

LEAF HYDRAULIC VULNERABILITY IN TWO FIELD-GROWN COFFEE CULTIVARS UNDER SEVERE DROUGHT CONDITIONS¹

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ABSTRACT: Hydraulic traits such as leaf hydraulic conductance (K_{leaf}) define the xylem operational limits at the leaf level. Leaf hydraulic vulnerability was assessed in two field-grown coffee cultivars under a severe drought to test coffee susceptibility to hydraulic dysfunctions. Here we evaluated two field-grown coffee cultivars under severe (natural) drought and subsequent recovery with the following objectives: (1) to assess naturally occurring Ψ_{min} and determine reliable hydraulic safety margins and (2) to test whether loss of conductivity is significant in leaves expanded in the drought season. The large negative Ψ_1 experienced were sufficient to cause hydraulic failure and leaf loss. Leaf vulnerability curves followed a sigmoidal behaviour with K_{leaf} declining gradually as a function of Ψ_1 . The Hybrid 12 displayed lower (more negative) P_{50} (26%) than Catuaí. We concluded that the coffee tree operates under narrow safety margins even under well-watered growth conditions and it is subjected to hydraulic failure upon severe drought events. Leaves that did not cross a given threshold for leaf abscission, likely to be the Ψ_1 at P_{88} , recovered from hydraulic loss presenting K_{leaf} similar to the new leaves that were developed over the rainy season

KEYWORDS: coffee, hydraulic vulnerability, drought tolerance.

INTRODUCTION

From all environmental resources, water is likely the most important resource determining plant distribution, growth, yield and survival (Zhao and Running, 2010). Plants are constantly threatened by the risk of desiccation through exposure to a drying soil or atmosphere and survival hinges on finding a strategy to optimise water loss and carbon gain (Brodrribb et al., 2014). As water lost has to be replenished, xylem has a challenging task in supplying water to allow stomata aperture at the same time that physical tension increases its susceptibility to cavitation (Tyree et al., 1994). The risk of hydraulic failure depends on the extent that minimum xylem water potentials occurring under growth conditions (Ψ_{min}) are close to the water potential representing 50% loss of hydraulic conductivity (P_{50}) reflecting the hydraulic safety margin (HSM) for a given species (Meinzer et al., 2009). The hydraulic vulnerability assessment coupled to future climate modelling can provide more robust predictions of changes in species' distribution or elaboration of climatic risk zoning for cultivated plants.

Coffee, a tropical woody crop, is one of the most important commodities in the international agricultural trade, representing a significant source of income to several Latin American, African and Asian countries. It is also one of most threatened species by global climate changes; depending on the climate change scenarios, extinction of native populations in Ethiopia (Davis et al., 2012) as well as remarkable reductions (up to 50%) of suitable areas for coffee cultivation have been predicted (Bunn et al., 2014). In Brazil, the world's largest coffee producer, as well as in several other coffee growing countries, drought is considered to be the major environmental stress affecting coffee growth and production (DaMatta, 2004). Despite the growing body of information on the morphological and physiological mechanisms through which the coffee tree cope with drought stress (DaMatta, 2004; Pinheiro et al. 2005; Cavatte et al. 2012), little is known on how hydraulic vulnerability, HSM and lethal water potentials are correlated in coffee.

Recently, leaf vulnerability was assessed in four potted coffee cultivars by Nardini et al. (2014) who found P_{50} ranging from -0.6 to -1.0 MPa, which would imply that the coffee tree is highly vulnerable to drought. We herein suggest that such P_{50} values are highly unlikely to be observed under real field conditions given that coffee leaves reach Ψ_1 as low as -1.1 MPa on a daily basis at field capacity (Dias et al., 2007). Alternatively, cavitation and repair could be routine in coffee plants acting as a trigger for stomata closure as suggested by Nardini et al. (2014). These conflicting results urge for a proper evaluation of hydraulic vulnerability in coffee plants grown under real field conditions not only to assess the ecological importance of cavitation but also to resolve the risk of death by hydraulic failure.

Here we evaluated two field-grown coffee cultivars under severe (natural) drought and subsequent recovery with the following objectives: (1) to assess naturally occurring Ψ_{min} and determine reliable hydraulic safety margins and (2) to test

whether loss of conductivity is significant in leaves expanded in the drought season. Assessment of these points will allow a better comprehension on how the coffee tree is affected by drought aiming at envisaging the importance of hydraulic failure for the plant survival and functioning.

MATERIAL AND METHODS

The experiment was carried out under field conditions on a Cambic Podzol, in Viçosa (20°45'S, 42°15'W, 650 m a.s.l.), southeastern Brazil. The site is characterised by a subtropical climate, with a mean annual temperature of 20°C, and receives an average rainfall of 1200 mm, mainly distributed from September to March (growing season). Two coffee cultivars, Catuaí (*C. arabica* L. 'Catuaí Vermelho IAC 44') and Hybrid 12 (a hybrid of *C. arabica* x *C. racemosa* Lour.), were investigated. The trees have been cultivated at full sunlight conditions with no supplemental irrigation, as is usually performed in most coffee farms in southeastern Brazil. The coffee trees were grown at a spacing of 2.0 x 1.0 m in north-south oriented hedgerows. Six to seven trees, with approximately 2-m tall, were selected for uniformity and vigour and assigned in a completely randomised design. The experimental plot was one tree per hole.

Leaf hydraulic measurements (K_{leaf}) were made in January 2015 in leaves developed in the wet season (expected to present maximum K_{leaf}) according to the standard protocol outlined by Brodribb and Cochard (2009). Detached shoots with at least three expanded leaf pairs from at least three plants were bench dried, and K_{leaf} was determined at intervals of 0.25-0.5 MPa (Ψ_1) by measuring the rehydration flux of water into leaves using a hydraulic flowmeter. The relationship between Ψ_1 and K_{leaf} was used to determine the P_{50} (Ψ_1 , at 50% loss of K_{leaf}) from a sigmoidal curve fitted to the pooled data from each genotype. Measurements of Ψ_1 and instantaneous gas-exchange parameters were simultaneously performed using completely expanded leaves from the third or fourth pair from the apex of plagiotropic branches at 0800 h, 1200 h and 1600 h (Ψ_1 was additionally assessed at predawn). Leaf Ψ_1 was measured using the above mentioned pressure chamber. In October and January, fully expanded leaves were detached to produce pressure-volume curves, exactly as described in Pinheiro et al. (2005). From these curves, the osmotic potential at full turgor ($\Psi_{\pi(100)}$), the Ψ_1 at the turgor loss point (Ψ_{TLP}), the relative water content at the turgor loss point (RWC_{TLP}), and the bulk modulus of elasticity (ϵ) were estimated. Hydraulic safety margins (HSM) were calculated as described in Choat et al. (2012) using the average of the four lowest Ψ_1 measured under drought or control conditions as the value for Ψ_{min} . Then, a standard HSM based on P_{50} (HSM_{50}) were calculated as: $\text{HSM}_{50} = \Psi_{\text{min}} - P_{50}$.

The data from the experiment were analyzed using a completely randomized design with six replicates. The data were subjected to an analysis of variance (two-way ANOVA with all main factors evaluated as fixed factors) that was performed using the general linear models (GLM) procedure of SAS (version 9.1.) adopting $\alpha = 0.05$. When any interaction was found significant, the Slice statement of GLM was used to interpret the dependency effect between factors.

RESULTS AND DISCUSSION

Leaf vulnerability curves followed a sigmoidal behaviour with K_{leaf} declining gradually as a function of Ψ_1 . The Hybrid 12 displayed lower (more negative) P_{50} (26%) than Catuaí. In contrast, there were no cultivar differences in maximum fitted K_{leaf} which averaged on *c.* 2.6 mmol H₂O m⁻² s⁻¹ MPa⁻¹ (Fig. 1). Predawn Ψ_1 was *c.* -1.5 MPa in the dry season and was above -0.1 MPa when the rainy season started. Under drought, Catuaí and Hybrid 12 had their lowest Ψ_1 values (*c.* -4 MPa) at midday and Ψ_1 recovery was observed at the afternoon; such a recovery was more prominent in Catuaí. In the recovery and control conditions Ψ_1 markedly increased in all time periods with the lowest values observed at midday (*c.* -1.6 MPa). In the recovery phase, there was no difference in Ψ_1 from the morning to the afternoon for both cultivars. Catuaí exhibited the same previous behaviour under control conditions; the Hybrid 12, in turn, displayed a decreasing Ψ_1 until midday, followed by a Ψ_1 recovery in the afternoon (Fig. 1).

The variables of tissue water relations based on pressure-volume curves were similar for both cultivars under a same condition (drought or control) with the exception of ϵ , which was 50% higher in Catuaí in comparison with the Hybrid 12 under drought conditions (Table 1). Ψ_{TLP} was *c.* -2.3 and -1.9 MPa under drought and control conditions, respectively; however, osmotic and elastic adjustments were significant only in Catuaí. K_{leaf} values measured by the evaporative flux method (averaged on 2.9 mmol H₂O m⁻² s⁻¹ MPa⁻¹) did not differ between cultivars or leaf age (Fig. 1). Hydraulic safety margins (HSM) based on P_{50} or P_{88} were negative, implying that loss of conductivity occurred, for both cultivars under drought. Positive HSM was found in the recovery and control conditions, but they were narrower in Catuaí (by approximately 0.5 MPa) than in the Hybrid 12 (Table 1).

The P_{50} values of coffee leaves is in the lower range reported for montane rainforest species (Blackman et al., 2010), which suggests that coffee should behave as a moderately tolerant species to hydraulic dysfunctions. Most importantly, our vulnerability curves revealed a sigmoidal behaviour, which is in sharp contrast with the linear behaviour proposed recently for coffee by Nardini et al. (2014). Hence, as opposed to the suggestion of Nardini et al. (2014), major cavitation events should not be routine in coffee under well-watered conditions. This statement is in line with what has empirically been

observed in coffee plantations under field conditions (fast vigour recovery after long-term droughts) as well as with the proposed role for cavitation representing a threat to the mechanism of sap ascent that must be avoided (Delzon and Cochard, 2014). In any case, it must be emphasised that, under drought conditions, both cultivars operated with negative safety margins implying that hydraulic failure probably occurred in line with the findings of Choat et al. (2012) (Fig. 1 and Table 1). Indeed, Catuaí, the less drought tolerant cultivar, presented high rates of defoliation whereas the Hybrid 12 was able to retain much of its foliage (visual observation).

Table 1. Water potential at the turgor loss point (Ψ_{TLP} , MPa), osmotic potential at full turgor ($\Psi_{\pi(100)}$, MPa), relative water content at the turgor loss point (RWC_{TLP} , %), bulk modulus of elasticity (ϵ , MPa), leaf hydraulic conductance measured with the evaporative flux method (K_{leaf} EFM, $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$), and hydraulic safety margins ($\Psi_{min} - P_x$, MPa) based on P_{50} or P_{88} of coffee plants. When underlined, means for drought-stressed plants differ from those for control plants; * denotes differences between cultivars ($P \leq 0.05$). For the K_{leaf} measurements, drought refers to a leaf expanded under drought, but measured at the same time as the leaves that developed upon the wet season.

	Catuaí		Hybrid 12	
	Drought	Control	Drought	Control
Ψ_{TLP}	-2.29	<u>-1.87</u>	-2.21	-1.96
$\Psi_{\pi(100)}$	-1.89	<u>-1.44</u>	-1.79	-1.63
RWC_{TLP}	91.8	89.2	93.0	89.7
ϵ	24.1*	<u>15.5</u>	16.1	15.2
K_{leaf} EFM	3.10	3.24	3.31	2.31
$\Psi_{min_drought} - P_{50}$		-1.83		-1.38
$\Psi_{min_drought} - P_{88}$		-0.54		-0.13
$\Psi_{min_wet} - P_{50}$		0.52		1.06
$\Psi_{min_wet} - P_{88}$		1.81		2.31

Coffee seems to behave as an anisohydric species (Tausend et al, 2000), thus allowing midday Ψ_1 to decline as soil Ψ declines with drought (Fig. 1) in order to maintain photosynthetic activity. Although such strategy maximises WUE, it may come at a cost of increased hydraulic dysfunction, reflecting the trade-off involving efficiency and safety (McDowell et al., 2008). As coffee has an important share of leaf resistances outside the xylem (Gascó et al., 2004) it is expected that loss of turgor cause a significant decline in K_{leaf} by affecting extra-xylary pathways (Brodribb and Holbrook, 2006). Such decline is simpler in nature to be reverted given that it does not depend on positive pressure to refill emboli (Holloway-Phillips and Brodribb, 2011). On the other hand, cavitation *per se* may have play a role in K_{leaf} loss at more negative Ψ_1 ; This statement well agrees with earlier attempts to measure stem hydraulic vulnerability in coffee which would predict coffee death to happen at -6 to -8 MPa stem Ψ (Tausend et al., 2000). In fact, this also corroborates experimental observation of predawn Ψ_1 as low as -4 MPa in coffee plants without leading to plant death (DaMatta and Ramalho, 2006).

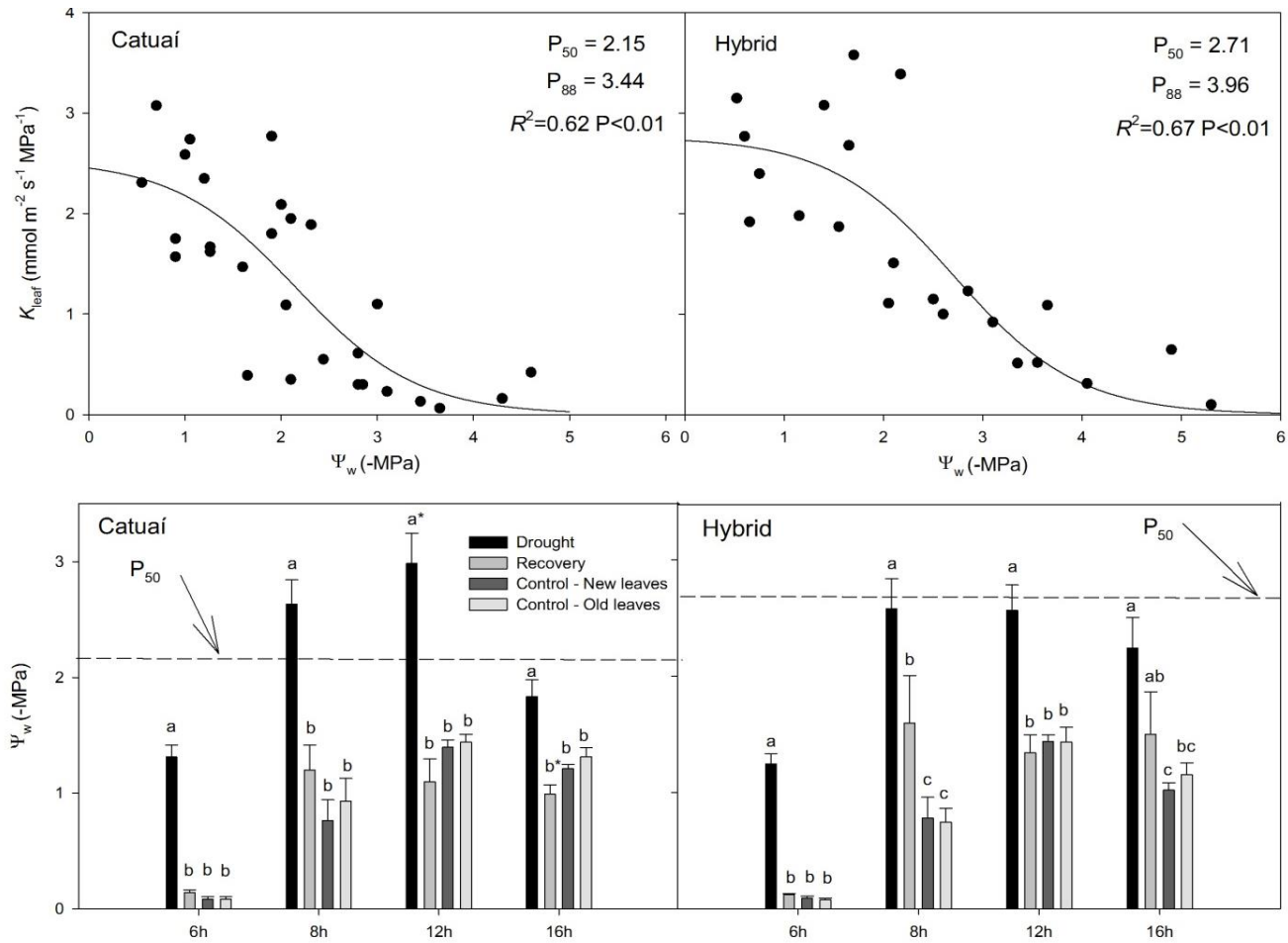


Figure 1. Responses of leaf hydraulic conductance (K_{leaf}) to declining leaf water potential (Ψ_l) during dehydration and the diurnal Ψ_l time-course in two coffee cultivars. Curves fitted are sigmoidal functions. Horizontal dotted lines indicate P_{50} in the diurnal Ψ_l time-course graphs. Only significant differences among means for water regimes within time are shown, as denoted by different letters; * denotes difference between cultivars within a given water regime ($P < 0.05$). Each bar represents the mean ($n = 6$) \pm SE.

CONCLUSION

As other angiosperms, the coffee tree operates under narrow safety margins even under well-watered growth conditions and it is subjected to hydraulic failure upon severe drought events. Difference in the level of foliage retention after drought between cultivars appears to be related to lower leaf vulnerability to hydraulic dysfunction, despite minor alteration in gas exchange parameters. In any case, leaves that did not cross a given threshold for leaf abscission, likely to be the Ψ_l at P_{88} , recovered from hydraulic loss presenting K_{leaf} similar to the new leaves that were developed over the rainy season, but constrained at their maximum realizable g_s . The advantage of such risky strategy is the maximisation of WUE, which may come at the cost of foliage loss. Further studies to determine hydraulic vulnerability in stems and roots as well in other varieties, are of utmost importance for a proper assessment of the impact climate changes will have for the coffee crop.

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