Plant water stress and previous herbivore damage affect insect performance

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Abstract. 1. Short-term changes in plant resistance traits can be affected by abiotic factors or damage by herbivores, although how the combined effects of abiotic factors and previous damage affect subsequent insect larval development is not well understood.

2. Complementary glasshouse and field experiments were conducted to evaluate whether plant water stress and previous herbivore damage influenced monarch (*Danaus plexippus*) larval development on common milkweed, *Asclepias syriaca.*

3. In the glasshouse, water stress altered a suite of *A. syriaca* functional traits but did not affect nutrient content, whereas herbivore damage increased leaf nitrogen (N) and reduced the carbon:nitrogen (C:N) ratio. A bioassay experiment showed that monarch larval survival was lower on well-watered plants that were previously damaged by monarch larva than on damaged and drought-stressed plants. Bioassay larvae consumed less leaf tissue of previously damaged plants, whereas monarch larval mass was affected additively by water stress and previous damage, after correcting for the amount of leaf tissue consumed.

4. In a 2-year field experiment, monarch larval performance was higher on previously damaged *A. syriaca* plants that received experimentally reduced rainfall, relative to plants receiving ambient rainfall.

5. Collectively, these results from glasshouse and field experiments suggest that insect performance was highest on previously damaged plants under water stress and highlight the additive and interactive roles of abiotic and biotic factors on herbivore performance.

Key words. Induced defence, milkweed, monarch, plant drought stress, plant−insect interaction.

Introduction

Development and performance of insect herbivores depend critically on the quality of their host plants, such as nutrient content and resistance traits (Awmack & Leather, 2002; Carmona *et al.*, 2011; Wetzel *et al.*, 2016). Changes in abiotic conditions, such as soil nutrients or other environmental stressors, can affect short-term changes in plant nutrition and expression of resistance traits (Bazzaz *et al.*, 1987; Donaldson *et al.*, 2006; Hakes & Cronin, 2011). Herbivore damage can also induce changes in plant traits, such as plant nutrition (Huang *et al.*, 2013) and chemical or physical resistance, which usually, but not always, reduces the performance of subsequent herbivores feeding on damaged plants (Agrawal, 1998; Karban *et al.*, 1997). Although the degree to which abiotic factors and herbivore damage individually contribute to levels of plant resistance and

herbivore performance is well documented, less is known about the combined effects of abiotic factors and previous herbivory on the subsequent performance of larval insects. Most current theories of plant defence predict that inducible resistance should be lower under stressful conditions, because stressed plants should have fewer resources available to allocate to induced resistance (Herms & Mattson, 1992; Orrock *et al.*, 2015). As such, herbivore performance should only be affected by previous damage when plants are not stressed. However, current evidence is mixed regarding how herbivore performance is affected by abiotic stress and previous damage, and most studies have been conducted using only nutrient stress under glasshouse conditions (e.g., Glynn *et al.*, 2003; Burghardt, 2016).

Water stress to host plants can have substantial effects on insect performance through changes in plant quality and resistance traits (Chaves *et al.*, 2003; Huberty & Denno, 2004; Grinnan *et al.*, 2013). However, the combined effects of water stress and previous herbivory is often overlooked in studies that examine the effect of water stress on insect performance. Several

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studies have shown that water-stressed plants are better able to induce resistance traits and reduce insect performance compared with well-watered plants (Tariq *et al.*, 2013; Kleine & Muller, 2014). Another study, however, showed that drought can inhibit the induction of resistance traits (Halpern *et al.*, 2010), while others found no or mixed effects of drought on inducibility (Hale *et al.*, 2005; Gutbrodt *et al.*, 2012; Weldegergis *et al.*, 2015). Of the studies that found mixed results, one found that water stress and previous damage acted independently on plant nutritional and resistance status; each factor had unique effects on the performance of different herbivores (Hale *et al.*, 2005). Another study that found mixed results showed that water-stressed plants and previously damaged plants received higher rates of oviposition by a generalist lepidopteran, but subsequent performance of the larvae was not affected by either treatment (Weldegergis *et al.*, 2015). Finally, the effects of water stress on insect performance may be driven largely by changes in plant nutrition, rather than by changes in induced resistance (Hale *et al.*, 2005; Gutbrodt *et al.*, 2012). Overall, while there is evidence that water stress and previous damage to plants can both, in isolation, affect insect performance, no clear patterns have emerged with regard to how water stress and previous damage interact to affect insect performance.

In this study, we conducted complementary glasshouse and field experiments to test how water stress and previous herbivory on common milkweed, *Asclepias syriaca*, influence monarch (*Danaus plexippus*) larval performance. *Asclepias syriaca* is a long-lived herbaceous perennial plant that occurs in much of the eastern part of North America. It is attacked by several species of specialist insect herbivores and exhibits a variety of resistance traits, such as trichomes, latex, and cardenolides (Agrawal, 2005). Monarch larvae have evolved behavioural and physiological mechanisms to overcome milkweed resistance (Zalucki & Brower, 1992; Petschenka *et al.*, 2013), although milkweed resistance traits are still effective at killing or reducing monarch larval growth (Zalucki & Brower, 1992; Zalucki & Malcolm, 1999; Agrawal, 2005). Constitutive levels of plant resistance traits can be affected by water stress. For example, cardenolides increase in concentration in water-stressed plants, whereas latex and trichome production declines with water stress (Agrawal *et al.*, 2014b; Couture *et al.*, 2015). Cardenolides and latex are also inducible following damage by foliar and root-feeding insects (e.g. monarchs, chrysomelid and cerambicid beetles; van Zandt & Agrawal, 2004; Bingham & Agrawal, 2010; Agrawal *et al.*, 2014a). However, it is unclear how herbivore performance might be influenced by induced plant resistance or nutritional traits that are mediated by water stress. We predicted that water stress would compromise *A. syriaca*'s ability to induce defences (Herms & Mattson, 1992; Orrock *et al.*, 2015), resulting in increased herbivore performance on previously damaged, water-stressed plants compared with previously damaged plants that did not experience water stress.

Materials and methods

Glasshouse experiment

To test the effects of water stress and previous herbivore damage on monarch larval performance, we conducted a 2×2 factorial experiment manipulating water stress (two levels; low-watering frequency to impose water stress and a high-watering frequency control treatment; see later for further detail) and previous damage by monarch larva (two levels; induced by herbivore damage or not induced). *Asclepias syriaca* seeds were purchased from Prairie Moon Nursery (Winona, Minnesota), cold-stratified in moist paper towels at 2 °C for approximately 2 weeks. The seeds were then planted in 0.5-litre pots filled with a 1 : 1 mixture of potting soil (Metromix 366P, BFG Supply, Burton, Ohio) and local field soil (silt loam) and allowed to germinate in a glasshouse ($n = 180$ plants total). Cotyledons began to emerge by 9 June 2015 and all plants had emerged by 15 June 2015. All plants were allowed to grow for approximately 1 month, received water every 1–2 days, and were fertilised once with an all-purpose fertiliser (Scotts Miracle-Gro Products, Inc., Marysville, Ohio). We randomly divided the 180 plants into two groups; plants in one group were used to measure several traits related to physiology (stomatal conductance, leaf water content, and $\delta^{13}C$), growth [total dry biomass, stem height, root:shoot, and specific leaf area (SLA)], and resistance or nutrition (trichome density, latex, leaf nitrogen, and leaf C:N; $n = 80$ trait plants), and plants in the other group were used as hosts for monarch larvae, so their performance could be assessed ($n = 100$ bioassay plants). Within these two groups, we randomly assigned plants to factorial combinations of the water stress and damage treatments.

We began the water-stress treatment on 7 July 2015, which was 22 days after all the plants had germinated and emerged above ground. At this time, plants had four to 12 leaves. We monitored water-stressed plants daily and added 150 ml of water to pots when plants began to show signs of wilting (approximately every 5–8 days). Well-watered plants received *ad libitum* water every 2–3 days throughout the course of the experiment. Approximately 5 weeks after initiating the drought treatment, we placed one freshly hatched monarch larva on half of the plants ($n = 40$ trait plants and $n = 50$ bioassay plants). Monarch eggs were ordered from Michigan Monarchs (Allenton, Michigan). We allowed the monarchs to feed for 4 days, which is long enough for *A. syriaca* plants to induce defences (Agrawal *et al.*, 2015). Using insects to damage plants is a common method to induce defences in *Asclepias* (and other) species and is more effective than mechanical damage (van Zandt & Agrawal, 2004; Rasmann *et al.*, 2009). At the end of the experiment (16 August 2015), we measured stomatal conductance using a portable leaf porometer (Decagon Devices, Inc. Pullman, Washington) and we collected latex on a pre-weighed 1.5-cm-diameter filter paper by clipping the tip (~5 mm) off one of the top fully expanded leaves. Latex was stored at −2 °C in a pre-weighed 28.4-g plastic container and reweighed within 2 days. Latex content was calculated as the fresh mass of latex (mg) collected from each plant. We collected the other top, fully expanded leaf to measure trichome density, leaf water content, and SLA. Leaves were stored in a moist paper towel at 2 °C for <2 days until trichomes could be counted. Trichomes were counted under a dissecting scope in a 33 mm² area of the leaf. The leaf was then scanned, weighed, dried at 60 °C for 2 days, and reweighed to the nearest mg. Leaf water content was calculated as the difference between leaf fresh

weight and leaf dry weight. Leaf area was calculated from the scanned leaf image using IMAGEJ (NIH, Bethesda, Maryland). Specific leaf area was calculated as the area (cm^2) per dry mass (mg). Percentage carbon (C), percentage nitrogen (N), and δ^{13}C (an indicator of water-use efficiency) was determined for a subset of plants ($n = 23$) via elemental analysis (Colorado Plateau Stable Isotope Laboratory, Flagstaff, Arizona). We also completely harvested, dried, and weighed all above- and below-ground biomass from a subset of plants ($n = 35$).

The bioassay plants ($n = 100$) received a new freshly hatched monarch larva that served as a bioassay monarch immediately following removal of the initial damaging monarch. Larvae were placed on the upper most leaves of uncaged plants and were allowed to roam over the entire plant. Individually potted plants were spaced so that the larvae could not move to adjacent plants. After 5 days of feeding, we collected the bioassay larva, waited 12 h so that they could void their guts, and then weighed the live larvae to the nearest 0.1 mg. We also estimated the amount of leaf tissue consumed by the bioassay larvae using a punched hole (33 mm^2 in diameter) as a guide to visually estimate the area of leaf tissue consumed to the nearest 16.5 mm^2. For plants that were previously damaged, we subtracted the amount of damage prior to the bioassay from the final amount of damage to the plant.

Field experiment

Our field experiment was conducted in a restored prairie in southern Wisconsin (latitude, 42.51199; longitude, −88.54890) over two growing seasons. In spring 2015, within uncultivated buffer areas next to a restored agriculture field, we haphazardly selected 36 established *A. syriaca* plants, ensuring that stems of replicate individuals were separated by >2 m. Some plants had multiple stems originating from within 50 cm of the focal stem, but no plants were part of an obvious large clonal patch. We avoided depressions and slopes. We randomly assigned one of the following treatments to each of the 36 plants: ambient rainfall ($n = 15$ plants), reduced rainfall via rainout shelters ($n = 15$ plants), and control shelter (rainout shelter with water added back to plot; $n = 6$ plants). For the control shelters, water was collected from the roof with a gutter that drained into a 18.9-litre bucket, and the water was added back to the plot within approximately 24 h of a rainfall event. We constructed rainout shelters (Yahdjian & Sala, 2002) in late May 2015. Shelters were 1.5 m × 1.5 m and centred over each individual *A. syriaca* plant assigned to the rainout or control treatments. The ambient treatment plants did not receive a shelter. Shelters were 1 m tall in the front and 1.4 m tall in the back (15° incline) oriented towards the direction of prevailing winds (which was from the south; P. G. Hahn, unpublished). Rainout shelter roofs were constructed out of seven 14-cm-wide slats of clear polycarbonate roofing materials, which covered approximated 65% of the 2.25-m^2 plots. The roofing materials allow >90% light transmission (Palram Americas Inc., Kudztown, Philadelphia). Several shelters were compromised in the second year of the study, which reduced the total sample size ($n = 13$ ambient plants; $n = 11$ rainout shelter plants).

We measured volumetric soil moisture (VSM) at three locations within a 25-cm radius of each plant throughout the growing season in 2015 and once in late July in 2016 using a Field-Scout TDR 100 soil moisture meter (Spectrum Technologies Inc., Aurora, Illinois). In 2015, the rainout shelters reduced VSM by about 9% averaged across the entire season, from 35.6% near ambient plants to 26.2% near plants under the rainout shelters (Appendix S1). In 2016, the rainout shelters reduced VSM by about 8%, from 25.6% near ambient plants to 17.5% near plants under the rainout shelters (Supporting Information). To determine whether the shelters altered microclimate, using a Thermo-Hygrometer (Spectrum Technologies Inc., Aurora, Illinois), we measured relative humidity and temperature under and immediately outside the shelters on 17 June 2015 . Relative humidity ($F_{1,16} = 2.2$, $P = 0.16$) and temperature ($F_{1,16} = 0.5$, $P = 0.50$) were not affected by the shelters. We also measured plant size and herbivore damage throughout the season in 2015 and once in 2016. Herbivore damage was estimated as the proportion of leaves with >5% tissue removed by leaf-chewing insects in 2015. The proportion of leaves damaged is often correlated with other metrics of herbivore damage and plant performance in *Asclepias* and other species of forbs (Agrawal, 2005; Hahn & Orrock, 2015). Presence or absence of herbivory was scored in 2016. Most folivory was caused by the specialist beetles *Labidomera clivicollis* and *Tetraopes tetrophthalmus*, although other herbivores that consume milkweed, such as monarch larva (*Danaus plexippus*), aphids (*Aphis asclepiadis*), milkweed tussock moth (*Euchaetes egle*), and milkweed stem weevils (*Rhyssematus lineaticollis*), were observed in low abundance (P. G. Hahn, unpublished).

In late (23–28) July 2015 and 2016, we conducted a bioassay experiment on all plants using freshly hatched monarch larvae (see earlier). We placed three larvae on each plant and enclosed them in a mesh insect sleeve. After 5 days, we collected all surviving larvae, allowed them to void their guts for 12 h, and weighed them to the nearest 0.1 mg.

Statistical analysis

For the glasshouse experiment, we conducted a two-way ANOVA using the watering treatment (high and low), induced defence treatment (induce and not induced) and their interaction as fixed-effect predictor variables. For plant traits (trichomes, latex, leaf water content, stomatal conductance, SLA, and stem height), we first conducted a MANOVA with plant traits as response variables, followed by univariate ANOVAS. Because of the smaller sample size for leaf nitrogen, C:N, δ^{13}C, and biomass, we did not include these traits in the MANOVA. We analysed each bioassay response variable separately (survival after 5 days, leaf area consumed, and mass after 5 days). Survival was modelled with a binomial error structure and leaf area consumed and mass were modelled with a normal error structure. When using larval mass as the response variable, we also conducted an additional analysis that included the amount of leaf area consumed as a covariate, which allowed us to quantify how leaf area consumed relates to larval mass. No transformations were necessary to meet assumptions of normal distributions or homogeneity of residuals.

For the field experiment, we conducted a repeated-measures ANOVA to evaluate the effect of the rainout treatment on larval performance over the 2 years. We also included plant ID as a random effect to account for the multiple larvae per plant. Mass of surviving larvae on each plant was natural-log-transformed prior to analysis. Plant height did not differ among the three rainfall treatments during the first year (2015) of the experiment ($F_{2,29} = 1.4$, $P = 0.26$) and so we did not consider using plant height as a covariate in these analyses. Herbivore damage, measured as the proportion of leaves damaged, did not differ among the three rainfall treatments in 2015 ($F_{2,29} = 1.3$, $P = 0.28$). The mean proportion of leaves damaged, pooled across all treatments, was 0.27 (± 0.20 SD). We assumed that all plants with previous damage had induced resistance traits. In 2015, the proportion of leaves damaged was negatively correlated with the final mass of the bioassay caterpillar, suggesting that more heavily damaged plants were induced to a greater extent. However, this trend was relatively weak and not significant ($\beta = -1.21$, $R^2 = 0.09$, $F_{1,21} = 3.0$, $P = 0.097$), so we did not include it as a potential covariate. Only two plants (one in the ambient and one in the rainout shelter treatment) did not receive any damage during the 2015 season and one plant in 2016. However, these plants may have been damaged below ground by the beetle *Tetraopes tetrophthalmus*, which was common at our site and feeds on *A. syriaca* roots as larvae and can induce resistance traits in above-ground tissue (Erwin *et al.*, 2014). Removing these two observations from the analysis of larval mass (see later) did not change the results, so we kept them in the analysis. All analyses were conducted in R 3.2.0 (R Core Team, 2017) or SAS 9.4 (SAS Inc., Cary, NC, USA). For all analyses, we considered $\alpha = 0.1$ as marginally significant and $\alpha = 0.05$ as significant. Data from all experiments and R code for analyses are provided in Appendices S2–S5.

Results

Glasshouse experiment

There was an overall significant effect of the watering treatment on eight plant traits for which we had complete data (MANOVA: Pillai's trace $F_{6,67} = 28.5$, $P < 0.001$), but there was no effect of the induction treatment on plant traits as a group (Pillai's trace $F_{6,67} = 0.6$, $P = 0.71$), or the interaction (Pillai's trace $F_{6,67} = 0.5$, $P = 0.81$). Univariate ANOVAs showed that the high water treatment significantly increased values of eight (stomatal conductance, leaf water content, δ^{13}C, biomass, stem height, root:shoot ratio, SLA, and trichome density) of the 11 measured plant traits compared with the low water treatment, whereas the damage treatment marginally increased leaf N and reduced C:N (Fig. 1). Note that the traits that were affected by the damage treatment (leaf N and C:N) as revealed by the univariate analyses were not included in the MANOVA, which revealed no effect of damage on trait values, due to lower sample size. Latex was the only trait not affected by either treatment (Fig. 1).

Survival of the bioassay larva was not affected by host plant water stress ($F_{1,91} = 1.7$, $P = 0.20$) or previous damage ($F_{1,91} = 0.1$, $P = 0.71$), but there was a trend towards a significant interaction between these treatments ($F_{1,91} = 3.7$,

$P = 0.057$, Fig. 2a). On previously damaged plants, larval survival was higher on water-stressed plants than on plants that received *ad libitum* water (linear contrast: $t = -2.1$, $P = 0.037$). Larvae that survived the 5-day bioassay consumed less plant tissue on plants with previous herbivore damage ($F_{1,70} = 11.4$, $P = 0.001$), but were not affected by the watering treatment ($F_{1,70} = 1.0$, $P = 0.32$) or the interaction between watering and previous damage ($F_{1,70} = 1.5$, $P = 0.23$l; Fig. 2b). Mass of larva that survived the 5-day bioassay was not affected by watering ($F_{1,71} = 2.4$, $P = 0.12$), previous damage ($F_{1,71} = 0.2$, $P = 0.65$), or the interaction ($F_{1,71} = 1.1$, $P = 0.31$; Fig. 2c). However, after controlling for the effect of leaf tissue consumed on larval mass ($F_{1,69} = 19.3$, $P < 0.001$), the watering ($F_{1,69} = 4.8$, $P = 0.032$) and induction ($F_{1,69} = 4.4$, $P = 0.039$) treatments had significant effects on larval mass, while the interaction was not significant ($F_{1,69} = 0.3$, $P = 0.57$, Fig. 2d). Area consumed was positively related to larval mass ($\beta = 0.04$, SE = 0.01, $P < 0.001$), but interactions between area consumed and the watering treatment or the damage treatment were not significant (all P-values > 0.8) and these were removed from the final model.

Field experiment

Monarch larval mass in the control shelter treatment was more similar to the ambient treatment than to the rainout treatment (data not shown). However, because these differences were not significant (perhaps due to low sample size of the control shelters; $n = 6$), we removed the control shelters from subsequent analyses. The rainout shelter treatment significantly affected monarch larval growth on milkweed plants ($F_{1,31.5} = 5.2$, $P = 0.029$). Larvae were larger on plants exposed to the rainout treatment than on plants exposed to ambient rainfall (Fig. 3). Larval growth was also affected by year, with larvae growing larger in 2015 than in 2016 ($F_{1,88.8} = 9.5$, $P = 0.003$), but the interaction between the rainout treatment and year was not significant ($F_{1,88.8} = 0.4$, $P = 0.53$).

Discussion

Plant resistance traits are well known to be affected by either abiotic factors, such as water stress (Chaves *et al.*, 2003; Huberty & Denno, 2004), or biotic factors, such as herbivore damage (Agrawal, 1998; Karban, 2011). Here we show that water stress can affect insect performance, using complementary glasshouse and field experiments. In the glasshouse we show that monarch larval survival was improved on water-stressed *A. syriaca* plants that were induced by previous herbivore damage, compared with undamaged water-stressed plants. Consumption was affected by previous damage, but not by water stress. In a 2-year field experiment, we also found effects of water reduction on monarch larval performance, where monarch larval growth was improved on damaged *A. syriaca* plants exposed to experimental reductions in rainfall, as compared with plants receiving ambient rainfall. However, because different metrics of insect performance were affected in the glasshouse (survival) and in the field (growth), different mechanisms may have driven these results.
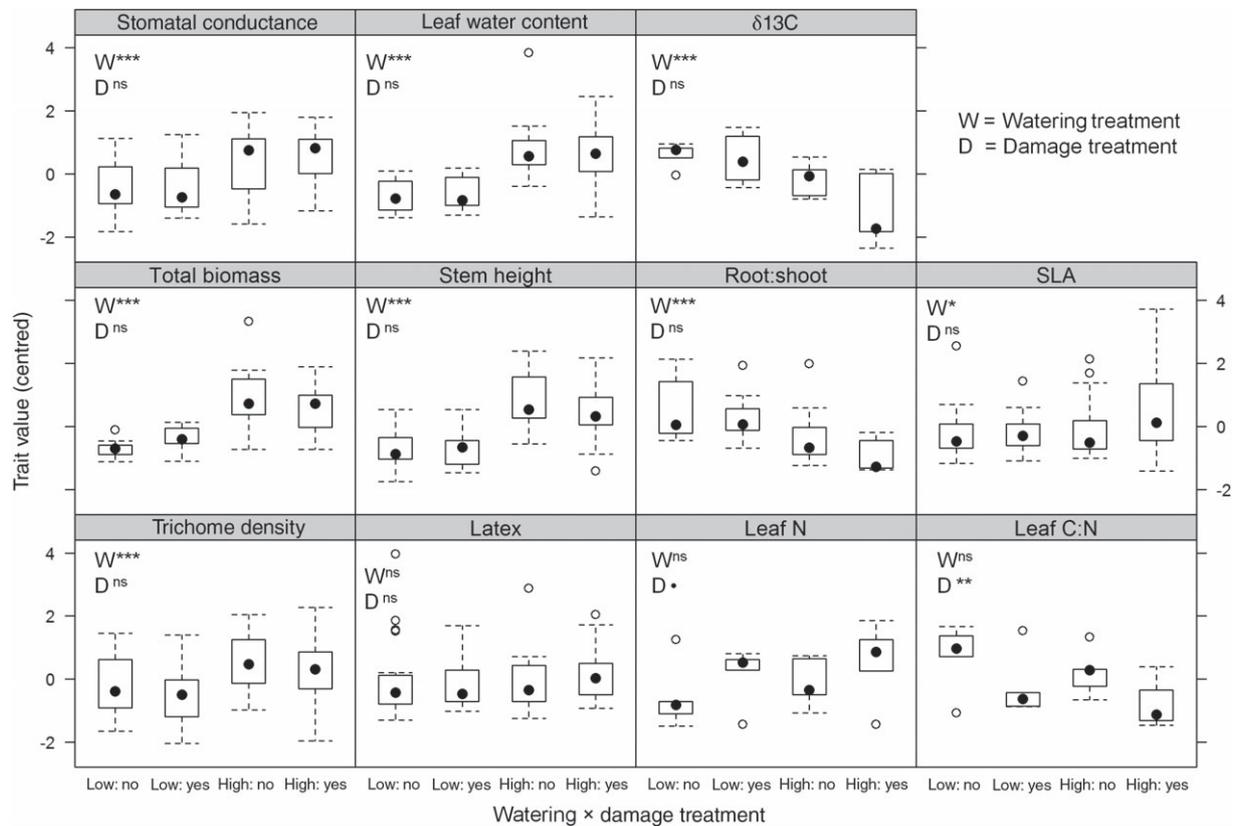
Fig. 1. Traits measured on plants exposed to two watering regimes (high and low) and two damage treatments (previously damaged and not previously damaged) in the glasshouse experiment. Trait values were centred to a mean of zero and scaled to a standard deviation of 1. Black dots show the median, boxes represent the interquartile range, whiskers represent 1.5 times the interquartile range, and open dots represent data points outside the whiskers. See the 'Materials and methods' for a description of trait measurements and units. SLA, specific leaf area.

Our experiments provide some evidence that water stress and previous herbivore damage can influence the performance of insects that subsequently feed on the plant (Herms & Mattson, 1992; Orrock *et al.*, 2015), although we did not find strong differences in the resistance traits we measured. In our glasshouse experiment we found that monarch larvae survived better on water-stressed plants that were previously damaged, compared with undamaged water-stressed plants. We also found that previous damage affected the nutrient status of the host plants, whereas the only resistance trait affected by the watering treatment was trichome production. It is possible that variation in cardenolide induction could drive our results, although we did not measure cardenolides in our study. Importantly, our experiment used freshly hatched (first-instar) larvae, which are most susceptible to mortality caused by *Asclepias* resistance traits (Zalucki & Brower, 1992; Zalucki & Malcolm, 1999). Water stress, in the absence of herbivore damage, typically increases the concentration of nutrients in plant tissue in the short term, which can result in increased insect performance (White, 1984; Behmer & Joern, 2012). However, this pattern can be reversed by prolonged water stress, which reduces plant growth and quality, resulting in reduced performance of insect herbivores (Chaves, 1991; Huberty & Denno, 2004). We found that persistent water stress reduced plant growth in our glasshouse study (Fig. 1), but

the watering treatment had no effect on larval survival (Fig. 2a) or amount of tissue consumed on non-damaged plants (Fig. 2b). Larval mortality was probably not related to plant nutrition as we found no effect of the watering treatment on leaf nitrogen or C:N, and previous herbivore damage actually increased leaf nitrogen and reduced C:N.

We also found that surviving larvae consumed significantly less leaf tissue on plants that were previously damaged (Fig. 2b), although there was no difference in larval mass at the end of the experiment. However, when compared with the mean level of leaf tissue consumed, larva grew faster on well-watered plants and on previously damaged plants (Fig. 2c). This suggests that plant quality improved because larval growth rate was greater, per unit area of tissue consumed, on well-watered plants and on previously damaged plants. This could be related to increases in leaf nitrogen, at least on previously damaged plants (Fig. 1). Recent studies have found similar results, where water stress had negative effects on plant quality and insect preference or performance (Grinnan *et al.*, 2013; Lenhart *et al.*, 2015). However, comparing among studies can be challenging because changes in drought duration and intensity can produce negative, positive, or neutral effects of water stress on insect performance (Huberty & Denno, 2004; Sconiers & Eubanks, 2017) and the direction may depend on the feeding guild or diet breadth of the
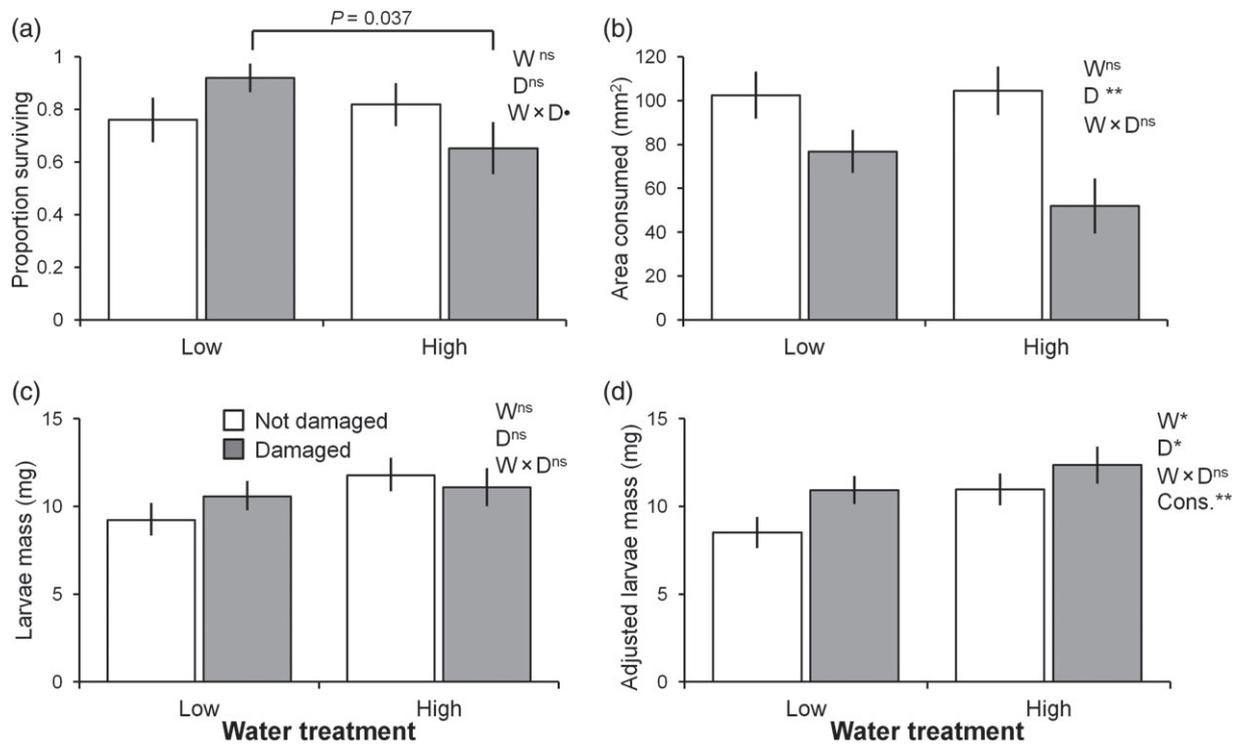
Fig. 2. Results of the 5-day bioassay glasshouse experiment. (a) Proportion of monarch larvae surviving; (b) area of leaf tissue consumed by surviving larvae; (c) larval mass; and (d) larval mass after adjusting for the amount of leaf tissue consumed. ANOVA results are provided next to each panel: W, watering treatment; D, damage treatment; cons., area leaf tissue consumed; $^{ns}P > 0.1$, $\bullet P = 0.057$, $*P < 0.05$, $**P < 0.01$. Bars are one standard error.
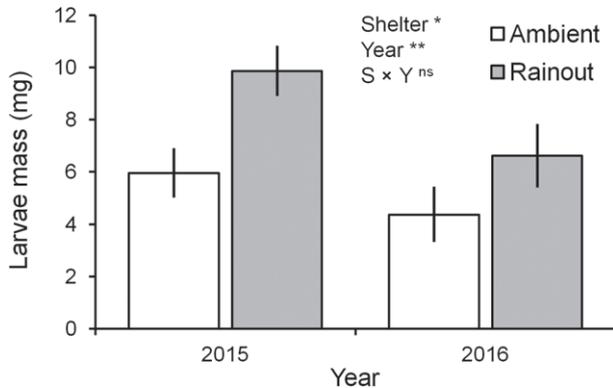

Fig. 3. Results from the bioassay experiment conducted on field plants that were exposed to ambient rainfall or a rainout shelter. ANOVA results are provided within the panel. $^{ns}P > 0.05$, $*P < 0.05$, $**P < 0.01$. Bars are one standard error.

herbivore (Huberty & Denno, 2004; Mody *et al.*, 2009). Other studies with *A. syriaca* found that water stress can decrease trichome density and latex production, but increase cardenolide production, although the responses were typically fairly small (Agrawal *et al.*, 2014b; Couture *et al.*, 2015). We did not find significant effects of water stress on latex content, although trichome production was lower for water-stressed plants and latex trended in this direction (Fig. 1). Leaf nitrogen was not

affected by the watering treatment (Fig. 1). As such, the small changes in resistance traits (e.g. trichomes) that we documented probably did not affect any metric of larval performance. Rather, other factors such as leaf water content, carbohydrates, or unmeasured resistance traits (e.g. cardenolides) probably increased larval growth per unit area of tissue consumed on well-watered plants (Fig. 2d).

The results of our field experiment are consistent with those from our glasshouse experiment in that there was higher larval growth on *A. syriaca* plants that experienced experimentally reduced rainfall compared with plants receiving ambient rainfall (Fig. 3). The majority of plants in the field were naturally damaged prior to our bioassay experiments, mainly by specialist beetles *Tetraopes tetraophthalmus* and *Labidomera clivicollis* (P. G. Hahn, unpublished). As both of these species can induce herbivore resistance in *A. syriaca* (van Zandt & Agrawal, 2004; Erwin *et al.*, 2014), it is reasonable to assume that plants in our field experiment had been induced. We also found that monarch performance was significantly greater in the first than in the second year of the experiment. It is unclear what factors were responsible for this year effect, although there was greater soil moisture availability in the second than in the first year of the experiment (Appendix S1). Other climatic factors, such as temperature and cloud cover, might also have contributed to the difference in larval performance.

Plastic responses of plants are important for persisting in variable environments (Ruel & Ayres, 1999; Bolnick *et al.*, 2011),

but responses to abiotic and biotic factors have historically been considered in isolation. Our field and glasshouse experiments show that water stress and previous herbivore damage interact to affect plant nutrients and insect performance. Collectively, this work provides additional empirical support for the growing interest in understanding how the abiotic environment can mediate interactions between plants and herbivores.

Acknowledgements

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/een.12468

Appendix S1. Results of soil moisture analysis from the field experiment.

Appendix S2. Data on plant traits from the glasshouse experiment.

Appendix S3. Data from the bioassay glasshouse experiment.

Appendix S4. Data from the field experiment.

Appendix S5. R code used for analyses.

References

Agrawal, A.A. (1998) Induced responses to herbivory and increased plant performance. *Science*, **279**, 1201–1202.

Agrawal, A.A. (2005) Natural selection on common milkweed (*Asclepias syriaca*) by a community of specialized insect herbivores. *Evolutionary Ecology Research*, **7**, 651–667.

Agrawal, A.A., Hastings, A.P., Patrick, E.T. & Knight, A.C. (2014a) Specificity of herbivore-induced hormonal signaling and defensive traits in five closely related milkweeds (*Asclepias* spp.). *Journal of Chemical Ecology*, **40**, 717–729.

Agrawal, A.A., Patrick, E.T. & Hastings, A.P. (2014b) Tests of the coupled expression of latex and cardenolide plant defense in common milkweed (*Asclepias syriaca*). *Ecosphere*, **5**, 1–11.

Agrawal, A.A., Hastings, A., Bradburd, G., Woods, E., Züst, T., Harvey, J. *et al.* (2015) Evolution of plant growth and defense in a continental introduction. *American Naturalist*, **186**, E1–E15.

Awmack, C.S. & Leather, S.R. (2002) Host plant quality and fecundity in herbivorous insects. *Annual Review of Entomology*, **47**, 817–844.

Bazzaz, F., Chiariello, N., Coley, P. & Pitelka, L. (1987) Allocating resources to reproduction and defense. *BioScience*, **37**, 58–67.

Behmer, S.T. & Joern, A. (2012) Insect herbivore outbreaks viewed through a physiological framework: insights from Orthoptera. *Insect Outbreaks Revisited* (ed. by P. Barbosa, D. K. Letourneau and A. Agrawal), pp. 3–29. Blackwell Publishing Ltd, Sussex, U.K.

Bingham, R.A. & Agrawal, A.A. (2010) Specificity and trade-offs in the induced plant defence of common milkweed Asclepias syriaca to two lepidopteran herbivores. *Journal of Ecology*, **98**, 1014–1022.

Bolnick, D.I., Amarasekare, P., Araújo, M.S., Bürger, R., Levine, J.M., Novak, M. *et al.* (2011) Why intraspecific trait variation matters in community ecology. *Trends in Ecology & Evolution*, **26**, 183–192.

Burghardt, K.T. (2016) Nutrient supply alters goldenrod's induced response to herbivory. *Functional Ecology*, **30**, 1769–1778.

Carmona, D., Lajeunesse, M.J. & Johnson, M.T.J. (2011) Plant traits that predict resistance to herbivores. *Functional Ecology*, **25**, 358–367.

Chaves, M.M. (1991) Effects of water deficits on carbon assimilation. *Journal of Experimental Botany*, **42**, 1–16.

Chaves, M.M., Maroco, J.P. & Pereira, J.S. (2003) Understanding plant responses to drought–from genes to the whole plant. *Functional Plant Biology*, **30**, 239–264.

Couture, J.J., Serbin, S.P. & Townsend, P.A. (2015) Elevated temperature and periodic water stress alter growth and quality of common milkweed (*Asclepias syriaca*) and monarch (*Danaus plexippus*) larval performance. *Arthropod-Plant Interactions*, **9**, 149–161.

Donaldson, J.R., Kruger, E.L. & Lindroth, R.L. (2006) Competition- and resource-mediated tradeoffs between growth and defensive chemistry in trembling aspen (*Populus tremuloides*). *New Phytologist*, **169**, 561–570.

Erwin, A.C., Züst, T., Ali, J.G. & Agrawal, A.A. (2014) Above-ground herbivory by red milkweed beetles facilitates above- and below-ground conspecific insects and reduces fruit production in common milkweed. *Journal of Ecology*, **102**, 1038–1047.

Glynn, C., Herms, D.A., Egawa, M., Hansen, R. & Mattson, W.J. (2003) Effects of nutrient availability on biomass allocation as well as constitutive and rapid induced herbivore resistance in poplar. *Oikos*, **101**, 385–397.

Grinnan, R., Carter, T.E. & Johnson, M.T.J. (2013) The effects of drought and herbivory on plant-herbivore interactions across 16 soybean genotypes in a field experiment. *Ecological Entomology*, **38**, 290–302.

Gutbrodt, B., Dorn, S. & Mody, K. (2012) Drought stress affects constitutive but not induced herbivore resistance in apple plants. *Arthopod-Plant Interactions*, **6**, 171–179.

Hahn, P.G. & Orrock, J.L. (2015) Land-use legacies and present fire regimes interact to mediate herbivory by altering the neighboring plant community. *Oikos*, **124**, 497–506.

Hakes, A.S. & Cronin, J.T. (2011) Environmental heterogeneity and spatiotemporal variability in plant defense traits. *Oikos*, **120**, 452–462.

Hale, B.K., Herms, D.A., Hansen, R.C., Clausen, T.P. & Arnold, D. (2005) Effects of drought stress and nutrient availability on dry matter allocation, phenolic glyosides, and rapid induced resistance of poplar to two lymantriid defoliators. *Journal of Chemical Ecology*, **31**, 2601–2620.

Halpern, S.L., Adler, L.S. & Wink, M. (2010) Leaf herbivory and drought stress affect floral attractive and defensive traits in nicotiana quadrivalvis. *Oecologia*, **163**, 961–971.

Herms, D. & Mattson, W. (1992) The dilemma of plants: to grow or defend. *Quarterly Review of Biology*, **67**, 283–335.

Huang, W., Siemann, E., Yang, X., Wheeler, G.S. & Ding, J. (2013) Facilitation and inhibition: changes in plant nitrogen and secondary metabolites mediate interactions between above-ground and below-ground herbivores. *Proceedings of the Royal Society B*, **280**, 20131318.

Huberty, A.F. & Denno, R.F. (2004) Plant water stress and its consequences for herbivorous insects: a new synthesis. *Ecology*, **85**, 1383–1398.

Karban, R. (2011) The ecology and evolution of induced resistance against herbivores. *Functional Ecology*, **25**, 339–347.

Karban, R., Agrawal, A. & Mangel, M. (1997) The benefits of induced defenses against herbivores. *Ecology*, **78**, 1351–1355.

Kleine, S. & Muller, C. (2014) Drought stress and leaf herbivory affect root terpenoid concentrations and growth of *Tanacetum vulgare*. *Journal of Chemical Ecology*, **40**, 1115–1125.

Lenhart, P.A., Eubanks, M.D. & Behmer, S.T. (2015) Water stress in grasslands: dynamic responses of plants and insect herbivores. *Oikos*, **124**, 381–390.

Mody, K., Eichenberger, D. & Dorn, S. (2009) Stress magnitude matters: different intensities of pulsed water stress produce non-monotonic resistance responses of host plants to insect herbivores. *Ecological Entomology*, **34**, 133–143.

Orrock, J.L., Sih, A., Ferrari, M.C.O., Karban, R., Preisser, E.L., Sheriff, M.J. *et al.* (2015) Error management in plant allocation to herbivore defense. *Trends in Ecology & Evolution*, **30**, 441–445.

Petschenka, G., Fandrich, S., Sander, N., Waqschal, V., Boppre, M. & Dobler, S. (2013) Stepwise evolution of resistance to toxic cardenolides via genetic substitutions in the Na^+/K^+-ATPase of milkweed butterflies (leidoptera: Danaini). *Evolution*, **67**, 2753–2761.

R Core Team (2017) *R: A language and environment for statistical computing*. R Foundation for Stastical Computing, Vienna, Austria. [WWW document]. URL http://www.R-project.org.

Rasmann, S., Johnson, M.D. & Agrawal, A.A. (2009) Induced responses to herbivory and jasmonate in three milkweed species. *Journal of Chemical Ecology*, **35**, 1326–1334.

Ruel, J.J. & Ayres, M.P. (1999) Jensen's inequality predicts effects of environmental variation. *Trends in Ecology and Evolution*, **14**, 361–366.

Sconiers, W.B. & Eubanks, M.D. (2017) Not all droughts are created equal? The effects of stress severity on insect herbivore abundance. *Arthropod-Plant Interactions*, **11**, 45–60.

Tariq, M., Rossiter, J.T., Wright, D.J. & Staley, J.T. (2013) Drought alters interactions between root and foliar herbivores. *Oecologia*, **172**, 1095–1104.

Weldegergis, B.T., Zhu, F., Poelman, E.H. & Dicke, M. (2015) Drought stress affects plant metabolites and herbivore preference but not host location by its parasitoids. *Oecologia*, **177**, 701–713.

Wetzel, W.C., Kharouba, H.M., Robinson, M., Holyoak, M. & Karban, R. (2016) Variability in plant nutrients reduces insect herbivore performance. *Nature*, **539**, 425–428.

White, T.C.R. (1984) The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia*, **63**, 90–105.

Yahdjian, L. & Sala, O. (2002) A rainout shelter design for intercepting different amounts of rainfall. *Oecologia*, **133**, 95–101.

Zalucki, M.P. & Brower, L.P. (1992) Survival of first instar larvae of *Danaus plexippus* (Lepidoptera: Danainae) in relation to cardiac glycoside and latex content of *Asclepias humistrata* (Asclepiadaceae). *Chemoecology*, **3**, 90–93.

Zalucki, M.P. & Malcolm, S. (1999) Plant latex and first-instar monarch larval growth and survival on three North American milkweed species. *Journal of Chemical Ecology*, **25**, 1827–1842.

van Zandt, P. & Agrawal, A.A. (2004) Specificity of induced plant responses to specialist herbivores of the common milkweed Asclepias syriaca. *Oikos*, **104**, 401–409.