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Alan W. Bowsher*, Ethan F. Milton and Lisa A. Donovan

Comparison of Desert-Adapted *Helianthus niveus* (Benth.) Brandegee ssp. *tephrodes* (A. Gray) Heiser to Cultivated *H. annuus* L. for Putative Drought Avoidance Traits at Two Ontogenetic Stages

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Abstract: Water availability is a major factor limiting plant productivity in both natural and agronomic systems. Identifying putative drought resistance traits in crops and their wild relatives may be useful for improving crops grown under water-limiting conditions. Here, we tested the expectation that a desert-dwelling sunflower species, *Helianthus niveus* ssp. *tephrodes* (TEPH) would exhibit root and leaf traits consistent with greater ability to avoid drought than cultivated sunflower *H. annuus* (ANN) in a common garden environment. We compared TEPH and ANN at both the seedling and mature stages under well-watered greenhouse conditions. For traits assessed at the seedling stage, TEPH required a longer time to reach a rooting depth of 30 cm than ANN, and the two species did not differ in root:total biomass ratio at 30 cm rooting depth, contrary to expectations. For traits assessed at the mature stage, TEPH had a higher instantaneous water use efficiency and photosynthetic rate on a leaf area basis, but a lower photosynthetic rate on a mass basis than ANN, likely due to TEPH having thicker, denser leaves. Contrary to expectations, ANN and TEPH did not differ in leaf instantaneous stomatal conductance, integrated water-use efficiency estimated from carbon isotope ratio, or nitrogen concentration. However, at both the seedling and mature stages, TEPH exhibited a lower normalized difference vegetative index than ANN, likely due to the presence of dense leaf pubescence that could reduce heat load and transpirational water loss under drought conditions. Thus, although TEPH root growth and biomass allocation traits under well-watered conditions do not appear to be promising for improvement of

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***Corresponding author: Alan W. Bowsher,** Department of Plant Biology, University of Georgia, Athens, GA, USA 30602, E-mail: bowsher@uga.edu
Ethan F. Milton, Lisa A. Donovan, Department of Plant Biology, University of Georgia, Athens, GA, USA 30602

cultivated sunflower, TEPH leaf pubescence may be promising for breeding for drought-prone, high radiation environments.

Keywords: abiotic stress, crop improvement, leaf pubescence, NDVI, rooting depth rate, water use efficiency

Introduction

Drought is a major stress that limits plant productivity in both natural and agronomic systems, and the duration, frequency and severity of drought is expected to increase in many regions worldwide (IPCC, 2007; Sheffield and Wood, 2008). Thus, gaining a better understanding of physiological traits associated with drought resistance has become a major focus for crop improvement (Passioura, 2006; Richards, 2006; Richards *et al.*, 2010; Tardieu, 2012). Numerous studies have investigated variation in traits putatively associated with drought resistance in existing crop germplasm in order to exploit that variation in breeding programs (e. g. Fereres *et al.*, 1986, Rauf and Sadaqat, 2007; Henry *et al.*, 2011; Wishart *et al.*, 2014). In addition, wild congeners native to drought-prone environments are also a potential source of drought resistance traits for improving crop productivity under water-limited conditions (Thompson *et al.*, 1981; Shimshi *et al.*, 1982; Jackson and Koch, 1997; Seiler *et al.*, 2006a, 2006b; Hajjar and Hodgkin, 2007; Seiler, 2008). Here, we evaluate putative drought avoidance traits in cultivated sunflower, *Helianthus annuus* (hereafter ANN), and its wild congener *H. niveus* ssp. *tephrodes* (hereafter TEPH), which grows in the arid Algodones Dunes (annual precipitation 62 mm) of the Sonoran desert (Barbour and Billings, 1988; Hickman, 1993; Seiler and Rieseberg, 1997). Given its arid habitat and production of dense leaf pubescence, TEPH has been hypothesized to be drought resistant and may serve as a germplasm resource for sunflower crop improvement (Heiser *et al.*, 1969; Seiler, 1992; Seiler *et al.*, 2006a, 2006b). However, compared to the numerous studies that have assessed variation in traits associated with drought resistance in other wild *Helianthus* species or interspecific hybrids (e. g. Sobrado and Turner, 1983a, 1983b, 1986; Seiler, 1994, 1998, 2008; El Midaoui *et al.*, 2003; Onemli and Gucer, 2010), very few have assessed drought resistance traits in TEPH (Milton *et al.*, 2013), due in part to the extremely low availability of achenes in germplasm repositories prior to recent large-scale collections (Seiler *et al.*, 2006a, 2006b).

Drought is generally defined as a prolonged absence of precipitation or supplemental water supply that results in declining soil water availability (Boyer, 1982). Plants may be able to avoid the adverse effects of drought by preventing or delaying low internal water potentials through traits that allow for continued water uptake as water availability declines, and/or traits that decrease water loss (Turner and Begg, 1981; Verslues *et al.*, 2006). Plants that avoid drought are often expected to exhibit either deep rooting, high root:total biomass ratio (RMR), or both, supporting the capacity to reach deep soil layers and extract soil moisture (Blum, 1996; Passioura, 2006; Reynolds *et al.*, 2007; Verslues *et al.*, 2006; Seiler, 2008). For example, in rice, increased expression of the quantitative trait locus *DEEPER ROOTING 1* (*DRO1*) results in deeper root distributions and higher yield under drought conditions (Uga *et al.*, 2013). However, even in crops with relatively deep taproot systems such as ANN, drought can strongly impact yield (Fereres *et al.*, 1986; Tahir and Mehdi, 2001; Rauf and Sadaqat, 2007; Alahdadi *et al.*, 2011). Previous work in sunflower has demonstrated that variation exists among cultivated ANN and interspecific hybrids of cultivated ANN and several wild *Helianthus* in primary root elongation rates and other rooting traits (Seiler, 1994, 1998, 2008), but rooting depth rate and RMR in desert-dwelling TEPH has not, to our knowledge, been previously examined.

In addition to root traits that increase water uptake, leaf traits may play an important role in drought avoidance by reducing transpirational water loss (Farquhar and Sharkey, 1982; Kramer and Boyer, 1995). Stomata regulate both water loss due to transpiration as well as diffusion of CO_2 into the leaf, thereby influencing plant water use efficiency (WUE; plant carbon assimilation per unit water loss), which can be assessed at the leaf level as either instantaneous WUE estimated from gas exchange, or as time-integrated WUE estimated from leaf carbon isotope ratio ($\delta^{13}C$) (Farquhar *et al.*, 1989; Donovan and Ehleringer, 1994). Smaller leaves generally have a small boundary layer, allowing for greater convective heat loss and cooler leaf temperatures, and resulting in reduced leaf transpiration and increased leaf WUE (Smith, 1978; McDonald *et al.*, 2003). In addition, smaller, thicker leaves concentrate photosynthetic machinery which can also facilitate greater WUE per unit leaf area (Craufurd *et al.*, 1999). However, although these leaf-level traits may confer greater drought avoidance, they could potentially negatively impact plant productivity under well-watered conditions. For example, although lower stomatal conductance (g) tends to decrease transpirational water loss, thereby increasing leaf-level WUE and drought avoidance, it also tends to reduce photosynthetic carbon assimilation (Héroult *et al.*, 2013 and references therein), potentially resulting in reduced productivity relative to genotypes with higher g. Similarly, although smaller leaf size can increase leaf-level WUE, it also reduces total leaf area available for photosynthetic carbon assimilation unless compensated by larger number of leaves, potentially resulting in unacceptable yield tradeoffs from a crop breeding perspective.

Leaf pubescence, the presence of dense hairs on the leaf surface, can also reduce transpirational water loss by reflecting excess radiation, thereby reducing leaf heat load (Gates, 1968; Ehleringer *et al.*, 1976; Ehleringer, 1984). The occurrence and extent of leaf pubescence generally increases with habitat aridity, and dense pubescence is often associated with desert species, suggesting the adaptive value of leaf pubescence in arid, high radiation habitats (Sandquist and Ehleringer, 1997). As with lower *g* and smaller leaf size, however, leaf pubescence may generate unacceptable tradeoffs for crop production, as greater reflectance of solar radiation may reduce the absorbance of wavelengths that drive photosynthetic carbon assimilation (Ehleringer *et al.*, 1976; Ehleringer and Mooney, 1978; Ehleringer, 1984). Although dense leaf pubescence has been observed for TEPH, the potential impact on gas exchange and WUE, has not, to our knowledge, been previously examined. Parameters such as normalized difference vegetation index (NDVI; a common parameter calculated from reflectance of both visible and near-infrared wavelengths) can provide insight into plants' ability to dissipate excess radiation driving photosynthesis (visible wavelengths) and radiation that does not (near-infrared wavelengths), both of which contribute to leaf heat load (Ehleringer and Björkman, 1978; Ehleringer and Mooney, 1978).

Here, we assessed the potential for TEPH to serve as a germplasm resource for sunflower crop improvement by comparing multiple accessions of cultivated ANN and TEPH for putative drought resistance traits at two ontogenetic stages (seedling and mature) under well-watered greenhouse conditions. We conducted our study under well-watered conditions for two reasons. First, high-resource conditions are generally expected to maximize variation among genotypes with contrasting resource-use strategies (Chapin *et al.*, 1993); thus, selection for drought resistance traits in crop breeding programs is generally most effective under well-watered conditions (Richards *et al.*, 2010). Second, although drought resistance traits may reduce crop yield losses under drought conditions, the capacity to achieve high photosynthetic rates under well-watered conditions is crucial for producing acceptable yields when moisture availability is high. Our evaluation of drought avoidance focused on three trait categories: root allocation and rooting depth rate (assessed at seedling stage), leaf traits which contribute to leaf heat load and transpirational water loss (assessed at both seedling and mature stages), and gas exchange parameters and WUE (assessed at mature stage). We addressed the following hypotheses: 1) TEPH seedlings will have a faster rooting depth rate and will allocate proportionally more biomass to the root system (higher RMR) than ANN; 2) TEPH seedlings and mature plants will have leaf characteristics consistent with greater drought avoidance than ANN (lower SLA, lower individual leaf area and total leaf area, lower NDVI); 3) mature TEPH plants will have greater WUE, but lower photosynthetic capacity, than ANN.

Materials and methods

Seedling study

The seedling study was conducted in the University of Georgia Plant Biology greenhouses, Athens, GA in spring 2012. All seed was obtained from the USDA National Plant Germplasm System of the National Genetic Resources Program, and accession names represent identifiers from the USDA Germplasm Resources Information Network (http://www.ars-grin.gov/). For ANN the accession identifiers are PI-642777 (HA-412-HO), PI-560141 (RHA-373), PI-578872 (HA-383) and PI-607506 (RHA 415). For TEPH the accession identifiers are PI-664653 (AMES-27850), PI-613758 (AMES-6852), PI-650018 (AMES-27422), and PI-664643 (AMES-27421). The experimental design was a randomized complete block design, with two species, four accessions per species and three replicates per accession in each of four spatial blocks, for a total of 96 seedlings. Plants for each accession within each block were averaged to a single data point to avoid pseudoreplication, giving a total analyzed sample size of n = 32. Seeds were scarified by excising the blunt end of each achene and then placed on moist filter paper in petri dishes. On 4 February 2012, germinated seedlings were transferred to 2.5 cm plugs containing Fafard 3B soil mix (Conrad Fafard Inc., Agawam, MA, USA) in a growth chamber (12/12 light cycle under 1,000 µmol at 22 °C). On 11 February, seedlings were transplanted into 2 liter tree pots measuring 30 cm deep, and filled with Fafard 3B soil mix. A hole was cut in the center of the bottom surface of each pot, and additional ~1 cm incisions were made at each bottom corner of the pot to form flaps that could be pulled back to examine when roots had reached the bottom of the pot. Plants were watered daily and fertilized three times weekly with a 30 ppm (based on nitrogen) Jack's Professional 20-20-20 fertilizer solution (J.R. Peters, Inc. Allentown, PA, USA).

Pots were examined daily, and individual plants were harvested on the first day that any root tip (whether from the primary root or a lateral root) was visible behind the flaps at the bottom of the pot. Although this procedure resulted in trait measurements being conducted on plants of different ages, it allows for comparison at a similar belowground developmental stage (roots of the two species exploring the same depth of soil; *sensu* Nicotra *et al.*, 2002). The number of days from scarification to roots reach the bottom of the 30 cm pot (days to bottom; DTB) was recorded as a proxy for root system growth rate. Each leaf of the most recently fully expanded leaf pair was then assessed for reflectance (*R*) of red (680 nm) and near-infrared (800 nm) wavelengths using a spectroradiometer (Unispec Spectral Analysis System, PP Systems Inc., Amesbury, MA, USA) and then averaged to give a single *R* value for both red and near-infrared wavelengths for the plant. The leaf reflectance parameter NDVI was calculated according to equation (1) for each plant following (Tucker, 1979):

$$\mathrm{NDVI} = (\mathrm{R}_{800} - \mathrm{R}_{680})/(\mathrm{R}_{800} + \mathrm{R}_{680}) \tag{1}$$

Following reflectance measurements, shoot (leaves and stems) and root systems were separated, and root systems were rinsed clean of substrate. The total number of true leaves, leaf area of the most recently fully expanded leaf pair, and total leaf area were assessed (LI 3100, LiCor Instruments, Lincoln, NE, USA). All biomass was dried at 60ºC for at least 48 hours and weighed. Specific leaf area (SLA; $\mathrm{cm}^2\ \mathrm{g}^{-1}$) was calculated as leaf area of the most recently fully expanded leaf pair divided by the mass of the leaf pair. Root mass ratio (RMR) was calculated as root biomass divided by total plant biomass.

Mature plant study

The mature plant study was conducted in the University of Georgia Plant Biology greenhouse in summer 2010. For both ANN and TEPH, the accessions in the seedling study (four each species, see above) were included. For TEPH, five additional accessions from the USDA National Plant Germplasm System were included that capture additional variation: PI-650017 (AMES-27420), PI-650019 (AMES-27830), PI-650020 (AMES-27831), PI-650021 (AMES-27832), and PI-664654 (AMES-27851). The initial experimental design was a randomized block design, with two species, nine accessions of TEPH (six replicates each) and four accessions of ANN (three replicates each), in each of three spatial blocks. We sampled a subset of 2–3 individuals per block for each accession, and replicate plants for each accession within each block were averaged to a single data point to avoid pseudoreplication. This resulted in a total analyzed sample size of $n = 39$. Seeds of ANN and TEPH were scarified and placed on moist filter paper in petri dishes. Germinated TEPH seedlings were transferred to 2.5 cm plugs containing Fafard 3B soil mix for approximately one week to allow establishment before being transplanted to 4 liter pots filled with the same soil mix. Germinated ANN seedlings were direct sown into the 4 liter pots. Plants were supplied 30 g of Osmocote 14-14-14 slow release fertilizer (Scotts, Marysville, OH, USA) and maintained under well-watered conditions with drip irrigation.

Leaf traits were assessed at the pre-reproductive/reproductive stage (hereafter “mature”, for brevity). Several TEPH plants had produced buds, while the majority of ANN plants were budding or beginning to flower. Although aboveground developmental stage can impact leaf-level trait measurements, a study of three diverse *Helianthus* species found that, in general, leaf-level traits fluctuated much more strongly between the juvenile and pre-reproductive stages than between the pre-reproductive and reproductive stages (Mason *et al.*, 2013). Therefore, our study design was expected to capture the major differences between ANN and TEPH in trait values, despite slight differences in aboveground developmental stage.

We measured instantaneous gas exchange on the most recently fully expanded leaf for each plant using a LI-6400 (LiCor Instruments, Lincoln, NE, USA). Gas exchange measurements were conducted within a growth chamber to ensure uniformity of ambient environmental conditions, and were completed over two days. Each plant was moved to the growth chamber and allowed to adjust to growth chamber conditions (photosynthetic photon flux of 1,000 µmol, air temperature of 30˚C and vapor pressure deficit of 2.0 kPa) for at least 20 minutes prior to measurements. LI-6400 chamber conditions were set to 2,000 µmol m^{-2} s^{-1} photosynthetically active radiation and 400 ppm CO_2, and block temperature and relative humidity were adjusted to ambient conditions. From instantaneous gas exchange measurements we obtained photosynthetic rate on an area basis (A_{area}; µmol m^{-2} s^{-1}), stomatal conductance (g; mmol m^{-2} s^{-1}), and instantaneous WUE estimated as A/g (mmol mol^{-1}). Photosynthesis on a mass basis (A_{mass}; µmol g^{-1} s^{-1}) was calculated as A_{area} divided by SLA (assessed below).

Following gas exchange measurements, plants were harvested. For each plant, the leaf used for gas exchange was excised, imaged using a flatbed scanner, and measured for leaf reflectance values as described for the seedling study. Leaf toughness was also assessed on the leaf used for gas exchange measurements as the average of eight measurements using a Chatillon force gauge (model DFE; Ametek Inc., Largo, FL, USA). Leaves were dried at 60ºC for at least 48 hours and weighed. Scanned leaf images were analyzed for leaf area with ImageJ (U.S. National Institute of Health, Bethesda, MD, USA) (Schneider *et al.*, 2012). Scanned leaves were ground to a fine powder using a ball mill, and assessed for leaf nitrogen and carbon isotopic ratio (leaf δ^{13} C) on a continuous flow mass spectrometer (University of Georgia Soil Ecology Laboratory, Athens, GA, USA).

Statistical analyses

Statistical analyses for both the seedling study and the mature plant study were carried out using general linear models (PROC GLM) in the statistical software package SAS v. 9.3 (SAS Institute Inc. 2011). For the traits measured in each study, differences among species were analyzed using analysis of variance (ANOVA) and $\alpha = 0.05$.

Results and discussion

Rooting and biomass allocation characteristics: seedling study

Because root development plays a critical role in supplying crops with adequate water, faster rooting depth rates could be beneficial for improvement of drought resistance in cultivated sunflower (Seiler, 1994, 1998, 2008). Contrary to our expectation that the desert dwelling species TEPH would have a faster rooting depth rate, ANN seedlings reached a rooting depth of 30 cm much more quickly than TEPH seedlings (Figure 1), indicating a faster rooting depth rate in ANN. This finding was unexpected, given that rapid growth of deep roots is expected to characterize species adapted to arid and semi-arid environments (Nicotra *et al.*, 2002). One possible explanation for our findings is that our study assessed root growth under non-water limiting conditions, typical of a well-irrigated crop field, supporting a high growth rate in ANN that has likely resulted from intense selection during domestication under high resource availability. However, interspecific comparisons of species native to high rainfall versus low rainfall sites under well-watered common garden conditions have found that species native to low rainfall sites tend to exhibit faster rooting depth rates than those native to high rainfall sites (Nicotra *et al.*, 2002). In any case,

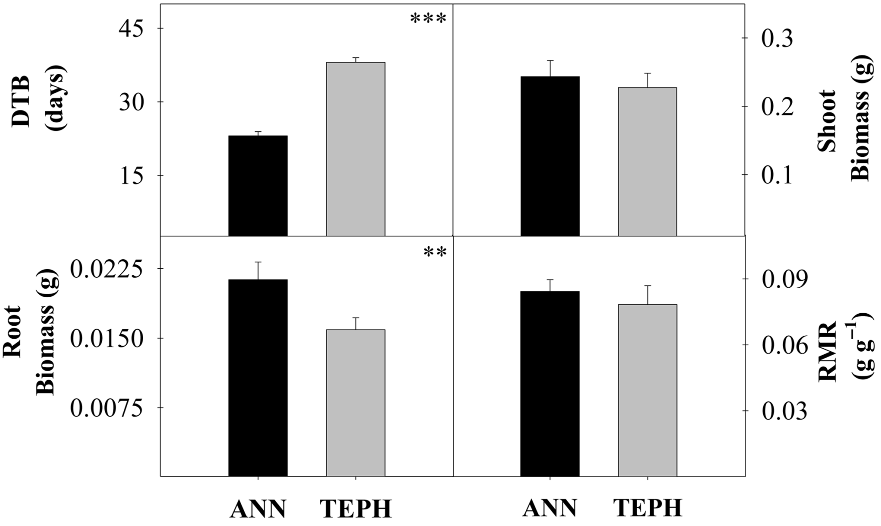

Figure 1: Root and biomass characteristics for *Helianthus annuus* (ANN) and *H. niveus* ssp. *tephrodes* (TEPH) assessed at the seedling stage. DTB (number of days required for root to reach the bottom of a 30 cm deep pot); RMR (root:total biomass ratio). Values which differed between ANN and TEPH are indicated by: (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.001$.

our finding of slower rooting depth rate in TEPH than in ANN suggests that the genetic basis of variation in this trait in TEPH may not be a valuable target for improvement of drought avoidance in cultivated sunflower. It is important to note, however, that rooting traits are clearly influenced by water availability in *Helianthus*, with studies reporting lower total root length, volume, and/or dry weight (El Midaoui *et al.*, 2003; Onemli and Gucer, 2010), altered root depth distributions (Sobrado and Turner, 1986; Comas *et al.*, 2013), and greater root-to-shoot biomass ratio (Sobrado and Turner, 1986) in response to drought or osmotic stress. In addition, our study of seedlings grown in 30 cm pots provides a somewhat limited understanding of root growth in TEPH and ANN, as root growth can be substantially deeper than 30 cm in both cultivated ANN and wild *Helianthus* species (e. g. Jones, 1984; Sobrado and Turner, 1986; Sadras *et al.*, 1989; Bartelme, 2014). Future studies examining root development under drought stress and without soil depth restrictions would help determine whether the differences detected in this study hold under conditions more representative of the field.

Although TEPH and ANN differed for seedling root biomass, they did not differ for shoot biomass, which was approximately ten-fold greater than root biomass for both species (Figure 1). As a result, TEPH and ANN did not significantly differ in RMR (Figure 1), contrary to the expectation that inherently high proportional allocation of the root system is adaptive for species found in arid habitats. Because RMR tends to decline as plants age (Poorter *et al.*, 2012), the lack of difference between the two species for RMR may be related to the nearly two-fold longer growing period for TEPH than ANN to reach a rooting depth of 30 cm (Figure 1). Time-course studies comparing TEPH and ANN would shed light on whether this lack of difference in RMR holds when accounting for plant age. It is important to note, however, that the most severe effects of drought are at particular developmental stage (flowering and seed set) in sunflower, rather than at a particular plant age *per se* (Karaata, 1991; Reddy *et al.*, 2003; cited in Rauf, 2008; Škorić, 2009). Thus, trait comparisons at a given developmental stage may be more agriculturally-relevant than comparisons at a similar plant age for established seedlings.

Leaf characteristics: seedling and mature plant studies

In addition to root and biomass allocation, leaf traits also strongly impact plant water use and the ability to avoid drought. At both the seedling and mature stages, TEPH produced smaller, tougher leaves with lower SLA than ANN (Figure 2). Smaller, thicker leaves are associated with reduced boundary layer resistance,

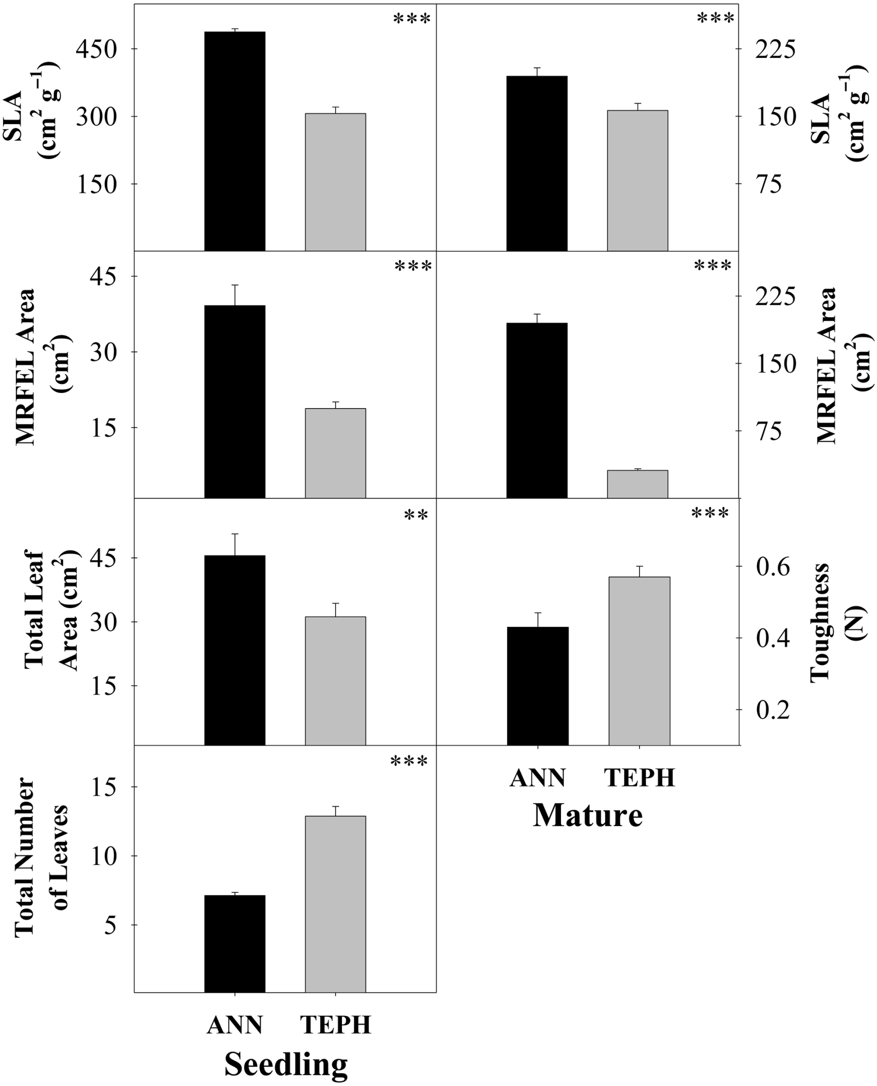

Figure 2: Leaf traits for *Helianthus annuus* (ANN) and *H. niveus* ssp. *tephrodes* (TEPH) assessed at the seedling and mature stages. Specific leaf area (SLA) was calculated from the most recently fully expanded leaf pair at the seedling stage, and from the single most recently fully expanded leaf at the seedling stage. Likewise, area of the most recently fully expanded leaf (MRFEL Area) was calculated as the average of two leaves at the seedling stage, and as the area of the single most recently fully expanded leaf at the mature stage. Values which differed between ANN and TEPH are indicated by: (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.001$.

thereby contributing to leaf cooling (Smith, 1978; McDonald *et al.*, 2003). Under mesic conditions, heat load is not as detrimental to plant metabolic function because transpiration can dissipate excess heat energy; however, as soil moisture decreases, plants cannot use water as freely for heat dissipation and thus smaller leaves serve as an alternative strategy to reduce heat load (McDonald *et al.*, 2003). TEPH produced a greater total number of leaves than ANN, but still produced significantly less total leaf area (Figure 2), likely reducing potential transpirational water loss at the whole-plant level (Blum, 1996). However, smaller total leaf area also reduces total leaf area available for photosynthetic carbon assimilation, and may be counterproductive for cultivated sunflower grown in high-resource conditions.

At both the seedling and mature stages, TEPH exhibited lower NDVI than ANN (Figure 3). This indicates that visible wavelengths comprise a relatively greater proportion of total reflectance (visible plus near-infrared) in TEPH, potentially due to its dense leaf pubescence. Leaf pubescence generally increases reflectance of visible wavelengths, but its effects on reflectance of near-infrared wavelengths are mixed (Ehleringer and Björkman, 1978; Grant, 1987; Slaton *et al.*, 2001; Sims and Gamon, 2002; Zhang *et al.*, 2012), possibly because reflectance of near-infrared wavelengths is more closely associated with leaf internal structure and light scattering than with leaf surface features (Sims and Gamon, 2002). Indeed, the pubescent-leaved TEPH had higher reflectance at 680 nm (visible wavelength) than ANN, whereas TEPH and ANN did not differ in reflectance at 800 nm (near-infrared wavelength) (Figure 3). Similar results have been seen in comparisons of spectral characteristics of cotton (*Gossypium barbadense*) cultivars, with the pubescent variety T1T1 exhibiting greater reflectance at 680 nm, but similar reflectance at 800 nm, in comparison to less pubescent varieties (Zhang *et al.*, 2012). Given that the majority of light absorption by leaves is in the visible spectrum, greater reflectance of visible wavelengths is expected to substantially reduce leaf heat load and transpirational water loss, which is particularly important in hot, arid environments such as where TEPH is found (Ehleringer *et al.*, 1976; Ehleringer and Björkman, 1978; Ehleringer and Mooney, 1978; Ehleringer, 1984). However, increased reflectance of visible (photosynthetically-active) wavelengths due to leaf pubescence could potentially result in an undesirable tradeoff with photosynthetic capacity under well-watered conditions (Ehleringer *et al.*, 1976; Ehleringer and Mooney, 1978; discussed below).

Photosynthesis, WUE and leaf chemistry: mature plant study

Although drought avoidance traits may reduce crop yield losses under drought conditions, the capacity for high photosynthetic rates when moisture availability

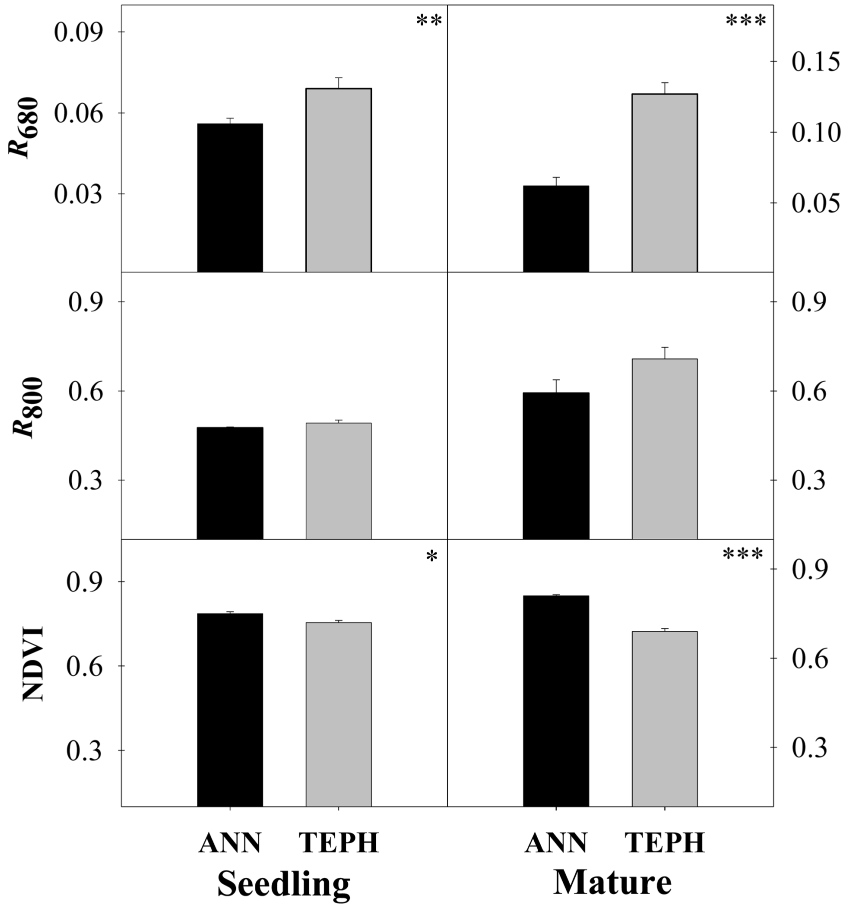

Figure 3: Normalized difference vegetation index (NDVI) and leaf reflectance values for *Helianthus annuus* (ANN) and *H. niveus* ssp. *tephrodes* (TEPH) assessed at the seedling and mature stages. NDVI was calculated from leaf reflectance at red (R_{680}) and near-infrared (R_{800}) wavelengths as described in the main text. Values which differed between ANN and TEPH are indicated by: (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.001$.

is high (i. e. following intermittent rains) is essential for generating acceptable yields. Surprisingly, the pubescent-leaved TEPH had a greater A_{area} than ANN (Figure 4). This is in contrast to a study of the desert shrub *Encelia farinosa*, which found that pubescent leaves exhibited lower A_{area} than less pubescent leaves, likely due to greater reflectance of visible (photosynthetic) wavelengths (Ehleringer *et al.*, 1976). However, ANN had a greater A_{mass} than TEPH (Figure 4) due to the greater SLA in ANN, suggesting faster carbon assimilation capacity per

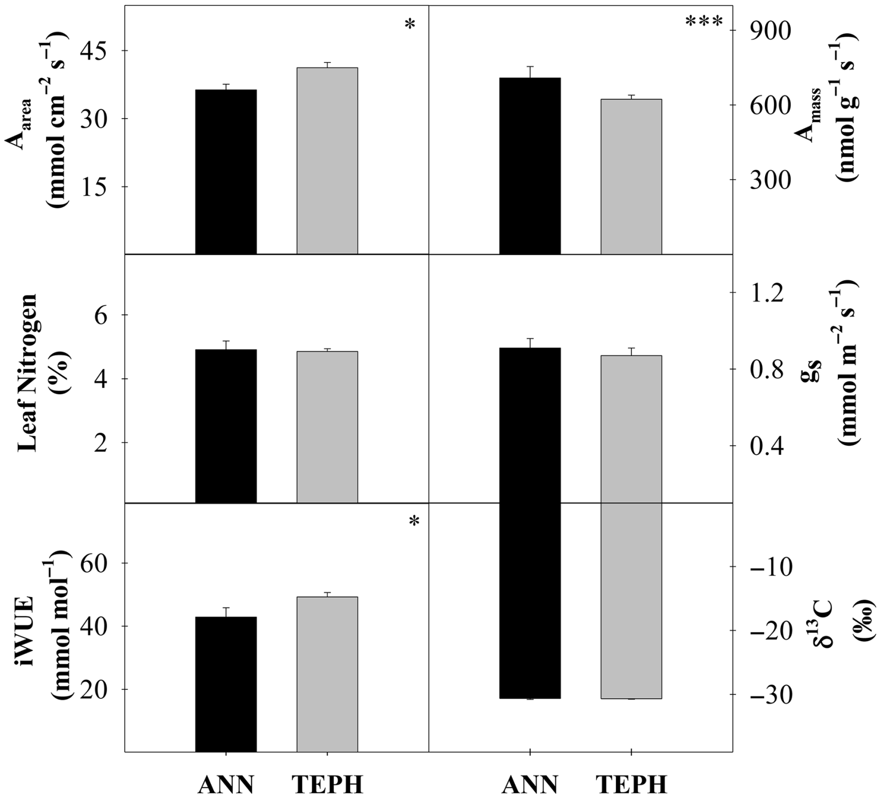

Figure 4: Leaf gas exchange, nitrogen concentration, and water-use efficiency for *Helianthus annuus* (ANN) and *H. niveus* ssp. *tephrodes* (TEPH) assessed at the mature stage. A_{area} (photosynthetic rate per unit leaf area); A_{mass} (photosynthetic rate per unit leaf mass); leaf nitrogen (leaf nitrogen concentration); g_s (stomatal conductance); $\delta^{13}C$ (proxy for integrated water-use efficiency). Values which differed between ANN and TEPH are indicated by: (*) $P < 0.05$; (**) $P < 0.01$; (***) $P < 0.001$.

unit leaf investment in ANN. Although this suggests that pubescent-leaved plants likely exhibit reduced yield under well-watered conditions relative to non-pubescent plants, the same may not be true under hot, droughted conditions. A tradeoff model for *E. farinosa* predicted that, as both temperature and aridity increase, greater reflectance of visible wavelengths by pubescent genotypes may actually result in faster photosynthetic rates than non-pubescent genotypes under hot, arid conditions, since high leaf temperatures can lead to reduced photosynthetic rates, and pubescent genotypes can maintain leaf temperatures closer to the optimum for photosynthesis (Ehleringer and Mooney, 1978). Thus, breeding for increased pubescence may only be desirable in hot, dry, high radiation environments that less pubescent varieties are unable to tolerate.

Although A_{mass} is positively correlated with both SLA and leaf nitrogen concentration in global-scale datasets of wild species (Wright *et al.*, 2004), TEPH and ANN did not differ in leaf nitrogen, despite the higher A_{mass} found in ANN (Figure 4), potentially because in species with high SLA, leaf nitrogen is distributed over a proportionally larger leaf area (Poorter and Evans, 1998). Additionally, although TEPH had a higher A_{area} than ANN, the two species did not differ in stomatal conductance (g), resulting in TEPH exhibiting a higher instantaneous WUE than ANN (Figure 4). However, TEPH and ANN did not differ in leaf $\delta^{13}C$ (a proxy for time-integrated WUE). This suggests the two species do not differ in integrated WUE under well-watered conditions, but that differences in instantaneous WUE may be detected under some environmental conditions, likely because instantaneous WUE can fluctuate in response to short-term changes in light quality and ambient relative humidity. It is important to note that, although integrated WUE is less susceptible to short-term environmental fluctuations since this parameter incorporates carbon uptake over the lifetime of the leaf, sustained drought can also impact integrated WUE (Richards, 1996). It would be interesting to test whether TEPH exhibits greater integrated WUE than ANN under drought conditions; a potential mechanism contributing to its tolerance of arid environments.

An important consideration of our study is that we examined trait variation in TEPH and ANN only under well-watered conditions. Although high resource availability is often expected to maximize variation among species with different resource-use strategies (Chapin *et al.*, 1993), species can differ in the response to resource limitations. This has been shown in a number of studies comparing drought responses of other wild *Helianthus* species and subspecies with ANN (e. g. Sobrado and Turner, 1983a, 1983b, 1986; Sobrado and Rawson, 1984; Milton *et al.*, 2013). For example, a recent study found that, although ANN and TEPH did not differ in germination rates in non-stressed control treatments, TEPH exhibited a sharper decline in germination rates in response to simulated drought stress than ANN (Milton *et al.*, 2013). Although this response may be adaptive for the desert-dwelling TEPH, reduced germination under drought stress is likely not beneficial for cultivated sunflower breeding (Milton *et al.*, 2013). Thus, comparisons of TEPH and ANN under drought conditions could reveal important interspecific differences not detected here. Another important consideration is that, although comparisons of crops and their wild relatives can provide insight into the variation that exists for traits of interest, evaluation of TEPH × ANN hybrids could demonstrate unique trait combinations and trait values relative to either parental species due to genetic recombination (i. e. transgressive segregation and/or heterosis) that were not detected in this study (e. g. deVicente and Tanksley, 1993, Singh and Ocampo, 1997; Xie *et al.*, 2008). Studies examining putative drought avoidance traits in TEPH × ANN hybrids under both well-watered and drought conditions would improve our understanding of the potential value of incorporating TEPH germplasm into sunflower breeding programs.

Conclusions

Overall, we found that under well-watered conditions, ANN develops deep roots more quickly at the seedling stage than TEPH, suggesting genetic variation for rooting depth rate in TEPH may not be beneficial for improvement of cultivated sunflower. However, TEPH produced smaller, thicker, more pubescent leaves than ANN, suggesting the ability of TEPH to decrease leaf heat load and transpirational water loss under water-limited, high radiation conditions. Additionally, at the mature stage, TEPH had a lower A_{mass} than ANN, suggesting a tradeoff between leaf traits that reduce heat load and those that regulate plant carbon assimilation under well-watered conditions. Thus, while incorporating TEPH germplasm into cultivated sunflower breeding programs may not be desirable for environments with high water availability due to yield tradeoffs, TEPH possesses favorable leaf morphology to offset heat load, suggesting this species could be a source of beneficial alleles for improved drought resistance for sunflower crops grown in drought-prone, high radiation environments. Future studies examining drought-resistance traits and performance of ANN and TEPH in such water-limited, high radiation conditions would inform on the potential for improvement of cultivated sunflower using the desert-dwelling TEPH.

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