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

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Authors: Aramee C. Diethelm, Michael Reichelt, Thomas E. Dilts, James P. Farlin, Audrey Marlar, and Elizabeth G. Pringle

Contact information of corresponding author: epringle@unr.edu

Appendix S1: Supporting Information

The following supporting information is available in this appendix:

Fig. S1 Correlations between mean annual climatic water deficit (CWD) and other climate means and coefficients of variation.

Fig. S2 Interaction plots of plant growth responses to water treatment by seed-source.

Fig. S3 Marginal mean plot of the plant biomass response to water treatment.

Fig. S4 Correlations between the total concentration of leaf UV-absorbent metabolites and the concentration of leaf C_{15} flavonol glycosides.

Fig. S5 Flavonol plasticity (difference between dry and control means per seed-source) by the climatic water deficit at the seed source.

Fig. S6 Pregnane glycoside concentrations by the climatic water deficit at the seed source.

Table S1 Locations of seed-source sites.

Table S2 Plant physical and physiological responses to the water treatment.

Table S3 Model selection results for plant growth and physiological traits.

Table S4 Model selection results for phytochemical traits.

Methods S1 Calculation of cumulative annual climatic water deficit (CWD).

Methods S2 Analytical Chemistry Supplementary Methods.

Fig. S1 Pair plots showing correlations among water-balance and climatic variables for the six seed-provenance sites. Plots above the diagonal show raw points; numbers below the diagonal show corresponding Pearson's correlation coefficients. CWD = mean annual climatic water deficit; Mean Temp = mean annual temperature; Mean Aug Temp = mean August temperature; PPT = mean annual precipitation; AET = mean annual actual evapotranspiration; PET = mean annual potential evapotranspiration; CWD_winyr_cv = coefficient of variation in climatic water deficit among months within years; TMean_winyr_cv = coefficient of variation in mean temperature among months within years; PPT_winyr_cv = coefficient of variation in precipitation among months within years; CWD_btyr_cv = coefficient of variation in climatic water deficit between years; TMean_btyr_cv = coefficient of variation in mean temperature between years; PPT_btyr_cv = coefficient of variation in precipitation between years. All summary variables based on climate normals from 2004–2016.

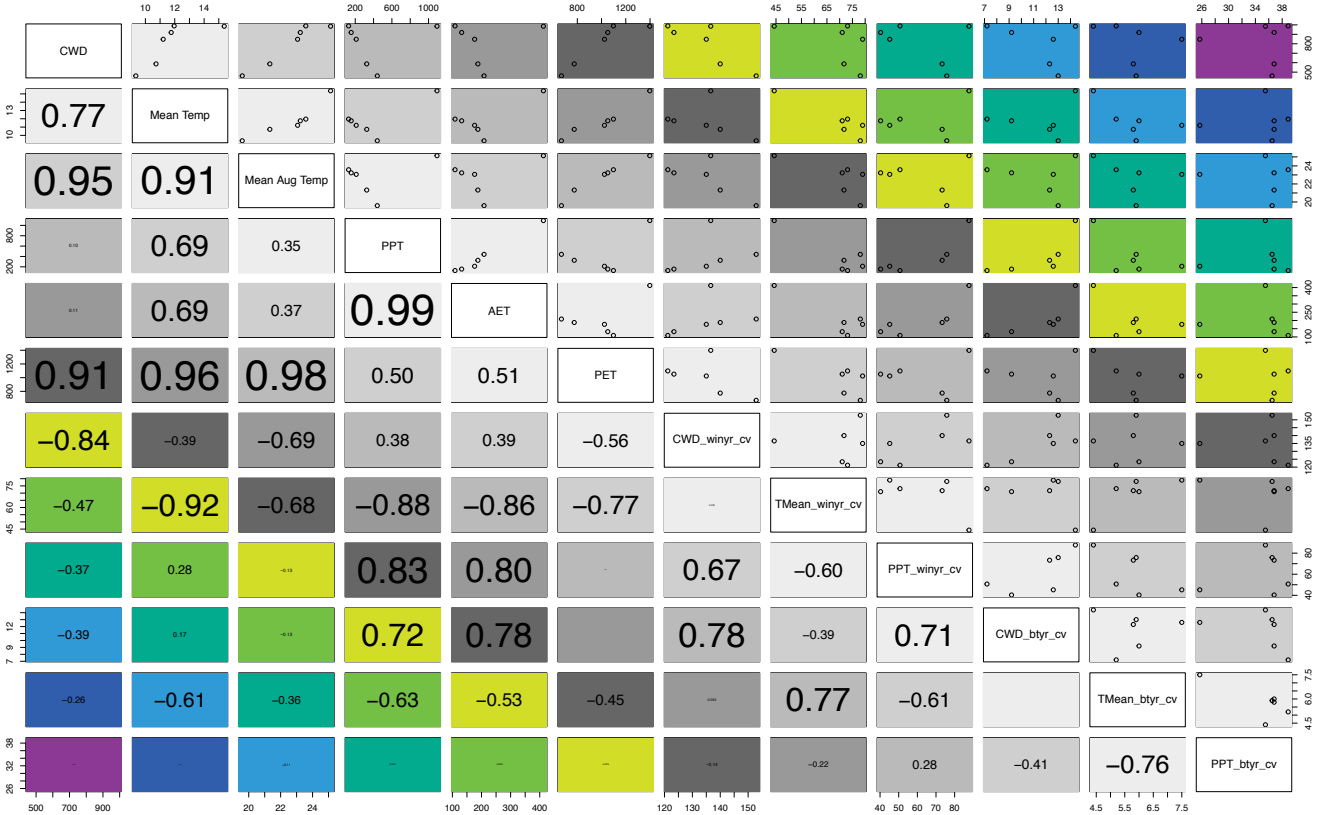

Fig. S2 Interaction plots showing plant growth responses to water treatment × climatic water deficit (CWD) at the seed source. (**a**,**b**) Dry biomass of roots and shoots (*i.e.*, whole-plant) and (**c**,**d**) root:leaf ratios in both species, (**a**,**c**) *A. fascicularis* and (**b**,**d**) *A. speciosa*. Points represent means and bars represent SE. The best general linear mixed models for each response retained the fixed predictors: (**a**) water treatment × CWD; (**b**) intercept only; (**c**) water treatment; and (**d**) intercept only.

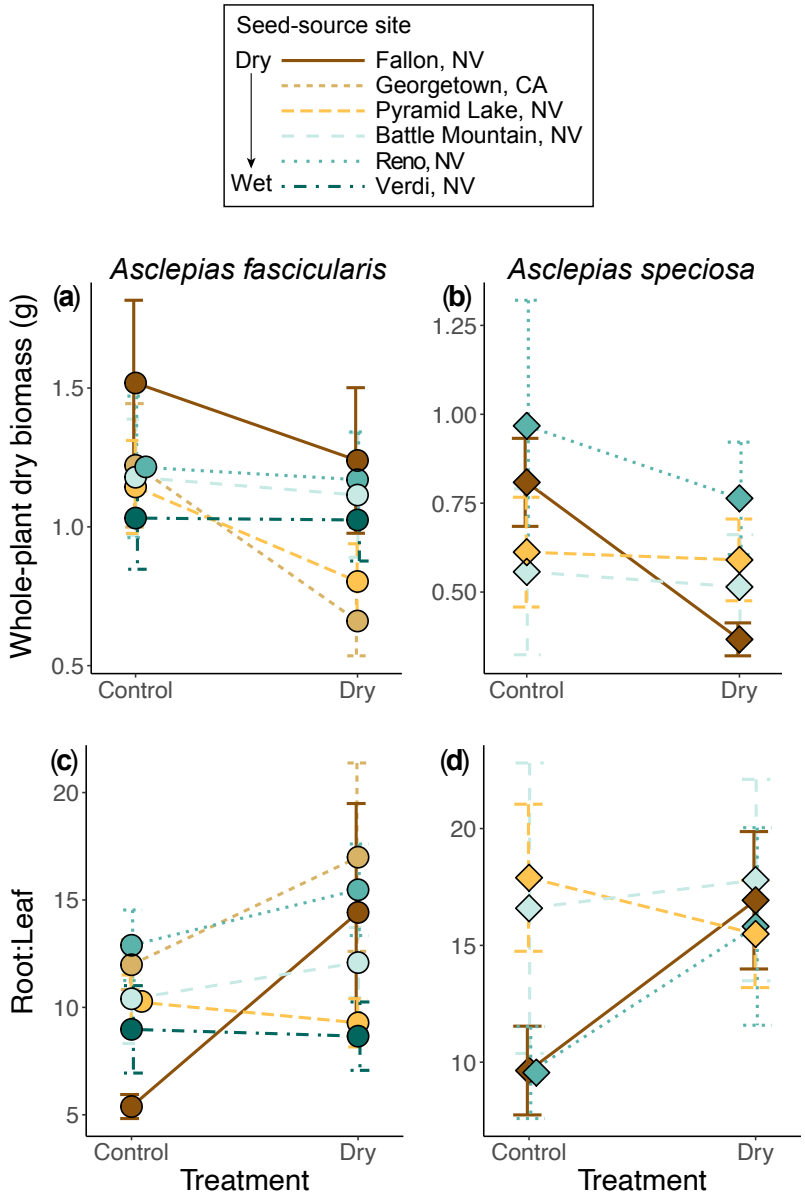

Fig. S3 Effect of the dry treatment on whole-plant dry biomass contingent on the mean fixed effect of seed-source CWD and random effect of plant genotype.

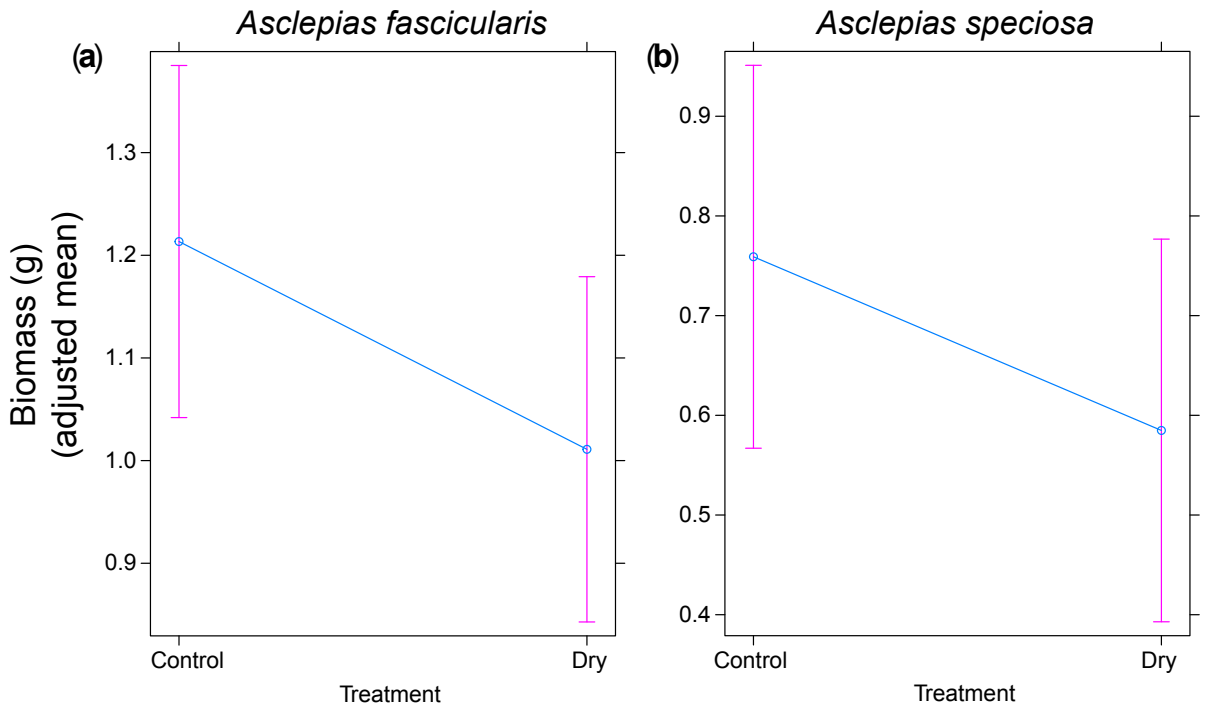

Fig. S4 Correlations between the total concentration of consistently identified UV absorbent metabolites and the concentration of C_{15} flavonol glycosides (QGR and KGR in *A. fascicularis*; QGR and QG in *A. speciosa*). These C_{15} flavonol glycosides were also the only four compounds in leaves that were induced upon water limitation.

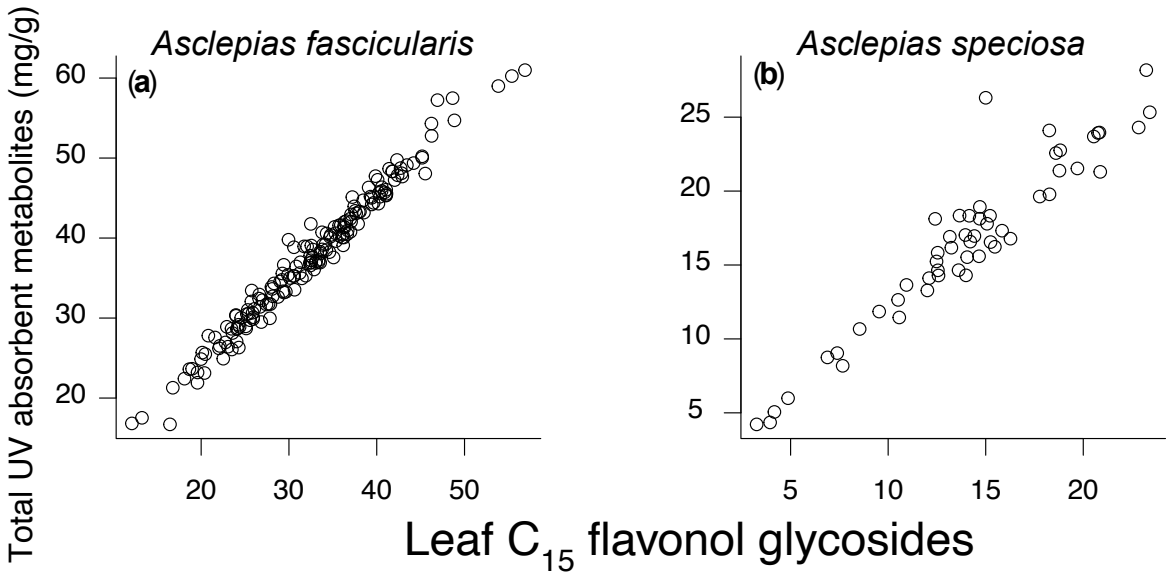

Fig. S5 Flavonol plasticity in response to acute water stress among seeds sourced from along a CWD gradient. Points show maternal-family mean differences between dry treatment and well watered concentrations and SE. SE error bars for climatic water deficits were calculated interannually from 2004–2016.

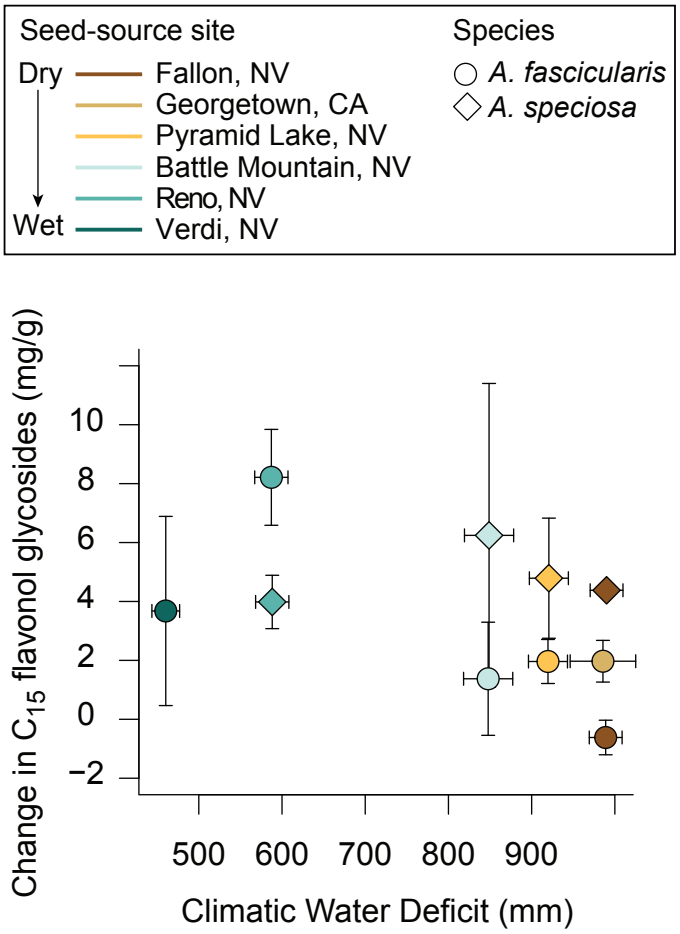

Fig. S6 Constitutive concentrations of pregnane glycoside compounds in the leaves and roots of control plants (*i.e.* well watered). Points show the mean concentration among all plants sourced from each site and bars show SE. SE for climatic water deficits was calculated interannually from 2004–2016. Note the differences in scale on the y-axis. Insets show representations of leaves and roots of each species; no pregnane glycosides were identified in the leaves of *A. speciosa.*

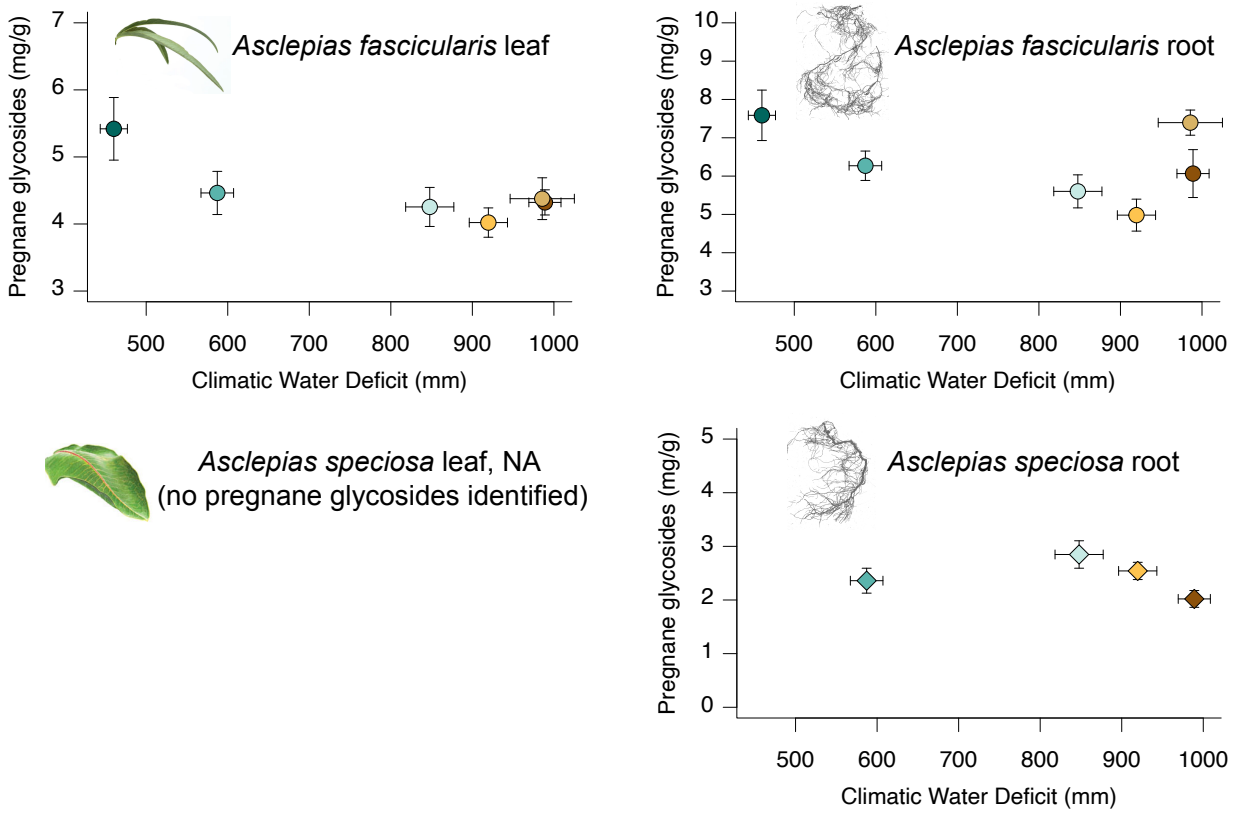

Table S1. Seed-provenance site locations.

Site	Latitude	Longitude
California	38.87178726	-120.8187756
Verdi	39.52318534	-119.9985313
Reno	39.50261419	-119.8986715
Pyramid Lake	39.86179074	-119.3868993
Fallon	39.47482708	-118.6575129
Battle Mountain	40.662576	-116.9320299

Table S2 Plant physical and physiological responses in the glasshouse drought experiment.

Response	Plant part	Treatment (mean ± SE (*N*))		Best model
		Control	Dry	
Asclepias fascicularis				
Height change (cm)	*Shoot*	0.8 ± 0.2 (*97*)	0.3 ± 0.2 (*103*)	Treatment
Biomass (g)	*Root*	0.90 ± 0.06 (*92*)	0.76 ± 0.05 (*96*)	Treatment x CWD
Biomass (g)	*Shoot*	0.31 ± 0.03 (*92*)	0.25 ± 0.03 (*96*)	Treatment x CWD
Root:shoot biomass ratio		4.11 ± 0.25 (*92*)	4.02 ± 0.25 (*96*)	Intercept
Root:leaf biomass ratio		10.0 ± 0.7 (*92*)	12.8 ± 1.2 (*96*)	Treatment
Stomatal conductance (mmol/m2/s)	*Leaf*	232.5 ± 17.2 (*44*)	168.4 ± 10.2 (*51*)	Treatment
LMA (mg/mm2)	*Leaf*	0.05 ± 0.004 (*95*)	0.04 ± 0.002 (*94*)	Treatment + CWD
Asclepias speciosa				
Height change (cm)	*Shoot*	0.5 ± 0.4 (*34*)	0.4 ± 0.5 (*33*)	Intercept
Biomass (g)	*Root*	0.61 ± 0.09 (*32*)	0.49 ± 0.06 (*32*)	Intercept
Biomass (g)	*Shoot*	0.14 ± 0.04 (*32*)	0.11 ± 0.03 (*32*)	CWD
Root:shoot biomass ratio		6.88 ± 0.67 (*32*)	8.19 ± 1.05 (*32*)	CWD
Root:leaf biomass ratio		13.5 ± 1.7 *(32*)	16.3 ± 1.9 (*32*)	Intercept
Stomatal conductance (mmol/m2/s)	*Leaf*	180.6 ± 17.2 (*21*)	190.8 ± 17.4 (*19*)	Intercept
LMA (mg/mm2)	*Leaf*	0.04 ± 0.002 (*33*)	0.03 ± 0.002 (*29*)	Treatment

Table S3. Model selection results for growth and physiological traits from the global GLMM. Parameters in the model (K), degrees of freedom error (df), Aikaike's Information Criterion for small sample sizes (AICc), the difference in AIC (dAIC), and variance of the random intercept terms are shown. All models include a random effect of plant genotype nested within seed source site. Only models with dAICc < 2 are shown. Marginal (fixed effects only; R^2_M) and conditional (fixed + random effects; R^2_C) R^2 values are also shown.

							Random			
Species	Model	Fixed effects	K	df (N)	AICc	dAIC	Family	Site	R^2_M	R^2_C
Asclepias fascicularis	Height change (cm) ~	Treatment	6	194	130	0	0.378	0.072	NA	NA
	Total dry biomass (g) ~	Treatment * CWD	7	181	536.9	0.0	0.000	0.000	0.05	0.05
		Treatment	5	183	538.5	1.6				
	Root:shoot ratio ~	Intercept	4	184	454.8	0.0	0.087	0.103	0	0.26
		CWD	5	183	456.7	1.9				
	Root:leaf ratio ~	Treatment	5	183	514.1	0.0	0.100	0.080	0.02	0.21
		Treatment + CWD	6	182	516.1	2.0				
	Stomatal conductance ~	Treatment	5	90	280.9	0.0	0.021	0.000	0.11	0.13
	(mmol m^{-2} s^{-1})	Treatment + CWD	6	89	282.0	1.1				
	Leaf mass per area ~	Treatment + CWD	6	183	552.4	0.0	0.009	0.066	0.07	0.14
	(mg mm^{-2})	Treatment * CWD	7	182	552.6	0.2				
		Treatment	5	184	553.3	0.8				
		CWD	5	184	554.0	1.6				
Asclepias speciosa	Height change (cm) ~	Intercept only	5	62	82.1	0	0.153	0.234	NA	NA
		CWD	6	61	82.8	0.7				
	Total dry biomass (g) ~	Intercept only	4	60	174.5	0.0	0.000	0.000	0	0
		CWD	5	59	176.0	1.5				
		Treatment	5	59	176.3	1.9				
	Root:shoot ratio ~	CWD	5	59	195.9	0.0	0.000	0.000	0.08	0.08
		Treatment + CWD	6	58	197.2	1.3				
	Root:leaf ratio ~	Intercept only	4	60	188.3	0.0	0.000	0.025	0	0.03
		Treatment	5	59	188.5	0.2				
		CWD	5	59	188.8	0.5				
		Treatment + CWD	6	58	188.8	0.5				
	Stomatal conductance ~	Intercept only	4	36	104.1	0	0.133	0.000	0	0.20
	(mmol m^{-2} s^{-1})									
	Leaf mass per area ~	Treatment	5	57	144.3	0.0	0.059	0.016	0.04	0.17
	(mg mm^{-2})	Intercept only	4	58	144.5	0.2				
		Treatment + CWD	6	56	145.2	0.8				
		CWD	5	57	145.4	1.1				

Table S4. Model selection results for chemical traits from the global GLMM. Parameters in the model (K), degrees of freedom error (df), Aikaike's Information Criterion for small sample sizes (AICc), the difference in AIC (dAIC), and variance of the random intercept terms are shown. All models include a random effect of plant genotype nested within seed source site. Only models with dAICc < 2 are shown. Marginal (fixed effects only; R^2_M) and conditional (fixed + random effects; R^2_C) R^2 values are also shown.

							Random			
Species	Model	Fixed effects	K	df (N)	AICc	dAIC	Family	Site	R^2_M	R^2_C
Asclepias fascicularis	Total UV absorbent conc (mg/g) ~									
	Leaves	Treatment * CWD	7	168	479.3	0.0	0.227	0.000	0.05	0.28
		Treatment	5	170	479.7	0.4				
		Treatment + CWD	6	169	480.9	1.6				
	Total UV absorbent conc (mg/g) ~									
	Roots	Intercept only	4	193	552.9	0.0	0.077	0.006	0	0.08
		CWD	5	192	553.4	0.6				
		Treatment	5	192	554.1	1.3				
		Treatment + CWD	6	191	554.7	1.9				
	QGR + KGR (flavonoids) (mg/g) ~	Treatment * CWD	7	168	481.4	0.0	0.192	0.000	0.07	0.26
	Leaves ONLY	Treatment	5	170	482.9	1.5				
		Treatment + CWD	6	169	483.1	1.8				
	Pregnane glycosides (mg/g)									
	Leaves	CWD	5	170	455.4	0	0.250	0.000	0.08	0.34
		Intercept only	4	171	457	1.6				
		Treatment + CWD	6	169	457.1	1.7				
	Pregnane glycosides (mg/g)									
	Roots	Intercept only	4	193	550.2	0	0.040	0.106	0	0.15
		Treatment	5	192	551.3	1.1				
		CWD	5	192	551.7	1.5				
Asclepias speciosa	Total UV absorbent conc (mg/g) ~									
	Leaves	Treatment + CWD	6	47	151.4	0.0	0.179	0.000	0.21	0.37
		Treatment	5	48	152.0	0.6				
		CWD	5	48	153.3	1.9				
	Total UV absorbent conc (mg/g) ~									
	Roots	CWD	5	61	189.1	0.0	0.139	0.000	0.09	0.23
		Intercept only	4	62	189.5	0.4				
	QGR + QG (flavonoids) (mg/g) ~	Treatment	5	48	153.1	0.0	0.186	0.000	0.13	0.31
	Leaves ONLY	Treatment + CWD	6	47	154.4	1.2				
	Pregnane glycosides (mg/g)	Intercept	4	62	192.4	0.0	0.109	0.000	0	0.11
	Roots ONLY	Treatment	5	61	193.1	0.6				
		CWD	5	61	193.1	0.7				
		Treatment + CWD	6	60	194	1.6				

Methods S1 Calculation of cumulative annual climatic water deficit (CWD)

To calculate monthly CWD for each seed-provenance site, we acquired: (i) total monthly precipitation and mean monthly temperature using the PRISM Data Explorer tool for the years 2004–2016 (PRISM Climate Group 2019); (ii) elevation from the 10-m National Elevation Dataset (USGS 2019), which was used to derive slope and aspect; and (iii) the predicted soil water holding capacity from USDA SSURGO (USDA 2018), following Dilts (2014). These monthly data were then summed annually and averaged over the period 2004–2016 for each site.

Literature Cited

Dilts, T. E. 2014. Climatic Water Deficit Toolbox for ArcGIS 10.1. https://www.arcgis.com/home/item.html?id=de9ca57d43c041148b815da7ce4aa3a0.

PRISM Climate Group. (2019). PRISM Climate Data. Oregon State University. http://prism.oregonstate.edu. Date Accessed: June 25, 2019.

USDA. (2018). Soil survey geographic (SSURGO) database (Soil Survey Staff, Natural Resource Conservation Service, U.S. Department of Agriculture). Available online at https://sdmdataaccess.sc.egov.usda.gov. Date Accessed May 18, 2018.

Methods S2 Analytical Chemistry Supplementary Methods

HPLC-UV

Analysis of *A. fascicularis* and *A. speciosa* leaves and roots from the glasshouse drought experiment used samples extracted in methanol with a 0.075 mg/mL digitoxin internal standard. Analysis was performed using a HPLC-UV system (Agilent 1100 Series instrument) equipped with a reversed phase column (Nucleodur Sphinx RP, 250 x 4.6 mm, 5 µm particle size; Macherey-Nagel, Düren, Germany). Mobile phases were water (A) and acetonitrile (B), starting with 20% B, followed by a gradient to 68% B in 24 min at a constant flow of 1 ml/min, followed by a washing and reequilibration cycle. The eluent was monitored by a photodiode array detector at 219 nm. All peaks were quantified as digitoxin equivalents based on the internal standard digitoxin applying a relative molar response factor of 1.0.

LC-IonTrap-MS (low resolution mass spectrometer)

Analysis was done by LC-MS using a Bruker Esquire 6000 ion trap mass spectrometer (Bruker Daltonics, Bremen, Germany) operated in alternating ionization mode in the range m/z 60–1,400 (capillary exit voltage, +110/-110 eV; capillary voltage, +4,000/-4,000V; nebulizer pressure, 35 psi; drying gas, 11 l min-1; gas temperature, 330°C) coupled to an Agilent 1100 series HPLC (Agilent Technologies, Waldbronn, Germany). Elution was accomplished using a Nucleodur Sphinx RP column (250 x 4.6 mm, 5 µm; Macherey- Nagel, Düren, Germany). Mobile phases were 0.2% formic acid (v:v) (A) and acetonitrile (B), starting with 20% , followed by a gradient to 68% B in 24 min followed by a washing and reequilibration cycle. MS2 spectra were recorded in positive and negative ionization mode in AutoMS modus.

LC-Q-ToF-MS (high resolution mass spectrometer)

To determine the exact mass of metabolites, ultra-high-performance liquid chromatography–electrospray ionization– high resolution mass spectrometry (UHPLC–ESI–HRMS) was performed with a Dionex Ultimate 3000 series UHPLC (Thermo Scientific) and a Bruker timsToF mass spectrometer (Bruker Daltonik, Bremen, Germany). UHPLC was used applying a reversed-phase Zorbax Eclipse XDB-C18 column (100 mm × 2.1 mm, 1.8 µm, Agilent Technologies, Waldbronn, Germany) with a solvent system of 0.1% formic acid (A) and acetonitrile (B) at a flow rate of 0.3 ml/min. The elution profile was the following: 0 to 0.5 min, 5% B; 0.5 to 11.0 min, 5% to 60% B in A; 11.0 to 11.1 min, 60% to100% B, 11.1 to 12.0 min,

100% B and 12.1 to 15.0 min 5% B. Electrospray ionization (ESI) in negative/positive ionization mode was used for the coupling of LC to MS. The mass spectrometer parameters were set as follows: capillary voltage 4.5 KV/3.5KV, end plate offset of 500V, nebulizer pressure 2.8 bar, nitrogen at 280°C at a flow rate of 8L/min as drying gas. Acquisition was achieved at 12 Hz with a mass range from m/z 50 to 1500. At the beginning of each chromatographic analysis 10uL of a sodium formate-isopropanol solution (10 mM solution of NaOH in 50/50 (v/v%) isopropanol water containing 0.2% formic acid) was injected into the dead volume of the sample injection for re-calibration of the mass spectrometer using the expected cluster ion m/z values.