RESEARCH PAPER

Contrasting drought tolerance strategies in two desert annuals of hybrid origin

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Abstract

Woody plants native to mesic habitats tend to be more vulnerable to drought-induced cavitation than those in xeric habitats. Cavitation resistance in herbaceous plants, however, is rarely studied and whether or not annual plants in arid habitats conform to the trends observed in woody plants is unknown. This question is addressed by comparing the hydraulic properties of annual plants endemic to relatively mesic and seasonally xeric habitats in the Great Basin Desert, in both native and experimental settings. Vulnerability to cavitation between species differed as predicted when vulnerability curves of similar-sized native individuals were compared. Contrary to expectations, *Helianthus anomalus* from the relatively mesic dune sites, on average, exhibited higher native embolism, lower soil-to-leaf hydraulic conductance (k_L) and lower transpiration rates, than its xeric analogue, *H. deserticola*. In transplant gardens, *H. anomalus'* vulnerability to cavitation was unaffected by transplant location or watering treatment. In *H. deserticola*, however, vulnerability to cavitation varied significantly in response to watering in transplant gardens and varied as a function of stem water potential (Ψ_{stem}). *H. deserticola* largely avoided cavitation through its higher water status and generally more resistant xylem, traits consistent with a short life cycle and typical drought-escape strategy. By contrast, *H. anomalus'* higher native embolism is likely to be adaptive by lowering plant conductance and transpiration rate, thus preventing the loss of root-to-soil hydraulic contact in the coarse sand dune soils. For *H. anomalus* this dehydration avoidance strategy is consistent with its relatively long 3–4 month life cycle and low-competition habitat. We conclude that variance of hydraulic parameters in herbaceous plants is a function of soil moisture heterogeneity and is consistent with the notion that trait plasticity to fine-grained environmental variation can be adaptive.

Key words: Adaptation, arid habitats, safety margin, sand dunes, sunflowers, water potential, xylem cavitation.

Introduction

For woody species, the observation that plants from mesic habitats are more vulnerable to drought-induced xylem cavitation than plants from xeric habitats holds at broad spatial scales and in evolutionary frameworks (Alder *et al.*, 1996; Mencuccini and Comstock, 1997; Davis *et al.*, 1999; Kolb and Sperry, 1999; Pockman and Sperry, 2000; Maherali *et al.*, 2004). However, recent work by Jacobsen *et al.* (2007) showed that aridity does not always predict cavitation resistance in woody shrubs, and while some species in a semi-arid climate had high cavitation resistance, the dominant species in an arid desert community were, on average, less resistant to drought-induced cavitation. In annual and herbaceous plants, drought-induced xylem cavitation is poorly described and whether or not herbaceous annuals from mesic habitats are more vulnerable to cavitation than those from xeric habitats is an open

question. This is surprising given that many annual crops are grown in non-irrigated and dryland ecosystems. Of the studies that have examined vulnerability to drought-induced xylem embolism and cavitation in herbaceous species (Milburn and McLaughlin, 1974; Tyree *et al.*, 1986; Neufeld *et al.*, 1992; McCully *et al.*, 1998; Buchard *et al.*, 1999; Stiller and Sperry, 2002; Kocacinar and Sage, 2003; Stiller *et al.*, 2003; Lo Gullo *et al.*, 2004; Li *et al.*, 2009) few have been completed in the field, particularly in arid ecosystems, where water availability varies in space and time.

Because vulnerability to cavitation has a genetic basis, species and population differences in cavitation resistance can be due to adaptive or ecotypic differentiation (Neufeld *et al.*, 1992; Kavanagh *et al.*, 1999; Kolb and Sperry, 1999; Maherali *et al.*, 2004). In addition, gene flow or phenotypic plasticity among populations may alter adaptive or ecotypic differentiation (Maherali *et al.*, 2002). Vulnerability to cavitation is also an environmentally plastic trait such that drought induced stress can temporarily diminish or 'weaken' xylem resistance to cavitation (i.e. a rapid loss of xylem conductivity at relatively high water potentials). This 'cavitation fatigue' phenomenon, which has been demonstrated in a few genera including *Helianthus* (Hacke *et al.*, 2001), complicates interpretations of field-based measures of cavitation resistance. Evidence suggests that plants may take several days to regain previous cavitation resistance levels following drought stress (Stiller and Sperry, 2002). The implication is that, in desert habitats which have spatially and temporally heterogeneous moisture availability, vulnerability to cavitation could change seasonally for a given individual. Recently, Jacobsen *et al.* (2007) demonstrated that cavitation resistance changed in 'wet' versus 'dry'' seasons in several woody species in the Chaparral, Coastal scrub, and Mojave Desert scrub habitats. Therefore both genetic and environmental effects need to be considered when comparing vulnerability to cavitation within and among species.

In order to clarify how variation in water availability affects drought-induced cavitation in herbaceous taxa, two species of wild desert sunflowers, *Helianthus anomalus* and *Helianthus deserticola*, were studied. They are an excellent species pair to investigate the relationship between hydraulic parameters and habitat water status in desert annuals because they are closely related (Rieseberg, 1991; Rieseberg *et al.*, 1991) and can be found in adjacent but divergent habitats that are well characterized (Schwarzbach *et al.*, 2001; Rosenthal *et al.*, 2005; Donovan *et al.*, 2007). The species are locally endemic to active sand dunes (*H. anomalus*) and the adjacent stabilized dunes and desert floor (*H. deserticola*) in the Great Basin Desert, USA. *Helianthus anomalus* is relatively long-lived for a desert annual, germinating in the early spring and remaining active until the first frost in late autumn, presumably an adaptation to the relatively mesic dune habitat. However, in the stabilized off-dune habitat, plant water availability decreases rapidly during summer droughts (Rosenthal *et al.*, 2005) and *H. deserticola* completes its life cycle within 2 months.

The sand dunes habitat of *H. anomalus* has significantly coarser textured soils than the adjacent stabilized dunes where *H. deserticola* thrives (Rosenthal *et al.*, 2005). Soil texture is relevant here since hydraulic failure can occur in the soil before xylem cavitation, either due to coarse-textured soil or to the low surface area of the absorbing roots (Sperry *et al.*, 1998; Hacke *et al.*, 2000). Therefore, edaphic differences between these habitats may differentially affect soil and plant water status (Rosenthal *et al.*, 2005) and this, in turn, would affect plant hydraulic properties. In general, the dune habitat of *H. anomalus* is considered to be relatively mesic for the entire growing season since soil moisture is available to plants throughout the summer provided their roots are deep enough (Rosenthal *et al.*, 2005). In addition, nutrient availability is also significantly lower in *H. anomalus* (Ludwig *et al.*, 2006). While the species in our study have identical hybrid origins, different suites of traits have facilitated ecological transitions in these sunflowers (Rieseberg *et al.*, 2003) and it is unknown how hydraulic parameters may contribute to the ecological differentiation.

In the present study, the hypothesis that the 'on-dune' species *H. anomalus* is more vulnerable to drought-induced xylem cavitation, than the 'off-dune' species *H. deserticola* was tested. This was done by measuring vulnerability to cavitation, native embolism, transpiration rate (E), predawn (Ψ_{pd}) and midday (Ψ_{md}) water potential, soil-to-leaf hydraulic conductance (k_L), and related hydraulic parameters in native plants during two summer growth seasons. In a second experiment, seedlings of each species were removed from native populations and reciprocally transplanted into common gardens in each of their respective habitats. Within each transplant garden, half the plants received additional water. It was hypothesized that resistance to cavitation would vary in response to water availability. If variance in vulnerability to cavitation for either species is an environmentally plastic trait driven by water availability then cavitation was expected to differ in watered versus unwatered treatments.

Materials and methods

Study site

The study was conducted in the Little Sahara Recreation Area, Juab County, Utah (latitude 39°44' N, longitude 112°18' W). The climate is typical of the Great Basin Desert with the majority of precipitation occurring as snow or rain during the cold winter and cool spring months. The summers are characterized by a significant drought usually beginning in June and July. Mean annual precipitation is 312 mm, making this an arid zone according to the UNESCO (1977) classification. During the two years preceding the common garden study, annual precipitation was well below average (2002, 110 mm; 2001, 191 mm) compared to 317 mm in an average year (Rosenthal *et al.*, 2005), reflecting a significant long-term drought in the area. Annual precipitation was slightly below average (287 mm) in 2003, the year of the common garden study, with 20% (58 mm) falling during the summer study period (June-August) (Fig. 1).

The dominant vegetation at this site has been described previously (Rosenthal *et al.*, 2005). Briefly, cover is significantly lower
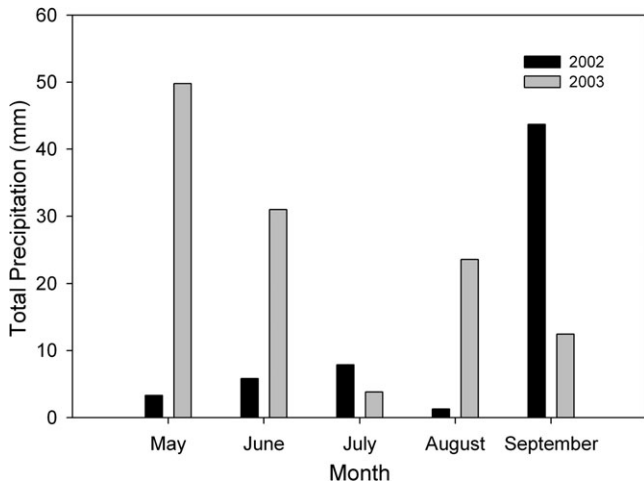
Fig. 1. Mean monthly precipitation at Little Sahara Sand Dunes for May through September of 2002 and 2003.

on-dunes (12.3%) than off-dunes (67.8%) the most common species on dunes are *Psoralidium lanceolatum* (Pursh) Rydb. (Dune scurfpea), *Salsola tragus* L. (Russian thistle), and *Achnatherum hymenoides* (Roem. & Schult.) Barkworth (Indian ricegrass). Common taxa off the dunes are *Bromus tectorum*, *Artemisia tridentata* var. *tridentata*, and several *Agropyron* sp. The proportion of soil covered by litter is also far lower on-dunes than off (6.4% versus 45.5%, respectively). The vast majority of off-dune litter was identifiable as *B. tectorum* (Rosenthal *et al.*, 2005).

Sampling of native plants

In the first year of our study, native plants of both species were collected simultaneously on two dates (July 2002 and August 2002). In the second year, plants of similar sizes and growth stages (i.e. at anthesis) were collected on two separate dates. *H. deserticola* plants were collected in July 2003 and *H. anomalus* plants were collected in August 2003 to coincide with measurements taken in the reciprocal transplant gardens as described below.

Reciprocal transplant experiment

Reciprocal transplant gardens were established in the *H. anomalus* and *H. deserticola* habitats in the Little Sahara Recreation Area, Juab County, UT, USA (latitude 39°44' N, longitude 112°18' W) (hereafter referred to as 'on-dune' and 'off-dune', respectively). One-hundred-and-twenty-five *H. anomalus* and *H. deserticola* seedlings were collected between 25 May and 27 May 2003 from naturally occurring populations at Little Sahara Sand Dunes. Individual plants were placed in temporary pots and 48 seedlings per species were randomly selected for the experiment. Half of the selected seedlings from each species were planted in the on-dune garden and off-dune garden, respectively. Within each transplant garden there were two irrigation treatments, watered (+H$_2$O) and unwatered (NO H$_2$O). The +H$_2$O and NO H$_2$O plants were in separate plots so that water additions would not affect control plants. To account for any heterogeneity within treatments, 2 blocks were nested within each treatment plot.

All plants were watered every other day for 14 d following the transplant. Previous studies indicated that this initial period of heavy watering would be necessary to minimize transplanting shock and facilitate seedling establishment (Ludwig *et al.*, 2004). Four weeks after transplanting, the +H$_2$O plants were supplemented with water equivalent to 33 mm of precipitation. Since it was not possible to know in advance what precipitation would occur, the average precipitation expected for that seasonal period (ten year average=35 mm) was added. The actual precipitation during the study was 22.4 mm. Therefore +H$_2$O plants received 55.4 mm precipitation (about 20.4 mm more than the average for

that period), which was administered four times at two-week intervals.

Helianthus deserticola blooms, sets seed, and frequently senesces before *H. anomalus* begins flowering. In order to compare cavitation resistance at similar growth stages in the experimental garden plants, *H. deserticola* was harvested and measured on 17 July and *H. anomalus* on 28 August. At these dates, both plants had terminated height growth and had initiated flowering.

Cavitation resistance and native embolism measurements

Vulnerability to xylem cavitation was quantified from curves based on the relationship between xylem pressure and the percentage loss of hydraulic conductivity (*PLC*) (Sperry *et al.*, 1988; Alder *et al.*, 1997). Hydraulic conductivity of stem segments was measured using a modification of the method of Sperry *et al.* (1988) from the flow rate of deionized and filtered (0.2 μm) water onto an electronic balance (BA210S, Sartorius, Goettingen, Germany). Whole plants were collected in the field, immediately sealed into humid plastic bags to minimize desiccation, and placed in coolers for the 2 h journey to the laboratory at the University of Utah. Several studies have shown that this treatment neither affects the level of native embolism nor the plants' vulnerability to xylem cavitation. In the laboratory, 0.14 m stem segments were cut from the main stem of each plant underwater and then the native hydraulic conductivity (k_{native}) of each stem was measured using a pressure head of 4–6 kPa. The stems were flushed at 100 kPa for 45 min to refill air-filled conduits to determine the stem maximum hydraulic conductivity (k_{max}). Native embolism or native per cent loss of conductivity (PLC_{native}) is given by:

$$PLC_{native} = ((k_{max} - k_{native})/k_{max}) \times 100$$

Once k_{native} and k_{max} were known, the stem segments were mounted in custom-built centrifuge rotors and spun for 4 min in a Sorval RC5C centrifuge to generate xylem embolisms. Xylem pressure is a function of the angular velocity and the distance from the centre of rotation to the stem ends, with the lowest pressures at the centre of the stem (Alder *et al.*, 1997). After spinning, stem hydraulic conductivity was remeasured. This process was repeated several times, spinning at incrementally higher speeds. Vulnerability curves were then derived from the plots of *PLC* versus xylem pressure.

Differences in cavitation resistance are frequently presented as the pressure required to cause a 50% loss of hydraulic conductivity (P50). However, sunflowers have been shown to suffer from 'cavitation fatigue' (Hacke *et al.*, 2001; Stiller and Sperry, 2002) and minor xylem tensions of only –1.0 MPa can cause a dramatic decrease in hydraulic conductivity. This shift is particularly prominent at the less negative (–1 or greater MPa) pressure end of the vulnerability curve. In order to correct vulnerability curves for cavitation fatigue it is not sufficient to use the conductivity at –0.5 MPa as the k_{max}. Therefore, to avoid confounding the effects of xylem weakening with inherent cavitation resistance, cavitation resistance at 75 *PLC* (P75) was compared as well as at 50 *PLC* (P50) (Sperry and Hacke, 2002).

Water relations and gas exchange measurements

Prior to harvesting plants for hydraulic measurements, plant predawn (Ψ_{pd}) and midday (Ψ_{md}) water potentials were determined with a pressure chamber (PMS instruments, Corvallis, Oregon, USA). Only fully expanded, mature, non-senescent leaves were used. Although soil Ψ and plant Ψ_{pd} may not equilibrate for some plants (Donovan *et al.*, 2001, 2003), night-time transpiration is similar and relatively low in *H. anomalus* and *H. deserticola* (Howard and Donovan, 2007). Therefore any deviation from equilibrium with soil Ψ would have small and similar effects on plant Ψ. Immediately following or preceding Ψ_{md}, transpiration rate (*E*) was measured on mature non-senescent leaves using a Li-Cor 6400 portable photosynthesis system (CO_2 concentration 360 ppm, ambient air temperature, photosynthetically active

radiation 1800 μmol m^{-2} s^{-1}). Transpiration measurements were done on clear days between 11.00 h and 13.00 h at similar air temperatures and *VPD*. Prior to measurement, ambient relative humidity and temperature were recorded and chambers were set to mimic ambient conditions.

Soil-to-leaf hydraulic conductance (k_L) was calculated using the following equation:

$$k_L = E/(\psi_{pd} - \psi_{md})$$

where k_L is defined by the ratio between flow rate (i.e. transpiration) and the 'driving force' (Kolb and Davis, 1994) defined by the difference between soil and leaf water potential. Field stem xylem pressure (Ψ_{stem}) was estimated as the midpoint between (Ψ_{pd}) and (Ψ_{md}):

$$\psi_{stem} = (\psi_{pd} + \psi_{md})/2$$

which corresponds to the xylem pressure at the midpoint of the soil-to-leaf continuum (Linton *et al.*, 1998) and is considered a better estimate of the xylem pressure in the shoots used to measure cavitation resistance.

After determining Ψ_{stem}, the safety margin against hydraulic failure was calculated. Plant Ψ_{crit} is defined here as a conservative estimate of the minimum Ψ_{stem} allowing xylem transport (see also Pockman and Sperry, 2000). If Ψ_{stem} were to reach Ψ_{crit}, all hydraulic transport would cease (i.e. 100% loss of hydraulic conductivity). The difference between the Ψ_{min} and Ψ_{crit} is considered the margin of safety against hydraulic failure (Sperry *et al.*, 1998). The safety margin for mesic species is generally small because water availability is usually high and predictable in those habitats. This margin is wider for species in arid habitats, presumably allowing plants to extract soil water at lower water potentials (Sperry, 1995). Ψ at P75 was conservatively selected as Ψ_{crit} and Ψ_{md} as Ψ_{min}. The safety margin (Ψ_{margin}) was calculated as the difference between Ψ_{crit} and Ψ_{md}.

Sampling and statistical methods

The pressure causing 50% and 75% loss in xylem conductivity (P50 and P75, respectively) was estimated for native and experimental populations by fitting a Weibull function to loss of hydraulic conductivity versus xylem tension for each individual stem (Neufeld *et al.*, 1992). Both, P75 and P50 were estimated since the former is less likely to be affected by drought-induced cavitation fatigue than the latter (Sperry and Hacke, 2002).

To test for overall differences in vulnerability curves between species in the native habitat, the response of per cent loss of stem conductivity (*PLC*) to change in xylem pressure (MPa) was modelled using a repeated measures analysis of variance (SAS, PROC GLM) with species as a fixed effect and xylem pressure as the repeated effect. For the reciprocal transplant experiment the ANOVA was done by species as we were primarily interested in the species response to watering, treatment (i.e. +H$_2$O versus NO H$_2$O) and habitat (i.e. on-dune and off-dune) were treated as fixed effects, and block nested within habitat was treated as a random effect (SAS, PROC MIXED). To compare between species across all experiments species summary statistics were generated and tested for significance of species level differences by comparing the measured values of Ψ_{pd}, Ψ_{md}, Ψ_{stem}, E, k_L, native embolism, P50, P75, and Ψ_{margin} between species with a MANOVA (PROC GLM option MANOVA).

Results

Native plants

In 2002, vulnerability curves were measured for both species in July and then again in August to assess seasonal variation. Within each species, entire vulnerability curves differed between sampling dates in 2002 when compared

using repeated measures ANOVA (PROC MIXED, (*H. deserticola* $F_{1,37}=10.6$, $P <0.01$; *H. anomalus*; $F_{1,29}=5.38$, $P <0.05$) (Fig. 2). Within sampling dates in
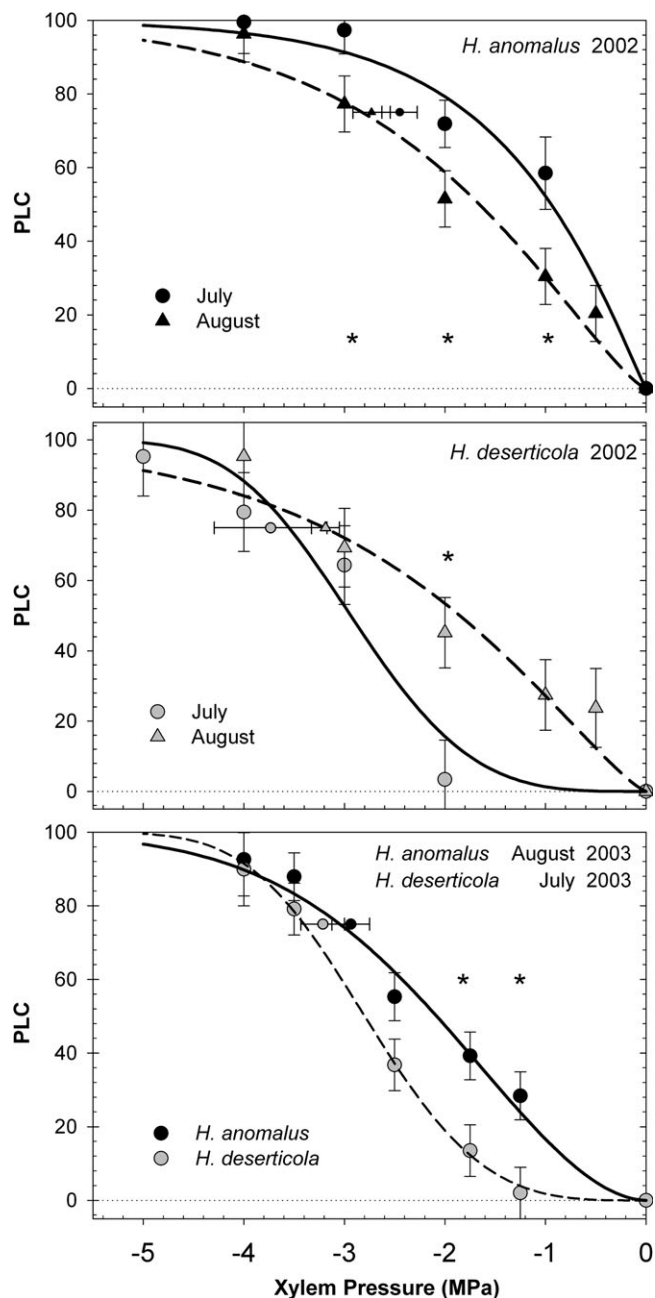

Fig. 2. Percent loss of hydraulic conductivity (*PLC*) as a function of xylem pressure for *H. anomalus* and *H. deserticola* plants collected in native habitats in 2002 and 2003 (Experiment 1). Sampling dates in 2003 were determined by the plants' growth stage (July for *H. deserticola* and August for *H. anomalus*). Larger symbols (*n*=4–7 stems at each xylem pressure ±SE) are adjusted means from repeated measure ANOVA. Smaller symbols with horizontal error bars represent mean P75 (±SE) as estimated from Weibull curves for each stem. Curves are Weibull functions fitted to the entire data set of each species at that time point. Asterisks denote means that are significantly different following *post hoc* tests.

2002, species vulnerability curves differed significantly in July ($F_{1,26}$=20.86, P <0.001) but not August. In 2003, native plant vulnerability curves differed significantly ($F_{1,48}$=12.01, P <0.005) (Fig. 2), but these were sampled at different times. Curves did not differ between years for a species, based on a comparison of the August data for *H. anomalus* and the July data for *H. deserticola*. The P75 was also less negative for *H. anomalus* than *H. deserticola*, but only significantly so in 2002. Overall, *H. deserticola* exhibited greater variability and range in susceptibility to xylem cavitation than did *H. anomalus* (Table 1). *H. anomalus* tended to be more vulnerable to cavitation and its water potential dropped much more during the summer (Ψ_{md} below –2 MPa) than *H. deserticola* (Ψ_{md} near –1.5 MPa; Fig. 3). Consequently, *H. anomalus* developed higher native embolism values at the end-of-summer August sampling dates in 2002 (39.2±8.9%) than *H. deserticola* (19.0±12.0%).

Table 1. Mean and range of xylem pressure at 75% loss of hydraulic conductivity (P75)

P75 was estimated for each individual then pooled for all native *H. anomalus* and *H. deserticola* collected in 2002 and 2003. Species means differ significantly in 2002 but are not significant in 2003. Note that native plants were sample at the same time in 2002 but not in 2003. See Materials and methods for details.

Species (year)	n	Mean	Standard error	Minimum	Maximum	Variance
2002 (*)						
H. anomalus	9	–2.663	0.133	–3.45	–2.15	0.142
H. deserticola	9	–3.421	0.250	–4.80	–2.90	0.438
2003 (ns)						
H. anomalus	8	–2.838	0.189	–3.65	–2.15	0.287
H. deserticola	12	–2.817	0.173	–3.90	–2.00	0.359

Reciprocal transplants

Helianthus anomalus cavitation resistance in experimental gardens did not differ significantly by garden (on-dune and off-dune), treatment (+H_2O or No H_2O) or their interaction when entire vulnerability curves were compared by repeated measures ANOVA (Table 2; Fig. 4). Nor were there any differences in P75. However, on-dune and off-dune, *H. anomalus* +H_2O (Fig. 3; ANY and AFY) had significantly greater Ψ_{pd} and Ψ_{md} than *H. anomalus* No H_2O (ANN and AFN) indicating their water status improved with additional watering (Table 3; Fig. 3).

In contrast to the low variability in cavitation resistance for *H. anomalus* in the gardens, *H. deserticola's* resistance to cavitation in transplant gardens differed significantly between on-dune and off-dune gardens based on whole-curve

Table 2. Repeated measures analysis of variance for the effects of habitat (on-dune and off-dune) and treatments (+H_2O and NO H_2O) and xylem pressure (MPa) on per cent loss of hydraulic conductivity (PLC) in common garden transplants of *H. anomalus* and *H deserticola*

Note that *H. deserticola* plants were sampled in July and *H. anomalus* plants were sampled in August 2003.

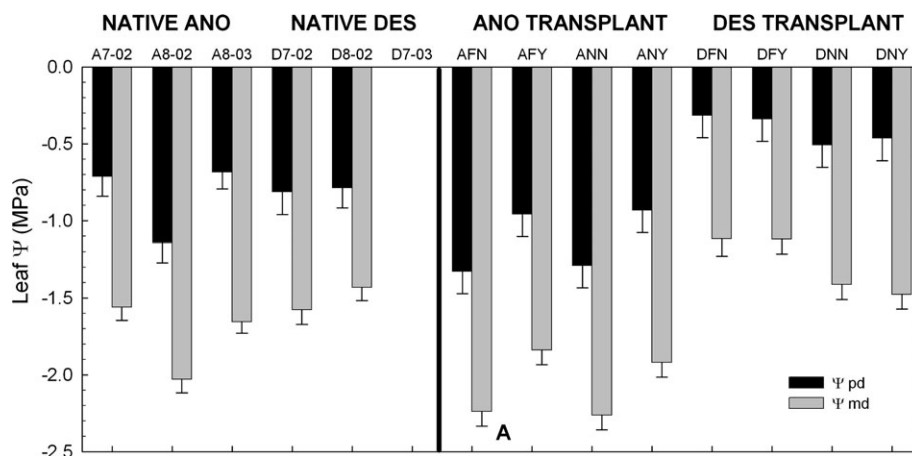| Effect	Ndf	*H. anomalus*			*H. deserticola*		
		Ddf	F	P	Ddf	F	P
Habitat	1	70	0.06	0.8082	61	12.09	**0.0009**
Treatment	1	70	2.63	0.109	61	0.36	0.5505
Habitat×Treatment	1	70	2.57	0.1135	61	7.56	**0.0078**
MPa	5	70	99.08	<0.0001	61	57.64	<0.0001
MPa×Garden	5	70	0.39	0.8555	61	0.66	0.6515
MPa×Treatment	5	70	0.46	0.8019	61	0.88	0.5019
MPA×Garden×Treatment	5	70	0.06	0.9974	61	0.22	0.9523

Fig. 3. Summary of leaf Ψ for *H. anomalus* and *H. deserticola*. Data for native plants Experiment (1), and the two transplant garden experiments (Experiment 2) are separated by a solid black line. The first letter of all abbreviations refer to species (*H. anomalus*=A and *H. deserticola*=D) and for Experiment 1 the month and year are noted. For Experiment 2, the second and third letters refer to treatments: off-dune and NO H_2O (=FN); off-dune and +H_2O (=FY); on-dune and +H_2O (=NY); on-dune and NO H_2O (=NN). Bars are means (n=4–7 for Experiment (1) and n=4 for Experiment (2) ±SE). Standard errors are based on a pooled estimate of the error variance for a one-way ANOVA. Note that *H. deserticola* transplant data were collected in July 2003 and *H. anomalus* transplant data were collected in Augusr 2003.
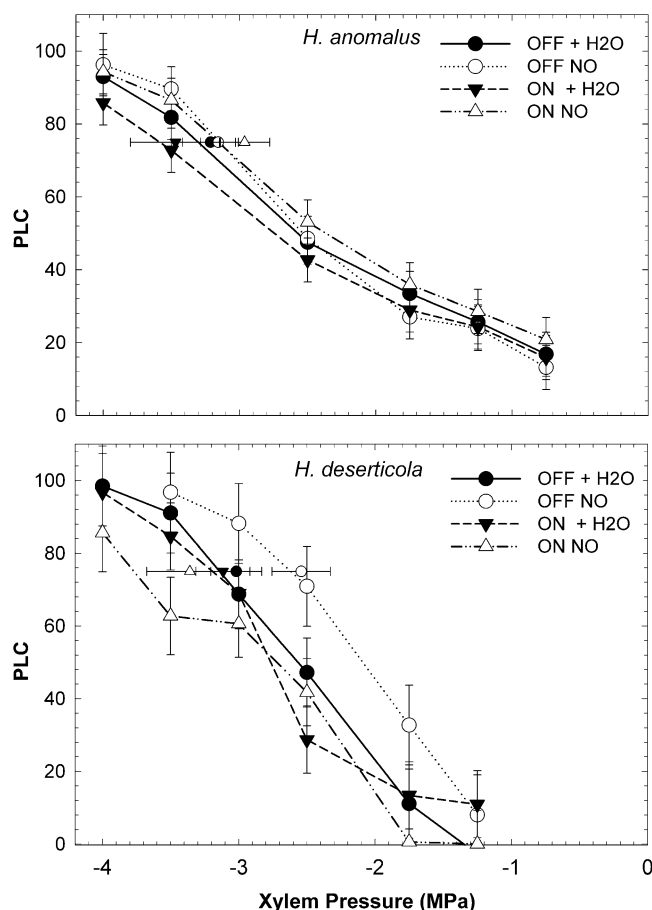
Fig. 4. Percent loss of hydraulic conductivity as a function of xylem pressure for *H. anomalus* and *H. deserticola* in Experiment (2) in on-dune (circles) and off-dune gardens (triangles) subjected to additional water (+H$_2$O) or no additional water (NO H$_2$O). Curve data were collected in July 2003 for *H. deserticola* and in August 2003 for *H. anomalus* when plants were at a comparable growth stage. Smaller symbol with horizontal error bars represent mean P75 ($\pm$SE) as estimated from Weibull curves for each stem.

ANOVA comparisons and P75 data. The magnitude of the difference varied depending on watering treatment (Table 2; Fig. 4). In the off-dune transplant garden *H. deserticola* was significantly less vulnerable to cavitation only when watered (Fig. 4) and both Ψ_{pd} and Ψ_{md} were significantly greater in the off-dune gardens (Table 3; Fig. 3).

Species trends

To compare species' hydraulic parameters response to plant water status in the broadest sense, data from native and experimental garden plants were combined and compared to Ψ_{stem} (i.e. $[(\Psi_{pd} + \Psi_{md})/2]$, Fig. 5). Overall, *H. anomalus* developed significantly lower water potentials and higher native embolism than *H. deserticola* (Fig. 5A; Table 4). Consequently, *H. anomalus* had, on average, an approximately three times lower soil-to-leaf hydraulic conductance (k_L) than *H. deserticola* and a similarly lower average transpiration rate (Fig. 5B, C; Table 4).

The P75 exhibited a significant relationship with Ψ_{stem} in *H. deserticola*, such that plants exposed to more negative

Table 3. ANOVA results for leaf predawn (Ψ_{pd}) and midday (Ψ_{md}) water potential for the same plants that were used to generate vulnerability curves in the common garden 2003 (see Fig. 2)

H. deserticola individuals were sampled in July and *H. anomalus* in August, habitat (on-dune versus off-dune) and treatments (+H$_2$O and NO H$_2$O)

H. anomalus	Ψ_{pd}			Ψ_{md}		
	df	*F*	*P*	df	*F*	*P*
Habitat	1	0.17	0.69	1	0.72	0.42
Treatment	1	23.28	**<0.005**	1	35.31	**<0.001**
Habitat×Treatment	1	0.01	0.94	1	0.2	0.67
Block (Habitat×Treatment)	4	6.9	**<0.05**	4	8.23	**<0.01**
Error	8			8		
H. deserticola	Ψ_{pd}			Ψ_{md}		
	df	*F*	*P*	df	*F*	*P*
Habitat	1	18.08	**<0.005**	1	11.4	**<.01**
Treatment	1	0.53	0.49	1	0.40	0.55
Habitat×Treatment	1	0.1	0.76	1	1.72	0.23
Block (Habitat×Treatment)	4	0.34	0.84	4	1.30	0.36
Error	7			7		

xylem pressure tended to be more resistant to cavitation. There was no correlation between Ψ_{stem} and P75 in *H. anomalus* in spite of its much wider range of Ψ_{stem} (Fig. 5D). When all native and experimental plants were pooled, the mean P50 was significantly higher (more vulnerable) in *H. anomalus* (2.16$\pm$0.1 versus 2.67$\pm$0.1 MPa; P <0.01) than in *H. deserticola* (Table 4). Differences in P75 followed similar trends with *H. anomalus* being more vulnerable than *H. deserticola*, but these differences were not statistically significant (2.98$\pm$0.09 versus 3.17$\pm$0.1; P >0.05).

Discussion

In support of our hypothesis native *H. anomalus* plants were significantly more vulnerable to cavitation than *H. deserticola* when similar-sized native plants were sampled early and late in the growth season of 2002. This trend held for P50 and P75 but was not significant for the latter when all plants were pooled. *Helianthus anomalus* plants have a smaller margin of safety (i.e. P75–Ψ_{md}) than *H. deserticola* (1.08 MPa versus 1.79 MPa) consistent with the notion that the active on-dune habitat is mesic relative to off-dune habitats (Rosenthal *et al.*, 2005). A wider safety margin is more typical of xeric species as a hedge against variable soil moisture (Sperry, 1995). By contrast, and contrary to our expectations, *H. anomalus* experienced more than twice the native embolism of *H. deserticola* (25% versus 10%) which caused *H. anomalus* to operate at lower Ψ, and to have roughly a three times lower soil-to-leaf hydraulic conductance than *H. deserticola*. This was especially true when the native embolism of native plants was compared at similar times in 2002 (39.2$\pm$8.9% versus 19.0$\pm$12.0%) for *H. anomalus* and *H. deserticola*, respectively.
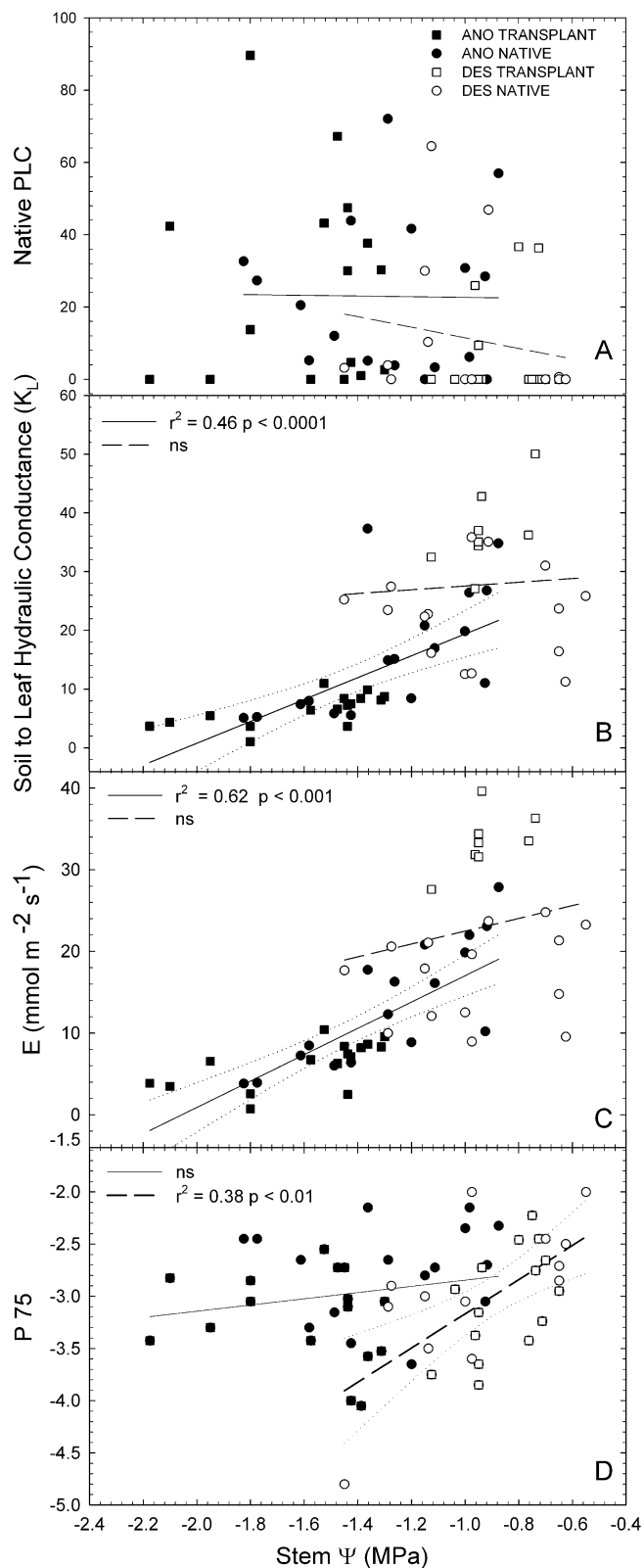
Fig. 5. Native embolism (*PLC*) (A), soil-to-leaf hydraulic conductance (B), transpiration (C), and P75 (D) for native and transplanted *H. anomalus* (black symbols, solid lines) and *H. deserticola* (white symbols, dashed lines) as a function of stem Ψ. Symbols are values for individual plants and 95% confidence intervals are only shown for regressions that are significant.

Table 4. Species summary statistics of hydraulic parameters measured in both native and field-grown *H. anomalus* and *H. deserticola*

Several *H. deserticola* individuals had missing data for E and k_L (see Fig. 3). Means and standard errors are from ANOVAs. Significant differences between species were calculated with a MANOVA testing for the effect of species for all variables.

Variables	*H. anomalus*		*H. deserticola*		df	P
	Mean	SE	Mean	SE		
Ψ_{pd} (MPa)	−0.97	0.052	−0.54	0.06	**1,52**	**<0.0001**
Ψ_{md} (MPa)	−1.90	0.05	−1.37	0.06	**1,52**	**<0.0001**
Stem Ψ (MPa)	−1.44	0.05	−0.96	0.06	**1,52**	**<0.0001**
E (mmol m^{-2} s^{-1})	10.06	1.170	24.27	2.428	**1,48**	**<0.0001**
k_L (mmol m^{-2} s^{-1} MPa^{-1})	11.33	1.560	30.36	2.311	**1,48**	**<0.0001**
Native embolism (%)	24.24	3.69	10.16	3.87	**1,52**	**<0.01**
P50 (MPa)	−2.16	0.10	−2.67	0.10	**1,52**	**<0.01**
P75 (MPa)	−2.98	0.09	−3.17	0.10	**1,52**	**ns**
Ψ margin (MPa)	−1.08	0.09	−1.79	0.11	**1,52**	**<0.0001**

Why would *H. anomalus* maintain a relatively high native embolism in spite of being in the putatively more mesic habitat? It is well known that the root–soil interface is especially vulnerable to severe desiccation in coarse soils where water is easily displaced by air in the large pore spaces (Bristow *et al.*, 1984; Hacke *et al.*, 2000). Previously, it was demonstrated that soil Ψ decreases precipitously as a function of water content in these dune habitats (Rosenthal *et al.*, 2005). In spite of this, native plant water status remains high in this dune habitat relative to adjacent off-dune habitats (Rosenthal *et al.*, 2005) presumably because plants in coarser soils have deep roots that are able to mine soil moisture. This has been shown for woody species, which developed deeper roots in coarse textured soils to reach wetter soils (Hacke *et al.*, 2000; Jackson *et al.*, 2000; Sperry and Hacke, 2002). We have previously argued that *H. anomalus* can maximize access to soil moisture by having deep roots (Ludwig *et al.*, 2004; Donovan *et al.*, 2007, 2009). However, a recent study has shown that, while native *H. anomalus* did have some deep roots (>130 cm deep), more than 88% of its root biomass can be in the top 25 cm of soil (Ludwig *et al.*, 2006). The significant increases in Ψ_{pd} in the watered (+H_2O) *H. anomalus* transplant garden are consistent with this observation and suggest that *H. anomalus* may not always have the capacity to develop a sufficiently extensive deep root system to meet its water requirements. As a result, higher native embolism in *H. anomalus* would decrease the rate of water consumption by lowering hydraulic conductivity and transpiration, extending the time of hydraulic contact between water in the coarse soil and the root system (Sperry *et al.*, 1998). This makes adaptive sense on the dunes because low plant cover means inter-specific competition is minimal. This resource conservation strategy is consistent with the notion that *H. anomalus'* adaptation to the dune habitat consists of a dehydration avoidance and stress tolerating strategy (Brouillette *et al.*, 2006; Donovan *et al.*, 2009).

Interestingly, our results revealed that both species suffered from 'cavitation fatigue' (Hacke *et al.*, 2001; Stiller and Sperry, 2002). However, this apparent increase in cavitation vulnerability at less negative xylem pressures was much more pronounced in *H. anomalus*, which also was able to recover from it (compare 60% embolism at –1 MPa in July 2002 versus 30% embolism at –1 MPa in August 2002; Fig. 2). In order to avoid confounding the effects of cavitation fatigue with inherent cavitation resistance, cavitation resistance at 75% embolism (P75) as well as at 50% embolism (P50) were compared (Sperry and Hacke, 2002).

A growing body of work suggests that temporal or ontogenetic changes in physiology or morphology in response to fine grained environmental variation can be adaptive (Winn, 1999; Miner and Vonesh, 2004; Picotte *et al.*, 2007; Maherali *et al.*, 2009). *H. deserticola* showed significantly more variability both within and between curves than *H. anomalus*, evidence of a greater plasticity in cavitation resistance. The variance in *H. deserticola*'s hydraulic parameters was correlated with Ψ_{stem}, such that plants experiencing lower Ψ_{stem} also had lower P75 (Fig. 5). For instance, in 2002 with a meagre 9 mm of precipitation in July and August, *H. deserticola* had significantly lower P75 than *H. anomalus* but this was not the case in 2003 when July and August precipitation was much greater (27.4 mm). This makes adaptive sense for a cavitation-avoiding species like *H. deserticola*. Avoidance of cavitation by *H. deserticola* by maintaining a more cavitation-resistant xylem in drier times would contribute to its higher soil-to-leaf hydraulic conductance and transpiration rates. These traits may be beneficial in its higher-cover habitat with greater potential for inter-specific competition, higher soil moisture heterogeneity, and, where finer-textured soils permit, a higher rate of water uptake per root area. The drought-escaping strategy is also consistent with its short 1–2 month life cycle. In sharp contrast, *H. anomalus* did not show any adjustment in P75 with Ψ_{stem}, consistent with a strategy of maintaining a consistently higher level of native embolism regardless of stem Ψ.

Three commonly described general drought adaptations are dehydration tolerance, dehydration avoidance, and drought escape (used here as defined by Ludlow, 1989). So what drought-adaptation strategies apply to these desert annuals? If *H. deserticola* is a cavitation-avoiding drought escapist, then *H. anomalus* is an embolism-maintaining dehydration avoider. Desert annuals are frequently characterized as drought escapists, however, a growing number of studies of functional traits in annuals have shown that annual species exhibit a range of adaptations from dehydration avoidance to dehydration escape (Geber and Dawson, 1990, 1997; Stanton *et al.*, 2000; McKay *et al.*, 2003; Heschel and Riginos, 2005; Sherrard and Maherali, 2006). *Helianthus deserticola*'s increasing resistance to xylem cavitation in response to decreasing water availability ensures that it will maintain hydraulic continuity at a greater range of soil water potentials even at higher transpiration rates. Thus, *H. deserticola* maximizes the length of time that resources are favourable consistent with the idea that annual drought-escapers may only reap fitness benefits when resources are favourable (Sherrard and Maherali, 2006). This is consistent with Donovan *et al.* (2007) in that higher fitness is not always associated with direct selection for lower water use efficiency in native *H. deserticola* populations (Donovan *et al.*, 2007, 2009). By contrast, *H. anomalus* persists throughout the summer growing season and continues flowering until late in the autumn. If, as mentioned earlier, *H. anomalus* does not always have an extensive deep root system it may avoid dehydration by maintaining high native embolism and low transpiration rates. Indeed, a recent study suggested that nutrient limitation, not water use efficiency, appears to have been the driving selective force on *H. anomalus* populations (Donovan *et al.*, 2009).

There still remains unexplained variation in cavitation resistance in these species, suggesting other factors beyond the scope of this study, such as root cavitation, variance in growth rate, and nutrient status, all with potential influences on the cavitation phenotype. Ultimately, the cavitation phenotype in herbaceous and woody species appears to be a complex function of inter-vessel pit structure and number (Hacke *et al.*, 2004; Sperry and Hacke, 2004) the details of which need to be more fully studied.

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