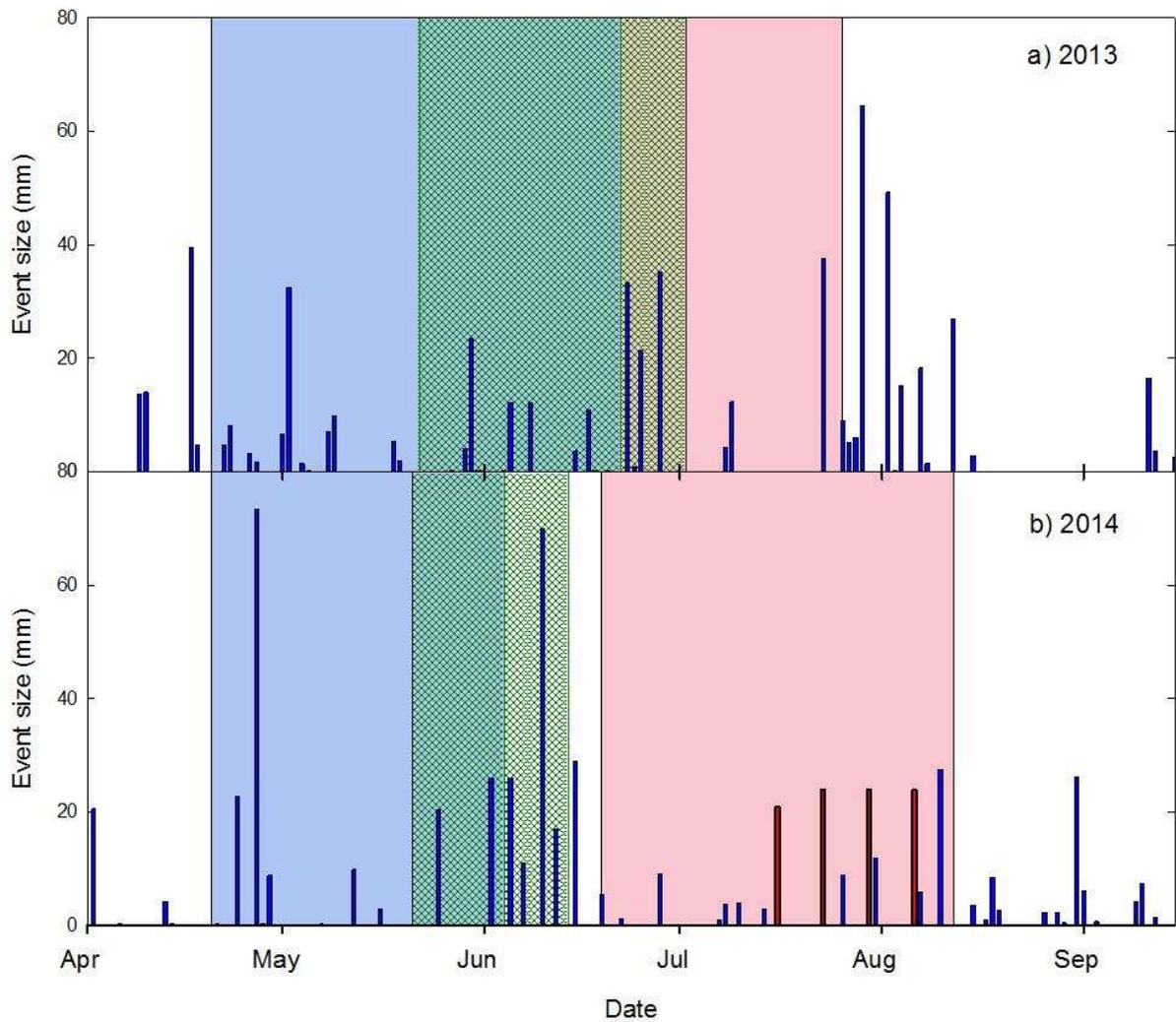
Supplementary Table 5. Repeated measures ANOVA describing the effect of drought timing treatment and date on ecophysiological parameters -- leaf water potential measured before dawn (Pre-dawn ψ_{leaf}), leaf water potential measured between 11:00am and 3:00pm (Mid-day ψ_{leaf}), and net CO₂ assimilation rate (A_{net}). Drought timing treatment (Trt) was the fixed effect, and Date was the repeated effect. d.f. = numerator degrees of freedom, denominator degrees of freedom.

Effect	Pre-dawn ψ_{leaf}			Mid-day ψ_{leaf}			A_{net}		
	d.f.	F-value	P-value	d.f.	F-value	P-value	d.f.	F-value	P-value
Trt	3, 112	24.76	<.0001	3, 161	22.44	<.0001	3, 131	2.74	0.046
Date	5, 112	4.96	0.0004	7, 161	31.57	<.0001	7, 131	63.94	<.0001
Trt*Date	15, 112	7.33	<.0001	21, 161	5.86	<.0001	21, 131	2.89	0.0001

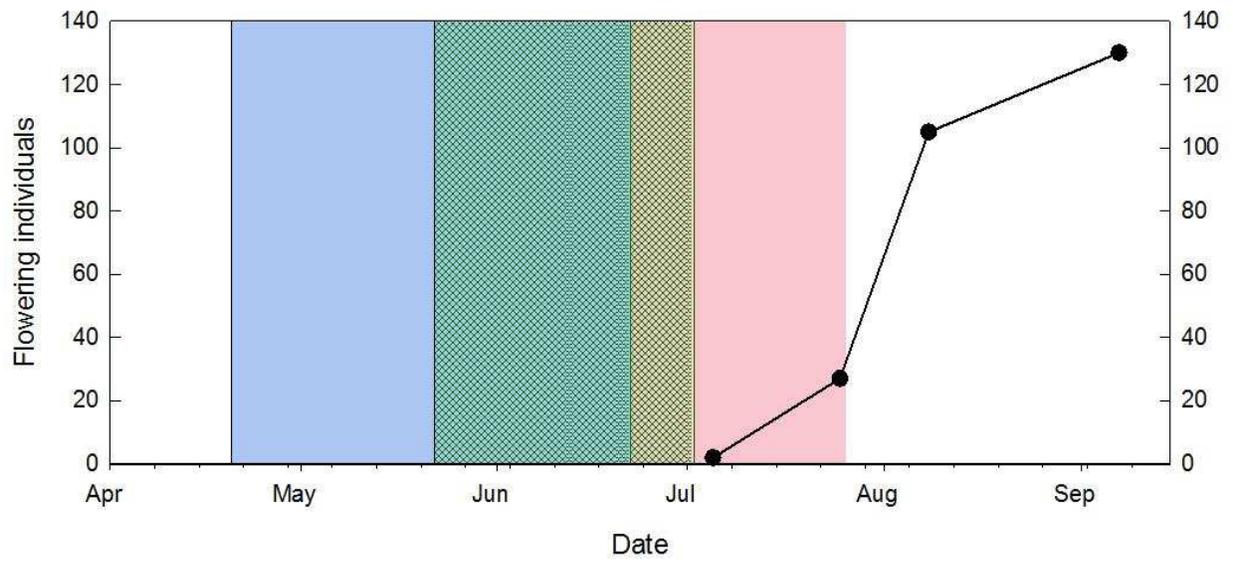
SUPPLEMENTARY FIGURES



Supplementary Figure 1. Long term flowering record for *A. gerardii* at the Konza Prairie Biological Station for the a) 2013 and b) 2014 study sites (4b and 4a watersheds, respectively). Data collection began in 1982 for the 4b watershed, while it did not begin until 1994 for the 4a watershed (see Konza Prairie Long-term Ecological Research website for details on methodology of data collection; dataset: PRE022). Red diamonds indicate years in which the watershed was burned in the spring (~mid-April). Inset: overall means of flowering stalk biomass for burn years and non-burn years.



Supplementary Figure 2. Rain events over the course of two growing seasons 2013 and 2014. Rainfall exclusion periods are indicated by different background colors. Red bars indicate supplementary watering applied during the 2014 growing season to the control and all drought treatments, excluding the mid-summer drought treatment.



Supplementary Figure 3. Number of individuals of *A. gerardii* committed to flowering during the 2013 growing season. Rainfall exclusion periods are indicated by the different background colors.