

Research

Timing of short-term drought structures plant–herbivore dynamics

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Drought is an important stressor that affects plant growth, survival and physiology and, through plant responses, alters plant–herbivore interactions and herbivore population dynamics. Short-term drought can occur at different times during a growing season, affecting herbivore populations and plants at various stages of development and growth. As phenology influences drought response, drought timing could strongly structure plant–herbivore interactions. We grew common milkweed plants with or without its dominant aphid herbivore, allowing both plants and herbivore populations to develop over time as in a typical growing season. To determine how drought timing affects plants and aphid populations, we applied short-term (1 week) drought at different times. Plants and aphids were sensitive to drought timing, with a few weeks difference shifting the effects of drought from minimal to causing massive declines in plant growth, plant survival and herbivore population density. Aphid herbivory added to or overrode drought consequences in plants, with different plant fitness components responding distinctly to herbivory. Drought timing also affected whether plant and herbivore responses were parallel or divergent, suggesting that shifts in the seasonal timing of drought have the potential to disrupt plant–herbivore interactions and affect the larger ecological community. Our results show drought timing has important and disparate consequences for herbivores and their host plants and indicates the necessity of considering phenology and timing in assessing drought response.

Keywords: *Aphis nerii*, *Asclepias syriaca*, drought, herbivory, invertebrates, phenology, plants

Introduction

Drought is a major cause of stress in many terrestrial ecosystems, and the impact of drought is becoming increasingly important with current and expected changes in climate (Stocker et al. 2013). Drought frequently arises from declines in rainfall (Stocker et al. 2013). The duration, frequency and timing of rainfall events are arguably more important in drought than total or mean precipitation, and these aspects of precipitation are likely to change with warming temperatures (Trenberth et al. 2003), altering drought occurrence and impacting plants and herbivores at various times

during the growing season. Given the increasing variability of rainfall and the unpredictability of drought (Field et al. 2014), the influence of rainfall and drought timing on plants and herbivores is an increasingly important question.

Rainfall is one of the main limitations on primary productivity (Tezara et al. 1999, Chaves et al. 2002), and water stress can induce a variety of responses in plants, from reversible stomatal closure to longer-term physiological acclimation (Yordanov et al. 2000), including increased root growth (Pang et al. 2011, Xu et al. 2015). Seasonal timing of rainfall and drought can alter impacts of water stress. For instance, early season droughts reduced shortgrass biomass more than late season drought (Lemoine et al. 2018), and reduced winter precipitation decreased sagebrush steppe vegetation production (Bates et al. 2006). Late season droughts impact larger and more mature plants, which are frequently more tolerant, as shown in *Aristotelia chilensis* (González-Villagra et al. 2018) and cabbage (Levitt 1985). However, early season drought may affect smaller plants with lower water requirements (Zotz et al. 2001), which can more easily acclimate to the stress, as observed in rice (Pandey and Shukla 2015) and wheat root growth (Selote and Khanna-Chopra 2010), reducing the impacts of earlier drought. The timing of when rainfall and drought occurs clearly affects the impact on plants, though it is not known if early or late season drought is more harmful.

The timing of rainfall and water stress also has important implications for plant–herbivore interactions. Herbivory can significantly reduce plant biomass (Kucharik et al. 2016), alter resource allocation (McNaughton 1983, Kucharik et al. 2016) and induce costly defences (Strauss et al. 2002). Additionally, water stress may restrict plant compensatory responses to herbivory (Hilbert et al. 1981, Wise and Abrahamson 2007). Combined drought and herbivory stresses were highly detrimental to plants in Chinese rye grass (Gao et al. 2008), and *Brassica* (Tariq et al. 2013), although

not all studies find a strong interactive effect of drought and herbivory (Grinnan et al. 2013a). In particular, early season drought impacts younger and less defended plants (Barton and Boege 2017), often preferred by herbivores, including the aphid species *Aphis nerii* (Hall and Ehler 1980) and *Myzus persicae* (Tomczak and Müller 2017). Younger plants may be more vulnerable to the combined impact of drought and herbivory, although the consequences of interactions remain uncertain.

The pattern of rainfall and water stress can determine herbivore population growth and persistence. For instance, phloem-feeding invertebrate herbivore response depends on the duration and severity of water stress, with shorter, pulsed droughts encouraging population outbreaks (White 1984, Mattson and Haack 2006) while continuous droughts decrease population performance (Huberty and Denno 2004). The timing of rainfall is also expected to impact population dynamics. Multivoltine invertebrate herbivores may be disproportionately harmed by early season drought, as the smaller early-season populations have a higher risk of local extinction (Lande 1993). Alternatively, late droughts may be more devastating, as older and lower quality plants are often unable to support the larger populations of outbreaking species, causing high plant and herbivore mortality (Grainger et al. 2018). These different possibilities raise the potential for drought timing to impact herbivore populations and their host plants in distinct ways.

In this study, we tested the effect of timing in rainfall-induced drought on plants and their invertebrate herbivores. We manipulated the timing of short-term (1-week) drought experienced by common milkweed *Asclepias syriaca* with and without oleander aphid herbivores *Aphis nerii* by modelling a typical growing season. Our goal was to test how short-term cessation of water input at critical periods in the season altered the impacts of drought on plants and their aphid populations as they typically co-vary in a seasonal

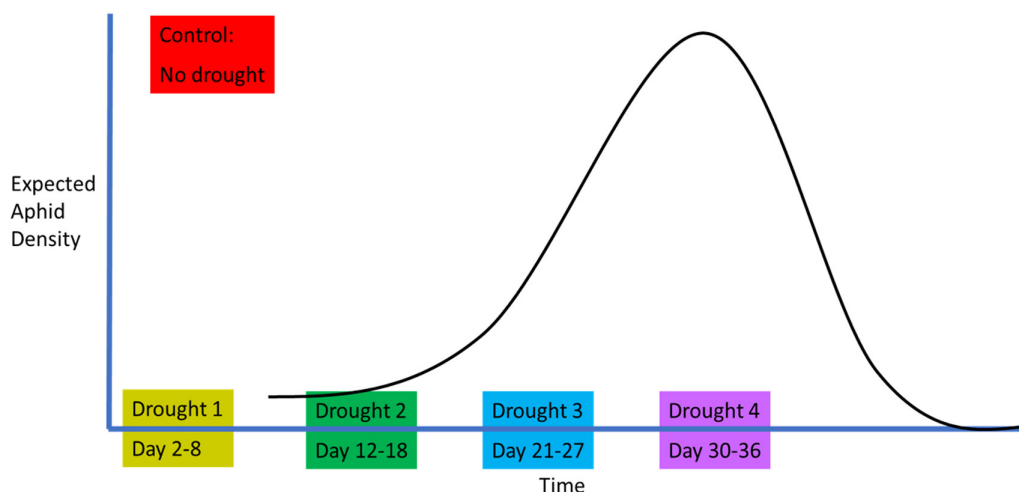


Figure 1. Timing of drought treatments relative to expected aphid population growth. Expected aphid density is drawn as an idealized population, with the timing of increases and peak population sizes corresponding to previous research on milkweed and *A. nerii* in non-drought conditions (Grainger et al. 2018).

environment (Fig. 1). We addressed four main questions regarding plant and herbivore responses: 1) does the timing of drought alter the impact on plant growth and survival? 2) Does the presence of the aphid herbivore change the effects of drought timing on plants? 3) Do the effects of drought on aphid population performance depend on timing? 4) Does drought timing differentially impact plants and herbivores and the nature of their interactions? As plant size and herbivore population dynamics impact drought response, we hypothesized timing would significantly affect the consequences of drought to plants, their invertebrate herbivores and plant–herbivore interactions. We show that the timing of drought is important to both plants and aphids, and that plants and aphids can have disparate responses, impacting interactions.

Methods

Study system

Common milkweed *Asclepias syriaca* is a perennial plant found in open fields and meadows throughout much of eastern North America. Young milkweed plants germinate in early summer, increasing in size throughout the growing season. During the first year, milkweed plants build root systems, and flowers do not usually emerge until the second or third year. The plant uses a variety of defences, including the secretion of latex when damaged and the presence of trichomes, both of which can be induced by invertebrate herbivory (Van Zandt and Agrawal 2004a, Grainger et al. 2018). For the study, milkweed plants were grown from commercially obtained seeds at 20–25°C and 65–75% humidity, with 16 h of light. Plants were transferred to cylindrical pots (1840 cm³) after four weeks and 100 ml water was provided to the bottom of pots twice per week, with 50 ml fertilizer additions (3 g l⁻¹ Plant-Prod 20–20–20 Classic) provided once per week.

The oleander aphid *Aphis nerii* is an important invertebrate herbivore of milkweed that shows strong seasonal trends in population dynamics (Smith et al. 2008). Aphids are phloem feeders and reproduce asexually through viviparous parthenogenesis (Powell et al. 2006), with nymphs reaching sexual maturity in 7–10 days (Özderl and Sağlam 2013). Aphids migrate north each spring, arriving on plants in our study area (southern Ontario) approximately 9–10 weeks after milkweed germination. Populations commonly show exponential growth over much of the season, followed by rapid decline (Grainger et al. 2018). As a result, new plants and their aphid populations tend to covary in size over much of the growing season. A winged morph is expressed when populations become stressed due to high densities or low nutritional quality of plants, allowing for greater dispersal (Müller et al. 2001, Grainger et al. 2018). Aphids were collected from wild populations at the Koffler Scientific Reserve in southern Ontario, Canada (44°2'N, 79°30'W). Populations were maintained on potted milkweed plants at 20–25°C with 16 h of light prior to the experiment.

Experimental setup

The study was conducted in a controlled growth chamber at 25°C with 16 h of light. Two weeks prior to experiment commencement, chamber humidity was lowered to and maintained at 50–55%, and a cloth mesh bag was placed around individual milkweed plants to prevent aphid movement among plants. During the experiment, plants were provided with 100 ml of water three times a week and 50 ml fertilizer (Plant-Prod 20–20–20 Classic 3 g l⁻¹) on days 2, 12, 21 and 30. Plants were approximately eight weeks old when the experiment began (day 0).

Plants were organized into 10 blocks according to size, with block 1 containing the smallest plants and block 10 containing the largest. As plant size often correlates to plant water requirements and drought tolerance, the blocking design was implemented in this way to reduce the influence of plant size differences other than those caused by seasonal development. Each block contained 10 plants and plants within a block were randomly grouped into pairs. Five unwinged aphids were introduced to one plant in each pair on day 9 and examined on day 10 and 11 to ensure successful establishment, with additional aphids added as necessary to ensure a starting population of 5 individuals. Each pair within a block was randomly assigned to one of five drought treatments, creating a fully factorial design between drought treatments and aphid presence nested within blocks. In total, we had 10 treatment combinations of 10 replicates each.

To mimic a natural growing season, plants and aphid populations were allowed to grow without interference following the initial establishment of aphids. Four drought treatments plus a 'no drought' control were conducted (Fig. 1). Drought treatments manipulated water input into the system, mimicking changes in rainfall expected to induce water stress and similar to short droughts commonly observed at our field site over a typical growing season (Fausto et al. 2015, Wazneh et al. 2017). Treatments were imposed by withholding water from plants for one week at crucial intervals: prior to aphid introduction (day 2–8); during early aphid establishment (day 12–18); during rapid population growth (day 21–27); and during peak aphid population density (day 30–36). Since the 'no drought' treatment did not have water withheld, plants in that treatment received 200 ml more water than drought treatment plants over the course of the experiment.

Data collection

Photosynthesis was determined on the first, fourth and last day of each treatment using a LCpro-SD photosynthesis system (ADC BioScientific Limited) to measure change in carbon dioxide ($\mu\text{mol m}^{-2} \text{s}^{-1}$). Measurements were conducted on a subsample of four plants per treatment and, due to the infeasibility of measuring aphid-infested plants without altering herbivory, only on plants without aphids. One leaf per plant was measured, with three measurements taken one minute apart. Data were reviewed for each plant, and any

measurement that differed from others for the same day by more than $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ was removed. Averages were calculated for each plant per day.

Aphid counts were conducted twice weekly, with the density of winged and unwinged individuals present on each plant recorded. Due to multiple observers conducting counts, a quality control check was conducted by recounting populations that were much higher or lower than the average for a particular treatment. No observations were removed.

On day 47, all plants were harvested and the state (dead or alive) recorded. Plants were recorded as dead if all leaves were wilted or lost, the stem was dry and brown, and no other signs of growth were present. All plants were separated into aboveground (shoot) and belowground (root) biomass, gently washed to remove soil and aphids, and dried to determine biomass. Data were also collected on leaf area, leaf mass, latex content and trichome density. We present aphid population, plant survival and plant mass data, and include the additional data and results in the Supporting information.

Data analysis

Our general approach was to first test the effect of drought timing treatments against the effect of 'no drought', and to subsequently test whether differences in drought timing caused significantly different responses. For plant responses, the presence or absence of aphids was also included as an independent variable. We detail the specific responses and tests below. All statistical analyses were conducted using R ver. 3.5.2 (<www.r-project.org>) with the 'lme4' (Bates et al. 2015), 'lmerTest' (Kuznetsova et al. 2017), 'emmeans' (Lenth 2020) and 'nlme' (Pinheiro et al. 2014) packages.

To examine plant productivity, the average photosynthesis rate of all plants experiencing drought was calculated separately from all other plants for each day and compared graphically. Plant responses for survival, total dry biomass (root mass + shoot mass) and resource allocation (relative allocation to root mass: $\ln(\text{root mass}/\text{shoot mass})$) were considered through linear mixed effects models, with the drought treatment and aphid presence as predictor variables and block as a random effect. A log-transformation was applied to total biomass data to meet assumptions of normality, and two measurements in different treatments which showed abnormally low root biomass (approaching zero), likely indicating errors in measurement, were removed from comparison for resource allocation. Finally, plant survival was compared between treatments by fitting a generalized linear mixed model with a binomial distribution to the plant state on day 47 (dead or alive).

For aphid population responses, we considered initial per capita growth rates (at 10 days after aphid introduction,

calculated as $\frac{\ln(N_2) - \ln(N_1)}{t_2 - t_1}$), the peak population densities (maximum aphids/plant for each plant in the experiment), and the time until populations reached their peak (experimental day when maximum population density

was recorded). As winged and unwinged aphid populations showed similar patterns (Supporting information), only the total aphid densities (sum of winged and unwinged densities) was considered, and day 21 and day 30 drought treatments were excluded from initial growth rate analysis due to lack of relevance, as treatments occurred after the period of interest.

Type III ANOVA was conducted on the linear models. Results with $p < 0.05$ are referred to as significant, and degrees of freedom were estimated using the Satterthwaite method (Satterthwaite 1946). To test the effect of specific drought treatments, each treatment was compared with the control (no drought treatment) using a Dunnett's test. We additionally conducted separate ANOVAs that excluded the control to test for significant differences among drought timing treatments; the results of these tests corroborated our initial findings. Details for all follow-up tests are provided in the Supporting information. No differences in significance were found between ANOVA tests with and without a control. All model assumptions were verified by visually examining plots of residuals.

Results

Photosynthesis rates generally declined during drought treatments, with the decline noticeably increased in severity with later drought treatments (Fig. 2a). Photosynthesis rates in plants not experiencing drought conditions increased from an average $36.12 \mu\text{mol m}^{-2} \text{s}^{-1}$ on day 2 to $82.88 \mu\text{mol m}^{-2} \text{s}^{-1}$ on day 21, followed by a decline to $32.51 \mu\text{mol m}^{-2} \text{s}^{-1}$ by day 36. The photosynthesis rates of plants in the drought treatments were lower than the non-drought average by the end of each drought treatment in all except the earliest drought (Fig. 2a). An additional consideration of treatment-level differences showed that photosynthesis was similar among treatments except when treatments were exposed to drought (Supporting information).

Plant survival declined with later drought treatments and the presence of aphid herbivores (Fig. 2b; drought $\chi^2 = 11.54$, $df = 4$, $p = 0.021$; herbivory $\chi^2 = 16.79$, $df = 1$, $p < 0.0001$; interaction not significant, $p = 0.99$). In the absence of herbivory, survival decreased from 100% with day 2 droughts to 50% with day 30 droughts. The presence of aphids caused an additional 40% mortality though did not alter the relative impact of drought treatments. A Dunnett's post hoc test showed that only day 30 differed significantly from the control (Supporting information). Further analysis showed the effect of drought on survival to differ over time, with high mortality occurring during drought periods, particularly in late droughts (Supporting information).

Total biomass was impacted significantly by aphid herbivory alone and in interaction with drought treatments (Fig. 2c; herbivory $F_{1,81} = 242.29$, $p < 0.0001$; drought: $F_{4,81} = 0.46$, $p = 0.76$; interaction $F_{4,81} = 2.78$, $p = 0.032$). When aphids were absent, biomass of plants experiencing day 2 droughts was nearly double compared to day 30 droughts (6.36 g versus 3.82 g). The control treatment was similar on average to

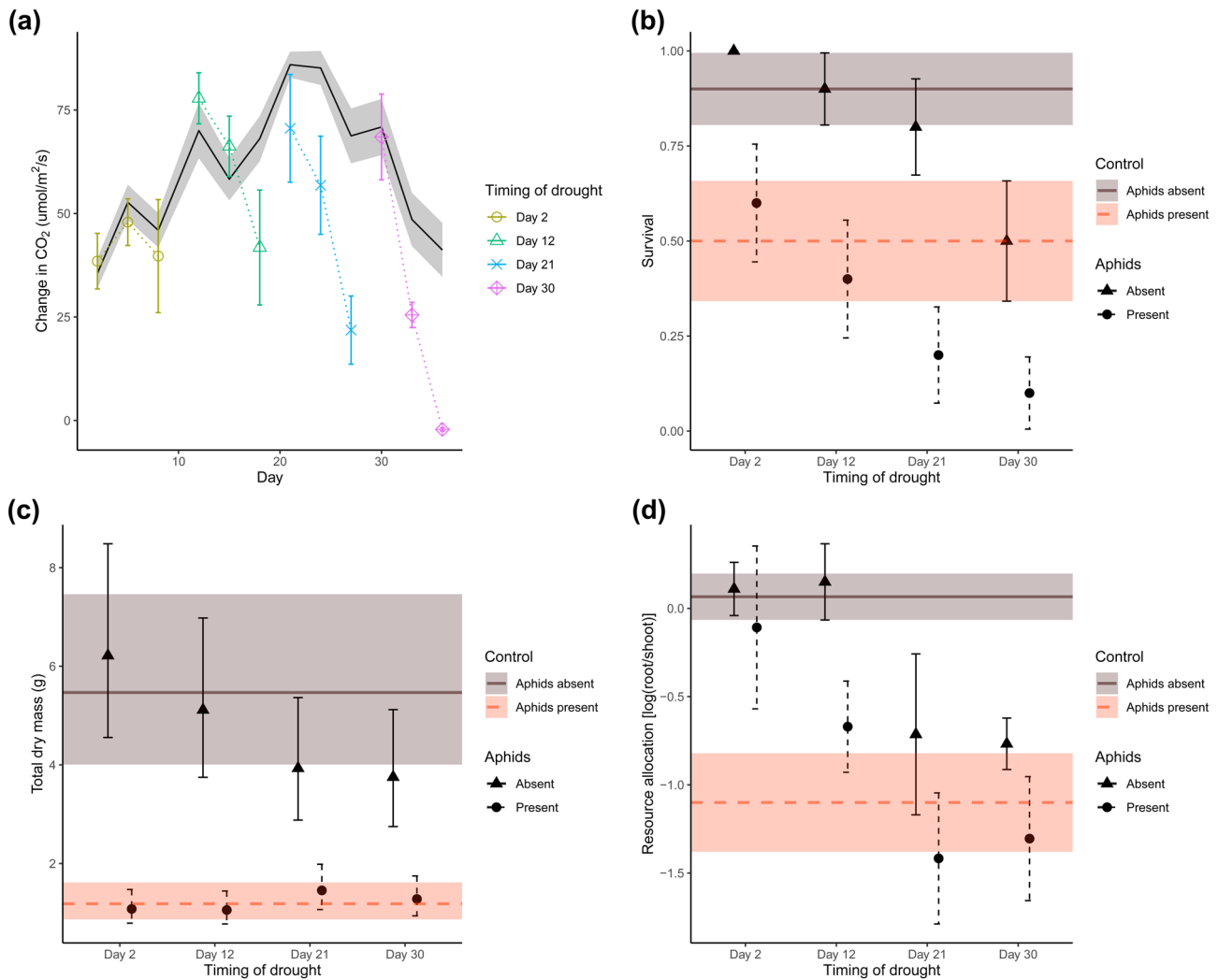


Figure 2. (a) Photosynthesis rate, measured as the change in carbon dioxide ($\mu\text{mol m}^{-2} \text{s}^{-1} \text{CO}_2$), for plants experiencing drought (coloured points, 4 replicates) and non-drought plants (black line, 16 replicates). Data are from plants without aphids. (b) Proportion of plants surviving by the end of the experiment for each drought and aphid treatment (day 47). (c) Total dry plant biomass of all plants at the end of the experiment. Values were back-transformed from fitted models. (d) Resource allocation on day 47 for all drought and aphid treatments. Points (b–d) represent the average of 10 replicates for all drought treatments, and horizontal lines represent the average for 10 control (no drought) replicates. Bars and shaded areas represent standard errors for drought treatments and controls, respectively.

the early drought treatments (6.06 g), although we found no significant difference between the control and any drought timing treatment (Dunnnett's test, Supporting information). In contrast, biomass was similar in all treatments when aphids were present, ranging from 1.13 g in day 2 droughts to 1.73 g in day 21 droughts. Aphid herbivory reduced biomass by more than half in all drought treatments, with the greatest reduction in early drought (Fig. 2c) and no treatments significantly different than the control (Dunnnett's test, Supporting information). The lack of significant differences from the controls, coupled with the ANOVA tests (Supporting information), indicate a significant difference exists between one or more of the drought treatments (Fig. 2c).

Resource allocation to root mass declined significantly with later drought and aphid herbivory (Fig. 2d; drought

$F_{4,80} = 4.95$, $p = 0.0012$; herbivory $F_{4,80} = 13.32$, $p = 0.00047$; interaction not significant $p = 0.59$). When aphids were absent, root mass was 10% greater than shoot mass in day 2 drought treatments (log-ratio 0.110), while shoot mass was nearly double root mass in day 30 drought treatments (-0.767). Aphid herbivory further decreased allocation to roots by 20–50% although herbivory did not significantly alter relative impacts of drought treatments. Control plants were more similar to early drought treatments when aphids were absent (0.066), and to late droughts when aphids were present (-1.100). Post hoc comparisons found no significant differences in resource allocation between drought treatments and the control (Supporting information), although ANOVA tests indicate differences between one or more of the drought treatments (Supporting information). An

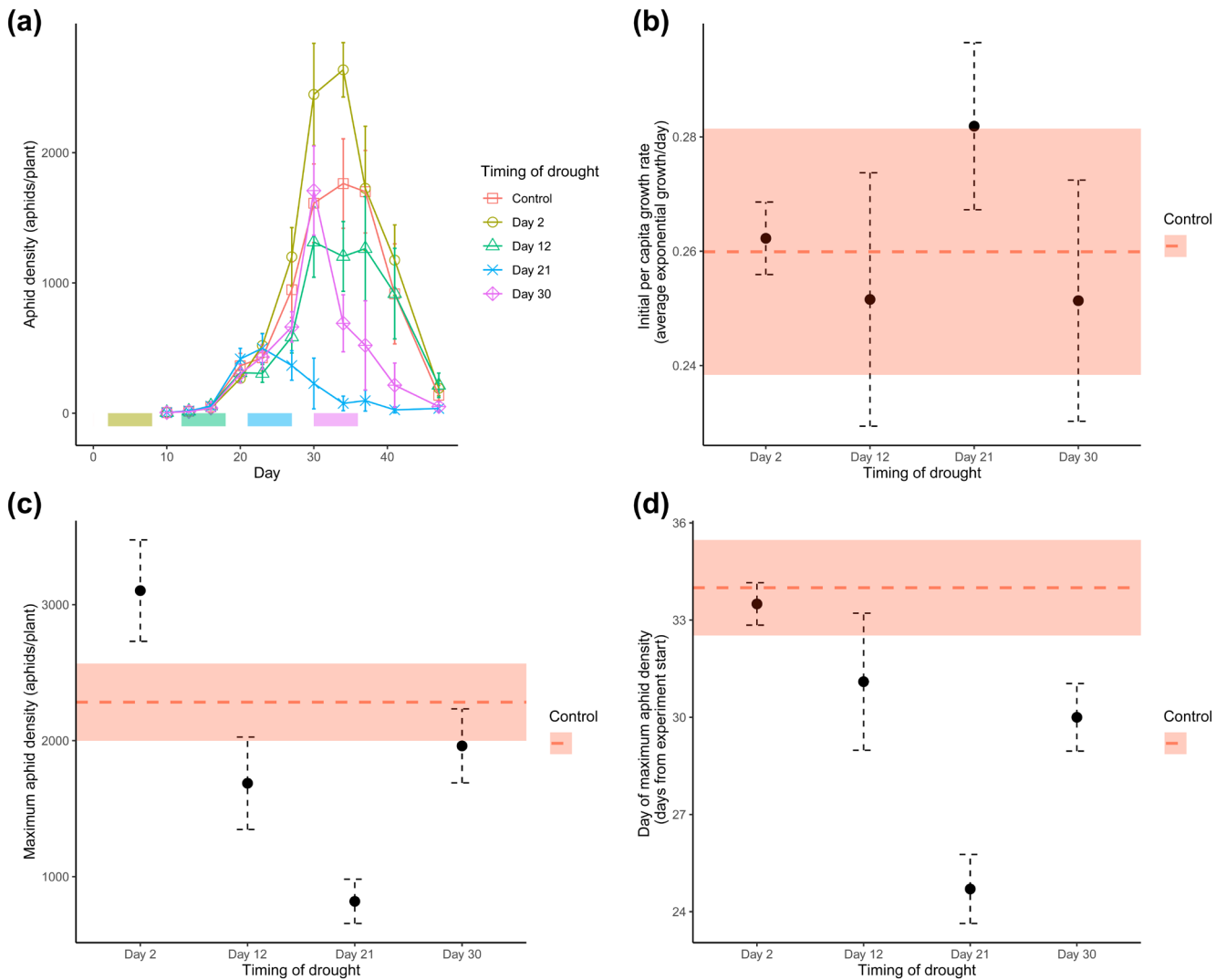


Figure 3. (a) Total aphid population counts per day, by treatment. Shaded rectangles represent the time and duration of drought treatments. (b) Per capita growth rate of aphids from day 10 to day 20 (the first 10 days after aphid populations were established). Day 21 and day 30 droughts are not shown as initial growth rates were calculated prior to these treatments. (c) Peak aphid density reached for each treatment. (d) Day peak aphid population was reached for each treatment. Points represent the average of 10 replicates for all drought treatments, and the dashed line represents the average for 10 control (no drought) replicates. Bars and shaded areas represent standard errors for drought treatments and controls, respectively.

additional analysis supported the results presented for plant biomass and resource allocation when only considering the subset of plants that were alive on the last day of the experiment (Supporting information).

All aphid populations peaked during the experiment, showing exponential growth followed by decline (Fig. 3a). Drought treatments had no effect on initial growth rates (Fig. 3b; $F_{4,18} = 0.1188$, $p = 0.89$); however, treatments significantly affected the peak aphid population densities (Fig. 3c; $F_{4,36} = 12.27$, $p < 0.0001$) and the time until peak abundance was reached (Fig. 3d; $F_{4,36} = 22.11$, $p < 0.0001$). The highest population density occurred in day 2 drought

treatments (3103 individuals plant⁻¹), roughly 50% higher than in control treatments (2283 individuals plant⁻¹) and nearly quadruple the density reached in day 21 drought treatments (819 individuals plant⁻¹; Fig. 3c). Populations in day 21 drought treatments also reached their peak the earliest (day 24.7), over a week earlier than populations in the control treatment (day 34.0) and approximately 5 days earlier than in day 30 drought treatments, the next population to reach a peak (day 30.0; Fig. 3d). Post hoc comparisons showed both peak aphid abundances and the day peak abundances were reached differed between the control treatment and drought treatment at day 21 (Supporting information).

Discussion

The timing of water input and short-term drought has profound impacts on plants and their aphid herbivores as they typically occur over the growing season. Plant survival and growth, the impacts of herbivory on plants, and the population dynamics of herbivores all showed significant responses to drought timing. Indeed, our results show that two reductions in water input of identical length occurring one month apart can reduce survival and biomass of young plants by half (Fig. 2) and a difference of twenty days can cause nearly a four-fold decrease in the herbivore populations supported (Fig. 3). Below, we consider the direct and indirect effects of rainfall and drought timing on plant performance and corresponding herbivore populations.

Direct effects of drought timing on plants

In the absence of herbivores, milkweed plants had 50% lower survival and growth when drought treatments occurred late in the season (Fig. 2b–c), with drought more severely reducing photosynthesis rates in the larger and older plants (Fig. 2a). The results suggest larger size alone does not confer an advantage to drought tolerance, and implies early drought to be less detrimental, contrary to field studies in other prairie systems (Lemoine et al. 2018). The results may reflect younger plants acclimating more easily to stress, similar to acclimation in *Arabidopsis* (Harb et al. 2010) and *Euphorbia* (Sivakumaran and Hall 1978). Evidence of acclimation is further supported by the heightened resource allocation to root mass in earlier drought treatments (Fig. 2d), much as young *Medicago* (Pang et al. 2011) and rice plants (Xu et al. 2015) increase root mass to maximize water uptake.

The higher survival and growth of plants in early drought treatments may also reflect lower water requirements in younger and smaller plants. Transpiration generally scales with plant size (Zotz et al. 2001), and as water addition was consistent throughout our experiment, larger plants likely depleted soil water content faster, increasing water stress severity and causing declines in plant performance. Our photosynthesis data suggest this is the case, as photosynthesis of non-drought plants peaked on day 21 (Fig. 2a), indicating productivity was more constrained beyond this time. Thus, while the length of rainfall droughts were identical, late droughts were likely more severe. The influence of later water limitation is captured in the plants not experiencing an imposed drought, likely one constraint on growth and resource allocation. Lower soil water content later in the season is consistent with water dynamics in many ecosystems (Koster and Suarez 2001). Interestingly, a large decrease in soil moisture later in the growing season, even in the absence of noticeable drought, would cause early acclimation to water stress to be beneficial. Our experiment was not designed to test this hypothesis, though it remains an important topic for further research.

Combined effects of aphid herbivory and drought timing on plants

The addition of aphid herbivores was highly detrimental to plants, in some cases adding to the effects of drought treatments and in others overwhelming them. Aphid presence caused a 40% drop in survival across all treatments (Fig. 2b), an effect size nearly equivalent to the difference between late and early droughts. This large effect of aphids is consistent with other experiments testing the impacts of aphids on young milkweed (Wong et al. 2017, Grainger et al. 2018), and even larger milkweed are extremely sensitive to aphids (Grainger and Gilbert 2017). For plant survival, aphid herbivory is an additional stress, and the additive effects of herbivory and drought were consistent with those observed in other research (Levine and Paige 2004, Bansal et al. 2013, Grinnan et al. 2013b). In contrast, our results for biomass showed herbivory to have an overwhelming impact on plants (Fig. 2c), with any influence of drought timing becoming undetectable when aphids were present. Herbivory is clearly a strong pressure on plants, and the influence of herbivore population dynamics needs to be considered to fully understand the consequences of abiotic stress on plant performance.

An unresolved question in ecology is whether multiple stressors have additive, synergistic or sub-additive impacts on species performance. Goldenrod, another open field species that commonly occurs with milkweed, showed both additive and synergistic effects of the herbivory and drought on growth (Shibel and Heard 2016). In contrast, while our study showed additive effects for survival, the effects of drought and herbivory on biomass were sub-additive, with aphid presence weakening drought impact. As plant growth frequently captures an individual's long-term performance, especially for perennial plants (Younginger et al. 2017), both survival and biomass have implicit consequences for plant fitness.

An additional impact of herbivores can arise when herbivory induces phenotypic changes that are maladaptive to drought. Increased biomass allocation to roots during drought is one phenotypic response considered adaptive and widely observed (Eziz et al. 2017). Our experiment caused this physical acclimation in plants, particularly with early drought (Fig. 2d). However, herbivory reduced allocation to roots, likely as compensation for aphid infestation (McNaughton 1983, Kucharik et al. 2016). This response to herbivory effectively dampens the phenotypic response to drought, and a similar impact of herbivory on plant biomass allocation has been found in other grasslands species (Haag et al. 2004), suggesting that herbivory commonly reduces plant acclimation ability. Thus, the combined impacts of drought and herbivory may be complicated by competing plant responses.

Effects of drought timing on herbivore population dynamics

As with plants, aphid population dynamics responded strongly to drought timing. Drought was most damaging to aphid populations when drought occurred during the rapid

growth phase (day 21), and a decrease in population density was also observed when drought occurred as populations approached their peak (day 30). Both treatments impacted larger aphid populations and caused a rapid decline in aphid density during the drought (Fig. 3). As even in the absence of drought, aphid populations frequently exceed the ability of plants to support them (Grainger et al. 2018), the declines with late droughts at day 21 and day 30 are likely a result of water stress decreasing plant condition and, through impacts on plants, causing herbivore population collapse. Clearly, the timing of drought relative to population growth vastly alters population responses.

The outsized impact of late droughts on aphid populations may also reflect water stress severity. Early droughts impacted smaller plants with lower water requirements (Zotz et al. 2001), resulting in less severe water stress compared to late droughts. Under intermittent droughts where turgor pressure was less affected, phloem-feeders have benefited from drought-induced changes in plant physiology (White 1984, Mattson and Haack 2006). Similarly, our results show drought prior to aphid introduction was of a slight benefit to populations, which reached a higher density than on non-drought plants. In contrast, late droughts often resulted in wilting of larger plants, indicating low turgor pressure and causing a detriment to aphid feeding, resulting in decreased populations, similar to invertebrate responses under severe and continuous drought (Mattson and Haack 2006). Although our experiment was unable to fully disentangle water stress severity and drought timing, these results indicate slight changes in conditions can result in divergent consequences for herbivore populations.

Our results also offer important insights into longer-term aphid population dynamics. Long-term aphid fitness depends on the colonization of new plants (Hodgson 1991, Lombaert et al. 2006), and, in the case of our study species *A. nerii*, on seasonal movement for overwintering (Hall and Ehler 1980). Dispersal range (Loxdale et al. 1993) and success rate (Ward et al. 1998) of aphid migration are reported to be low, and peak aphid abundance have been shown to correlate with aphid dispersal (Müller et al. 2001, Grainger and Gilbert 2017). As larger aphid populations also supported a greater abundance of winged morphs for long-distance dispersal (Supporting information), the nearly four-fold decrease in peak aphid abundance per plant caused by late drought compared to early drought has strong implications for how drought timing could impact the long-term performance of the species.

Phenology is an important component of species interactions, affecting insect fitness (Tikkanen and Lyytikäinen-Saarenmaa 2002, Chen et al. 2009) and plant susceptibility to herbivory (Benning et al. 2019). Consequently, the results presented here are likely common to many interactions. Our results indicate the timing of drought stress can not only alter plant and herbivore response, it can also disrupt plant–herbivore interactions. Indeed, our study shows plant and aphid responses to diverge dependent on relative timing. For example, while early droughts were favorable for

both plant performance and aphid populations, the most extreme reductions in aphid population peaks occurred with day 21 drought, while plants were most affected by day 30 droughts. This suggests an advantage to either plants or herbivores can appear dependent on the timing of drought occurrence. The immediate responses observed can impact long-term plant (Younginger et al. 2017) and aphid fitness (Hall and Ehler 1980, Dixon and Howard 1986, Hodgson 1991, Lombaert et al. 2006), and the consequences may be felt for many seasons. Additionally, outbreaks in invertebrate populations often affect the performance of other species using similar resources or preying on the outbreaking species (Tack et al. 2009, Ekholm et al. 2020), altering species abundance and community structure (Van Zandt and Agrawal 2004b, Timms and Smith 2011). Thus, the differing responses of plants and herbivores will likely cause further disparities between communities.

Conclusion

We found both plants and their aphid herbivores to be highly sensitive to within-season variation in the timing of short-term drought, with aphid herbivory causing additional or interactive effects to plants. The responses of plants and herbivores differed slightly, with the last drought treatment more harmful to plants than aphids. Dependent on time, plant and herbivore responses were either parallel, as observed in early droughts, or conflicting, as seen with late droughts, suggesting that year to year variation in the timing of drought can have large impacts on plant–herbivore interactions even when total rainfall is consistent. The disruptions to plant–herbivore interactions could have widespread effects on the larger ecological community, particularly as outbreaking species frequently alter the trophic structure and distribution of species in the community. The impact of the timing of drought on plants and their herbivores raises new challenges for understanding how climatic fluctuations affect ecological communities, and suggests that any within-season shifts in resource timing are likely to cascade through food webs.

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Author contributions

Ruiping Luo: Conceptualization (lead); Data curation (equal); Formal analysis (equal); Investigation (lead); Methodology (equal); Validation (equal); Visualization (equal); Writing – original draft (lead); Writing – review and editing (equal). **Benjamin Gilbert:** Conceptualization (supporting); Data curation (equal); Formal analysis (equal); Investigation (supporting); Methodology (equal); Validation

(equal); Visualization (equal); Writing – original draft (supporting); Writing – review and editing (equal).

Data availability statement

Data are available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.3n5tb2rjk>> (Luo and Gilbert 2021).

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