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A STAGE-BASED STUDY OF DROUGHT RESPONSE IN *CRYPTANTHA FLAVA* (BORAGINACEAE): GAS EXCHANGE, WATER USE EFFICIENCY, AND WHOLE PLANT PERFORMANCE¹

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Models of global climate change predict an increase in the frequency of major droughts, yet we know little about the consequences of drought for the demography of natural populations. This study examined a population of the semi-desert perennial *Cryptantha flava* (Boraginaceae) to determine how plants of different developmental stages respond to drought through changes in leaf gas exchange, leaf water potential, water use efficiency, growth, and reproduction. In two of the four years, drought was applied using rainout shelters, and a severe natural drought occurred in another. Small, presumably younger, plants sometimes had lower rates of maximum photosynthesis, lower leaf water potentials, and lower instantaneous or integrated water-use efficiency than large plants. Small plants also had higher relative growth rates and lower reproductive effort. Large plants with evidence of shrinkage from a previously larger size often produced less growth and reproduction than large healthy plants, suggesting a decline in plant vigor with age. Drought depressed gas exchange and leaf water potentials equally in all plant stages. Thus, leaf-level physiological attributes provide no clues for why drought reduces growth more strongly in large plants. The results point to several additional avenues of research relevant to understanding stage-dependent or age-dependent plant performance under drought conditions.

Key words: Boraginaceae; *Cryptantha flava*; drought; plant aging; relative growth rate; water use efficiency.

Global climate change models predict an increasing frequency in the occurrence of extreme weather events, including drought (Palmer and Räsänen, 2002; Ackerly, 2003; Diffenbaugh et al., 2005). Yet, surprisingly few studies have determined how drought impacts the demography of natural populations. Far more is known about morphological and physiological adaptations of plants to consistently arid environments (Larcher, 2003) than about plant responses to temporal variation in water availability. If drought response varies with plant size (Casper, 1996), drought can have important but predictable repercussions for the size structure of the population and/or the relationship between plant size and fecundity (Caswell, 2001; Gurevitch et al., 2002; Bruna and Oli, 2005).

This study builds on the observation that in natural populations of the herbaceous perennial *Cryptantha flava* (A. Nels.) Payson (Boraginaceae) large plants shrink during drought while small plants continue to grow (Casper, 1996). The goals were to determine whether plants of different developmental stages respond differently to drought through changes in growth or reproduction and to examine leaf-level physiological attributes that might explain such differences. Basal-rosette forming perennials, like *C. flava*, are particularly common in cold deserts (McLaughlin, 1996). Understanding how plants of this growth form respond to drought is a step

toward understanding how climate change will alter the structure of desert vegetation.

Consistent with the observations of *C. flava*, several types of evidence suggest that susceptibility to drought varies with plant size or stage of development. These range from size-specific growth during drought in other species (Franco et al., 1994; Toft, 1995) to stage-specific differences in photosynthesis, transpiration, or water use efficiency (Nobel, 1987; Knapp and Fahnestock, 1990; Donovan and Ehleringer, 1991; Anderson and Tomlinson, 1998; Cavender-Bares and Bazzaz, 2000). Stage-specific drought responses are also demonstrated in annual crops when final seed yield is affected by the timing of water stress within the growing season (Blum, 1996; Lopez et al., 1996; Winkel et al., 1997).

Moreover, common garden studies have linked plant performance under drought conditions to leaf-level gas exchange or water potential. Such studies often explore genotypic variation in and selection on physiological attributes (Arntz and Delph, 2001; Geber and Griffen, 2003), usually in annuals, and the experimental approach maintains different but constant water levels throughout the growing season (Dudley, 1996a, b; Heschel and Riginos, 2005). How plants of different stages respond to drought is not normally a focus.

Here, we examine three developmental stages of *C. flava* for differences in leaf water potential, leaf level photosynthesis (A_{\max}), stomatal conductance (g_{\max}), instantaneous water use efficiency (A_{\max}/g_{\max}), and integrated water use efficiency, determined from carbon isotopic ratios. We assess whether there are stage-specific responses to drought in these physiological traits or in growth and reproduction. Over this 4-year study, we created drought experimentally by installing rainout shelters during the growing season in 2 years, and in another year we followed plants through a severe natural drought.

We used the physiological measurements and plant performance to test specific hypotheses regarding water

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requirements and drought responses for the different developmental stages of *C. flava*. Our first hypothesis dealt with water uptake: with increasing developmental age, plants have greater difficulty meeting their water requirements and have lower rates of photosynthesis during drought. This is consistent with the root/shoot ratio declining in many species as plants grow (Coleman et al., 1994; Gedroc et al., 1996; Muller et al., 2000). Direct measurements of root responses are not feasible for *C. flava* because the diffuse root system extends laterally as much as a meter (Peek and Forseth, 2005) and because the ephemeral fine roots, which provide most of the absorptive surface area, are difficult to recover. Alternatively, leaf-level physiological measurements should reveal whether large plants have more difficulty meeting their water requirements than small plants. If so, large plants should have lower leaf water potentials and possibly lower rates of transpiration and photosynthesis, and/or experience greater decline in these physiological parameters under drought conditions.

Our second hypothesis involved water use efficiency, often considered a measure of drought tolerance (Donovan and Ehleringer, 1994; Dudley, 1996a; Heschel et al., 2002). By examining both instantaneous water use efficiency and carbon isotopic ratio, which integrates water use efficiency over the period the carbon was assimilated, we tested the hypothesis that water use efficiency increases with increasing developmental age. We based this hypothesis on research with this species (Casper et al., 2005) and others (Knapp and Fahnestock, 1990; Donovan and Ehleringer, 1991, 1992; Cavender-Bares and Bazzaz, 2000) in which older or larger plants had greater water use efficiency. However, an increase in water use efficiency in larger, older plants is inconsistent with increasing susceptibility to drought with age.

Finally, we examined plant growth and reproduction to test the hypothesis that drought reduces the overall performance of large plants more than of small plants. We recognized that if relative growth rate normally declines with plant size, then drought could result in negative growth in large plants and positive growth in small plants even if drought depresses growth equally in plants of all sizes. We evaluated how drought affected growth and reproduction, both adjusted for plant size (relative growth rate and reproductive effort), and used regression analyses to compare the relationship between relative growth rate and plant size among years differing in precipitation. The latter approach allowed us to determine whether drought alters the slope of the relationship in a way that reflects disproportionately less growth in larger plants.

MATERIALS AND METHODS

The study system—*Cryptantha flava* grows in sandy soils from central Wyoming, throughout eastern Utah, and into northern Arizona and New Mexico, USA. A plant consists of one to >70 basal leaf rosettes supported by a branched woody underground stem (caudex) connected to a single taproot. Smaller roots branch off the taproot and may extend laterally as much as a meter before turning downward (Peek and Forseth, 2005; B. Casper, personal observation). The suberized covering of the woody root system is interrupted at intervals where ephemeral, fine roots are produced in clusters. This arrangement allows for a rapid increase in absorptive root surface following precipitation but minimizes surface exposure to dry soils. The narrow, nearly vertical, oblanceolate leaves (6.0–9.0 cm long) first appear in mid-April. New leaves are produced and old ones die throughout the spring and early summer growing season (Casper et al., 2001), and new rosettes arise from axillary buds. Additional leaves may appear in response to late summer rains (Casper et al.,

2001), but most growth occurs in spring. Seedlings emerge either in October and overwinter as green plants, or in the spring.

A vegetative meristem may continue to produce leaves for several years until it bolts, producing an erect stalk with evenly distributed leaves and a terminal inflorescence, usually bearing more than 30 flowers. Flowering is initiated in spring, with the first flowers opening by mid-May. Flower stalks die when seeds ripen in mid-June to early July, and the remaining leaves on vegetative rosettes senesce a few weeks later. The shrinkage of plants through the mortality of vegetative rosettes is particularly common in older plants (L. Spindler, University of Pennsylvania, and B. Casper, unpublished data) and in dry years (Casper, 1996).

The study population is located adjacent to Red Fleet State Park in Uintah County in northeastern Utah at 1730 m a.s.l. (40°30' N, 109°22'30" E), where vegetation is dominated by sagebrush, *Artemisia tridentata* Nutt., rabbitbrush, *Chrysothamnus nauseosus* (Pallas) Britt., and the small tree *Juniperus osteosperma* (Torr.) Little.

Climatic data—Precipitation and temperatures during the study were compared to long term climatic means from data collected at the Maeser 9 weather station, located ~18 km SW of the site, at 1950 m a.s.l. (Western Regional Climate Center, <http://www.wrcc.dri.edu>).

Experimental design—In early spring 1997, 18 5 × 5 m study plots, spatially arranged in blocks of three plots, were demarcated within a natural population of *C. flava* using at least 1 m between plots and several meters between blocks. Within each block, one randomly selected plot was covered with a rainout shelter during the 1998 growing season and another was covered in 1999. The six remaining plots received ambient precipitation all years.

In each plot, at least three small, young plants (≤12 rosettes) and at least six large plants (>12 rosettes) were selected arbitrarily from among those growing in full sun (>0.5 m from a shrub) for measures of gas exchange and plant performance. Large plants were further separated, depending on the presence or absence of dead vegetative rosettes, which is evidence of shrinkage from a previously larger size. Thus, there were three size/condition categories: small, large healthy, or large with dieback. Although absolute plant age could not be known, small plants were essentially juveniles, developmentally younger than large plants, and easily distinguished from large plants that had shrunk in size. We judged that plants with evidence of dieback were, on average, the oldest because a 20-yr study of a single cohort showed that most individuals shrink before they die (L. Spindler, University of Pennsylvania, and B. Casper, unpublished data), and there had been no recent severe drought to cause the shrinkage. A plant was moved from the adult healthy to the adult dieback category when it lost more than 30% of its vegetative rosettes. When large plants shrank to ≤12 rosettes they were removed from the study. Additional small plants were selected in 1999 to replace those that had grown out of the small size category or had died. Plants were marked with flags on short stakes and buried metal tags that could be relocated with a metal detector.

Drought treatments—Two different types of rainout shelters were used. In 1998, six plots were covered only during rain events between 1 March and 17 June by unrolling 6 m × 6 m (0.5 m plot overhang to reduce edge effects) opaque canvas tarpaulins over metal frames slanted from 1 m to 2.5 m in height to allow runoff. Covers were extended only during rainfall to minimize the effects on plot microclimate. In 1999, the frames were moved to six different plots, and the tarpaulin was replaced with stationary polyethylene roofing material that transmits both photosynthetically active (400–700 nm) and infrared radiation (Reynolds et al., 1999). The roofing material was left in place continuously between 1 March and 23 May. Ambient precipitation was measured at the field site during the growing seasons of 1998 and 1999, in order to calculate the amount of rainfall excluded by the shelters (Casper et al., 2001). Air temperatures were measured continuously under the shelters in 1999 and in unsheltered areas nearby.

Gas exchange—Maximum daily photosynthetic rates (A_{\max}) and stomatal conductances (g_{\max}) were measured on fully exposed leaves from marked plants between 1000 to 1400 hours MST approximately biweekly from mid-May to July in all 4 years of the study. Instantaneous water use efficiency was calculated as the ratio of A_{\max}/g_{\max} . Photosynthesis was measured by enclosing one to three fully expanded, healthy leaves from the same vegetative rosette in a 0.25-L chamber of a LiCor 6200 closed photosynthesis system (LiCor, Lincoln, Nebraska, USA) and calculating CO₂ depletion in the chamber over time. Leaf area was determined from length × width of the enclosed portion of

each leaf. It was not possible to sample plants in all 18 plots on a single day, but all marked plants from at least one plot from each drought treatment (sheltered in 1998, sheltered in 1999, and never sheltered) were measured on the same day to maintain balanced comparisons. For each year, the three measures, A_{\max} , g_{\max} , and A_{\max}/g_{\max} , were separately analyzed as a function of the size/condition category and drought treatment, both fixed effects, using ANCOVA with date as the covariate and block as a random factor.

We expected either that rates of gas exchange would be inherently lower in large plants or that our drought treatments would depress gas exchange more in large plants than in small plants, which would result in a significant size/condition category \times drought treatment interaction in 1998 and 1999—the years when the drought treatments were applied. No differences in gas exchange as a function of drought treatment should be evident in 1997, prior to the first drought application.

Water potential—Predawn and midday leaf water potentials were measured using a pressure chamber (Plant Moisture Systems, Corvallis, Oregon, USA) and/or a Peltier-type thermocouple psychrometer (Tru-Psi Water Potential Meter, Model SC10X, Decagon Devices, Pullman, Washington, USA). Because these are destructive measurements, leaves were taken from unmarked small and large plants within the study plots. Three to four times each year we collected one randomly selected, fully expanded, healthy leaf from at least three to five plants per size category in each treatment. We did not distinguish large healthy plants and dieback plants, and the treatment categories were either the drought treatment of the current year or controls, i.e., two drought treatments in 1998 and 1999 but none in 1997 and 2000. For each year, predawn and midday leaf water potentials were separately analyzed as a function of size category (large vs. small) and drought treatment, if applicable, using date as a covariate in ANCOVA.

As soil water potentials decreased in the latter part of the growing season, leaves often broke in the pressure chamber prior to water potential equilibrium, preventing an accurate determination of midday values. Therefore, in 1999 and 2000, a psychrometer was used in conjunction with the pressure chamber after first using the two methods on different leaves of the same plant to check for instrumental bias, which was nonsignificant. For psychrometer measurements, leaf surfaces were abraded with fine grit (600) silicon carbide paper, and leaves were placed in a stainless steel sample cup. Leaf water potential was plotted vs. time to determine suitable equilibration for these measurements.

Integrated water use efficiency—The carbon isotopic ratio δ ($^{13}\text{C}/^{12}\text{C}$) of leaf tissue was used as an integrated measure of water use efficiency (Farquhar et al., 1989). Each June, two to four leaves were collected from every living marked plant, dried, and ground in liquid nitrogen before analyzing isotopic ratios relative to standards by mass spectrometry at the Stable Isotope Ratio Facility for Ecological Research, University of Utah, Salt Lake City, Utah, or at the Department of Geology, University of Maryland, College Park, Maryland. Fifty samples were used to cross calibrate measurements from both facilities to ensure consistent results. Carbon isotope ratio values were converted to discrimination values (Δ) by the equation (Farquhar et al., 1989):

$$\Delta = (\delta_a - \delta_p)/(1 + \delta_p/1000)$$

where, δ_a = carbon isotope ratio of CO_2 in the atmosphere (assumed to be -8‰) and δ_p = measured carbon isotope ratio of the plant tissue. Lower values of Δ indicate higher water use efficiency. For each year, Δ was examined as a function of drought treatment and plant size/condition category, with block as a random factor, using ANOVA.

Whole plant performance—Vegetative and flowering rosettes on marked plants were counted once annually, in the middle of the growing season, to determine the effect of drought treatment on growth and reproduction. Growth was expressed as relative growth rate (RGR), (total no. rosettes in year₊₁ – total no. rosettes in year_t)/total no. rosettes in year_t. Reproduction was expressed as reproductive effort (RE), the number of flowering rosettes divided by the total number of rosettes. For each year_t, RGR and RE were separately analyzed as a function of drought treatment and plant size category in year_t, with block as a random factor in ANOVA.

Then to assess whether the severe natural drought in 2000 reduced growth in large plants differentially, we compared RGR graphed as a function of plant size (actual number of rosettes) among different years. For each year, we graphed RGR between year_t and year₊₁ as a function of actual plant size in year_t, and used linear regression to test for a significant relationship. Because

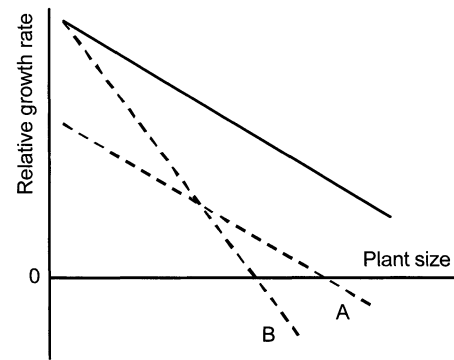


Fig. 1. The expected relationship between relative growth rate and size of plants (solid line) and two hypothetical changes in the relationship after drought. Line A indicates that growth is depressed equally in plants of different sizes, while line B illustrates a disproportionate reduction in growth for larger plants. In both cases, larger plants have negative growth.

RGR normally declines with plant size (Evans, 1972), we expected a negative slope for the regression (Fig. 1, solid line). Should all plants be equally affected by drought, a dry year should produce the same slope but a smaller y-intercept (Fig. 1, Line A), with larger plants potentially having negative growth. If large plants were more severely affected by drought, then the negative slope of the regression line would be of greater magnitude in dry years (Fig. 1, line B). We included all marked plants in the control treatment only and used ANOVA to compare slopes of significant regressions. We tested our specific prediction that the regression of RGR on plant size would produce a negative slope of the greatest magnitude 1999–2000 because 2000 was the driest year.

Statistical analyses—Physiological data involving ANOVA and ANCOVA were analyzed using STATISTICA (version 6.0, StatSoft, Tulsa, Oklahoma, USA). Post-hoc comparisons were made using the Fisher least significant difference test ($P < 0.05$). Data were transformed, when necessary, to meet homogeneity of variance. For some data that were still not normal, the Kruskal–Wallis test was applied. Regression analyses of RGR were performed using ANOVA in SAS (version 8.2, SAS Institute, Cary, North Carolina, USA).

RESULTS

Climate patterns—At the Maeser 9 NW climate station, annual precipitation averaged 357 mm from 1971 to 2003, and mean monthly temperature ranged from -6.2°C in January to 20.4°C in July. From 1997 to 1999, precipitation in winter and spring, when 60% of the annual precipitation normally falls, was above the long-term mean (Fig. 2). Precipitation from October 1999 to February 2000 was well below normal, resulting in dry soils for the initiation of spring growth; precipitation was also below normal in May and June 2000 (Fig. 2).

Temperatures during the study differed from normal in two obvious ways. From 1998 through 2000, mean monthly temperatures were generally warmer than long-term means in the winter and spring months but especially so in January 1999, when the average temperature exceeded the 30-yr mean by 4°C (Fig. 2). An exception to the warmer trend occurred between April and June 1999, when temperatures were considerably below the long-term mean. There were 13 dates between 1 April and 15 May 1999 when ambient nighttime air temperatures at the study site dropped below 0°C .

Effects of shelters—As reported in detail elsewhere (Casper et al., 2001), in 1998 the retractable rainout shelters reduced precipitation between 1 March and 17 June, 1998 by roughly

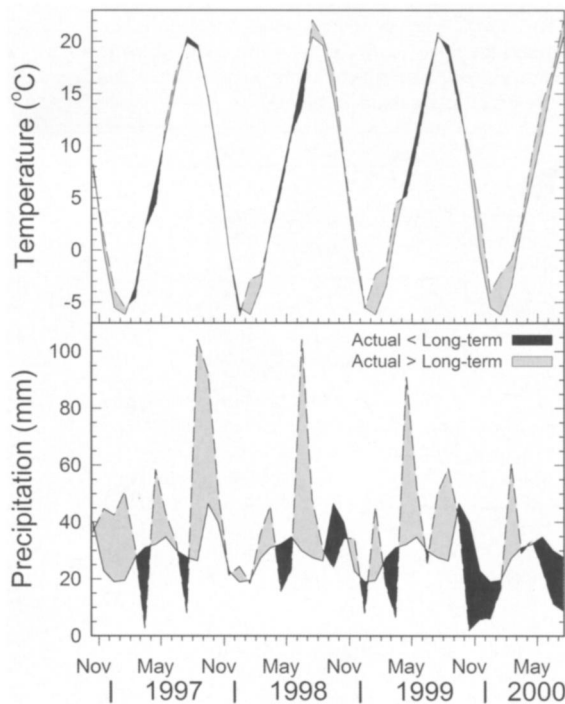


Fig. 2. Monthly mean temperatures and precipitation at the Maeser, Utah, weather station in relation to the 30-yr mean for the period from November 1996 to July 2000.

50%, while in 1999 shelters eliminated all precipitation between 1 March and 23 May 1999. In 1999, shelters also had a moderating influence on nighttime air temperatures, which were warmer than ambient by $2.6 \pm 0.1^\circ\text{C}$ (mean \pm SE). When air temperatures under shelters dropped below freezing, they remained so for shorter periods (1.5 ± 0.6 h) compared to those in the open (4.5 ± 0.8 h). Daytime air temperatures under shelters, in contrast, were always within 0.5°C of ambient.

Gas exchange—Gas exchange rates were reduced in sheltered plants and, in some years differed among plant size/condition categories, but the consistent lack of a treatment \times category interaction for A_{max} , g_{max} or $A_{\text{max}}/g_{\text{max}}$ means that gas exchange in different plant categories was equally affected by our drought treatments. The gas exchange values confirm that the drought treatment was more effective in 1999, when shelters substantially reduced both A_{max} and g_{max} , than in 1998, when sheltering plants reduced only g_{max} (Figs. 3–5, Table 1). Instantaneous water use efficiency differed among drought treatments only in 1999, when surprisingly, plants sheltered in 1998 exhibited a higher $A_{\text{max}}/g_{\text{max}}$ than those sheltered in 1999 and those never sheltered.

In years when there was a difference among size/condition categories in gas exchange, small plants had the lowest rates, with significantly lower values of A_{max} than large healthy plants in 1997 and lower $A_{\text{max}}/g_{\text{max}}$ than both categories of large plants in 1999 and 2000 (Figs. 3–5, Table 1). Large dieback plants did not differ from large healthy plants except in 1999, when they had lower g_{max} than either of the other two size categories.

There was more variation among years in gas exchange parameters than between treatments. The natural drought of

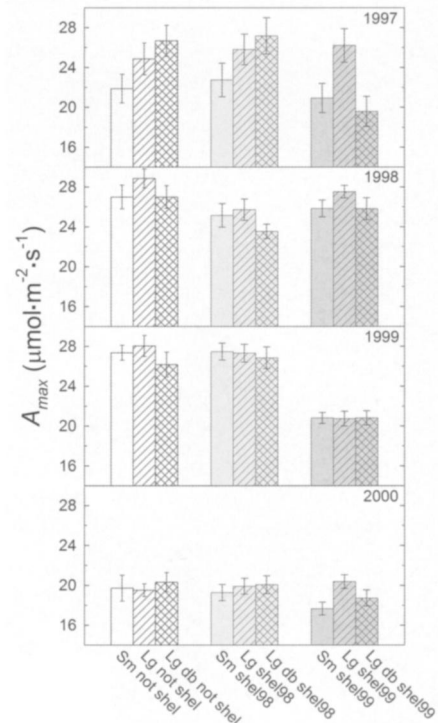


Fig. 3. Values of A_{max} (mean \pm SE) for plants of *Cryptantha flava* of different sizes/conditions (sm = small, lg = large, lg db = large with dieback) and drought treatments (shel98 = sheltered in 1998, shel99 = sheltered in 1999, or not shel = controls) for each of the 4 years.

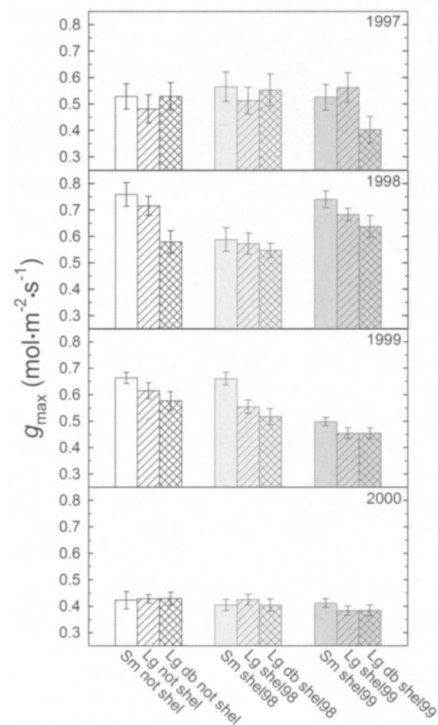


Fig. 4. Values of g_{max} (mean \pm SE) for plants of *Cryptantha flava* of different sizes/conditions and drought treatments for each of the 4 years.

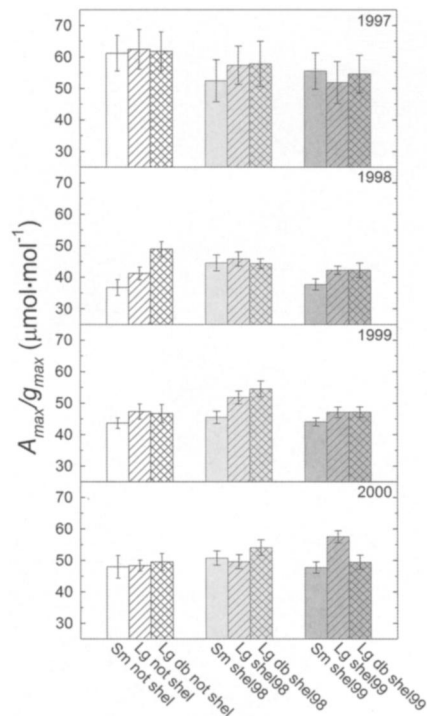


Fig. 5. Values of A_{\max}/g_{\max} (mean \pm SE) for plants of *Cryptantha flava* of different sizes/conditions and drought treatments for each of the 4 years.

2000 reduced A_{\max} and g_{\max} slightly more than did our experimental drought treatments in 1998 or 1999 (Figs. 3, 4). Date as a significant covariate indicated a decline in A_{\max} over the growing season in 1998, 1999, and 2000 and a similar decline in g_{\max} in 1998, 1999, and 2000. The changes in these two parameters resulted in a significant increase in A_{\max}/g_{\max} during the 1998 and 1999 seasons. Block was significant as a main effect, indicating spatial variation independent of our drought treatments, only for g_{\max} in 1997 and for A_{\max}/g_{\max} in 1999. A significant treatment \times block interaction term for A_{\max} in 1998 means that the 1998 drought treatment reduced A_{\max} more in some blocks than others.

Water potential—Plant water potentials were reduced by drought treatment and in some years were lower in small plants. Sheltering plants reduced both predawn and midday leaf water potentials to a greater extent in 1999 than in 1998 (Fig. 6, Table 2). Small plants had lower predawn water potentials than large plants in 1999 and 2000 and lower midday water potentials in 2000. In most years, both measures of water potential declined over the growing season, except predawn values did not have a seasonal decline in 1998.

Integrated water use efficiency—Carbon isotopic ratios (Δ) were much lower in 2000, the year of natural drought, than in other years (Fig. 7). There were differences in Δ among plant size/condition categories only in 1997, when small plants had significantly higher ($F_{2,10} = 7.7$; $P < 0.01$) values, indicating lower water use efficiency, than the other two size/condition categories. The block \times treatment term was significant ($F_{10,16} = 3.22$; $P < 0.02$) for values in 2000, indicating, in some blocks,

TABLE 1. ANOVA mean square values for gas exchange parameters in *Cryptantha flava* analyzed as a function of current size, drought treatment, and date as a covariate. In some years, sufficient data were collected in fewer than six blocks.

Year	Effect	df	A_{\max}	g_{\max}	A_{\max}/g_{\max}
1997	Date	1	177.7	1.075***	1219.4
	Category 1997 (Cat97)	2	224.2*	0.033	44.5
	Treatment (Treat)	2	148.6	0.057	1060.4
	Block	4	319.7	0.590*	3555.5
	Cat97 \times Treat	4	121.2	0.081	115.0
	Cat97 \times Block	8	43.2	0.076	714.6
	Treatment \times Block	8	80.0	0.058	378.0
	Cat97 \times Treat \times Block	16	67.5	0.030	751.4
	Error	149	47.5	0.052	743.0
	1998	Date	1	459.4***	0.095
Category 1998 (Cat98)		2	68.1	0.168	154.4
Treatment (Treat)		2	96.0	0.311*	236.2
Block		5	36.4	0.080	567.0
Cat98 \times Treat		4	8.5	0.013	70.8
Treat \times Block		10	97.2*	0.091	297.7
Cat98 \times Block		10	49.6	0.051	103.7
Cat98 \times Treat \times Block		12	33.7	0.052	155.3
Error		466	31.3	0.044	141.8
1999		Date	1	1396.8***	4.748***
	Category 1999 (Cat99)	2	14.1	0.627**	853.1**
	Treatment (Treat)	2	1178.9***	1.680**	786.9*
	Block	5	30.2	0.479	692.4*
	Cat99 \times Treat	4	2.5	0.057	84.0
	Cat99 \times Block	10	29.3	0.051	96.9
	Treatment \times Block	8	46.0	0.158	174.3
	Cat99 \times Treat \times Block	14	24.4	0.085	76.1
	Error	355	22.9	0.066	119.2
	2000	Date	1	2931.8***	1.584***
Category 2000 (Cat00)		2	28.5	0.011	$P < 0.05^a$
Treatment (Treat)		2	10.9	0.002	NS
Block		5	140.4	0.181	—
Cat00 \times Treat		4	34.6	0.018	—
Cat00 \times Block		10	42.4	0.029	—
Treatment \times Block		10	90.3	0.057	—
Cat00 \times Treat \times Block		16	32.6	0.020	—
Error		462	28.3	0.018	—

Note: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS = not significant.

^a Kruskal–Wallis test, $H = 8.64$.

significantly lower values for plants sheltered in 1999. Otherwise, there were no other differences in Δ among size/condition categories or among drought treatments. Plants sheltered in 1999 tended to have lower Δ values that year than the other two treatment groups, but the difference was not significant ($P < 0.11$).

Whole plant performance—On average, the greatest RGR occurred 1999–2000 and the lowest from 1998–1999. Small plants always had higher RGR than large healthy plants. Large healthy plants had higher RGR than large plants with dieback in all years except 1998–1999, when they did not differ (Fig. 8, Table 3). In both 1997–1998 and 2000–2001, the two categories of large plants had, on average, zero or negative RGR, while small plants produced positive growth. The experimental drought treatments did not affect RGR any year.

There were differences among size/condition categories in RE in 1997, 1999, and 2000 with small plants consistently having lower RE than large healthy plants (Fig. 9; Table 4); many small plants did not flower at all. Large dieback plants

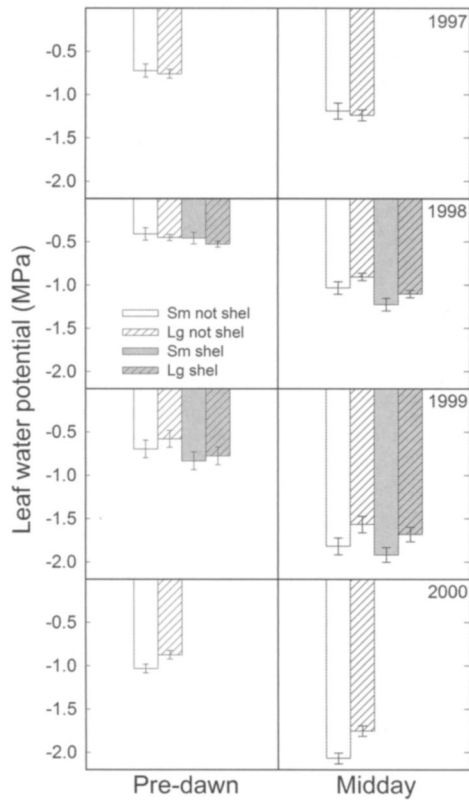


Fig. 6. Predawn and midday leaf water potential values (means \pm SE) for small and large plants of *Cryptantha flava*, separately presented for plants sheltered and those not sheltered in each of the four years.

had intermediate levels of RE, differing significantly from both small and large healthy plants in 1997 ($P < 0.05$), differing only from small plants in 1999 ($P < 0.01$) and differing from neither small nor large healthy plants in 2000.

Drought treatment affected RE only in 1999 when plants that had been sheltered in 1998 had higher RE than those sheltered in 1999 ($P < 0.001$) or controls ($P < 0.05$). Overall, RE in 1999 was lower than in other years, and many flowering stalks on unsheltered plants were visibly frost damaged while those under shelters were not.

Graphing RGR between years t and $t + 1$ provides support for the idea that drought affects large plants more severely than small plants (Fig. 10). The regression relationships are significant for each of the four years but explain more variation in 1999–2000 ($r^2 = 0.24$, $P < 0.001$) and 2000–2001 ($r^2 = 0.27$, $P < 0.001$) than in 1997–1998 ($r^2 = 0.16$, $P < 0.01$) or 1998–1999 ($r^2 = 0.12$, $P < 0.05$), which could reflect the severe 2000 drought. The slopes of the relationships, although always negative, differ across the four years ($F_{3,173} = 10.44$; $P < 0.001$). The steepest slope occurred for RGR from 1999 to 2000, but this is partly accounted for by greater growth of some small plants compared to other years. The slope of the regression for RGR 1999–2000 is only marginally different from RGR 2000–2001 ($F_{1,91} = 3.20$; $P < 0.08$), which has the next steepest slope, but significantly different from RGR 1997–1998 ($F_{1,96} = 8.80$; $P < 0.001$); 1997 and 1998 are both years without either natural drought or exceptionally cold spring temperatures.

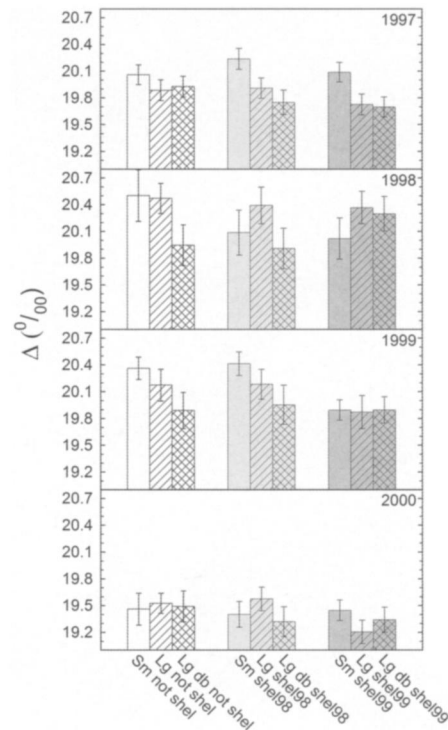


Fig. 7. Carbon isotopic ratios (Δ , means \pm SE) for plants of *Cryptantha flava* of different size/conditions and drought treatments.

DISCUSSION

Hypothesis 1—We found no support for our hypothesis that plants of increasing developmental age have greater difficulty meeting their water requirements and lower leaf-level photosynthetic rates under drought conditions. In years when leaf water potentials differed among size/developmental categories, small plants had lower values, suggesting that they adjust leaf water potentials while maintaining the same transpiration rate as large plants. This adjustment could be

TABLE 2. ANCOVA mean square values for predawn and midday (1200–1400 hours) water potential for *Cryptantha flava*, with date as the covariate and plant size and plot cover as fixed effects. Error df in parentheses.

Year	Effect	Predawn	Midday
1997	Date	11.45***	1.293***
	Plant Size	11.43	0.003
	Error	0.17 (62)	0.047 (42)
1998	Date	0.014	2.853***
	Plant Size	0.021	0.006
	Plot Cover	0.113*	0.310**
	Size \times Cover	0.001	0.025
	Error	0.018 (67)	0.030 (50)
1999	Date	21.032***	36.792***
	Plant Size	0.463	0.502*
	Plot Cover	1.715***	1.069***
	Size \times Cover	0.024	0.026
2000	Date	0.138 (139)	0.090 (175)
	Plant Size	9.692***	5.307***
	Error	0.199*	0.296**
2000	Date	0.033 (172)	0.029 (117)
	Plant Size	0.199*	0.296**
	Error	0.033 (172)	0.029 (117)

Note: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

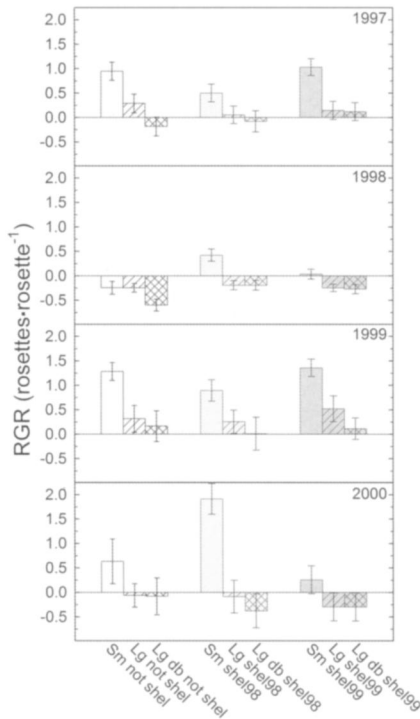


Fig. 8. Relative growth rate from year *t*, the year shown in panel, to year *t* + 1 (means ± SE) presented for plants of *Cryptantha flava* of different sizes/conditions and drought treatments.

necessary if their roots are less developed or draw from shallower, drier soil layers. In some years, small plants also had lower A_{max} or lower A_{max}/g_{max} , indicating greater water loss per unit of carbon gained. Importantly, our experimental drought treatments reduced gas exchange equally in plants of different sizes and developmental stages.

Hypothesis 2—Differences in Δ as a function of plant size existed only in the first year of the study, when small plants had higher Δ values, indicating lower water use efficiency, than large plants. This result supports our second hypothesis that water use efficiency increases with increasing developmental age, but fails to explain why large plants shrink during drought. The fact that stage-specific differences in Δ were not maintained in subsequent years could be due to the small plants selected in 1997 becoming more similar in size to large plants over the course of the 4 years. Consistent with this interpretation, there was a slight, nonsignificant trend for differences in Δ among plants of different sizes and conditions ($P < 0.11$) in 1999,

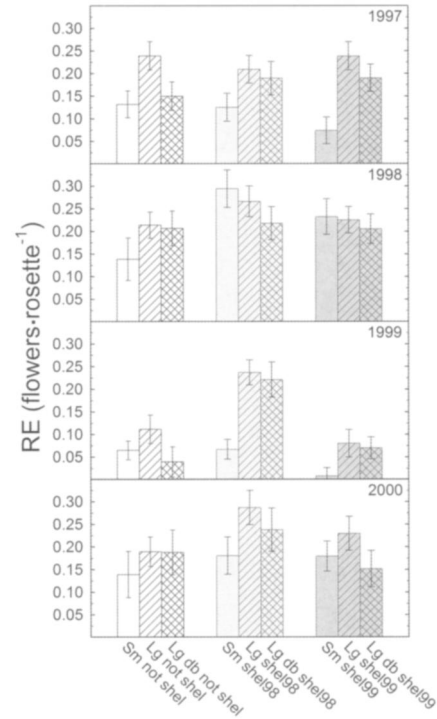


Fig. 9. Annual reproductive effort presented separately for plants of *Cryptantha flava* of different sizes/conditions and drought treatments.

when we replaced some plants that had outgrown the small size category. Differences in water use efficiency among plants of different stages might also depend on soil water status, occurring only in some years (Cavender-Bares and Bazzaz, 2000). It is noteworthy that in our study plants of different sizes did not differ in Δ in 2000, the driest year.

Changes in water-use physiology with ontogeny is a common pattern in perennial species. Larger or older plants often have higher instantaneous water use efficiency or integrated water use efficiency as measured by Δ (Knapp and Fahnestock, 1990; Donovan and Ehleringer, 1991; Donovan and Ehleringer, 1992). In red oaks (Cavender-Bares and Bazzaz, 2000), drought causes a greater increase in water use efficiency for adults and a greater depression of photosynthesis in seedlings. Those differences suggest that older plants are more competent than younger plants to cope with temporal variation in water availability.

Hypothesis 3—We examined whole plant performance to test the specific hypothesis that drought reduces the overall

TABLE 3. ANOVA mean square values for relative growth rate (RGR) (Year_{*t*} to Year_{*t*+1}) in *Cryptantha flava* analyzed as a function of size category and drought treatment by year. Error df is in parentheses.

Effect	df	1997-98	1998-99	1999-2000	2000-01
Size Category (Cat)	2	6.189***	1.480***	5.731**	5.681**
Treatment (Treat)	2	1.165	1.019	0.289	0.406
Block	5	0.674	0.248	0.532	1.095
Cat × Treat	4	0.210	0.291	0.071	0.664
Cat × Block	10	0.331	0.095	0.678	0.622
Treat × Block	10	0.424	0.367	1.060	0.863
Cat × Treat × Block	20	0.326	0.139	0.867	0.354
Error		0.246 (98)	0.219 (74)	0.250 (95)	0.339 (83)

TABLE 4. ANOVA mean square values for reproductive effort (RE) (Year_t to Year_{t+1}) in *Cryptantha flava* analyzed as a function of size category and drought treatment in Year_t. Error df in parentheses.

Effect	df	1997-98	1998-99	1999-2000	2000-01
Size Category (Cat)	2	4.424**	0.022	<i>P</i> < 0.001 ^a	<i>P</i> < 0.01 ^c
Treatment (Treat)	2	0.001	0.039	<i>P</i> < 0.001 ^b	NS
Block	5	0.005	0.121	—	—
Cat × Treat	4	0.016	0.013	—	—
Cat × Block	10	0.020	0.019	—	—
Treat × Block	10	0.034	0.029	—	—
Cat × Treat × Block	20	0.015	0.024	—	—
Error		0.016 (103)	0.018 (92)	—	—

Note: NS = not significant.
^a Kruskal-Wallis test, *H* = 43.31.
^b *H* = 18.58.
^c *H* = 12.42.

performance of large plants more strongly than small plants. Even in the absence of drought, RGR decreased and RE increased with plant size, which is typical (Evans, 1972; Franco et al., 1994). RGR and RE are presumably related because reproduction should reduce the amount of energy available for growth. The negative relationship between plant size and RGR may also reflect the fact that canopy level assimilation, expressed on a leaf area basis, cannot scale directly with measurements of photosynthesis made on individual leaves held perpendicular to incident radiation. This is due to variable leaf angles and leaf temperatures and self-shading within the leaf canopy (Forseth and Norman, 1993; Larcher, 2003).

Because RGR normally declines with plant size, a change in the slope of RGR graphed as function of plant size is required to demonstrate that drought more strongly affects growth in large plants. For *C. flava*, the slope of the relationship did change among years. The slope was steepest for RGR 1999–2000 with 2000 being the driest year of the study, but overall, RGR 1999–2000 was actually higher than in other years, and the steeper slope was largely driven by generally higher RGR of small plants compared to other years. Some repercussions of the 2000 drought were likely expressed in RGR 2000–2001, the year with the next steepest slope, but even then RGR

overall was not the lowest of the study. The regression analysis, therefore, provides limited support for our hypothesis that drought reduces growth more strongly in large plants. Because each regression line essentially represents a single data point, these analyses should be replicated across more years in order to evaluate more fully whether drought differentially affects the growth of small and large plants.

Should the pattern hold that drought reduces growth disproportionately in large plants despite size-independent effects of drought on leaf level assimilation, *A*_{max}, there will be the need to understand carbon budgets and water acquisition in whole plants as a function of plant size and environmental conditions. Further investigations should include (1) whole plant carbon assimilation, carbon allocation (Franco et al., 1994), and year round respiratory demands (Wyka, 1999), (2) the possibility that plants shrink due to hydraulic failure in a localized portion of the plant (Sperry et al., 1991; Cruiziat et al., 2002; Sperry et al., 2002; Vesk and Westoby, 2003), and (3) the possibility that leaf surface area is adjusted to maintain favorable root to shoot ratios regardless of hydraulic failure (Sharp and Davies, 1985; Sperry, 2000; Chaves et al., 2002). There is evidence from agricultural species that roots can detect dry soils and signal the shoot, which can adjust development, even before unfavorable leaf water potentials occur (Sharp and Davies, 1985; Zhang and Davies, 1987). It is also possible that the daily or seasonal duration of assimilation differs between small and large plants in ways that are not indicated by instantaneous gas exchange measurements made at the height of the growing season as they were here.

Additional observations—Whole plant performance also varies with the condition of the individual. This was indicated by adults with previous shrinkage having RGR and RE intermediate in magnitude between small and large healthy plants. As relative measures of performance, RGR and RE adjust for differences in absolute plant size, so plants with previous dieback produce less growth and fewer flowering stalks than comparably sized plants without prior shrinkage. Data from a 20-yr longitudinal study of a single cohort of *C. flava* at this site show that most plants shrink and decrease flower stalk production for a few years before they die (L. Spindler, University of Pennsylvania, and B. Casper, unpublished data), suggesting an age-related decline in physiology (Watkinson, 1992; Partridge and Barton, 1993) or hydraulic conductivity. We have no direct evidence for hydraulic failure in *C. flava*, but we did observe lower stomatal

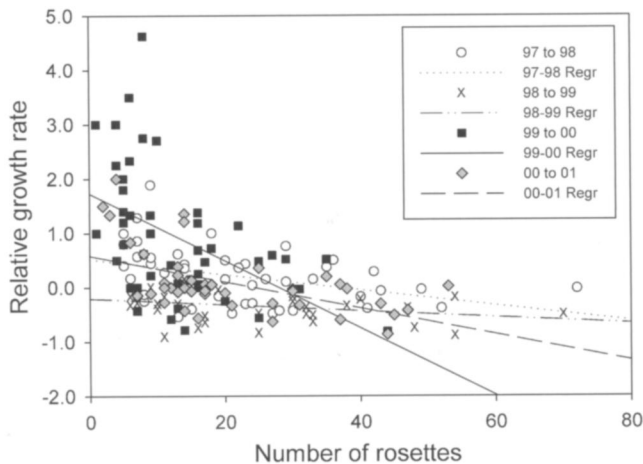


Fig. 10. Relative growth rate (year *t* to year *t* + 1) graphed as a function of size in year *t* for plants of *Cryptantha flava* in the control treatment. All plants, healthy and with dieback, are included. All regression relationships are significant (*P* < 0.05).

conductance for plants with dieback in 1999, which could reflect impedance of water transport.

Interestingly, our study shows that the biological consequences of drought extended beyond the drought year itself. There were two carryover effects of the 1998 drought treatment in the following year. In 1999, the plants that had been sheltered in 1998 had higher A_{\max}/g_{\max} and higher RE than plants sheltered in 1999 or controls. Drought has been shown to trigger flowering in some species (Southwick and Davenport, 1986; Nakajima et al., 1993), so the increase in RE we observed the year following the drought treatment is not surprising. Any residual effects from the 1999 drought treatment might have been masked by the severe natural drought in 2000.

Annual variation in flowering and growth appears to be influenced by springtime temperatures as well as precipitation. RGR from 1998 to 1999 and RE in 1999 were the lowest of the 4 years. Neither 1998 nor 1999 were particularly dry years, but the spring of 1999 was exceptionally cold. That year, freezing nighttime temperatures occurred after the initiation of plant growth, visibly damaging developing flowering stalks that were not protected by rainout shelters, which elevated nighttime temperatures.

Conclusions—Our findings that small plants sometimes had lower water potentials, lower rates of gas exchange, and lower water use efficiency than large plants were directly opposite the results expected, given our observations that drought reduces growth more strongly in large plants. Moreover, drought affected water potentials, gas exchange, and water use efficiency equally in plants of different sizes. Results suggest that a decline in overall vigor occurs with plant age. Explanations for size- and age-related phenomena, including response to drought, require a better understanding of whole plant assimilation, respiration, and hydraulic conductivity under a variety of environmental conditions. The ability to predict how natural populations will respond demographically to increasingly frequent extreme weather events relies on this information.

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