

Plant water-use strategies as mediators of herbivore drought response: Ecophysiology, host plant quality and functional traits

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Abstract

1. The response of herbivorous insects to plant drought stress can range from positive to negative, and it has been challenging to understand the causes of this variation. We tested whether plant trait values associated with aridity gradients might underlie this variation and how such effects vary between two insect feeding guilds.
2. Here, we propose that plants trait values associated with adaptation to arid environments would result in positive effects of experimental drought on herbivores, with such plant species adaptively shifting resources away from resistance to maintain performance under stress. In contrast, plants with trait values associated with adaptation to mesic environments would result in negative effects of drought because such species lose vigour and thus decrease their host quality.
3. We tested these predictions using experimental manipulations in 13 milkweed species (genus *Asclepias*) adapted to a wide range of environmental conditions and the herbivore performance of a specialist leaf-chewer (monarch butterfly; *Danaus plexippus*) and sap-feeder (oleander aphid; *Aphis nerii*). We exposed plants to physiologically calibrated species-specific watering regimes to maximize (100%) or reduce (50%) stomatal conductance and then monitored herbivore performance.
4. The effects of drought stress on herbivore survival ranged from strongly positive (50% increase) to strongly negative (80% decrease) among milkweed species, but these effects were inconsistent between the two herbivores.
5. Plant trait values associated with adaptation to aridity were correlated with monarch survival in the predicted manner, such that milkweed species with high water-use efficiency (WUE) and low relative water content (RWC) increased monarch survival under drought. In contrast, aphid survival was unrelated to arid-adapted trait values.

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6. Drought-induced changes in plant quality were negatively associated with both herbivore survivals. Specifically, milkweed species that increased cardenolides concentration under drought conditions decreased monarch and aphid survival.
7. *Synthesis.* We demonstrated that the indirect effects of drought on herbivores varied tremendously among closely related plant species and between co-occurring insect herbivores. We in turn present evidence that some of this variation is explained by plant traits associated with adaptation to arid versus mesic environments for leaf-chewers but not sap-feeders and that such effects are mediated by drought-induced changes in chemical defences.

KEYWORDS

Aphis nerii, aridity gradients, *Asclepias* genus, *Danaus plexippus*, host plant quality, plant stress hypothesis, plant water-use strategies, plant vigour hypothesis

1 | INTRODUCTION

Drought stress is common in many ecosystems (Kramer, 1983) and can affect virtually every plant function (English-Loeb et al., 1997), but its effects on herbivores are notoriously variable and difficult to predict (Gely et al., 2020). Indeed, the response of herbivores to plant drought stress can range from instigating herbivore outbreaks (Mattson & Haack, 1987) to population declines (i.e. Carnicer et al., 2019). Hundreds of empirical studies and many hypotheses have sought to characterize the effects of drought on herbivores, but the specific mechanisms driving herbivore response to drought stress in plants are still poorly understood. Climate change is predicted to intensify drought events in many parts of the world (Sheffield & Wood, 2008) with significant consequences for ecosystem functioning and services, as well as economic activities such as forestry and agriculture. Thus, to better address the ecological and economic impacts of climate change, it is imperative that we develop a conceptual framework that allows us to predict herbivore response to plant drought stress.

The high variability of drought effects on herbivores has led to competing hypotheses: the plant stress hypothesis (PSH) and the plant vigour hypothesis (PVH). These hypotheses offer opposing predictions and have both received some empirical support (i.e. Bauerfeind & Fischer, 2013; Grinnan et al., 2013). The PSH (White, 1969) was proposed to explain patterns of insect outbreaks following drought events. This theory posits that herbivores benefit from drought-stressed plants due to (1) an increase in plant nutritional quality as plants flush nutrients (i.e. carbohydrates, nitrogen) to maintain osmotic potential under drought and (2) a decrease in plant defences as plants divert resources (i.e. carbon and nitrogen) from costly defence mechanisms to enhance survival under drought (Gutbrodt et al., 2011). In contrast, the PVH states that reduced plant performance from drought-stressed plants negatively affects herbivore fitness (Price, 1991). Low water availability reduces photosynthetic rate, above-ground dry mass and leaf turgor (Eck et al., 2001; Gutbrodt et al., 2011;

Ryan, 2011) and these effects are predicted to negatively affect insects that preferentially feed from fast-growing vigorous plants (Grinnan et al., 2013).

Multiple review papers and meta-analysis have tested and refined these predictions (Gely et al., 2020; Huberty & Denno, 2004; Jactel et al., 2012; Koricheva & Haukioja, 1997; Larsson, 1989; Mattson & Haack, 1987). In general, there is consensus that herbivore feeding guilds respond differently to drought-stressed plants, since herbivores that feed from different plant tissues can differentially experience drought-induced changes in plant nutrition, chemistry, and growth. These studies have also revealed that the effects of drought on herbivores are often nonlinear and depend on the mode and duration of the drought. Most feeding guilds are expected to initially benefit from a moderate drought as plant nutritional quality increases, but severe and prolonged drought typically have debilitating effects on both, plants and herbivores (Gely et al., 2020; Mattson & Hack, 1987). However, different drought types (i.e. short, long, moderate, or severe) are often not well defined by researchers, and experimental studies often fail to provide direct evidence that plants are indeed water stressed (Huberty & Denno, 2004; Larsson, 1989). Huberty and Denno (2004) suggested that plants must experience intermittent periods of turgor recovery to allow nutrients to become available to sap-feeders (pulse stress hypothesis). Several other factors have also been considered to mediate herbivore response to drought, including herbivore diet breadth such as specialist versus generalist (Gely et al., 2020; Gutbrodt et al., 2011), as well as herbivore sub-guild such as senescence feeders versus flush feeders (White, 2009). Despite these advances, there is still considerable unexplained variation in the drought response of certain feeding guilds (i.e. leaf-chewers and leaf miners; Gely et al., 2020) and we have yet to unify these hypotheses under a single predictive framework.

Surprisingly, the role of plant traits values associated with water-use strategies in mediating drought effects to herbivores is largely understudied. It is widely recognized that plant

physiological responses to drought vary greatly within and among species depending on their drought adaptation or acclimation capacity (Lopez-Iglesias et al., 2014; Mody et al., 2009; Ordoñez et al., 2009; Reich, 2014; Turtola et al., 2005). Despite this variation, most drought experiments have been based on individual plant species and, while a diversity of plant–herbivore systems have been studied, we are aware of no work using a consistent methodology to explicitly test for how plant adaptation to aridity influences herbivore–plant dynamics under water-deficit conditions.

We propose that plant traits values associated with aridity gradients can provide a means of integrating the PSH and PVH, and thus improve predictions of herbivore responses to plant drought stress. Plant traits that underlie adaptation to arid versus mesic conditions include multiple morphological, physiological, and biochemical adaptations to avoid or tolerate drought (i.e. stomatal regulation, low specific leaf area [SLA], high water-use efficiency [WUE], nitrogen and phosphorous content (Ordoñez et al., 2009; Passioura, 1996; Reich, 2014; Wright et al., 2001, 2005)). We hypothesized that herbivores respond positively to drought-stressed plant species with aridity-associated traits values because these plants adaptively shift resources away from costly defensive compounds and increase nutrient content to maintain homeostasis under water-limiting conditions (PSH). In contrast, herbivores respond negatively when feeding on drought-stressed plants with mesic-associated traits because these plants are unable to maintain homeostasis, lose vigour and decline in quality (PVH) (Figure 1). Our predictions are underlain by the more general observation that costly physiological investments lead to trade-offs in plant response to conflicting stresses (i.e. abiotic vs. abiotic) and that plants adapted to different environments evolve unique positions along such trade-off axes that are appropriate to their specific environments (Agrawal, 2020; Blumenthal et al., 2020; Coley et al., 1985). Indeed, recent evidence suggests that drought-adapted species are genetically predisposed to prioritize investment in response to drought stress (i.e. proline and abscisic acid responsible for stomatal regulation) over their investment to face herbivore attack (i.e. jasmonic and salicylic acid) (Montesinos-Navarro et al., 2020).

To test our hypothesis, we imposed a physiologically calibrated water stress treatment on 13 milkweed species (*Asclepias* spp.) and tested for effects of aridity adaptation on the performance of two specialist herbivores from distinct feeding guilds, a leaf-chewer (monarch caterpillars; *Danaus plexippus*) and a sap-feeder (oleander aphids; *Aphis nerii*). The *Asclepias* genus has diversified into wet and arid habitats, while herbivore defence traits remained consistent among species across habitats (Agrawal, Fishbein, Halitschke, et al., 2009), thus allowing us to mechanistically investigate how water stress indirectly affects herbivores. By taking a comparative approach and measuring nine functional traits, we sought to explicitly link traits values based on habitat affiliations with herbivore drought response. To our knowledge, this study represents the first formal test for how plant traits

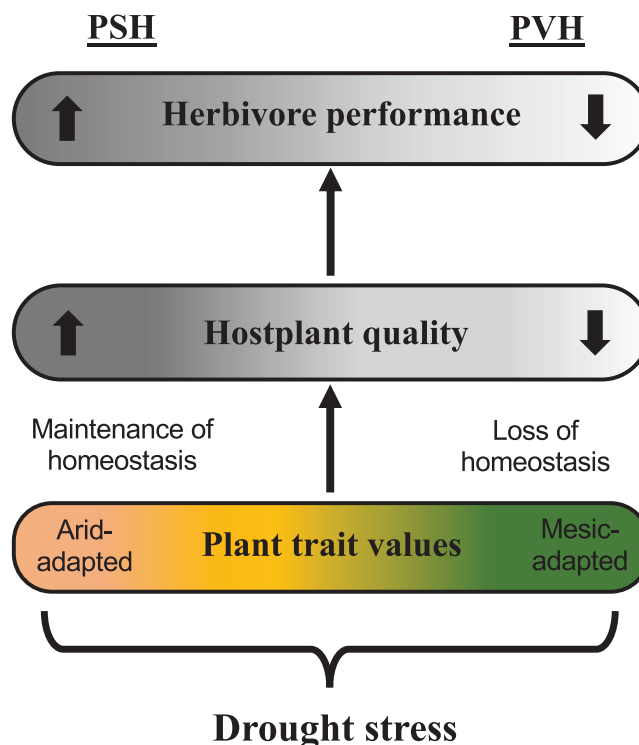


FIGURE 1 Schematic showing the mechanisms and hypotheses linking drought adaptation traits and herbivore response to drought in arid and mesic-adapted species. PSH indicates the plant stress hypothesis, and PVH indicates the plant vigour hypothesis. Plant trait values are represented by a gradient from arid (light orange) to mesic (green). Plant quality and herbivore performance shown from low (light grey) to high (dark grey). Black thick arrows indicate an increase (pointing up) or decrease (pointing down) in performance.

values associated with aridity gradients mediate drought effects on herbivores.

2 | MATERIALS AND METHODS

2.1 | Study system

Milkweed (*Asclepias* genus, Apocynaceae) is a large plant genus consisting of over 140 known species widely spread on the American continent and the Caribbean (Woodson, 1954). *Asclepias* species have diversified into a variety of habitats including tropical and temperate forests, deserts, wetlands, prairies and savannas (Agrawal, Fishbein, Jetter, et al., 2009). Plants in this genus have evolved a combination of antiherbivore defence strategies (defence syndrome), latex and cardenolides (cardiac glycosides) being the most characteristic defensive types (Agrawal & Fishbein, 2006). Latex is exuded by plant tissues to deter further feeding damage by entangling herbivore's mouth parts (Agrawal et al., 2014). Cardenolides (cardiac glycosides) are a group of highly toxic steroidal compounds that impact the function of Na⁺/K⁺-ATPases (Agrawal et al., 2014). Both types of defence have been shown to decrease survival and slow

growth in monarch caterpillars (Agrawal et al., 2014; Agrawal & Fishbein, 2006; Zalucki et al., 2001) and the development and fecundity of oleander aphids (Birnbaum & Abbot, 2018). The monarch larvae and the oleander aphid are highly specialized to feed on all milkweed species despite their toxicity; they are broadly distributed and interact strongly in nature (Agrawal & Konno, 2009; Dobler et al., 2011). Thus, the *Asclepias* genus allows one to compare herbivore response to plant drought stress in species adapted to contrasting environments but with consistent defence types.

2.2 | Study design

We selected 13 *Asclepias* species from a wide range of habitats, from arid (i.e. *A. californica*) to humid tropical environments (i.e. *A. curassavica*), which vary tremendously in traits associated with adaptation to mesic and dry habitats (Figure S1). The selected species were distributed throughout the *Asclepias* phylogeny (Figure S2), increasing opportunities to effectively control for non-independence and test for evolutionary convergence between plant traits and indirect drought effects on herbivores. We grew plants from seeds for 2 months in a greenhouse and watered them to saturation twice a week. All plant species were grown in 10 cm² pots fill with potting soil and fertilized every 2 weeks with 'All-purpose Miracle Gro' fertilizer (see Supplementary material 1.1 for details about germination and greenhouse protocols). For each plant species, 8–10 healthy plants were randomly divided into control or drought treatment. Replication among the 26 species-by-treatment combinations (13 species, 2 water treatments) ranged from 3 to 5 plants, with a total sample size of 121 plants.

2.3 | Water treatment

To impose a consistent physiological stress among species adapted to different moisture environments, we standardized water treatments based on the stomatal response to water availability (Cowan, 1978). We developed response curves for each *Asclepias* species by taking measurements of stomatal conductance with a leaf porometer (Decagon Devices; model SC-1) over a gradient of soil moisture conditions (see Supplementary material 1 for a detailed description of how response curves were constructed). Using these curves, we determined the amount of water needed to achieve the target pot weight at which a species reached its maximum stomatal conductance (100%; control) and a 50% reduction in stomatal conductance (drought) (Figure S3) (Skelton et al., 2015). Plants were watered twice a week, with each watering restoring the pot to its respective target weight. We imposed this treatment for 2 weeks. This duration was used because it imposes plants to drought stress while avoiding confounding effects of drought acclimation such as morphological adaptations in new leaves and roots (Touchette et al., 2007).

2.4 | Herbivore bioassay

After 2 weeks in their respective water regimes, we placed a single monarch larva and one aphid on each intact plant. Adult monarchs obtained from the University of California-Irvine campus and a captive colony housed at University of California-Davis were caged and oviposited on *Asclepias curassavica* as a source of neonate caterpillars. Oleander aphids were similarly collected from the University of California-Irvine campus and reared on *Asclepias fascicularis*. For aphids, we placed a gravid female on each plant, which was then removed after reproducing, leaving one aphid nymph per plant. After 7 days, we exhaustively searched each plant for caterpillars and aphids, assuming that any missing herbivores had died. This assumption is supported by the fact that neonate movement among plants is extremely difficult (both in greenhouse and field conditions), and we found only a single instance of two herbivores on the same plant (two monarch larvae on *A. humistrata*). We maintained plants in their respective water regimes during the bioassay period for a total of 3 weeks (2 weeks prior to bioassays and 1 week during the bioassay) under the water manipulation treatments.

2.5 | Plant trait selection and measurements

2.5.1 | Plant trait selection

We selected a set of leaf traits, each associated with water-use strategies and host quality to herbivores. For water-use-associated traits, we selected SLA, relative water content (RWC), intrinsic WUE and maximum stomatal conductance ($g_{s\ max}$), all traits that have previously been associated with drought tolerance and/or avoidance strategies (Passioura, 1996; Taiwo et al., 2020; Touchette et al., 2007; Volaire et al., 2014). Low SLA (or high leaf mass area, its inverse) acts as a drought avoidance strategy to prevent water loss (Reich, 2014; Wright et al., 2001, 2005). High $g_{s\ max}$ values are associated with fast-growing acquisitive plant species that maximize photosynthesis and are less drought tolerant, while species with low $g_{s\ max}$ are typically slow-growing, water conservative, and thus are more drought tolerant (Reich, 2014; Wright et al., 2001). Plant species with drought tolerance strategies can also operate at low water content through resilience in plant metabolism (Taiwo et al., 2020). Species with high WUE, defined as the amount of water used (through transpiration) per unit dry matter produced (Touchette et al., 2007), are able to operate under water-deficit conditions (Lopez-Iglesias et al., 2014; Touchette et al., 2007; Volaire et al., 2014). However, intrinsic WUE has also been associated with adaptation to aridity gradients and growth strategies (Moreno-Gutiérrez et al., 2012). For host-quality traits, we selected traits shown to affect herbivore performance under drought: nutrients content (protein, non-structural carbohydrates and nitrogen; Couture et al., 2015; English-Loeb et al., 1997; Gutbrodt et al., 2011, 2012; Lenhart et al., 2015; Mattson & Haack, 1987) and defensive traits (latex and cardenolides; Couture et al., 2015; Hahn & Maron, 2018). We recognize many other host

quality traits could be affected by drought and could have been included in our analysis. For example, phenolic compounds have been found to increase under plant drought stress in several studies (Sarker & Oba, 2018; Turtola et al., 2005, among others) and these changes influence herbivore performance under drought (i.e. English-Loeb et al., 1997; Estiarte et al., 1994). To reduce type 1 error, we limited the number of traits used in our analysis to the defence traits most representative of the milkweed system and nutritional traits that have been found to play an important role in herbivore drought response.

2.5.2 | Plant trait measurements

Trait measurements were obtained from 3 to 5 newly expanded and undamaged leaves collected from each experimental plant at the conclusion of the greenhouse experiment. Excised leaves were transferred to a paper envelope and immediately frozen at -20°C for later characterization of plant traits. Due to the relatively short duration of the experiment, all leaves were formed prior to the initiation of the drought treatment. For water-use-associated traits, we measured leaves from plants in the control treatment as representative of their constitutive trait values because we were interested in testing whether plant adaptation to aridity gradients—not plant drought response—mediate herbivore response to drought. To measure SLA, leaf area (cm^2)/dry mass (mg), frozen leaves were scanned, weighed, dried at 60°C for 2 days, and reweighed to the nearest mg. Leaf water content was calculated as percentage, estimated from the difference between leaf fresh weight and leaf dry weight. Dried leaves were pulverized using a Mixer Mill (Retsch MM 400) for stable isotope and macronutrient analyses. Foliar carbon isotopes ratio (^{13}C and ^{12}C) was estimated from pulverized foliar tissue as an indicator of WUE for a subset of samples ($n = 46$) at the UC-Irvine Mass Spectrometry facility. Plants with high WUE tend to be less fractionated in ^{13}C and therefore have less negative $\delta^{13}\text{C}$ values (Moreno-Gutiérrez et al., 2012). Maximum stomatal conductance ($g_{s\text{ max}}$) was the raw stomatal conductance values achieved by each species when at its maximum level of stomatal conductance.

To quantify drought-induced changes in host plant quality, we measured plant defences and nutritional content of plants in control and drought treatments. Latex exudation and cardenolides concentration were measured as the typical plant defensive traits in milkweeds (Agrawal & Fishbein, 2006). Latex exudation was estimated by excising 2–3 mm off the tip of a new, undamaged, fully extended leaf. The latex produced within 30 seconds was collected on pre-weighed 1 cm^2 filter paper discs which were then placed in pre-weighed 2 ml Eppendorf tubes. The tubes were immediately reweighed to estimate the mass of wet latex collected (Mooney & Agrawal, 2008). This procedure was carried out in the greenhouse after leaf collection to avoid inducing plant chemical defences. Cardenolides concentrations were analysed from a subsample of plants ($n = 47$) from pulverized foliar tissue. We determined the concentration of cardenolides (mg / g dry tissue) from pulverized foliar

tissue ($n = 47$) by high-performance liquid chromatography following the methods of Züst et al. (2019) in the Ecology and Evolutionary Biology department at Cornell University (Supplementary material 1).

The nutritional profiles of milkweed were characterized from pulverized leaf tissue by quantifying nitrogen, protein and total non-structural carbohydrate concentrations for a subset of samples ($n = 46$). An increase in nitrogen and carbohydrates as a result of osmotic adjustments under drought is the main proposed mechanism for increased herbivore performance under drought (Bauerfeind & Fischer, 2013; Lenhart et al., 2015; Mattson & Haack, 1987). Nitrogen content (mg/g of dry tissue) was estimated during stable isotope analysis at the UC-Irvine Mass Spectrometry facility. Soluble carbohydrates and carbohydrates from starch were extracted separately following protocols described by Chow and Landhausser (2004) and quantified colourimetrically using the phenol-sulfuric acid assay (DuBois et al., 1956) optimized for microplate reading (Masuko et al., 2005). Protein was extracted by sonication following protocols described in Lenhart et al. (2015) and quantified colourimetrically using the Bio-Rad Bradford micro assay (Bradford, 1976) in a microplate reader. Total carbohydrates and protein content were also estimated in a per mass basis (mg/g dry mass). Refer to the supplemental material for a detailed description of carbohydrate and protein extraction and analyses (Supplementary material 1).

2.6 | Statistical analysis

2.6.1 | Overview

Our general approach to the analysis was to (i) measure trait values associated with aridity gradients in all milkweed species, (ii) quantify variation in drought effects on host plant quality and herbivore response with an effect size metric (log response ratio of drought vs. control), (iii) test for milkweed species variation in herbivore response to our imposed drought stress, and (iv) explore the mechanisms underlying this variation separately for each herbivore through species correlations between drought effect size on herbivores and plant species traits related to adaptation to aridity (four traits) and plant quality (five traits) (Figure 1). Therefore, this study addressed the same hypotheses with respect to two separate herbivore species a plant traits. Furthermore, these traits could reasonably be assessed based on either variation in constitutive values (i.e. under the control treatment) or changes in those trait values induced by drought. We took several approaches to address the concern for inflated type 1 error given the large number of tests (i.e. 13 species, two treatments, nine traits) while not unnecessarily reducing statistical power (Garcia, 2004).

We chose to assess the effects of constitutive values (only) for traits underlying adaptation to arid versus mesic environments, and we did not assess whether drought altered these traits or whether such changes in traits explained variation in herbivore response. This approach is consistent with our hypothesis that variation in

the adaptation of milkweed species to drought drives variation in herbivore response to drought. Although we explored reducing the dimensionality of the trait data through principal component analysis (PCA), the resultant PCs did a poor job of explaining variation in drought adaptation traits (Supplemental material 2). Consequently, we tested our hypotheses with trait-by-trait analyses.

In contrast to aridity-associated traits, we choose to assess changes in trait values for plant quality traits. This approach is consistent with our hypothesis that variation in drought effects on herbivores is mediated by changes in the quality of milkweed species in response to drought. Here, to reduce type 1 error, we performed a single multivariate test to determine whether there was species variation in the response of host plant quality traits to drought, but then performed a trait-by-trait analysis to associate those changes with herbivore response.

We tested for phylogenetic signal for each set of traits in the 'phylotools' R package (Revell, 2012) and corrected for potential effects of phylogenetic signal using a phylogenetic independent contrasts approach (PIC; Freckleton, 2000).

Finally, we explored the overall pattern in our results using the *dbinom* R function to quantify the probability of the observed number of significant ($\alpha = 0.05$) associations between plant traits and herbivore response based on the total number of tests conducted. All statistical analyses were performed in R studio (R Studio Team, 2015; version 3.5).

2.6.2 | Drought effects on host plant quality traits

To test whether plant defensive traits and plant nutritional content were affected by the water treatment, we first conducted a PerMANOVA. Then we identified which specific traits were affected by drought by conducting nonparametric ANOVAs based on permutations using cardenolides, latex, nitrogen, protein and carbohydrate content as the representative plant defences and nutritional quality traits.

2.6.3 | Drought effects on herbivore survival

We used logistic regression models to test for the main drought effect and the interactive effect of drought and *Asclepias* species on the survival of both herbivores. To examine whether *Asclepias* species transmit similar drought effects to both herbivores, we first calculated effect sizes (log response ratios, Ln R) using the formula $\text{Ln R} = \ln(\bar{x}_1 / \bar{x}_2)$, where $\bar{x}_1$ represents the average herbivore survival for a given species in the drought treatment and $\bar{x}_2$ was the average herbivore survival of given species in the control or the well-watered treatment (Cohen, 1988). A positive Ln R indicates that drought increased herbivore survival, 0 indicates no effects and a negative Ln R indicates that drought decreased herbivore survival in a given species. We then conducted correlations of these effect size values to detect associations between

the drought effect on monarchs and the drought effect on oleander aphids.

2.6.4 | Drought effect on herbivores as mediated by drought adaptation traits

We tested for correlations between each plant trait associated with aridity gradients (SLA, WUE, RWC and $g_{s \max}$) and drought effects on the survival of the two herbivores (Ln R).

2.6.5 | Drought effect on herbivores as mediated by changes in host plant quality

Once we identified which traits were affected by drought, we examined whether herbivore survival was associated with drought-induced changes in host plant quality. We first estimated the effect sizes (Ln R) for each host plant quality using the same formula, $\text{Ln R} = \ln(\bar{x}_1 / \bar{x}_2)$, but here $\bar{x}_1$ represented the average trait value under drought treatment and $\bar{x}_2$ was the average trait value under the control treatment. Then we tested for correlations between drought effects on each plant quality trait affected by the drought treatment (Ln R) and drought effects on the survival of the two herbivores (Ln R).

3 | RESULTS

3.1 | Variation in aridity-associated plant traits and drought effect on host plant quality

Plant trait values associated with aridity are presented in Figure S1 (Carvajal-Acosta, 2022a). Milkweed species varied considerably in SLA, WUE, RWC and $g_{s \max}$, but did not co-varied as a coordinated syndrome (Supplemental material 2). Our PerMANOVA results revealed that the differences among the values of host plant quality traits were driven by the water treatment ($p = 0.001$) and that this treatment effect also varied among *Asclepias* species (species $\times$ treatment interaction: $p = 0.001$). Our subsequent trait-by-trait analysis showed that all plant quality traits were affected by drought ($p < 0.05$) except for latex flow ($p = 0.152$; Table 2). However, the magnitude and direction of the effects differed among milkweed species. For each of the four affected plant traits, approximately half of the species increased and half decreased host plant quality trait values, with the exception of cardenolides, where most species increased concentrations under drought (eight increased vs. four decreased) (Figure S5; Carvajal-Acosta, 2022b).

3.2 | Drought effect on herbivore survival

Monarch survival was 71% across all *Asclepias* species and water treatments. The effect of drought treatment on monarch survival

differed among *Asclepias* species (plant species $\times$ treatment interaction, $p = 0.043$; main effects: species $p = 0.09$ and treatment $p = 0.28$) (Figure S5-A; Carvajal-Acosta, 2022a, 2022b). Oleander aphid survival was 56% across all *Asclepias* species and water treatments. Similarly, the effect of drought on aphid survival differed among host species (plant species $\times$ treatment interaction, $p = 0.09$) and was strongly influenced by *Asclepias* host species ($p = 0.002$) (Figure S5-B).

We found no correlation between drought effects (Ln R) on monarch and oleander aphid survival among plant species ($r^2 = 0.02$, $p = 0.612$) indicating that *Asclepias* species differentially transmitted drought effects to these herbivores in contrasting feeding guilds (Figure 3 and Figure S5). Milkweed species occurred in all quadrants of the correlation plane, with five milkweed species transmitting concordant drought effects to the two herbivores (three positive, two negative), two species transmitted contrasting effects (i.e. positive effects on one herbivore but negative effects on the other) and four species transmitted no effect to one herbivore and either positive or negative effects on the other (Figure 2).

3.3 | Drought effect on herbivore survival as mediated by plant traits associated with aridity gradients

Phylogenetic signal was weak for all plant traits associated with aridity gradients (Table 1); therefore, we interpreted our results based on RAW correlations. For completeness, we report both RAW and PIC results in Table 1 and PIC correlations are shown in Figure S6. Drought effects on monarch survival (Ln R) varied from positive to negative and survival was positively correlated with two traits associated with drought tolerant strategies, WUE and RWC. Specifically, species with higher WUE (less negative $\delta^{13}\text{C}$ values) led to higher survival of monarchs under drought conditions (Ln R) ($p = 0.015$, $r^2 = 0.42$). The effects of drought on monarch survival (Ln R) were negatively correlated with RWC ($p = 0.058$, $r^2 = 0.28$; Table 1) so that monarch performed better under drought conditions in those species with low RWC (Figure 4). Conversely, SLA and $g_{s_{\max}}$ values

were not correlated with monarch survival ($p = 0.586$ and $p = 0.782$, respectively).

Drought effects on oleander aphid survival (Ln R) also ranged from positive to negative but were uncorrelated with *Asclepias* aridity-associated traits. We found no significant correlations between SLA, water content, WUE, or $g_{s_{\max}}$ and drought effects on oleander aphid survival (Ln R) (Table 1, Figure 3; PIC correlations shown in Figure S6).

3.4 | Drought effect on herbivore survival as mediated by changes in host plant quality traits

Phylogenetic signal was weak for most drought-induced changes in plant-quality traits except for changes in total carbohydrate content (Table 3). Thus, we interpreted our results based on RAW correlations but report both PIC and RAW results. Drought effect on both herbivores survival was uncorrelated with drought-induced changes in nutritional content (nitrogen, protein, and total carbohydrates). Latex flow was excluded from this analysis because it was not affected by drought (Table 2). We detected a significant correlation between drought effect on cardenolide concentrations and drought effects on both, monarch and oleander aphid survival (Ln R; $p = 0.037$ and $p = 0.005$, respectively; Table 3). Specifically, species where drought increased cardenolides concentrations, were associated with negative drought effects on oleander and (Ln R; Figures 3 and 4). PIC correlation shown in Figure S7.

3.5 | Inflated type 1 error

Overall, we tested for 8 associations between plant traits and the performance of each herbivore, for 16 total associations, and detected five significant results. The probability of five significant ($\alpha = 0.05$) associations out of 16 tests is low ($p = 0.0008$). Inspecting the responses of monarch and oleander aphids separately, the probability of detecting four significant associations in monarchs by chance alone is low ($p = 0.0004$), while one significant association in

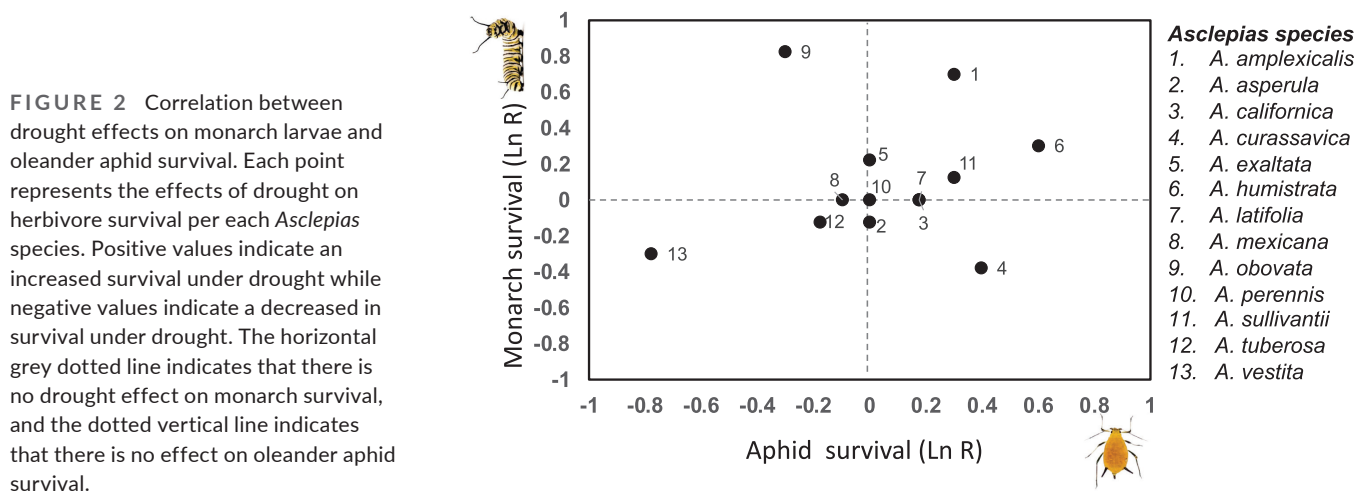


TABLE 1 Effect of drought on herbivore survival mediated by plant trait values associated with aridity gradients

Species traits	Phylogenetic signal	Monarch (<i>p</i> -values, <i>r</i> ²)				Aphid (<i>p</i> -values, <i>r</i> ²)			
		Raw (<i>p</i>)	Raw (<i>r</i> ²)	PIC (<i>p</i>)	PIC (<i>r</i> ²)	Raw (<i>p</i>)	Raw (<i>r</i> ²)	PIC (<i>p</i>)	PIC (<i>r</i> ²)
SLA	$\lambda = 7.34 \times 10^{-5}$ ^a	0.586	0.02	0.20	0.066	0.436	0.04	0.63	0.02
RWC	$\lambda = 7.34 \times 10^{-5}$ ^a	0.019*	0.28	0.058	0.28	0.362	0.07	0.57	0.03
<i>g</i> _{s max}	$\lambda = 7.34 \times 10^{-5}$ ^a	0.782	0.007	0.053	0.29	0.568	0.03	0.39	0.06
WUE	$\lambda = 4.92 \times 10^{-5}$ ^a	0.042*	0.32	0.015*	0.42	0.937	0.006	0.10	0.21

Note: Latex was not found to be affected by drought and therefore was not included in this analysis. *g*_{s max} is maximum stomatal conductance; RWC stands for relative water content; SLA stands for specific leaf area, and WUE stands for water-use-efficiency measured as $\delta^{13}\text{C}$.

^aPhylogenetic signal test performed on the effect sizes of traits. $\lambda = 0$ indicates no phylogenetic signal, whereas $\lambda = 1$ indicates a strong phylogenetic signal.

*Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

aphids could have been detected by change ($p = 0.28$), out of eight respective set of tests. Thus, while we have evidence that our measured traits mediated drought effects on monarchs, it is less clear for aphids.

4 | DISCUSSION

In this study, we proposed that aridity-associated plant traits may underlay herbivore response to plant drought stress. We tested this hypothesis in the *Asclepias* genus and two herbivore specialists with distinct feeding strategies. The species in the *Asclepias* plant genus have diversified into a variety of environments and employ similar herbivore defensive mechanisms. With a physiologically customized treatment, we show that the effects of drought on herbivores varied dramatically among these closely related plant species—in both magnitude and direction—ranging from strong decreases to increases in the survival of both herbivore species on drought stressed compared to control plants (Figure 2 and Figure S5). Past attempts at predicting drought stress effects on insects have struggled to unify varying empirical results without regard to plant traits, focusing on herbivore feeding guild or the characteristics of the drought. While such factors are undoubtedly important, our findings clearly establish that plant traits associated with aridity gradients can play a central role in mediating drought effects on herbivores.

The effects of drought on herbivores varied tremendously among milkweed species but were uncorrelated between our two species in different feeding guilds (Figure 2), suggesting that the effects of drought may be driven by mechanisms that differ between both herbivores. These results are consistent with previous studies indicating that feeding guilds respond to plant drought stress differently (Gely et al., 2020; Huberty & Denno, 2004; Koricheva & Haukioja, 1997). This is because certain feeding guilds may be more responsive to water-stressed plants than others because they differentially experience changes in plant nutrition, allelochemistry and growth (the insect performance hypothesis; Larsson, 1989). It is worth noting that this study focuses on the effect of drought on only two species representing distinct feeding guilds. Although it

is possible that different herbivore species belonging to the same feeding guild may perform differently under plant drought stress, previous metanalysis have shown that species belonging to the same feeding guild tend to exhibit similar responses to drought (Huberty & Denno, 2004; Jactel et al., 2012; Koricheva & Haukioja, 1997). Similarly, we investigated the effects of drought in highly specialized herbivore species. It has been suggested that plant drought stress may affect specialized insects differently than generalist, with specialist benefiting from moderate drought and generalist from severe droughts (Gely et al., 2020). White (2009) suggested that trophic sub-guild may also be an important factor on herbivore drought response, as senescence feeders may respond positively to drought, while flush feeders may be negatively affected. Thus, it would be important to test the generality of these finding in other less specialized systems, multiple herbivore species, as well as different herbivore sub-guilds.

For leaf-chewing monarchs, we found evidence that the variation in drought effects was associated with plant traits values associated with aridity gradients. These results are consistent with our proposed hypothesis that species with plant traits values associated with aridity adaptation transmit positive drought effects to herbivores, whereas plant traits values associated with mesic environments transmit negative drought effects. Intrinsic WUE and RWC were each associated with drought effects on monarch survival in the predicted manner, indicating that species with drought tolerant strategies transmit positive drought effects to monarchs (Table 1, Figure 3). In contrast, SLA and *g*_{s max} were uncorrelated with monarch survival. If the selected traits co-vary as a coordinated syndrome that confer arid-adapted species drought resistance, we would expect a correlation between monarch survival and all aridity associated traits. However, the lack of co-variation of these traits in our PCA (Supplementary material 2) suggests that *Asclepias* species may have adapted unique water-use strategies when diversifying into different habitats. Alternatively, it is also likely that SLA and *g*_{s max} values do not represent a drought adaptation strategy in this genus. Thus, although intrinsic WUE and RWC explained monarch response in drought stressed *Asclepias*; this may not be the case in other plant taxa with different water-use strategies.

FIGURE 3 Raw correlations between plant trait values associated with aridity gradients and drought effects on monarch larvae (top panel) and aphid (lower panel) survival. Each point represents the mean constitutive trait values of each milkweed species. The grey dotted line indicates that there is no effect of drought on herbivore survival. *Asclepias* species are numbered as follows: 1. *A. amplexicalis*, 2. *A. asperula*, 3. *A. californica*, 4. *A. curassavica*, 5. *A. exaltata*, 6. *A. humistrata*, 7. *A. latifolia*, 8. *A. mexicana*, 9. *A. obovata*, 10. *A. perennis*, 11. *A. sullivantii*, 12. *A. tuberosa*, 13. *A. vestita*. PIC correlations are reported in Figure S6.

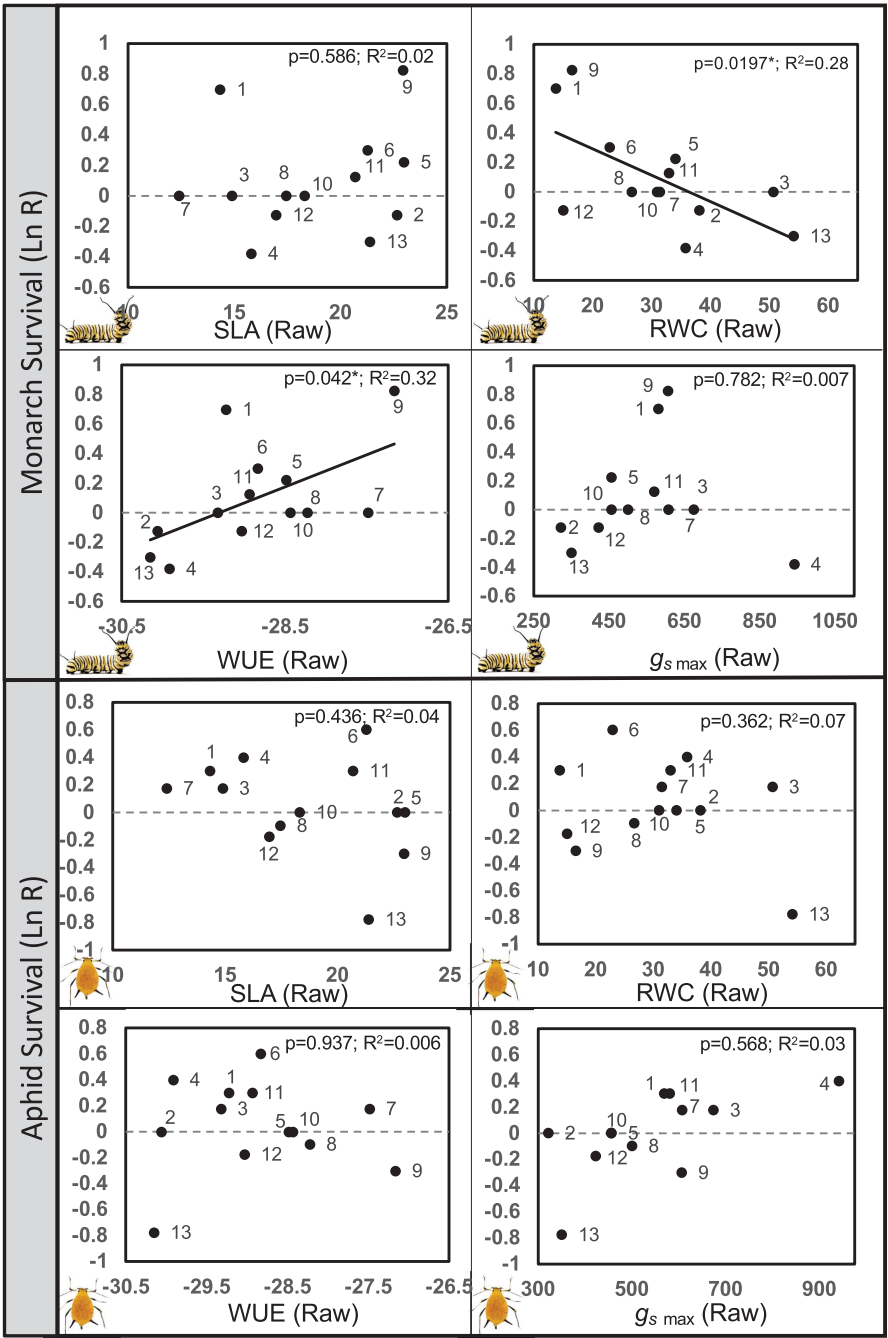


TABLE 2 Effect of drought on plant traits values associated with plant quality

Trait	Species	Treatment	Species × Tmt
Cardenolides	<0.001***	<0.001***	<0.001***
Latex	<0.001***	0.1528	0.958
Nitrogen	0.002***	0.0506	0.002***
Protein	<0.001***	<0.001***	<0.001***
Total carbohydrates	<0.001***	<0.001***	<0.001***

Note: Significance codes: 0 (*) 0.001 (***) 0.01 (*) 0.05 (.) 0.1 (.) 1.

We proposed that plant species with traits values associated with aridity maintain homeostasis and increase in quality transmitting positive drought effects to herbivores whereas plants with mesic traits values are expected to decline in quality transmitting negative drought effects. Indeed, monarch survival was driven by drought-induced changes in chemical defences, with monarch survival decreasing on milkweed species that increased cardenolides concentrations under drought (Table 3; Figure 3). Our results are in agreement with past research suggesting that plant secondary chemistry plays a major role in driving herbivore drought responses (i.e. Couture et al., 2015; English-Loeb et al., 1997; Gutbrodt et al., 2011;

TABLE 3 Effect of drought on herbivore survival mediated by drought-induced changes in plant quality traits

Species traits (ln R)	Phylogenetic signal	Monarch (<i>p</i> -values, <i>r</i> ²)				Aphid (<i>p</i> -values, <i>r</i> ²)			
		Raw (<i>p</i>)	Raw (<i>r</i> ²)	PIC (<i>p</i>)	PIC (<i>r</i> ²)	Raw (<i>p</i>)	Raw (<i>r</i> ²)	PIC (<i>p</i>)	PIC (<i>r</i> ²)
Cardenolides	$\lambda = 7.34\text{e-}05^{\text{a}}$	0.037*	0.33	0.584	0.02	0.058	0.29	0.005*	0.52
Nitrogen	$\lambda = 7.34\text{e-}05^{\text{a}}$	0.301	0.12	0.29	0.10	0.852	0.003	0.214	0.13
Protein	$\lambda = 7.34\text{e-}05^{\text{a}}$	0.529	0.03	0.402	0.06	0.743	0.01	0.126	0.19
Total carbohydrates	$\lambda = 0.91879^{\text{a}}$	0.819	0.004	0.571	0.03	0.390	0.06	0.983	<5 ^{-e05}

Note: Latex was not found to be affected by drought and therefore was not included in this analysis.

^aPhylogenetic signal test performed on the effect sizes of traits. $\lambda = 0$ indicates no phylogenetic signal whereas $\lambda = 1$ indicates a strong phylogenetic signal.

*Significance codes: 0 '****' 0.001 '***' 0.01 '**' 0.05 '.' 0.1 '' 1.

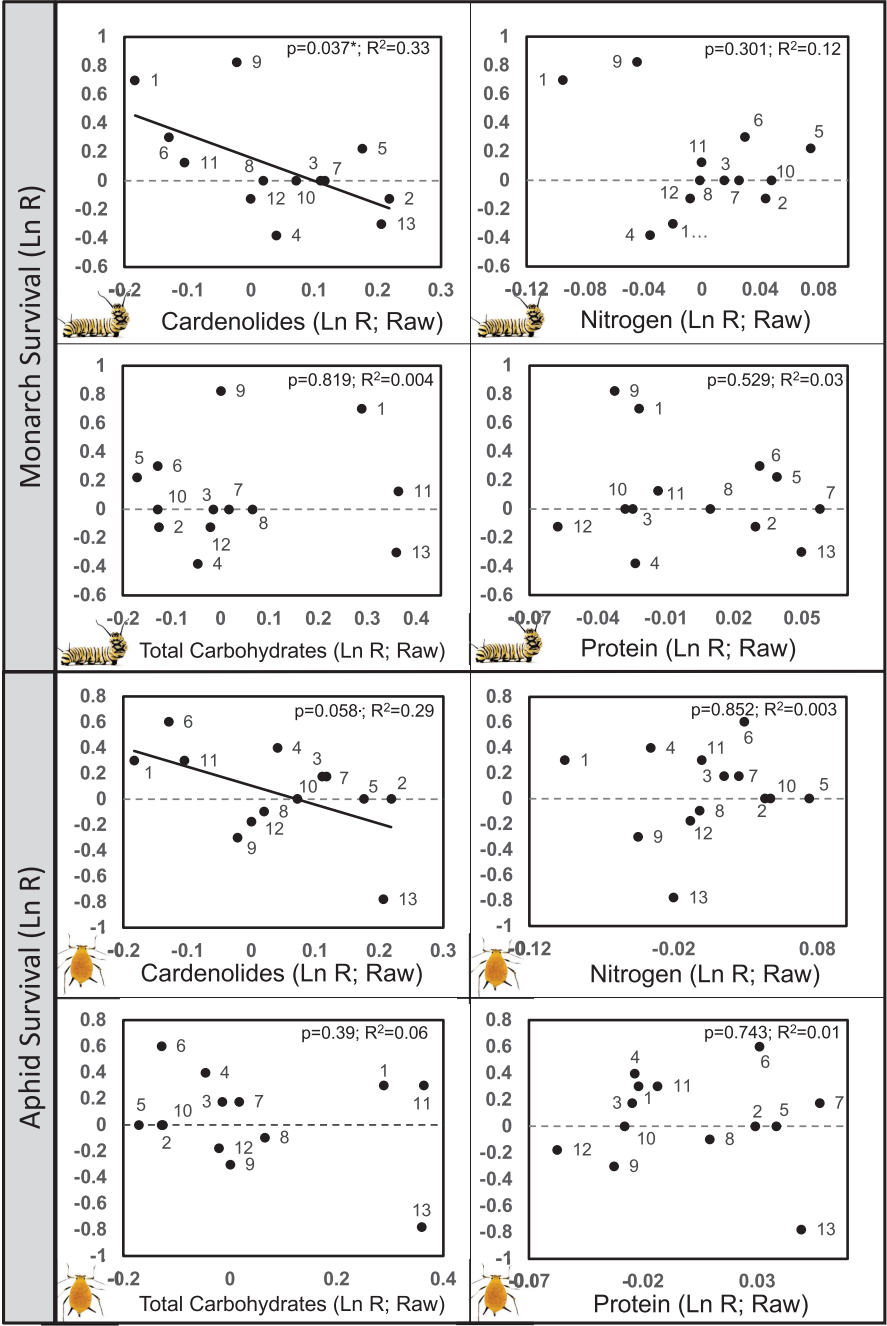


FIGURE 4 Raw correlations between drought effects on plant quality traits and drought effects on monarch (top panel) and aphid (lower panel) survival. Each point represents the effect size of drought on plant quality trait values of each milkweed species. The grey dotted line indicates that there is no effect of drought on the survival of oleander aphids. 1. *A. amplexicalis*, 2. *A. asperula*, 3. *A. californica*, 4. *A. curassavica*, 5. *A. exaltata*, 6. *A. humistrata*, 7. *A. latifolia*, 8. *A. mexicana*, 9. *A. obovata*, 10. *A. perennis*, 11. *A. sullivantii*, 12. *A. tuberosa*, 13. *A. vestita*. PIC correlations are reported in Figure S7.

Kuczyk et al., 2021), although drought stress altered plant chemistry and herbivore performance in contrasting ways. Regarding our finding about the lack of drought effect on latex flow, although we expected drought to negatively affect latex flow, we were not surprised by these results. Past drought experiments on *A. syriaca* show that water stress had either no effect (Hahn & Maron, 2018) or caused a very small decrease in latex production (Couture et al., 2015).

Conversely, drought-induced changes in milkweed nutritional quality (total carbohydrates, nitrogen and protein content) appeared to have no effect on monarch response to plant drought stress (Table 2; Figure 5). Increases in nutritional compounds in drought-stressed plants have been proposed as the main mechanism driving herbivore performance under drought conditions (PSH; White, 1969), but empirical evidence has been mixed regarding their importance (i.e. Lenhart et al., 2015; Walter et al., 2012). This includes studies involving *Asclepias* species. For example, Hahn and Maron (2018) found no evidence that monarch mortality in drought-stressed *A. syriaca* was associated with changes in nutritional content (nitrogen) and speculate that monarch's drought response was driven by changes in cardenolides, but this trait was not measured in this study. In contrast, Couture et al. (2015) found that monarch larvae performed better in *A. syriaca* subjected to intermittent water stress and was associated with an increase in nitrogen foliar content. One plausible explanation for the lack of association between nutrients and monarch survival is that our assessment of fitness was based on survival, and changes in nutritional content may be more evident in other components of fitness such as growth or reproduction. In addition to survival, larval weight is an important component of herbivore fitness that influences survival and adult reproductive success (Coley et al., 2006; Grafen, 1988; Travers-Martin & Müller, 2008). However, we were unable to explore the effects of drought on larval mass because the effects of drought on monarch mortality were so strong in some species that replication was insufficient. In this regard, it has been suggested that, while an increase in nutrient content during drought can make more nutrients available to herbivorous insects, increased production of plant defences is likely to offset potential positive effects on plant nutritional quality (Gely et al., 2020). Therefore, it is also possible that changes in cardenolides concentrations may have a stronger effect due to higher herbivore mortality than drought-induced changes in nutritional content, which is more likely to affect growth and reproduction.

In contrast to the monarch's findings, our hypothesis was not supported for the oleander aphid, whose performance under drought was not correlated with plant drought traits (Table 1; Figure 4). However, the performance of oleander aphids was associated with drought-induced changes in cardenolides concentrations in the same manner as monarchs. Sap-feeders have traditionally been predicted to benefit from higher nutrient content in drought-stressed plants (Larsson & Björkman, 1993) but, similar to monarchs, drought-induced changes in plant nutritional quality (carbohydrates, protein and nitrogen) did not influence oleander aphid survival. One potential explanation for the lack of response to changes in nutritional content is that our drought treatment simulated a continuous rather

than an intermittent drought; thus, nutrient effects might have become apparent only with a return of plant turgor as suggested by the pulse stress hypothesis (Huberty & Denno, 2004). As mentioned previously, it is also likely that other components of fitness not measured here were impacted, or that aphids responded more strongly to changes in chemical defences than in nutrient concentrations. It is interesting to note the consistent response to drought-induced changes in cardenolides in both feeding guilds despite the distinct correlation patterns with aridity-associated traits. Unlike monarchs, oleander aphid survival was more strongly affected by the identity of the host species than the drought treatment. Therefore, it is possible that intra-specific variation in traits not measured in this study (i.e. trichomes) better explained aphid survival than aridity-related traits.

We note that our interpretation of the findings is based on raw and not phylogenetically corrected trait values as we detected no phylogenetic signal in our analysis for most water-use and host quality-associated traits (Tables 1 and 3). We recognize that estimating phylogenetic signal can be problematic with small datasets or highly under-sampled phylogenies (Münkemüller et al., 2012) and including a larger number of species may have yielded different results. However, previous studies have successfully estimated phylogenetic signal in the milkweed system consistent with Brownian motion ($\lambda = 1$) with 10–20 *Asclepias* species (Rasman & Agrawal, 2011). Nevertheless, our phylogenetically corrected and raw trait analysis do not differ significantly, and we report both for comparison.

Unstudied here, but potentially important, is how variation in drought characteristics mediate herbivore responses (Sconiers & Eubanks, 2017). For instance, intermittent versus continuous drought events have been shown to have contrasting effects on insect herbivores, not only for sap-feeders (Huberty & Denno, 2004; Koricheva et al., 1998) but also for leaf chewers (Mody et al., 2009). Similarly, variation in the duration, intensity and timing of the drought may have nonlinear effects on plant nutrients and secondary chemistry, and consequently on herbivore preference or performance (English-Loeb et al., 1997; Gutbrodt et al., 2011, 2012; Lenhart et al., 2015; Luo & Gilbert, 2021). In this study, we standardized the drought treatment to elicit a comparable physiological response to drought across species. However, given the nonlinearity of drought effects, a more intense (or less intense) drought can yield different results. Furthermore, recent research suggests that drought sensitivity increases with dryness as plants from more arid environments have faster and stronger responses to drought than their counterparts along an aridity gradient (Xu et al., 2021). An aspect worth exploring in future studies is whether arid- versus mesic-adapted species differentially respond to various drought durations and/or intensities. Although the characteristics of the drought do matter, our goal was to assess the importance of aridity-gradient modulated traits in mediating drought effects on herbivores while avoiding confounding effects of acclimation or compensation (i.e. through the production of new leaves with altered traits). Thus, we measured herbivore performance within a relatively short drought period so that insects responded to rapid changes in

plant physiological responses and not compensation or acclimation. Finally, it should be noted that our drought treatment elicited a physiological response in all milkweed species, as drought affected almost all plant traits associated with host plant quality except for latex flow (Table 2 and Figure S4). We also noted other signs of plant physiological stress, such as leaf shedding. However, milkweed species exhibited a wide range of responses to drought stress, with some *Asclepias* species either increasing or decreasing in host plant quality (i.e. toxicity and nutrients) under drought conditions (Figure S4).

To our knowledge, this represents the first study to systematically test for the role of plant traits in mediating drought response to herbivores by comparing herbivore drought response on multiple plant species adapted to contrasting environments and ensuring that all plant species experienced the same level of drought stress. We showed that plant trait variation associated with aridity gradients could be the key to predicting the effects of drought on herbivores. For two distinct feeding guilds, we observed the dynamics predicted by both, the PSH and PVH (drought increasing and decreasing herbivore performance, respectively) depending on host plant species; thus, plant traits may be important in resolving the apparent conflict between these hypotheses. It is worth noting that we tested our hypothesis with respect to recently evolved trait variation among a genus of herbaceous plants and it will be important to test whether these dynamics hold in a community context with co-existing plant species and multiple leaf-feeding and sap-feeding taxa. However, this may be challenging as more distantly related plant species may vary not just in the magnitude of trait expression, but also in the type of traits. Another aspect worth exploring is whether the characteristic of drought (duration or intensity) interacts with the adaptation of the plant to aridity gradients. In this sense, it would be important to test our framework within a wider range of drought types and/or water-use strategies (i.e. acclimation). In conclusion, resolving some of the variation in how drought stress impacts herbivores can be achieved by linking plant drought adaptation strategies to functional traits that span abiotic and biotic interactions. Progress in this field is important given projections for the increasing frequency and severity of drought with climate change.

AUTHOR CONTRIBUTIONS

Kailen Mooney conceived the ideas, designed methodology and contributed to data analysis and interpretation. Nayeli Carvajal contributed to conception of experimental design, conducted greenhouse experiment and plant nutrient analyses, collected and analysed the data and led the writing of the manuscript. Anurag Agrawal contributed to the experimental design, plant seed material, chemical analyses and manuscript editing. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

PEER REVIEW

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/1365-2745.14059>.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are available in the Dryad Digital Repository at <http://doi.org/10.7280/D16989> (Carvajal-Acosta, 2022b) and <http://doi.org/10.7280/D12H6C> (Carvajal-Acosta, 2022a).

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