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Helianthus porteri, a Granite Outcrop Endemic, Does Not Have More Drought Resistant Traits Than Congeners

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Abstract: *Helianthus porteri*, a sunflower endemic to drought prone granite outcrops, has been hypothesized to be drought resistant. We compared *H. porteri* to three *Helianthus* species (*H. annuus, H. agrestis, H. carnosus*) from less drought prone habitats for traits associated with drought avoidance and tolerance in greenhouse experiments. Under well-watered conditions, *H. porteri* had high specific root length suggestive of high capacity for water uptake, but intermediate root mass ratio (RMR) and shallow rooting inconsistent with enhanced capacity for drought avoidance. In response to mild drought, none of the species exhibited osmotic adjustment, and *H. porteri* had no change in RMR and no greater capacity to increase water-use efficiency, again, inconsistent with greater drought avoidance. In response to cessation of watering, *H. porteri* wilted at a leaf water potential similar to a wet habitat species, inconsistent with greater drought tolerance. Overall, under the conditions assessed, we found no evidence that *H. porteri* possesses traits that confer a unique ability to avoid or tolerate drought as compared to congeners.

Keywords: drought, sunflower, root mass ratio, rooting depth, stomatal conductance, water potential, water-use efficiency

Introduction

Rocky outcrops are home to unique assemblages of endemic plants that are adapted to shallow soils, high light availability and highly variable soil moisture availability (McVaugh, 1943; Mellinger, 1972; Baskin and Baskin, 1988; Poot

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et al., 2012). Shallow soils and quick water run-off combined with high temperatures and evapotranspiration in the summer often cause water to be limiting on the outcrops (Cumming, 1969; Sharitz and McCormick, 1973; Shure and Ragsdale, 1977; Lugo and McCormick, 1981). The development of deeper soils that provide increased capacity for soil moisture availability have been shown to drive ecological interactions and plant succession in these unique habitats (Burbanck and Platt, 1964; McCormick and Platt, 1964). However, the hypothesized drought resistance mechanisms of granite outcrop species have received relatively little attention. Here we explore traits putatively associated with drought resistance for a wild sunflower endemic to granite outcrops in the Southeastern US, *Helianthus porteri* (A. gray) Pruski (Confederate Daisy), formerly *Viguiera porteri*.

Drought resistance in plants can be achieved by traits that allow plants to either escape, avoid, and/or tolerate low soil water availability (Levitt, 1980; Ludlow, 1989; Verslues *et al.*, 2006). Drought escape is generally characterized by either completing the reproductive cycle or undergoing dormancy before the onset of drought. Drought avoidance is typically portrayed by the ability to delay declines in plant-water potential by maximizing water uptake and/or minimizing water loss. Traits associated with enhanced ability to take up water include deeper rooting depth, higher allocation to root biomass (high root mass ratio, RMR) or greater surface area for water uptake (higher specific root length, SRL). Traits associated with minimizing water loss are the ability to decrease water loss through stomata (lower stomatal conductance, g_s) and increase water use efficiency (WUE). Drought tolerance is generally characterized by traits that allow plants to continue metabolic function at lower (more stressful) plant water potentials. The trait most often associated with tolerance is the ability to osmotically adjust and thus decrease the water potential at which cells lose turgor and wilt, but the ability to wilt at a less negative water potential and survive in a wilted state has also received attention (Levitt, 1980; Ludlow, 1989; Verslues *et al.*, 2006). Plants that grow through the summer on granite outcrops are expected to have some traits associated with drought avoidance or tolerance.

Helianthus porteri is found primarily within the lichen-annual and annual-perennial communities on the outcrops where soil depths range from 7 to 41 cm deep (Burbanck and Platt, 1964; Burbanck and Phillips, 1983). These communities experience wide fluctuations in soil moisture ranging from water-logged to drought conditions during the growing season, depending on drainage and weather (Lugo, 1969; Baskin and Baskin, 1988). *Helianthus porteri* is one of the few granite outcrop annuals that persists vegetatively through drought-prone summer months and reproduces in late summer through fall. In extreme years, some populations experience 100 % mortality before flowering due to drought, and population persistence is achieved through the substantive seedbank (Mellinger, 1972; Houle and Phillips, 1988; Bowsher *et al.*, 2016). In most years, however, at least some plants survive to reproduce, and survival is related soil water availability (Mellinger, 1972; Bowsher *et al.*, 2016), suggesting that drought could be a key selective pressure for this species. Although there is no evidence of differential adaptation to drought among populations of *H. porteri* (Gevaert *et al.*, 2013; Bowsher *et al.*, 2016), we hypothesize that it may have traits associated with greater drought resistance compared to congeners native to less drought-prone habitats. *Helianthus porteri* has been reported to wilt at a higher soil water content than weedy Composite species not native to outcrops, to remain viable in the wilted state for longer periods of time than other outcrop species, and to recover quickly and grow rapidly following rainfall (Shelton, 1963; Mellinger, 1972; McCormick *et al.*, 1974). This led to the suggestion that tolerance of long-term wilting, followed by rapid growth, allows this species to persist through the extreme fluctuations in resource availability that occur on granite outcrops (Shelton, 1963; Lugo and McCormick, 1981). Additionally, *H. porteri* may root deeply into cracks at the rock surface to access moisture, effectively avoiding drought (Shelton, 1963). However, there have been no comparisons of *H. porteri* to other non-outcrop sunflower species in order to determine if it has traits consistent with greater drought resistance.

In order to explore the putative drought resistance of *H. porteri*, we compared it to three congeners from non-outcrop habitats (Timme *et al.*, 2007): *H. annuus* L. (Common sunflower), *H. agrestis* Pollard (Southeastern sunflower), and *H. carnosus* Small (Lakeside sunflower). H*elianthus annuus* is a weedy erect annual that occupies a wide range of habitats throughout much of North America, including high and low rainfall regions (Heiser, 1969; Seiler and Rieseberg, 1997). In addition, this species is the wild progenitor of cultivated *H. annuu*s. *Helianthus agrestis* is an erect, branched annual found throughout central Florida. It grows in mucky, wet soils and can be found in standing water in some areas (Heiser, 1969). *Helianthus carnosus* is a rare, basal rosette-forming perennial species found in Northeastern Florida on wet, sandy soils. Among these species, *H. porteri* consistently occupies the most drought-prone habitat and *H. agrestis* occupies the least drought-prone habitat.

The broad objective of this study was to compare *H. porteri* to three other *Helianthus* species in order to determine whether it has traits associated with greater drought resistance. Specifically, we asked the following: (1) When water is not limiting, does *H. porteri* exhibit higher RMR and greater rooting depth than other *Helianthus* species, consistent with an ability to avoid declines in plant water status? (2) When exposed to mild drought, does *H. porteri* have a greater ability than other *Helianthus* species to increase RMR and WUE, consistent with an ability to avoid declines in plant water status, or a greater ability to osmotically adjust, consistent with a greater ability to tolerate drought? (3) When exposed to severe drought, does *H. porteri* differ from other *Helianthus* species in leaf water potential at wilting or ability to survive in a wilted state, consistent with a greater ability to tolerate drought? We addressed these questions in a series of four greenhouse studies that varied in treatments applied and traits assessed.

Methods

Methodology common to all four experiments

Helianthus porteri was compared to *H. agrestis, H. carnosus* and *H. annuus* in a series of greenhouse experiments at the Plant Biology greenhouses, University of Georgia, Athens GA. Achenes (hereafter seeds) were collected either directly from wild populations or obtained from the USDA Germplasm Resources Information Network (GRIN) from sites that span the range of each species. For *H. porteri*, seeds were collected from three populations in Georgia (CR, 33°14′N, −85°8′W; HR, 33°32′N, −82°16′; PM, 33°38′N, −84°10′W). For *H. annuus*, seeds were collected from one population in Utah (LS, 39°41′N, −112°22′W) and seeds of two additional populations were obtained from GRIN (TX, PI494567; NE, PI586870). For *H. agrestis*, seeds were collected from two populations in Florida (FB, 28° 21′N, −80°51′W; SC, 28°47′N, −81°51′W) and seeds of additional populations were obtained from GRIN (GL, PI673202; SC, PI673205) due to a limited availability of wild seed. For *H. carnosus*, seeds were collected from one population in Florida (FC, 29°30′N, −81°15′W) and seeds of two additional populations were obtained from GRIN (DE, AMES28375; FE, PI64956).

Seeds were germinated by excising the blunt end with a razor blade and placing the seeds on wet filter paper in Petri dishes. Seeds were kept in the dark for 48 hours and then moved to a 12h day/12h night light schedule for three to five days and transplanted to experimental pots when root hairs were observed. For all experiments, a potting mixture of 1:1 sand and calcined clay (Turface Athletics, MVP ®, Buffalo Grove, IL) was used, and pots were fertilized with Osmocote Plus 15-9-12 (Scotts, Marysville, OH). Greenhouse temperatures were generally set to maintain temperatures in the range of 21 to 30 C. However, greenhouse temperatures likely exceeded that range during summer months.

Species and treatment effects on traits were assessed with analysis of variance procedures (ANOVA) using PROC GLM in SAS (v.9.2 SAS Institute

Inc., Cary, NC, USA) with alpha of 0.05. For experiments where multiple populations were used, populations were nested within species for the ANOVA. Data were transformed as necessary to meet assumptions of ANOVA. Species least-squares means were compared with the Tukey-Kramer multiple comparison method. Additional analyses specific to each experiment (if any) are described below.

Methodology specific to each experiment

Experiment 1: Species comparison under well-watered conditions at 30 cm rooting depth stage. The planned experimental design was a randomized complete block with 4 species, 3 populations per species (*H. agrestis* FB, GL, SC; *H. annuus* LS, NE, TX; *H. carnosus* FC, FE, DE; and *H. porteri* CR, HR and PM), and 6 replicates for each population divided among 2 spatial blocks, for a total of 72 plants. However, one of the *H. porteri* populations (CR) was removed from the design due to low germination, and additional replicates were included for the *H. porteri* HR and PM populations ($n = 9$ each). Seeds were germinated on 2 April 2012 and seedlings were transplanted into 12.7 × 12.7 cm wide and 30 cm deep and 5 L volume Treepots (Stuewe and Sons, Inc., Tangent, OR). The pots were prepared for observations of root growth at 30 cm depth by insertion of a screen mesh at the bottom of each pot before being filled, and making cuts along the four corners so that a 2.5 × 2.5 cm flap could be peeled back to expose the soil and any associated roots. Slow-release fertilizer (15 g Osmocote Plus) was applied one week after transplant to each pot and plants were watered to field capacity daily.

Pots were checked daily for the presence of roots at the bottom of the pot. When a plant reached the stage where roots were observed at the bottom of the pot, that plant was measured for shoot height and harvested. Aboveground biomass was separated into stems, leaves, and roots. Leaves were digitally scanned and assessed for total plant leaf area using the freeware Image J (Schneider *et al*., 2012). Roots were excavated, rinsed, spread out in a thin layer of water, and digitally scanned for assessment of total root length and surface area (WinRHIZO v. 2002c, Regent Instruments, Quebec, Canada). In addition, a 5 cm section starting at the top of the root system (i. e., just below the root collar) was assessed for the number of lateral roots branching from the main taproot. Stem, leaves and roots were then dried at 60 °C and weighed. Root mass ratio (RMR) was calculated as total root biomass/total plant biomass and specific root length (SRL) was calculated as total root length/total root biomass.

Experiment 2: Species comparison under well-watered conditions after two months of growth. The experimental design was a randomized compete block with 3 species, one population each, (*H. annuus* LS, *H. carnosus* FE, and *H. porteri* PM) and 12–24 replicates per species ($n = 24$ for *H. porteri* and *H. carnosus*, and $n = 12$ for *H. annuus*) divided among 2 spatial blocks, for a total of 60 plants. *Helianthus agrestis* was excluded from this study because of insufficient seedling survival. Seeds were germinated on 3 October 2013 and seedlings were transplanted into clear cylindrical tubes 7.62 cm wide and 120 cm deep (Uline, Pleasant Prairie, WI, USA) fertilized with 15 g Osmocote Plus. The bottom of each tube was capped and holes were drilled to allow for drainage. Tubes were placed at a 45° angle so that plant roots would grow along the bottom side of the tube to facilitate viewing (Latta *et al.*, 2004). Heavy black plastic sheeting was placed around the tubes so that roots were not exposed to daylight. Plants were watered daily to field capacity using an automatic irrigation system and drip emitters located at each 15 cm depth intervals in each tube. The irrigation system supplied water for 5 minutes every six hours (0.63 L each watering cycle) to keep the soil near field capacity.

The black plastic around the tubes was removed at night so that roots could be observed with a dim green/blue headlamp ($< 1\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$) to avoid light responses. For each tube, the location of the deepest root was marked on the exterior of the tube and dated in order to assess rooting depth relative to the soil surface. *Helianthus annuus* root growth reached the bottom of the 120 cm deep tubes after 62 days, at which time all plants in the experiment were harvested. Biomass was divided into aboveground and belowground components, dried to a constant mass at 60 °C and weighed. Root mass ratio (RMR) was calculated as total root biomass divided by total plant biomass.

Rooting depths were analyzed with repeated-measures ANOVA (Von Ende, 1993). The between subjects effect was species, and within subjects effects were time and the time by species interaction. Univariate analyses and Greenhouse-Geisser (G-G) adjusted *p* values were used for testing within-subjects effects. Mauchly's criterion did indicate significant departure from compound symmetry, but the G-G adjusted *p* concurred with unadjusted *p* values. Results of additional multivariate analyses concurred with the univariate results (Von Ende, 1993).

Experiment 3: Species comparison under well-watered and mild drought conditions. The experimental design was a randomized complete block with 4 species and 1 population each (*H. agrestis* SC, *H. annuus* LS, *H. carnosus* FE, and *H. porteri* PM), 2 treatments (well-watered (WW) and mild sustained drought (DR)), and 12 replicates divided among 3 blocks, for a total of 96 plants. Seeds were germinated on 15 May 2013, and seedlings transplanted into 4 L pots (24.7 cm diameter; Hummert International, Earth City, MO). Plants were fertilized with 20 g Osmocote Plus and grown for approximately 40 days under well-watered conditions (watered to field capacity twice daily). At that time the WW and DR treatments were implemented using an automatic irrigation system to maintain soil moisture at predetermined set points (Nemali and Van Iersel, 2006). Based on preliminary studies, we chose 20 % soil moisture as the well-watered treatment and 14 % soil moisture as the mild drought treatment that was expected to allow plants to persist under drought without wilting or death. Soil water content was monitored with an ECH_2O-5 soil moisture probe (Decagon Devices, Pullman, WA) in each pot, and the average value per species and treatment was compared to the set-point every 30 seconds. When the average soil moisture for a given species and treatment dropped below the set point, a signal was sent to open a solenoid valve and deliver water for 30 seconds. A problem occurred with the irrigation system that affected the water delivery to the well-watered *H. annuus* early in the experiment, resulting in a brief exposure to drought. We expected that the unintended early drought likely led to reduced biomass accumulation in the well-watered *H. annuus* so we excluded biomass data for this species and treatment from our analyses. However, we included *H. annuus* gas exchange and osmotic adjustment measurements in our analyses because this species has been shown to recover photosynthetic rate and stomatal conductance within 9 days from a soil dry-down (Cechin *et al.*, 2006) and water treatments were maintained at target levels for 11 days before physiological measurements.

Twenty-two days after initiation of the drought treatment, a subset of plants was assessed for osmotic potential at full hydration (π_o), osmotic potential at turgor loss point (π_{tlp}), and relative water content at turgor loss point (RWC_{tlp}). These traits were assessed from pressure-volume curves constructed using a pressure chamber (PMS Instrument Company; Albany, OR, USA) and the bench dry method (Boyer, 1995) for rehydrated stems with leaves for *H. agrestis, H. annuus* and *H. porteri*. Due to its growth form, it was not possible to collect pressure-volume curve data for *H. carnosus* because there was no stem tissue and repeated measurements damaged the leaf. Traits values were estimated using the Pressure Volume Curve analysis spreadsheet tool (http://prometheus wiki.publish.csiro.au).

After pressure-volume curve analysis, 6 plants for each species and treatment were measured for leaf level gas exchange with a Li-Cor 6400 portable photosynthesis system (Lincoln, NE, USA): photosynthetic rate (A_{max}), stomatal conductance (g_s) and intrinsic water-use efficiency (intrinsic WUE, A_{max}/g_s). Due to cloudy weather, plants were moved to a growth chamber and acclimated to higher light conditions (photosynthetic photon flux density of approximately 600 µmol m^{-2} s^{-1}) for one hour before measurements. Gas exchange was measured on the most recently fully expanded leaf, which was produced under treatment conditions. The Li-Cor chamber conditions were set to photosynthetic photon flux density of 2000 µmol m^{-2} s^{-1}, sample CO_2 of 400 ppm, and sample H_2O of 25 mmol H_2O mol^{-1}. After gas exchange measurements, above ground biomass was collected and soil was washed away to collect roots. All biomass was dried to a constant mass at 60 °C and weighed.

Experiment 4: Species comparison under a soil dry-down followed by re-watering. The experimental design was a randomized compete block with 4 species, 3 populations per species (*H. agrestis* FB, GL, SC; *H. annuus* LS, NE, TX; *H, carnosus* FC, FE, DE; and *H. porteri* CR, HR and PM), and 9 replicates divided among 3 blocks for a total of 108 plants. The experiment had three separate stages: initial growth under well-watered conditions, a dry-down period with no watering, and a recovery period under well-watered conditions. The species were germinated on different dates (30 July–14 August 2012) in order to compensate for anticipated differences in seedling growth rate and minimize species differences in RMR at the start of the dry-down. After germination, seedlings were transplanted into 4 L pots (24.7 cm diameter) fertilized with 20 g Osmocote Plus.

Immediately prior to initiation of the dry-down (12 September 2012), three replicate plants per species and population were measured for shoot height and stem diameters, and then destructively harvested for biomass components (leaf, stem and root biomass) in order to assess RMR at the initiation of the dry-down. For the remaining plants, three replicates per species and population were measured for stomatal conductance with the Li-Cor 6400. Chamber conditions were set to photosynthetic photon flux density of 2000 µmol m^{-2} s^{-1}; sample CO_2 of 400 ppm; sample H_2O of 22.9 mmol H_2O $mol^{-1,}$ and block temperature of 27 °C. The dry-down phase was then initiated by withholding water from all plants until wilting. Daily before dawn, each plant was visually assessed for wilting, defined as having curled leaves and a petiole that was no longer stiff (i. e., the leaves were floppy). As each plant wilted, plant pre-dawn Ψ_{leaf} was measured with a pressure chamber to estimate leaf turgor loss point. Additionally, the plants that had been measured for stomatal conductance prior to the dry-down were re-measured for stomatal conductance when wilted to provide an estimate of cuticular conductance when stomata are maximally closed (Howard and Donovan, 2007). After wilting, each plant remained wilted for a preassigned number of days (3, 5, 7, 9, 11 or 13; one replicate per species per day) before being re-watered to field capacity and returned to daily watering schedule to determine plant survival.

Results

Experiment 1: species comparison under well-watered conditions at 30 cm rooting depth stage

After growing under well-watered conditions, plants were harvested when they reached 30 cm rooting depth. The *Helianthus* species differed for the number of Days to Harvest ($F_{3,56} = 71.76$, $P < 0.001$; Figure 1A), which serves as a proxy for root growth rate. *Helianthus porteri* took longer to reach 30 cm rooting depth than *H. annuus*, but did not differ from *H. agrestis*, and reached 30 cm depth much sooner than *H. carnosus*. The species also differed for plant size and root characteristics at the 30 cm rooting depth stage. *Helianthus porteri* was the tallest species ($F_{3,56} = 83.08$, $P < 0.001$; Figure 1B), and, along with *H. carnosus*, had a higher number of leaves ($F_{3,56} = 17.55$, $P < 0.001$; Figure 1C). *Helianthus porteri* had intermediate total biomass ($F_{3,56} = 17.29$, $P < 0.001$; Figure 1D) and RMR ($F_{3,56} = 12.81$, $P < 0.001$; Figure 1E) compared to the other species.

The species also differed for root traits at the 30 cm depth rooting stage. *Helianthus porteri* and *H. agrestis* had a higher number of lateral roots in the top 5 cm of soil ($F_{3,56} = 11.61$, $P < 0.001$; Figure 1F), and both of these species, along with *H. carnosus*, had higher total root length than *H. annuus* ($F_{3,56} = 16.79$, $P < 0.001$; Figure 1G). Additionally, *H. porteri* had relatively small diameter fine roots ($F_{3,56} = 7.35$, $P < 0.001$; Figure 1H) and a high root surface area similar to *H. agrestis* and *H. carnosus* ($F_{3,56} = 10.68$, $P < 0.001$; Figure 1I). The high root length combined with thin roots and lower root biomass give *H. porteri* the highest SRL ($F_{3,56} = 38.21$, $P < 0.001$; Figure 1J).

Experiment 2: species comparison under well-watered conditions after two months of growth

Over two months of growth in 120 cm tubes under well-watered conditions, *H porteri* exhibited much shallower rooting depth than *H. annuus*, and slightly deeper rooting depth than *H. carnosus* ($F_{10,180} = 41.13$, $P < 0.001$; Figure 2). This

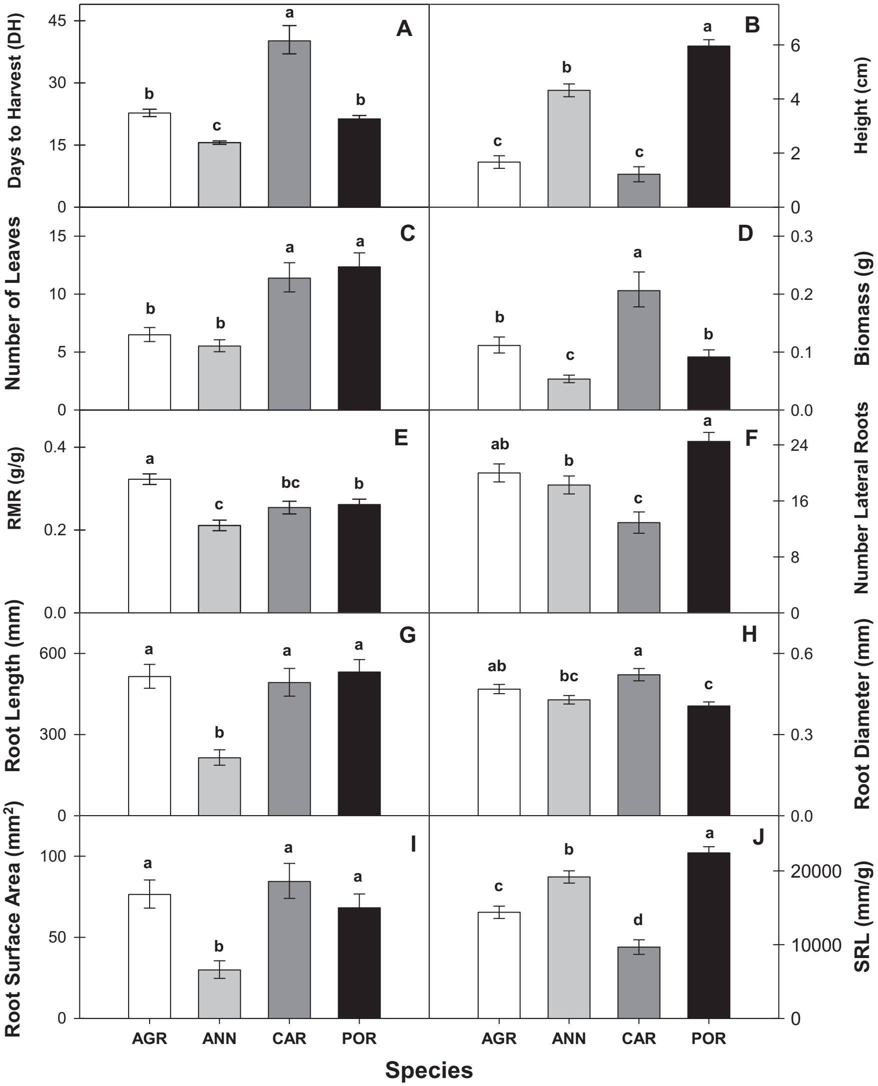

Figure 1: Plant traits (means ± SE) at 30 cm rooting depth for *Helianthus agrestis* (AGR, n = 18), *H. annuus* (ANN, n = 18), *H. carnosus* (CAR, n = 14) and *H. porteri* (POR, n = 18): Days to harvest (A), Height (B), Number of Leaves (C), Total Biomass (D), Root Mass Ratio (RMR; E), Number of Lateral Roots in the top 5 cm of soil (F), Root Length (G), Root Diameter (H), Root Surface Area (I), and Specific Root Length (SRL; J). For each trait, different letters indicate significant species differences based on a multiple comparison test.

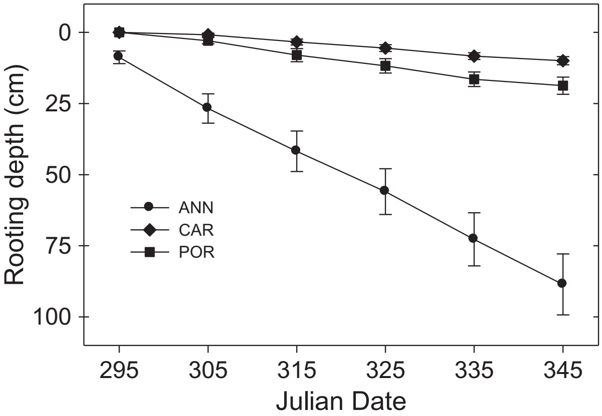

Figure 2: Rooting depth (mean ± SE) sampled at 10 day intervals over a two-month growing period for well-watered *Helianthus annuus* (ANN, n = 9), *H. carnosus* (CAR, n = 16), and *H. porteri* (POR, n = 14). See text for statistical results of repeated measures ANOVA.

ranking of species rooting depth at each sampling date is consistent with the time each species took to reach 30 cm depth in experiment 1: *H. annuus* faster, *H. carnosus* slower, and *H. porteri* intermediate.

After 2 months of growth, *H. porteri* total biomass was not different from *H. carnosus*, but was substantially less than *H. annuus* ($F_{2,52} = 20.99$, $P < 0.001$; Figure 3A). In this experiment, RMR did differ by species ($F_{2,52} = 6.55$, $P = 0.003$; Figure 3B), and the species ranking in this experiment was inconsistent with that found when plants were harvested at the same 30 cm depth stage in experiment

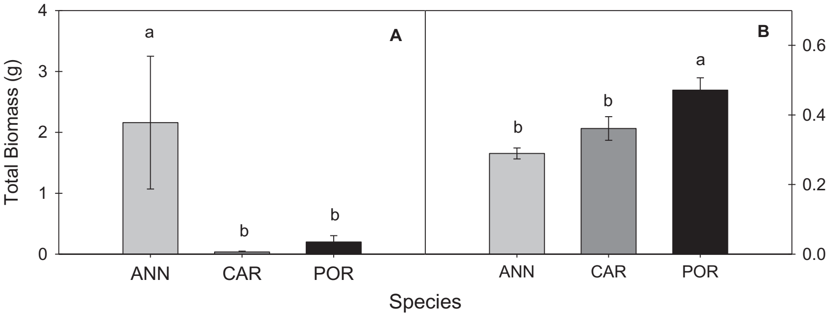

Figure 3: Biomass traits (means ± SE) for *Helianthus annuus* (ANN, n = 9), *H. carnosus* (CAR, n = 16) and *H. porteri* (POR, n = 14) harvested after two months of growth under well-watered conditions in 120 cm deep pots: Total Biomass (A) and Root Mass Ratio (RMR; B).

1. The differences in species ranking for RMR are likely due to the differences in growth period among species in experiment 1.

Experiment 3: species comparison under well-watered and mild drought conditions

In this experiment, the species differed for total biomass ($F_{2,57} = 56.93$, $P < 0.001$; Figure 4A) and there was a trend for a decreased biomass in response to 22 day mild drought treatment ($F_{1,57} = 3.62$, $P = 0.063$), but no significant interaction of species and treatment ($F_{2,57} = 0.49$, $P = 0.614$). *Helianthus porteri* had higher biomass than *H. agrestis* and *H. carnosus* in both treatments. For RMR, the species effect was not significant ($F_{2,57} = 0.39$, $P = 0.680$; Figure 4B) but treatment ($F_{1,57} = 8.00$, $P = 0.007$) and interaction were significant ($F_{2,57} = 3.05$, $P = 0.056$). *Helianthus agrestis* and *H. carnosus* responded to the mild drought treatment with increases in RMR while *H. porteri* did not.

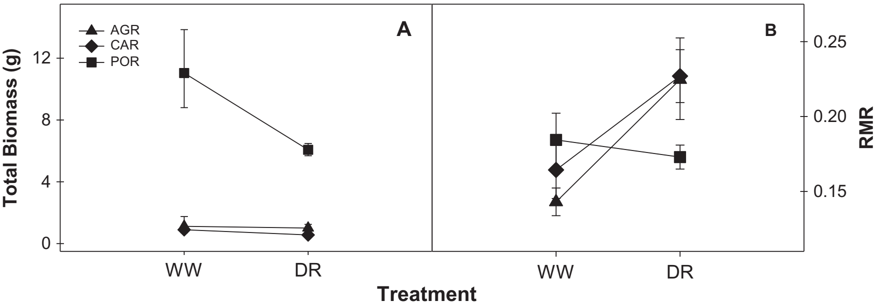

Figure 4: Biomass traits (means ± SE) for *Helianthus agrestis* (AGR, n = 7–10), *H. carnosus* (CAR, n = 10–12) and *H. porteri* (POR, n = 10–10) grown under well-watered (WW) and mild drought (DR) treatments: Total Biomass (A) and Root Mass Ratio (RMR; B).

For photosynthesis (A_{max}), there were species differences, ($F_{3,40} = 7.44$, $P < 0.001$; Figure 5A), and a general decrease in response to the mild drought treatment ($F_{1,40} = 4.19$, $P = 0.048$), but no significant interaction of species and treatment ($F_{3,40} = 1.15$, $P = 0.345$). For stomatal conductance (g_s), there were also species differences ($F_{3,40} = 13.17$, $P = 0.001$; Figure 5B) and a response to the mild drought treatment ($F_{1,40} = 37.06$, $P = < 0.007$), and significant interaction of species and treatment apparently driven by *H. carnosus* ($F_{3,40} = 7.17$, $P = 0.008$). This resulted in significant species ($F_{3,40} = 4.16$, $P = 0.013$; Figure 5C) and treatment ($F_{1,40} = 23.26$, $P < 0.001$) effects for instantaneous WUE, and a significant

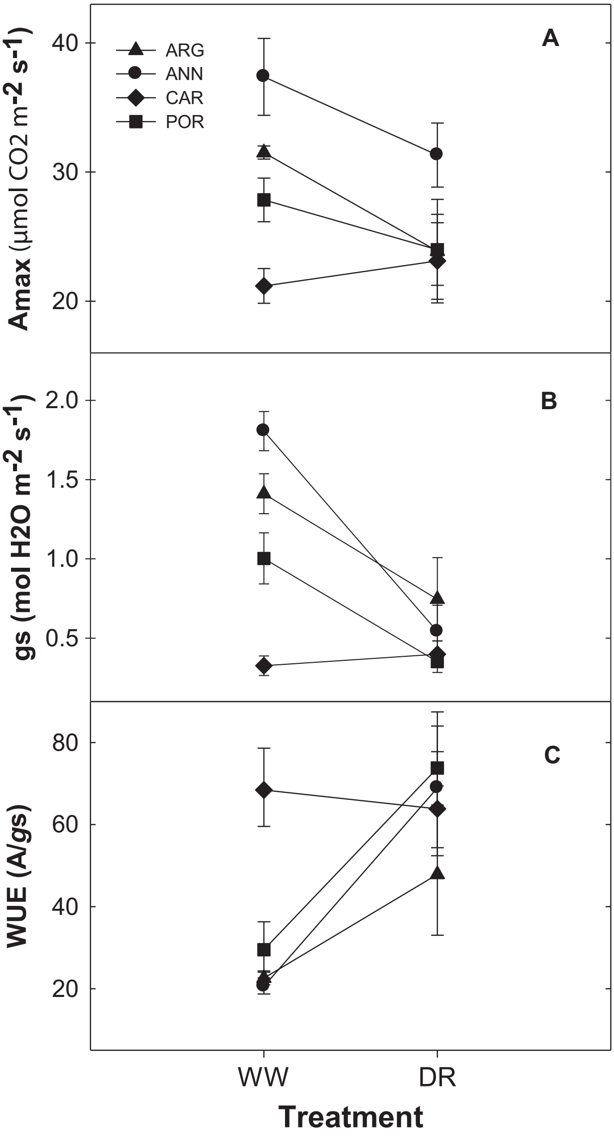

Figure 5: Gas exchange traits (mean ± SE) for *H. annuus* (ANN, n = 12–11), *H agrestis* (AGR, n = 7–10), *H. carnosus* (CAR, n = 10–12) and *H. porteri* (POR, n = 10–10) grown under well-watered (WW) and mild drought (DR) treatments: Maximum Photosynthesis (A_{max}; A), Stomatal Conductance (g_s; B), and Intrinsic Water-Use Efficiency (WUE; C).

interaction ($F_{3,40} = 3.55$, $P = 0.025$) again, largely driven by *H. carnosus* ($F_{2,40} = 3.55$, $P = 0.025$). *Helianthus porteri* was intermediate in gas exchange responses.

In the well-watered treatment, there were no significant differences among the three species for pressure-volume curve parameters (osmotic potential at full rehydration (π_o), turgor loss point (π_{tlp}), and relative water content at turgor loss point (RWC_{tlp}), Table 1) and for the osmotic potential at full rehydration using the vapor pressure osmometer (π_o VPO). In addition, there were no treatment differences between the well-watered and drought treatments for π_o which would be indicative of an increased accumulation of compatible solutes. Thus, there was no evidence that the mild drought induced osmotic adjustment in any of the species.

Experiment 4: species comparison under a soil dry-down followed by re-watering

The final experiment was designed to compare species responses to a more substantive soil dry-down. Plant traits were measured prior to the initiation of the dry-down and again at plant wilting, and plants were then re-watered and monitored for recovery. Germination times were staggered in this experiment in an effort to achieve similar species RMR, and thus similar ratio of root biomass for water uptake and shoot biomass, at the start of the dry-down. The staggered germination did result in a similar RMR ($F_{3,20} = 0.78$, $P = 0.518$; Figure 6A) and stem diameter ($F_{3,20} = 2.40$, $P = 0.099$; Figure 6B) at the initiation of the dry-down, but *H. porteri, H. agrestis*, and *H. annuus* were taller than *H. carnosus* ($F_{3,20} = 27.56$, $P < 0.001$; Figure 6C) at this time. The higher biomass of *H. carnosus* in this comparison was likely due to being germinated first ($F_{3,20} = 17.12$, $P < 0.001$; Figure 6D). Under these well water conditions prior to dry-down initiation, *H. porteri* had an intermediate g_s, ($F_{3,22} = 19.77$, $P < 0.001$; Figure 6E), consistent with Experiment 3.

Following the initiation of the dry-down, i. e., cessation of watering, the species differed by length of time until wilting ($F_{3,54} = 124.14$, $P < 0.001$; Figure 6F). At wilting, g_s of *H. porteri* was similar to *H. agrestis* and *H. carnosus*, and much lower than *H. annuus* ($F_{3,22} = 25.67$, $P < 0.001$; Figure 6G). In addition, *H. porteri and H. agrestis* had a higher (less negative) ψ_{PD} ($F_{3,55} = 89.28$, $P < 0.001$; Figure 6H) at wilting than *H. carnosus* and *H. annuus*. After persisting in a wilted state for pre-assigned lengths of time (3–13 days) before re-watering, all individuals survived and exhibited new above ground tissue growth after re-watering (data not presented), indicating no species difference in ability to persist in a wilted state for the treatment periods tested here.

Table 1: Water relations traits (means ± SE) for *Helianthus agrestis* (AGR), *H. annuus* (ANN), and *H. porteri* (POR) grown under well-watered (WW) and controlled mild drought (DR) treatments: Osmotic potential at full rehydration obtained from a pressure-volume curve (π_o PV), turgor loss point obtained from a pressure-volume curve (π_{tlp} PV), relative water content at turgor loss point (RWC_{tlp}), and the osmotic potential at full rehydration using a vapor pressure osmometer (π_o VPO) (N = 6 for every species/treatment except for N = 5 for POR WW). F- and P-values are presented for species, treatment and the species*treatment interaction.

		Well-Watered (WW)	Mild Drought (DR)	Species		Treatment		Species*Treatment	
				F	***P***	***F***	***P***	***F***	***P***
π_o PV	AGR	−0.663 ± 0.051	−0.680 ± 2.053	0.39	0.683	2.75	0.108	0.52	0.602
	ANN	−0.538 ± 0.077	−0.689 ± 0.065						
	POR	−0.585 ± 0.043	−0.688 ± 0.087						
π_{tlp} PV	AGR	−0.829 ± 0.060	−0.884 ± 0.048	0.51	0.484	1.50	0.238	0.16	0.696
	ANN	−0.768 ± 0.097	−0.884 ± 0.053						
	POR	−0.807 ± 0.029	−0.867 ± 0.093						
RWC_{tlp}	AGR	91.316 ± 0.048	92.967 ± 1.369	3.78	0.070	1.36	0.261	0.55	0.471
	ANN	89.412 ± 1.932	90.839 ± 0.631						
	POR	89.848 ± 0.459	90.294 ± 0.629						
π_o VPO	AGR	−0.762 ± 0.048	−0.773 ± 0.022	0.30	0.590	1.59	0.224	1.26	0.277
	ANN	−0.719 ± 0.015	−0.766 ± 0.029						
	POR	−0.752 ± 0.028	−0.820 ± 0.024						

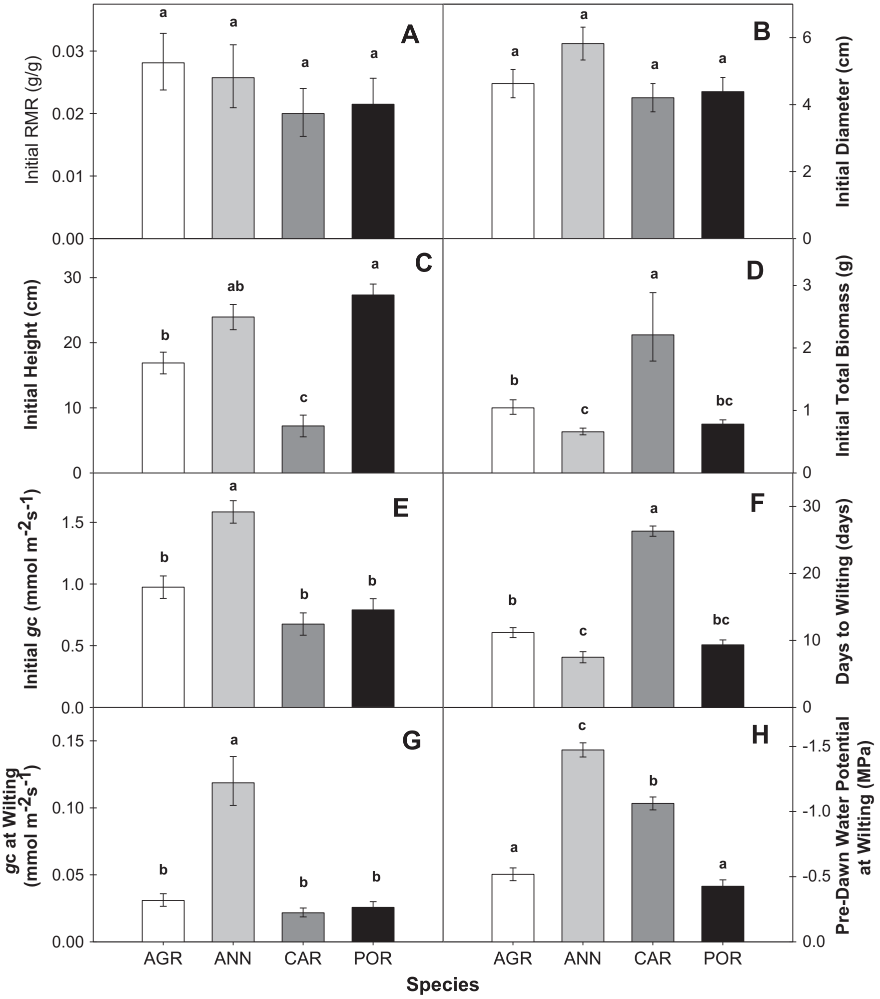

Figure 6: Traits (mean ± SE) at initiation of the dry-down treatment and at wilting for *H. agrestis* (AGR), *H. annuus* (ANN), *H. carnosus* (CAR), and *H. porteri* (POR). Traits collected prior to initiation of dry-down (n = 3 for each species) include Initial Root Mass Ratio (RMR, A), Initial Stem Diameter (B), Initial Height (C), Initial Total Biomass (D), and Initial Stomatal Conductance (g_c; G). Traits collected at wilting (n = 9 for each species) include Days to Wilting (F), Stomatal Conductance at wilting (G), and Leaf Pre-dawn Water Potential at wilting (H). For each trait, different letters indicate significant species differences based on a multiple comparison test.

Discussion

Based on endemism in a habitat where drought is major factor driving ecological interactions, and a phenology that makes it susceptible to frequent summer droughts, we expected *H. porteri* to have traits associated with greater drought resistance as compared to other congeners from less drought-prone habitats. We did find that *H. porteri* traits differed from those of other *Helianthus* species, but did not find any substantive evidence of greater capacity for drought avoidance or tolerance under the conditions presented.

In the well-watered conditions across the four experiments, the species were compared at various stages and ages. In general, the ability of *H. porteri* ability to accumulate biomass and achieve a tall stature was more similar to the fast growing *H. annuus*, although it should be noted that traits such as height and number of leaves may not be informative metrics of comparative performance in species such as *H. porteri* and *H. carnosus*, which exhibit drastically different growth habits (erect branched growth vs. basal-rosette growth, respectively). This is consistent with the anecdotal observations of the capacity of *H. porteri* for fast growth under high resource conditions and rapid recovery of growth after rains return following drought (Shelton, 1963; Mellinger, 1972; Lugo and McCormick, 1981). However, when compared at both a similar stage (30 cm rooting depth) and age (120 days), *H. porteri* exhibited a slower rooting depth rate and shallower rooting capacity than *H. annuus*, and was more similar to the wetter habitat species *H. agrestis* and *H. carnosus*. Rapid root growth in *H. annuus* may reflect the fast-growing, resource acquisitive strategy typically exemplified by this species, and could be related to the relatively large seed size (and likely relatively high carbon and nutrient resources in the seed) in *H. annuus*, although it should be noted that seed size prior to the experiments was not measured in this study. The shallow rooting depth in *H. porteri* is consistent with an adaptation to shallow soils on granite outcrops (Poot *et al.*, 2012), and does not support the hypothesis that *H. porteri* has the capacity to root deeply in cracks in the granite to access moisture (Shelton, 1963). It may, however, help explain why *H. porteri* is a poor competitor when transplanted into communities off of the outcrops (Shelton, 1963; Mellinger, 1972). The moderately high RMR for *H. porteri* compared to the other species under well-watered conditions, combined with highest specific root length, suggests that it has a higher capacity for water and nutrient uptake per unit mass compared to the other species. This high capacity for resource acquisition may play a key role in supporting the rapid growth of *H. porteri* when soil moisture is available on the granite outcrops.

In response to the mild drought treatment at a comparable soil moisture, controlled with soil moisture probes, there was trend for decrease in biomass across species, as expected. Although drought resulted in an approximately 50 % increase in RMR for both *H. carnosus* and *H. agrestis*, drought did not affect RMR in *H. porteri*, despite the expectation that increased RMR under drought is a common drought avoidance response. As a result, *H. porteri* exhibited a trend towards lower RMR than *H. carnosus* and *H. agrestis* under the mild drought conditions (in contrast to the relatively high RMR of *H. porteri* under well-watered conditions described above). It may be that increasing RMR in response to drought would not provide much of a selective advantage in outcrops, given the relatively small soil volume available on outcrops. The mild drought also resulted in lowered stomatal conductance and increased WUE in response to mild drought, as expected, for all of the study species except *H. carnosus*. Thus, there is no evidence that *H. porteri* has a greater capacity than congeners to avoid drought through greater stomatal control or WUE. The mild drought was not sufficient to induce changes in π_o and π_{tlp} in any of the species, indicating no osmotic adjustment in this experiment. A more substantive controlled drought would be needed to test for species differences in capacity for osmotic adjustment.

When water was withheld for an extended period of time causing a more severe drought, *H. porteri* wilted at a leaf water potential similar to *H. agrestis*, the species from the wettest habitat, and at a less negative water potential (i. e., a higher plant water status) than that of *H. annuus* and *H. carnosus*. This pattern is consistent with previous reports of *H. porteri* wilting at a higher soil water content than weedy Composite species not native to outcrops (Shelton, 1963). It was suggested that early wilting in response to drought and long-term persistence in the wilted condition allows this species to survive until rains return (Shelton, 1963; Lugo and McCormick, 1981). However, the fact that *H. porteri* wilted at a similar leaf water potential as the wet site species *H. agrestis* does not support the interpretation of a less negative wilting point as a mechanism for drought resistance in *Helianthus*. It would be interesting to investigate the factors underlying the less negative wilting point, since it suggests that *H. porteri* and *H. agrestis* may have either have less capacity to osmotically adjust than the other species, or higher cell wall stiffness that allows cells to lose turgor but maintain a high relative water content (RWC, %), facilitating persistence in the wilted state (Verslues *et al.*, 2006). We were unable to test whether *H. porteri* can persist in a wilted condition longer than the other species because all four species survived the maximum experimental period of 13 days in the wilted state, and exhibited new shoot growth after re-watering. A longer period of sustained drought before re-watering would be needed to test whether a tolerance of long-term wilting is indeed a mechanism for drought resistance in *H. porteri*.

Contrary to our expectation, these experiments did not identify a unique ability of *H. porteri* to avoid or tolerate drought as compared to three closely-related sunflower species. This lack of greater drought resistance in *H. porteri* is surprising, given the major role that water limitation plays in determining the performance of individual plants, populations, and species in its native habitat (Burbanck and Platt, 1964; McCormick and Platt, 1964; Cumming, 1969; Mellinger, 1972; Sharitz and McCormick, 1973; Shure and Ragsdale, 1977; Lugo and McCormick, 1981; Houle and Phillips, 1989; Bowsher *et al*., 2016). We cannot rule out the possibility that controlled studies comparing these species under more severe or sustained drought may reveal unique drought resistance mechanisms in *H. porteri* not detected here. Similarly, it may be informative for future studies to examine the drought performance and drought resistance traits of the offspring of the plants assessed in the present study, given that drought can sometimes, but not always, exert maternal effects on the next generation (Metz *et al*., 2015 and references therein). In general, however, the highly variable soil moisture availability of outcrop soils appears to have favored an alternate strategy of prolific growth and reproduction when water is available, and persistence as a seed bank in extreme droughts.

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