



ARTICLE

Climate Ecology

Climatic history, constraints, and the plasticity of phytochemical traits under water stress

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Funding information

Alexander von Humboldt-Stiftung; University of Nevada, Reno; NSF Graduate Research Fellowship Program

Handling Editor: Debra P. C. Peters

Abstract

Environmental stress can induce changes in organismal traits and in resulting intraspecific variation. The nature of such effects will depend on the plasticity of trait expression and on any ecological constraints to such expression. Plants can mitigate abiotic stress, like drought, by changing their chemistry, but the ability to induce costly metabolites may be under strong local selection and ecologically constrained. Here, we asked whether climate at the seed source predicts plant chemical plasticity in response to water stress and what the consequences are for intraspecific variation in phytochemical traits. To this end, we used common gardens of two widespread species of western milkweed (*Asclepias fascicularis* and *Asclepias speciosa*) that had been collected from sites across an aridity gradient. Both species produce high concentrations of leaf flavonols, which are hypothesized to mitigate water stress by functioning as antioxidants. These compounds were found in higher constitutive concentrations in plants sourced from drier sites, and both species responded to water stress in the common garden by increasing leaf flavonol concentrations. Interestingly, flavonol plasticity was higher in plants sourced from wetter sites in *A. fascicularis*, with similar, but weaker, patterns in *A. speciosa*. These opposing patterns in constitutive and induced flavonol expression reduced the variation between populations in leaf flavonol concentrations under water stress. These results suggest that local adaptation in plants can shape phytochemical strategies for water limitation but that the cost of metabolite production may ultimately limit the range of phytochemical variation.

KEYWORDS

co-gradient plasticity, constraints, drought, intraspecific variation, milkweed, phytochemistry, resource availability

INTRODUCTION

Intraspecific variation may increase diversity and resilience in ecological communities (Bolnick et al., 2011), but

it remains unclear what environmental factors favor such variation (Kuppler et al., 2020). For example, theory can alternatively predict stressful environmental conditions to increase or to decrease intraspecific variation (Hoffmann &

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Merilä, 1999). Empirically, stress appears to reduce genetically determined phenotypic variance (i.e., heritability) within populations, but this effect is stronger in some traits than in others (Charmantier & Garant, 2005). Stress can also influence the magnitude of phenotypic variation among populations, but if there is a directional link, it is not yet clear (Matesanz & Ramírez-Valiente, 2019). Given that global change is exerting increasing stress on organisms worldwide (Orr et al., 2020), a better understanding of how such conditions will affect phenotypic variation within species is necessary.

Phenotypic variation in the same environment may be driven either by variation in fixed genetic traits or by genetic variation in plasticity (i.e., gene-by-environment interactions, $G \times E$). Within a set of genotypes, then, any changes in phenotypic variation with environmental stress will be the result of $G \times E$. The mechanisms that favor more plastic genotypes may depend on spatial scale: Differential selection and drift are more likely among populations than within them. Among populations, for example, higher plasticity could be adaptive in populations that have experienced more, and more predictable, climatic variation (Leung et al., 2020; Pratt & Mooney, 2013). On the other hand, resource availability may consistently constrain plasticity and associated trait expression among individuals regardless of their evolutionary history (Valladares et al., 2007; Van Buskirk & Steiner, 2009). If so, then phenotypic variation may be lower among populations under stress compared with variation among populations in less stressful conditions, similar to an apparent trend of reduced heritability under stress within populations (Charmantier & Garant, 2005). The consequences of such reduced variation could include both reduced survival of individuals and reduced resilience of the ecosystem under rapid environmental change (Fox et al., 2019; Wilcox et al., 2020).

A recent meta-analysis reported that among-population variation in trait plasticity (i.e., $P \times E$) is common in terrestrial plants (occurring in 77% of 151 case studies) (Matesanz & Ramírez-Valiente, 2019). Among the 20 studies that had assessed whether such $P \times E$ effects led to more or less variation among populations in stressful environments, however, there was no clear pattern (Matesanz & Ramírez-Valiente, 2019). Population differentiation in stressful environments was found to be higher, lower, or equivalent to differentiation in less stressful environments in roughly equal proportions. These results should be considered in light of at least two contextual variables: (1) how do we define stress? and (2) which traits?

Stress can be defined most broadly as any time an organism experiences conditions outside its fundamental niche (Steinberg, 2012), but such a broad definition can conflate novelty with unfavorable conditions (Schlichting,

2008). Within populations, unfavorable environmental conditions appear to reduce trait variation, whereas novel conditions do the opposite (Charmantier & Garant, 2005). This distinction may be similarly important among populations. Here, we define stress as severe resource limitation, with the potential for trade-offs in allocation of the resource or its products among biosynthetic pathways (Auld et al., 2010). This definition lends itself, in turn, to a specific consideration of the biochemical traits of individuals, which may provide direct insight into such patterns of allocation (Bradshaw, 1965).

Plants provide particularly interesting study systems for investigating biochemical traits due to their extraordinary chemical diversity, which can drive associated variation in terrestrial food webs and biogeochemical cycles (Hunter, 2016). Phytochemical plasticity can mitigate harm to plants from both abiotic and biotic stresses (Agrawal, 1998; Chaves et al., 2003). Both the production of stress-mitigating secondary metabolites (i.e., trait expression) and the mechanisms allowing for induction of such metabolites (i.e., plasticity) can be costly (Agrawal, 2001; Baldwin, 1998). The magnitude of induced phytochemical responses to an abiotic stress, such as drought, is thus likely to depend both on past selection by the local environment and on present resource availability. Yet, few studies have investigated how stress impacts the magnitude of intraspecific variation in phytochemical trait expression.

Severe and unprecedented droughts are predicted by climate models for the western United States (Cook et al., 2015), where water availability is already a key selective agent in natural plant populations. The Great Basin Desert, a 540,000-km² watershed in the western United States, receives ~80% of its annual precipitation between October and March. Water deficits for plants are thus both variable and predictable within years: low in spring when water is plentiful and temperatures are cooler, and high in late summer when there has been little precipitation for several months and temperatures are high. Indeed, this marked seasonality causes plants at relatively wetter sites to experience higher intra-annual variation in water deficits. The Great Basin also contains hundreds of ranges and drainages, which create heterogeneous water availability on the landscape that favors strong local adaptation in plants (Baughman et al., 2019; Svejcar et al., 2017). Plasticity is likely to be important to plant survival in this heterogeneous environment, and climate change may only amplify its importance (Hendry, 2016).

Here, we asked whether two widely distributed Great Basin plant species exhibit population-specific phytochemical trait expression and plasticity in response to acute water stress. We also asked how these patterns

affect intraspecific variation in phytochemical traits among populations. To answer these questions, we collected seeds of the two most widespread species of western milkweed (*Asclepias speciosa* and *Asclepias fascicularis*) from six sites spanning a 500-mm range in climatic water deficits (i.e., the evaporative demand of vegetation not met by available water; Stephenson, 1998). We define “constitutive” chemistry as the concentrations of metabolites produced under well-watered conditions and “induced” chemistry as that produced under water stress. We hypothesized that climate at the seed-source location would shape constitutive phytochemistry, as well as the degree of phytochemical plasticity in response to acute water stress. With the understanding that producing high concentrations of secondary metabolites can be costly, we predicted that plants from wetter sites would produce lower constitutive concentrations of water-stress-mitigating metabolites. However, because wetter sites can exhibit higher variation than drier sites in water deficits within years, we predicted that plants sourced from wetter sites would also exhibit higher plasticity in the production of such metabolites. Finally, we predicted that higher metabolic costs under water stress would constrain variation in induced phytochemical trait expression relative to that under non-stressful conditions.

MATERIALS AND METHODS

Study system

Asclepias speciosa (showy milkweed) and *A. fascicularis* (narrowleaf milkweed) are widespread in the western United States (Dilts et al., 2019; Woodson, 1954). The two species exhibit distinct morphologies—*A. fascicularis* has narrow, glabrous leaves, whereas *A. speciosa* has wide, pubescent leaves (Agrawal, Fishbein, et al., 2009)—but both can be found across a wide range of water availabilities, including in very dry locations (down to at least 100 mm of annual precipitation). Neither species produces many of the toxic cardenolides for which milkweeds are best known (Rasmann & Agrawal, 2011), but both species produce a broad range of other UV-absorbent secondary metabolites, including flavonol glycosides, small phenolics, and pregnane glycosides (Mundim & Pringle, 2020). We predicted that flavonol glycosides would be particularly likely to respond to drought because flavonols can act as antioxidants, mitigating water stress by scavenging reactive oxygen species (Kaminska-Rozek & Pukacki, 2004). The biological roles of pregnane glycosides in milkweeds are unknown, although they could act as herbivore deterrents, especially in plants with low cardenolide content (Zehnder & Hunter, 2007).

Seed sources

To seed the experiments, we collected seeds from six sites spanning 385 km of the Great Basin Desert, USA, in 2016 (Figure 1). To estimate the typical drought stress at each of the sites, we calculated the cumulative annual climatic water deficit (CWD) (Figure 1; Appendix S1: Methods S1). Climatic water deficit explicitly accounts for how temperature and precipitation interact to affect plant water balance, with temperature driving water demand through increased potential evapotranspiration and precipitation driving the amount of water available in the system (Stephenson, 1990, 1998). Climatic water deficit is the single most important climate variable for predicting the distribution of many plants in the Great Basin (Dilts et al., 2015). The seed-source sites with high CWD (hereafter dry) experience more water limitation on an annual basis than the sites with low CWD (hereafter wet) (Figure 1). From highest to lowest CWD (in millimeters), our seed-source sites were as follows: Fallon, NV (FN, 988.98 mm); Georgetown, CA (CA, 985.68 mm); Pyramid Lake, NV (PL, 919.62 mm); Battle Mountain, NV (BM, 847.74 mm); Reno, NV (RN, 587.19 mm); and Verdi, NV (VE, 460.3 mm) (Appendix S1: Table S1).

Prior to using mean annual CWD as our main predictor, we explored its relationship to other bioclimatic and water-balance variables (Appendix S1: Figure S1). Climatic water deficit was positively related to mean annual temperature (Pearson's $r = 0.77$, $df = 4$, $p < 0.08$) and mean August temperature ($r = 0.95$, $df = 4$, $p < 0.004$). In contrast, CWD was not strongly related to precipitation ($r = 0.10$, $df = 4$, $p = 0.8$), suggesting that water deficits can still be high at wetter sites when temperatures are high. Wetter sites had higher variation than drier sites in water deficits within years ($r = -0.84$, $df = 4$, $p < 0.04$), but all sites showed similar variation in CWD and precipitation between years ($r = -0.39$, $df = 4$, $p = 0.4$ and $r = -0.06$, $df = 4$, $p = 0.9$, respectively).

Experimental design

To determine whether intraspecific trait variation could be predicted by seed-source CWD and how such variation is affected by water limitation, we conducted a drought experiment with *A. fascicularis* and *A. speciosa* in a glasshouse. We germinated 12 *A. fascicularis* seeds from each of three maternal families from all six sites ($N = 216$), and 12 *A. speciosa* seeds from each of three maternal families from four of the sites (FN, PL, BM, and RN; $N = 144$). We randomly assigned six plants from each maternal family to the control (well-watered) treatment and the other six plants to the dry treatment ($n = 36$ per

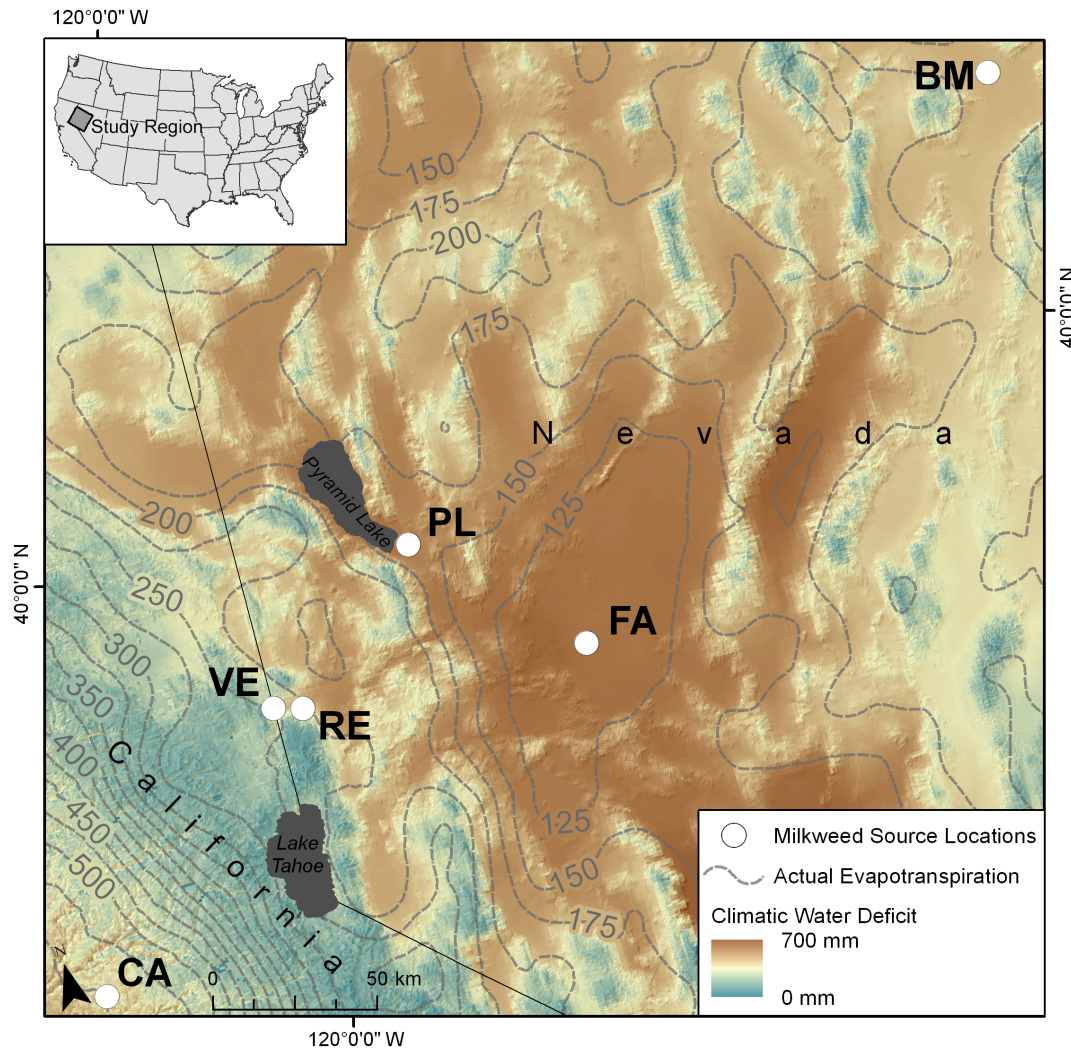


FIGURE 1 Six study sites described by water-balance variables, based on 1981 to 2010 climate normal. Colors on the map show the climatic water deficit gradient, with blue representing less arid areas and brown representing more arid areas. The isolines show actual evapotranspiration (simultaneous availability of water and energy) in millimeters. Sites are (bottom-left to top-right) California (CA), Verdi (VE), Reno (RE), Pyramid Lake (PL), Fallon (FA), and Battle Mountain (BM).

seed-source site). Prior to beginning the dry treatments, mortality was higher for *A. speciosa* than for *A. fascicularis*, such that the final average n was ~ 33 for *A. fascicularis* but ~ 17 for *A. speciosa*.

We used a gravimetric dry-down treatment to expose plants to drought stress for 4 weeks in March–April 2017. Seeds were germinated in petri dishes under lights (L18: D6) at 25°C in November 2016. Plants were grown in 10 × 24 cm treepots containing 1500 g of a mixture of sand, peat moss, and composted bark (2:1:1). Pots were completely randomized on tables in the glasshouse and fertilized weekly with 24:8:16 N:P:K fertilizer. We calculated gravimetric soil water content using 13 treepots with 1500 g of the same soil. Saturated mass was measured 2 h after fully saturating the pots; dry mass was measured after oven drying for 48 h at 90°C. A 100% soil

saturation was estimated as follows: saturated mass – dry mass. We allowed control plants to dry to 70% soil saturation and dry plants to 10% soil saturation. These treatments are, respectively, what these milkweeds might experience at the weedy edge of an irrigated agricultural field in Nevada (Irmak et al., 2007) and the plants' wilting point. In the fourth and last week of the experiment, we increased the dry treatment to 30% soil saturation to maintain plant survival.

Plant traits

To verify the efficacy of our drought treatment and to explore differences in physiological responses to drought, which may mediate the plant's metabolic allocation, we

measured the following plant traits: change in plant height; whole-plant dry biomass of roots and shoots; root:shoot ratio; leaf mass per area (LMA); and stomatal conductance. Plant height was recorded prior to beginning the dry treatment and again prior to harvesting the plants. Roots, stems, and leaves were harvested separately, washed, and dried at 60°C for 72 h before weighing. Tissues were weighed in microcentrifuge tubes, and the weight of the tube was subtracted. Prior to weighing, 41 microcentrifuge tubes that had contained stem tissue were accidentally discarded; we thus present results on both root:shoot ratios and root:leaf ratios. A leaf in position 3 of the phyllotaxis was collected for LMA, which was estimated by dividing the dry weight of the leaf in milligrams by the estimated leaf area in square millimeters (length \times width). Stomatal conductance was measured between 12:00 PM and 2:00 PM using an SC1 porometer (Decagon).

To determine how water stress affected phytochemical trait expression, we measured plant UV-absorbent secondary chemistry. Prior to harvest, leaves and fine roots were collected and stored at -80°C . These tissues were later freeze-dried, ground, and extracted in 100% methanol with a cardenolide internal standard (digitoxin). The UV-absorbent peaks were measured on a high-performance liquid chromatography system with a diode array detector recording peaks that absorbed between 200 and 330 nm (see additional methods in Appendix S1: Methods S2). We retained peaks for our analysis that could be consistently identified from mass fragments using low-resolution mass spectrometry and/or that were present in a majority of the samples of a given species (*A. fascicularis* or *A. speciosa*) and respective tissue (leaf or root).

Statistical analysis

All analyses were conducted in R version 4.0.5 (R Core Team, 2021).

To determine whether trait plasticity under acute water stress depended on climatic history at the seed source, we used generalized linear mixed models (GLMMs) from the *glmmTMB* package (Brooks et al., 2017). Preliminary analyses showed strong trait differences and a lack of correspondence in phytochemical compounds between species, such that we ran separate models for each species. Each saturated model started with the fixed effects of water treatment, seed-source CWD, the water \times CWD interaction, and the random intercept effects of plant maternal family nested within the seed-source site. To compare effect sizes among response variables, we normalized responses and CWD values using the *BBmisc* package (Bischl et al., 2017),

and we report beta coefficients (β) with standard errors. We assessed the residuals of each fitted model, and we square-root or log-transformed response variables when these transformations provided a better fit to the Gaussian distribution.

To understand how the dry treatment affected growth, we used GLMMs with log-transformed plant height as a Tweedie-distributed variable, log-transformed biomass as a Gaussian-distributed variable, and log-transformed root:shoot and root:leaf ratios as Gaussian-distributed variables. To understand how the dry treatment affected physiology, we used GLMMs with stomatal conductance at the beginning and end of the experiment as Gaussian-distributed variables and log-transformed LMA as a Gaussian-distributed variable. To understand how the dry treatment affected phytochemistry, we first explored variation among unique compounds and their relationship to total concentrations using graphical data exploration. We then analyzed the responses of total concentration, the concentration of water-responsive flavonol glycosides, and the concentration of pregnane glycosides using GLMMs with Gaussian-distributed variables. We also assessed constitutive patterns in the dominant leaf flavonol in each species using GLMMs with control-plant flavonol concentrations as the response and seed-source CWD as the sole fixed predictor.

The best model for each response was selected based on the lowest sample-size-corrected Akaike information criterion (AIC_c), and any marginal predictors ($\leq 2 \Delta \text{AIC}_c$) were evaluated using log-likelihood ratio tests (LRT) in the *lmtest* package (Zeileis & Hothorn, 2002). Marginal and conditional R^2 values were calculated for each best model in the *MuMIn* package. Marginal means were calculated in the *effects* package (Fox, 2003; Fox & Weisberg, 2019).

To test whether the dry treatment affected the magnitude of intraspecific variation in phytochemistry, we first calculated coefficients of variation among populations in the control and dry treatment using the *cvqcv* package (Beigy, 2019) and Mahmoudvand-Hassani confidence intervals (Mahmoudvand & Hassani, 2009). We then also compared among-population coefficients of variation between the dry and control treatments using modified signed-likelihood ratio tests (Krishnamoorthy & Lee, 2014) in the *cvequality* package (Marwick & Krishnamoorthy, 2019).

Finally, to explore the potential value of flavonol plasticity to plant performance, we investigated the relationship between plasticity in leaf flavonols and the maintenance of plant biomass in the dry treatment. Plasticity was estimated as the change in mean trait value per maternal family between the dry and control treatments. We then applied a simple linear model with change in biomass as a function of change in flavonol concentration to data from both species. Plant species and average seed

mass were examined as potential covariates but neither improved model fit (not shown).

RESULTS

Growth and physiological responses to water stress

The dry treatment produced evidence of water limitation for both species. Water limitation tended to reduce plant growth and biomass (Appendix S1: Tables S2 and S3). The dry treatment reduced *A. fascicularis* plant biomass by ~17%, but this effect was stronger in plants whose seeds originated from drier sites (Appendix S1: Figure S2a; $\beta_{\text{water} \times \text{CWD}} = -0.33 \pm 0.14$, $z = -2.44$, $p < 0.02$). In *A. speciosa*, the dry treatment reduced plant biomass by 20% on average (Appendix S1: Figure S3); this effect was not significant (LRT water: $\chi^2 = 0.50$, $df = 1$, $p = 0.5$) but was most pronounced in plants originating from the driest site (Appendix S1: Figure S2b).

Seed-source CWD had a stronger effect on root:shoot ratios in *A. speciosa* than in *A. fascicularis*, but both species increased their root:leaf ratios in the dry treatment (Appendix S1: Tables S2 and S3). *Asclepias speciosa* produced higher root:shoot ratios than *A. fascicularis* (Welch's $t = -5.36$, $p < 0.0001$). The root:shoot ratio of *A. speciosa* plants was higher in plants sourced from drier sites ($\beta_{\text{CWD}} = 0.36 \pm 0.15$, $z = -2.40$, $p < 0.02$), and the root:leaf ratio was marginally higher in the dry treatment (Appendix S1: Figure S2d; LRT water: $\chi^2 = 2.18$, $df = 1$, $p = 0.1$). *Asclepias fascicularis* plants also increased their root:leaf ratio in the dry treatment (Appendix S1: Figure S2c; $\beta_{\text{water}} = 0.28 \pm 0.13$, $z = 2.21$, $p < 0.03$), but this effect was not apparent in the root:shoot ratio (LRT water: $\chi^2 = 0.00$, $df = 1$, $p = 0.9$) and did not depend on seed-source CWD (LRT CWD: $\chi^2 = 0.14$, $df = 1$, $p = 0.7$).

Plasticity in the physiological traits of stomatal conductance and LMA was stronger in *A. fascicularis* than in *A. speciosa* (Appendix S1: Tables S2 and S3). Stomatal conductance at the end of the experiment was lower in dry *A. fascicularis* plants than in controls ($\beta_{\text{water}} = -0.70 \pm 0.21$, $z = -3.39$, $p < 0.0008$) but was not different between control and dry *A. speciosa* plants (LRT water: $\chi^2 = 0.28$, $df = 1$, $p = 0.6$). Water limitation reduced LMA in both species, but the uncertainty in LMA response was higher in *A. speciosa* (*A. fascicularis*: $\beta_{\text{water}} = -0.28 \pm 0.14$, $z = -1.94$, $p < 0.06$; *A. speciosa*: $\beta_{\text{water}} = -0.28 \pm 0.17$, $z = -1.62$, $p = 0.1$). In *A. fascicularis*, control plants originating from wetter sites also had higher LMA than control plants originating from drier sites ($\beta_{\text{CWD}} = -0.24 \pm 0.12$, $z = -1.96$, $p < 0.06$), such that the direction of plasticity in LMA paralleled the constitutive decline in LMA with seed-source CWD in this species.

Phytochemical responses to water stress

Flavonol glycosides present in the leaves of both species dominated the total concentration of UV-absorbent secondary metabolites. In total, we retained 9 UV-absorbent secondary metabolites from *A. fascicularis* leaves, 12 from *A. fascicularis* roots, 6 from *A. speciosa* leaves, and 10 from *A. speciosa* roots for our analysis (Appendix S2). Fifteen, or ~40%, of these 37 unique compounds were putatively identified as pregnane glycosides present in *A. fascicularis* leaves and roots and *A. speciosa* roots. We also identified one putative cardenolide in *A. speciosa* roots, one putative saponin in *A. fascicularis* leaves, and two putative small phenolics in *A. fascicularis* roots. Flavonol glycosides (henceforth flavonols) were present only in the leaves of both species. Moreover, the leaves of each species contained a single dominant flavonol that comprised ~77% of the total UV-absorbent metabolite concentration: a quercetin-glucoside-rhamnoside (QGR) in *A. fascicularis* leaves and a quercetin-glucoside (QG) in *A. speciosa* leaves (Appendix S1: Figure S4).

The dominant flavonol in each species tended to be found in higher constitutive concentrations in plants sourced from drier sites (Figure 2). In particular, well-watered plants originating from drier sites had higher concentrations of QGR in the leaves of *A. fascicularis* than plants originating from wetter sites ($\beta_{\text{CWD}} = 0.33 \pm 0.14$, $z = -2.38$, $p < 0.02$). There was no statistical relationship between QG concentrations and the CWD of the seed source in well-watered *A. speciosa* leaves (LRT CWD: $\chi^2 = 0.60$, $df = 1$, $p = 0.4$), but this lack of pattern was driven mostly by plants sourced from Reno, NV, which contained relatively high concentrations of the flavonol despite their relatively wet source location compared with plants sourced from the other three sites (Figure 2).

Water limitation drove increases in the total concentration of UV-absorbent secondary metabolites in the leaves of both milkweed species, and this induction was driven by increases in flavonols (Figure 3; Appendix S1: Table S4; Appendix S2). In *A. fascicularis*, the magnitude of the flavonol response depended on seed-source CWD, such that only plants originating from wetter sites increased expression of flavonols under water limitation (Figure 3a; $\beta_{\text{water} \times \text{CWD}} = -0.26 \pm 0.13$, $z = -1.99$, $p < 0.05$; Appendix S1: Figure S5, Table S4). In *A. speciosa*, flavonols increased in the dry treatment ($\beta_{\text{water}} = 0.70 \pm 0.25$, $z = 2.77$, $p < 0.006$), but this response did not depend statistically on seed-source CWD (LRT CWD: $\chi^2 = 0.94$, $df = 1$, $p = 0.3$; Appendix S1: Table S4). Nevertheless, the *A. speciosa* leaves of plants sourced from wetter sites also tended to show larger increases in flavonol expression under water stress

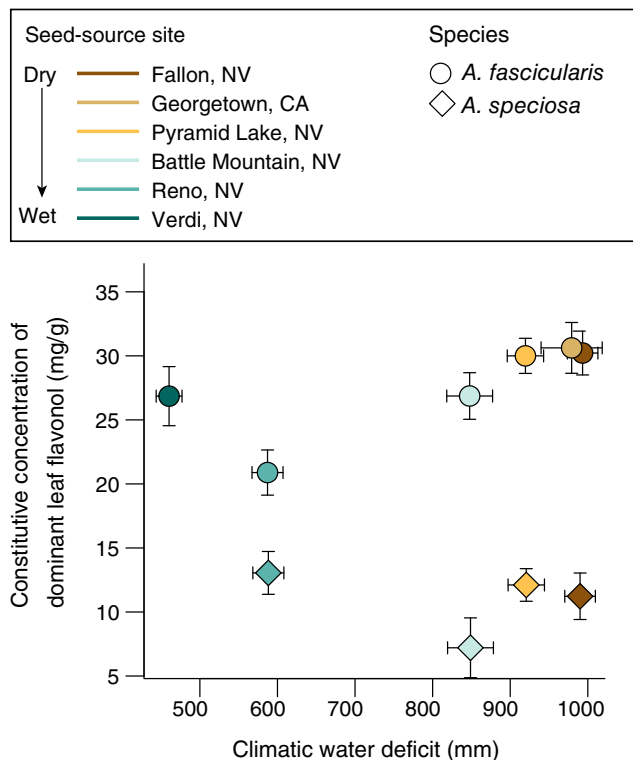


FIGURE 2 Leaf concentration (in milligrams per gram) of the dominant flavonol in control (well-watered) *Asclepias fascicularis* and *A. speciosa* plants. Points show the mean concentration of quercetin–glucoside–rhamnoside in *A. fascicularis* and of quercetin–glucoside in *A. speciosa*; bars show SE. The SE for climatic water deficits was calculated interannually from 2004 to 2016.

(Figure 3b; Appendix S1: Figure S5), except for plants from Reno, NV, which contained higher constitutive flavonols. Because plants from drier sites tended to contain higher constitutive flavonols, whereas plants from wetter sites tended to increase flavonols more under drought, among-population intraspecific variation in flavonol concentrations was higher in well-watered plants than in water-stressed plants in both species (Figure 3c,d). This change was marginally significant by a modified signed-likelihood ratio test for *A. fascicularis* ($F = 3.38$, $df = 5$, $p < 0.07$) but not for *A. speciosa* ($F = 1.11$, $df = 3$, $p = 0.3$). Finally, higher flavonol plasticity was weakly associated with better biomass maintenance in the dry treatment across both species (Figure 4; $\beta = 0.35 \pm 0.19$, $t = 1.79$, $p < 0.09$).

Roots, unlike leaves, did not contain flavonols, and the total concentration of UV-absorbent compounds in roots did not respond to water limitation in either species (Appendix S1: Table S4). Likewise, the diverse pregnane glycosides did not respond to water limitation, although they were generally found in higher concentrations in plants from wetter sites (Appendix S1: Figure S6, Table S4).

DISCUSSION

To predict the ecological consequences of drought and other stressors, we must understand how acute stress to organisms interacts with past selection by the local environment to affect trait expression and resulting variation. Here, we have shown that two widespread species of western milkweeds show phytochemical trait expression consistent with local adaptation to seed-source water deficits. In particular, the same chemical compounds that were induced upon acute water stress exhibited higher constitutive concentrations in plants sourced from drier sites (i.e., plasticity occurred in a “co-gradient” direction; Lusk et al., 2008). Interestingly, however, plants sourced from wetter sites tended to increase the concentrations of these putatively stress-mitigating secondary metabolites more than plants sourced from drier sites in response to acute water stress. Acute water stress thus reduced intraspecific variation in the concentrations of these dominant metabolites. We also found a weak but positive correlation between phytochemical plasticity and biomass maintenance under reduced water. Together, these results suggest that despite the potential for induced chemical responses to mitigate plant water stress, such responses may be constrained by the cost of metabolite production. The consequence may be reduced phytochemical variation among populations as environmental stress becomes more severe.

Our predictions for trait expression and plasticity were based on a definition of stress as severe resource limitation, and plant growth and physiological traits suggested that our dry treatment successfully produced such limitation in both species. Nevertheless, the two species appeared to regulate their physiology differently. In particular, *A. fascicularis* appears to be relatively more isohydric than *A. speciosa*. *Asclepias fascicularis* leaves were unique in their regulation of stomatal conductance in the dry treatment, and we have also found that *A. fascicularis* plants maintain a more constant leaf water potential than *A. speciosa* (Pringle, unpublished data). In contrast, *A. speciosa* produced higher root:shoot ratios than *A. fascicularis*, and constitutive *A. speciosa* root:shoot ratios also varied positively with water deficits at the seed-source site. Higher allocation to roots may allow *A. speciosa* to better tolerate drought, whereas isohydricity in *A. fascicularis* may indicate more drought avoidance (McDowell et al., 2008). Changes in the respective traits of each species in the dry treatment, however, suggested the potential for allocation trade-offs that could affect phytochemical trait expression.

In this study, phytochemical trait expression and plasticity were associated with climatic history at the seed-source site as characterized by the annual cumulative

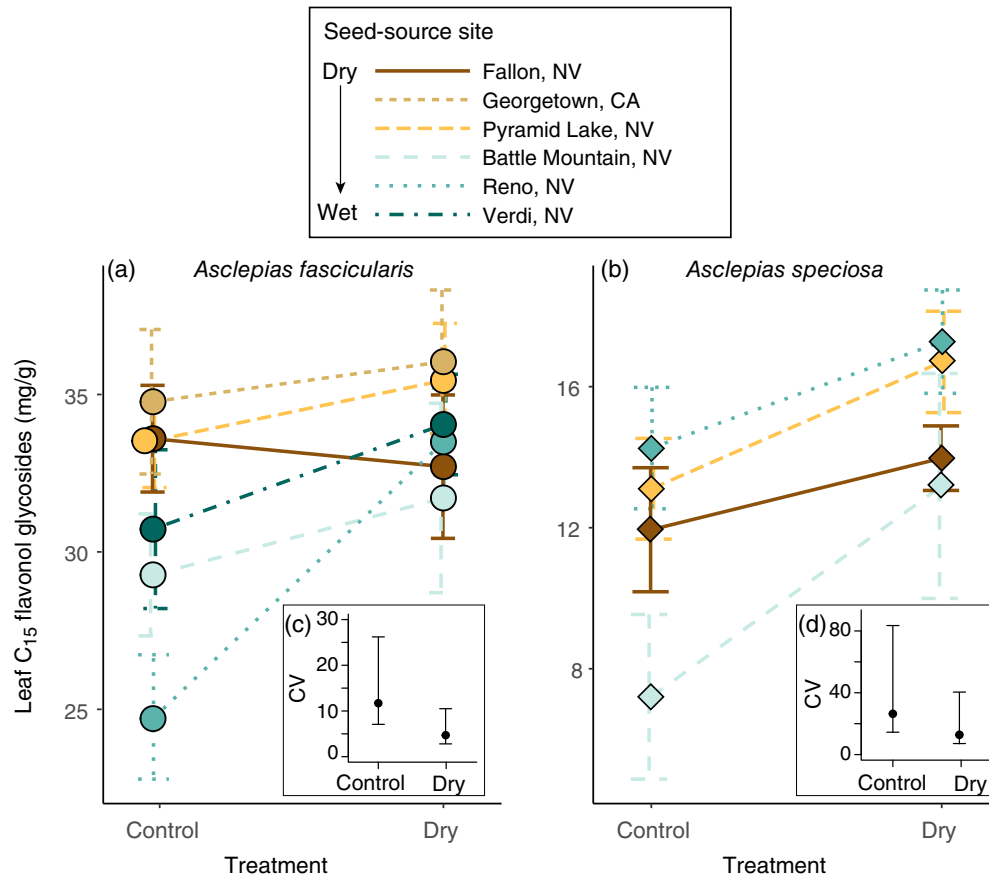


FIGURE 3 Interaction plots showing the responses of leaf C₁₅ flavonol glycosides to water treatment × climatic water deficit (CWD) at the seed source. (a) Responses in *Asclepias fascicularis* leaves and (b) responses in *A. speciosa* leaves. Interaction plots show reaction norms separately by source location, colored by their location along a gradient of annual cumulative CWD from dry to wet. Points represent means, and bars represent standard errors. The best general linear models for each response retained the fixed predictors: (a) water treatment × CWD and (b) water treatment. Estimated among-population coefficients of variation (CV) with confidence intervals for flavonol concentrations in (c) *A. fascicularis* and (d) *A. speciosa* plants in the control and dry treatments.

CWD. Importantly for regions with highly seasonal precipitation, this statistic was correlated not with precipitation, but with late-summer temperatures. Moreover, CWD at our source sites is driven by low late-summer water supply in combination with high temperatures. Predicted higher summer temperatures under climate change (Hegewisch et al., 2021) may thus be sufficient—irrespective of changes in precipitation—to increase the duration and frequency of future drought stress for plants in our study region. Late summer may also be a critical time for the *A. fascicularis* and *A. speciosa* milkweeds examined here because it is when these plants begin their reproductive investment in fruit. Summer temperature and its effects on plant water balance may thus exert strong selection on drought-mitigating phytochemistry.

The patterns in phytochemical trait expression that we observed are consistent with local adaptation to CWD because the direction of plasticity paralleled constitutive differences in phytochemical traits, with clinal variation

along the water-deficit gradient. Although a true test of whether plasticity is adaptive requires an association between plasticity and a reliable measure of fitness (van Kleunen & Fischer, 2005), patterns resulting from adaptive plasticity would (1) produce reaction norms driving traits closer to the values favored by selection in the new environment (Ghalambor et al., 2007) and (2) vary clinally along environmental gradients (Muir & Angert, 2017). Given how little we know about phytochemical responses to drought in most species (Mundim & Pringle, 2018), including the two milkweeds examined here, we took an untargeted approach to the measurement of UV-absorbent secondary metabolites. This discovery-oriented approach led to the clear identification of flavonols as phenolic compounds that both dominated the overall concentration of UV-absorbent metabolites and responded most strongly to the dry treatment. This finding is consistent with an important role for flavonols in reducing oxidative stress under water deficits,

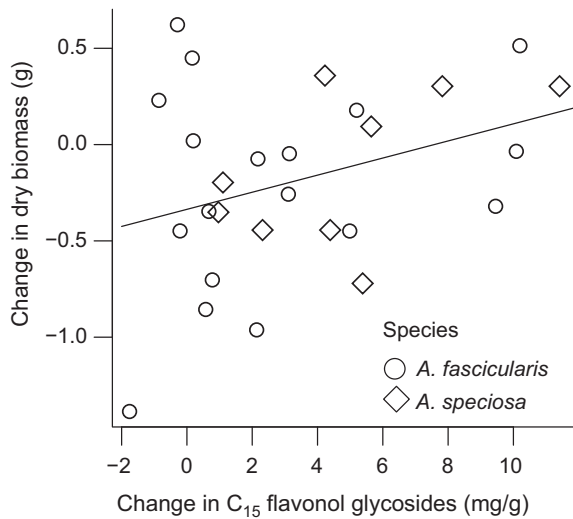


FIGURE 4 Relationship between the change in biomass of experimental plants and the change in C₁₅ flavonol glycoside concentrations (dry - control). Most plants at the end of the experiment were smaller in the dry treatment than in the control, so change in biomass is referred to in the text as biomass maintenance. Points represent maternal families. Circles represent *Asclepias fascicularis* plants, and diamonds represent *A. speciosa* plants. The regression line represents the marginal ($p < 0.09$) relationship across species described in the text.

preventing damage to cell membranes from reactive oxygen species (Kaminska-Rozek & Pukacki, 2004). Moreover, the positive relationship between flavonol plasticity and biomass maintenance is preliminary evidence of a fitness-related benefit of these compounds. The conclusion that flavonols may represent important, adaptive phytochemicals in these milkweeds is also consistent with phylogenetic conservatism in these compounds across the broader milkweed phylogeny (Agrawal, Salminen, et al., 2009) and with the higher concentration of these compounds among milkweed species whose leaf traits are adapted to more arid environments (Agrawal, Fishbein, et al., 2009).

Focusing on flavonols, then, as putative water-stress-mitigating metabolites, our results generally supported our predictions: Plants sourced from drier sites contained higher constitutive concentrations of these compounds, whereas plants sourced from wetter sites exhibited higher plasticity. These results are thus also consistent with the hypotheses underlying these predictions; namely, (1) there is a cost to flavonol production; and (2) given this cost, plasticity is favored in predictably variable environments. In particular, constitutively higher production of flavonols is avoided by plants sourced from locations with plentiful early-season water, but these plants also experience larger annual variation in water deficits due to the bigger differential between wet springs and dry

summers. These conclusions fit the patterns observed in *A. fascicularis*, but they require some allowances in *A. speciosa*. Indeed, neither the constitutive expression nor the plasticity of flavonols was statistically dependent on seed-source CWD in *A. speciosa*. Nevertheless, we suspect that flavonols play fundamentally similar roles in the two species. Statistical p values are context-dependent (Hartig & Barraquand, 2022), and we had lower power to assess the effect of seed-source CWD in *A. speciosa*, due to fewer source sites and an ~50% lower sample size per site than in *A. fascicularis*. In many respects, *A. speciosa* plants sourced from the wettest site for this species (Reno, NV) behaved more like plants from much drier sites, whereas the patterns among plants sourced from the other three sites were more similar to those in *A. fascicularis*. We can only speculate that perhaps the microenvironment of the seasonal ditch along which *A. speciosa* seeds were collected in Reno, NV, was distinct from the site's broader climatic patterns (McLaughlin et al., 2017) or that plants in this relatively urban environment were once planted from other sources (Auffret et al., 2014). The possibility that the observed patterns in water-stress-mitigating phytochemicals, which were statistically supported in *A. fascicularis*, are common to other widespread Great Basin species thus merits further study.

Ultimately, we are interested in how commonly such population-dependent patterns in constitutive and drought-induced phytochemicals will reduce intraspecific phytochemical variation if our study area continues to aridify. Again suggesting a cost to flavonol production, plants that were sourced from the driest sites and produced the highest constitutive concentrations of leaf flavonols also produced little, if any, additional flavonols in the dry treatment. On average, these plants also lost more biomass in the dry treatment, a consequence that did not depend on the maternal effect of seed size, suggesting that resource availability constrained flavonol production across populations under acute water stress. Such ecological limits on trait expression and/or plasticity may be commonplace (Auld et al., 2010; Valladares et al., 2007). We know of only three other studies that have examined the effect of resource stress on among-population phytochemical variation. Two of these studies examined primary metabolites: Nutrient stress reduced variation in leaf nutrient concentrations (Andivia et al., 2012), whereas drought stress reduced variation in glucose:sucrose ratios but increased variation in the concentration of soluble carbohydrates (Lázaro-Nogal et al., 2016). A third study examined between-population expression of secondary metabolites under drought and found a pattern similar to ours: In a perennial grass, in which isoprenes are proposed to act as critical antioxidants,

drought stress reduced the between-population difference in leaf isoprene concentrations (Ahrar et al., 2017). The generality of these results deserves further study, especially considering the possible consequences of changes in phytochemical variation for plant populations and ecological communities.

To the extent that phytochemistry mediates plant responses to stress, changes in phytochemistry may also provide early indicators of ecosystem responses to global change. Phytochemical diversity is hypothesized to regulate the structure and diversity of food webs and the dynamics of biogeochemical cycles (Hunter, 2016; Wetzel & Whitehead, 2020). It will thus be important to determine: (1) on what scales (among species, among populations, and/or among individuals) global change stressors tend to change phytochemical variation, and (2) whether and how phytochemical variation, or lack thereof, impacts ecological dynamics. For example, lower diversity in plant communities has been shown to reduce functional resilience to disturbance (Wilcox et al., 2020) and variation in soil mineralization processes (Rewcastle et al., 2022). Future work should examine whether such patterns can be traced back to phytochemistry, and whether the processes that drive phytochemical variation at the community level parallel, or not, the intraspecific patterns that we have begun to document here.

ACKNOWLEDGMENTS

This work was funded by an NSF Graduate Research Fellowship and a Hitchcock Chemical Ecology Fellowship to Aramee C. Diethelm, by the University of Nevada, Reno (UNR), and by an Alexander von Humboldt Fellowship to Elizabeth G. Pringle. We thank the Pringle Lab at UNR, the Plant-Insect Group (“PIG”) at UNR, and the Biochemistry Department at the Max Planck Institute for Chemical Ecology for their help and feedback. We thank Elizabeth Leger and Felipe Barrios Masias for loaning equipment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT


Data (Diethelm et al., 2022) are available from Dryad: <https://doi.org/10.5061/dryad.djh9w0w2p>.

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How to cite this article: Diethelm, Aramee C., Michael Reichelt, Thomas E. Dilts, James P. Farlin, Audrey Marlar, and Elizabeth G. Pringle. 2022. “Climatic History, Constraints, and the Plasticity of Phytochemical Traits under Water Stress.” *Ecosphere* 13(8): e4167. <https://doi.org/10.1002/ecs2.4167>