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Authors

Acosta-Rangel, Aleyda
Ávila-Lovera, Eleinis
De Guzman, Mark E
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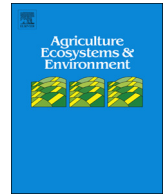
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Evaluation of leaf carbon isotopes and functional traits in avocado reveals water-use efficient cultivars



Aleyda Acosta-Rangel^{a,*}, Eleinis Ávila-Lovera^a, Mark E. De Guzman^a, Luis Torres^a, Roxana Haro^a, Mary Lu Arpaia^a, Eric Focht^a, Louis S. Santiago^{a,b}

^a Botany & Plant Sciences, University of California, 2150 Batchelor Hall, Riverside, CA, 92521, USA

^b Smithsonian Tropical Research Institute, PO Box 0843-03092, Balboa, Ancon, Panama, Panama

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ABSTRACT

Plant water-use efficiency (*WUE*) describes the ratio of carbon gain to water loss during photosynthesis. It has been shown that *WUE* varies among crop genotypes, and crops with high *WUE* can increase agricultural production in the face of finite water supply. We used measures of leaf carbon isotopic composition to compare *WUE* among 24 cultivars of *Persea americana* Mill (avocado) to determine genotypic variability in *WUE*, identify potentially efficient cultivars, and to better understand how breeding for yield and fruit quality has affected *WUE*. To validate carbon isotope measurements, we also measured leaf photosynthetic gas exchange of water and carbon, and leaf and stem functional traits of cultivars with the highest and lowest carbon isotope composition to quantify actual *WUE* ranges during photosynthesis. Our results indicate large variation in *WUE* among cultivars and coordination among functional traits that structure trade-offs in water loss and carbon gain. Identifying cultivars of subtropical tree crops that are efficient in terms of water use is critical for maintaining a high level of food production under limited water supply. Plant functional traits, including carbon isotopes, appear to be an effective tool for identifying species or genotypes with particular carbon and water economies in managed ecosystems.

1. Introduction

Functional traits have now been used extensively in ecological studies as easy-to-measure proxies for more complex processes (Cornelissen, 1999; Westoby, 1998). The study of functional traits arose from earlier efforts to place numerous species into fewer and more tangible functional groups (Grime, 1977, 1974; Smith et al., 1997), or to place species along axes of ecological strategy variation (Reich et al., 1997; Westoby, 1998; Westoby et al., 2002). Functional traits have mostly been used to advance plant biology through simplified representations of complex processes and their use in managed systems is increasing in importance for identifying differences among species, genotypes or documenting responses to environmental change (Gleason et al., 2016; Vitoria et al., 2016; Wood et al., 2015). This trend coincides with the expansion of managed and human impacted terrestrial ecosystems across the globe. For example, at large scales, functional traits have important linkages to ecosystem and landscape processes, such as water and carbon exchange between vegetated surfaces and the atmosphere (Ainsworth and Long, 2005; Baldocchi et al., 2004), or nutrient absorption and cycling by crop plants (Chapin, 1980; Wendling

et al., 2016). As climate change proceeds and the growing range of crops changes (Challinor et al., 2015; Kenny and Harrison, 1992; Lobell et al., 2006), functional traits may offer a way forward to organizing crop species along axes of trait variation that also reflect habitat suitability. Functional traits may also be applied to crop cultivars to select for particular traits that promote efficiency of resource use (Farquhar and Richards, 1984; Lauteri et al., 1997; Zhang et al., 2009). All of these applications benefit from emphasizing the main strengths of trait approaches, which center on quantifiable traits that are continuous and comparable across plant species or genotypes (Westoby, 1998).

In agriculture, functional traits that relate to water consumption are increasingly important because water is a major limiting resource for agriculture. In many parts of the world, the water resources available for agriculture are declining in availability or quality, or increasing in expense due to droughts, floods, or political disagreements (Fu et al., 2013; Lenihan et al., 2003; Mendelsohn and Dinar, 2003; Rosegrant et al., 2009). Thus, there is great interest in identifying cultivars with high water-use efficiency, which is normally expressed as yield or productivity divided by water consumed in the process (Cernusak et al., 2007). At the leaf scale, photosynthetic water-use efficiency is

* Corresponding author.

E-mail address: aacos011@ucr.edu (A. Acosta-Rangel).

expressed as photosynthetic rate, divided by stomatal conductance (Richards et al., 2002). A proxy for long-term time-integrated plant water-use efficiency can be obtained by the measurement of bulk leaf carbon isotope composition ($\delta^{13}\text{C}$). This relationship exists because conditions that cause the plant to reduce stomatal aperture cause an increase in water-use efficiency and also a reduction of CO_2 concentration at the site of carboxylation, forcing Rubisco to assimilate more $^{13}\text{CO}_2$ (Farquhar et al., 1982; Farquhar and Richards, 1984). Thus, a significantly larger $\delta^{13}\text{C}$ value is interpreted as greater water-use efficiency (Cernusak et al., 2013). Furthermore, water supply to leaves by stems creates coordination of leaf traits with stem traits, such as wood density (Santiago et al., 2004). Plants often achieve low-density wood through construction of large xylem vessels, which tend to have high water transport capacity and can sustain high rates of transpiration at the leaf level, but also tend to be more vulnerable to drought-induced xylem cavitation (Gleason et al., 2016; Pockman and Sperry, 2000; Wheeler et al., 2005). Therefore, further information about the regulation of leaf carbon and water economy can be obtained by considering water relations, and certain stem traits, along the transpiration pathway.

Stable isotope analysis of carbon has been used extensively as a key functional trait in agricultural and forestry systems to provide information on long-term time-integrated water-use efficiency (Brendel et al., 2002; Brugnoli et al., 1988; Farquhar and Richards, 1984; Lauteri et al., 1997; Monclus et al., 2005). This has allowed researchers to identify water-use efficient crop cultivars (Brugnoli et al., 1988; Farquhar and Richards, 1984; Hubick et al., 1986), investigate relationships between *WUE* and productivity (Marguerit et al., 2014; Martin and Thorstenson, 1988; Monclus et al., 2005), and link genotypic and phenotypic responses to water deficit by experimentally mapping quantitative trait loci (Brendel et al., 2008, 2002; Brugnoli et al., 1988; Hausmann et al., 2005; Marguerit et al., 2014). Such studies that provide a long-term integrated signal for *WUE* are important because they differ from short-term traits associated with photosynthetic carbon assimilation. In agroecosystems, information on both short- and long-term traits associated with carbon assimilation and water-use efficiency, as well as knowledge of the relationships among them, is critical for crop selection and crop breeding. Thus studies on crop cultivar $\delta^{13}\text{C}$ have contributed to identification of water-use efficient varieties of wheat, peanut, tomato, barley, cowpea, coffee and rice (Farquhar and Richards, 1984; Hall et al., 1990; Hubick et al., 1986; Hubick and Farquhar, 1989; Martin and Thorstenson, 1988; Meinzer et al., 1990; Zhao et al., 2004). The combined analysis of $\delta^{13}\text{C}$ with leaf and stem functional traits has emerged as a useful tool to identify water-use efficient crop genotypes.

We investigated the use of leaf $\delta^{13}\text{C}$ in combination with leaf functional traits in *Persea americana* (avocado), a meso-American tree species with a global yield of 5,028,756 kg from 547,849 ha across 70 countries in 2014 (FAO, 2017). Like many crops, avocado cultivars have been selected for yield or fruit quality but not for being water-use efficient or having other physiological traits that allow survival and production under scarce resources. There is a growing interest in water-use efficient avocado cultivars because of reduced water quality and availability. For example, the most recent California drought lasted four years and was the driest three-year record in California history (Department of Water Resources, 2015), putting half of the state in a category of exceptional drought (US Drought Monitor, 2015). This situation increased water supply costs and reduced water quality for food producers throughout California. Cultivation of citrus, avocado and other subtropical tree crops have been especially impacted (Campbell, 2011; Spann, 2014). With further scarcity of water resources predicted, crop varieties or cultivars that are especially efficient in water use may play an increasing role in securing food production in the future. Although there are seven avocado cultivars grown commercially in California, about 95% of California avocado production is based on a single cultivar, *Hass*. This study aims to identify water-use efficient avocado

cultivars using an integrated trait analysis of leaf carbon isotope composition and leaf and stem functional traits across 24 cultivars. Our main objectives were to: 1) analyze the variation in $\delta^{13}\text{C}$ among avocado cultivars; 2) use measurements of instantaneous water-use efficiency to determine what physiological factors are related to $\delta^{13}\text{C}$ in avocado leaves; 3) evaluate relationships between physiological and proxy traits; 4) describe relationships among leaf and stem traits.

2. Materials and methods

2.1. Study site and plant material

The study was conducted in the University of California South Coast Research and Extension Center (REC), Irvine, California, United States (33°41'18"N, 117°43'20"W), at an elevation of 124 m. The site has a mean annual precipitation of 165 mm with 56% of rainfall occurring from November to February and an average daily temperature range of 29–17 °C in July and 18–7 °C in January over the past three years (CIMIS, 2016). Sample collection and measurements were conducted between June and September 2016 on 24 cultivars of *P. americana* (avocado) that are part of the Avocado Breeding Program of the University of California and the California Avocado Commission. Irrigation rates are determined using an irrigation scheduling calculator (Hofshi and Hofshi, 2007), that is based on the Irvine 75 CIMIS station (CIMIS, 2016). Fertilizer is applied twice per year with a granular application of 21:7:14 NPK in April and a liquid application of 17:0:0 NPK in November. Trees were not pruned. The trees came from two experimental plots, an established 40-year old plot and a newer 5-year old plot. The plots are located in an open flat (0–2° slope) area and share the same deep, moderately sloped, alluvial fan soil. All trees were randomly planted at 6 m row spacing and 4.5 m tree spacing. The 5-year old trees were grafted onto *Dusa* rootstocks and the 40-year old trees were grafted onto *Thomas* rootstocks, except *Floccosa*, which was not grafted, and there were five additional cultivars with unknown rootstocks (Table 1). All trees were physiologically mature during sample collection and measurements.

Table 1

Scion and rootstock cultivar and number of individuals sampled for avocado study trees at South Coast Research and Extension Center, Irvine, California, USA.

Scion	Rootstock	Sample size
<i>UC05-1</i>	<i>Dusa</i>	6
<i>UC99-1</i>	<i>Dusa</i>	6
<i>UC99-2</i>	<i>Dusa</i>	6
<i>UC99-3</i>	<i>Dusa</i>	6
<i>UC99-4</i>	<i>Dusa</i>	3
<i>UC00-1</i>	<i>Dusa</i>	6
<i>UC00-2</i>	<i>Dusa</i>	6
<i>Flavia</i>	<i>Dusa</i>	6
<i>Eugenin</i>	<i>Dusa</i>	6
<i>AO.48</i>	<i>Dusa</i>	6
<i>UCBL</i>	<i>Dusa</i>	6
<i>Carmen</i>	<i>Dusa</i>	6
<i>Fairchild</i>	<i>Thomas</i>	3
<i>Floccosa</i>	<i>Floccosa</i>	2
<i>Gem</i>	<i>Dusa</i>	6
<i>Hass</i>	<i>Dusa</i>	6
<i>LT01</i>	<i>Thomas</i>	6
<i>Mother Hass</i>	<i>Dusa</i>	6
<i>XX3</i>	<i>Dusa</i>	6
<i>Walden</i>	Unknown	3
<i>Nahlat</i>	Unknown	2
<i>Maoz</i>	Unknown	2
<i>Thomas</i>	Unknown	6
<i>Simmons</i>	Unknown	2

2.2. Leaf functional traits

The leaf carbon isotope composition ($\delta^{13}\text{C}$) of 24 cultivars was determined using five newly formed mature leaves from the top of the canopy per individual to control for leaf variation. The number of individuals used per cultivar varied from 2 to 7 (Table 1). Leaves of each individual tree were pooled, dried at 65 °C for at least 48 h, ground and homogenized to a fine powder with a mill (3383L10 Wiley Mini-Mill, Swedesboro, New Jersey, USA). Leaf $\delta^{13}\text{C}$ was measured with an isotope ratio mass spectrometer (Delta V Advantage; Thermo Scientific, Bremen, Germany), interfaced with an elemental analyzer (ECS4010; Costech, Valencia, CA, USA) and reported in delta notation (‰) relative to the Pee Dee Belemnite standard. Isotope measurements were done at the University of California, Riverside Facility for Isotope Ratio Mass Spectrometry (FIRMS). Values of leaf $\delta^{13}\text{C}$ of the 24 cultivars were used to choose a subset of eight cultivars (*UC05-1*, *UC99-1*, *UC99-3*, *UC00-1*, *Carmen*, *Hass*, *Gem*, *XX3*) for further detailed functional trait measurements. This subset included cultivars with the highest and lowest $\delta^{13}\text{C}$ values, a range of intermediate $\delta^{13}\text{C}$ values, and cultivars that are particularly important in agriculture.

Gas exchange was measured between 1000 and 1200 h during sunny days with a portable infrared gas analyzer (Model LI-6400, Li-Cor Biosciences, Lincoln, NE, USA) on eight cultivars. Two newly formed mature exposed leaves from six individuals per cultivar were measured. The measurements were taken using a red/blue light source (6400-02B #SI-710, Li-Cor Biosciences) at 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photon flux density (PFD), 400 $\mu\text{mol mol}^{-1} \text{CO}_2$, 1.3–2.5 kPa of vapor pressure deficit, and 40–50% relative humidity. Leaf temperature was allowed to vary naturally from 24 to 30 °C. Maximum rate of photosynthetic CO_2 assimilation per unit leaf area (A_{area}) and corresponding stomatal conductance (g_s) and intercellular CO_2 concentration (C_i), as well as environmental conditions inside and outside the cuvette were recorded for each measurement once stable readings were achieved after 2–5 min. Intrinsic water-use efficiency (WUE_i) was calculated as A_{area} divided by g_s . We stratified measurements across cultivars by time so that one of each cultivar was measured in each round, before moving on to the next set of replicates. All measurements represent the maximum values that avocado cultivars achieve, given the conditions provided. Leaves used for gas exchange were harvested to determine specific leaf area (SLA). Specific leaf area was calculated as leaf area (cm^2) measured with a leaf area meter (LI-3100; Li-Cor Biosciences) divided by dry mass (g), after drying leaves at 65 °C for 48 h. SLA was used to calculate maximum rate of CO_2 assimilation per unit mass (A_{mass}).

2.3. Stem functional traits

Wood density (WD) was determined for the eight chosen cultivars by collecting six 1-cm diameter and 1-cm long stems per cultivar and separating sapwood from bark to measure its volume using the displacement method (Chave, 2005). On small diameter stems such as these, all wood, once the bark and phloem are removed, is considered to be sapwood. Then, the sapwood was dried at 65 °C for 48 h to measure dry mass, and WD was calculated as dry mass (g) divided by sapwood volume (cm^3). Leaf:sapwood area ratio (LA:SA) was measured on three terminal branches on each study individual as an index of hydraulic supply relative to transpiring area (Pivovarov et al., 2014). Six branches from each of the eight cultivars were collected and transported to the laboratory where sapwood diameter at the bottom of the branch was measured using a caliper to estimate sapwood area, and all leaves distal to this sapwood were removed and their total area measured using a leaf area meter (Li-3100, Li-Cor Biosciences). LA:SA was determined as total leaf area (cm^2) divided by sapwood area (cm^2).

2.4. Statistical analysis

We averaged trait data for each individual and used this data to

calculate and compare trait means among cultivars. The traits were tested for normality using a Shapiro-Wilk test, which showed that they were normally distributed ($p \leq 0.05$) or were only slightly non-normal, so no transformations were performed. Comparisons of functional traits among cultivars were done with a three-way ANOVA using the *cov* function in R, with cultivar and time since planting as fixed factors and rootstock as a random factor. Because there were no significant effects of rootstock ($F_{2,117} = 0.2524$; $p = 0.7773$) or time since planting ($F_{1,118} = 0.6722$; $p = 0.4130$), they were removed from the model and subsequent analyses were performed with one-way ANOVA using only cultivar as a factor. Tests for homoscedasticity were performed with the studentized Breusch-Pagan test using the *lmtest* package in R and the data for $\delta^{13}\text{C}$ failed the test indicating heteroscedasticity ($BP = 42.152$, $df = 23$, $p = 0.008715$). Data therefore underwent a Box Cox Transformation using the *caret* package in R, which resulted in homoscedasticity. Differences among cultivars were tested with Tukey's range test post-hoc using the *agricolae* package in R. Bivariate relationships among functional traits were first evaluated with a Pearson product-moment correlation to check for significance. If significant, standard major axis estimation (Model II Regression) was used to describe relationships using the *lmodel2* package in R (Legendre, 2014). Model II regression was chosen over linear regression (ordinary least-squares regression) because all functional traits were measured with error and our objective was to describe the relationships between traits, not to predict values of one trait from another one (Falster et al., 2003).

3. Results

3.1. Leaf and stem functional traits

There was substantial variation in carbon isotope composition among the 24 avocado cultivars ($F = 18.77$; $p < 0.0001$), with $\delta^{13}\text{C}$ ranging from -32.62 to -27.17% across the whole data set and a gradual distribution among cultivars (Fig. 1). Eight different significance groups of cultivars were detected, with *Carmen* having the highest mean $\delta^{13}\text{C}$ ($-27.86 \pm 0.29\%$) and *XX3* the lowest ($-31.93 \pm 0.22\%$). There were substantial differences in all leaf functional traits among avocado cultivars. Values for A_{area} were significantly greater in *UC00-1* than *XX3* with all other cultivars showing intermediate values (Table 2). For A_{mass} , *UC99-3* was significantly greater than *Carmen* and *XX3* with other cultivars showing intermediate values (Table 2). Although *XX3* showed the lowest values for A_{area} and WUE_i , it showed the highest values for g_s and C_i . In contrast, *Carmen* and *UC05-1* showed the highest values for WUE_i and the lowest values for g_s and C_i (Table 2). This opposite behavior of *Carmen* and *UC05-1* compared to *XX3* was consistent with their $\delta^{13}\text{C}$ values located in the extremes of Fig. 1. Values for SLA in *Carmen* were statistically indistinguishable from *UC00-1* and *XX3*, but significantly lower compared to all other cultivars (Table 2). There were no significant differences in WD among cultivars, but LA:SA showed significant variation among cultivars with *XX3* showing the highest mean value and *Carmen* showing the lowest (Table 2). This corresponds to their $\delta^{13}\text{C}$, where *Carmen* had the highest value and *XX3* the lowest (Fig. 1).

3.2. Trait relationships

There was a strong relationship between $\delta^{13}\text{C}$ and WUE_i demonstrating the utility of $\delta^{13}\text{C}$ as a time-integrated proxy for WUE_i (Fig. 2). Measurements of $\delta^{13}\text{C}$ and WUE_i were significantly correlated with g_s , C_i and LA:SA (Fig. 3), but not with A_{area} , or A_{mass} (Table 3), indicating that variability in $\delta^{13}\text{C}$ is more strongly correlated to variability in leaf water loss than photosynthetic rate (Table 3). Enrichment in ^{13}C resulted in cultivars that were more water-use efficient whereas cultivars with high values of C_i and g_s were relatively depleted in ^{13}C (Figs. 2 and 3). g_s was strongly linked to $\delta^{13}\text{C}$, given its negative relationship with WUE_i . Values for LA:SA were strongly related to WUE_i and $\delta^{13}\text{C}$,

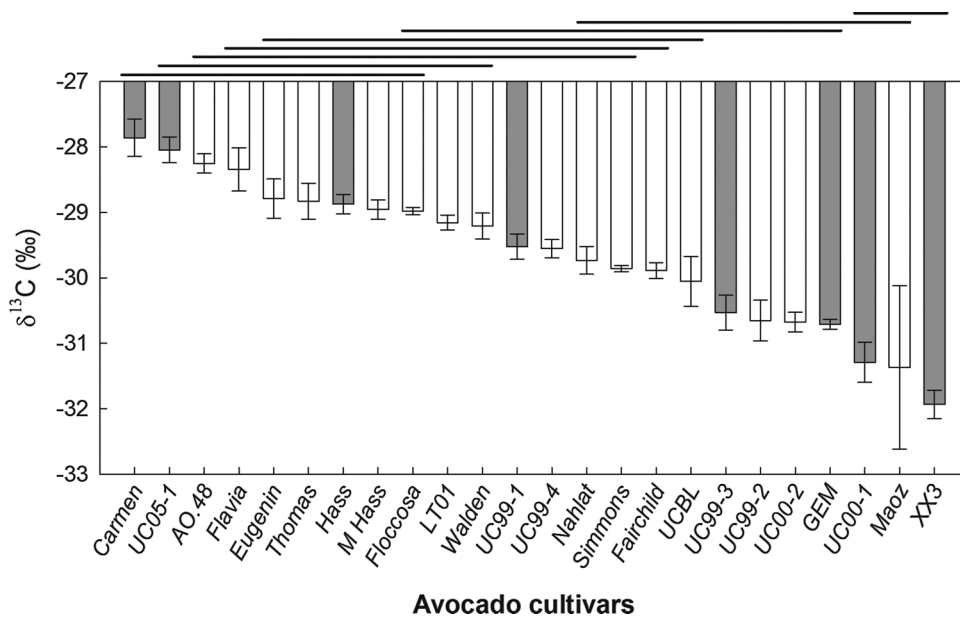


Fig. 1. Mean (± 1 SE) leaf carbon isotope composition ($\delta^{13}\text{C}$) of 24 agricultural cultivars of avocado growing at South Coast Research and Extension Center, Irvine, California, USA. There was significant variation among cultivars ($F = 18.77$; $p < 0.0001$). Cultivars that are overlapped by the same solid horizontal lines are not significantly different at an alpha of 0.05. Cultivars with grey bars were selected for further trait measurements.

indicating that cultivars that supply more leaf area with a given amount of sapwood are less conservative in their water use. Finally, C_i had a positive association with g_s ($r^2 = 0.87$; $p < 0.001$) and $LA:SA$ ($r^2 = 0.56$; $p < 0.032$) showing coordination between gas exchange, water loss and morphology.

4. Discussion

Our results indicate that WUE_i , as well as several key underlying functional traits showed strong variation among cultivars of avocado investigated in this study. The nature of functional trait relationships that describe photosynthetic gas exchange behavior in avocado are generally consistent with patterns found in natural vegetation at global scales (Farquhar et al., 1989; Maire et al., 2015; Wright et al., 2004). Our results also clearly demonstrate the link between leaf $\delta^{13}\text{C}$ and WUE_i described by standard photosynthetic gas exchange measures and its utility in identifying cultivars that have the potential for efficient photosynthetic productivity while conserving critical water resources (Farquhar et al., 1982; Farquhar and Richards, 1984). Yet, we also show significant relationships of $\delta^{13}\text{C}$ with functional traits beyond the leaf, indicating that considering coordination between leaf and stem functional traits that describe hydraulic supply and transpiring area provides enhanced understanding of how stem hydraulic traits constrain leaf performance (Pivovarov et al., 2014). This implies that the functional trait approach is successful at evaluating the physiology and ecology of important crop species, analogous to the way that functional

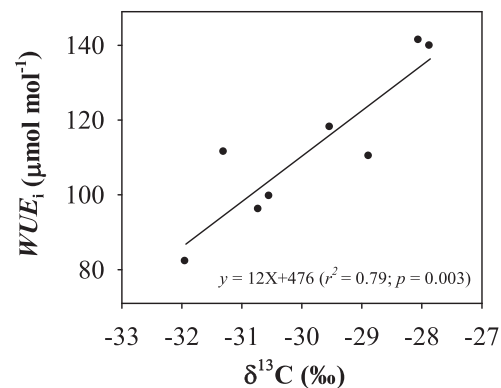


Fig. 2. Relationship between intrinsic water-use efficiency (WUE_i) and leaf carbon isotope composition ($\delta^{13}\text{C}$) for eight avocado cultivars growing at South Coast Research and Extension Center, Irvine, California, USA.

traits have been used to place wild species from natural ecosystems along axes of ecological strategy variation (Ackerly, 2004; Cornelissen, 1999; Westoby et al., 2002). Thus within a particular crop species, different cultivars can spread out along these axes and certain traits that are relatively easily measurable can be used to inform managers where along these ecological axes different cultivars fall.

Whereas most functional trait approaches to date have been directed at wild plants in natural ecosystems, it is clear that such

Table 2

Leaf functional traits of eight avocado cultivars measured at South Coast Research and Extension Center, Irvine, California, USA. Values with the same superscript letter are not significantly different at an alpha value of 0.05. Units: A_{area} ($\mu\text{mol m}^{-2} \text{s}^{-1}$), A_{mass} ($\text{nmol g}^{-1} \text{s}^{-1}$), g_s ($\text{mol m}^{-2} \text{s}^{-1}$), C_i ($\mu\text{mol mol}^{-1}$), WUE_i ($\mu\text{mol mol}^{-1}$), SLA ($\text{cm}^2 \text{g}^{-1}$), WD (g cm^{-3}), $LA:SA$ ($\text{cm}^2 \text{cm}^{-2}$).

Variety	A_{area}	A_{mass}	g_s	C_i	WUE_i	SLA	WD	$LA:SA$
UC05-1	15.2 \pm 2.3 ^{ab}	128 \pm 19 ^{abc}	0.12 \pm 0.04 ^b	134 \pm 51 ^b	141 \pm 33 ^a	84.2 \pm 7.8 ^a	0.31 \pm 0.01	3329 \pm 82 ^{abc}
UC99-1	14.1 \pm 2.1 ^{ab}	123 \pm 18 ^{bc}	0.13 \pm 0.05 ^{ab}	172 \pm 44 ^{ab}	118 \pm 28 ^{ab}	87.5 \pm 4.9 ^a	0.29 \pm 0.07	3969 \pm 2617 ^{ab}
UC99-3	16.3 \pm 2.4 ^{ab}	146 \pm 22 ^a	0.18 \pm 0.06 ^{ab}	197 \pm 44 ^a	99 \pm 29 ^b	89.6 \pm 4.5 ^a	0.32 \pm 0.09	4320 \pm 1869 ^{ab}
UC00-1	16.7 \pm 2.2 ^a	129 \pm 17 ^{abc}	0.17 \pm 0.06 ^{ab}	182 \pm 65 ^{ab}	111 \pm 40 ^{ab}	77.1 \pm 10.7 ^{ab}	0.36 \pm 0.07	4240 \pm 2090 ^{ab}
Carmen	16.1 \pm 2.4 ^{ab}	110 \pm 16 ^c	0.13 \pm 0.05 ^b	135 \pm 48 ^b	140 \pm 32 ^a	68.4 \pm 5.5 ^b	0.35 \pm 0.08	1897 \pm 629 ^c
Gem	15.1 \pm 1.8 ^{ab}	123 \pm 15 ^{bc}	0.17 \pm 0.06 ^{ab}	209 \pm 50 ^a	96 \pm 31 ^b	81.9 \pm 8.3 ^a	0.30 \pm 0.04	4354 \pm 1556 ^{ab}
Hass	15.7 \pm 2.5 ^{ab}	131 \pm 20 ^{ab}	0.16 \pm 0.07 ^{ab}	185 \pm 56 ^{ab}	110 \pm 35 ^{ab}	83.4 \pm 8.8 ^a	0.32 \pm 0.07	2696 \pm 665 ^{bc}
XX3	13.6 \pm 1.3 ^b	109 \pm 11 ^c	0.19 \pm 0.09 ^a	234 \pm 49 ^a	82 \pm 30 ^b	80.7 \pm 6.4 ^{ab}	0.36 \pm 0.01	4672 \pm 2283 ^a
F-value	3.04	6.12	3.42	7.22	6.44	5.80	1.24	2.26
p-value	< 0.01	< 0.0001	< 0.005	< 0.0001	< 0.0001	< 0.0001	0.302	< 0.05

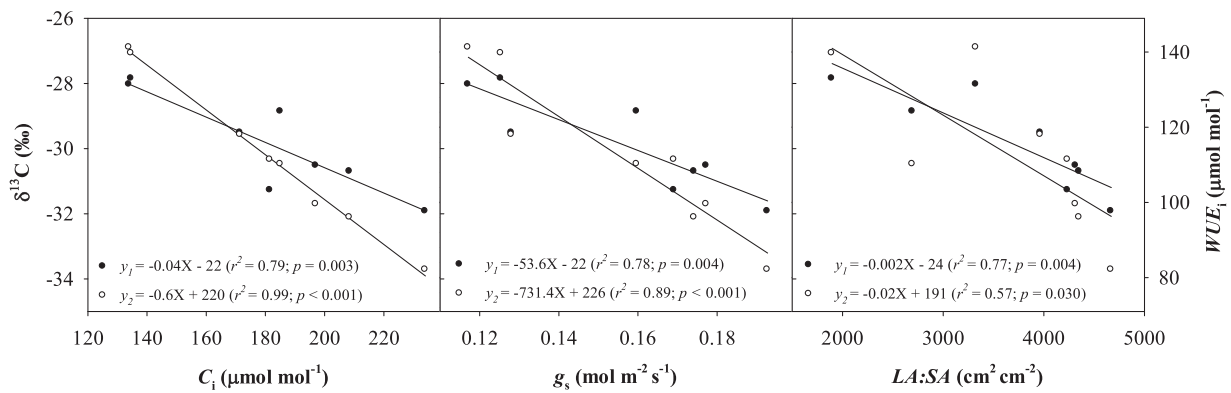


Fig. 3. Relationships between leaf carbon isotope composition ($\delta^{13}\text{C}$) and intrinsic water use efficiency (WUE_i) with intercellular CO_2 concentration (C_i); stomatal conductance (g_s); and leaf sapwood area ratio ($LA:SA$); for eight avocado cultivars growing at South Coast Research and Extension Center, Irvine, California, USA. Close symbols represent $\delta^{13}\text{C}$ values whereas open symbols represent WUE_i values.

approaches also represent a powerful tool for identifying cultivars with particular behavior regarding carbon and water economy (Brugnoli et al., 1988; Farquhar and Richards, 1984; Hubick and Farquhar, 1989; Meinzer et al., 1990). Because many of the trait relationships reflect trade-offs between high rates of resource consumption and fast growth on one end of the spectrum and low rates of resource consumption and slow growth on the other (Reich et al., 1997), it was not clear how this would play out within a species that has been subject to purposeful breeding by humans for productivity and yield. Thus, our findings that within a species, functional traits of cultivars align with known biophysical and metabolic constraints on leaf physiological function to produce patterns that are broadly analogous to global patterns on wild plants, illustrate the generality of these approaches and potential for selecting nuanced tendencies in resource use in agriculture, forestry or ecological restoration.

Because WUE_i is a composite variable based on both photosynthetic income (A) and rates of concomitant water loss (g_s), it is important to consider what factors control $\delta^{13}\text{C}$ to improve isotopic interpretation in other systems. We found that $\delta^{13}\text{C}$ was strongly correlated with g_s , but not A_{area} . There was 1.2-fold variation in values for A_{area} and 1.6-fold variation in g_s , suggesting that the greater range in stomatal behavior contributed to its strong relationship with $\delta^{13}\text{C}$ (Fig. 2). These findings are critical for calibration and interpretation of $\delta^{13}\text{C}$ values in other studies, as carbon isotopes are used to re-construct past climates and vegetation types and as monitors of ecological change (Dawson and Siegwolf, 2007; Graham et al., 2014). Thus, incorporating leaf $\delta^{13}\text{C}$ into trait assemblages for determining aspects of carbon and water economy represents a promising avenue for comparisons within a species, consistent with the original use for comparing WUE_i among wheat genotypes (Farquhar and Richards, 1984).

One of the most striking results of our study is that cultivars with high $LA:SA$ are less water-use efficient. This was shown with both long-term integrated and instantaneous measurements of WUE , yet this is

somewhat counterintuitive because cultivars with more leaves supplied by a given xylem area show more profligate water use. This result is likely related to cultivars with a high $LA:SA$ supporting high rates of water transport through high stem sapwood-specific hydraulic conductivity, as shown in woody plants from natural ecosystems (Pivovarov et al., 2014). High values for $LA:SA$ can also signify greater self-shading by leaves, which can affect radiation load and boundary layer conditions influencing photosynthesis and evaporative demand and therefore rates of leaf water loss (Ackerly, 1999). Yet, even with greater self-shading, there is still significantly lower WUE_i in cultivars with high $LA:SA$.

All of the traits measured have potential to be used in screening and selection of cultivars of avocado that use water efficiently and can withstand drought. Traits that are relatively easy to measure, as $\delta^{13}\text{C}$, SLA and WD , would be of great aid in assessing large accessions of cultivars, consistent with their use to characterize physiological processes for wild plants. Although the way these traits perform in predicting physiological processes varies across sites and species (Wright et al., 2005), our results indicate that within a single site and species they have potential to characterize nuanced physiological variation, and place cultivars or genotypes along known axes of ecological strategy variation for resource-based comparison. With such large variation in key ecological traits in relatively limited environmental conditions, we also expect that these traits show greater variation among more wide-ranging environmental conditions than within a single managed agroecosystem. The importance of variation in these traits in identifying suitable cultivars is likely more critical across sites than within this common garden experiment.

We found that variation in WUE exists across cultivars of avocado and that certain cultivars such as *Carmen*, *UC05-1* and *AO.48* are particularly water-use efficient. This knowledge can be used to improve efficiency of water use as water resources decline in quantity, quality, or become more expensive, and is promising to practitioners, but also

Table 3

Pearson product-moment correlation matrix of functional traits for eight avocado cultivars from South Coast Research and Extension Center, Irvine, California, USA. Correlation coefficients are in the upper right diagonal and p-values are in the lower left diagonal.

	A_{area}	A_{mass}	g_s	C_i	WUE_i	$\delta^{13}\text{C}$	SLA	WD	$LA:SA$
A_{area}		0.52	-0.06	-0.39	0.34	0.22	-0.27	0.22	-0.34
A_{mass}	0.19		0.13	0.02	-0.07	-0.04	0.68	-0.44	0.23
g_s	0.88	0.76		0.93	-0.94	-0.88	0.18	0.39	0.66
C_i	0.34	0.96	< 0.001		-0.99	-0.89	0.33	0.17	0.75
WUE_i	0.40	0.87	< 0.0005	< 0.0001		0.89	-0.35	-0.16	-0.75
$\delta^{13}\text{C}$	0.60	0.92	< 0.005	< 0.005	< 0.005		-0.22	-0.31	-0.88
SLA	0.52	0.06	0.68	0.42	0.40	0.60		-0.69	0.55
WD	0.60	0.28	0.35	0.69	0.70	0.46	0.05		-0.08
$LA:SA$	0.40	0.58	0.07	< 0.05	< 0.05	< 0.005	0.16	0.84	

raises further questions about its implementation in managed plant systems. First, though $\delta^{13}\text{C}$ is good indicator of long-term integrated *WUE*, short-term climatic perturbations such as the El Niño Southern Oscillation or episodic drought may produce effects that must be quantified using short-term physiological measurements, such as photosynthetic gas exchange. Thus, functional trait approaches that bridge disparate time scales are most likely to reveal key processes. Second, whereas high *WUE* with respect to carbon gain per unit water loss is relatively straightforward to evaluate using functional traits, the more important metric may be fruit yield per unit water loss, so combining short-term functional trait campaigns with long-term data on yield or growth from growers or land managers has the potential to add other dimensions to conclusions based on functional traits alone. Finally, because many agricultural species have been strongly bred for high productivity, in many cases it is not clear what stress-tolerance or efficiency traits are still fixed within the organism (Milla et al., 2014). Therefore, striking a tailored balance between efficiency, stress tolerance, productivity and yield appears to be a critical challenge for agriculture as availability of water and nutrient inputs decline.

Conflict of interest

None.

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