DROUGHT RESPONSE STRATEGIES OF *CLARKIA GRACILIS* (ONAGRACEAE) POPULATIONS FROM SERPENTINE AND NONSERPENTINE SOILS

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Editor: Hafiz Maherali

Premise of research.   The serpentine grassland system provides the opportunity to examine whether drought response traits may have contributed to differentiation in the stress-tolerant genus *Clarkia*. Commonly utilized drought responses might be indicative of traits important to species diversification in stressful environments.

Methodology.   We examined the drought response strategies of populations of *Clarkia gracilis* (Onagraceae) from both serpentine and nonserpentine soils. Physiological, morphological, and biomass data were collected under controlled greenhouse conditions.

Pivotal results.   Serpentine-derived plants exhibited faster growth and germination rates and larger leaf size than nonserpentine-derived plants under increasing water stress, indicating drought escape strategies. However, serpentine-derived plants also employed dehydration avoidance by increasing their water-use efficiency (decreased transpiration rate) under drought stress. In terms of biomass, serpentine-derived plants had a higher fitness potential than nonserpentine-derived plants. Nonserpentine-derived plants tended toward dehydration avoidance through decreased growth rate, decreased transpiration, and smaller leaf size.

Conclusions.   Our data support transpiration rate as a trait important to stress tolerance. Moreover, serpentine-derived plants avoid drought with drought escape strategies more than nonserpentine plants and may have a higher fitness potential, particularly in water-limiting conditions. Thus, serpentine populations may be competitively superior to nonserpentine populations under drought stress conditions.

Keywords: *Clarkia*, serpentine system, drought stress, transpiration rate, growth rate, plasticity.

Introduction

Globally, the frequency and/or intensity of ecosystem disturbances such as drought have increased, likely due to climate change (IPCC 2014). These disturbances will impact the biogeography of plant species as physiological tolerances of populations to stressors such as drought will determine their distribution and abundance (Hacker and Bertness 1995). Leaf-level adaptations to drought involve an interplay between leaf desiccation and gas exchange rates (Maherali et al. 2008); for example, many plants can minimize leaf water loss by closing their stomata and thereby decreasing stomatal conductance of water vapor and transpiration rates (Larcher 2003). By decreasing transpiration, a plant can increase water-use efficiency (WUE), which can increase fitness in drought conditions, despite the resulting lower rates of carbon assimilation due to reduced carbon dioxide intake through closed stomata (Dudley 1996;

Heschel et al. 2002; Caruso et al. 2006). Plants can minimize this trade-off between photosynthesis and water loss by increasing leaf size and root allocation (Heschel et al. 2004); for example, in the herbaceous annual *Impatiens capensis*, minimal increases in leaf size can help to reduce the trade-off between water conservation and photosynthesis while avoiding leaf overheating (Bibee et al. 2011). However, a combination of larger leaves and reduced transpiration rates can have negative implications for plant thermoregulation and photoinhibition (Heschel et al. 2014).

Plants can mediate these morphological and physiological trade-offs with phenotypic plasticity (Sultan et al. 1998; Viger et al. 2016). Behavioral shifts in growth and development can simultaneously maximize carbon assimilation and limit water loss (Heschel et al. 2014). These adjustments often involve multiple morphological and physiological traits; for example, plants that utilize dehydration avoidance tactics may attempt to withstand drought by having a shorter stature, smaller leaves, more cuticular wax, decreased stomatal density, decreased transpiration, and increased WUE (Bibee et al. 2011). Plasticity in multiple drought response traits can translate into increased plant fitness when plants experience drought stress (Heschel et al. 2004); this type of plasticity can be particularly important when plants

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experience drought in combination with other stressors (Sultan et al. 1998).

Drought response plasticity in multiple traits can be important to the distribution of species that inhabit both serpentine and nonserpentine soils (Brady et al. 2005). In California grasslands, serpentine is often a tectonically derived rock composed of at least 70% mafic minerals (Brooks 1987) that forms soils rich in magnesium and iron and low in calcium and other primary nutrients, particularly nitrogen, phosphorus, and potassium (Whittaker 1954; Brooks 1987). Serpentine or ultramafic soils are typically dry and often contain increased concentrations of heavy metals, including chromium, cobalt, and nickel (Proctor 1971; Brooks 1987; Brady et al. 2005). Low moisture in these soils can be due to substrate depth and sandy composition, which can be challenging for plant establishment (Huenneke et al. 1990; Harrison et al. 2015). The combination of these factors can create a soil environment with depressed nutrient levels and low moisture availability (Walker 1954; Brooks 1987).

Serpentine plant communities have been shown to exhibit a greater number of traits associated with adaptation to stress than communities on fertile soils (Fernandez-Going et al. 2013). In order to survive a combination of drought, metals, and nutrient stresses, populations of serpentine-tolerant species can use a combination of morphological and physiological plasticity. For example, some populations of *Lasthenia californica* allocate more biomass to roots, while others flower earlier and shift their growth rates (Rajakaruna et al. 2003). Moreover, these same populations have differences in metal uptake rates and sodium tolerance (Rajakaruna and Whitton 2004). Taken together, herbaceous species can persist in serpentine systems with combinations of morphological and physiological plasticity.

Here, we examined drought response plasticity in multiple traits for the herbaceous annual *Clarkia gracilis* ssp. *gracilis*. Some species of *Clarkia* are endemic to serpentine systems in California, making them interesting subjects for understanding how plants can persist under multiple soil stressors. This study addressed the following questions: (1) Do serpentine and nonserpentine populations of *C. gracilis* exhibit morphological and physiological plasticity in response to drought stress? (2) Do these serpentine and nonserpentine populations have different biomass accumulation under dry versus moist conditions? (3) Are observed drought-adaptation traits correlated with biomass accumulation under dry versus moist conditions?

Methods

Study Species and Source Populations

Within Northern California (the location of our source populations), there are approximately 44 species of the genus *Clarkia* (Lewis and Lewis 1955); many of these species are able to persist in both serpentine and nonserpentine soils. *Clarkia gracilis* ssp. *gracilis* ((Piper) A. Nelson & J.F. Macbr.), or slender clarkia, is an annual herb native to California, Washington, and Oregon. *Clarkia gracilis* can be found growing within coastal, foothill, valley, and low-elevation montane habitats. Self-pollination may occur autonomously or when pollinators move between flowers on the same individual; however, geitonogamy may be infrequent because *C. gracilis* plants are small

and rarely have more than two or three flowers open at a time (Jones 1996).

Clarkia gracilis fruits were collected from three serpentine (S) and three nonserpentine (NS) populations near Yreka (Siskiyou County), California. This area is located approximately 760 m above sea level and is classified as having a hot summer Mediterranean climate (mean annual precipitation is approximately 50 cm, and mean annual temperature is 11°C). These S sites are typically drier than the NS sites (L. Ruane, personal observation), but soil moisture conditions can overlap between these sites. During a dry spring (April/May 2013), the soil volumetric water content (VWC) for S sites ranged from 2.5% to 12%, while the VWC for NS sites ranged from 3% to 20% (note: 10–12 VWC readings were taken at each of the three S and three NS sites, for a total of about 70 measurements). VWC was measured at 12 cm of soil depth with a TDR moisture probe (Campbell Scientific). Soil from these S sites had higher Ni and Mg levels and lower Ca and K levels than the NS sites (average ppm values for S sites: Ni = 5.8, Mg = 2259.3, Ca = 560.7, K = 67.7; average ppm for NS sites: Ni = 0.3, Mg = 265.7, Ca = 1286.4, K = 220.4).

After field collection, the fruits from the six populations were transferred to Christopher Newport University, where they were grown in a common growth chamber environment for one generation to minimize maternal effects. In this Conviron chamber, plants experienced 23°/13°C (day/night) temperatures and 15 h of halogen incandescent light. Seeds resulting from autonomous self-pollinations were sent to Colorado College, where they were cold stratified at 4°C for 7 mo. Following stratification, the seeds were planted into MetroMix 360 soil (Scotts) and placed in a growth chamber (Percival AR36L) for 3 wk, during which they were exposed to a 12-h photoperiod (incandescent and fluorescent light with a neutral red : far-red ratio, generating 200 µmol photons m^{-2} s^{-1} of light intensity). The temperature in the growth chamber was 22°C, humidity was between 40% and 60%, and the plants were misted daily. After 3 wk, the seedlings were transplanted into Ray Leach Cone-tainers (21 cm × 3.8 cm; Stuewe) containing MetroMix 360 in the Colorado College greenhouse, where they were exposed to natural light (July–August 2014), temperatures between 23° and 29°C, and 50%–60% humidity.

Experimental Design

Plants were divided into two treatment categories: dry and moist. In the dry treatment, no water was provided for 1.5 wk after the seedlings were about 4 wk old. The moist treatment, however, provided adequate water to the seedlings throughout the experiment. Within each treatment there were four blocks (nested within treatment, such that entire blocks were either dry or moist), and each block contained six plants from each of the six populations, for a total of 288 plants (i.e., 2 treatments × 4 blocks [nested] × 36 plants). Blocks were bottom-watered to provide equivalent amounts of water to plants in a given treatment. Dummy pots were placed along the edges of each block to control for edge effects and to monitor soil moisture (with a Delta-T TDR moisture probe). For these dummy pots without plants, the average VWC for the moist treatment was 39.7%, while the average VWC for the dry treatment was 23.9%.

Before treatments began, we scored germination date and measured cotyledon area. Germination date was defined by the first day that the seedling was visible above the surface of the soil (i.e., emergence date). Cotyledon areas were estimated by creating digital images of seedling cotyledons and calculating area with ImageJ (NIH). The cotyledon areas of plants assigned to dry and moist treatments were statistically equivalent ($F = 0.17$, df $= 1$, $P = 0.68$). During the drought treatments, we measured the following traits on the most recently fully expanded leaf: leaf area, carbon assimilation rate (A), transpiration rate (E), WUE, and leaf temperature. Leaf area was determined by scanning traced leaves and calculating the area of these traces with ImageJ. Photosynthesis, transpiration, and WUE were measured using an ADC LCA4 infrared gas analyzer (IRGA) with an *Arabidopsis* leaf chamber, and leaf surface temperature was measured with an IR temperature probe (ExTech). The ADC IRGA was an open system, such that E was calculated as a function of differences in air water vapor entering and leaving the leaf chamber; also, the leaf chamber was very small with a low flow rate, so boundary layer disruption was reduced. At thermal equilibrium, this chamber had average ambient conditions of 26°C, 62% relative humidity, and 0.19 Δe (vapor pressure deficit). WUE was calculated by dividing carbon assimilation by transpiration rates. IRGA measurements were taken between 10 a.m. and 3 p.m., with the adaxial leaf surface exposed to supplemental diode light at about 800 µmol photons m^{-2} s^{-1}. The temperature of each clamped leaf was taken immediately before gas exchange measurements.

After 1.5 wk of drought treatment, all the plants were watered and grown for another week before harvest. Growth rate was measured during the week following the drought treatment as the height divided by the number of days since a given plant germinated. To estimate biomass at the conclusion of the experiment, we dried aboveground plant parts at 65°C for 24 h, then at 40°C until weighed. Plants were grown for about 6 wk and had just started to flower, which represents a short growing season; due to this short growing period, reproductive success could not be determined.

Statistical Analysis

All statistical analyses were conducted with JMP, version 4.0 (SAS Institute, Cary, NC). Population differences in germination timing and cotyledon area were examined with one-way ANOVA models. Growth rate and carbon assimilation rate were log$_{10}$ transformed to meet assumptions of normality. Carbon assimilation rate, transpiration rate, and leaf temperature were adjusted for effects of day and time (Heschel and Riginos 2005). These trait values were regressed against day and time, and the residuals from these models were added to their respective grand means. We used a nested ANOVA (standard least squares, mixed model) to determine any interactions between drought treatment, S/NS, and population; block (random effect) was nested within treatment, and population was nested within S/NS. Residual variation was normally distributed for these models. Pairwise Pearson correlations were used to determine associations between traits and biomass within the dry and moist treatments, separately; these correlations were used to determine which traits might predict biomass under dry versus moist soil conditions.

Results

All plants survived the level of drought stress inflicted by the dry experimental treatment (note: dry conditions in dummy pots never fell below 10% VWC and averaged 23.9% VWC). Both S- and NS-derived plants utilized both drought escape and dehydration avoidance to cope with the drought stress (table 1). Leaf temperature was the only trait with precisely the same mean values between NS and S plants across all treatments.

Physiology

Both S- and NS-derived plants demonstrated plasticity in WUE between the dry and moist treatments (i.e., increased WUE; fig. 1*A*). However, the S-derived plants exhibited greater plasticity than the NS-derived plants, as reflected by a significant treatment by S/NS interaction term for transpiration (*E*; table 2). Carbon assimilation rate (*A*), or photosynthesis, was statistically equivalent across all plants and treatments, and there were no significant interactions between treatment and S/NS or population (table 2; fig. 1*B*). However, photosynthetic rate was marginally higher for NS populations than for S-derived plants, regardless of treatment (table 2; fig. 1*B*).

Morphology

S-derived plants had a larger average leaf area than NS-derived plants (significant population effect; table 2). Within the S populations, the plants in the moist treatment had signifi-

Table 1

Trait Response Summary for the Effects of Dry/Moist Treatment and Population Source (Serpentine/Nonserpentine)

	Moist and dry treatment responses		Population differences
Trait	Nonserpentine	Serpentine	
Growth rate	Decreased with drought (plastic)	Same (fixed)	S > NS overall
Leaf area	Same (fixed)	Decreased with drought (plastic)	S > NS overall
A	Same (fixed)	Same (fixed)	NS > S overall
E	Decreased with drought (plastic)	Decreased with drought (plastic)	S < NS in drought
Leaf temperature	Same (fixed)	Same (fixed)	Same (fixed)

Note. S = serpentine; NS = nonserpentine; *A* = carbon assimilation rate; *E* = transpiration rate.
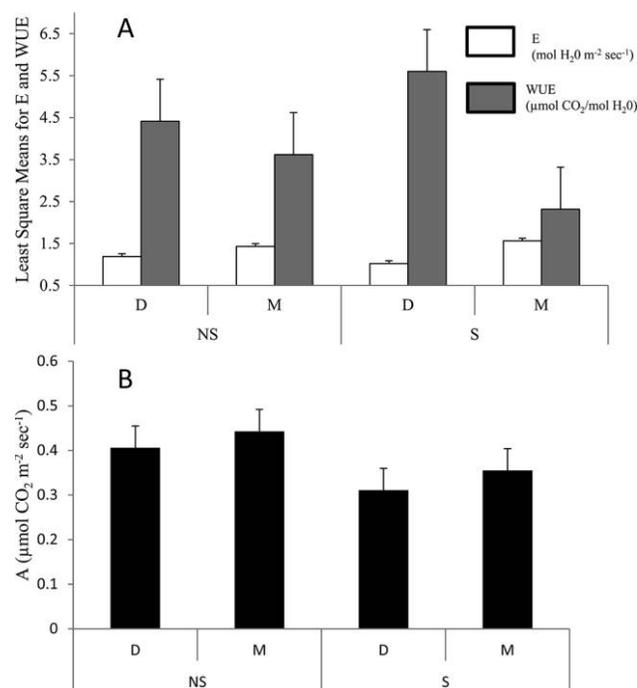
Fig. 1 Least square means (± 1 SE) of transpiration rate (E), water-use efficiency (WUE), and carbon assimilation rate (A) during dry (D) and moist (M) treatments for nonserpentine- (NS) and serpentine- (S) derived plants.

cantly larger leaf areas than the dry treatment plants (table 2; fig. 2A). Also, leaf area for S-derived plants plastically changed with drought, while NS plants had consistently small leaves between treatments (significant treatment by S/NS interaction; table 2).

Growth Rate

S-derived seedlings germinated more quickly than NS-derived seedlings (significant S/NS population effect on germination date; $F = 16.38$, df $= 1$, $P = 0.0003$). Growth rate of the *Clarkia gracilis* plants was also significantly faster for the S

populations than the NS populations (significant S/NS effect; table 2), although there was variation by population (fig. 3). Furthermore, within the S populations, growth rate did not differ between the dry and moist treatments. In the NS populations, however, the growth rate in the moist treatment was marginally faster (table 2; fig. 3). Nonetheless, there was no statistically significant interaction between S/NS and treatment for growth rate (table 2).

Biomass and Response Strategies

Aboveground biomass was significantly higher in the S populations than the NS populations for both treatments (fig. 2B). Also, for both S and NS populations, aboveground biomass was higher in moist than dry conditions. However, this increase was greater in the S than in the NS populations (table 2; fig. 2B), which may indicate greater reproductive potential in S populations. Cotyledon area, leaf area, growth rate, A, E, and WUE were significantly associated with biomass (and potentially fitness; table 3). Plants with increased leaf area, cotyledon area, and growth rate produced more biomass, regardless of treatment. Increased WUE and decreased transpiration translated into biomass gains only when plants were exposed to drought (table 3). Therefore, plants with faster growth rates, larger leaves, and increased WUE may have greater fitness potential in dry conditions.

Discussion

When dealing with drought stress, two avenues utilized by plants to cope with water limitation are drought escape and dehydration avoidance (Verslues et al. 2006). Both S and NS populations of *Clarkia gracilis* utilized drought escape and dehydration avoidance strategies in order to survive the drought stress from this experiment (table 1). Like other serpentine-tolerant species, *Clarkia* populations may be preadapted to the serpentine system because they are tolerant of exposed, open habitats (Armbruster 2014); also, the S populations studied here had only slightly drier field conditions than the NS sites. While plants from both soil types avoided dehydration with increased WUE, on average more S-derived plants attempted to escape drought with accelerated phenology (cf. Bull-

Table 2

ANOVA Results for the Effects of Dry/Moist Treatment and Population Source (Serpentine/Nonserpentine) on Traits

| Effect	Growth rate	Leaf area	A	E	WUE	Biomass
Treatment	1.44	9.15**	.69	35.82***	4.39*	17.91***
Block (treatment)	14.77***	23.22***	1.3	2.71*	2.46*	51.14***
S/NS	42.43***	18.53***	3.52+	.064	.013	43.56***
Population (S/NS)	45.11***	.84	.63	1.21	1.39	18.08***
Treatment × S/NS	.22	5.46*	.0059	5.04*	1.41	2.42
Treatment × population (S/NS)	2.80*	.78	.99	1.66	1.14	1.76

Note. F values are reported. S = serpentine; NS = nonserpentine; A = carbon assimilation rate; E = transpiration rate; WUE = water-use efficiency. Cotyledon areas not included because young seedlings were not subjected to dry and moist treatments.
  * $P < 0.05$.
  ** $P < 0.01$.
  *** $P < 0.001$.
  + $P < 0.10$.
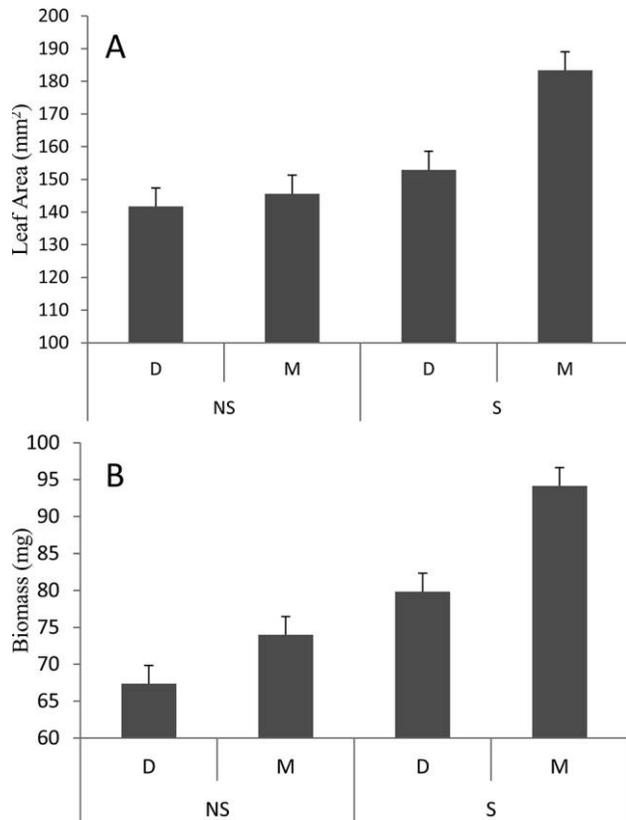
Fig. 2 Least square means (± 1 SE) of leaf area (most recent fully expanded leaf) and aboveground biomass during dry (D) and moist (M) treatments of nonserpentine- (NS) and serpentine- (S) derived plants.

Herenu and Arroyo 2009). In general, the way that the S/NS populations responded to edaphic stress was different for certain traits, which provides clues about how serpentine soil conditions affect the evolution of plant growth, physiology, and fitness potential.

Physiological and Morphological Traits

All plants in this experiment survived the stress caused by the dry treatment. Because carbon assimilation rates were similar across treatments and populations, there may be an inherent homeostasis that allows *C. gracilis* to continue photosynthesis in stressful conditions. Also, there was no difference in mean leaf temperature between treatments or populations, which points to *C. gracilis* having an additional homeostatic mechanism that maintains a minimal temperature load on leaves regardless of water availability (cf. Heschel et al. 2014). However, there were changes in other functional traits that indicate how the plants from S/NS populations responded to the drought stress, namely, WUE and growth rate.

Both S/NS populations decreased transpiration rates (increased WUE) in response to the dry treatment. This plastic response in transpiration was greater within the S populations than the NS, which may indicate that serpentine plants are better equipped to cope with intense drought conditions while also taking advantage of ephemeral soil moisture (Heschel et al. 2002).

However, this plastic response (i.e., the degree of transpiration regulation) may be lessened by low potassium levels at S sites (Larcher 2003). S populations also exhibited drought escape strategies; S-derived plants germinated more quickly and had consistently larger leaves than NS-derived plants. Therefore, S populations seemed to utilize both drought escape (e.g., faster growth) and dehydration avoidance (e.g., increased WUE) to withstand drought stress.

Traits that allow plants to escape drought may also help them escape heavy metal toxicity, especially when plant roots lack the ability to prevent the uptake of heavy metals (Kazakou et al. 2008). If plants with faster germination and growth rates also have accelerated flowering, then S-derived plants may avoid both drought and heavy metal toxicity (Maruyama et al. 2016). Faster growth and early germination should allow plants to reproduce earlier in the season, potentially avoiding the consequences of accumulating metals while still producing offspring. One NS population had relatively high growth rates, potentially because this NS site has also occasionally experienced drought stress in the field.

Plants derived from serpentine soils had larger leaf areas than plants derived from nonserpentine soils. The larger leaf area of plants derived from S populations also suggests serpentine drought escape strategies; however, leaf size was plastic for the S populations. Consistently greater leaf areas may indicate that in the field, S populations experience stressful conditions early in life, perhaps due to both limited water availability and a high concentration of metals. Greater leaf areas could contribute to increased photosynthetic/growth potential and drought escape strategies of S plants. In contrast, the consistently smaller leaves of NS-derived plants suggest the utilization of dehydration avoidance strategies. In fact, treatment did not affect the leaf size of plants derived from NS populations. Along with increased WUE, the consistently smaller leaves of NS-derived plants implies a readiness to endure (not escape) drought stress, as smaller leaves provide less surface area for water loss (Bibee et al. 2011). The smaller leaves may have also been a preadaptation to high light, warm temperatures, and drought in open habitats (Armbruster 2014), but we lack SLA data to determine whether smaller leaves also contained significant amounts of hydrenchyma tissue.

Fitness Potential and Competition

Biomass data revealed that S-derived plants had greater early-season fitness potential than NS-derived plants, no matter how much soil moisture was available in our experiment. Within-environment correlations indicated that leaf area, WUE, and growth rate were positively correlated with biomass. Thus, the larger leaves, increased WUE, and faster growth rate of S populations might translate into a higher fitness potential than the NS *C. gracilis*, particularly in drought conditions. This faster growth and greater fitness potential may also translate into a competitive advantage in this system. However, it should be noted that the biomass advantage for S-derived plants observed here was for a relatively short growing season and a single drought pulse.

Some studies with *Clarkia* in serpentine systems concur with our findings (e.g., Anacker 2014), while studies with other species have found that individuals from serpentine soils are at a
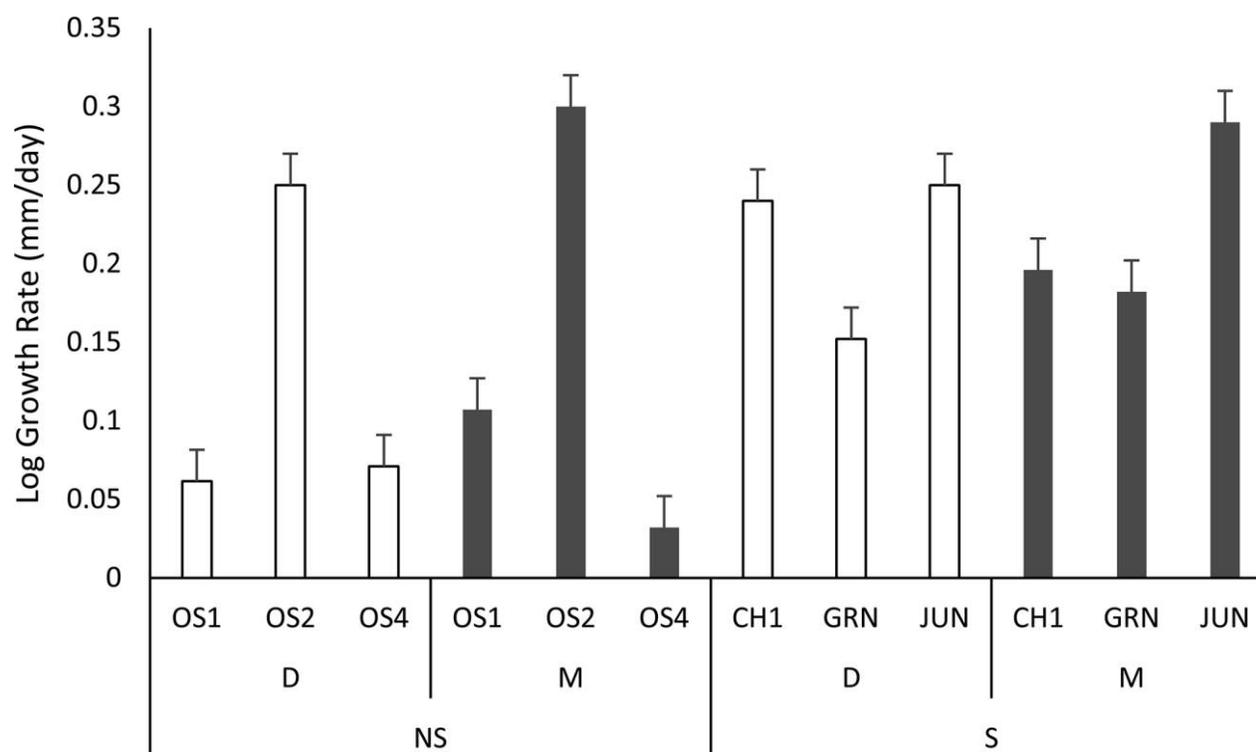
Fig. 3   Least square means (± 1 SE) of growth rate (height/days since germination) during dry (D) and moist (M) treatments for nonserpentine- (NS) and serpentine- (S) derived populations.

disadvantage when competing against nonserpentine plants on nonserpentine soils (e.g., Kruckeberg 1951; Harrison 1999; Moore and Elmendorf 2012). Interestingly, Anacker (2014) suggested that some serpentine races might be at a competitive advantage on both serpentine and nonserpentine soils by producing more biomass than nonserpentine races regardless of soil type. The results here suggest that S plants may have greater early-season competitive ability with both dehydration avoidance and drought escape. Interestingly, early-season dehydration avoidance traits are often disadvantageous due to trade-offs between WUE, carbon assimilation, and growth rate (Heschel and Riginos 2005). However, the serpentine system, with its combination of differentiation for drought and

metal/nutrient ion responses, may exemplify a situation where trade-offs between reduced water loss and photosynthesis are minimized in accumulated biomass.

Generally, serpentine endemics are considered to be inferior competitors but highly stress tolerant (Brooks 1987). This ability to endure edaphic stress may make plant communities on serpentine soils more resilient to changes in local water availability (Harrison et al. 2015). Our data suggest that a mixed suite of drought tolerance/avoidance strategies (here, drought escape and dehydration avoidance) has evolved to increase survivorship and population persistence across a range of environmental conditions in this system. Moreover, the ability of serpentine plants to exhibit plasticity in drought response traits

Table 3

Values for Pairwise Pearson Correlations with Biomass Are Reported for Nonserpentine- and Serpentine-Derived Plants within Dry (D) and Moist (M) Treatments

S/NS, treatment	Growth rate	Cotyledon area	Leaf area	A	E	WUE
NS, D	.5195***	.2323[+]	.6744***	(−.3477)**	(−.4652)***	.3494**
NS, M	.5316***	.2195[+]	.7140***	.0211	(−.0188)	.1048
S, D	.6718***	.3095*	.8060***	(−.0120)	(−.4247)***	.2722*
S, M	.7091***	.2726*	.4883***	.0823	.1877	.0899

Note.   S = serpentine; NS = nonserpentine; A = carbon assimilation rate; E = transpiration rate; WUE = water-use efficiency.

* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.
[+] $P < 0.10$.

might further increase fitness potential, especially when under stress (Dudley 1996; Heschel et al. 2002; Caruso et al. 2006). With plasticity in a mixed suite of traits, plants able to survive and grow under low soil moisture and other limiting factors in serpentine systems may have a competitive advantage over nonserpentine plants in stressful conditions. Thus, in evolving adaptations to cope with multiple stressors, S populations may have obviated trade-offs that limit stress-adapted species to particular environments.

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