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SPECIAL ISSUE ON INTRASPECIFIC VARIATION IN PLANT FUNCTIONAL TRAITS

Patterns of intraspecific trait variation along an aridity gradient suggest both drought escape and drought tolerance strategies in an invasive herb

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- **Background and Aims** In water-limited landscapes, some plants build structures that enable them to survive with minimal water (drought resistance). Instead of making structures that allow survival through times of water limitation, annual plants may invoke a drought escape strategy where they complete growth and reproduction when water is available. Drought escape and resistance each require a unique combination of traits and therefore plants are likely to have a suite of trait values that are consistent with a single drought response strategy. In environments where conditions are variable, plants may additionally evolve phenotypically plastic trait responses to water availability. Invasive annual species commonly occur in arid and semi-arid environments and many will be subject to reduced water availability associated with climate change. Assessing intraspecific trait variation across environmental gradients is a valuable tool for understanding how invasive plants establish and persist in arid environments.
- **Methods** In this study, we used a common garden experiment with two levels of water availability to determine how traits related to carbon assimilation, water use, biomass allocation and flowering phenology vary in California wild radish populations across an aridity gradient.
- **Key Results** We found that populations from arid environments have rapid flowering and increased allocation to root biomass, traits associated with both drought escape and tolerance. Early flowering was associated with higher leaf nitrogen concentration and lower leaf mass per area, traits associated with high resource acquisition. While trait values varied across low- and high-water treatments, these shifts were consistent across populations, indicating no differential plasticity across the aridity gradient.
- **Conclusions** While previous studies have suggested that drought escape and drought resistance are mutually exclusive drought response strategies, our findings suggest that invasive annuals may employ both strategies to succeed in novel semi-arid environments. As many regions are expected to become more arid in the future, investigations of intraspecific trait variation within low water environments help to inform our understanding of potential evolutionary responses to increased aridity in invasive species.

Key words: Drought escape, drought resistance, California wild radish, *Raphanus*, common garden, functional traits, leaf economics, biomass allocation, intraspecific trait variation.

INTRODUCTION

Water availability strongly impacts plant performance and survivorship and is thus a major driver of species distributions (Angert, 2009; Cornwell and Ackerly, 2009; Bartlett *et al.*, 2012; Moles *et al.*, 2014; Louthan *et al.*, 2015). In arid and semi-arid environments, investment in structures that allow a plant to withstand periods of moderate to severe water limitation can inhibit a plant's ability to rapidly acquire resources, resulting in a trade-off between carbon gain and plant water status (Schwinning and Ehleringer, 2001; Angert *et al.*, 2007; Huxman *et al.*, 2008; Gremer *et al.*, 2013). In response to drought, plants close their stomata to decrease water loss, which results in lower CO₂ influx into the leaf. To avoid reduced carbon gain, plants adapted to low water conditions can increase allocation to photosynthetic enzymes, leading to high leaf nitrogen concentration, photosynthetic rate and water-use efficiency (WUE) (Wright *et al.*, 2001; Brouillette *et al.*, 2014). A high-WUE strategy may

also include robust tissues that minimize water loss, such as leaves with high leaf mass per area (LMA), or facilitate water capture, such as dense roots or increased allocation to roots (Chapin *et al.*, 1993; Wright *et al.*, 2001; Reich *et al.*, 2003; Poorter and Markesteijn, 2008). The leaf economic spectrum (LES) predicts that leaf traits are coordinated with each other, such that species with a 'slow-return' strategy have long-lived, high-investment leaves and species with a 'fast-return' strategy have short-lived, low-investment leaves (Wright *et al.*, 2004). The extent to which above- and belowground traits are coordinated (Freschet *et al.*, 2010; Liu *et al.*, 2010) and how these traits are linked to a species' drought response strategy (Kooyers, 2015) have been less intensely studied.

Evolution of phenotypic plasticity may also play a role in response to aridity. Intraspecific variation via phenotypic plasticity is generally predicted to increase in environments characterized by strong climate variability (Molina-Montenegro and

Naya, 2012; Lázaro-Nogal *et al.*, 2015; Grewell *et al.*, 2016; Li *et al.*, 2016), facilitating the maintenance of fitness across variable conditions. Therefore, we may expect to observe high intraspecific trait variation in annual plants in arid and semi-arid ecosystems characterized by high intra- and interannual variation in precipitation (Loik *et al.*, 2004). Plasticity in the physiological and morphological traits underlying growth and reproductive output allows fitness homeostasis (Sultan, 2001; Matesanz *et al.*, 2012). Thus, plants occurring in regions with strong climate variability might display high plasticity in traits pertaining to water use and acquisition. Characterizing intraspecific variation and trait responses to water availability along environmental gradients builds our understanding of drought response strategies (Albert *et al.*, 2011; Bolnick *et al.*, 2011) and will help predict how species respond to environmental shifts, particularly in the context of climate change (Kimball *et al.*, 2016).

While we generally observe high-WUE traits associated with aridity, species may differ in their adaptation to limited water availability. Instead of investing resources in structures that allow high WUE (drought resistance), annual species may cope with limited water availability by completing their life cycle during times when water is available, resulting in earlier flowering (drought escape) (Levitt, 1980; Volaire, 2018). In order to obtain enough resources to flower and produce seed within the growing season, species with a drought escape strategy should have high rates of resource acquisition and growth (Kooyers, 2015) aligned with a fast-return economic strategy (Wright *et al.*, 2004). Several traits have been linked to early flowering in annuals including rapid growth, high leaf nitrogen concentration, high photosynthetic capacity and low WUE (Sherrard and Maherali, 2006; Franks, 2011; Campitelli *et al.*, 2016; Nguyen *et al.*, 2016). While fewer studies have examined how root traits align with drought escape, annual plants can increase root allocation in response to drought and across aridity gradients (Heschel *et al.*, 2004; Larson and Funk, 2016; Li *et al.*, 2016; Eziz *et al.*, 2017).

Many problematic invasive species have an annual life history [53 % of the world's worst weeds are annuals (Holm *et al.*, 1997; Holt *et al.*, 2013)] and occur in arid and semi-arid regions of the world (Funk *et al.*, 2016). As these regions get drier or have greater interannual variation in rainfall due to climate change (IPCC, 2013), there is an urgent need to predict how invasive species will respond to variation in water availability. While we may expect that invasive annuals will exhibit a drought escape strategy characterized by fast-return traits, studies of intraspecific trait variation across environmental gradients can elucidate the role that traits associated with drought response strategies play in adaptation to climate, and can allow the identification of traits involved in invasiveness and population persistence across landscapes (Sakai *et al.*, 2001; Rice *et al.*, 2013). Studies of intraspecific trait variation in invasive species can also improve predictive models of range expansion and species response to climate change (Moran and Alexander, 2014).

In this study, we use a common garden approach to compare functional traits across six populations of California wild radish (Brassicaceae), an annual weedy plant species. Populations were sampled along an aridity gradient ranging from high water availability (1199 mm annual precipitation)

and low temperature (average 11.5 °C) in northern California to low water availability (289 mm annual precipitation) and high temperature (average 17 °C) in southern California. We ask three questions. First, how do traits potentially associated with drought response vary among populations across an aridity gradient in California wild radish? Theory suggests that, in this annual species, populations in more arid regions will display traits aligned with drought escape (early flowering, rapid growth, high rates of carbon assimilation). Secondly, are there suites of traits that are correlated and do these correlations vary across the aridity gradient? We expect to observe correlations between traits consistent with the LES, independent of aridity. Finally, is there trait plasticity in response to water availability across populations (population by treatment interaction)? Given that the entire aridity gradient occurs in an area of high interannual precipitation variation, we expect that California wild radish will exhibit a plastic response to drought.

MATERIALS AND METHODS

Study system

California wild radish is a hybrid between *Raphanus raphanistrum* (wild radish or jointed charlock), a weed native to Eurasia that occurs in disturbed areas and agricultural fields (Holm *et al.*, 1997; Snow and Campbell, 2005) and *Raphanus sativus* (cultivated radish), a crop selected for colourful swollen roots and delayed flowering. Both *R. raphanistrum* and *R. sativus* were introduced into California in the 19th century (Panetsos and Baker, 1967). California wild radish is self-incompatible (Warwick and Francis, 2005) and pollinated by various insects (bumbees, halictid bees, syrphid flies, honeybees and butterflies) (Stanton *et al.*, 1986; Lee and Snow, 1998; Sahli and Conner, 2007). California wild radish has displaced *R. raphanistrum* in California, such that pure *R. raphanistrum* is no longer present (Baack, 2005; Hegde *et al.*, 2006; Snow *et al.*, 2010). Ongoing hybridization with *R. sativus* is likely occurring and concentrated in regions with substantial agriculture. California wild radish has been used as a model system in plant evolutionary ecology to evaluate the ecological effects of crop–wild gene flow (Klinger and Ellstrand, 1994; Snow *et al.*, 2001) and to answer fundamental questions in pollination biology, floral evolution and ecological genetics (Stanton *et al.*, 1986; Ellstrand and Devlin, 1989; Conner, 1997; Strauss and Irwin, 2004). The range of California wild radish spans California and extends southward onto the Baja peninsula in northern Mexico through California and into southern Oregon (Ellstrand and Marshall, 1985; Nason and Ellstrand, 1995; DiTomaso and Healy, 2007). A reciprocal transplant study demonstrated that populations from both northern and southern California exhibit local adaptation (Ridley and Ellstrand, 2010).

Seeds of California wild radish were collected from six populations in California between August and September of 2017 (Fig. 1; Supplementary Data Table S1). Seeds were collected from up to 30 maternal plants per site along a ~30-m transect, sampling the nearest plant to the transect every 1 m. Five crop varieties were also included ('French Breakfast', 'Cherry Belle', 'Round Black Spanish', 'White Icicle' and

‘Mantanghong Watermelon’). All crop varieties were purchased from Botanical Interests (Broomfield, CO, USA). We include the crop in this study because California wild radish is a hybrid derivative of crop radish, and ongoing gene flow from nearby crop populations may play a role in the evolution of California wild radish.

To understand how traits differ among populations we conducted a common garden study outdoors in full sun at Chapman University (Orange, CA, USA). Trait measures were conducted over two years (2017–18 and 2018–19 growing seasons; Table 1). During the first year, we measured LMA, linear growth rate, days to flowering, leaf nitrogen concentration, root:shoot biomass ratio, photosynthetic rate and WUE on a limited number of individuals (2–19 per population per treatment) for six populations. During the second year, we measured the same traits with the exception of photosynthesis and WUE on a larger number

of individuals ($N = 26\text{--}30$ per population per treatment) for two populations representing the extreme ends of the gradient (Eureka, San Diego) to increase our statistical power, as mortality resulted in low sample sizes for some populations in year 1.

Climate data

We used BioClim-modelled climate variables to describe the aridity gradient at 30 arc resolution (~ 1 km) for each population (Fick and Hijmans, 2017). We calculated an aridity index for each site using mean temperature (BIO1), maximum temperature (BIO5), temperature standard deviation (BIO4), annual precipitation (BIO12), precipitation of the wettest month (BIO13) and coefficient of variation of precipitation (BIO15) (Harouna and Carlson, 1994).



FIG. 1. We sampled seeds from six populations of California wild radish (*Raphanus sativus* \times *raphanistrum*) distributed across the state of California, USA. Sample sizes for populations range from $n = 9\text{--}34$.

TABLE 1. Description of collection sites.

Population	Code	Latitude	Distance to coast (km)	Elevation (m)	Site description
San Diego	SAN	32.906293	4.5	17	Riparian area near creek
Santa Barbara	SBA	34.413238	0.737	6	Near lagoon co-occurring with <i>Carpobrotus edulis</i>
San Luis Obispo	SLO	35.247684	8.7	32	Roadside
Vallejo	VAL	38.158908	2.16	9	Roadside
Mendocino	MEN	39.305097	0.240	5	Coastal park
Eureka	EUR	40.729528	0.256	39	Roadside

Year 1: multi-population survey

Prior to germination, each seed was weighed for seed mass. To coincide with an average start to the California growing season, which is highly variable between years, seeds were germinated in January 2018 on moist filter paper in Petri dishes and then transplanted into 4-L (10 cm wide × 25 cm deep) pots containing moistened field-collected soil. While the growing season is likely to begin at different times across populations, a standard start time was necessary for this experiment. Soil was collected from a fallow agricultural field at the South Coast Research Extension Center in Irvine, CA, USA. Nutrient levels of the collected soil were 16 and 32 mg kg⁻¹ soil for NO₃-NH₄ and P, respectively.

Plants were split into two watering treatments. The ‘high water’ plants were watered every 2 d to saturation (~30 % soil volumetric water content). The ‘low water’ plants were watered every 3–5 d to achieve an average soil volumetric water content of 10 %. Soil volumetric water content was measured with an ML3 Theta Probe Soil Moisture Sensor (Delta-T Devices, Cambridge, UK). We planted 20 replicate pots per population per treatment (up to one individual from each maternal family was included in each treatment group) but had fewer replicates for some due to mortality caused by high winds immediately following planting (Table 1). Equal numbers of individuals from each population and treatment were assigned to each block, and position within the block was randomly determined.

Plant size was measured at four time points during the growing season (13 February, 20 February, 13 March, 28 March). We also counted the number of live and senesced leaves at each time point. Leaf length and width were measured on the most recently mature leaf on each plant. Linear growth rate was calculated as [(leaf length × width)^{1/2} × leaf number] × d⁻¹ as done in Dlugosch *et al.* (2015). Senesced leaves were excluded from size calculations; however, including them did not change the findings.

Gas exchange measurements were conducted on 20 and 21 March 2018 between 0800 and 1100 h using an LI-6400 portable photosynthesis system (LI-COR, Lincoln, NE, USA). Chamber conditions were controlled at 25 °C, with CO₂ at 400 μL L⁻¹, light at 2000 μmol photons m⁻² s⁻² and relative humidity at 35–52 %. Measures included photosynthetic rate (A_{mass}; nmol CO₂ g⁻¹ s⁻¹) and WUE (mmol CO₂ mmol⁻¹ H₂O).

Plants were harvested when flowering began, which occurred from 27 March to 27 July. During harvest, plants were separated into shoot (stem and leaves) and root fractions. The most recently mature leaf from each plant was used to determine LMA (g m⁻²) and leaf nitrogen concentration (%). The harvested leaves were scanned (CanoScan LiDE 210) to obtain total leaf area (ImageJ) and dried at 60 °C for at least 48 h to obtain leaf mass. Dried leaf material was ground (Thomas Wiley Mill, 40 mesh screen) and leaf nitrogen concentration was determined using an elemental analyser (ECS 4010 CHNSO Analyzer, Costech Analytical Technologies). Leaves, shoots and roots were cleaned, dried at 60 °C for at least 48 h, and weighed to obtain a ratio of belowground dry mass to aboveground dry mass (root:shoot ratio, g root g⁻¹ shoot).

Year 2: intensive sampling of two populations

In the second growing season, we grew larger numbers of plants from two populations (Eureka, San Diego) in high and

low water treatments and grown within blocks at Chapman University, as described above. Because the field soil used in year 1 did not hold much water, we used a different soil (50 % peat, 50 % sand). However, this medium had lower nutrient availability than the field soil used in year 1 (4 and 2 mg kg⁻¹ soil for NO₃-NH₄ and P, respectively). Soil nutrient availability in disturbed California soils populated by annual invaders is variable, with nitrogen ranging from 5 to 2000 mg kg⁻¹ and phosphorus ranging from 30 to 600 mg kg⁻¹ (Funk *et al.*, 2016; Valliere *et al.*, 2017). Thus, soil nutrient levels in both years were on the low end of these ranges and this could have impacted plant performance. For example, flowering occurred earlier in the growing season in year 2 and may have resulted from lower nutrient levels. However, because all plants were exposed to the same soil in a given year, the low nutrient conditions should not affect our interpretation of population differences. An analysis of the Eureka and San Diego populations grown across years (described below) suggested few differences in trait values across the different nutrient conditions. Thus, our experimental design allows us to examine relative differences across populations but may not represent trait values observed under higher nutrient conditions in the field.

Seeds for the second-year intensive study were planted on 2 December 2019. Plant size was measured at two time points during the growing season (7 December and 28 January) using the method described above. Plants were harvested when flowering began, from 28 February to 29 April. LMA, leaf nitrogen concentration, shoot biomass and root biomass were measured as described above.

Analysis

Data were ln-transformed to meet assumptions of multivariate normality and linearity. Differences in traits among populations and water treatments were evaluated with a two-factor analysis of variance. *Post hoc* tests were conducted using Tukey contrasts. Pearson correlation coefficients were used to examine relationships among all traits. A linear model was used to evaluate the relationship between the aridity index scores and trait values. Coefficients of variation (CVs; standard deviation/mean) for each trait across water treatments were calculated to describe the extent of phenotypic plasticity among traits. An ordinary least squares linear regression was used to test for correlations between phenotypic plasticity and the aridity index. A linear mixed-effects model including populations present in both years of the study (Eureka and San Diego) with year as a fixed effect and water treatment as a random effect was used to determine what traits varied between years of the study using the lme4 R package (Bates *et al.*, 2015). All analyses were completed using R (<http://www.r-project.org>, version 3.3.2).

RESULTS

Days to flowering, root to shoot biomass allocation and photosynthetic rate were the only traits that varied significantly across populations in the multi-population survey (Fig. 2, Supplementary Data Table S1). Plants from the San Diego, San Luis Obispo and Vallejo populations flowered earlier than plants

from the other three populations (Supplementary Data Table S2). San Diego and San Luis Obispo had higher root:shoot biomass ratios than the two northernmost populations (Eureka, Mendocino; Fig. 2, Supplementary Data Table S2). Plants from Vallejo had higher photosynthetic rates than Eureka. Across populations, water stress resulted in lower photosynthetic rate and LMA but higher leaf nitrogen (Fig. 2, Supplementary Data Table S1). The highest relative root allocation was seen in the crop progenitor; however, this was caused by the swollen crop root, which was a root system notably different from that observed in all wild populations. The crop species allocated more biomass belowground (higher root:shoot ratio), had lower LMA, and delayed flowering relative to the wild populations (Fig. 2, Supplementary Data Table S1).

In the multi-population survey (year 1), we found that populations with earlier flowering had high root:shoot allocation (all groups) and lower LMA and leaf nitrogen (some groups; Table 2). In some of the population–treatment groups, we found trait relationships consistent with a fast-return economic strategy. For example, leaf N concentration and photosynthetic rate were negatively correlated with LMA in northern populations. However, several trait correlations contradicted our expectations. Under both water conditions, high photosynthetic rates did not translate into faster rates of growth. Furthermore, while not statistically significant, photosynthetic rate was negatively associated with leaf nitrogen in southern populations under conditions of low water availability.

Aridity was negatively correlated with days to maturity ($R^2 = 0.11$, $P < 0.001$), positively correlated with root:shoot allocation ($R^2 = 0.08$, $P = 0.002$), and positively correlated with A_{mass} ($R^2 = 0.04$, $P = 0.054$). No other traits were significantly associated with aridity. Coefficients of variation were not significantly associated with aridity for any trait.

In our second-year intensive survey, we found several trait differences across a representative southern (San Diego) and northern (Eureka) population. The northern population had higher LMA, lower leaf N, lower root:shoot ratio and delayed flowering relative to the southern population (Fig. 3, Supplementary Data Table S1). Across these two populations, water stress resulted in lower LMA, earlier flowering and lower root:shoot ratio (Fig. 3, Supplementary Data Table S1). The only trait that differed significantly between the year 1 multi-population survey and year 2 intensive sampling was leaf nitrogen concentration, with average leaf nitrogen concentration increasing in the year 2 study ($P = 0.012$).

In the second-year intensive survey of the San Diego and Eureka populations, we found similar trait correlation patterns as in year 1. Plants that flowered earlier had trait values consistent with high resource acquisition: high leaf nitrogen concentration, low LMA and high growth rate, although some of these correlations were restricted to certain groups (Table 3). Plants with early flowering (drought escape) tended to allocate more biomass belowground (drought tolerance). Following predictions of the leaf economic spectrum, leaf nitrogen concentration was negatively associated with LMA in all groups. Under high water conditions, we found that growth rate was negatively associated with leaf nitrogen concentration (San Diego) and positively associated with LMA (Eureka, San Diego).

DISCUSSION

In this study, we found strong evidence of intraspecific trait variation in California wild radish associated with aridity. When grown in a common environment, populations from more arid regions generally flowered earlier than northern populations and had high allocation to root tissues. Interestingly, some of the more mesic populations flowered later than the crop progenitor, which has been artificially selected for delayed flowering. Earlier flowering and higher rates of carbon assimilation associated with increasing aridity are consistent with a drought escape strategy and previous studies that have demonstrated evolution of earlier phenology in populations from more arid portions of climate gradients (Petrů *et al.*, 2006; Franks *et al.*, 2007; Bull-Hereñu and Arroyo, 2009). However, increased allocation to root tissue in populations from more arid regions is consistent with a drought resistance strategy (e.g. Sack *et al.*, 2003; Poorter and Markesteijn, 2008) and studies finding increased allocation to root tissue along aridity gradients (Heschel *et al.*, 2004; Larson and Funk, 2016; Li *et al.*, 2016; Eziz *et al.*, 2017). Thus, it appears that California wild radish succeeds in these novel arid environments by employing trait values consistent with both drought escape and tolerance strategies.

We found that individuals from the most arid populations (although this was only statistically significant in the intensive study) displayed decreased LMA and increased leaf nitrogen concentration in association with early flowering. While LMA increases along aridity gradients at a global scale (Wright *et al.*, 2005), annuals exhibiting a drought escape strategy might benefit more from maximizing rates of carbon assimilation and growth in order to flower and seed before the onset of the summer drought (Mason and Donovan, 2015). Thus, creating thinner leaves (low LMA) to maximize carbon assimilation is advantageous, which is consistent with our observations in this study. High leaf nitrogen in more arid regions is consistent with predictions of drought resistance via high WUE (Wright and Westoby, 2002; Brouillette *et al.*, 2014) and drought escape (Kooyers, 2015). As noted above, soil nitrogen and phosphorus availability were low in both years of our study, and therefore trait values may not represent those of field-grown plants, particularly in high-nutrient disturbed soils, where wild radish is often found. However, trait differences among Eureka and San Diego populations were largely similar in both years, with the exception of leaf nitrogen. This may suggest that the second-year plants were more stressed, and may have increased leaf nitrogen concentration to achieve higher rates of carbon assimilation and growth in anticipation of a shorter growing time.

Drought resistance and escape are thought to be mutually exclusive strategies that are unlikely to evolve together (Geber and Dawson, 1997; McKay *et al.*, 2003). In this study, we observed a correlation between early flowering (drought escape) and both high relative allocation to root tissue (drought resistance) and low LMA (drought escape). These results demonstrate a lack of trade-off between drought escape and drought resistance, suggesting that drought response in California wild radish may not align with a single drought response strategy. In light of recent work on belowground traits, perhaps this result is not surprising. While most studies linking traits to drought

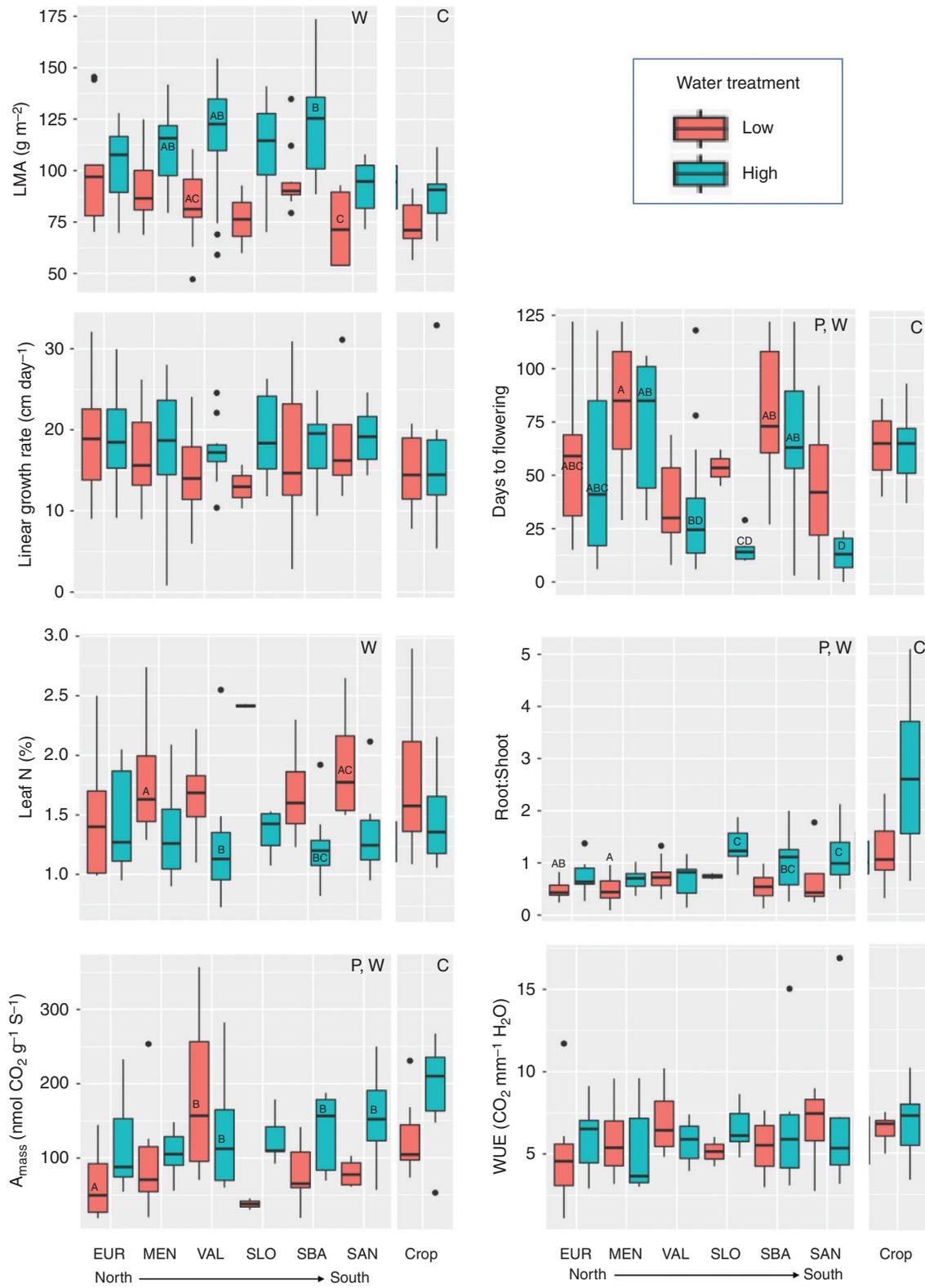


FIG. 2. Box and whisker plots demonstrate trait values for each population in the multi-population survey and the results of a two-way ANOVA. Differences between groups as determined by a Tukey's HSD *post hoc* test are demonstrated by letters within the boxes. Groups that are not different from any other groups have no letters. 'P' denotes that populations significantly differ in their trait value. 'W' denotes that for a particular trait there is a significant effect of water treatment. 'C' denotes that the crop differs significantly from the wild populations. Boxplots show the median and first and third quartiles of the data with whiskers demonstrating the 5 and 95 percentiles. Dots are statistical outliers.

TABLE 2. Trait correlations for northern (EUR, MEN, VAL) and southern (SLO, SBA, SAN) populations separated by water treatment from the year 1 multi-population survey. High water treatment correlations are shaded in grey and low water treatments correlations are unshaded. Statistically significant correlations ($P < 0.05$) are in bold type

		High water						
Northern populations		Nitrogen(%)	LMA(g m ⁻²)	Root:shoot ratio	Growth rate (cm d ⁻¹)	Days to flowering	Amass(nmol CO ₂ g ⁻¹ s ⁻¹)	WUE(CO ₂ mm ⁻¹ H ₂ O)
Low water	Nitrogen		-0.63	-0.44	-0.15	0.64	0.39	-0.05
	LMA	-0.61		0.26	0.01	-0.24	-0.70	0.12
	Root:shoot ratio	0.01	-0.22		-0.08	-0.39	0.19	0.07
	Growth rate	-0.23	0.15	-0.28		-0.25	0.17	-0.04
	Days to flowering	0.27	0.12	-0.48	-0.11		0.05	-0.18
	Amass	0.23	-0.55	0.25	-0.48	-0.12		-0.12
	WUE	0.07	-0.20	0.24	-0.29	-0.23	0.63	
Southern populations		High water						
		Nitrogen	LMA	Root:shoot ratio	Growth rate	Days to flowering	Amass	WUE
Low Water	Nitrogen		-0.31	-0.19	-0.03	0.22	0.32	-0.16
	LMA	-0.36		-0.03	-0.01	0.42	-0.58	0.45
	Root:shoot ratio	-0.11	-0.26		0.42	-0.61	-0.18	0.03
	Growth rate	-0.13	-0.01	0.23		-0.33	-0.23	0.10
	Days to flowering	0.16	0.42	-0.65	-0.17		-0.20	0.11
	Amass	-0.63	0.36	-0.51	-0.13	0.16		-0.24
	WUE	-0.62	0.21	-0.11	-0.28	-0.22	0.48	

response have focused on aboveground traits, several studies have found weak coordination among leaf, stem and root traits (Fortunel *et al.*, 2012; Bowsher *et al.*, 2016; Silva *et al.*, 2018). Despite recent renewed interest in drought response strategies (Volaire, 2018), more studies are needed to determine how traits align with the evolution of drought response strategies and how well strategies predict species response to drought. Interestingly, growth rates were not widely associated with flowering time; earlier flowering time (drought escape) was only positively correlated with growth rate in the low water treatment in the Eureka population (year 2). While growth rates were largely consistent across populations in our experiment, it is possible that differences in growth rate occur in the field, as abiotic and biotic factors influence growth rate through changes in germination timing or competition, for example.

Trait correlations observed in this study were largely consistent with predictions of the leaf economic spectrum. Across populations and water treatments, leaves with high photosynthetic rates and nitrogen concentration generally had low LMA. However, in year 2 under high water availability, high growth rate was correlated with low leaf nitrogen and high LMA in the northern population. One reason for a negative relationship between leaf-level carbon assimilation (low LMA, high nitrogen) and growth rate could be that successful individuals diluted leaf nitrogen to make more leaves rather than increasing photosynthetic enzymes. While this strategy decreases leaf-level carbon assimilation rates, it may increase plant-level assimilation (Reich *et al.*, 1989; Steers *et al.*, 2011; Funk and Wolf, 2016). This is further supported by a significant negative correlation between leaf number and leaf nitrogen ($r = -0.26$, $P = 0.01$). Alternatively, this may be an artefact of harvest time; plants that flowered later were harvested when older and may have had thicker leaves with lower nitrogen at the time (Anten *et al.*, 1998). Collectively, these results suggest that California wild radish largely follows LES predictions, but that leaf traits are

decoupled from growth rates, which may limit the ability of traits to predict invasiveness and how species respond to environmental variation.

Significant differences in multiple traits between water treatments (leaf nitrogen, LMA, photosynthesis, root:shoot ratio and days to flowering in the multi-population comparison and LMA, root:shoot ratio and days to flowering in the intensive comparison) point to plastic responses to water availability in California wild radish. Despite these plastic responses, we found no evidence for evolution of differential plasticity between populations. This result contrasts with those from a number of studies finding population-level variation in plasticity, including several invasive plant species (Molina-Montenegro and Naya, 2012; Grewell *et al.*, 2016; Li *et al.*, 2016). Our results suggest that either no differential selection for plasticity exists across this latitudinal gradient or that there has not been sufficient evolutionary time or heritable variation for differential plasticity to evolve.

In this study, we ask how traits vary within California wild radish along an aridity gradient; however, sites include complex combinations of abiotic and biotic factors that may influence trait evolution. For example, although the Santa Barbara population exists in a region characterized by low water availability and high temperature, it is located in a marshy microsite (Table 1) and does not have traits consistent with our predictions based on the temperature and precipitation in this region. Across all populations, non-climate site characteristics including biotic interactions such as pollinator limitation (Sandring and Ågren, 2009) and herbivore pressure (Siemann *et al.*, 2006) are likely, in concert with abiotic factors, to play a role in trait adaptation. Adaptation may also be constrained by pleiotropic effects, which are a known factor in the evolution of traits associated with drought response. For example, in *Arabidopsis thaliana* flowering time genes are also known to affect WUE and herbivore resistance (McKay *et al.*, 2003; Weinig *et al.*, 2003), and in *Mimulus guttatus* alleles

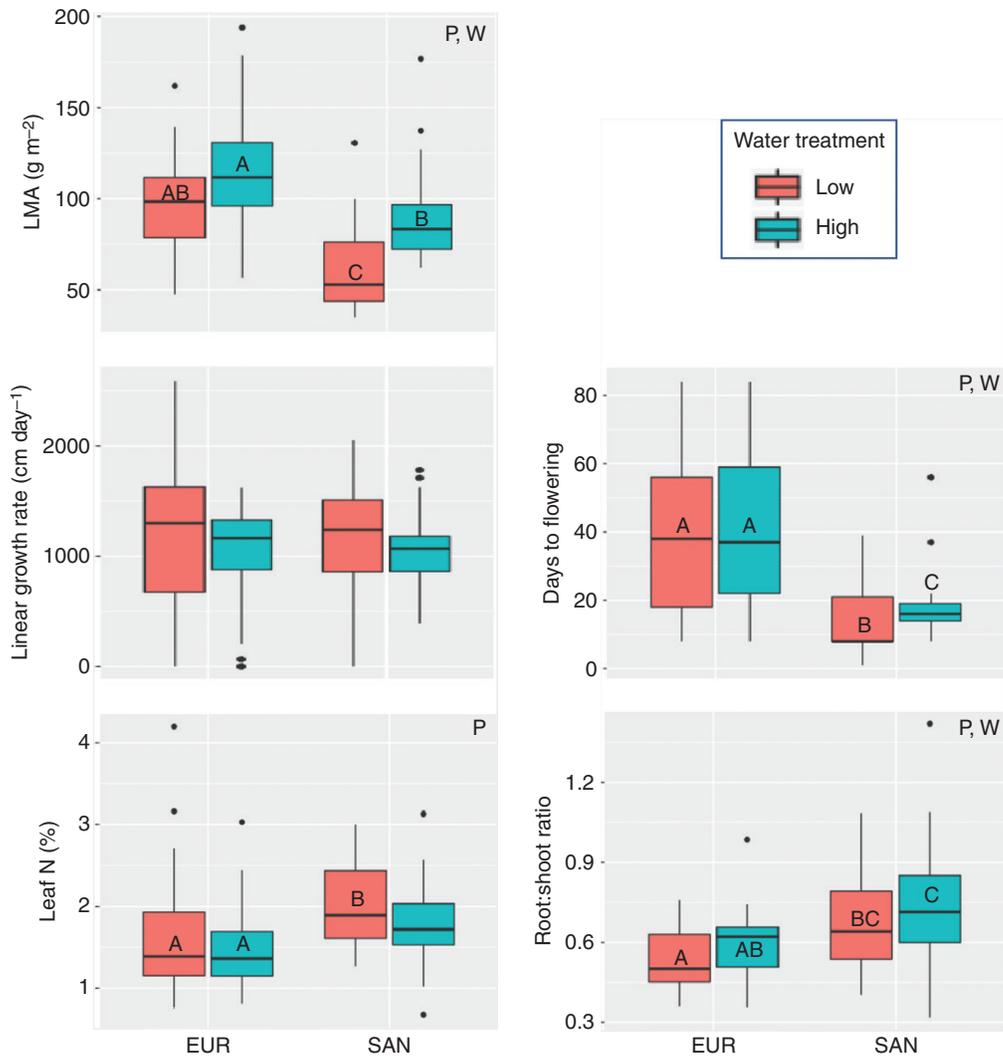


FIG. 3. Box and whisker plots demonstrate trait values for Eureka (EUR) and San Diego (SAN) in the intensive sampling survey and the results of a two-way ANOVA. Differences between groups as determined by a Tukey’s HSD *post hoc* test are demonstrated by letters within the boxes. Groups that are not different from any other groups have no letters. ‘P’ denotes that the populations significantly differ in their trait value. ‘W’ denotes that for a particular trait there is a significant effect of water treatment. Boxplots show the median, first and third quartile of the data with whiskers demonstrating the 5 and 95 percentiles. Dots are statistical outliers.

TABLE 3. Trait correlations for a representative northern (EUR) and southern (SAN) populations separated by water treatment from the year 2 intensive sampling. High water treatment correlations are shaded in grey and low water treatments correlations are unshaded. Statistically significant correlations ($P < 0.05$) are in bold type

		High water				
EUR		Nitrogen(%)	LMA(g m ⁻²)	Root:shoot ratio	Growth rate(cm d ⁻¹)	Days to flowering
Low water	Nitrogen		-0.44	0.14	-0.19	-0.39
	LMA	-0.72		-0.68	0.33	0.52
	Root:shoot ratio	0.04	-0.15		-0.05	-0.54
	Growth rate	0.10	-0.18	0.14		0.03
	Days to flowering	-0.34	0.60	0.01	-0.51	
		High water				
SAN		Nitrogen	LMA	Root:shoot	Growth rate	Days to flowering
Low water	Nitrogen		-0.58	-0.18	-0.49	-0.30
	LMA	-0.66		0.15	0.48	0.71
	Root:shoot ratio	0.16	0.03		0.08	0.02
	Growth rate	0.04	-0.04	0.05		0.26
	Days to flowering	-0.59	0.64	0.01	-0.20	

that affect flower size also delay flowering (Kelly, 2003). Due to the close genetic relationship between California wild radish and crop radish, gene flow from nearby crop populations may also impact trait variation (Klinger *et al.*, 1992). Additionally, the traits observed could be influenced by maternal effects. However, maternal effects have stronger impacts on early life traits such as germination than traits relating to vegetative growth and reproduction (Bischoff *et al.*, 2008). Additional studies are needed to understand how maternal environment impacts phenology (Gaudinier and Blackman, 2020).

While theory suggests that invasive annuals are likely to invoke resource-acquisitive traits associated with a drought escape strategy (Leishman *et al.*, 2007; Holt *et al.*, 2013; Kooyers, 2015), here we demonstrate that California wild radish responds to increasing aridity with trait values consistent with both drought escape and resistance. Invasive species that simultaneously invoke multiple drought response strategies may be better prepared for future, drier climates but studies of more species are needed. This result also suggests that single drought response strategies or LES traits may fail to predict how plant species will respond to environmental variation. Including a diversity of traits, particularly root traits, in future studies may help elucidate mechanisms of drought escape and resistance. Invasive species, like California wild radish, are strong model systems to investigate adaptation to climate because they commonly exist across broad climate ranges (Colautti and Barrett, 2013). Furthermore, studies of adaptation to climate are critical to modelling range expansion under climate change. As prevention and early detection of invasive species are the most effective forms of management (Westbrooks, 2004), improving predictive models of habitat suitability will enhance control efforts. While some invasive species are predicted to expand their ranges and become increasingly problematic with climate change (Kriticos *et al.*, 2003; Thuiller *et al.*, 2006), these models largely do not consider adaptation (Funk *et al.*, 2020; Clements and Ditommaso, 2011). Studies, such as this one, that examine the extent to which invasive species adapt to changing climate are urgently needed to enhance distribution models for invasive species (Peters *et al.*, 2014).

SUPPLEMENTARY DATA

Supplementary data are available online at <https://academic.oup.com/aob> and consist of the following. Table S1: *P* values from the analysis of variance for the multi-population and intensive two-population survey. Table S2: results of Tukey HSD *post hoc* tests from the multiple population ANOVA in which population was a significant factor.

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