ECOLOGICAL AND EVOLUTIONARY PATTERNS AMONG STEM AND LEAF FUNCTIONAL TRAITS IN *HELIANTHUS*

by

ALEX JOHN PILOTE

(Under the Direction of Lisa A. Donovan)

ABSTRACT

Plant functional traits are hypothesized to co-vary and have been often interpreted as reflecting resource strategies for acquisition, transport, and use of carbon, water, and nutrients. These trait combinations are expected to range along a continuum from “fast”, resource-acquisitive trait values to “slow”, resource-conservative values. This dissertation focuses on leaf and stem functional traits related to tissue structure and water transport for the sunflower genus, *Helianthus,* which encompasses wild species from diverse habitats across the North American continent and cultivated *H. annuus*. Using a comparative approach and common garden greenhouse studies, expected stem and leaf trait co-variation was examined from three complementary perspectives: evolutionary diversification of wild species, evolutionary responses to artificial selection, and ecological responses. At the evolutionary scale, a comparison of stem and leaf traits for 14 wild species provided evidence of correlated trait evolution and adaptive differentiation associated with habitat climate. The effects of crop domestication were assessed by comparison of two varieties of domesticated *H. annuus* (ancient landraces and modern improved cultivars) with its wild progenitor. This comparison revealed that this suite of leaf and stem traits did not shift in a coordinated fashion in response to the artificial selective pressures of crop domestication. Additionally, trait shifts were found to be inconsistent in comparison of these two forms of domestications (i.e. wild to ancient landraces v. wild to improved cultivar). In response to the abiotic stress of water limitation, leaf and stem traits of six wild sunflower species shifted in a coordinated fashion towards more resource-conservative trait values. In conclusion, this dissertation provides evidence for correlated evolution of a suite of stem and leaf functional traits and the plastic responses of this suite of traits are observed to co-vary when species are subjected to water stress; however, these traits are not found to co-vary during the artificial selective process of crop domestication. This suggests that co-variation of these traits across wild taxa may be primarily due to selective pressures rather than hypothesized biophysical or genetic constraints.

INDEX WORDS: resource strategy, hydraulic anatomy, xylem, leaf economics spectrum, phylogenetic comparison, *Helianthus*, drought, water limitation, crop domestication

ECOLOGICAL AND EVOLUTIONARY PATTERNS AMONG STEM AND LEAF FUNCTIONAL TRAITS IN *HELIANTHUS*

by

ALEX JOHN PILOTE

BS, Tennessee Technological University, 2010

MS, The University of Tennessee, 2012

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2017

ECOLOGICAL AND EVOLUTIONARY PATTERNS AMONG STEM AND LEAF FUNCTIONAL TRAITS IN *HELIANTHUS*

by

ALEX JOHN PILOTE

Major Professor:	Lisa Donovan
Committee:	Shu-Mei Chang Marc van Iersel Chris Peterson Robert Teskey

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
May 2017

DEDICATION

To my husband, who has been a steady presence of patience, optimism, and reassurance over the past five years.

To my teachers, who inspired me to pursue a career in science education. Special thanks to: Delores Doyle, John Dusenberry, Darrick Bowman, and Christy Carter.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Lisa Donovan, who has provided mentorship, support, and opportunities to advance my career. I would like to thank my committee members: Shu-Mei Chang, Marc van Iersel, Chris Peterson, and Robert Teskey, who have provided assistance and constructive criticism towards the production of this work. I would also like to greatly thank the staff of the Plant Biology department, who were integral in my success and timely completion: Susan Watkins, Stephanie Chirello, Chelsea Harvey, Gretchen Bowen, Shannon Kennedy, Mike Boyd, Kevin Tarner, Greg Cousins, Richard Hare, et al.

I owe a large debt of gratitude to the greater Donovan lab group, who provided valuable feedback on experimental designs, manuscripts, and countless conversations that improved my work: Elise Bartelme, Alan Bowsher, Torey Burns, Kayleigh Davis, Eric Goolsby, Karolina Heyduk, Caitlin Ishibashi, Kelly Kerr, Rishi Masalia, Chase Mason, Ethan Milton, Ashley Rea, Jess Stephens, and Andries Timme. Thanks to Kelly Bettinger for all of her help and assistance. I would also like to thank undergraduate students, Kayla Smith and Amna Jamshad, and a high school intern, Lucy Delaney, who greatly assisted in the experimental setup and measurements for my fourth chapter.

Thanks to Sigma Xi Grants in Aid of Research and UGA Plant Biology Palfrey small research grants for funding portions of this research.

Lastly, I would like to thank my family and friends, who have provided endless support of my goals, and who have helped me maintain perspective through this process.

TABLE OF CONTENTS

Page

APPENDICES

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Understanding co-variation among functional traits within and among plant species and taxonomic groups is among the primary goals of plant ecologists. When two or more ecologically important traits are correlated across species, they may be thought of as forming a strategic dimension of trait variation (Wright et al. 2007). Identification and interpretation of such patterns of trait co-variation may help to explain the physiological and structural basis determining the distribution of plant taxa and the ecological roles played by these taxa within an ecosystem or across a landscape (Grime et al. 1997, Reich et al. 1999, Ackerly 2004). The identification and study of suites of plant functional traits is the basis for trait-based ecology, which examines the relationships of plant functional traits with each other and the environment.

Trait-based plant ecologists have long sought to determine the functional traits that contribute to species distributions and success in varying environments and to characterize the environmental factors that result in repeated evolution of similar phenotypes. Grime (1977) hypothesized that plant taxa fall into a continuum of three categories (competitors, stress-tolerants, and ruderals), which describe variation among a suite of broad plant traits, including growth-rate, lifespan, and fecundity, which are continuous between categories and help determine which environments are associated with greatest success for a given species. This work complemented that of Bloom et al. (1985), who began describing the balance of carbon acquisition and use in terms of economic theory. This analogy applied the logic that increased carbon investment might contribute to increased stress-resistance of a given tissue, providing a longer “return on investment” in terms of tissue lifespan, at the cost of slower growth rates: much like a low interest savings account will provide slow but steady returns. The idea of faster growth being associated with lower tissue carbon investment and the goal of identifying suites of plant traits that define a species’ niche were combined and further expounded upon extensively in the 1990s (Lambers and Poorter 1992, Chapin et al. 1993, Grime et al. 1997). The work of these researchers described suites of physiological and anatomical traits hypothesized to co-vary through evolutionary time, forming resource strategies that may confer greater success in given environments. These traits are associated with faster or slower resource acquisition and use across taxa that ranged across habitats with varying degrees of competition and environmental stress. It was hypothesized that traits, such as high rate of photosynthesis, growth, and nutrient acquisition, would be favored by natural selection in resource-rich environments, while “slower” trait values would be favored in resource-poor environments.

A result of investigations into the expectation of fast-to-slow resource strategies among plant taxa was the identification and description of strikingly consistent co-variation among leaf traits, referred to as the worldwide leaf economics spectrum (Wright et al. 2004). This framework explains co-variation observed across species with “fast” resource-acquisitive trait combinations, including high specific leaf area, high leaf N and P concentrations, high rates of gas exchange, and short leaf lifespans, to species with “slow” resource-conservative trait combinations of opposing values (Wright et al. 2004). Recently, there has been a push to expand this framework across organ types and resources, through to whole plant economic relationships (Chave et al. 2009, Reich 2014). Reich (2014) proposed that selection along trait dimensions, along with biophysical constraints, results in convergence on strategies with high, medium, or low rates of resource acquisition and processing, beyond the scope of leaf economic traits. He thus proposes that this LES “fast v. slow” (i.e. resource-acquisitive v. resource-conservative) spectrum should extend to stem and fine root traits through traits related to acquisition and use of resources, whether they be carbon, nutrients or water.

Similar to the leaf economics spectrum, patterns of co-variation among stem functional traits have been found along dimensions of productivity, lifespan, and resistance to stress. The water-conducting elements of plants (xylem) provide biomechanical support for woody stems that suspend photosynthetic tissues above the ground and conduct water and nutrients along the soil-plant-atmosphere continuum (Rowe and Speck 2005). These functions place important constraints on the architecture of stems and have led to covariance in traits associated with hydraulic efficiency and mechanical strength (Tyree and Zimmermann 2002, Sperry et al. 2008). A potential “wood economics spectrum” has been hypothesized to explain this co-variation in the context of variation in functional trait values of species with low-density wood, fast growth rate, high stem hydraulic conductance, large xylem lumen area, low xylem density, high leaf area to sapwood ratio, and high total leaf area as compared to species with opposing trait values (Chave et al. 2009). Thus far however, these patterns have been studied in woody taxa, with a notable lack of investigations into the vascular anatomy and stem tissue properties of herbaceous species (Nolf et al. 2016).

This dissertation investigates the correlations of leaf and stem functional traits at three scales: evolutionary patterns of co-variation across inter-generic taxa, their response to the artificial selective processes of crop domestication, and their plastic responses to water stress. Previous studies have found co-variation among stem and leaf functional traits in broad samplings of field-collected data (Mendez-Alonzo et al. 2012, de la Riva et al 2015). A common garden assessment of congeneric species of known phylogenetic relatedness in a common garden setting would account for trait correlations that are the product of taxa relatedness, as well as environmentally-induced trait variation. This would provide a robust assessment of the co-variation of these functional traits through the evolutionary diversification of this genus. An assessment of these traits at the smaller evolutionary scale of crop domestication would allow for tests of trait co-variation through evolutionary time, but under artificial selective pressures. This analysis may provide insight into how selection for productivity in high-input systems, such as agricultural fields, may affect the evolution of resource strategies. Lastly, an assessment of the plastic responses of these functional traits to an environmental stress, such as water limitation, may provide insight into the evolutionary question: "do ecological responses follow similar trajectories as evolutionary patterns?" in regard to the co-variation of stem and leaf functional traits. The herbaceous genus *Helianthus* is an excellent model system for these analyses, as its species range across the United States in habitats that include desert sand dunes, roadsides, granite outcrops, and wetlands. Additionally, *Helianthus*, includes both annual and deciduously perennial species, including the wild progenitor of the crop sunflower (*Helianthus annuus*), and has recently been described using modern phylogenetic tools allowing phylogenetically-explicit analyses to be conducted within this genus (Stephens et al. 2015).

Researchers have begun to make inferences that stem and leaf functional traits should follow patterns of co-variation at the evolutionary scale among wild taxa (Edwards 2006, Freschet et al. 2010, Mendez-Alonzo et al. 2012, de la Riva et al. 2015). Freschet et al. (2010) found co-variation among organ economics across root, stem, and leaf traits, including specific leaf area and dry matter content and chemical composition of organ-specific tissues within subarctic flora. This study presented a single, multi-trait axis that supported a "fast v. slow" axis of trait variation across organ types. (Mendez-Alonzo et al. 2012) has provided further evidence for continuity among leaf and stem traits, observing co-variation among stem anatomical, mechanical, and functional traits along with leaf mass per area, leaf retention time, and leaf dry matter content in tropical dry forest trees. However, these patterns have previously been described in field settings, introducing an unknown amount of environmental variation into the dataset. Thus, investigations that assess these patterns in a common garden and phylogenetically-explicit setting are needed to provide a robust context for determining the degree of genetically based trait co-variation among leaf and stem traits and whether they have played a role in adaptation of taxa to native habitats.

According to resource strategy theory, crop domestication would be expected to result in "faster", more resource-acquisitive trait values because crops encounter selective pressures in artificially resource-rich environments. However, the artificial selective pressures of crop domestication focus on specific plant organs, tissues, or anatomical features instead of whole suites of plant traits. Thus, we might find shifts in trait values in comparisons of crop species and their wild progenitors that do not follow patterns of trait shifts observed among wild taxa (i.e. shifts towards faster values among some traits and slower values in others rather than an integrated shift towards more resource-acquisitive trait values in an agricultural setting). Research has begun to assess patterns of functional trait shifts between crop species and their wild progenitors, focusing on leaf and root traits due to their integral role in agricultural settings, e.g. carbon, nutrient, and water acquisition for plant growth (Pujol et al. 2008, Milla et al. 2014, Martin et al. 2015). Findings from these works support the idea that the artificial selection of crops may result in functional trait shifts that do not align with patterns observed across wild taxa, i.e. findings of shifts towards both more resource-acquisitive trait values and more resource-conservative trait values among both leaf and root functional traits. Thus far, stem hydraulic traits have not been analyzed in the context of crop domestication, and studies comparing crop species with their wild progenitors have focused on patterns between these two extremes along the process of domestication. Thus, a study that analyzes stem and leaf functional traits related to water transport and use and makes use of a primitive domesticated plant populations (such as ancient landraces) may provide novel insights into how functional traits evolve and co-vary during the process of crop domestication.

Functional traits that form resource strategies are additionally hypothesized to co-vary in their plastic response to environmental stress (Chapin 1991; Grime and Mackey 2002). Plant water transport and use has been observed to co-vary across environmental gradients of water availability in field studies, with traits shifting towards reduced water transport and increased leaf-level water use efficiency in regions with reduced water availability (Gleason et al. 2013). These patterns may be the result of local adaptation or varying success by individuals with more suitable trait values across a given ecological gradient. However, patterns of trait co-variation are further hypothesized to remain consistent at the ecological scale of phenotypic plasticity, with findings of trait shifts towards "slower" values when taxa are exposed to external stress (Grime and Mackey 2002, Plavcova and Hacke 2012). Specifically in relation to water stress, plants respond with alterations to anatomical and physiological parameters across tissues, with hormone signaling from roots driving alterations to leaf function, increasing water use efficiency, which has been observed to associate with alterations to stem hydraulic anatomy (Chapin 1991, Chapin et al. 1993, Grime and Mackey 2002). Thus, we may expect the suite of leaf and stem functional traits addressed in this dissertation to shift in a coordinated fashion towards "slower" trait values in response to water limitation in a controlled irrigation experiment.

For this dissertation, I specifically asked the following questions: 1) Have leaf and stem functional traits evolved in a correlated fashion, following expected trends of resource strategy, ranging from resource-acquisitive to resource-conservative trait combinations?; 2) Have these stem and leaf functional traits shifted in a coordinated fashion in response to the artificial selective pressures of crop domestication towards more resource-acquisitive trait values in agricultural settings? And 3) Do the plastic responses of these leaf and stem functional traits co-vary in their response to water stress?

REFERENCES

Ackerly, D. D. 2004. Adaptation, niche conservatism, and convergence: Comparative studies of leaf evolution in the California chaparral. American Naturalist 163:654-671.

Bloom, A. J., F. S. Chapin, and H. A. Mooney. 1985. Resource limitation in plants -- An economic analogy. Annual Review of Ecology and Systematics 16:363–392.

Chapin, F. S. 1991. Integrated responses of plants to stress. Bioscience 41:29–36.

Chapin, F. S., K. Autumn, and F. Pugnaire. 1993. Evolution of suites of traits in response to environmental stress. American Naturalist 142:S78-S92.

Chave, J., D. Coomes, S. Jansen, S. L. Lewis, N. G. Swenson, and A. E. Zanne. 2009. Towards a worldwide wood economics spectrum. Ecology Letters 12:351-366.

de la Riva, E. G., A. Tosto, I. M. Perez-Ramoz, C. M. Navarro-Fernandez, M. Olmo, N. P. R. Anten, T. Maranon, and R. Villar. 2015. A plant economics spectrum in Mediterranean forests along environmental gradients: is there coordination among leaf, stem, and root traits. . Journal of Vegetation Science 27:187–199.

Edwards, E. J. 2006. Correlated evolution of stem and leaf hydraulic traits in Pereskia (Cactaceae). New Phytol 172:479-789.

Freschet, G. T., J. H. C. Cornelissen, R. S. P. van Logtestijn, and R. Aerts. 2010. Evidence of the 'plant economics spectrum' in a subarctic flora. Journal of Ecology 98:362-373.

Gleason, S. M., D. W. Butler, and P. Waryszak. 2013. Shifts in leaf and stem hydraulic traits across aridity gradients in eastern Australia. International Journal of Plant Sciences 174:1292-1301.

Grime, J. P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. American Naturalist 111:1169–1194.

Grime, J. P., and J. M. L. Mackey. 2002. The role of plasticity in resource capture by plants. Evolutionary Ecology 16:299–307.

Grime, J. P., K. Thompson, R. Hunt, J. G. Hodgson, J. H. C. Cornelissen, I. H. Rorison, G. A. F. Hendry, T. W. Ashenden, A. P. Askew, S. R. Band, R. E. Booth, C. C. Bossard, B. D. Campbell, J. E. L. Cooper, A. W. Davison, P. L. Gupta, W. Hall, D. W. Hand, M. A. Hannah, S. H. Hillier, D. J. Hodkinson, A. Jalili, Z. Liu, J. M. L. Mackey, N. Matthews, M. A. Mowforth, A. M. Neal, R. J. Reader, K. Reiling, W. RossFraser, R. E. Spencer, F. Sutton, D. E. Tasker, P. C. Thorpe, and J. Whitehouse. 1997. Integrated screening validates primary axes of specialisation in plants. Oikos 79:259-281.

Lambers, H., and H. Poorter. 1992. Inherent variation in growth-rate between higher-plants - A search for physiological causes and ecological consequences. Advances in Ecological Research 23:187–261.

Martin, A. R., M. E. Isaac, and P. Manning. 2015. REVIEW: Plant functional traits in agroecosystems: a blueprint for research. Journal of Applied Ecology 52:1425-1435.

Mendez-Alonzo, R., H. Paz, R. C. Zuluaga, J. A. Rosell, and M. E. Olson. 2012. Coordinated evolution of leaf and stem economics in tropical dry forest trees. Ecology 93:2397-2406.

Milla, R., J. Morente-Lopez, J. M. Alonso-Rodrigo, N. Martin-Robles, and F. S. Chapin, 3rd. 2014. Shifts and disruptions in resource-use trait syndromes during the evolution of herbaceous crops. Proc Biol Sci 281.

Nolf, M., A. Rosani, A. Ganthaler, B. Beikircher, and S. Mayr. 2016. Herb Hydraulics: Inter- and Intraspecific Variation in Three Ranunculus Species. Plant Physiology 170:2085-2094.

Plavcova, L., and U. G. Hacke. 2012. Phenotypic and developmental plasticity of xylem in hybrid poplar saplings subjected to experimental drought, nitrogen fertilization, and shading. Journal of Experimental Botany 63:6481–6491.

Pujol, B., J.-L. Salager, M. Beltran, S. Bousquet, and D. McKey. 2008. Photosynthesis and leaf structure in domesticated cassava (Euphorbiaceae) and a close wild relative: have leaf photosynthetic parameters evolved under domestication? Biotropica 40:305–312.

Reich, P. B. 2014. The world-wide 'fast-slow' plant economics spectrum: a traits manifesto. Journal of Ecology 102:275-301.

Reich, P. B., D. S. Ellsworth, M. B. Walters, J. M. Vose, C. Gresham, J. C. Volin, and W. D. Bowman. 1999. Generality of leaf trait relationships: A test across six biomes. Ecology 80:1955-1969.

Rowe, N., and T. Speck. 2005. Plant growth forms: an ecological and evolutionary perspective. New Phytologist 166:61–72.

Sperry, J. S., F. C. Meinzer, and K. A. McCulloch. 2008. Safety and efficiency conflicts in hydraulic architecture: scaling from tissues to trees. Plant Cell and Environment 31:632–645.

Stephens, J. D., W. L. Rogers, C. M. Mason, L. A. Donovan, and R. L. Malmberg. 2015. Species tree estimation of diploid Helianthus (Asteraceae) using target enrichment. American Journal of Botany 102:910-920.

Tyree, M. T., and M. H. Zimmermann. 2002. Xylem Structure and the Ascent of Sap. Springer, Berlin, Germany.

Wright, I. J., D. D. Ackerly, F. Bongers, K. E. Harms, G. Ibarra-Manriquez, M. Martinez-Ramos, S. J. Mazer, H. C. Muller-Landau, H. Paz, N. C. A. Pitman, L. Poorter, M. R. Silman, C. F. Vriesendorp, C. O. Webb, M. Westoby, and S. J. Wright. 2007. Relationships among ecologically important dimensions of plant trait variation in seven Neotropical forests. Annals of Botany 99:1003-1015.

Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. C. Cornelissen, M. Diemer, J. Flexas, E. Garnier, P. K. Groom, J. Gulias, K. Hikosaka, B. B. Lamont, T. Lee, W. Lee, C. Lusk, J. J. Midgley, M. L. Navas, U. Niinemets, J. Oleksyn, N. Osada, H. Poorter, P. Poot, L. Prior, V. I. Pyankov, C. Roumet, S. C. Thomas, M. G. Tjoelker, E. J. Veneklaas, and R. Villar. 2004. The worldwide leaf economics spectrum. Nature 428:821-827.

CHAPTER 2

EVIDENCE OF CORRELATED EVOLUTION AND ADAPTIVE DIFFERENTIATION OF STEM AND LEAF FUNCTIONAL TRAITS IN THE HERBACEOUS GENUS, *HELIANTHUS* [1]

---

[1] Pilote, A.J. and L.A. Donovan. 2016. *American Journal of Botany* 103(2): 2096–2104. Reprinted here with permission of the publisher.

ABSTRACT

Premise of the Study– Patterns of plant stem traits are expected to align with a “fast-slow” plant economic spectrum across taxa. Although broad patterns support such tradeoffs in field studies, tests of hypothesized correlated trait evolution and adaptive differentiation are more robust when taxa relatedness and environment are taken into consideration. Here we test for correlated evolution of stem and leaf traits and their adaptive differentiation across environments, in the herbaceous genus, *Helianthus*.

Methods– Stem and leaf traits of 14 species of *Helianthus* (28 populations) were assessed in a common garden greenhouse study. Phylogenetically independent contrasts were used to test for evidence of correlated evolution of stem hydraulic and biomechanical properties, correlated evolution of stem and leaf traits, and adaptive differentiation associated with source habitat environments.

Key results– Among stem traits, there was evidence for correlated evolution of some hydraulic and biomechanical properties, supporting an expected tradeoff between stem theoretical hydraulic efficiency and resistance to bending stress. Population differentiation for suites of stem and leaf traits was found to be consistent with a “fast-slow’ resource-use axis for traits related to water transport and use. Associations of population traits with source habitat characteristics supported repeated evolution of a resource-acquisitive “drought-escape” strategy in arid environments.

Conclusions– This study provides evidence of correlated evolution of stem and leaf traits consistent with fast-slow spectrum of trait combinations related to water transport and use along the stem to leaf pathway. Correlations of traits with source habitat characteristics further indicate that the correlated evolution is associated, at least in part, with adaptive differentiation of *Helianthus* populations among native habitats differing in climate.

Key Words: Hydraulic anatomy, leaf economics spectrum, correlated evolution, functional traits, phylogenetic comparison

INTRODUCTION

When two or more ecologically important traits are found to consistently correlate across taxa, they may be thought of as a strategic dimension of trait variation (Wright et al., 2007; Freschet et al., 2010; Reich, 2014). Through identifying and understanding such trait dimensions, we can begin to understand the coordination of functional diversity among plants (Grime et al., 1997; Reich et al., 1999; Ackerly, 2004). Co-variation of functional traits governing resource acquisition, use, and transport is often studied in an ecological context, assessing patterns among field populations from sites varying in environmental characteristics and thus incorporating an unknown and potentially large amount of environmentally induced trait variation (Wright et al., 2004; Chave et al., 2009; Freschet et al., 2010). While these studies form a good basis for scaling up ecological consequences of trait variation, tests of hypothesized correlated trait evolution and adaptive differentiation are more robust when taxa relatedness is taken into consideration (Ackerly and Donoghue, 1998; Ackerly, 2000; Willson, Manos, and Jackson, 2008; Creese, Benscoter, and Maherali, 2011). In addition, comparisons among native populations includes an unknown mix of environmentally induced variation, while comparing taxa with a common garden approach allows for comparison of traits in response to a similar environment (Donovan et al., 2014; Poorter, Lambers, and Evans, 2014). This study will test for correlated trait evolution and adaptive differentiation of stem and leaf traits in herbaceous *Helianthus* species using a common garden approach.

Traits describing stem hydraulic efficiency and biomechanical support have been observed to covary with plant productivity, lifespan, and resistance to stress in broad field studies (Baas et al., 2004; Chave et al., 2009). Vascular tissue conducts water and nutrients along the soil-plant-atmosphere continuum while providing mechanical support for stems that suspend photosynthetic tissues above the ground. In woody angiosperms, it is hypothesized these functions are met by xylem conduit dimensions driving hydraulic traits (e.g. stem hydraulic conductivity) and fiber conduit dimensions driving biomechanical traits (e.g. resistance to bending and breaking stress) (Wagner, Ewers, and Davis, 1998; Jacobsen et al., 2005). This leads to the expectation of correlated evolution among stem traits governing each of these functions: water transport capacity (e.g. low xylem vessel density [Nv] associated with high xylem lumen fraction [Fx] and high theoretical hydraulic conductivity [Kt]), and stem biomechanical properties (e.g. low stem-specific density [SD] associated with low modulus of elasticity [MOE] (i.e. low resistance to bending), low fiber density [Nf], and high fiber lumen fraction [Ff]) (Wright et al., 2007; Chave et al., 2009). Stem water transport capacity is associated with larger xylem vessels and lower tissue density in woody angiosperms, and has thus been found to correlate negatively with biomechanical strength (MOE) in studies of woody angiosperms, but with mixed findings in regards to the strength of this relationship (Wagner, Ewers, and Davis, 1998; Jacobsen et al., 2005; Mendez-Alonzo et al., 2012). Herbaceous stem anatomy and function has been markedly less well studied than that of woody taxa, though large differences exist between herbaceous and woody stems (Nolf et al., 2016). Herbaceous stems are composed of smaller proportions of heavily lignified and conductive vascular tissue, and xylem and fiber cells are distinct within vascular bundles. Thus, large xylem vessels that support higher water transport may be expected to have large effects on the biomechanical strength of herbaceous stems.

In contrast to stems, covariation in leaf functional traits has received a lot of recent attention exemplified by the worldwide leaf economics spectrum (LES) describing a spectrum from resource acquisitive strategies (higher specific leaf area, leaf N and P, gas exchange rates, and short leaf lifespans) to resource conservative strategies (opposing trait value combinations) (Wright et al., 2004). Recent efforts have extended the resource acquisitive vs. conservative LES conceptual framework to a fast vs. slow plant economic spectrum that includes resource related stem and fine root traits (Chave et al., 2009; Reich, 2014). It has been argued that strong selection on resource use traits and biophysical constraints have resulted in coordination of resource acquisition, transport, and use across organs, because high resource use in one organ would only be advantageous under conditions where high resource acquisition and transport were found in other organs due to interdependence of organ function (Reich, 2014). This sets up the expectation for correlated evolution of leaf and stem traits, such as rapid metabolic rate in leaf tissue (e.g. higher photosynthetic rate) with higher capacity for water transport in stem tissue (e.g. higher hydraulic conductivity). Thus far, this expectation has found support among field studies across broad groups of woody taxa, with findings of correlated trait evolution among stem and leaf hydraulic parameters (Edwards, 2006), tissue elemental compositions (Freschet et al., 2010), and broader suites of functional and anatomical traits (Mendez-Alonzo et al., 2012; de la Riva et al., 2015). Differentiation of these traits would be expected to provide an adaptive advantage to populations across gradients of temperature, precipitation, and other environmental factors, with fast resource use and transport generally expected to be advantageous in environments that provide ample resources. For some life forms, however, rapid use of limited resources may be favored, e.g. a “drought escape” strategy observed in species growing in areas with short periods of water availability (Levitt, 1972; Verslues et al., 2006).

The sunflower genus, *Helianthus*, naturally occurs across North America, with populations found across a wide range in local habitat, including deserts, prairies, and wetland ecosystems. This genus exhibits high diversity in both morphology and phenology, with annual and deciduously perennial species. A well-resolved phylogeny recently developed for diploid, non-hybrid *Helianthus* has provided an opportunity for testing of correlated trait evolution of resource related traits (Stephens et al., 2015). A recent common garden study with this genus provided evidence for correlated evolution of some “fast” LES traits, and association of the “fast” leaf traits with drier and higher fertility source habitats (Mason and Donovan, 2015). This is consistent with a “drought-escape” strategy associated with fast growth and completion of reproduction during intervals when water is available (Levitt, 1972; Blum, 1988; Kramer and Boyer, 1995; Verslues et al., 2006). However, a common garden study of root traits with the same species did not find consistent evidence for a single fast-slow axis of both leaf and root traits (Bowsher et al., 2016).

The broad objective of this study is to use phylogenetic comparative methods and a common garden approach to assess correlated trait evolution and adaptive differentiation of stem and leaf traits, using the herbaceous *Helianthus* study system. First, we tested for evidence of correlated evolution among the stem anatomical traits, with an expectation of positive correlations among traits governing water transport capacity (e.g. high Kt, low Nv, and high Fx) and among traits governing the biomechanical strength of stem tissue (e.g. high SD, high MOE, high Nf, and low Ff), and a negative correlation between water transport capacity and biomechanical strength (traits outlined in Table 2.1). Second, we tested for evidence of correlated trait evolution between stem traits and leaf traits related to the leaf economics spectrum, with an expectation of strong correlations between traits governing stem water transport (Kt, Nv, and Fx) and leaf-level water use (iWUE and gs), as well as between traits characterizing carbon investment of stems (SD, Nf, and Ff) and leaves (LDMC, LMA,). Third, we tested for adaptive differentiation of suites of stem and leaf functional traits, with an expectation of strong correlations between traits representing resource-use strategy (i.e. Amass, iWUE, Kt) and environmental characteristics describing habitat temperature and water availability (i.e. mean annual temperature, mean annual precipitation, global aridity index, and potential evapotranspiration).

MATERIALS AND METHODS

Experimental design**–** The high resource (well-watered, fertilized) common garden study was conducted at the University of Georgia BioSciences greenhouse facility during July–November of 2014. The experimental design was a randomized complete block with 14 *Helianthus* species (Figure 2.1), 2 populations per species chosen from disparate parts of species' range to capture variation in temperature and precipitation (Dataset A1), and n=8 individuals per population. The species include six annual and eight perennial diploid non-hybrid species, representing the three major clades identified by Stephens et al. (2015), with natural ranges spanning the continental United States (Appendices S1 and S2). Among perennials, basal rosette species were excluded because stem tissue exists only during the reproductive stage of growth and, thus, does not supply water and nutrient to leaves.

For each population, achenes (hereafter "seeds") were either collected from the wild in 2007-2013 or obtained from the USDA National Genetic Resources Program (www.ars-grin.gov/npgs; Dataset A1). Beginning on 7 July 2014, seeds were scarified and germinated on wet filter paper in petri dishes and transferred to seedling trays until the emergence of the first true leaf pair. Seedlings were then planted individually into 20.3 cm diameter x 20 cm depth pots (2.92 L) filled with a 3:1 sand:calcined clay substrate. Mortality reduced the sample size for some populations: n=7 for *H. giganteus* (IRW and BUR), *H. maximiliani* (LAW), and *H.*

atrorubens (FMF), and n=4 for *H. angustifolius* (MAN). To ensure high nutrient conditions, each pot received 20g of Osmocote Plus 15-9-12 (7% NH_4, 8% NO_3) slow-release nine-month fertilizer with micronutrients (Scotts, Marysville, OH) at planting, and a monthly application of balanced liquid fertilizer containing supplemental calcium, iron, and magnesium. To ensure well-watered conditions, each pot received drip irrigation to field capacity multiple times daily for the entirety of the study. No supplemental lighting was used for extension of day length, thus light and temperature levels were ambient during the growth period.

Leaf trait measurement– Leaf functional traits were measured at the 3-5 true leaf pair ontogenetic stage. Due to differential timing of when populations reached this stage, measurements were conducted on three separate days, with all individuals of a population and both populations of a species were measured on the same day (*H. annuus, H. argophyllus, H. grosseserratus, H. microcephalus,* and *H. porteri* were measured 26 August; *H. petiolaris, H. debilis, H. giganteus, H. maximiliani,* and *H. agrestis* were measured 28 August; *H. atrorubens, H. silphioides, H. angustifolius,* and *H. floridanus* were measured 5 September). Days were chosen with clear skies, maximum temperatures varying +/- 1.1^{o} C, and morning relative humidity varying +/- 4% for optimal comparison across populations. The most recently fully expanded leaf was measured for photosynthetic rate (Aarea) and stomatal conductance (gs) at 400 ppm CO_2 and 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity, using a LiCor 6400 Portable Photosynthesis System (Li-Cor Biosciences, Lincoln, NE). The measured leaf was removed before dawn on the following morning, measured for fresh weight, and scanned to obtain a digital leaf images. Leaves were then dried at 60° C and weighed again for calculation of leaf dry matter content (LDMC, g·g). Leaf images were processed with Image J software (Rasband, 1997-2012) to obtain leaf area and allow calculation of leaf mass per unit area (LMA, $g \cdot cm^{-2}$) and rate of photosynthesis per unit dry mass (Amass). Instantaneous water use efficiency (iWUE) was calculated as the rate of photosynthetic carbon gain relative to the rate of stomatal conductance (Aarea/gs).

Stem trait measurement– Stem functional traits were measured during plant harvest, which occurred when each individual produced its first bud to ensure comparison at a similar ontogenetic stage. Due to differential timing of when individuals reached this stage, not all individuals of a population were harvested on the same day. Plant harvest occurred between September 1 and November 23, 2014, with ranges for populations noted in Data S1. At harvest, the first order stem was stripped of all leaves and/or lateral branches and sectioned into 2 segments: a 10 cm segment, 1 cm distal to the first true-leaf pair or branch, and a 15 cm segment immediately distal to the first. Volume of the 10 cm segment was measured via the water displacement method, after which this segment was oven-dried for at least 72 h at 60°C. Stem density (SD, $g \cdot cm^{-3}$) was then determined as dry mass per unit of fresh volume.

Modulus of elasticity (MOE, MPa) was measured on the 15 cm segment via the method outlined in Cooley, Reich, and Rundel (2003). The proximal end of the segment was fixed into a bored rubber stopper and secured to a table edge. Mass was incrementally added to the distal end while the stem segment's angle of deflection was noted. MOE is a measure of a material's resistance to bending per unit area, and was calculated using a standard equation, modified by Chazdon (1986) to account for tapering of a linear segment: $MOE = (M \cdot L^3\ [t_0/t_l])/(3 \cdot \delta \cdot I_0)$, where M is the total added mass at a given vertical deflection (δ), L is the distance between the fixed end of the segment and the point of mass addition, t_0 and t_l are the segment thicknesses at the fixed end and point of addition, respectively, and I_0 is the second moment of area of a cross-section at the fixed end of the stem segment. I_0 was calculated using an equation modified for elliptical cross-sections: $I_0 = (\pi/4) \cdot a^3 \cdot b$, where a is the radius of the transverse axis and b is the radius of the longitudinal axis (Niklas, 1992).

Xylem anatomy was assessed for 5 randomly selected individuals of each population on a ~2.5 mm cross-section was sliced from the 10 cm segment prior to measurement of fresh volume. The cross-sections were fixed in 10% alcoholic formalin (Cancer Diagnostics, Inc., Durham, NC) and processed at the University of Georgia Veterinary Histology Laboratory, where each sample was embedded in paraffin, sliced with a sledge-microtome, mounted to a slide, and stained with Toluidine blue. Slides were then imaged with a camera-mounted Zeiss light microscope using ZEN software (Carl Zeiss Microscopy, Oberkochen, Germany). Two xylem bundles per cross-section were imaged at 100x, and cortex fiber bundles were imaged at 200x, capturing two bundles or until greater than 75 fiber conduits per cross-section were imaged. The images were analyzed using Image J software (Rasband, 1997-2012). The selection tool was used to isolate xylem vessels or fiber cells from ground tissue within each image, and then lumen diameter (calculated as the diameter of a circle of equal area to the measured conduit) and lumen area of individual conduits in each cross-sectional image were measured. Vessel density and fiber density (Nv and Nf; $N \cdot mm^{-2}$) were calculated as number of conduits per unit area. Xylem lumen fraction and fiber lumen fraction (Fx and Ff; %) were determined as the ratio of total lumen area to total xylem area and total fiber area, respectively. Theoretical hydraulic conductivity (Kt, $kg \cdot s^{-1} \cdot m^{-1} \cdot MPa^{-1}$) for each sample was calculated, based on the Hagen-Poiseuille equation for ideal capillaries assuming laminar flow, as $Kt = ([\pi \cdot \rho]/[128 \cdot \eta \cdot A]) \cdot (\Sigma D_V^4)$; where ρ is the density of water (998.2 $kg \cdot m^{-3}$ at 20^{o}C); η is the viscosity of water (1.002×10^{-9} MPa·s at 20^{o}C); A is the total measured area (m^2); and D_X is the xylem lumen diameter for i=1 to n conduits for all conduits measured per sample (m) (Tyree and Ewers, 1991;

Santiago et al., 2004). Percent of vascular tissue (%) was calculated from total stem cross-sectional area using the selection and measurement tools in Image J.

***Population Source Site Environmental Data*–** Data related to climatic conditions for the native site of each population was extracted from the WorldClim database (Hijmans et al., 2005), including mean annual temperature (MAT; °C), and mean annual precipitation (MAP; mm/month). Mean annual potential evapotranspiration (PET; mm/month) and global aridity index (GAI; unitless, calculated as MAP/PET, with higher values indicative of more arid habitat) were extracted from the CGIAR Global Aridity and PET database (Data S1) (Zomer et al., 2008).

***Statistical Analysis*–** Population means were used to assess patterns across the studied species. Data were transformed as necessary to meet the assumptions of normality: gs, iWUE LMA, MOE, Kt, Nv, Nf, and Ff were log-transformed prior to data analysis. Phylogenetic analyses were conducted using the most recent phylogeny of *Helianthus* (Stephens et al., 2015), which was based on 170 nuclear genes that were sampled from individuals representative of all populations used for this study. This phylogeny is well-resolved and derived substitution branch lengths using maximum likelihood on a fixed coalescent topology (Stephens et al., 2015). Phylogenetically independent contrasts of trait-trait, within and across organ type, and trait-environment relationships were conducted using PhyloPars web-based software, which utilizes a phylogenetic mixed model for trait evolution and allows for intra-specific variation between populations (Bruggerman, Heringa, and Brandt, 2009). This model incorporates assumptions and models of phylogenetic comparison laid out in Felsenstein (2008), while treating population means as independent with a species node and allowing calculation of missing data (Bruggerman et al., 2009). Incorporating within-species variation has been shown to reduce bias in the analysis of macroevolutionary covariance (Sivestro et al., 2015). Previous work in *Helianthus* found that the Brownian motion model of trait evolution, employed by PhyloPars, was favored over single-optimum Ornsein-Uhlenbeck models for most leaf traits (Mason and Donovan, 2015). Pearson correlations and principal component analyses of leaf and stem traits were conducted using JMP Pro 10 software (JMP, Version 10, SAS Institute Inc., Cary, NC, 1989-2007). Principle component analysis was run using the correlation matrix to remove the influence of differences in scaling among the measured stem and leaf traits.

RESULTS

Evidence of correlated evolution among stem traits, supporting tradeoff between stem hydraulic efficiency and resistance to bending stress– There was substantive variation in stem traits among populations of this herbaceous genus. Coefficients of variation for these traits ranged from 0.117–0.720 (Table 2.1). Phylogenetically independent contrasts found significant pairwise correlations among stem traits (Table 2.2; ahistorical correlations can be seen in Appendix S3). As predicted, strong correlations were found among traits related to water transport (low vessel density [Nv], high xylem lumen fraction [Fx], and high theoretical hydraulic conductivity [Kt]) and among traits related to stem biomechanical properties (high modulus of elasticity [or resistance to bending stress; MOE], low fiber lumen fraction [Ff], and high fiber density [Nf]) (Table 2.2). Strong associations were found between xylem and fiber conduit dimensions, with vessel and fiber densities (Nv and Nf, respectively) and xylem and fiber lumen fractions (Fx and Ff, respectively) correlating positively. Additionally, a strong negative correlation was observed between Kt and MOE, suggesting that a tradeoff exists such that higher water transport capacity is associated with lower stem biomechanical strength as assessed by resistance to bending stress among these populations. Stem density (SD) was not found to covary strongly with the majority of stem traits, deviating from the expectation that SD would correlate strongly with other stem biomechanical properties. Rather SD was found to correlate with the percentage of stem cross-sectional area composed of vascular tissue (Vascular %; Figure 2.2) and whole plant leaf area (Table 2.2).

***Evidence of correlated evolution of stem and leaf traits, supporting spectrum of fast-slow resource use and transport*–** Phylogenetically independent contrasts found significant pairwise correlations among leaf traits (Table 2.2), supporting previous findings among species in this genus described in detail by Mason and Donovan (2015). Additionally, strong correlations were found between these leaf traits and stem traits, as expected. Stem traits describing hydraulic capacity (Fx, Nv, and Kt) strongly associated with leaf level water use efficiency (iWUE) (Table 2.2). Stem fiber traits (Nf and Ff) were further found to correlate well with leaf mass per unit area (LMA) and leaf dry matter content (LDMC), providing evidence of correlation between organ-specific tissue densities. SD and total leaf area (LA) were strongly correlated ($R= 0.788, p<0.001$), though neither was found to correlate with other leaf or stem traits. A principal components analysis including all stem and leaf functional traits revealed a predominant axis of trait covariation across both organs (Figure 2.3; Table 2.3). This axis ranges from populations with high Kt, large lumen fractions in both xylem and fiber cells, and high rates of gas exchange, to populations with high vessel and fiber densities, high MOE (i.e. higher resistance to bending), greater iWUE, and greater LMA. Perennial species were generally more resource-conservative (more positive for principle component 1) relative to annual species (Figure 2.3).

***Evidence for adaptive differentiation, with resource-acquisitive traits favored in arid habitats* –** Stem and leaf traits were correlated with source site environmental characteristics related to temperature and water availability. As expected, repeated appearance of a resource-acquisitive strategy was observed among herbaceous populations associated with more arid habitats. The first principal component of stem and leaf trait covariation (positive values indicative of a resource-acquisitive strategy) correlated negatively with local habitat potential evapotranspiration and temperature (Table 2.4). Populations from locations with higher potential evapotranspiration (a factor of a location's temperature and precipitation) were characterized by traits indicative of higher water transport capacity and use (i.e. lower Nv, LMDC, iWUE, and higher LA) (Table 2.4). Of note, a number of correlations among environmental characteristics were significant and have been included in Appendix S4.

DISCUSSION

We used a common garden approach and phylogenetically-informed comparisons to detect evidence of correlated evolution and adaptive differentiation of stem and leaf traits within the herbaceous genus, *Helianthus*. Among stem traits, there were strong, consistent correlations, with associations between hydraulic traits (Nv, Fx, and Kt), fiber conduit dimensions (Nf and Ff), and MOE (resistance to bending stress). Coordination among hydraulic traits was consistent with the expectation of wide xylem conduits being able to transport water more efficiently than many, narrow conduits because water flow is a product of a conduit's diameter to the fourth power (Ewers and Fisher, 1989; Ewers, Fisher, and Chiu, 1990; Sperry, Hacke, and Pittermann, 2006). These hydraulic parameters had a strong negative correlation with MOE or resistance to bending stress, suggesting a tradeoff between potential stem water transport capacity and stem mechanical strength in *Helianthus*, i.e. stems capable of high water transport may be more vulnerable to structural damage caused by wind or other stem mechanical stressors. Study of this tradeoff in woody angiosperms has been met with mixed results, with findings of correlated evolution of a tradeoff between water transport capacity and biomechanical properties among dry tropical species (Mendez-Alonzo et al., 2012), but only weak correlations were observed among California chaparral species (Wagner, Ewers, and Davis, 1998; Jacobsen et al., 2005). The weak relationships were attributed to differences in fiber and xylem conduit function, with xylem conduit dimensions driving hydraulic traits (i.e. stem water flow capacity), and fiber conduit dimensions driving biomechanical traits (i.e. resistance to bending and breaking stress). We, however, found strong correlations between xylem traits (Nv and Fx), fiber traits (Nf and Ff), and MOE in our dataset (Table 2.2), suggesting that there may be less differentiation between these two vascular cell types in these herbaceous populations than in previously measured woody species.

Helianthus stem density (SD) was only weakly correlated with xylem and fiber traits (Table 2.2), suggesting that variation in water transport capacity may be attained through an increased number of larger xylem vessels with the possibility of little effect on overall SD. Findings for this comparison have been mixed in woody angiosperms, with studies that show that a wide range of vessel dimensions may be possible within a narrow range of wood density. However, the lack of a significant relationship between SD and MOE among *Helianthus* species differs from strong associations between SD and MOE across woody taxa (Niklas, 1995; Poorter et al., 2008; Chave et al., 2009). Further analysis into the traits underlying both SD and MOE in *Helianthus* revealed that the proportion of stem tissue composed of vascular tissue resulted in the strongest association with SD (Figure 2.2). This suggests that SD may be gained through an increase in overall vascular tissue area, while MOE is a product of the conduit dimensions within this vascular tissue. Rather, SD was found to correlate positively with overall leaf area, suggesting that a greater investment in the highly lignified vascular tissue of these herbaceous stems may allow for greater leaf area, rather than providing greater resistance to bending stress.

Why do the relationships among stem traits in herbaceous *Helianthus* differ from those previously described primarily for woody species? One factor may be the composition of herbaceous stems, such as the greater percentage of stem tissue composed of ground versus vascular tissue. Additionally, however, it is important to keep in mind that our common garden approach reduces the effect of environmental variation on trait values, whereas most of the woody plant studies to date come from field studies that may include a lot of environmentally induced variation in stem traits that may obscure underlying patterns of correlated trait evolution (Jacobsen et al., 2007; Gleason, Butler, and Waryszak, 2013; Bai et al., 2015; Laughlin et al., 2015). Conversely, common garden growth may introduce novel environmental conditions to populations, potentially resulting in phenotypes not found in natural settings. Additional studies that link field-gathered trait values to those observed in common garden assessments of the same populations would allow further disentanglement of genetically- and environmentally-induced trait variation and provide unique insight into the correlated evolution of functional traits.

When leaf and stem traits were considered together for *Helianthus*, they extended patterns from a broader survey of *Helianthus* species that found covariation in many (but not all) LES traits consistent with the worldwide LES (Mason and Donovan, 2015). For the multivariate analyses in the current study, the predominant axis ranges from populations with high stem water transport capacity (high Kt and Fx, low Nv) and low resistance to bending stress (low MOE and Nf, high Ff), associated with high rates of gas exchange (Amass and gs) and low LMA, to populations with opposing trait values (Figure 2.3; Table 2.3). These patterns were additionally found to span from perennial species to annual species, with perennials generally having more resource-conservative trait combinations than annuals. Looking at bivariate relationships, there was evidence for correlated evolution of traits governing leaf-level water use (iWUE and gs) with those governing stem water transport (Kt, Nv, and Fx) consistent with the hypothesis that it is advantageous for a plant to maintain high water transport capacity while there is also high demand for its use at the leaf level, especially in species from arid environments (Reich, 2014). These patterns are consistent with previous field observations across woody angiosperm species in patterns of water transport and use, tissue chemical composition, and other functional trait values of stem and leaf tissue (Freschet et al., 2010; Mendez-Alonzo et al., 2012; de la Riva et al., 2015). Thus, the evidence continues to build for an evolutionary basis to a spectrum of resource-related strategies across aboveground tissues. The evidence of the extension of a consistent fast-slow axis of resource-related traits across roots as well is less evident for *Helianthus* (Bowsher et al., 2016).

Evidence of correlated trait evolution consistent with a fast-slow economics spectrum lends supports to the argument that some combination of selection and biophysical constraints (or as we have argued elsewhere, selection and genetic constraints) (Donovan et al., 2011) has contributed to the trait patterns (Wright et al., 2004; Reich, 2014). A finding of correlated trait evolution does not it and of itself provide any insight into the relative strength of selection vs. constraints, or the selective pressures involved. However, evidence of the association of traits in a common garden study with the environmental characteristics of each population's source site, combined with physiological insights, can provide inference for selection and selective pressures. We found that populations from habitats with greater potential evapotranspiration had more resource-acquisitive strategies, with higher LA, and lower Nv, LDMC, and iWUE under common garden conditions (Table 2.4). This represents the repeated evolution of a "fast" strategy populations in warmer, drier habitats, consistent with expectations of selection towards rapid growth in habitats characterized by short periods of water availability. The fast water transport likely supports a greater capacity for photosynthetic rate, suggesting selection for faster growth and ability to complete reproduction during favorable conditions, i.e. a “drought escape” strategy (Levitt, 1972; Verslues et al., 2006). This reinforces the recent evidence for repeated evolution of more resource-acquisitive leaf traits in more arid and higher fertility habitats in a *Helianthus* study that included more species and several of the same traits and environmental measures (Mason and Donovan, 2015). Additionally, it is consistent with the association of resource-acquisitive leaf traits with drier and lower fertility habitats for populations of the desert annual species, *H. anomalus* (Brouillette et al., 2014).

In summary, this study provides novel insight into the evolution of stem of biomechanical and hydraulic traits and leaf functional traits using common garden design and strong phylogenetic context in the herbaceous genus, *Helianthus*. Our data provides evidence for correlated evolution of stem and leaf traits consistent with fast-slow spectrum of trait combinations related to water transport and use along the stem to leaf pathway. Correlations of traits with source habitat characteristics further indicate that the correlated evolution is associated, at least in part, with adaptive differentiation of *Helianthus* populations among native habitats differing in climate. Additional studies of taxa representing other life forms and ranges of habitats will be needed to build a broader understating of the evolutionary basis of the worldwide fast-slow plant spectrum.

REFERENCES

ACKERLY, D. D. 2000. Taxon sampling, correlated evolution, and independent contrasts. *Evolution* 54: 1480-1492.

ACKERLY. 2004. Adaptation, niche conservatism, and convergence: Comparative studies of leaf evolution in the California chaparral. *American Naturalist* 163: 654-671.

ACKERLY, D. D., AND M. J. DONOGHUE. 1998. Leaf size, sapling allometry, and Corner's rules: Phylogeny and correlated evolution in maples (Acer). *American Naturalist* 152: 767-791.

BAAS, P., F. W. EWERS, S. D. DAVIS, AND E. A. WHEELER. 2004. Evolution of xylem physiology. *In* A. R. Hemsley AND I. Poole [eds.], The evolution of plant physiology from whole plants to ecosystems, xiii, 492 p. Published for the Linnean Society of London by Elsevier Academic Press, Amsterdam.

BAI, K. D., C. X. HE, X. C. WAN, AND D. B. JIANG. 2015. Leaf economics of evergreen and deciduous tree species along an elevational gradient in a subtropical mountain. *Aob Plants* 7: 15.

BLUM, A. 1988. Plant Breeding for Water-Limited Environments. Springer Science & Business Media, LLC, New York, NY.

BOWSHER, A. W., C. M. MASON, E. W. GOOLSBY, AND L. A. DONOVAN. 2016. Fine root tradeoffs between nitrogen concentration and xylem vessel traits preclude unified whole-plant resource strategies in Helianthus. *Ecology and Evolution* 6: 1016-1031.

BROUILLETTE, L. C., C. M. MASON, R. Y. SHIRK, AND L. A. DONOVAN. 2014. Adaptive differentiation of traits related to resource use in a desert annual along a resource gradient. *New Phytologist* 201: 1316–1327.

BRUGGERMAN, J., J. HERINGA, AND B. W. BRANDT. 2009. PhyloPars: estimation of missing parameter values using phylogeny. *Nucleic Acids Research* 37: W179–W184.

CHAVE, J., D. COOMES, S. JANSEN, S. L. LEWIS, N. G. SWENSON, AND A. E. ZANNE. 2009. Towards a worldwide wood economics spectrum. *Ecology Letters* 12: 351-366.

CHAZDON, R. L. 1986. The costs of leaf support in understory palms - economy versus safety. *American Naturalist* 127: 9-30.

COOLEY, A. M., A. REICH, AND P. RUNDEL. 2003. Leaf support biomechanics of neotropical understory herbs. *American Journal of Botany* 91: 573–581.

CREESE, C., A. M. BENSCOTER, AND H. MAHERALI. 2011. Xylem function and climate adaptation in Pinus. *American Journal of Botany* 98: 1437-1445.

DE LA RIVA, E. G., A. TOSTO, I. M. PEREZ-RAMOZ, C. M. NAVARRO-FERNANDEZ, M. OLMO, N. P. R. ANTEN, T. MARANON, AND R. VILLAR. 2015. A plant economics spectrum in Mediterranean forests along environmental gradients: is there coordination among leaf, stem, and root traits. *Journal of Vegetation Science* 27: 187–199.

DONOVAN, L. A., H. MAHERALI, C. M. CARUSO, H. HUBER, AND H. DE KROON. 2011. The evolution of the worldwide leaf economics spectrum. *Trends in Ecology & Evolution* 26: 88-95.

DONOVAN, L. A., C. M. MASON, A. W. BOWSHER, E. W. GOOLSBY, AND C. D. A. ISHIBASHI. 2014. Ecological and evolutionary lability of plant traits affecting carbon and nutrient cycling. *Journal of Ecology* 102: 302-314.

EDWARDS, E. J. 2006. Correlated evolution of stem and leaf hydraulic traits in Pereskia (Cactaceae). *New Phytol* 172: 479-789.

EWERS, F. W., AND J. B. FISHER. 1989. Variation in vessel length and diameter in stems of 6 tropical and sub-tropical lianas. *American Journal of Botany* 76: 1452-1459.

EWERS, F. W., J. B. FISHER, AND S. T. CHIU. 1990. A survey of vessel dimensions in stems of tropical lianas and other growth forms. *Oecologia* 84: 544-552.

FELSENSTEIN, J. 2008. Comparative methods with sampling error and within-species variation: contrasts revisited and revised. *American Naturalist* 171: 713–725.

FRESCHET, G. T., J. H. C. CORNELISSEN, R. S. P. VAN LOGTESTIJN, AND R. AERTS. 2010. Evidence of the 'plant economics spectrum' in a subarctic flora. *Journal of Ecology* 98: 362-373.

GLEASON, S. M., D. W. BUTLER, AND P. WARYSZAK. 2013. Shifts in leaf and stem hydraulic traits across aridity gradients in eastern Australia. *International Journal of Plant Sciences* 174: 1292-1301.

GRIME, J. P., K. THOMPSON, R. HUNT, J. G. HODGSON, J. H. C. CORNELISSEN, I. H. RORISON, G. A. F. HENDRY, et al. 1997. Integrated screening validates primary axes of specialisation in plants. *Oikos* 79: 259-281.

HIJMANS, R. J., S. E. CAMERON, J. L. PARRA, P. G. JONES, AND A. JARVIS. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25: 1965-1978.

JACOBSEN, A. L., F. W. EWERS, R. B. PRATT, W. A. PADDOCK, AND S. D. DAVIS. 2005. Do xylem fibers affect vessel cavitation resistance? *Plant Physiology* 139: 546-556.

JACOBSEN, A. L., L. AGENBAG, K. J. ESLER, R. B. PRATT, F. W. EWERS, AND S. D. DAVIS. 2007. Xylem density, biomechanics and anatomical traits correlate with water stress in 17 evergreen shrub species of the Mediterranean-type climate region of South Africa. *Journal of Ecology* 95: 171-183.

KRAMER, P. J., AND J. S. BOYER. 1995. Water Relations of Plants and Soils. Academic Press, San Diego, California, USA.

LAUGHLIN, D. C., S. J. RICHARDSON, E. F. WRIGHT, AND P. J. BELLINGHAM. 2015. Environmental filtering and positive plant litter feedback simultaneously explain correlations between leaf traits and soil fertility. *Ecosystems* 18: 1269-1280.

LEVITT, J. 1972. Responses of Plants to Environmental Stresses. Academic Press, New York, New York, USA.

MASON, C. M., AND L. A. DONOVAN. 2015. Evolution of the leaf economics spectrum in herbs: Evidence from environmental divergences in leaf physiology across Helianthus (Asteraceae). *Evolution* 69: 2705-2720.

MENDEZ-ALONZO, R., H. PAZ, R. C. ZULUAGA, J. A. ROSELL, AND M. E. OLSON. 2012. Coordinated evolution of leaf and stem economics in tropical dry forest trees. *Ecology* 93: 2397-2406.

NIKLAS, K. J. 1992. Plant Biomechanics: an engineering approach to plant form and function. University of Chicago Press, Chicago, Illinois, USA.

------. 1995. Size-dependent allometry of tree height, diameter, and trunk-taper. *Annals of Botany* 75: 217-227.

NOLF, M., A. ROSANI, A. GANTHALER, B. BEIKIRCHER, AND S. MAYR. 2016. Herb hydraulics: inter- and intraspecific variation in three Ranunculus species. *Plant Physiology* 170: 2085-2094.

POORTER, H., H. LAMBERS, AND J. R. EVANS. 2014. Trait correlation networks: a whole-plant perspective on the recently criticized leaf economic spectrum. *New Phytologist* 201: 378-382.

POORTER, L., S. J. WRIGHT, H. PAZ, D. D. ACKERLY, R. CONDIT, G. IBARRA-MANRIQUES, K. E. HARMS, et al. 2008. Are functional traits good predictors of demographic rates? Evidence from five Neotropical forests. *Ecology* 89: 1908-1920.

RASBAND, W. S. 1997-2012. ImageJ. website: imagej.nih.gov/ij/.

REICH, P. B. 2014. The world-wide 'fast-slow' plant economics spectrum: a traits manifesto. *Journal of Ecology* 102: 275-301.

REICH, P. B., D. S. ELLSWORTH, M. B. WALTERS, J. M. VOSE, C. GRESHAM, J. C. VOLIN, AND W. D. BOWMAN. 1999. Generality of leaf trait relationships: A test across six biomes. *Ecology* 80: 1955-1969.

SANTIAGO, L. S., G. GOLDSTEIN, F. C. MEINZER, J. B. FISHER, K. MACHADO, D. WOODRUFF, AND T. JONES. 2004. Leaf photosynthetic traits scale with hydraulic conductivity and wood density in Panamanian forest canopy trees. *Oecologia* 140: 543-550.

SIVESTRO, D., A. KOSTIKOVA, G. LITSIOS, P. B. PEARMAN, AND N. SALAMIN. 2015. Measurement errors should always be incorporated in phylogenetic comparative analysis. *Methods in Ecology and Evolution* 6: 340–346.

SPERRY, J. S., U. G. HACKE, AND J. PITTERMANN. 2006. Size and function in conifer tracheids and angiosperm vessels. *American Journal of Botany* 93: 1490-1500.

STEPHENS, J. D., W. L. ROGERS, C. M. MASON, L. A. DONOVAN, AND R. L. MALMBERG. 2015. Species tree estimation of diploid Helianthus (Asteraceae) using target enrichment. *American Journal of Botany* 102: 910-920.

TYREE, M. T., AND F. W. EWERS. 1991. The hydraulic architecture of trees and other woody-plants. *New Phytologist* 119: 345-360.

VERSLUES, P. E., M. AGARWAL, S. KATIYAR-AGARWAL, J. H. ZHU, AND J. K. ZHU. 2006. Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. *Plant Journal* 45: 523-539.

WAGNER, K. R., F. W. EWERS, AND S. D. DAVIS. 1998. Tradeoffs between hydraulic efficiency and mechanical strength in the stems of four co-occurring species of chaparral shrubs. *Oecologia* 117: 53-62.

WILLSON, C. J., P. S. MANOS, AND R. B. JACKSON. 2008. Hydraulic traits are influenced by phylogenetic history in the drought-resistant, invasive genus Juniperus (Cupressaceae). *American Journal of Botany* 95: 299-314.

WRIGHT, I. J., D. D. ACKERLY, F. BONGERS, K. E. HARMS, G. IBARRA-MANRIQUEZ, M. MARTINEZ-RAMOS, S. J. MAZER, et al. 2007. Relationships among ecologically important dimensions of plant trait variation in seven Neotropical forests. *Annals of Botany* 99: 1003-1015.

WRIGHT, I. J., P. B. REICH, M. WESTOBY, D. D. ACKERLY, Z. BARUCH, F. BONGERS, J. CAVENDER-BARES, et al. 2004. The worldwide leaf economics spectrum. *Nature* 428: 821-827.

ZOMER, R. J., A. TRABUCCO, D. A. BOSSIO, AND L. V. VERCHOT. 2008. Climate change mitigation: a spatial analysis of global land suitability for clean development mechanism afforestation and reforestation. *Agriculture, Ecosystems, & Environment* 126: 67–80.

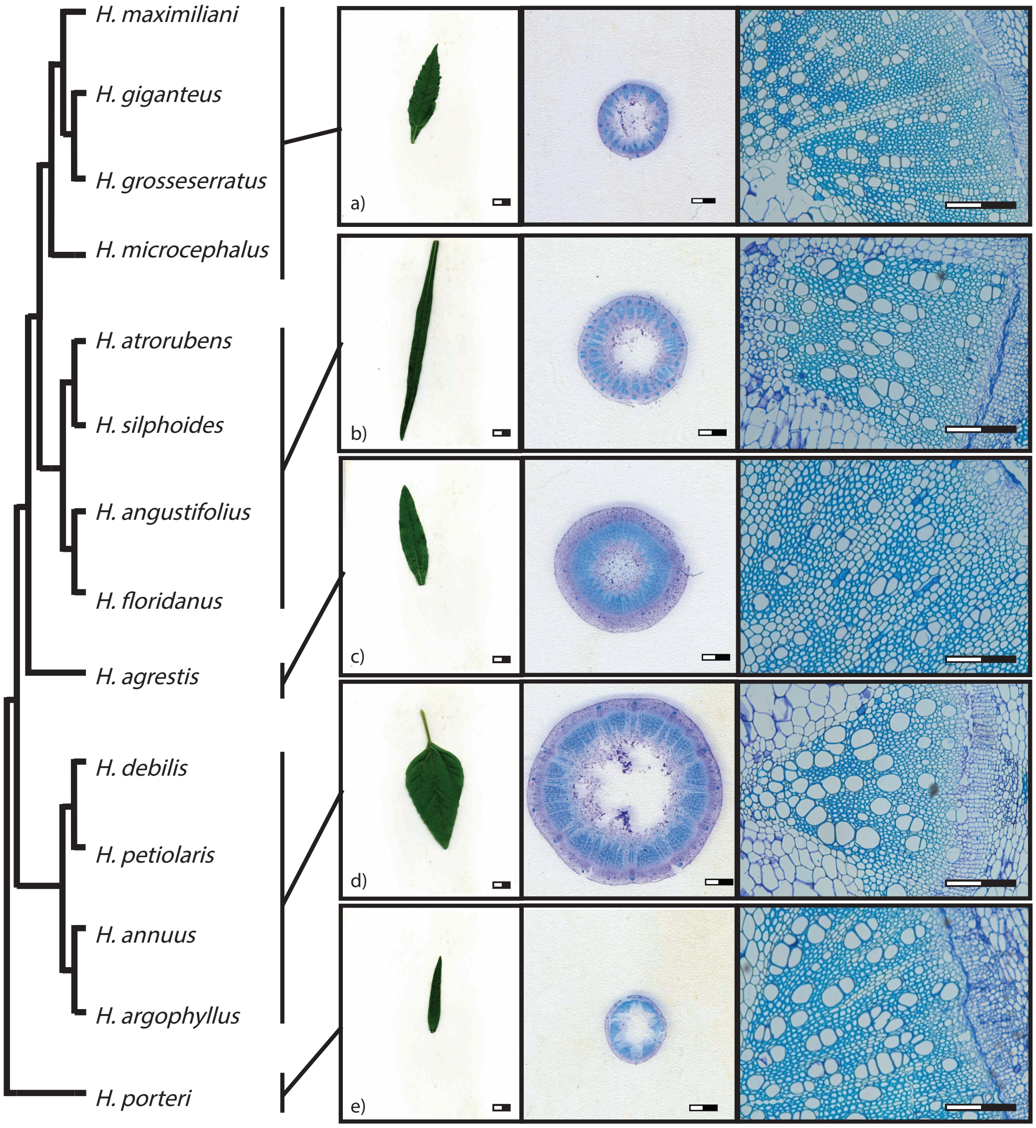


Figure 2.1. Cladogram representative of the *Helianthus* phylogeny, with images of leaves, stem cross-sections, and vascular bundles from each clade or individual species. Scale bars represent 2 cm, 2mm, and 0.2 mm in the first, second, and third columns, respectively. Images are: a) *H. giganteus,* b) *H. angustifolius,* c) *H. agrestis,* d) *H. annuus,* and e) *H. porteri,* consistent across rows. Species included in the clades designated as a) and b) are perennial. Species included in c), d), and e) are annual.

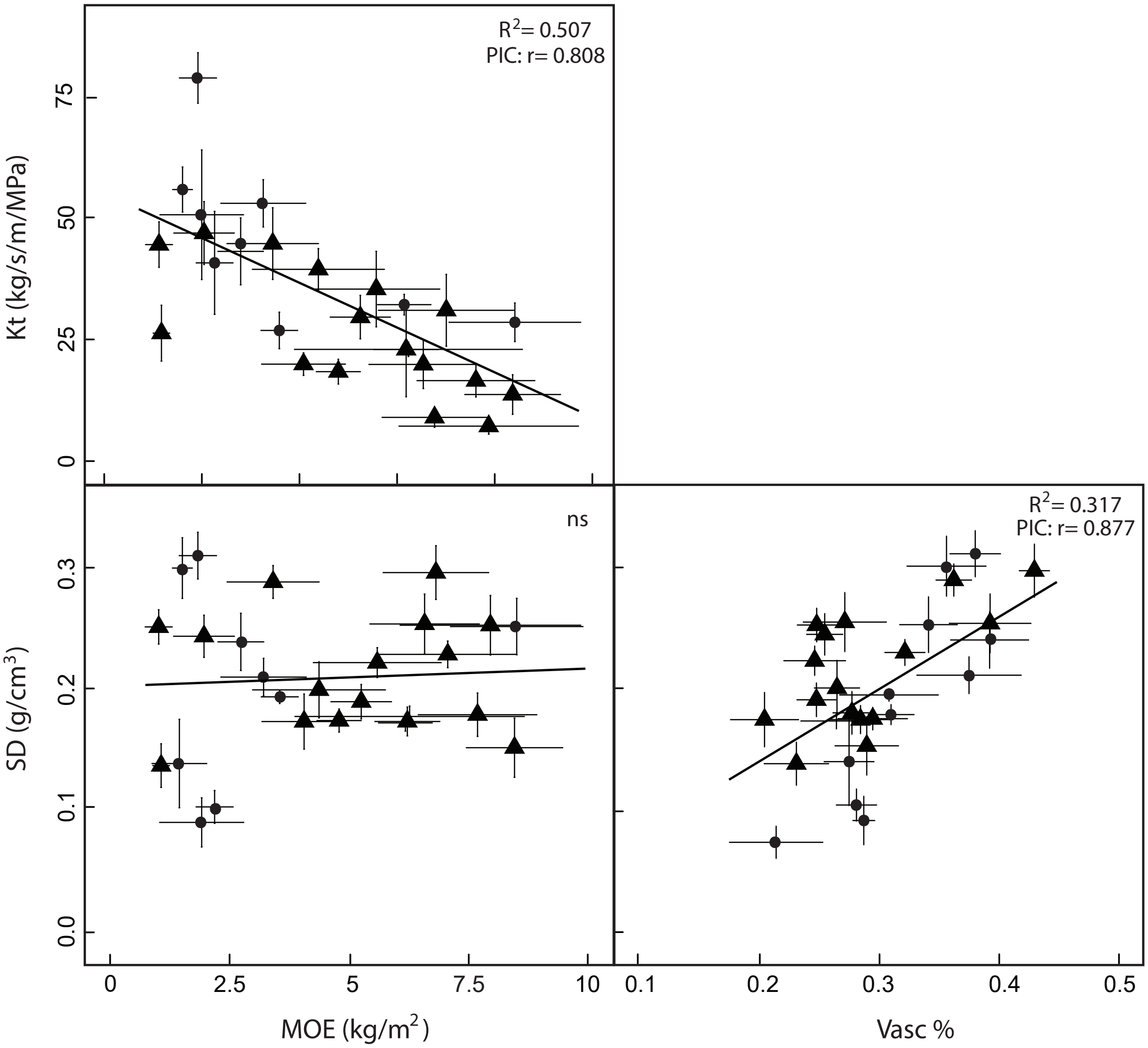


Figure 2.2. Comparison of traits describing stem biomechanical properties. Data points are ahistorical population means, with phylogenetically-corrected r values noted. Circles represent annual species, triangles represent perennial species. Kt, theoretical stem hydraulic conductivity; MOE, modulus of elasticity; SD, stem density; Vasc %, percentage of stem cross-sectional area composed of vascular tissue.

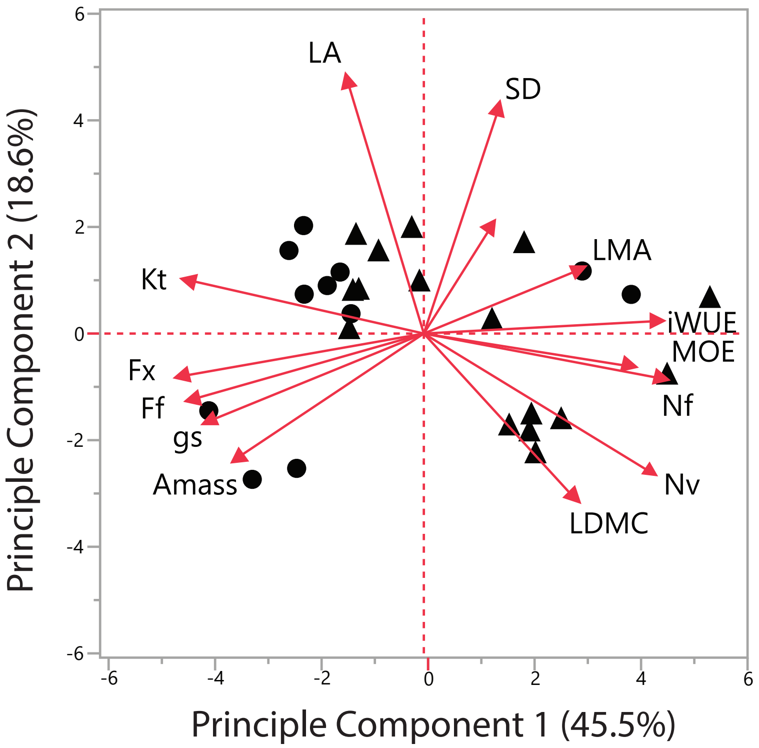


Figure 2.3. Principal components analysis of stem and leaf traits, run using population means for all traits. Circles represent annual species, triangles represent perennial species. Amass: photosynthetic rate per unit mass; Ff: fiber lumen fraction; Fx: xylem lumen fraction; gs: rate of stomatal conductance; iWUE: instantaneous water use efficiency; Kt: theoretical stem hydraulic conductance; LA: total leaf area; LDMC: leaf dry matter content; LMA: leaf mass per unit area; MOE: modulus of elasticity; Nf: fiber density; Nv: vessel density; SD: stem-specific density, Vasc %: percentage of stem cross-sectional area composed of vascular tissue.

Table 2.1. Trait definitions and descriptive statistics on populations for measured stem and leaf functional and anatomical traits.

Trait	Abbreviation	Units	Mean	Minimum	Maximum	Coefficient of Variation
Stem traits						
Vessel density	Nv	No.·mm^{-2}	213.27	65.96	732.22	0.771
Fiber density	Nf	No.·mm^{-2}	293.26	204.93	448.40	0.248
Xylem lumen fraction	Fx	%	0.455	0.351	0.553	0.117
Fiber lumen fraction	Ff	%	0.234	0.097	0.486	0.428
Theoretical hydraulic conductance	Kt	kg·s^{-1}·m^{-1}·MPa	36.799	7.053	92.417	0.584
Modulus of elasticity	MOE	kg·m^{-2}·10^{8}	5.305	1.098	12.177	0.637
Stem density	SD	g·cm^{-3}	0.202	0.073	0.310	0.312
Percentage stem cross-section vascular tissue	Vasc %	%	0.305	0.205	0.430	0.203
Leaf traits						
Total leaf area per plant at harvest	LA	cm^{2}	1234.35	255.94	2472.58	0.597
Leaf mass per unit area	LMA	g·m^{-2}	4.673	3.000	11.549	0.477
Photosynthetic rate per unit mass	Amass	nmol·g^{-1}·s^{-1}	8.156	1.845	12.344	0.322
Stomatal conductance	gs	mol·m^{-2}·s^{-1}	1.158	0.609	1.934	0.328
Instantaneous water use efficiency	iWUE	ratio of A/gs	31.509	20.876	46.205	0.222
Leaf dry matter content	LDMC	mg·g^{-1}	119.73	93.31	146.29	0.135

Table 2.2. Macroevolutionary correlations (r-values) among stem functional traits using population means in a phylogenetic mixed-model that accounts for intraspecific variation. Notes: All listed values $p<0.05$. SD: stem-specific density, MOE: modulus of elasticity, Nv: vessel density, Nf: fiber density, Fx: xylem lumen fraction, Ff: fiber lumen fraction, Kt: theoretical hydraulic conductivity, Vasc %: percentage of stem cross-sectional area composed of vascular tissue, LA: total leaf area per plant at harvest, LDMC: leaf dry matter content, LMA: leaf mass per unit area, Amass: photosynthetic rate per unit mass, gs: stomatal conductance, iWUE: instantaneous water use efficiency

	SD	MOE	Nv	Nf	Fx	Ff	Kt	Vasc %	LA	LDMC	LMA	Amass	gs
MOE	-												
Nv	-	0.545											
Nf	-	0.732	0.743										
Fx	-0.561	-0.659	-0.568	-									
Ff	-0.548	-0.858	-0.537	-0.720	0.804								
Kt	-	-0.808	-0.823	-	0.889	0.796							
Vasc %	0.861	-	-	-	-	-0.558	-						
LA	0.788	-	-0.659	-	-	-	-	-					
LDMC	-	-	0.776	0.580	-	-	-0.568	-	-0.627				
LMA	-	-	-	0.727	-	-0.567	-	0.599	-	-			
Amass	-	-0.553	-	-0.675	0.545	0.763	-	-	-	-	-0.903		
gs	-	-0.577	-	-0.745	0.590	0.666	-	-	-	-	-	0.929	
iWUE	-	-	0.595	0.723	-0.635	-	-0.534	0.604	-	0.570	0.803	-0.824	-0.921

Table 2.3. Loading values for stem and leaf traits in the principle component analysis displayed in Figure 2.3. Notes: SD: stem-specific density, MOE: modulus of elasticity, Nv: vessel density, Nf: fiber density, Fx: xylem lumen fraction, Ff: fiber lumen fraction, Kt: theoretical hydraulic conductivity, Vasc %: percentage of stem cross-sectional area composed of vascular tissue, LA: total leaf area per plant at harvest, LDMC: leaf dry matter content, LMA: leaf mass per unit area, Amass: photosynthetic rate per unit mass, gs: stomatal conductance, iWUE: instantaneous water use efficiency.

	PC1	PC2
SD	0.263	0.793
MOE	0.724	-0.115
Nv	0.793	-0.485
Nf	0.837	-0.158
Fx	-0.850	-0.150
Ff	-0.814	-0.231
Kt	-0.826	0.187
Vasc %	0.241	0.382
LA	-0.267	0.884
LDMC	0.526	-0.574
LMA	0.542	0.222
Amass	-0.659	-0.439
gs	-0.754	-0.307
iWUE	0.818	0.044

Table 2.4. Macroevolutionary correlations (r-values) of stem and leaf functional traits with environmental characteristics of seed-source sites, using population means in a phylogenetic mixed-model that accounts for intraspecific variation. Notes: All listed values $p<0.05$. MAP: mean annual precipitation; GAI: global aridity index (calculated as MAP/PET, with higher values indicate more mesic conditions); PET: potential evapotranspiration; MAT: mean annual temperature. SD: stem-specific density, MOE: modulus of elasticity, Nv: vessel density, Nf: fiber density, Fx: xylem lumen fraction, Ff: fiber lumen fraction, Kt: theoretical hydraulic conductivity, Vasc %: percentage of stem cross-sectional area composed of vascular tissue, LA: total leaf area, LDMC: leaf dry matter content, LMA: leaf mass per unit area, Amass: photosynthetic rate per unit mass, gs: rate of stomatal conductance, iWUE: instantaneous water use efficiency, PC1: first principle component outlined in Table 2.3.

	MAP	GAI	PET	MAT
SD	-	-	-	0.548
MOE	-	0.487	-	-
Nv	-	-	-0.754	-0.819
Nf	-0.627	-	-	-0.508
Fx	-	-0.509	-	-
Ff	-	-	-	-
Kt	-	-	-	-
Vasc %	-	-	-	-
LA	0.711	-	0.796	0.896
LDMC	-	-	-0.800	-
LMA	-	-	-	-
Amass	-	-	-	-
gs	-	-0.503	-	-
iWUE	-	-	-0.583	-0.649
PC1	-	-	-0.419	-0.547

CHAPTER 3

DOMESTICATION OF SUNFLOWER INVOLVES SHIFTS OF LEAF AND STEM FUNCTIONAL TRAITS INCONSISTENT WITH PATTERNS OBSERVED ACROSS WILD TAXA[1]

---

[1] Pilote, A.J. and Donovan, L.A. To be submitted to *International Journal of Plant Sciences.*

ABSTRACT

It is hypothesized that natural selection in high resource environments should favor plant functional traits associated with fast resource acquisition, transport, and use. Thus, improvement of crop species in agricultural settings would be expected to entail coordinated shifts in trait values towards more resource-acquisitive trait combinations. However, crop domestication emphasizes overall yield and involves artificial selection on specific plant tissues, organs, or traits; whereas natural selection operates on whole plant fitness, which may drive evolution of entire suites of plant functional traits. Thus, shifts of suites of functional traits in response to artificial selection may not be consistent with patterns observed across wild taxa associated with habitats differing in resource availability. In an effort to assess whether leaf and stem functional traits shifted in a coordinated fashion during the process of domestication, wild populations of common sunflower (*Helianthus annuus*) were compared to ancient landraces and improved cultivars. Leaf and stem traits related to tissue density, gas exchange, hydraulic efficiency, and vascular anatomy, were measured and compared between wild and domesticated (including ancient landraces and improved cultivars) populations. In general, the domesticated sunflowers in this study exhibited reduced investment in carbon-costly tissues, with anatomical shifts towards reduced stem density and vascular tissue composition of stem tissue. However, leaf traits, including photosynthetic rate, stomatal conductance, and water use efficiency, and stem hydraulic conductivity were found to shift inconsistently across the wild to domesticated comparison. Additionally, the two domesticated groups, landraces and improved cultivars, did not shift in a similar direction for all traits. Ancient landraces exhibited lower water use efficiency and faster water transport than improved cultivars, potentially due to conditions in which these plants are grown. This study provides evidence that stem and leaf functional traits, which have been observed to co-vary across wild taxa, do not co-vary during the domestication process of crop sunflower from its wild progenitor.

Key words: Crop domestication, functional trait, plant ecological strategy, stem hydraulic conductivity, leaf economics, xylem anatomy

INTRODUCTION

Crop domestication is a process by which humans impose artificial selective pressures to produce greater yield of specific plant products (Dempewolf et al. 2008). The selective process and resulting plant form varies among taxa, but often involves selection towards greater productivity in unnaturally high-resource settings (Evans 1993, Milla et al. 2014). Research into plant resource strategy among wild taxa has provided evidence that resource-rich environments favor selection of resource-acquisitive trait combinations, which include rapid resource acquisition, transport and use across plant tissues (Chapin et al. 1993, Grime 2006). Thus, crop species may be expected to exhibit functional traits that facilitate rapid acquisition, transport, and use of resources, such as increased leaf metabolism and stem water transport and reduced investment in carbon-costly tissues, in comparison with their wild progenitors. However, as artificial selective pressures act on individual plant traits, tissues and/or organs, we may observe patterns of trait co-variation that differ from those observed across wild taxa. Here, we compare two forms of domesticated sunflower (*Helianthus annuus*) to determine whether leaf and stem functional traits shift consistently towards more resource-acquisitive trait values, as compared to wild populations.

Differentiation across plant taxa has been observed among functional traits linked to the acquisition, transport, and use of resources that include plant nutrients and water (Chapin et al. 1993, Grime et al. 1997). These traits are hypothesized to co-vary from “fast”, resource-acquisitive trait combinations, including high rates of leaf gas exchange, stem water transport, and root nitrogen, to “slow”, resource-conservative trait combinations with opposing trait values (Reich 2014). Within this framework, fast growth and acquisition of resources comes at the cost of decreased carbon investment and stress resistance of plant tissue (Grime 2006). Resource-rich habitats are generally expected to favor selection of “fast” resource-acquisitive trait combinations that include rapid photosynthetic metabolism and nutrient acquisition, enabling high growth rate and productivity in wild taxa (Chapin et al. 1993, Grime 2006). Thus, it is hypothesized that domesticated crops should be characterized by traits indicative of resource-acquisitive trait combinations in their artificially resource-rich habitats. However, crop domestication involves artificial selection towards greater yield of specific plant products, rather than selection based on fitness, which may or may not entail involve co-variation of entire suites of functional traits, such as those hypothesized to comprise resource strategies (Martin et al. 2015). Thus, crop domestication may involve shifts in functional trait values, from wild progenitor to domesticated crop, that do not occur in a similar pattern to those identified across wild taxa (Milla et al. 2014).

Leaf traits, in particular, have been observed to co-vary in a strikingly consistent fashion across wild taxa, ranging from resource-acquisitive trait combinations, including high rates of photosynthesis, low leaf mass per unit area, and low leaf lifespan, to resource-conservative combinations with opposing trait values (Wright et al. 2004). Additionally, in samplings of wild taxa, strong positive correlations have been observed between resource-acquisitive leaf traits and “fast” stem traits, such as low stem density, high water transport capacity, and low investment in carbon-costly vascular tissue (Mendez-Alonzo et al. 2012, de la Riva et al. 2015). Recent assessments of the traits that define the leaf economics spectrum and of additional leaf and stem functional traits in *Helianthus* have found these functional traits to have evolved in a correlated fashion, ranging from resource-acquisitive to resource-conservative trait combinations across the wild species of this genus (Mason and Donovan 2015, Pilote and Donovan 2016).

Co-variation of functional traits, which forms fast to slow resource strategies, is hypothesized to be driven by some combination of selective pressures and biophysical and/or genetic constraints (Wright et al. 2004, Donovan et al. 2011, Reich 2014). It has been hypothesized that the high resource environments in which crop species are grown may result in reduced or "relaxed" selective pressures, which may result in trait combinations inconsistent with those observed across wild taxa (Milla et al. 2014). Initial comparisons of leaf and root traits between crop species and their wild progenitors suggests that leaf and root traits may not see consistent shifts towards more-resource acquisitive trait values, observing little change in rates of leaf and root resource uptake from wild populations to domesticated plants (Evans 1993, Milla and Matesanz 2017). Additionally, these studies report a lack of coordinated shifts among leaf traits that had been previously found to be tightly interrelated in studies of wild taxa. Little research has yet been focused on stem functional traits of crop species, though a survey of crop manioc did observe a reduction in stem biomechanical properties, including resistance to bending stress, in comparison with its wild progenitor (Menard et al. 2013).

Helianthus annuus is a model study organism for investigating patterns of trait variation as wild *H. annuus* is found across the continental United States and has adapted to a wide range of environments. Native Americans domesticated sunflowers, generating numerous ancient landraces for both consumption and dye production (Whiting 1939, Heiser 1951). Further selection of sunflower in fertilized agricultural fields for high yield of seed for consumption and oil production produced modern improved cultivars that are now grown across the North American and European continents. Ancient landraces have been grown in Native American gardens for centuries, preserving a primitive domesticated product that is genetically distinct from modern cultivars though phenotypically quite similar (Wills and Burke 2007). According to historical records, Native American growth of sunflower occurred in small plots and consisted of small irrigated gardens in the desert southwest (Whiting 1939, Heiser 1951) while improved cultivars are grown in modern agricultural settings with only 6% of sunflower cropfields irrigated globally (Portmann, 2010). Domestication syndromes characterize the phenotype of crop plants, with varying syndromes for plants selected for greater production of seed oil, tubers, leaf area, or other plant products (Dempewolf et al. 2008). The sunflower domestication syndrome involves selection towards reduced branching and time to flowering and increased seed set and floral display. Thus, we may use ancient landraces and improved cultivars to compare functional trait evolution through two forms of crop domestication that have resulted in similar phenotypes, i.e. domestication syndromes, but may have experienced unique selective pressures during the domestication process.

Here, we make use of this *Helianthus annuus* system of wild populations, ancient landraces, and improved cultivars to assess the evolution of stem and leaf functional traits through the process of crop domestication. We specifically ask: 1) Have leaf and stem functional traits evolved in a coordinated fashion towards more resource-acquisitive trait combinations through divergence from wild populations to domesticated products including ancient landraces and improved cultivars? 2) Have leaf and stem traits of ancient landraces and improved cultivars shifted to a similar extent?

METHODS

***Experimental design*–** This high resource (well-watered, fertilized) common garden study was conducted at the University of Georgia BioSciences greenhouse facility, using a randomized complete block design with eight individuals from each of four wild populations, three landraces, and four cultivars of *Helianthus annuus* (11 plant groups x 8 replicates, totaling 88 plants). Wild populations were chosen to represent a longitudinal gradient across the United States: FRE (California; 36°36’14”N, 120°3’46”W), UTA (Utah; 39°42’58”N, 112°12’25”W), KON (Kansas; 39°6’8”N, 96°36’37”W), and GRN (Mississippi; 33°21’23”N, 91°0’54”W). Ancient landraces were chosen from those publically available: Havasupai (Arizona; Coconino county), Hopi (Arizona; Navajo county), and Pueblo (New Mexico; McKinley county). Four improved cultivars were selected, representing each of the four quadrants of a principal component analysis of genetic diversity across 433 lines, which was based on 37 single-sequence repeats(Mandel et al. 2011). In an effort to account for differing selective pressures among *H.annuus* cultivars, we chose two lines bred for seed-oil production and two lines bred for seed consumption. For each population, landrace, and line, achenes (hereafter “seeds”) were either wild collected or from accessions established with the USDA National Genetic Resources Program (www.ars-grin.gov/npgs; outlined in Dataset B1). Beginning on 31 May 2016, seeds were scarified and germinated on wet filter paper in petri dishes and transferred to seedling trays until the emergence of the first true leaf pair. Seedlings were then planted individually into 20.3 cm diameter x 20 cm depth pots (2.92 L) filled with a 3:1 sand:calcined clay substrate. To ensure high nutrient conditions, each pot received 20g of Osmocote Plus 15-9-12 (7% NH_4, 8% NO_3) slow-release nine-month fertilizer with micronutrients (Scotts, Marysville, OH) at planting. To ensure well-watered conditions, each pot received drip irrigation to field capacity multiple times daily for the entirety of the study.

Leaf trait measurement– Leaf functional traits were assessed at the 3-5 true leaf pair stage of growth on 1 July 2016. The most recently fully expanded leaf was measured for photosynthetic rate (Aarea) and stomatal conductance (gs) at 400 ppm CO_2 and 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity, using a LiCor 6400 Portable Photosynthesis System (Li-Cor Biosciences, Lincoln, NE). The measured leaf was removed before dawn on the following morning, measured for fresh weight, and scanned to obtain a digital leaf images. Leaves were then dried at 60° C and weighed again for calculation of leaf dry matter content (LDMC, g·g). Leaf images were processed with Image J software (Rasband 1997-2012) to obtain leaf area and allow calculation of leaf mass per unit area (LMA, g·cm^{-2}). Instantaneous water use efficiency (iWUE) was calculated as the rate of photosynthetic carbon gain relative to the rate of stomatal conductance (Aarea/gs). Total leaf area (LA) was measured during harvest by removing all leaves from an individual and measuring area using an LI-3100 leaf area meter (Li-Cor Biosciences, Lincoln, NE).

Stem trait measurement– Plants were harvested following the production of an individual's first bud in order to control for ontogenetic stage in stem trait measurement (7–28 July 2016). At harvest, the first order stem was stripped of all leaves and/or lateral branches and a 15 cm segment was cut under water from roughly 1 cm distal to the first true-leaf pair or branch. Stem segments remained under water during transport to the Donovan lab for measurement of hydraulic conductivity. In the lab, stem segments were placed under vacuum in filtered (0.2 μm), distilled water for 30 minutes to dissolve native xylem embolism. Then, stem segments were trimmed under water to roughly 13 cm and fitted to a 'Sperry tubing apparatus' to measure maximum hydraulic conductivity (Sperry et al. 1988). Hydraulic conductivity, defined as mass flow rate of solution through a segment divided by the pressure gradient along the segment, was measured under gravity-induced pressure head with deionized, filtered (0.2 μm), and acidified (20 mmol KCl) solution using an electric balance to calculate rate of flow. Stem-specific hydraulic conductivity (Ks; kg·m^{-1}·MPa^{-1}·s^{-1}) was calculated as hydraulic conductivity divided by the cross-sectional area of xylem tissue within the segment. Fresh volume of the stem segment was then measured via the water displacement method for calculation of stem density (SD, $g \cdot cm^{-3}$), as dry mass per unit of fresh volume, after all tissue was dried in drying ovens for at least 72 hours at 60°C.

Modulus of elasticity (MOE, MPa) was measured on the stem segment prior to drying via the method outlined in Cooley et al. (2003). The proximal end of the segment was fixed into a bored rubber stopper and secured to a table edge. Mass was incrementally added to the distal end while the stem segment's angle of deflection was noted. MOE is a measure of a material's resistance to bending per unit area, and was calculated using a standard equation, modified by Chazdon (1986) to account for tapering of a linear segment: $MOE = (M \cdot L^3 [t_0/t_l])/(3 \cdot \delta \cdot I_0)$, where M is the total added mass at a given vertical deflection (δ), L is the distance between the fixed end of the segment and the point of mass addition, t_0 and t_l are the segment thicknesses at the fixed end and point of addition, respectively, and I_0 is the second moment of area of a cross-section at the fixed end of the stem segment. I_0 was calculated using an equation modified for elliptical cross-sections: $I_0 = (\pi/4) \cdot a^3 \cdot b$, where a is the radius of the transverse axis and b is the radius of the longitudinal axis (Niklas 1992).

Immediately following hydraulic conductivity measurements, a ~2.5 mm cross-section was cut from stem tissue most proximal (i.e. the stem tissue that would provide water flow to the stem segment measured for said flow). Sections were fixed in formalin and sent for processing at the University of Georgia Veterinary Histology Laboratory, where each sample was embedded in paraffin, sliced with a sledge-microtome, mounted to a slide, and stained with Toluidine blue. Slides were then imaged with a camera-mounted Zeiss light microscope using ZEN software (Carl Zeiss Microscopy, Oberkochen, Germany). Three xylem bundles and three fiber bundles per cross-section were imaged at 100x.

Stem cross-section anatomical analyses were carried out using Image J software (Rasband 1997-2012). The selection tool was used to isolate xylem or fiber cells from ground tissue within each image, and then lumen diameter (calculated as the diameter of a circle of equal area to the measured conduit) and lumen area of all individual conduits in each cross-sectional image were measured. Vessel density and fiber density (N_X and N_F; $N \cdot mm^{-2}$) were calculated as number of conduits per unit area. Xylem lumen fraction and fiber lumen fraction (F_X and F_F) were determined as the ratio of total lumen area to total conduit area (%). Percent of vascular tissue (%) was calculated from total stem cross-sectional area using the selection and measurement tools in Image J. Vessel diameters and the double wall thickness between adjacent vessels were measured using the selection tool in ImageJ for at least 100 vessels per cross-section. The hydraulically-weighted vessel diameter (Dh) was calculated as $Dh = (\Sigma D^5)/(\Sigma D^4)$, based on all sampled vessels of a given stem cross-section. Vessel implosion resistance [$(t/b)_h^2$;Hacke et al. (2001)] was calculated for the vessels of a cross-section whose diameters fell within ±5 µm of the calculated Dh, with t as the double-wall thickness of adjoining vessels and b as the lumen diameter of a given vessel.

Statistical Analysis–

Comparisons of wild populations, ancient landraces, and improved cultivars were conducted using a two-way ANOVA, using a nested design that accounted for variation within each wild population, ancient landrace, and improved cultivar.

Tukey post-hoc analyses were used to determine the relative rank of each group. Principal components analyses (PCA) were run with data input from 14 focal stem and leaf traits, representing a hypothesized resource-use and transport axis of co-variation defined by previous work with the *Helianthus* genus (Pilote and Donovan 2016). PCA were run using the LSMeans of each trait for each wild population, ancient landrace, and improved cultivar (i.e. one value representing each wild population, landrace, and cultivar for a total of 11 values for a given trait). Additionally PCA analyses were based on the correlation matrix to minimize the effect of differences in scaling among the measured leaf and stem traits. Principal component analyses and ANOVA were conducted in JMP Pro 10 software (JMP, Version 10, SAS Institute Inc., Cary, NC, 1989-2007).

RESULTS

In comparison with wild populations, domesticated populations (ancient landraces and improved cultivars) exhibited significant (ANOVA; $p<0.05$) reductions in stem traits involved in carbon investment, including stem density (SD) and the percentage of stem cross-sectional area composed of vascular tissue (Vasc %) (Figure 3.1; all traits described in Table B1). Ancient landraces additionally displayed significantly higher xylem lumen fraction (Fx) than wild populations, and improved cultivars were found to exhibit significantly higher vessel density (Nv). These patterns are found to be consistent with reduced investment in carbon-costly tissue within these domesticated populations, which may be indicative of more resource-acquisitive trait values.

Leaf traits were, however, not found to be consistent with faster, more resource-acquisitive trait values in comparison of wild and domesticated populations. Ancient landraces exhibited faster leaf trait values than wild populations, including higher stomatal conductance (gs) and lower instantaneous water use efficiency (iWUE), than wild populations, while also exhibiting slower values, including reduced photosynthetic rate on a mass basis (Amass) and higher leaf dry matter content (LDMC) (Figure 3.2). Improved cultivars were found to exhibit reduced leaf mass per unit area (LMA), indicative of a faster strategy, but were also characterized by reduced stomatal conductance, indicative of a slower strategy.

When stem and leaf traits were condensed to principal components of variation, differentiation between wild populations and domesticated populations (ancient landraces and improved cultivars) occurred primarily along the second principal component (PC2, Figure 3.4, Table 3.1). This axis primarily encompasses co-variation of tissue densities, with landraces and improved cultivars characterized by anatomical traits indicative of lower carbon investment in tissue (reduced SD, Vasc %, LMA, and resistance to stem bending stress [MOE]), whereas the wild populations occurred across a wide range of range of values along PC2. Traits describing stem water transport (Ks) and leaf physiology, including Amass, gs, and iWUE, were found to contribute more substantially to the first principal component than the second. Thus, neither principal component represents a single expected “fast to slow” resource transport and use axis of co-variation for these populations.

Stem and leaf traits can additionally be compared between the two regimes of domestication: ancient landraces and improved cultivars. These domesticated populations diverged from their wild progenitor primarily along principal component 2, which incorporates variation among stem and leaf tissue carbon-investment (SD, Vasc %, MOE, LMA). However, ancient landraces were found to display significantly reduced iWUE and KS, and increased gs in comparison with improved cultivars (Figure 3.2).

DISCUSSION

The study investigated the effects of artificial selection on a suite of leaf and stem functional traits during the domestication process of crop sunflower (*Helianthus annuus*). We specifically discuss here evidence that leaf and stem traits have not shifted along a fast-slow resource transport and use axis that has been observed across wild *Helianthus* species and broader samplings of wild taxa (Mendez-Alonzo et al. 2012, de la Riva et al. 2015, Pilote and Donovan 2016). Additionally, we discuss findings that domestication of ancient landraces resulted in shifts of traits governing water transport and use that differ in direction and degree when compared to the domestication of improved cultivars.

In comparison with populations of the wild progenitor of cultivated *H. annuus*, domesticated populations (ancient landraces and improved cultivars) exhibited decreased stem tissue density (SD), decreased vascular tissue composition within stem cross-sectional area (Vasc %), and a trend of decreased resistance to stem bending stress (MOE) (Figure 3.1). These represent a shift towards decreased investment in carbon-costly stem tissue for domesticated lines, consistent with an expectation that domestication would be associated with selection for "cheap" tissues that may allow for more rapid growth and expansion at the cost of resistance to external stress. Research in manioc found similar results, with reduced modulus of elasticity and more brittle stem tissue in domesticated lines (Menard et al. 2013). Additionally, domesticated plants in our study exhibited a reduced Vasc %, while not experiencing shifts towards reduced stem-specific hydraulic conductivity (Figure 3.2). This consistency in hydraulic conductivity may partially be explained by an increase in the density of vessels (Nv) that do not significantly differ in their hydraulically weighted vessel diameter (Dh) among domesticated populations. Among domesticated populations in this study, we find decreased investment in carbon-costly stem tissue and reduced resistance to bending stress to be consistent with a "fast", resource transport strategy that is consistent with those patterns observed across wild taxa (Mendez-Alonzo et al. 2012, de la Riva et al. 2015).

In contrast, however, shifts in leaf functional traits from wild progenitor to domesticated populations did not consistently occur towards more resource-acquisitive values. Previous studies have hypothesized that fast, resource-acquisitive strategies observed across wild taxa include relatively high rates of photosynthesis (Amass) and stomatal conductance (gs), associated with reduced leaf-level water use efficiency (iWUE) (Mendez-Alonzo et al. 2012, de la Riva et al. 2015). However, in our study, ancient landraces display reduced Amass, despite increased gs and reduced iWUE, and improved cultivars display reduced gs and no significant difference from wild populations in Amass or gs (Figure 3.2). Thus, leaf traits of domesticated sunflower populations did not show the consistent shift towards resource-acquisition expected for crop species. Previous studies have similarly observed crop species to exhibit either reduced or similar photosynthetic rates in comparison with their wild progenitors (Evans 1993, Jackson and Koch 1997, Milla and Matesanz 2017).

When leaf and stem traits were condensed into principal components, domesticated populations were found to fall towards the negative end of principal component 2 (Figure 3.3). This axis represents reductions in leaf mass per unit area (LMA), stem tissue carbon-investment (SD and Vasc %), and resistance to bending stress (MOE), following expectations that domesticated populations would produce “cheaper” tissues that may allow for more rapid growth and/or resource transport. However, these shifts in tissue composition were not associated with alterations to leaf carbon capture, in the form of Amass, or water transport and use, in the form of Ks, gs, and iWUE. This represents inconsistent shifts along the expected resource strategy axis towards more resource-acquisitive trait combinations. Recent common garden analyses of the wild species of *Helianthus* provided evidence for correlated evolution of traits that comprise the leaf economics spectrum (Mason and Donovan 2015) and the leaf and stem functional traits assessed within this study (Pilote and Donovan 2016). This co-variation of traits along a fast-slow resource axis among wild taxa is hypothesized to be the product of some combination of selection and biophysical and/or genetic constraints (Wright et al. 2004, Donovan et al. 2011, Reich 2014). Our study found a lack of co-variation in the evolution of leaf and stem functional traits that have been found to evolve in a correlated fashion across wild *Helianthus*. This suggests that selective pressures may play a greater role in the co-variation of these traits across wild taxa than biophysical tradeoffs or genetic mechanisms, such as pleiotropy. It has been hypothesized that, among wild taxa, the consistent integration of phenotypes that define a resource strategy, is driven by selective pressures in the form of external stress (Milla et al. 2014). Thus, during the process of domestication, which involves growing plants in artificially resource-rich environments with reduced external stress, selective pressures are "relaxed" and trait values are allow to shift towards combinations that are not readily observed among wild taxa.

In addition to comparing wild sunflower populations to domesticated populations, this study was able to compare trait values that resulted from two domestication processes, i.e. those of ancient landraces grown by Native Americans and of improved cultivars grown in modern agricultural settings. Domestication of both ancient landraces and improved cultivars were observed to entail shifts among tissue carbon investment, primarily among stems (Figure 3.3). However traits involving leaf physiology (Amass, gs, iWUE) and stem water transport (Ks) were not found to shift similarly from wild populations to ancient landraces and from wild populations to improved cultivars. Ancient landraces were found to exhibit significantly higher rates of stem water transport and lower leaf-level water use efficiency than improved cultivars. These differences in directional selection of leaf physiology and stem water transport may be due to the selective pressures of the environments in which ancient landraces and improved cultivars are, and have historically been, grown. Native Americans grew ancient landraces for consumption and dye production; however, reports from the early 1900's suggest that this growth occurred in small irrigated gardens, rather than in row crop settings, in the desert southwest (Whiting 1939). Conversely, much of the land used for sunflower harvest is not irrigated, with only 6% of sunflowers harvested from irrigated land globally in 2000, which may result in continued selection for increased water use efficiency among improved cultivars in modern agricultural settings (Portmann et al. 2010).

While both forms of artificial selection result in a "sunflower domestication syndrome": i.e. reduced branching, increased seed set and floral display, and reduced time to flowering, the underlying functional traits did not shift in a coordinated fashion towards more resource-acquisitive trait values. Additionally, this study found inconsistent trait shifts among leaf and stem functional traits that have been observed to co-vary across wild taxa. These results suggest that selective pressures may play a greater role in the co-variation of these traits across wild taxa than biophysical mechanisms, such as mechanical tradeoffs, or genetic constraints, such as pleiotropy, that would necessitate their co-variation under the artificial selective pressures of crop domestication.

REFERENCES

Chapin, F. S., K. Autumn, and F. Pugnaire. 1993. Evolution of suites of traits in response to environmental stress. American Naturalist 142:S78-S92.

Chazdon, R. L. 1986. The costs of leaf support in understory palms - economy versus safety. American Naturalist 127:9-30.

Cooley, A. M., A. Reich, and P. Rundel. 2003. Leaf support biomechanics of neotropical understory herbs. American Journal of Botany 91:573–581.

de la Riva, E. G., A. Tosto, I. M. Perez-Ramoz, C. M. Navarro-Fernandez, M. Olmo, N. P. R. Anten, T. Maranon, and R. Villar. 2015. A plant economics spectrum in Mediterranean forests along environmental gradients: is there coordination among leaf, stem, and root traits. . Journal of Vegetation Science 27:187–199.

Dempewolf, H., L. H. Rieseberg, and Q. C. Cronk. 2008. Crop domestication in the Compositae: a family-wide trait assessment. Genetic Resources and Crop Evolution 55:1141–1157.

Donovan, L. A., H. Maherali, C. M. Caruso, H. Huber, and H. de Kroon. 2011. The evolution of the worldwide leaf economics spectrum. Trends in Ecology & Evolution 26:88-95.

Evans, L. T. 1993. Crop evolution, adaptation, and yield. Cambridge University Press, New York, New York.

Grime, J. P. 2006. Plant Strategies, Vegetation Processes, and Ecosystem Properties. Wiley.

Grime, J. P., K. Thompson, R. Hunt, J. G. Hodgson, J. H. C. Cornelissen, I. H. Rorison, G. A. F. Hendry, T. W. Ashenden, A. P. Askew, S. R. Band, R. E. Booth, C. C. Bossard, B. D. Campbell, J. E. L. Cooper, A. W. Davison, P. L. Gupta, W. Hall, D. W. Hand, M. A. Hannah, S. H. Hillier, D. J. Hodkinson, A. Jalili, Z. Liu, J. M. L. Mackey, N. Matthews, M.

A. Mowforth, A. M. Neal, R. J. Reader, K. Reiling, W. RossFraser, R. E. Spencer, F. Sutton, D. E. Tasker, P. C. Thorpe, and J. Whitehouse. 1997. Integrated screening validates primary axes of specialisation in plants. Oikos 79:259-281.

Hacke, U. G., J. S. Sperry, W. T. Pockman, S. D. Davis, and K. A. McCulloch. 2001. Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. Oecologia 126:457-461.

Heiser, C. B. J. 1951. The Sunflower among the North American Indians. Proceedings of the American Philosophical Society 95:432–448.

Jackson, L. E., and G. W. Koch. 1997. The Ecophysiology of crops and their wild relatives.*in* H. A. Mooney and L. E. Jackson, editors. Ecology in Agriculture. Academic Press.

Mandel, J. R., J. M. Dechaine, L. F. Marek, and J. M. Burke. 2011. Genetic diversity and population structure in cultivated sunflower and a comparison to its wild progenitor, *Helianthus annus* L. Theoretical Applied Genetics 123:693–704.

Martin, A. R., M. E. Isaac, and P. Manning. 2015. REVIEW: Plant functional traits in agroecosystems: a blueprint for research. Journal of Applied Ecology 52:1425-1435.

Mason, C. M., and L. A. Donovan. 2015. Evolution of the leaf economics spectrum in herbs: Evidence from environmental divergences in leaf physiology across Helianthus (Asteraceae). Evolution 69:2705-2720.

Menard, L., D. McKey, G. S. Muhlen, B. Clair, and N. P. Rowe. 2013. The evolutionary fate of phenotypic plasticity and functional traits under domestication in manioc: changes in stem biomechanics and the appearance of stem brittleness. PLoS One 8:e74727.

Mendez-Alonzo, R., H. Paz, R. C. Zuluaga, J. A. Rosell, and M. E. Olson. 2012. Coordinated evolution of leaf and stem economics in tropical dry forest trees. Ecology 93:2397-2406.

Milla, R., and S. Matesanz. 2017. Growing larger with domestication: a matter of physiology, morphology or allocation. Plant Biology 19.

Milla, R., J. Morente-Lopez, J. M. Alonso-Rodrigo, N. Martin-Robles, and F. S. Chapin, 3rd. 2014. Shifts and disruptions in resource-use trait syndromes during the evolution of herbaceous crops. Proc Biol Sci 281.

Niklas, K. J. 1992. Plant Biomechanics: an engineering approach to plant form and function. University of Chicago Press, Chicago, Illinois, USA.

Pilote, A. J., and L. A. Donovan. 2016. Evidence of correlated evolution and adaptive differentiation of stem and leaf functional traits in the herbaceous genus, Helianthus. Am J Bot 103:2096-2104.

Portmann, F. T., S. Siebert, and P. Doll. 2010. MIRCA2000–Global monthly irrigated and rainfed crop area around the year 2000: A new high-resolution data set for agricultural and hydrological modeling. Global Biogeochemical Cycles 24:GB1011.

Rasband, W. S. 1997-2012. ImageJ. U.S. National Institutes of Health, Bethesday, Maryland, USA.

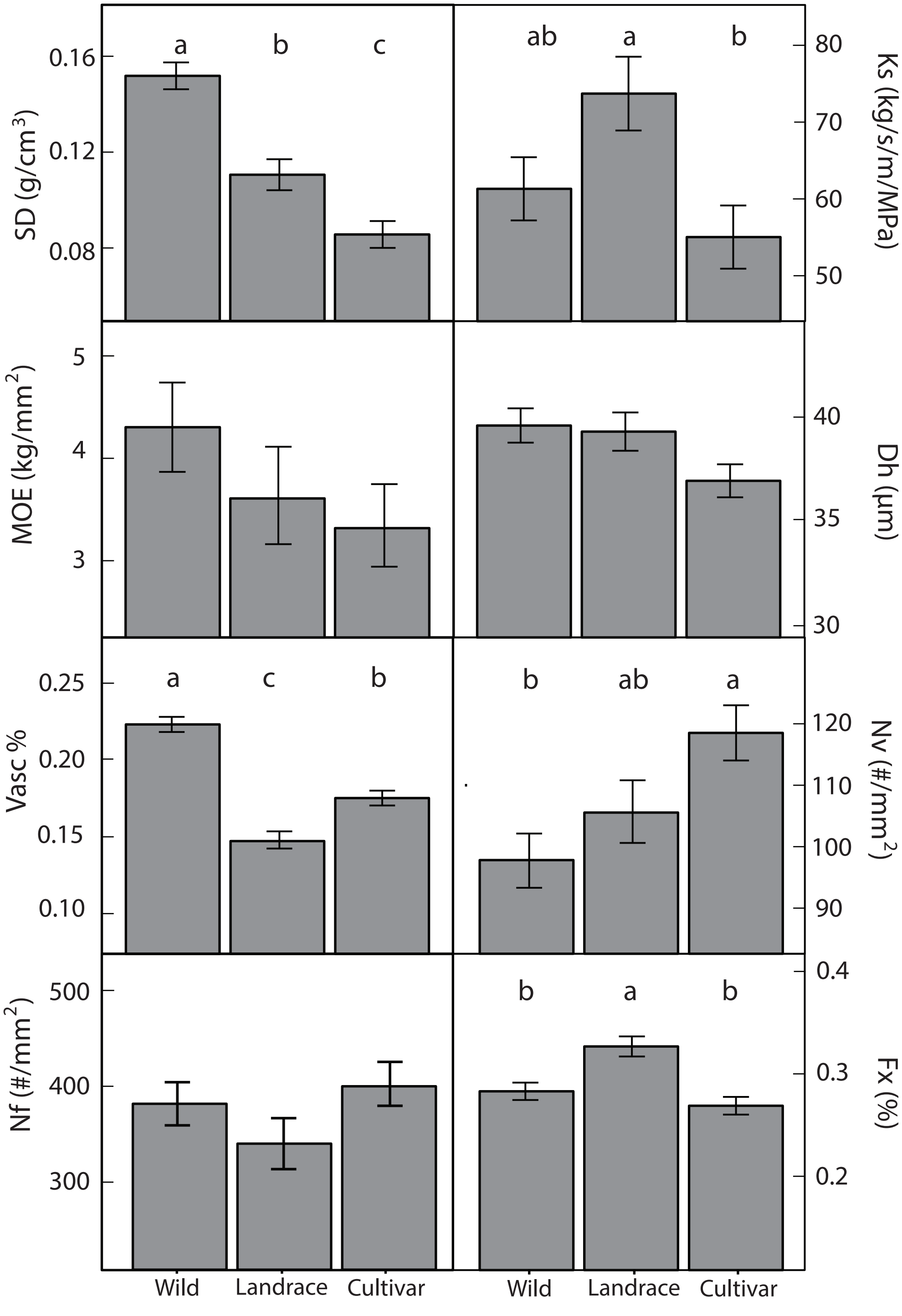
Reich, P. B. 2014. The world-wide 'fast-slow' plant economics spectrum: a traits manifesto. Journal of Ecology 102:275-301.

Sperry, J. S., J. R. Donnelly, and M. T. Tyree. 1988. A method for measuring hydraulic conductivity and embolism in xylem. Plant Cell Environ 11:35–40.

Whiting, A. F. 1939. Etnobotany of the Hopi. Museum of Northern Arizona Bulletin 15.

Wills, D. M., and J. M. Burke. 2007. Quantitative trait locus analysis of the early domestication of sunflower. Genetics 176:2589–2599.

Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. C. Cornelissen, M. Diemer, J. Flexas, E. Garnier, P. K. Groom, J. Gulias, K. Hikosaka, B. B. Lamont, T. Lee, W. Lee, C. Lusk, J. J. Midgley, M. L. Navas, U. Niinemets, J. Oleksyn, N. Osada, H. Poorter, P. Poot, L. Prior, V. I. Pyankov, C. Roumet, S. C. Thomas, M. G. Tjoelker, E. J. Veneklaas, and R. Villar. 2004. The worldwide leaf economics spectrum. Nature 428:821-827.



SD (g/cm^3)
0.16
0.12
0.08
a
b
c
Ks (kg/s/m/MPa)
80
70
60
50
ab
a
b
MOE (kg/mm^2)
5
4
3
Dh (µm)
40
35
30
Vasc %
0.25
0.20
0.15
0.10
a
c
b
Nv (#/mm^2)
120
110
100
90
b
ab
a
Nf (#/mm^2)
500
400
300
Fx (%)
0.4
0.3
0.2
b
a
b
Wild
Landrace
Cultivar
Wild
Landrace
Cultivar

Figure 3.1. Comparison of stem trait values for wild populations, ancient landraces, and improved cultivars. Bars represent LSMeans and error bars represent standard error. Bars that share a lowercase letter are not significantly ($p<0.05$) different from each other. SD, stem density; MOE, modulus of elasticity; Vasc %, percentage of stem cross-sectional area composed of vascular tissue; Nf, Fiber density; Ks, stem-specific hydraulic conductivity; Dh, hydraulically weighted mean vessel diameter; Nv, vessel density; Fx, xylem lumen fraction.

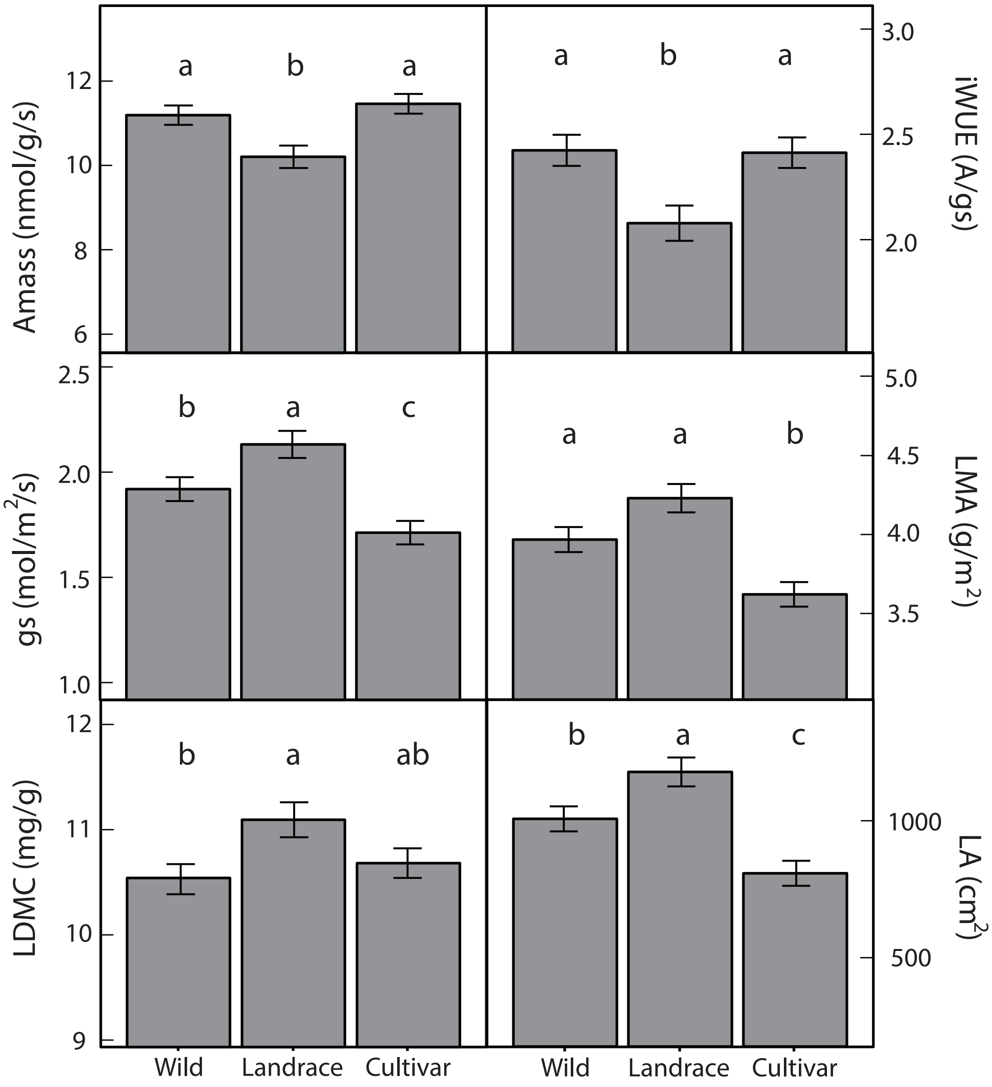


Figure 3.2. Comparison of leaf trait values of wild populations, ancient landraces, and improved cultivars. Bars represent LSMeans and error bars represent standard error. Bars that share a lowercase letter are not significantly ($p<0.05$) different from each other. Amass, photosynthetic rate on a mass basis; gs, stomatal conductance; iWUE, instantaneous water use efficiency; LMA, leaf mass per unit area; LDMC, leaf dry matter content; LA, leaf area

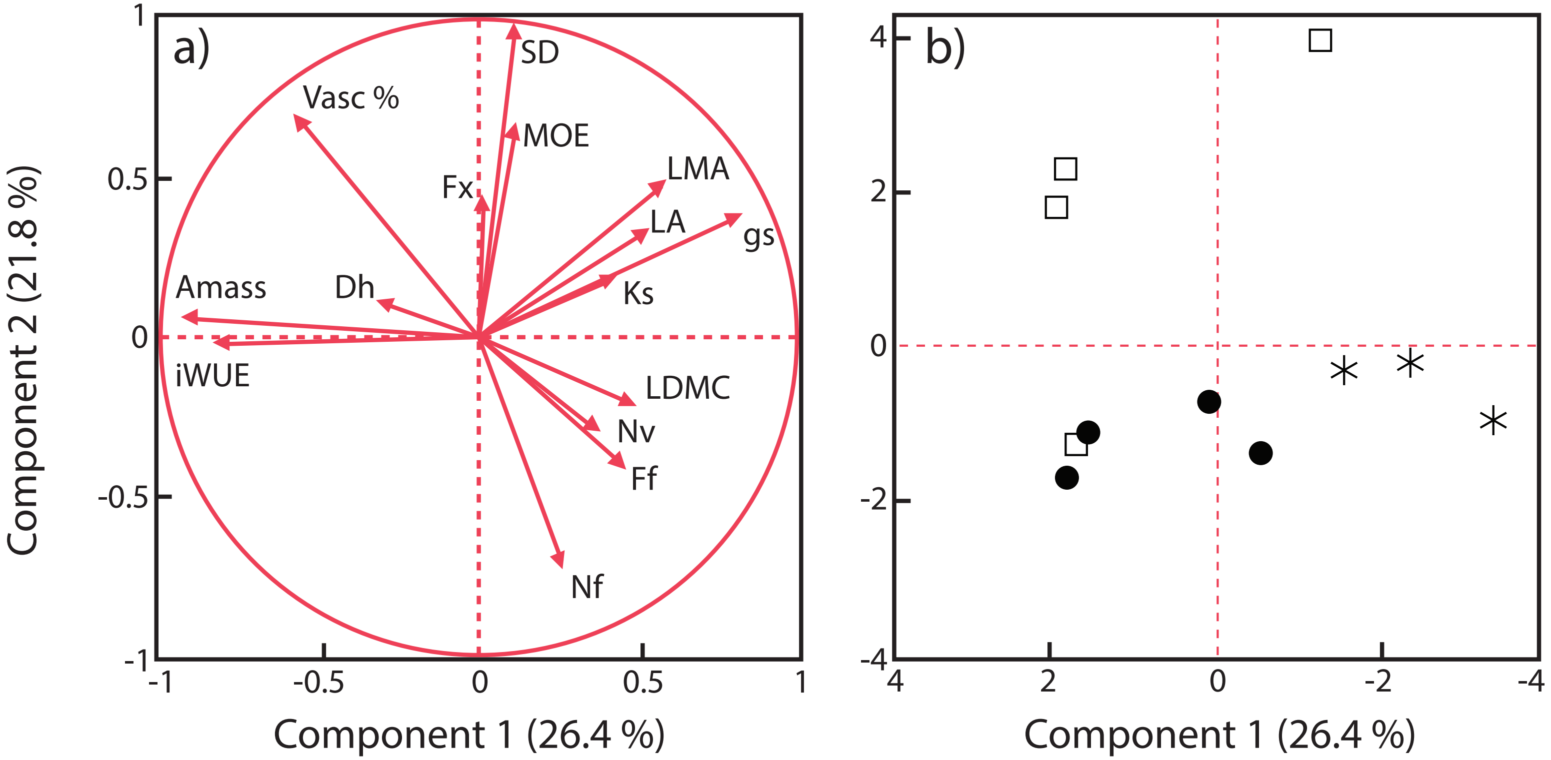

a)
SD
Vasc %
MOE
LMA
Fx
LA
gs
Ks
Amass
Dh
iWUE
LDMC
Nv
Ff
Nf
1
0.5
0
-0.5
-1
-1
-0.5
0
0.5
1
Component 2 (21.8 %)
Component 1 (26.4 %)
b)
4
2
0
-2
-4
4
2
0
-2
-4
Component 1 (26.4 %)

Figure 3.3. Principal component analysis of 13 focal stem and leaf traits, previously hypothesized as a “fast-slow” axis of trait co-variation among wild *Helianthus* species. a) Trait loadings for stem and leaf traits. b) Wild population (squares), ancient landrace (asterisks), and improved cultivar (circles) values along principal components 1 and 2. LSMeans from a nested ANOVA were used to calculate principal components. Amass, photosynthetic rate on an mass basis; gs, stomatal conductance; iWUE, instantaneous water use efficiency; LMA, leaf mass per unit area; LDMC, leaf dry matter content; LA, total leaf area; SD, stem density; MOE, modulus of elasticity; Ks, stem-specific conductivity; Nv, vessel density; Fx, xylem lumen fraction; Nf, fiber density; Ff, fiber lumen fraction; Dh, hydraulically weighted mean vessel diameter; Vasc%, percentage of stem cross-section composed of vascular tissue.

Table 3.1. Trait loadings for principle components analyses, depicted in Figure 3.3. Amass, photosynthetic rate on an mass basis; gs, stomatal conductance; iWUE, instantaneous water use efficiency; LMA, leaf mass per unit area; LDMC, leaf dry matter content; LA, total leaf area; SD, stem density; MOE, modulus of elasticity; Ks, stem-specific hydraulic conductivity; Nv, vessel density; Fx, xylem lumen fraction; Nf, fiber density; Ff, fiber lumen fraction; Dh, mean hydraulically-weighted vessel diameter; Vasc %, percent of stem cross-sectional area composed of vascular tissue.

	PC 1	PC 2
	(26.4%)	(21.8%)
Amass	-0.908	0.058
gs	0.805	0.373
iWUE	-0.810	-0.022
LMA	0.575	0.475
LDMC	0.473	-0.206
LA	0.523	0.329
SD	0.114	0.960
MOE	0.115	0.646
Ks	0.413	0.178
Fx	0.016	0.421
Nv	0.365	-0.289
Ff	0.447	-0.397
Nf	0.261	-0.704
Dh	-0.559	0.104
Vasc %	-0.295	0.678

CHAPTER 4

PLASTIC RESPONSES OF LEAF AND STEM FUNCTIONAL TRAITS OCCUR ALONG AN AXIS OF FAST-SLOW TRAIT CO-VARIATION IN RESPONSE TO WATER STRESS IN *HELIANTHUS*

---

[1] Pilote, A.J. and Donovan, L.A. To be submitted to *American Journal of Botany*.

ABSTRACT

Plant functional traits have been observed to co-vary across wild taxa and are hypothesized to form resource strategies that range from “fast, resource-acquisitive to resource-conservative trait combinations. Specifically, co-variation of leaf and stem functional traits, related to the investment of carbon in tissues and the transport and use of water, has been observed in field and common garden settings. It is hypothesized that the plastic responses of a suite of functional traits will co-vary in shifts towards more resource-conservative trait values when plants are subjected to environmental stress. For this study, we quantified the plastic responses of leaf and stem functional traits to water limitation, in order to assess whether plastic responses of this particular set of functional traits co-vary along a fast to slow resource axis. Six *Helianthus* species were grown under well-watered and water-limited treatments in a controlled irrigation, common garden experiment. Stem tissue responded to water limitation with shifts in xylem conduit parameters towards lower theoretical hydraulic conductivity and greater resistance to vessel implosion. Stem and leaf traits co-varied in their plastic response along a “fast to slow” resource strategy axis, with traits of water stressed plants characterized more resource-conservative values. Additionally, traits associated with tissue carbon-investment, including stem density, leaf mass per unit area, and leaf dry matter content, exhibited a greater degree of plasticity than traits associated with water transport and use, including leaf-level water use efficiency and theoretical hydraulic conductivity. This study suggests that the ecological response of this suite of leaf and stem functional traits may occur as shifts along a hypothesized axis of resource transport and use strategy that has been observed at an evolutionary scale across wild taxa.

Keywords: stem hydraulic anatomy, phenotypic plasticity, drought, plant functional traits

INTRODUCTION

Plant functional traits are hypothesized to co-vary to form a continuum of resource strategies from fast, resource-acquisitive trait combinations to slow, resource-conservative trait combinations (Reich 2014). Leaf functional traits have been found co-vary with striking consistency across plant taxa, ranging from fast combinations, consisting of low leaf mass per unit area, high photosynthetic rate, high leaf nitrogen, and low leaf lifespan, to slow combinations of opposing trait values (Wright et al. 2004). This pattern of resource-acquisitive and resource-conservative trait combinations has been hypothesized to extend beyond leaf tissue to that of stems and fine roots across traits functionally linked to resource acquisition, transport, and use, to form whole plant resource strategies (Reich 2014). Recent studies within *Helianthus* have found fast to slow trait co-variation among the traits of the leaf economics spectrum and of broader leaf and stem functional traits, which occurred through the evolutionary divergence of this genus (Mason and Donovan 2015, Pilote and Donovan 2016). It is hypothesized that ecological responses should follow a similar trajectory as this evolutionary pattern resulting in co-variation among the plastic responses of functional traits towards more resource-conservative trait values in response to environmental stress (Schlichting 1989, Chapin 1991). Here we assess the phenotypic plasticity of leaf and stem functional traits in response to water limitation to determine whether plastic responses occur in an integrated fashion towards more resource-conservative trait values.

Stem vascular anatomy plays a central role in maintaining plant water balance, as the dimensions of xylem conduits within the vascular system of plant stem tissue govern the rate at which water flows through the stem, and have been found to correlate with a plant’s vulnerability to drought-induced xylem embolism (Bryukhanova and Fonti 2012, Corcuera et al. 2012). On an evolutionary scale, greater vessel density, thinner vessel diameters, and greater vessel resistance to implosion (the ratio of a vessel's wall thickness to lumen diameter) have been observed to be associated with more arid regions among woody angiosperm taxa in field samplings (Chave et al. 2009, Barnard et al. 2011). On an ecological scale, these hydraulic anatomical properties exhibit large plastic responses to variation in water availability. Woody taxa have been observed to display reduced water flow, narrower vessels, and an increase in the ratio of xylem cell wall to lumen area (vessel implosion resistance) in response to reduced water supply (Bryukhanova and Fonti 2012, de Silva et al. 2012, Plavcova and Hacke 2012). The vascular anatomy of the herbaceous life form has been markedly less well studied thus far, though similar plastic responses, including reduced water flow and increased resistance to embolism have been observed in the response of common beans to variation in light availability (Matzner et al. 2014).

Stem traits, including hydraulic conductivity and stem-specific density, have been observed to co-vary with leaf traits, including leaf mass per unit area and rates of stomatal conductance and photosynthesis (Mendez-Alonzo et al. 2012, Gleason et al. 2013, de la Riva et al. 2015). Additionally, studies have observed co-variation among leaf and stem functional traits across ecological gradients of habitat water availability, with taxa found in habitats of reduced water availability exhibiting increased leaf water use efficiency, and decreased photosynthetic rate and stem water transport (Gleason et al. 2013, Aranda et al. 2015, Bai et al. 2015). This coordination predominantly follows a "fast-slow" axis of trait co-variation from resource-acquisitive to resource-conservative trait combinations that play a role in species' performance and role in a given environment, whether the resource in question be water or nutrient supply (Wright et al. 2004, Reich 2014). It is additionally hypothesized that a "fast-slow" axis of trait co-variation should be observed in the form of integrated plastic responses of plant functional traits to environmental stress (Schlichting 1989, Chapin 1991). Thus, we seek to assess here whether leaf and stem functional traits respond to external stress, specifically water limitation, with coordinated shifts among leaf and stem functional traits towards more resource-conservative trait values.

Here, we assessed the effects of water-limitation stress on leaf and stem functional traits of six congeneric herbaceous species to test expectations of coordination of response among traits of these organs and potential for differentiation in the degree of response among these traits. Specifically we sought to assess plastic responses to water limitation in the context of a hypothesized “fast-slow” resource use and acquisition spectrum, with an expectation that plastic responses will occur consistently towards more resource-conservative trait combinations.

MATERIALS AND METHODS

Study system– *Helianthus* provides an excellent study system of herbaceous species, with species located across North America, ranging from wetlands and roadsides to rock outcrops and desert sand dunes. *Helianthus* includes both annuals and deciduous perennial species that die back to a root ball over winter (i.e. aboveground tissue does not persist through winter months). The herbaceous habit and short lifespan of this genus allows for assessment of individuals over a significant portion of their aboveground lifespans and increased control of environmental conditions in large common garden studies. For this study, six species were chosen from across the *Helianthus* phylogeny (Stephens et al. 2015), representing species from habitats ranging substantially in source-site water availability (described in Supplemental Figure C1). Habitat water availability was determined from source-site annual precipitation, growth season rainfall (that which occurs between May and October) (Hijmans et al. 2005), and global aridity index

(Zomer et al. 2008). These six species were all directly collected from populations in the field: *H. annuus* (Utah; 39°42’57.6”N, 112°12’25.2”W), *H. argophyllus* (Florida; 29°15’14.0”N, 81°1’14.0”W), *H. debilis* (Florida; 29°48’21.6”N, 85°18’7.6”W), *H. petiolaris* (Illinois; 41°3’13.7”N, 90°56’5.6”W), *H. grosseserratus* (Iowa; 42°0’31.0”N, 96°1’41.0”W), *H. microcephalus* (South Carolina; 34°15’44.7”N, 82°39’45.9”W). All are erect, branched herbaceous species: *H. annuus, H. argophyllus, H. debilis, H. petiolaris* are annuals, and *H. grosseserratus* and *H. microcephalus* are perennials (Heiser et al. 1969). For each population, achenes were either wild collected or from accessions established with the USDA National Genetic Resources Program (www.ars-grin.gov/npgs; outlined in Dataset C1).

***Growth conditions and watering treatments*–** On 13 May 2015, the blunt end of each achene was scarified and the remainder placed on moist filter paper for germination before transfer to seedling trays until the emergence of the first true leaf pair. At such time, seedlings were transferred to 20.3 cm diameter, 14 cm height, 2.92 L pots filled with a 3:1, sand : calcined clay substrate. Individuals were arranged in a randomized complete block design in the UGA Biological Science greenhouse facility (6 species, 2 treatments, 4 blocks, 2 replicates per block, 96 total sample size). To provide high nutrient availability, 20g of Osmocote Plus 15-9-12 (7% NH_4, 8% NO_3) slow-release nine-month fertilizer with micronutrients (Scotts, Marysville, OH) was mixed into the substrate while pots were being filled. All pots received drip irrigation to field capacity twice daily until all individuals reached the 3-5 true leaf pair stage.

At the 3-5 true leaf pair stage of growth (12 June 2015), irrigation treatments were initiated; previously designated well-watered individuals were watered to 35% soil moisture consistently through the experimental growth period and water-limited individuals were watered to 20% soil moisture (determined as the lowest soil moisture not resulting in continuous wilt of plant tissue). To control pot soil moisture, one soil-moisture probe (Decagon EC-5, Decagon Devices, Pullman, WA) was placed into the substrate of each pot and connected via multiplexer (AM416, Campbell Scientific, Logan, UT) to a datalogger (CR23X, Campbell Scientific, Logan, UT). Soil moisture was averaged over the eight individuals of each species-treatment group (i.e. soil moisture values for all *H. annuus* individuals in the well-watered treatment) and compared to the 20% or 35% soil moisture set-points. If the average fell below said set-point, a solenoid was triggered via relay driver (SDM-CD16/AC-16 Channel AC/DC Controller, Campbell Scientific, Logan, UT) to deliver drip irrigation to those specific eight pots. Thus, 6 species x 2 treatments resulted in 12 individual irrigation lines. This irrigation method allows for direct control of substrate moisture for the entirety of treatment application (Nemali and van Iersel 2006). Treatments were maintained for 24 days, before trait measurement and harvest of plant material. Height and stem diameter were measured three times each week in order to calculate relative growth rates (HRGR, $\mathrm{mm \cdot cm^{-1} \cdot day^{-1}}$; StemRGR, $\mathrm{\mu m \cdot mm^{-1} \cdot day^{-1}}$) for assessment of the effectiveness of the water limitation treatment on plant growth.

Leaf trait measurement– Plant traits were measured systematically across blocks over three days to ensure that intensive measurement of individual traits could occur at the same time of day (i.e. 32 plants were measured each day, including two or three individuals from each species/treatment combination sampled across blocks). For measurement of gas exchange, all plants to be harvested on a given day were transferred to a growth chamber (Conviron, Winnipeg, Canada) and allowed to acclimate to set conditions (25°C, 60% RH, 800 $\mathrm{\mu mol \cdot m^{-2} \cdot s^{-1}}$ light intensity) for one hour. The most recent fully expanded leaf for each individual was tagged and measured for photosynthetic rate (Aarea) and stomatal conductance (gs,) at 400 ppm CO_2 and 2000 $\mathrm{\mu mol \cdot m^{-2} \cdot s^{-1}}$ light intensity, using a LiCor 6400 Portable Photosynthesis System (Li-

Cor Biosciences, Lincoln, NE). Tagged leaves were harvested, weighed for fresh mass and scanned with a digital flatbed scanner to obtain leaf images, and then dried for at least 72 hours at 60° C. Leaf images were processed with Image J software (Rasband 1997-2012) to obtain leaf area and allow calculation of leaf mass per unit area (LMA, $g\cdot cm^{-2}$) and leaf dry matter content (LDMC, $g\cdot g^{-1}$). Instantaneous water use efficiency (iWUE) was calculated as the rate of photosynthetic carbon gain relative to the rate of stomatal conductance (Aarea/gs). Total leaf area was measured during harvest by removing all remaining leaves from an individual, measuring area using an LI-3100 leaf area meter (Li-Cor Biosciences, Lincoln, NE), and adding the area of previously measured leaves taken for LMA.

Stem trait measurement–At harvest, a 10 cm segment was cut from stem tissue of each individual, beginning 5 cm distal to the height that the individual had reached prior to initiation of watering treatment. This height was chosen to ensure that only stem tissue grown under treatment was used for calculation of anatomical parameters. The stem segment was rehydrated overnight to allow comparison of turgid tissue across treatments. The volume of the segment was measured via the water displacement method and the segment was then oven-dried for at least 72 hours at 60^{o}C. Stem density (SD, $g\cdot cm^{-3}$) was then determined as dry mass per unit fresh volume. A ~2.5 mm stem cross-section was sliced from stem tissue directly proximal to the 10 cm stem segment. Sections were fixed in 10% alcoholic formalin (Cancer Diagnostics, Inc., Durham, NC) and sent for processing at the University of Georgia Veterinary Histology Laboratory, where each sample was embedded in paraffin, sliced with a sledge-microtome, mounted to slides, and stained with Toluidine blue. Slides were then imaged with a camera-mounted Zeiss light microscope using ZEN software (Carl Zeiss Microscopy, Oberkochen, Germany). Three xylem bundles and three cortex fiber bundles, selected randomly from each third of a stem cross-section, were imaged at 100x.

Cross-section anatomical analyses were carried out using Image J software (Rasband 1997-2012). The selection tool was used to isolate xylem or fiber cells from ground tissue within each image, and then lumen diameter (calculated as the diameter of a circle of equal area to the measured conduit) and lumen area of all individual conduits in each cross-sectional image were measured. Vessel density and fiber density (N_X and $N_{F;}$ $N\cdot mm^{-2}$) were calculated as number of conduits per unit area. Xylem lumen fraction and fiber lumen fraction (F_X and F_F) were determined as the ratio of total lumen area to total xylem and total fiber area, respectively (%). Theoretical hydraulic conductivity (K_t, $kg\cdot s^{-1}\cdot m^{-1}\cdot MPa^{-1}$) for each sample was calculated, based on the Hagen-Poiseuille equation for ideal capillaries assuming laminar flow, as $Kt = ([\pi\cdot\rho]/[128\cdot\eta\cdot A]) \cdot (\Sigma D_V^4)$; where ρ is the density of water (998.2 $kg\cdot m^{-3}$ at 20°C); η is the viscosity of water ($1.002x10^{-9}$ MPa·s at 20°C); A is the total measured area (m^2); and D_v is the xylem lumen diameter for i=1 to n conduits for all conduits measured per sample (m) (Tyree and Ewers 1991, Santiago et al. 2004). Percent of vascular, pith, and cortex tissue (%) were calculated from total stem cross-sectional area using the selection and measurement tools in Image J. Vessel diameters and the double wall thickness between adjacent vessels were measured using the selection tool in ImageJ for at least 100 vessels per cross-section. The hydraulically-weighted vessel diameter (Dh) was calculated as $Dh = (\Sigma D^5)/ (\Sigma D^4)$, based on all sampled vessels of a given stem cross-section. Vessel implosion resistance [$(t/b)_h^2$; Hacke et al. (2001)] was calculated for the vessels of a cross-section whose diameters fell within ±5 μm of the calculated Dh, with t as the double-wall thickness of adjoining vessels and b as the lumen diameter of a given vessel.

Statistical Analysis–Differences among species and between treatments were tested using a two-way ANOVA and Tukey post-hoc analyses were used to determine significant differences between species-treatment subsets. Principal components analyses were run with data input from 15 focal stem and leaf traits, representing a hypothesized resource-use and transport axis of co-variation defined by previous work with the *Helianthus* genus (Pilote and Donovan 2016). All individuals across species and treatment were used in this analysis. Principal components analyses were run using the correlation matrix to minimize the effect of differences in scaling among the measured leaf and stem traits. Principal component analyses and ANOVA were conducted in JMP Pro 10 software (JMP, Version 10, SAS Institute Inc., Cary, NC, 1989-2007).

The relative distance plasticity index (RDPI) was calculated to test for plasticity of individual traits according to Valladares et al. (2006). RDPI calculates the relative distance in trait value between two individuals: individual *j* of species x under condition *i* with a second individual *j'* of the same species x growing under a second condition *i'*, i.e. the absolute value of $x_{i'j'} – x_{ij}$. This distance is then divided by the sum ($x_{i'j'} + x_{ij}$) to obtain a relative distance. Computing these relative distances between all individuals of species x in treatments *i* and *j* will allow calculation of a mean RDPI value for a given trait: RDPI = $\Sigma(|x_{i'j'} – x_{ij}|/[x_{i'j'} + x_{ij}])/n$, where n is the total number of distances. This index ranges from 0 (no plasticity) to 1 (maximal plasticity).

RESULTS

All six species had reduced growth in the water-limited treatment as compared to the well-watered treatment demonstrating that the water stress treatment was effective (Table 4.1; data for all measured traits described in Table C1). Watering treatment additionally resulted in significant differences among stem hydraulic anatomical parameters across all species (ANOVA; $p<0.05$), including reduced theoretical hydraulic conductivity (Kt) and hydraulically-weighted vessel diameters (Dh), and increased vessel densities (Nv) and vessel implosion resistance ($(t/b)_h^2$) (Figure 4.1; additional effects on xylem described in Figure C2). Differences in trait values from well-watered to water-limited treatments were consistent with our expectation that water-limited individuals would exhibit lower theoretical hydraulic conductivity and higher vessel-implosion resistance, indicative of greater overall xylem safety from drought-induced cavitation and lower water transport capacity.

When leaf and stem traits were condensed to principal components of variation (Figure 4.2a), the major axis of trait co-variation was found to be generally consistent with the previously hypothesized “fast-slow” axis of resource transport and use (principal component 1 of Table 4.2and Figure 4.2). This first principal component ranges from well-watered groups with higher rates of photosynthesis and stomatal conductance, lower water use efficiency, higher water transport capacity, and lower investment in tissue densities across both organs to water-limited groups with opposing trait values consistently across all species pairs. This is consistent with our expectation that leaf and stem traits would shift along this fast-slow resource use and acquisition axis towards more resource-conservative trait values. Additionally, this treatment effect, representing a shift along principal component 1, is significant across all species, with no significant difference in species response (ANOVA; $p<0.05$; Table 4.1; Figure 4.3).

On an individual trait basis, the majority of stem and leaf functional traits shifted significantly under water limitation stress (Table 4.2). However, multiple traits concerning tissue composition and structure, including leaf mass per unit area (LMA), leaf dry matter content (LDMC), and Vasc %, did not shift significantly or consistently across the studied species pairs.

To assess the degree of phenotypic plasticity of these traits, we calculated relative distance plasticity indices (RDPI), representing the degree of phenotypic plasticity of a single trait across all individuals of a species across both water treatments. RDPI analysis revealed large variance across measured leaf and stem traits, from 0.06-0.11 in LMA to 0.33-0.61 in Kt (Figure 4.4). Traits which describe water transport and use (gs, iWUE, Kt, and Dh) were found to have relatively large RDPI values, ranging from 0.14–0.28 in Dh to 0.33–0.61 in Kt. In contrast, traits which describe tissue density and carbon investment in leaves and stems (LDMC, LMA, SD, Vasc%) were found to have relatively low RDPI values, ranging from 0.06-0.11 in LMA to 0.09-0.24 in SD.

DISCUSSION

This study assessed the plastic responses of leaf and stem functional traits to water stress in order to determine whether these ecological responses follow a similar trajectory as evolutionary patterns of fast to slow trait co-variation of traits that define resource strategy. This study utilized a common garden, controlled irrigation experimental approach to induce well-watered and water-limited treatments on six species from across the *Helianthus* phylogeny. Here we discuss these responses in detail, specifically in regards to the response of herbaceous stem hydraulic anatomy, coordination in stem and leaf functional plastic responses, and differential plastic response of traits that describe tissue carbon investment and traits that describe water transport and use.

The stem vascular anatomy of all six species responded to water limitation by reducing water transport capacity (Kt) and increasing vessel resistance to implosion $(t/b)_h^2$, which was accomplished with shifts towards a greater density of lower diameter xylem vessels (Figure 4.2).

These shifts represent an alteration of the stem hydraulic system towards more hydraulically "safe" xylem conduits, which are potentially less vulnerable to drought-induced cavitation, as $(t/b)_h^2$ is strongly associated with a species' resistance to cavitation (Sperry et al. 2006, Bryukhanova and Fonti 2012). Similar shifts towards reduced water transport capacity and increased vessel implosion resistance have been previously observed among studies of woody angiosperms in response to experimentally and naturally altered water availability, shading, nutrient supply, and heavy metal accumulation in woody taxa (Bryukhanova and Fonti 2012, de Silva et al. 2012, Plavcova and Hacke 2012), and in response to light availability in common beans (Matzner et al. 2014). It is hypothesized that acclimation of developing xylem conduits to environmental conditions contributes to a greater ability to continue growth, as decreased conduit diameters and increased vessel implosion resistance are associated with reduced vulnerability to embolism (Fichot et al. 2009, Gleason et al. 2013).

In addition to alterations of stem hydraulic anatomy, water limitation stress resulted in increased leaf-level water use efficiency via decreases in the rate of stomatal conductance relative to the rate of photosynthetic carbon gain across all studied species (Table 4.1). Principal component analysis revealed that the plastic responses of leaf and stem functional traits were consistent with shifts along a single hypothesized "fast-slow" resource use and transport axis of trait co-variation (Figure 4.3). Previous research has observed leaf and stem traits to co-vary across broad samplings of taxa in field settings (Freschet et al. 2010, Mendez-Alonzo et al. 2012, de la Riva et al. 2015), and a recent common garden analysis found evidence for correlated evolution of these traits across *Helianthus* species, ranging from resource-acquisitive to resource-conservative trait combinations (Pilote and Donovan 2016). The species studied here were found to be characterized by significantly different resource strategies, but were not found to significantly differ in their response towards more resource-conservative trait values along a fast to slow resource transport and use axis (Figure 4.3). This provides evidence that the ecological response of these traits follows a similar pattern of fast-to-slow trait co-variation as the evolutionary pattern previously observed. This agrees with hypotheses that the traits which define a taxa' resource strategy should respond to environmental stress with an integrated shift towards more resource-conservative trait values (Schlichting 1989, Chapin 1991). Co-variation among the plastic responses of functional traits has been hypothesized to be the result of a number of potentially interrelated causes, including shared genetic control, shared functional roles, shared resource base (Schlichting 1989), and/or centralized mechanism of stress response involving hormonal responses by tissues, which results in a cascade response across a whole plant (Chapin 1991).

While trait variation was found to occur consistent with integrated plastic responses towards more resource-conservative values, individual traits displayed varying degrees of phenotypic plasticity. We calculated relative distance plasticity indices to quantify these plastic responses and found that traits governing water transport and use, such as Kt, Dh, gs, and iWUE, exhibited substantially greater plasticity than traits that are associated with tissue carbon investment, such as LMA, LDMC, SD, and Vasc %. Thus, large alterations to plant water transport and use do not appear to be accompanied by a similarly large increase in tissue carbon investment. This may ensure maintenance of plant tissue structure while altering hydraulic anatomy and function to limit plant water loss under water limitation stress. High ecological lability of hydraulically-related traits, including Kt, has been previously observed in a comparison of deciduous and evergreen woody species (Scholz et al. 2014).

In conclusion, we found that water limitation resulted in significant alteration to the majority of the leaf and stem functional traits measured here across six congeneric herbaceous species. This stress resulted in plastic responses of leaf and stem functional traits that co-varied along a "fast-slow" resource-use and acquisition axis towards more resource-conservative trait values. This pattern of co-variation from fast, resource-acquisitive trait combinations to slow, resource-conservative strategies has previously been observed as an evolutionary pattern of correlated evolution across *Helianthus*. Thus, this study suggests the ecological responses of these functional traits follows a similar trajectory as the evolutionary pattern observed across wild taxa.

REFERENCES

Aranda, I., F. J. Cano, A. Gasco, H. Cochard, A. Nardini, J. A. Mancha, R. Lopez, and D. Sanchez-Gomez. 2015. Variation in photosynthetic performance and hydraulic architecture across European beech (Fagus sylvatica L.) populations supports the case for local adaptation to water stress. Tree Physiol 35:34-46.

Bai, K. D., C. X. He, X. C. Wan, and D. B. Jiang. 2015. Leaf economics of evergreen and deciduous tree species along an elevational gradient in a subtropical mountain. Aob Plants 7:15.

Barnard, D. M., F. C. Meinzer, B. Lachenbruch, K. A. McCulloh, D. M. Johnson, and D. R. Woodruff. 2011. Climate-related trends in sapwood biophysical properties in two conifers: avoidance of hydraulic dysfunction through coordinated adjustments in xylem efficiency, safety and capacitance. Plant Cell Environ 34:643-654.

Bryukhanova, M., and P. Fonti. 2012. Xylem plasticity allows rapid hydraulic adjustment to annual climatic variability. Trees 27:485-496.

Chapin, F. S. 1991. Integrated responses of plants to stress. Bioscience 41:29–36.

Chave, J., D. Coomes, S. Jansen, S. L. Lewis, N. G. Swenson, and A. E. Zanne. 2009. Towards a worldwide wood economics spectrum. Ecology Letters 12:351-366.

Corcuera, L., E. Gil-Pelegrin, and E. Notivol. 2012. Differences in hydraulic architecture between mesic and xeric Pinus pinaster populations at the seedling stage. Tree Physiol 32:1442-1457.

de la Riva, E. G., A. Tosto, I. M. Perez-Ramoz, C. M. Navarro-Fernandez, M. Olmo, N. P. R. Anten, T. Maranon, and R. Villar. 2015. A plant economics spectrum in

Mediterranean forests along environmental gradients: is there coordination among leaf, stem, and root traits. . Journal of Vegetation Science 27:187–199.

de Silva, N. D. G., E. Cholewa, and P. Ryser. 2012. Effects of combined drought and heavy metal stresses on xylem structure and hydraulic conductivity in red maple (*Acer rubrum* L.). Journal of Experimental Botany 63:5957–5966.

Fichot, R., F. Laurans, R. Monclus, A. Moreau, G. Pilate, and F. Brignolas. 2009. Xylem anatomy correlates with gas exchange, water-use efficiency and growth performance under contrasting water regimes: evidence from Populus deltoides x Populus nigra hybrids. Tree Physiol 29:1537-1549.

Freschet, G. T., J. H. C. Cornelissen, R. S. P. van Logtestijn, and R. Aerts. 2010. Evidence of the 'plant economics spectrum' in a subarctic flora. Journal of Ecology 98:362-373.

Gleason, S. M., D. W. Butler, and P. Waryszak. 2013. Shifts in leaf and stem hydraulic traits across aridity gradients in eastern Australia. International Journal of Plant Sciences 174:1292-1301.

Hacke, U. G., J. S. Sperry, W. T. Pockman, S. D. Davis, and K. A. McCulloch. 2001. Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. Oecologia 126:457-461.

Heiser, C. B. J., D. M. Smith, S. B. Clevenger, and W. C. J. Martin. 1969. The North American sunflowers: *Helianthus*. . Memoirs of the Torrey Botanical Club 22:1–218.

Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2005. Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25:1965-1978.

Mason, C. M., and L. A. Donovan. 2015. Evolution of the leaf economics spectrum in herbs: Evidence from environmental divergences in leaf physiology across Helianthus (Asteraceae). Evolution 69:2705-2720.

Matzner, S. L., D. D. Rettedal, D. A. Harmon, and M. R. Beukelman. 2014. Constraints to hydraulic acclimation under reduced light in two contrasting Phaseolus vulgaris cultivars. Journal of Experimental Botany 65:4409-4418.

Mendez-Alonzo, R., H. Paz, R. C. Zuluaga, J. A. Rosell, and M. E. Olson. 2012. Coordinated evolution of leaf and stem economics in tropical dry forest trees. Ecology 93:2397-2406.

Nemali, K. S., and M. W. van Iersel. 2006. An automated system for controlling drought stress and irrigation in potten plants. Scientia Horticulturae 110:292–297.

Pilote, A. J., and L. A. Donovan. 2016. Evidence of correlated evolution and adaptive differentiation of stem and leaf functional traits in the herbaceous genus, Helianthus. Am J Bot 103:2096-2104.

Plavcova, L., and U. G. Hacke. 2012. Phenotypic and developmental plasticity of xylem in hybrid poplar saplings subjected to experimental drought, nitrogen fertilization, and shading. Journal of Experimental Botany 63:6481–6491.

Rasband, W. S. 1997-2012. ImageJ. U.S. National Institutes of Health, Bethesday, Maryland, USA.

Reich, P. B. 2014. The world-wide 'fast-slow' plant economics spectrum: a traits manifesto. Journal of Ecology 102:275-301.

Santiago, L. S., G. Goldstein, F. C. Meinzer, J. B. Fisher, K. Machado, D. Woodruff, and T. Jones. 2004. Leaf photosynthetic traits scale with hydraulic conductivity and wood density in Panamanian forest canopy trees. Oecologia 140:543-550.

Schlichting, C. D. 1989. Phenotypic integration and environmental change. Bioscience 39:460–464.

Scholz, A., S. Jansen, B. Choat, and A. Stein. 2014. How drought and deciduousness shape xylem plasticity in three Costa Rican woody plant species. IAWA Journal 35:337-355.

Sperry, J. S., U. G. Hacke, and J. Pittermann. 2006. Size and function in conifer tracheids and angiosperm vessels. American Journal of Botany 93:1490-1500.

Stephens, J. D., W. L. Rogers, C. M. Mason, L. A. Donovan, and R. L. Malmberg. 2015. Species tree estimation of diploid Helianthus (Asteraceae) using target enrichment. American Journal of Botany 102:910-920.

Tyree, M. T., and F. W. Ewers. 1991. The hydraulic architecture of trees and other woody-plants. New Phytologist 119:345-360.

Valladares, F., D. Sanchez-Gomez, and M. A. Zavala. 2006. Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. Journal of Ecology 94:1103-1116.

Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. C. Cornelissen, M. Diemer, J. Flexas, E. Garnier, P. K. Groom, J. Gulias, K. Hikosaka, B. B. Lamont, T. Lee, W. Lee, C. Lusk, J. J. Midgley, M. L. Navas, U. Niinemets, J. Oleksyn, N. Osada, H. Poorter, P. Poot, L. Prior, V. I. Pyankov, C. Roumet,

S. C. Thomas, M. G. Tjoelker, E. J. Veneklaas, and R. Villar. 2004. The worldwide leaf economics spectrum. Nature 428:821-827.

Zomer, R. J., A. Trabucco, D. A. Bossio, and L. V. Verchot. 2008. Climate change mitigation: a spatial analysis of global land suitability for clean development mechanism afforestation and reforestation. Agriculture, Ecosystems, & Environment 126:67–80.

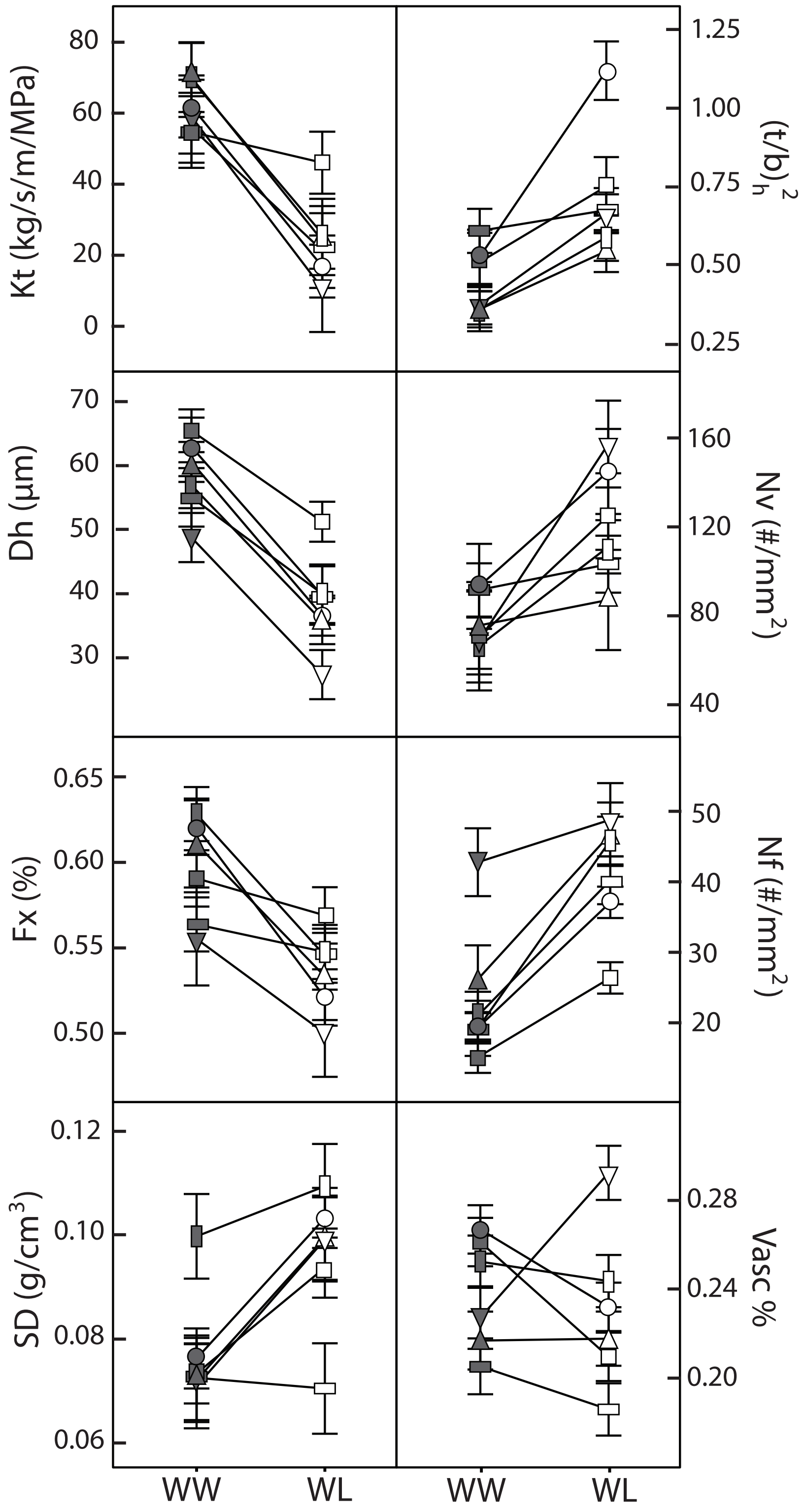

Kt (kg/s/m/MPa)
80
60
40
20
0
(t/b)$_h^2$
1.25
1.00
0.75
0.50
0.25
Dh (µm)
70
60
50
40
30
Nv (#/mm^2)
160
120
80
40
Fx (%)
0.65
0.60
0.55
0.50
Nf (#/mm^2)
50
40
30
20
SD (g/cm^3)
0.12
0.10
0.08
0.06
Vasc %
0.28
0.24
0.20
WW
WL
WW
WL

Figure 4.1. Response of vascular anatomy to water treatment (WW, well-watered [filled shapes]; WL, water-limited [unfilled shapes). Symbols represent LSMeans (± standard error) of each species. Circles represent *H. annuus*, squares represent *H. argophyllus*, horizontal rectangles represent *H. debilis*, upward triangles represent *H. grosseserratus*, downward-pointing triangles represent *H. microcephalus,* vertical rectangles represent *H. petiolaris*. Traits include: Kt, theoretical conductivity; Dh, mean hydraulically-weighted vessel diameter; Fx, xylem lumen fraction; SD, stem density; $(t/b)_h^2$, vessel resistance to implosion; Nv, vessel density; Nf, fiber density; Vasc%, percentage of stem cross-section composed of vascular tissue.

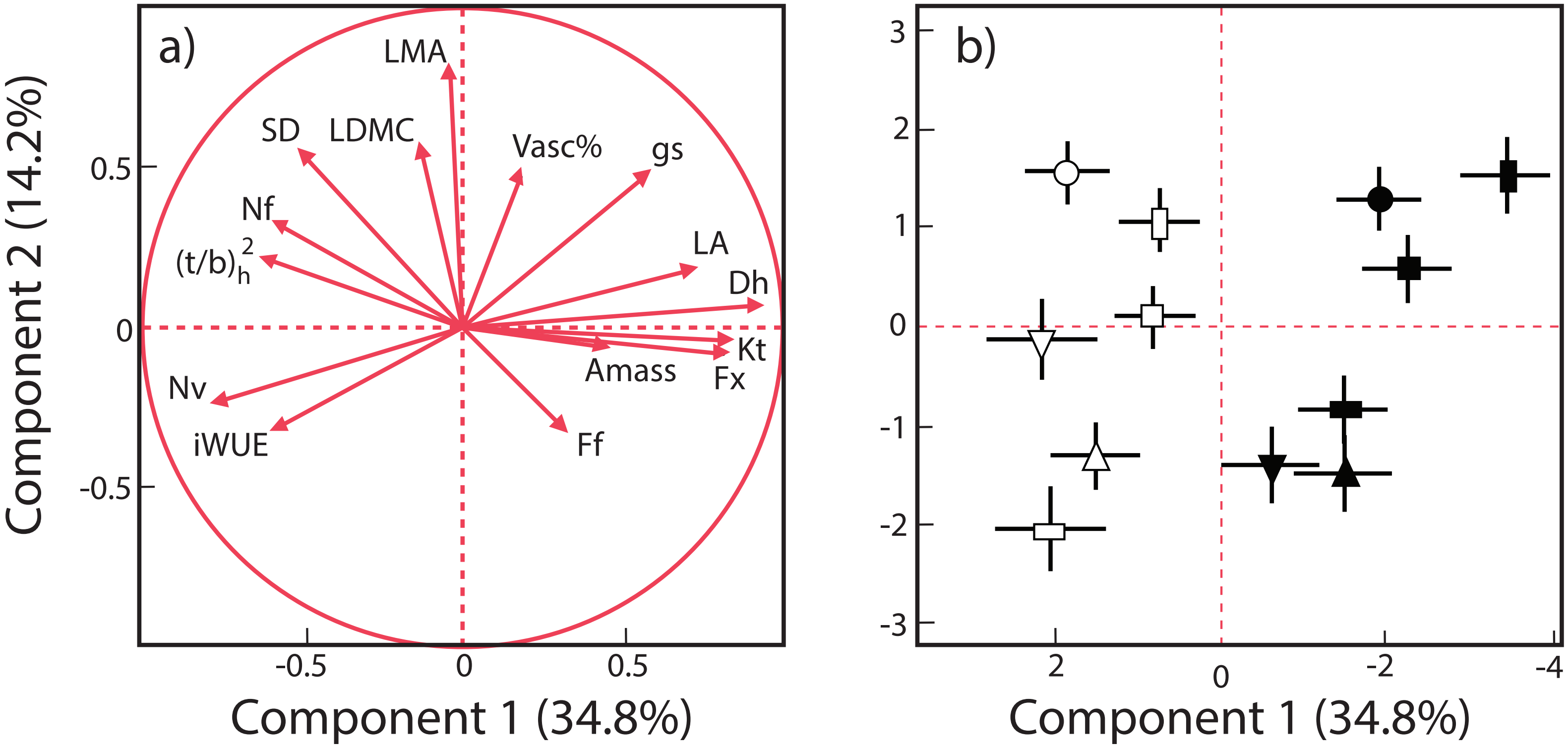

a)
LMA
SD
LDMC
Vasc%
gs
Nf
$(t/b)_h^2$
LA
Dh
Kt
Fx
Amass
Nv
iWUE
Ff
0.5
0
-0.5
-0.5
0
0.5
Component 2 (14.2%)
Component 1 (34.8%)
b)
3
2
1
0
-1
-2
-3
2
0
-2
-4
Component 1 (34.8%)

Figure 4.2. Principal component analysis of stem and leaf traits, run with all individuals across each species and treatment. a) Trait loadings for 15 stem and leaf traits, including: Amass, photosynthetic rate on an mass basis; gs, stomatal conductance; iWUE, instantaneous water use efficiency; LMA, leaf mass per unit area; LDMC, leaf dry matter content; LA, total leaf area; SD, stem density; Kt, theoretical conductivity; Nv, vessel density; Fx, xylem lumen fraction; Nf, fiber density; Ff, fiber lumen fraction; Dh, mean hydraulically-weighted vessel diameter; $(t/b)_h^2$, vessel resistance to implosion; Vasc%, percentage of stem cross-section composed of vascular tissue. b) LSMeans (± standard error) of species-treatment group principal component values (well-watered, filled shapes; water-limited, unfilled shapes) for principal components 1 and 2. Circles represent *H. annuus*, squares represent *H. argophyllus*, horizontal rectangles represent *H. debilis*, upward triangles represent *H. grosseserratus*, downward-pointing triangles represent *H. microcephalus,* vertical rectangles represent *H. petiolaris.*

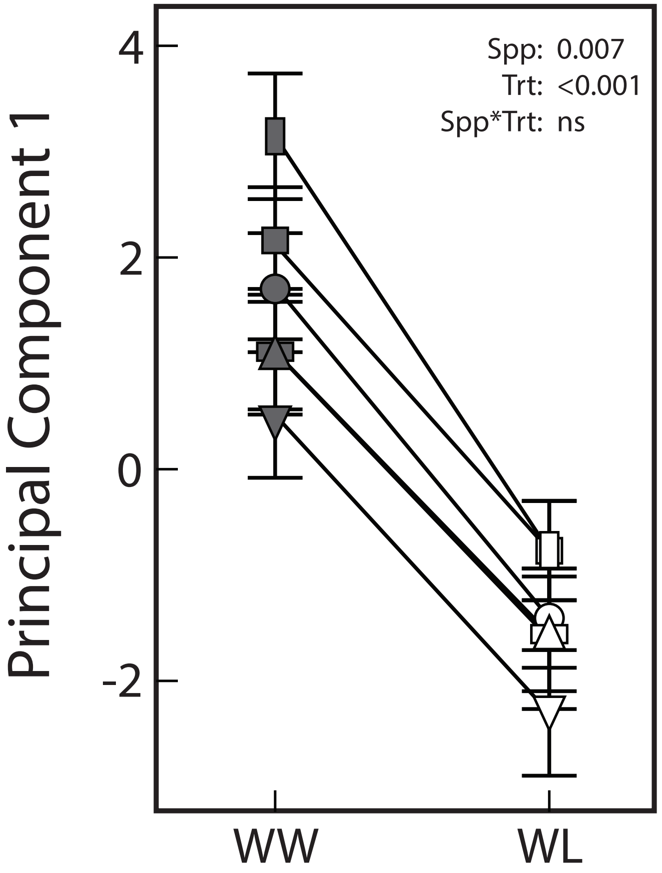


Figure 4.3. Reaction norms of species along principal component 1, described in Table 4.2 and Figure 4.2. Filled shapes represent well-watered treatment, unfilled shapes represent water-limited treatment. Circles represent *H. annuus*, squares represent *H. argophyllus*, horizontal rectangles represent *H. debilis*, upward triangles represent *H. grosseserratus*, downward-pointing triangles represent *H. microcephalus,* vertical rectangles represent *H. petiolaris*.

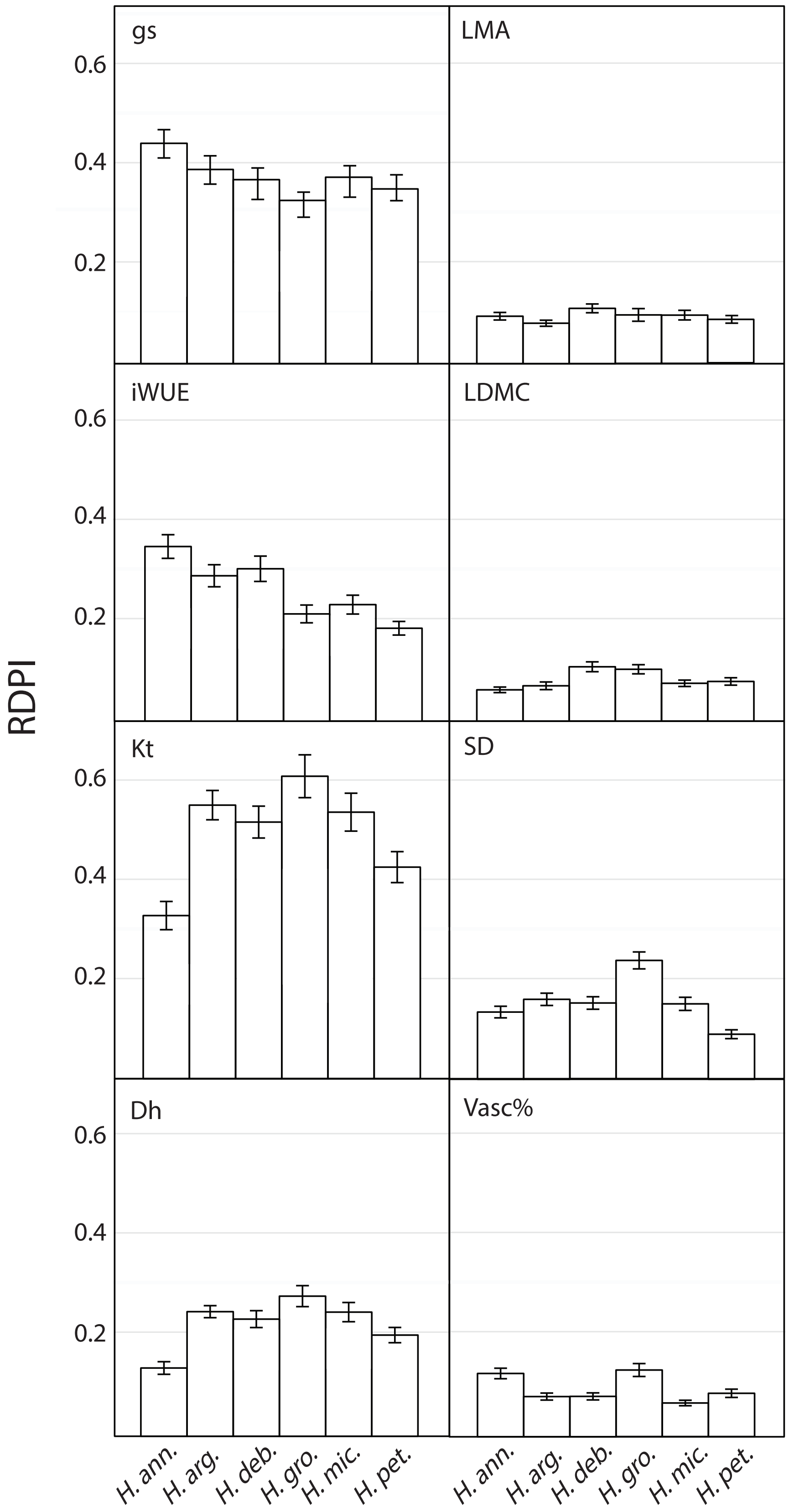

RDPI
gs
LMA
iWUE
LDMC
Kt
SD
Dh
Vasc%
0.6
0.4
0.2
0.6
0.4
0.2
0.6
0.4
0.2
0.6
0.4
0.2
H. ann.
H. arg.
H. deb.
H. gro.
H. mic.
H. pet.
H. ann.
H. arg.
H. deb.
H. gro.
H. mic.
H. pet.

Figure 4.4 Relative distance plasticity (RDPI) indices of leaf and stem traits. RDPI scales from 0 (no plasticity) to 1 (maximal plasticity) and is unitless. gs: stomatal conductivity, iWUE: instantaneous water use efficiency, Kt: theoretical stem hydraulic conductivity, Dh: hydraulically-weighted vessel diameter, LMA: leaf mass per unit area, LDMC: leaf dry matter content, SD: stem density, Vasc%: percentage of stem cross-sectional area composed of vascular tissue.

Table 4.1. Statistics summary from ANOVA analysis of treatment (well-watered and water limited), species, and treatment*species interactions. Arrows represent directional shift in trait value from well-watered treatment to water-limited. Bold indicates significant effect ($p<0.05$). Amass, photosynthetic rate on an mass basis; gs, stomatal conductance; iWUE, instantaneous water use efficiency; LMA, leaf mass per unit area; LDMC, leaf dry matter content; LA, total leaf area; SD, stem density; Kt, theoretical conductivity; Nv, vessel density; Fx, xylem lumen fraction; Nf, fiber density; Ff, fiber lumen fraction; Vasc %, percent of stem cross-sectional area composed of vascular tissue; Dh, hydraulically-weighted vessel diameter; $(t/b)_h^2$, vessel resistance to implosion; Biomass, total dry biomass at harvest; HRGR, height relative growth rate; StemGR, growth rate relative to stem diameter; PC 1, principal component one, described in Table 4.2.

		Treatment		Species		Species*Treatment	
		F	P	F	P	F	P
Leaf Traits							
Amass	⇩	**7.34**	**.008**	**2.89**	.019	0.58	.713
gs	⇩	**23.1**	**<.001**	**13.4**	**<.001**	1.26	.291
iWUE	⇧	**34.4**	**<.001**	**6.96**	**<.001**	0.62	.686
LDMC		0.25	.616	**3.13**	**.012**	1.19	.320
LMA		1.54	.218	**30.0**	**<.001**	**2.76**	**.024**
LA	⇩	**326.3**	**<.001**	**29.4**	**<.001**	**8.24**	**<.001**
Stem Traits							
SD	⇧	**18.3**	**<.001**	**4.06**	**.002**	1.32	.265
Kt	⇩	**42.9**	**<.001**	0.74	.599	1.08	.375
Nv	⇧	**67.1**	**<.001**	**10.7**	**<.001**	1.92	.100
Fx	⇩	**26.5**	**<.001**	2.24	.058	1.51	.197
Nf	⇧	**17.7**	**<.001**	1.27	.286	1.27	.286
Ff	⇩	**4.56**	**.036**	0.45	.809	1.29	.275
Vasc %		1.38	.240	**7.86**	**<.001**	**5.48**	**<.001**
Dh	⇩	**78.20**	**<.001**	**5.46**	**<.001**	0.58	.711
$(t/b)_h^2$	⇧	**37.09**	**<.001**	**6.68**	**<.001**	**2.80**	**.022**
Performance Traits							
Biomass	⇩	**28.87**	**<.001**	**129.42**	**<.001**	**8.77**	**<.001**
HRGR	⇩	**6.77**	**<.001**	**104.82**	**<.001**	3.10	.013
StemRGR	⇩	**25.40**	**<.001**	**228.61**	**<.001**	2.11	.072
PC 1	⇩	**3.47**	**.008**	**107.9**	**<.001**	0.46	.804

Table 4.2. Trait loadings for principle component analysis, displayed in Figure 4.2. Amass, photosynthetic rate on an mass basis; gs, stomatal conductance; iWUE, instantaneous water use efficiency; LMA, leaf mass per unit area; LDMC, leaf dry matter content; LA, total leaf area; SD, stem density; Kt, theoretical conductivity; Nv, vessel density; Fx, xylem lumen fraction; Nf, fiber density; Ff, fiber lumen fraction; Dh, mean hydraulically-weighted vessel diameter; $(t/b)_h^2$, vessel implosion resistance ; Vasc%, percent of stem cross-sectional area composed of vascular tissue.

	PC1 34.8%	PC2 14.2%
Amass	0.439	-0.061
gs	0.573	0.476
iWUE	-0.574	-0.311
LDMC	-0.129	0.556
LMA	-0.038	0.803
LA	0.712	0.180
SD	-0.495	0.537
Kt	0.823	-0.041
Nv	-0.762	-0.231
Fx	0.815	-0.083
Nf	-0.573	0.321
Ff	0.311	-0.311
Dh	0.921	0.069
$(t/b)_h^2$	-0.603	0.211
Vasc %	0.182	0.501

CHAPTER 5

CONCLUSIONS

This dissertation explored correlations of leaf and stem functional traits at three scales: evolutionary patterns of co-variation across inter-generic taxa, their response to artificial selective processes of crop domestication, and their responses to the stress of water limitation within the herbaceous genus, *Helianthus*. Using controlled environment, common garden investigations, we measured anatomical and physiological traits across many species of this herbaceous genus. These traits were expected to co-vary along a “fast-to-slow” axis of resource transport and use in the context of evolutionary divergence across the wild taxa of this genus, in response to the artificial selective pressures of crop domestication, and in ecological response, forming predominant axes of co-variation from resource-acquisitive to resource-conservative trait values. First, we assessed patterns of co-variation across 14 species of *Helianthus* in a phylogenetically explicit greenhouse context in order to determine and interpret genetically based trait correlations. We found that, among stem traits, water transport capacity (theoretical hydraulic conductivity) negatively correlated with resistance to bending stress (modulus of elasticity), suggesting that greater water transport comes at the cost of reduced resistance to mechanical stress among the stems of this genus. These stem traits, along with those describing vascular anatomy, co-varied strongly with leaf traits across the genus, with principal components analyses identifying a primary axis of co-variation including traits of both organs. This axis ranges from “faster”, more resource-acquisitive traits, including high rates of gas exchange, low leaf-level water use efficiency, low leaf and stem tissue density, low resistance to stem bending stress, and hydraulic anatomical properties indicative of high water transport capacity and low investment in vascular tissue, to “slower”, more resource-conservative traits with opposing trait values. Additionally the trait combinations that describe this primary axis of co-variation were found to correlate with source-site temperature and potential evapotranspiration, consistent with the hypothesis that different combinations of leaf and stem traits contribute to adaptive differentiation of these species to habitats ranging widely in temperature and water availability.

We then sought to determine how this suite of leaf and stem traits shifts at a smaller evolutionary scale: in response to the artificial selective pressures of crop domestication. Due to the history of domestication in cultivated sunflower, we were able to compare wild *Helianthus annuus* to the products of two forms of its domestication: Native American-domesticated ancient landraces and modern improved cultivars. It is hypothesized that traits should shift towards more resource-acquisitive values during the process of crop domestication, as they have faced selection towards productivity in artificially resource-rich environments. However, artificial selection occurs on specific plant parts and not on plant fitness, as in the process of natural selection. Comparisons of wild *H. annuus* with ancient landraces and improved cultivars found that this suite of leaf and stem functional traits did not shift in a coordinated fashion towards resource-acquisitive values under either form of domestication (i.e. ancient landrace or improved cultivar). Traits that were found to account for the primary variance between wild populations and domesticated populations were related to leaf and stem tissue density and resistance to stress (e.g. leaf mass per unit area and stem density and resistance to bending stress). However, traits related to stem water transport and leaf physiology, including photosynthetic rate, water use efficiency, and stomatal conductance, were not found to shift consistently from wild populations to domesticated populations. Thus, while domesticated sunflowers (including both landraces and cultivars) all shifted towards increased seed set, reduced branching, reduced time to flowering, and other sunflower “domestication syndrome” traits, the leaf and stem functional traits that accompanied this shift were not consistent between the two groups.

Studies of resource strategy have predominantly concerned patterns observed across ecological gradients or through evolutionary history. However, little research has specifically looked at how traits that describe taxa’s resource strategy respond plastically to external stress and whether they shift along the “fast-to-slow” axis of trait co-variation. To address this, we induced a water limitation experiment on six species of *Helianthus*. One goal of this experiment was to determine how the vascular anatomy of these herbaceous species responded to water limitation, as herbs have been relatively understudied in regards to their stem cross-sectional anatomy. We observed reductions in theoretical hydraulic conductivity, hydraulically weighted vessel diameter, and vascular lumen fraction, and increases were observed in vessel wall to span ratio. This suggests that these herbs respond to drought with decreased water transport capacity and increased resistance to vessel implosion, implying greater safety against drought-induced embolism when introduced to water stress. These alterations to vascular anatomy were further observed to occur in tandem with alterations to leaf-level gas exchange and water use efficiency, supporting the hypothesis these leaf and stem traits shift in a coordinated fashion, in response to water stress, towards more resource-conservative trait values. We additionally quantified the degree of plasticity among these traits and determined that traits associated with hydraulic function exhibited substantially higher plasticity than those associated with carbon investment in tissues. This suggests that proportionally higher carbon investment is not necessarily required to support greater stress response by plant tissues.

Taken together, these results provide evidence for correlated evolution and plastic response of these leaf and stem functional traits, which follows expectations of trait co-variation along a “fast-to-slow” axis or resource transport and use among the wild species of *Helianthus*. However, we additionally found that the evolutionary process of crop domestication within *Helianthus annuus*, which formed ancient landraces and improved cultivars, did not produce one axis of co-variation among these leaf and stem functional traits, such as that found across wild taxa. Co-variation of functional traits, which is hypothesized to form resource strategies, across wild taxa is hypothesized to be the result of a combination of selective pressures and biophysical constraints, such as mechanical or functional tradeoffs, and/or genetic mechanisms, such as pleiotropy. The findings of this dissertation suggests that co-variation among leaf and stem functional traits across wild *Helianthus* may be the product of selective pressures moreso than biophysical or genetic constraints that would result in co-variation of these traits, even under the artificial selective pressures of crop domestication. Further investigation of these resource transport and use traits, such as their response to additional ecological stressors and analyses into the genetic basis of these functional traits, may help to elucidate the basis of their coordination.

APPENDIX A

SUPPORTING INFORMATION FOR CHAPTER 2

Dataset A1. Location, environmental characteristics, and population means for all studied populations and focal traits.

Species	Population	Life History	State	County	GRIN Accession #	Harvest Dates	Lat	Long
H.agrestis	GLA	annual	FL	Glades	PI 673202	10/1-11/2	26.95098	-81.1349
H.agrestis	HEN	annual	FL	Hendry	PI 673201	9/27-10/9	26.42305	-81.2484
H.angustifolius	BAS	perennial	LA	Morehouse	PI 673154	9/23-10/23	32.98596	-91.76962
H.angustifolius	MAN	perennial	GA	Douglas	PI 649937	10/4-11/2	33.7595	-84.8555
H.annuus	FIR	annual	CA	Fresno	PI 649859	9/3-9/6	36.89028	-120.50278
H.annuus	UT	annual	UT	Juab	PI 673305	9/1-9/6	39.716	-112.207
H.argophyllus	DAY	annual	FL	Volusia	PI 468651	10/23-11/6	29.25389	-81.02056
H.argophyllus	MUS	annual	TX	Nueces	PI 673306	9/23-10/30	27.8351	-97.05253
H.atrorubens	FMF	perennial	SC	Berkeley	PI 664731	9/23-11/6	33.19444	-79.52556
H.atrorubens	WAR	perennial	AL	Blount	PI 649940	10/12-11/23	33.89056	-86.82583
H.debilis	CDK	annual	FL	Levy	PI 673213	9/17-10/16	29.1832	-83.0171
H.debilis	PSJ	annual	FL	Gulf	PI 673311	9/23-10/9	29.806	-85.3021
H.floridanus	APL	perennial	FL	Franklin	PI 673197	9/27-10/16	29.7147	-85.02516
H.floridanus	VOL	perennial	FL	Volusia	PI 673204	10/16-10/26	28.67504	-80.97614
H.giganteus	BUR	perennial	NC	Yancey	PI 664710	9/6-9/27	35.81167	-82.19722
H.giganteus	IRW	perennial	OH	Lucas	PI 673312	9/6-9/23	41.65711	-83.78203
H.grossesserratus	ONA	perennial	IA	Monona	PI 613793	9/6-9/13	42.00861	-96.02806
H.grossesserratus	SAN	perennial	IL	Kankakee	PI 673315	9/6-9/13	41.06907	-87.67554
H.maximillianii	KON	perennial	KS	Riley	Ames 32178	9/17-10/12	39.11001	-96.56251
H.maximillianii	LAW	perennial	IA	Woodbury	PI 613794	9/10-9/20	42.4597	-96.19417
H.microcephalus	MTR	perennial	SC	Oconee	PI 673317	9/10-9/27	34.9475	-83.08917
H.microcephalus	SUN	perennial	SC	Pickens	PI 664703	9/20-10/30	34.96111	-82.845
H.petiolaris	GAR	annual	IN	Lake	PI 673325	9/1-9/6	41.618	-87.2686
H.petiolaris	OQK	annual	IL	Henderson	PI 673327	9/1-9/6	41.0538	-90.9349
H.porteri	CMR	annual	GA	Heard	PI 673331	9/6-9/10	33.2507	-85.1466
H.porteri	PM	annual	GA	Henry	Ames 32745	9/6-9/10	33.63628	-84.16951
H. silphioides	COL	perennial	LA	Ouachita	PI 673156	10/16-11/13	32.32553	-92.20832
H. silphioides	WEP	perennial	MO	Howell	PI 664793	10/19-11/13	36.66333	-91.69555

Species	Population	altitude	Mean Annual Temperature	Mean Annual Precipitation	Potential Evapotranspiration	Global Aridity Index
H.agrestis	GLA	8	22.7	1188	1591	7462
H.agrestis	HEN	12	22.9	1321	1597	8271
H.angustifolius	BAS	47	17.3	1395	1390	10035
H.angustifolius	MAN	305	15.5	1359	1324	10271
H.annuus	FIR	44	16.6	211	1510	1397
H.annuus	UT	1580	9.8	306	1228	2508
H.argophyllus	DAY	6	21.2	1250	1400	8921
H.argophyllus	MUS	5	22	848	1267	6692
H.atrorubens	FMF	14	17.9	1306	1336	9775
H.atrorubens	WAR	272	15.5	1461	1327	11009
H.debilis	CDK	12	20.7	1162	1545	7514
H.debilis	PSJ	8	20.1	1490	1362	10939
H.floridanus	APL	4	20.2	1459	1361	10727
H.floridanus	VOL	6	21.8	1324	1431	9271
H.giganteus	BUR	856	11	1391	1138	12223
H.giganteus	IRW	206	9.5	845	1003	8424
H.grossesserratus	ONA	314	9.7	739	1067	6925
H.grossesserratus	SAN	197	9.7	964	1028	9377
H.maximillianii	KON	349	12.2	863	1167	7429
H.maximillianii	LAW	382	8.9	701	1034	6769
H.microcephalus	MTR	700	13	1839	1219	15176
H.microcephalus	SUN	299	15.1	1519	1304	11748
H.petiolaris	GAR	178	9.9	939	995	9437
H.petiolaris	OQK	171	10.3	891	1057	8429
H.porteri	CMR	251	16.2	1389	1401	9914
H.porteri	PM	212	16.3	1260	1316	9650
H. silphioides	COL	56	18	1362	1424	9571
H. silphioides	WEP	280	13	1137	1269	8967

Species	**Population**	**Amass (nmol/g/s)**	**gs (mol/m2/s)**	**iWUE (ratio of A/gs)**	**LMA (g/m2)**	**LDMC (mg/g)**	**LA (cm2)**
H.agrestis	GLA	7.7177	1.2537	24.5272	3.3924	9.4948	1678.7013
H.agrestis	HEN	8.5909	1.3440	24.0713	3.8240	10.2327	1298.8700
H.angustifolius	BAS	1.8446	0.6401	46.2053	11.5488	10.4734	1134.0325
H.angustifolius	MAN	7.9573	0.9293	29.3736	3.6483	9.7419	1542.0325
H.annuus	FIR	12.3439	1.9345	22.0901	3.4058	11.7115	681.7788
H.annuus	UT	10.2907	1.6890	27.0434	4.1373	12.9472	255.9388
H.argophyllus	DAY	10.4082	1.5141	26.5215	3.8429	11.0815	2472.5813
H.argophyllus	MUS	9.9311	1.5290	26.0434	3.9910	12.2897	2004.4138
H.atrorubens	FMF	8.8532	1.0273	31.8509	4.2279	9.9579	1493.2657
H.atrorubens	WAR	5.7600	0.8996	30.7778	4.1340	12.0256	2351.5363
H.debilis	CDK	10.0330	1.6528	22.2956	3.6430	12.1778	2069.1538
H.debilis	PSJ	11.2359	1.3679	31.7850	3.7743	11.5326	1861.0588
H.floridanus	APL	6.2089	1.1517	20.8756	3.6321	9.3311	1672.7088
H.floridanus	VOL	8.2222	1.1620	26.9707	3.8426	10.0680	1787.6863
H.giganteus	BUR	8.4039	0.8693	32.5791	3.6255	14.1251	706.8383
H.giganteus	IRW	8.1142	0.7573	40.6853	3.6543	14.5413	483.4033
H.grossesserratus	ONA	7.7440	0.9096	37.2361	3.5375	13.5121	460.8275
H.grossesserratus	SAN	8.9969	0.9258	34.7137	3.1888	13.2095	511.0938
H.maximillianii	KON	5.3497	0.9063	45.1359	8.2208	14.6292	592.2188
H.maximillianii	LAW	5.6034	1.0486	37.9468	6.2519	14.1908	424.9529
H.microcephalus	MTR	10.1355	1.0802	29.4433	3.2334	13.9748	751.5188
H.microcephalus	SUN	9.6378	0.9093	30.1663	3.0001	13.1360	1611.1957
H.petiolaris	GAR	11.8164	1.8155	27.4214	4.2492	12.5868	264.8267
H.petiolaris	OQK	11.1741	1.8900	27.8096	4.6450	12.4714	269.8200
H.porteri	CMR	3.1712	0.6092	45.4777	9.4490	11.6983	662.6600
H.porteri	PM	3.6310	0.9110	34.8662	9.6876	12.7238	887.9638
H. silphioides	COL	6.3799	0.7889	35.9995	3.7765	10.5928	2211.8088
H. silphioides	WEP	8.8220	0.9100	32.3309	3.2860	10.7895	2418.8275

Species	Population	SD (g/cm3)	MOE (kg/m2*10^8)	Kt (kg/s/m/Mpa)	Nv (#/mm2)	Fx (%)	Nf (#/mm2)	Ff (%)	Vasc % (%)
H.agrestis	GLA	0.1936	3.5685	26.7149	84.4876	0.4817	232.5367	0.1522	0.3085
H.agrestis	HEN	0.1722	6.1859	32.0121	92.8803	0.4929	234.9410	0.1372	0.2647
H.angustifolius	BAS	0.1993	4.3870	39.2683	128.7991	0.4615	337.4828	0.2170	0.2652
H.angustifolius	MAN	0.2285	7.0680	30.8519	143.4014	0.4812	344.6339	0.2515	0.3218
H.annuus	FIR	0.1438	1.4782	92.4170	109.4855	0.5438	244.1228	0.3528	0.2753
H.annuus	UT	0.0725	-	83.8867	198.7620	0.5532	277.4390	0.3686	0.2145
H.argophyllus	DAY	0.3096	1.8637	78.5817	98.2030	0.5098	273.7662	0.1967	0.3804
H.argophyllus	MUS	0.2994	1.5407	55.6591	74.2699	0.4917	225.0081	0.3031	0.3564
H.atrorubens	FMF	0.1217	1.0985	26.1554	160.7406	0.4341	224.4716	0.3698	0.2314
H.atrorubens	WAR	0.2434	3.6251	46.7224	104.3436	0.4488	230.1308	0.2389	0.2550
H.debilis	CDK	0.2388	2.7587	42.9570	98.7063	0.4881	247.0175	0.3306	0.3924
H.debilis	PSJ	0.2098	3.2323	52.8294	65.9636	0.4830	277.3691	0.2809	0.3752
H.floridanus	APL	0.1896	5.2660	29.4522	128.5864	0.4599	242.9895	0.2147	0.2481
H.floridanus	VOL	0.2217	5.6001	35.2069	146.8044	0.4515	204.9332	0.2704	0.2466
H.giganteus	BUR	0.1734	6.2265	22.8113	370.2116	0.4406	429.8254	0.1577	0.2848
H.giganteus	IRW	0.1733	5.8824	19.7900	403.8176	0.4361	380.4742	0.2436	0.2048
H.grossesserratus	ONA	0.1741	4.8053	18.2493	521.4250	0.4208	408.3089	0.1335	0.4091
H.grossesserratus	SAN	0.1789	7.6889	16.4022	492.0214	0.4265	327.9247	0.1922	0.2777
H.maximillianii	KON	0.2957	10.8567	8.8554	417.8270	0.3505	354.9365	0.0969	0.4297
H.maximillianii	LAW	0.2524	10.6888	7.0534	732.2175	0.3609	394.3249	0.1369	0.3928
H.microcephalus	MTR	0.1518	8.4589	13.5514	304.5113	0.3748	302.7995	0.1722	0.2900
H.microcephalus	SUN	0.2535	7.9692	19.7355	178.4777	0.3675	286.1813	0.1409	0.2717
H.petiolaris	GAR	0.0904	1.9428	50.4819	131.1812	0.5302	209.2370	0.4855	0.2875
H.petiolaris	OQK	0.1032	2.2122	40.5950	117.8887	0.5175	236.9016	0.3656	0.2814
H.porteri	CMR	0.1775	12.1766	22.8241	197.3650	0.4096	448.4006	0.1028	0.3102
H.porteri	PM	0.2514	12.0179	28.3888	190.3617	0.4327	363.7424	0.1139	0.3415
H. silphioides	COL	0.2511	1.1967	44.3522	112.7654	0.4426	207.5439	0.3335	0.2483
H. silphioides	WEP	0.2880	3.4305	44.5583	166.1772	0.4443	263.9698	0.1862	0.3625

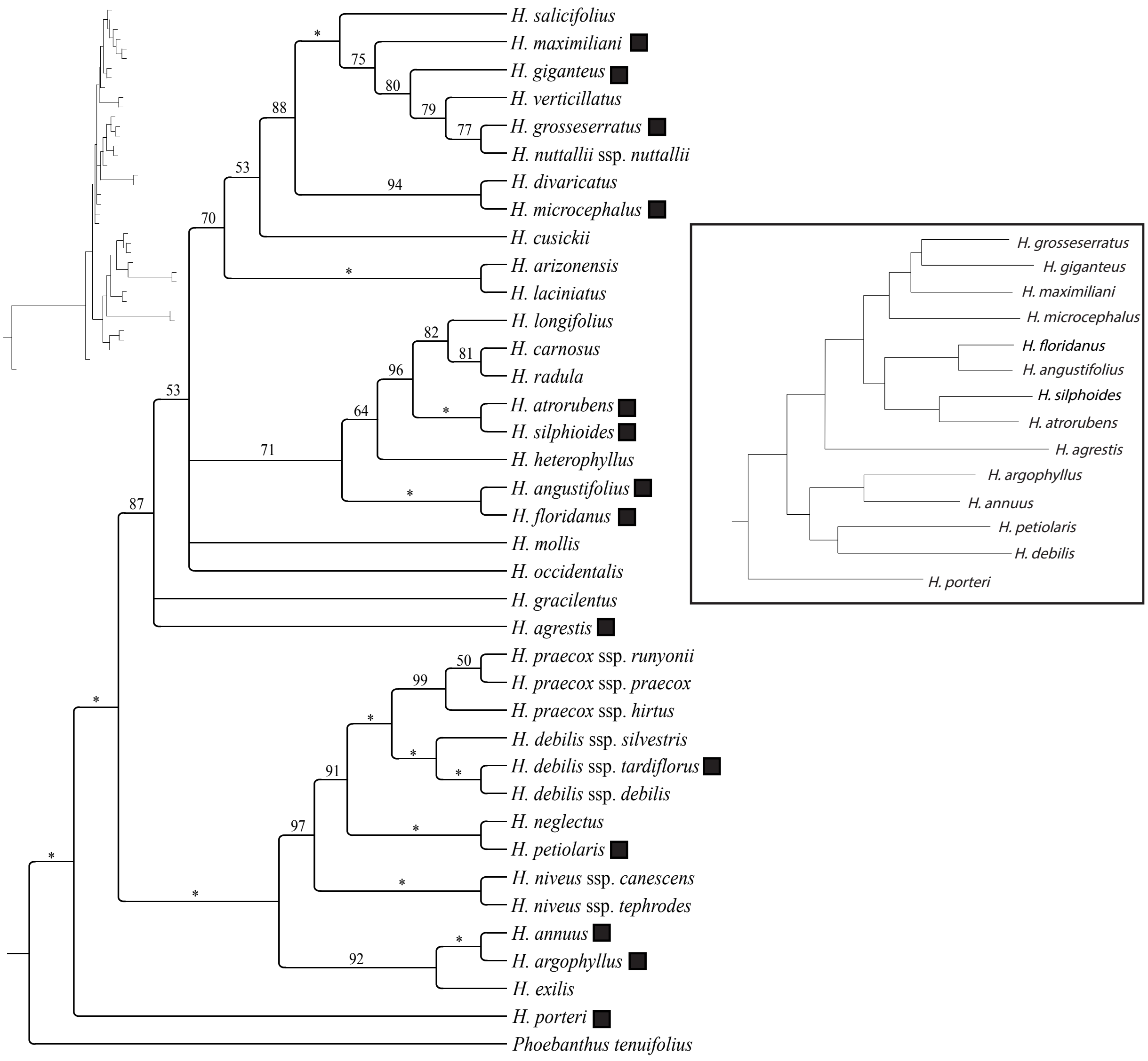


Figure A1. *Helianthus* phylogeny, constructed by Stephens et al (2015). Species used for this study are marked with a square to the right of species' names. Nodes are noted with bootstrap support, nodes with an asterisk indicate bootstrap support of 100. Box inset represents the trimmed phylogeny with branch lengths used for phylogenetic comparison across these 14 species.

Table A1. Ahistorical correlations (r values) of leaf and stem traits, based on population means. Notes: Bold indicates $p<0.05$. SD: stem-specific density, MOE: modulus of elasticity, Nv: vessel density, Fx: xylem lumen fraction, Nf: fiber density, Ff: fiber lumen fraction, Kt: theoretical hydraulic conductivity, LA: total leaf area, LDMC: leaf dry matter content, LMA: leaf mass per unit area, Amass: photosynthetic rate per unit mass, gs: rate of stomatal conductance, iWUE: instantaneous water use efficiency.

	Amass	gs	iWUE	LMA	LDMC	LA	SD	MOE	Kt	Nv	Fx	Nf
gs	**0.784**											
iWUE	**-0.635**	**-0.793**										
LMA	**-0.721**	**-0.392**	**0.627**									
LDMC	0.109	-0.090	**0.440**	0.136								
LA	-0.104	-0.132	-0.252	-0.210	**-0.626**							
SD	-0.287	-0.294	0.197	0.175	-0.057	**0.665**						
MOE	-0.327	**-0.407**	0.305	0.217	0.314	-0.226	0.085					
Kt	**0.375**	**0.492**	**-0.509**	-0.206	**-0.486**	0.298	-0.096	**-0.712**				
Nv	-0.266	**-0.460**	**0.595**	0.189	**0.699**	**-0.591**	-0.085	**0.589**	**-0.806**			
Fx	**0.515**	**0.697**	**-0.635**	-0.257	**-0.411**	-0.025	**-0.398**	**-0.599**	**0.864**	**-0.661**		
Nf	**-0.458**	**-0.594**	**0.723**	**0.434**	**0.526**	**-0.382**	0.089	**0.650**	**-0.555**	**0.698**	**-0.506**	
Ff	**0.572**	**0.558**	**-0.478**	**-0.398**	-0.274	-0.000	**-0.393**	**-0.737**	**0.654**	**-0.494**	**0.710**	**-0.669**

Table A2. Correlations among environmental characteristics, based on population means. Notes: Bold indicates p<0.05. MAT: mean annual temperature, PET: potential evapotranspiration, GAI: global aridity index, MAP: mean annual precipitation.

	MAT	PET	GAI
PET	**0.876**		
GAI	0.051	-0.089	
MAP	0.399	0.302	**0.918**

APPENDIX B

SUPPLEMENTAL INFORMATION FOR CHAPTER 3

Dataset B1. Population information for all wild populations, ancient landraces, improved cultivars in Chapter 3.

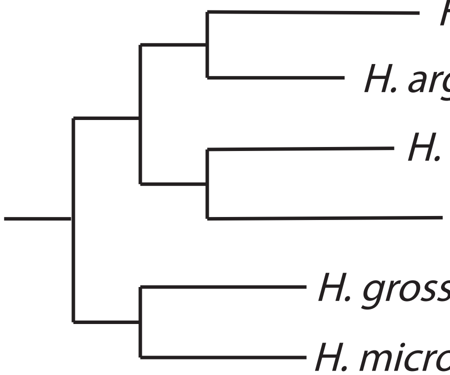
Population	Domestication Status	State	County	GRIN Accession Number	Latitude	Longitude
FRE	Wild	California	San Joaquin	PI 649858	36.604	-120.063
GRN	Wild	Mississippi	Washington	PI 664807	33.356	-91.015
KON	Wild	Kansas	Riley	Ames 32161	39.102	-96.61
UTA	Wild	Utah	Juab	PI 673305	39.716	-112.207
Havasupai	Landrace	Arizona	Cococino	PI 432511	36.237	-112.688
Hopi	Landrace	Arizona	Navajo	PI 432508	35.978	-110.672
Pueblo	Landrace	New Mexico	McKinley	PI 432515	unspecified	unspecified
599	Cultivar	-	-	PI 599753	-	-
655	Cultivar	-	-	PI 655011	-	-
597	Cultivar	-	-	PI 597364	-	-
561	Cultivar	-	-	PI 561918	-	-

Table B1. LSMeans for wild populations, ancient landraces, and improved cultivars. Trait values sharing an A or B are not significantly different from each other. A mass, photosynthetic rate on a mass basis; gs, stomatal conductance; iWUE, instantaneous water use efficiency; LMA, leaf mass per unit area; LDMC, leaf dry matter content; LA, leaf area; SD, stem density; MOE, modulus of elasticity; Ks, stem-specific conductivity; Fx, xylem lumen fraction; Nv, vessel density; Nf, fiber density; Ff, fiber lumen fraction; Vasc %, percentage of stem cross-sectional area composed of vascular tissue; Dh, hydraulically-weighted mean vessel diameter, $(t/b)_h^2$, vessel implosion resistance; HRGR, height relative growth rate; LMR, SMR, and RMR, leaf, stem, and root mass ratios, respectively.

		Wild	Landrace	Cultivated
Leaf Traits	Units			
Amass	nmol·g^{-1}·s^{-1}	11.5^{A}	10.2^{B}	11.2^{A}
gs	mol·m^{-2}·s^{-1}	1.92^{B}	2.13^{A}	1.71^{C}
iWUE	Aarea·gs^{-1}	2.42^{A}	2.08^{B}	2.41^{A}
LMA	g·m^{-2}	3.97^{A}	4.23^{A}	3.62^{B}
LDMC	mg·g^{-1}	10.5^{B}	11.1^{A}	10.7^{AB}
LA	cm^{-2}	1006^{B}	1178^{A}	808^{C}
Stem Traits				
SD	g·cm^{-3}	0.15^{A}	0.11^{B}	0.86^{C}
MOE	kg·m^{-2}·e^{-8}	4.30	3.64	3.34
Ks	kg·m^{-1}·MPa^{-1}·s^{-1}	61.3^{AB}	73.7^{A}	55.0^{B}
Fx	%	0.28^{B}	0.33^{A}	0.27^{B}
Nv	#·mm^{-2}	97.7^{B}	106^{AB}	119^{A}
Ff	%	0.32^{B}	0.42^{A}	0.35^{B}
Nf	#·mm^{-2}	382	340	403
Vasc %	%	0.22^{A}	0.15^{C}	0.18^{B}
Dh	µm	39.6	39.3	36.9
t/b	ratio	4.45^{B}	5.52^{A}	4.69^{AB}
Whole Plant Traits				
HRGR	µm·mm^{-1}·day^{-1}	1.50^{B}	2.16^{A}	1.39^{B}
LMR	g·g^{-1}	0.48^{A}	0.42^{B}	0.51^{A}
SMR	g·g^{-1}	0.29^{B}	0.39^{A}	0.26^{B}
RMR	g·g^{-1}	0.22^{A}	0.18^{B}	0.20^{A}

APPENDIX C

SUPPLEMENTAL INFORMATION FOR CHAPTER 4



	MAP	GAI	Avg. Monthly May-Oct. Precip.	Total May-Oct. Precip.
H. annuus	306	0.25	23.67	142
H. argophyllus	1250	0.89	137.67	826
H. petiolaris	891	0.84	94.33	566
H. debilis	1490	1.09	147.83	887
H. grosseserratus	739	0.69	87.83	527
H. microcephalus	1839	1.52	147.17	883

Figure C1. Cladogram of relatedness for species studied in Chapter 4, with environmental characteristics of their source habitats. MAP, mean annual precipitation; GAI, global aridity index.

Dataset C1. Species information for seed sources of populations used in Chapter 4.

Species	Life History	State	County	GRIN Accession Number	Latitude	Longitude
H. annuus	annual	Utah	Juab	PI 673305	39.716	-112.207
H. argophyllus	annual	Florida	Volusia	PI 468651	29.25389	-81.02056
H. debilis	annual	Florida	Gulf	PI 673311	29.806	-85.3021
H. grosseserratus	perennial	Iowa	Monona	PI 613793	42.00861	-96.02806
H. microcephalus	perennial	South Carolina	Oconee	PI 673317	34.9475	-83.08917
H. petiolaris	annual	Illinois	Henderson	PI 673327	41.0538	-90.9349

Table C1. LSMeans (± standard error) for all measured traits in Chapter 4 across each species and treatment (WW, well-watered; WL, water-limited). Amass, photosynthetic rate on a mass basis; gs, stomatal conductance; iWUE, instantaneous water use efficiency; LMA, leaf mass per unit area; LDMC, leaf dry matter content; LA, leaf area; SD, stem density; Kt, theoretical stem hydraulic conductivity; Nv, vessel density; Fx xylem lumen fractions; Nf, vessel fiber density; Ff, fiber lumen fraction; Dh, hydraulically-weighted mean vessel diameter; $(t/b)_h^2$, vessel implosion resistance; Vasc %, percentage of stem cross-sectional area composed of vascular tissue; Biomass, total dry biomass at harvest; HRGR, height relative growth rate; StemGR, growth rate relative to stem diameter.

	H. annuus		*H. argophyllus*		*H. debilis*	
	WW	WL	WW	WL	WW	WL
Leaf Traits						
Amass	6.11(.724)	5.84(.678)	5.93(.678)	5.81(.678)	6.66(.678)	5.24(.678)
gs	1.26(.145)	0.75(.145)	1.06(.145)	0.39(.145)	0.47(.145)	0.31(.145)
iWUE	3.98(.732)	5.69(.732)	4.01(.732)	7.73(.732)	6.23(.732)	8.05(.732)
LDMC	14.3(.71)	15.8(.71)	14.4(.71)	13.5(.71)	12.9(.71)	11.9(.71)
LMA	5.81(.289)	6.22(.27)	5.78(.27)	4.85(.27)	4.11(.27)	4.02(.27)
LA	1331(116)	336(116)	2584(116)	699(116)	1903(116)	511(124)
Stem Traits						
SD	0.08(.007)	0.10(.007)	0.07(.007)	0.09(.007)	0.07(.007)	0.07(.007)
Kt	61.9(9.43)	16.8(9.43)	54.7(10.9)	46.0(9.43)	55.9(9.43)	21.3(10.1)
Nv	193(34.4)	371(34.4)	151(34.4)	264(34.4)	210(39.7)	458(34.4)
Fx	0.62(.02)	0.52(.02)	0.59(.02)	0.57(.02)	0.56(.02)	0.55(.02)
Nf	93.2(17.2)	145(17.2)	70.5(18.4)	125(17.2)	92(17.2)	103(18.4)
Ff	0.54(.031)	0.54(.031)	0.57(.033)	0.47(.031)	0.52(.031)	0.5(.033)
Dh	60.0(3.82)	36.0(3.82)	64.9(4.08)	50.7(3.82)	54.5(3.82)	39.4(3.82)
$(t/b)_h^2$	0.52(.072)	1.12(.077)	0.51(.077)	0.75(.077)	0.61(.072)	0.67(.072)
Vasc %	0.27(.012)	0.23(.012)	0.26(.012)	0.21(.012)	0.21(.012)	0.19(.012)
Performance Traits						
Biomass	17.7(1.99)	7.47(1.99)	38.8(1.99)	10.6(1.99)	25.4(2.13)	9.37(1.99)
HRGR	1.64(.175)	0.93(.151)	1.37(.151)	1.07(.151)	2.19(.151)	1.24(.151)
StemRGR	0.25(.019)	0.09(.018)	0.40(.018)	0.18(.018)	0.27(.018)	0.13(.018)
Principal Components						
PC 1	1.91(.500)	-1.83(.500)	2.26(.540)	-0.79(.500)	1.49(.540)	-2.03(.661)
PC 2	1.29(.325	1.56(.325)	0.586(.351)	0.10(.325)	-0.85(.351)	-2.05(.429)

	H. petiolaris		*H. grosseserratus*		*H. microcephalus*	
	WW	WL	WW	WL	WW	WL
Leaf Traits						
Amass	8.14(.678)	6.45(.678)	5.52(.678)	4.51(.678)	7.88(.724)	5.97(.678)
gs	1.64(.145)	1.00(.155)	0.44(.145)	0.25(.145)	0.66(.145)	0.40a(.145)
iWUE	2.75(.823)	5.36(.732)	6.11(.732)	9.24(.732)	4.34(.732)	6.28(.732)
LDMC	13.7(.71)	13.7(.71)	13.3(.71)	14.8(.71)	13.1(.71)	13.2(.71)
LMA	5.41(.27)	6.31(.27)	3.89(.289)	4.39(.27)	3.27(.27)	3.67(.27)
LA	1998(124)	454(116)	1232(116)	258(116)	648(116)	86.3(116)
Stem Traits						
SD	0.10(.007)	0.11(.007)	0.07(.007)	0.1(.007)	0.07(.007)	0.1(.007)
Kt	69.4(10.1)	26.0(9.43)	70.0(9.43)	24.1(9.43)	59.0(10.1)	10.7(11.9)
Nv	210(34.4)	402(34.4)	262(36.8)	468(34.4)	428(36.8)	488(39.7)
Fx	0.63(.02)	0.55(.02)	0.61(.02)	0.53(.02)	0.56(.021)	0.5(.02)
Nf	66.4(18.4)	111(17.2)	75.6(17.2)	87.1(19.9)	69.0(19.9)	157(17.2)
Ff	0.53(.031)	0.52(.031)	0.58(.031)	0.52(.031)	0.56(.035)	0.47(.031)
Dh	62.4(3.82)	39.2(3.82)	56.4(3.82)	35.2(3.82)	48.2(4.08)	26.8(4.08)
$(t/b)_h^2$	0.36(.027)	0.59(.077)	0.36(.072)	0.54(.084)	0.04(.077)	0.66(.077)
Vasc %	0.25(.012)	0.24(.012)	0.22(.012)	0.22(.012)	0.23(.013)	0.29(.012)
Performance Traits						
Biomass	18.8(1.99)	7.07(1.99)	11.5(1.99)	2.93(1.99)	5.19(1.99)	1.08(1.99)
HRGR	2.57(.151)	1.22(.151)	1.74(.151)	0.83(.151)	1.95(.151)	0.69(.151)
StemRGR	0.23(.018)	0.09(.018)	0.21(.018)	0.08(.018)	0.17(.018)	0.04(.018)
Principal Components						
PC 1	3.46(.591)	-0.72(.500)	1.50(.591)	-1.50(.540)	0.62(.591)	-2.13(.661)
PC 2	1.53(.384)	1.06(.325)	-1.50(.384)	-1.30(.351)	-1.42(.384)	-0.13(.429)

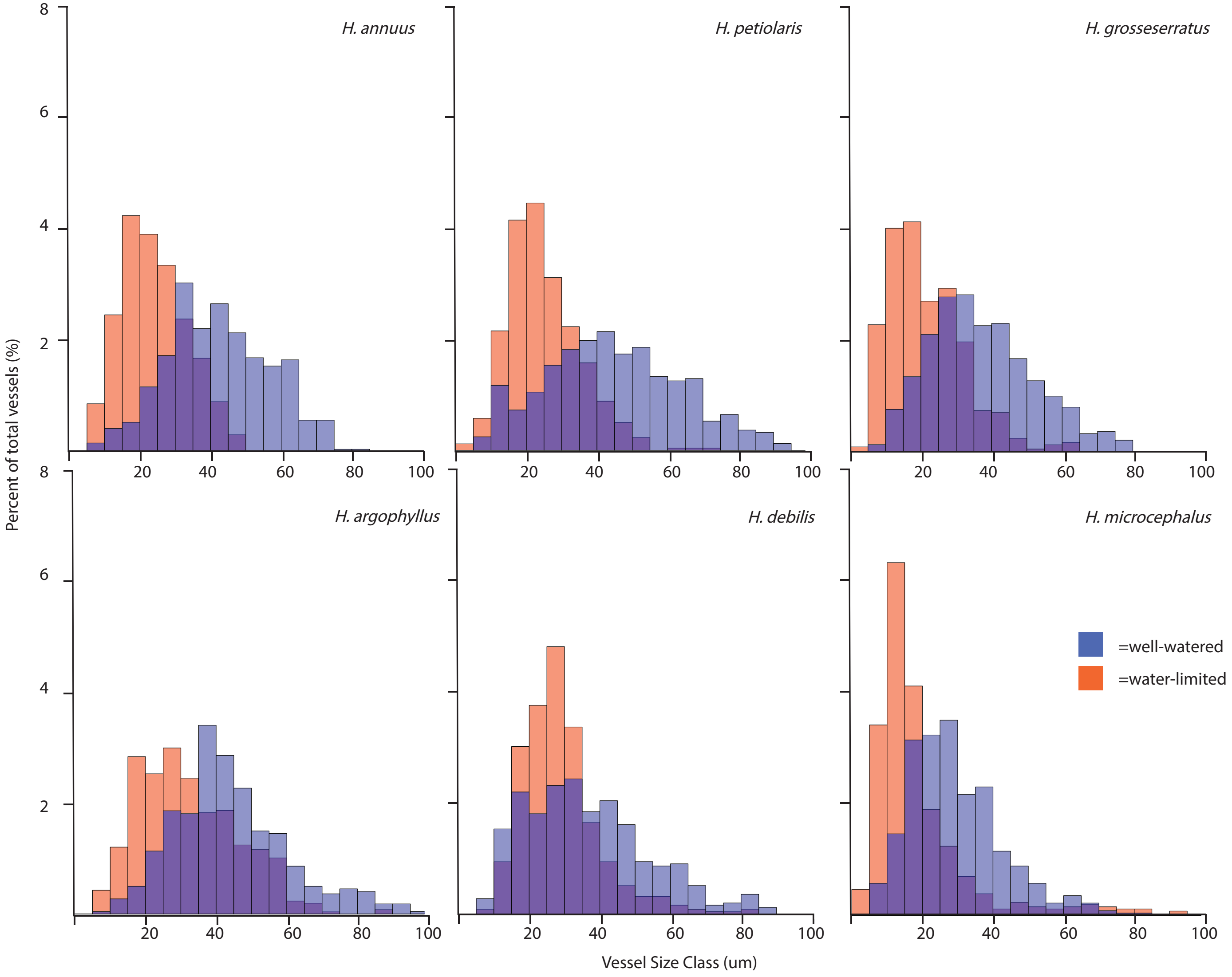


Figure C2. Vessel size class distributions for species, with shifts under water stress.