The Journal of Plant Hydraulics

The Journal of Plant Hydraulics 1: e0003

Soil water deficit decreases xylem conductance efficiency relative to leaf area and mass in the apple

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Submitted: February 14, 2014 **Published:** April 21, 2014.

Abstract

It is generally postulated that at the tree scale a drought-related decrease in hydraulic conductance is balanced by a decrease of leaf area. We hypothesized that, at the individual leaf scale, drought affects the allometry between leaf area or mass and hydraulics, leading to a non-linear relationships between these traits. The study was conducted on well-watered and on water-stressed shoots of several apple genotypes covering an extended range of leaf area. Working on dried leaves, we measured leaf lamina area and mass and analyzed their relationships with the maximal xylem hydraulic conductance of the water pathway through the parent shoot and the petiole connected to the leaf lamina. Drought decreased leaf area and mass in absolute values. It also changes the allometric relationships between these two variables: for a same decrease of leaf dry mass the water-stressed shoot had a lower decrease of leaf dry area than the well-watered shoot. Our study also showed that drought affected the stem-to-petiole hydraulics with a higher hydraulic efficiency in the well-watered shoot compared to the water-stressed shoot. We discuss that, compared to the well-watered condition, drought not only decreased leaf size, but also reduced xylem efficiency through the stem-to-petiole pathway with regard to the leaf area and mass supplied.

Introduction

Water availability is one of the major limitations to plant productivity (Wood 2005). Water use depends on genetic factors as well as on other factors such as the plant compartment, e.g. root vs. shoot (Regier et al. 2009), the combination between the variety and the rootstock for grafted perennials (Chaves et al. 2010), and more generally the environment, namely temperature and VPD (Chaves et al. 2010). Water deficit, hereafter referred to as drought for brevity, is particularly important for Mediterranean plants during late spring and summer and is likely to become of increasing importance in the future, not only in Mediterranean climates, but also in several parts of the middle and high latitudes. According to current climate change models for the 21st century, an increase in average temperature will likely occur in the near future, with more frequent extreme temperature and drought episodes (IPCC 2013).

Reactions induced by drought and affecting plant growth may be summarized as follows. Initially, there is a reduction of growth of expanding tissues related to a decrease of cell turgor and cell division rate (Tardieu 2013). Another short-term effect of drought is the stomatal closure resulting from combined processes of xylem hydraulics, sap composition, and chemical messages such as ABA (Tardieu 2013) entailing a reduction in carbon assimilation (Chaves et al. 2010). In the medium term, drought reduces organ size, namely internode and leaf, and whole-shoot growth through growth cessation (Chaves et al. 2010). This can be related or not to a decrease in length and/or in the number of leaves produced, depending on the intensity of stress (Wery 2005), which eventually leads to a decrease of overall gain in biomass (Guoth et al. 2009). Drought affects leaf expansion processes more than carbon assimilation, leading to higher concentrations of carbohydrates per unit of mass in water-stressed (WS) compared to well-watered (WW) plants (Dosio et al. 2011; McDowell 2011). Since leaf expansion becomes more limited by water fluxes as the leaf develops (Pantin et al. 2011), drought likely has a higher effect during the final phase of leaf growth than during the early stages.

Leaf size, shape, and function, are in part driven by the local hydraulic demand during the expansion phase (Zwieniecki et al. 2004), with a strong relationship between the major vein diameters and leaf area (Sack et al. 2012). There is also a positive relationship between leaf expansion, hydraulics and photosynthetic rate (Scoffoni et al. 2012). These results suggest that the leaf hydraulics and size, and its functioning, are intrinsically related in their responses to drought. As shown at the intra-specific level, leaf size is positively related to leaf hydraulic conductance, K_{leaf}, with a correlation between the theoretical axial hydraulic conductivity computed from the measurement of the number of xylem elements of the lamina and their diameters, and the leaf area to be supplied (Martre et al. 2001). Drought, by reducing leaf area, also decreases light interception and transpiration (Chaerle et al. 2005), affecting negatively vegetative biomass production, carbon supply to sinks and consequently fruit growth and yield, which is a main concern for horticultural plants (Corelli-Grappadelli and Lakso 2004; Naor 2006).

It is considered that, at the tree scale, the drought-related decrease in hydraulic conductance is balanced by a decrease of leaf area, allowing the tree to maintain "an almost unaffected leaf-specific hydraulic conductivity" when subjected to drought stress, resulting in a functional equilibrium (Limousin et al. 2009). Our hypothesis was that the effect of drought could not be summarized as a simple effect on biomass production, based on the assumption that traits such as leaf area, leaf mass and hydraulics are somehow linearly related as suggested by these results at the whole-tree level. Petiole xylem conductance ($K_{petiole}$) is positively related to the lamina area connected to that petiole, with no effect of the light environment, i.e. sun vs. shade, on the slope of the relationships (Sack et al. 2003). However, the water stress-induced decrease in K_{leaf} is related to leaf xylem embolism or collapse (Johnson et al. 2009) and also to changes in morpho-anatomical traits, namely smaller and denser conduits and veins, as well as increased conduit wall thickness (Nardini et al. 2012). We hypothesized that, beside the known decrease in leaf area and mass, the water-stress induced hydraulic and morpho-anatomical changes would also lead to a non-linearity of plant responses to drought and consequently to scaling relationships between hydraulic and morphological traits. In allometric terms, this means that a same increase of xylem efficiency would entail a different increase of a given leaf morphological trait in WS and WW plants.

The apple tree was chosen in our study as an example of perennial plant with a worldwide geographical and climatic distribution, and which is also cultivated in semi-arid regions where irrigation is crucial for regular cropping (Webster 2005). Among the complex interactions between morphology, anatomy, physiology and biochemistry that determine the aptitude of a genotype to adapt to drought, modifications of hydraulic relations play a major role (Bassett 2013). According to recent results, the mean value for the sap tension causing 50% loss of stem xylem hydraulic conductivity (P50) in the apple, ca. -4 MPa, is intermediate, between the values observed in two extreme genera, the highly resilient *Callitris* trees (ca. -12 MPa) and the highly vulnerable to drought-induced cavitation *Populus sp.* (ca. -2 MPa; see Lauri et al. 2011). For the present study, we made use of maximal xylem conductance of the water pathway through the stem towards the distal end of the petiole of the individual leaf, hereafter referred to as $K_{Stem\text{-}Petiole}$, measured at high pressure, i.e. between 0.4 and 0.5 MPa (see Materials and Methods). We relied on $K_{Stem\text{-}Petiole}$ as an easy-to-measure, integrated variable measuring the efficiency of water transport to the leaf lamina, with a good correlation between total petiole vessel cross-section area and hydraulic conductance of the petiole (Bucci et al. 2003), which has also been demonstrated at the stem level (Lauri et al. 2011). Based on this assumption, our objective was to get a better understanding of how drought affects biomass allocation into the leaf, namely mass and area, which are among the basic traits of the leaf investment strategies (Wright et al. 2004), in relation to the xylem water entering the leaf through the stem-to-petiole continuum.

First, we investigated, in a range of apple genotypes issued from a single progeny, the genotypic variations in leaf area and mass, and the effects of drought on these variables. Second, we analyzed in WS and WW shoots, across and within genotypes, the allometric coordination between leaf traits, and between these traits and $K_{Stem\text{-}Petiole}$, to assess a possible genotypic variability in these relationships. Third, we discussed the effects of the water regime on these allometric relationships from the point of view of the variation of water transport efficiency through the stem-to-petiole continuum.

Materials and Methods

Plant material and experimental setting

Two apple (*Malus domestica* Borkh.) cultivars, 'Starkrimson' and 'Granny Smith', and 15 hybrid genotypes issued from their progeny ('7', '23', '26', '35', '37', '38', '40', '41', '48', '54', '70', '96', '117', '121', '125') were chosen for their variability of leaf size (mean values between 25 and 80 cm²; Fig. 1a) and internode length (Segura et al. 2007). The two parent cultivars and the 15 hybrids are hereafter referred to as genotypes. In the beginning of February 2011, 12 scions per genotype were bench-grafted onto Pajam® 2 rootstock, a common rootstock in commercial orchards, and each composite tree was trained to a single shoot during the whole experiment. Young trees were placed in 4L plastic pots, each filled with an equal quantity of a potting mix composed of 40% brown peat, 30% composted pine bark, 20% disinfected soil and 10% pouzzolane (2/6mm), and grown in a greenhouse at Cirad Montpellier (43°37N, 03°52E), France. Photosynthetically active radiation (PAR), air temperature and humidity were recorded at one representative location in the greenhouse with PAR quantum sensor (SKP 215, Skye Instruments Ltd, Llandrindod Wells, UK) and humidity and temperature probe (HMP 155, Vaisala Oyj, Vantaa, Finland). Micrometeorological data were scanned every 30 seconds, averaged over 10-minute intervals and stored using a CR10X data logger (Campbell Scientific Ltd, Shepshed, UK). There was no artificial lighting during the whole trial. To avoid heat stress, shading was achieved by the use of 'paint on' shading liquid on upper windows from the beginning of June onwards. This shading limited solar radiation to a maximum of 740.2±348.5 µmoles m^{-2} s^{-1} in the greenhouse, which corresponded on average to 40% of the outside radiation.

During the pre-experiment period, from planting to the beginning of June 2011, plants were carefully drip-irrigated to prevent any drought stress. The fraction of transpirable soil water (FTSW) was measured to monitor root water (Wery 2005). For this, preliminary measurements were made on 15 pots containing young trees growing on the specific mix we used. As the maximum water content per pot was known, these conditions permitted an accurate control of water supply to the pots. Total transpirable soil water (TTSW) was calculated as the difference between the mass of pots at field capacity (FTSW=1.0) with full transpiration rate, and at the point where leaf transpiration rate fell below 10% and remained stable (with FTSW estimated as a rule of thumb at 0.1). Based on these preliminary data, the relative mass of water at these two thresholds was applied to each individual pot of the experiment, which was individually weighed at the beginning of the study. The mass of the pot at field capacity with the growing shoot at the beginning of the experiment was used, and the increase in mass due to shoot growth during the experiment period (ca. one month) was considered negligible. Based on previous results of one of our research teams (Regnard et al. 2008), the water deficit intensity, provoking a significant reduction of leaf area and stem growth across all genotypes, was set at FTSW=0.2. Pot mass was controlled every 2 days, and water added when necessary, so as to maintain the desired FTSW on the WS plants. Control-WW plants were maintained at FTSW=1.0 during the whole experiment by keeping the same drip-irrigation regime (ca. 0.5L daily amount) as before the beginning of the experiment. The experimental set-up was a completely randomized design with the combination of the two factors, genotype (x 17) and water regime (x 2), with six shoots per genotype x water regime.

The experiment began when the growing shoots reached 30 leaves unfolded on average and was carried out between the 6th of June and the 4th of July. For WS shoots, it included an eight-day period without any irrigation during which the amount of transpirable soil water decreased from FTSW=1.0 to FTSW=0.2, followed by 20 days of monitored water deficit at FSTW=0.2. Considering that apple leaves completely develop in 10 to 15 days in a temperature range of approximately 15°C to 34°C (Lauri and Térouanne 1995) WS plants were maintained at FSTW=0.2 during 15 days after July 4th to ensure that the last leaves completely developed under water stress. During the experiment, temperatures (mean±SD) remained between 16.1±2.3°C and 31.7±2.5°C, with leaf-to-air vapour pressure deficit (VPD, mean±SD) varying between 0.4±0.2 kPa and 3.1±0.7 kPa.

Stem-to-petiole hydraulics and leaf traits

Only the portion of shoots grown during the experiment was considered for both WW and WS shoots. At the end of the experiment, three to six fully developed leaves, depending on shoot growth, equally distributed along the shoot portion, were sampled, yielding to a total of 663 leaves. In the case of WS shoots, considering that on average three leaves were still folded at the beginning of the experiment and thus did not develop completely during the water stress period, only leaves located at the fourth rank and above were considered. Applying this rule excluded shoots with low growth, but sample size was always above 20 leaves for all genotype x water regime combinations.

First, the lamina of each leaf was separated from the petiole at the petiole-lamina junction and was immediately put in an oven (48H at 70°C) for later measurements of leaf traits. Hereafter, for convenience, leaf refers to the lamina only. Leaf dry area (LDA) was measured using an AM300 Portable Leaf Area Meter (ADC Bioscientific Ltd., Hoddesdon, UK), and leaf dry mass (LDM) was measured to the tenth of a milligram (balance Sartorius, model MC1, AC 210 P). Leaf traits were measured on dried leaves to avoid any bias in the measurement of fresh leaf area related to the difficulty of measuring leaf dimensions in a same state of turgor for all the leaves, even with repeated sampling. This procedure thus took into account shrinkage of leaf area compared to fresh leaf area (Blonder et al. 2012). Our assumption was that there was a linear relationship between areas measured on fresh and on dried leaves. However, as this relationship was probably dependent on the water regime, in relation to a change in leaf density and/or thickness, it was not possible to compare the leaf mass per unit area (LMA; ratio of leaf dry weight to leaf fresh area) in the two water regimes.

Second, maximum hydraulic conductance of the xylem pathway from the bottom of each sampled shoot portion to the distal end of the petiole of each removed lamina, hereafter referred to as maximum stem-to-petiole continuum conductance ($K_{\text{Stem-Petiole}}$; mmol s^{-1} MPa^{-1}), was measured. Our assumption was that there was a hydraulic integration in the one-year-old shoot portion due to the hydraulic communication between adjacent vessels through intervessel pits, and also due to the small length and the young age (less than six weeks at the time of hydraulic studies) of the shoot portion. We used a high pressure flow meter (HPFM, Dynamax, Houston, USA) apparatus, which is based on the perfusion of deionized and filtered water at a given pressure (P, 0.4 - 0.5 MPa) at the bottom of the cut shoot portion and measurement of the flow of water exudation (F, mmol s^{-1}) at each individual petiole distal end previously cut. Water exudation was measured using a weighed piece of dry cotton applied for 1 min to the petiole distal end cut surface. The difference in mass, to the nearest tenth of a milligram, before and after water exudation, gave the amount of water exuded (see the same procedure applied to buds in Lauri et al. 2008). In comparison with other studies (e.g. Sack et al. 2002), the petiole was not detached from the parent shoot in our study.

Data analysis

Two types of analyses were developed. First, the general effects of the genotype and of the water regime on LDA, LDM, $K_{\text{Stem-Petiole}}$ and on the ratios LDA/$K_{\text{Stem-Petiole}}$ and LDM/$K_{\text{Stem-Petiole}}$ were analyzed through a two-way ANOVA without interaction, our objectives being to assess the proper effects of each factor. The shoot effect was not significant for each variable and was not considered in the study. The effect of drought on these leaf and hydraulic traits was then assessed for each individual genotype with a t-test for paired data. The ordinary least square (OLS) regression between the leaf trait values in the two water regimes across genotypes was analyzed through the Pearson and the non-parametric Kendall-τ coefficients, giving a measure of the strength of the relationship and of the agreement between the ranking of the genotypes in the two water regimes, respectively. Statistical analyses were done using R software, version 2.14.0 (R Development Core Team 2012), with package 'car' (lm(), t.test() and correlation()).

Second, three covariations (Y vs. X) were explored. The first one described the relationship between the two leaf morphological traits: LDA vs. LDM. The other two investigated the relationships between LDA or LDM and $K_{\text{Stem-Petiole}}$. Our analyses made use of static allometry concepts, which deal with the covariation of traits of a given entity, here a leaf, within a particular ontogenetic stage, namely adult leaves. Allometric relationships usually refer to a power mathematical equation: $Y=b.X^a$, linearized as $\log_{10}(Y)=\log_{10}(b)+a.\log_{10}(X)$ where the intercept '$\log_{10}(b)$' with the dimension of Y is the allometric constant, and the exponent 'a' with the dimension of Y/X is the slope. When a=1, it indicates an isometric relationship, that is a proportional relationship whatever the values of X and Y. When $a\neq1$, it denotes an allometric relationship, either negative when a<1 (i.e. Y/X decreases when X increases) or positive when a>1 (i.e. Y/X increases when X increases) (Preston and Ackerly 2004). The standardized major axis (SMA) method was chosen for line fitting between the $\log_{10}$-transformed variables because it calculates the line of best fit without a priori hypothesis on the dependence of one variable on the other (Warton et al. 2006). The effects of the water regime were tested across all genotypes and then for each individual genotype. At these two scales, these relationships were analyzed in two steps. First, the effect of the water regime on the slope was tested with two alternatives, no difference in slopes or difference in slopes. In the former case, differences for Y-intercept and for shift along the lines with common slope could then be carried out (Warton et al. 2006). In the latter case, it indicated that the water regime affected the relationship between the two variables, and the other tests, on the Y-intercept and on the shift along the lines with common slope, were not relevant. The isometry (a=1) of the relationships was also tested against negative or positive allometry. All tests were computed using the SMATR software (Standardised Major Axis Tests and Routines, http://bio.mq.edu.au/ecology/SMATR/, accessed 1 September 2012; Falster et al. 2006).

A significance level of P<0.01 was used for all the analyses.

Results

Leaf area and mass, and $K_{\text{Stem-Petiole}}$ *variations across genotypes and water regimes*

There were highly significant effects of the genotype and the water regime on LDA (genotype: Df=16, F=14.9, P<0.001; water regime: Df=1, F=1084.4, P<0.001; Fig. 1a), LDM (genotype: Df=16, F=11.6, P<0.001; water regime: Df=1, F=1134.8, P<0.001; Fig. 1b) and $K_{\text{Stem-Petiole}}$ (genotype: Df=16, F=12.9, P<0.001; water regime: Df=1, F=1911.0, P<0.001). The three variables were always significantly higher in WW compared to WS shoots, with a 3 to 5.4 WW/WS ratio for LDA, a 2.7 to 4.8 WW/WS ratio for LDM and a WW/WS 2.5 to 8.1 ratio for $K_{\text{Stem-Petiole}}$ (Fig. 1a,b,c).

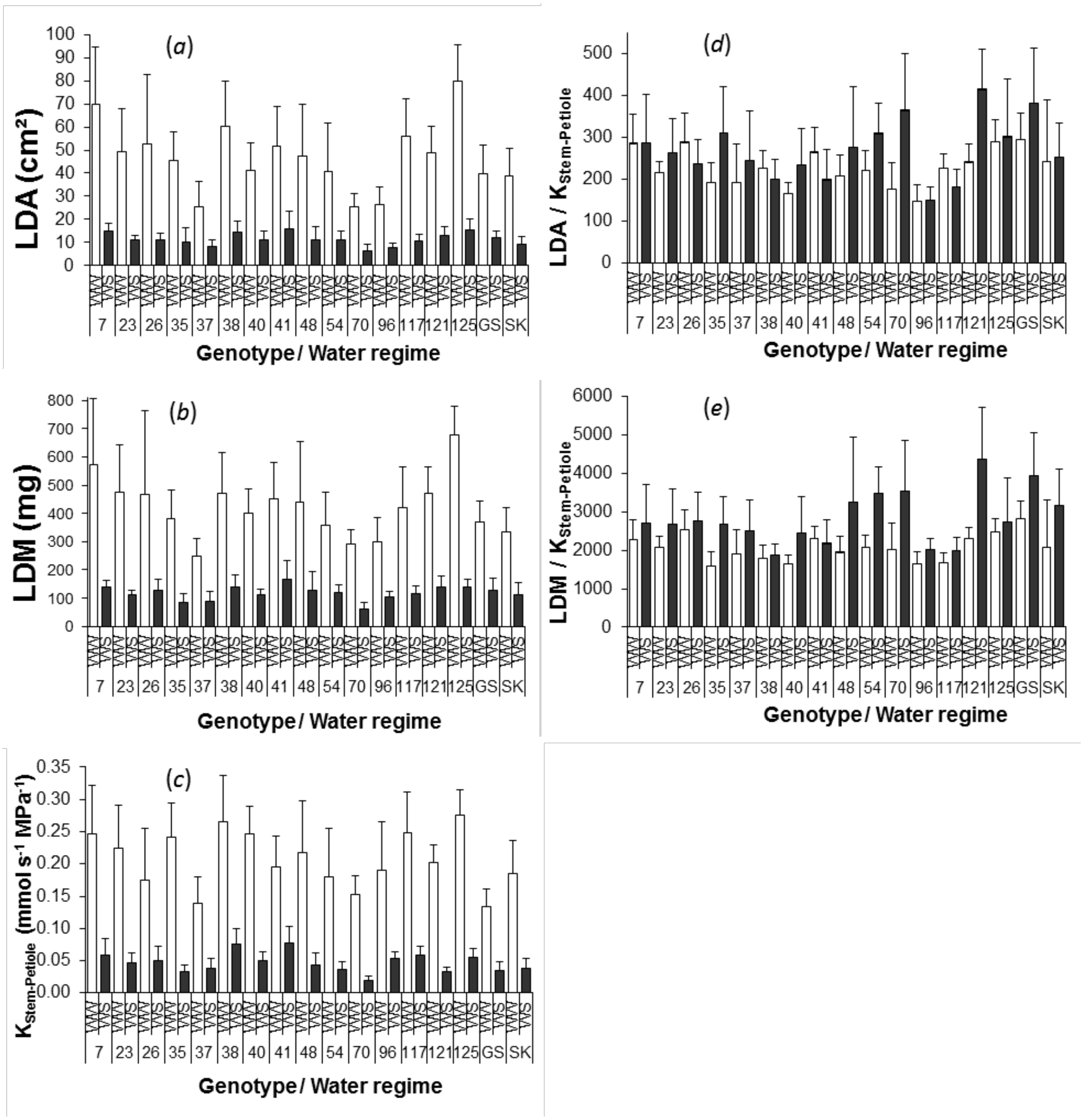

Figure 1: Variations of individual leaf traits across apple genotypes and water regimes, leaf dry area (LDA) (a), leaf dry mass (LDM) (b), $K_{\text{Stem-Petiole}}$ (c), and the two ratios LDA/$K_{\text{Stem-Petiole}}$ (d) and LDM/$K_{\text{Stem-Petiole}}$ (e). Data (mean+SD) are for 17 apple genotypes including the two parent cultivars, 'Starkrimson' (SK) and 'Granny Smith' (GS), and 15 of their progenies identified by their number, in two water regimes, well-watered (WW; white bars) and water-stressed (WS; black bars). For LDA, LDM and $K_{\text{Stem-Petiole}}$, each genotype shows a significant difference (t-test, $P<0.01$; data not shown) between WW and WS values. For LDA/$K_{\text{Stem-Petiole}}$ and LDM/$K_{\text{Stem-Petiole}}$ WW and WS values are similar or differed depending on the genotype (t-test, $P<0.01$; data not shown, see text).

There was a significant and positive relationship between WW and WS genotypes for LDA and LDM, and roughly the same ranking between genotypes, in the two water regimes (R and τ in Fig. 2a,b). This was not the case for $K_{\text{Stem-Petiole}}$ which was not significantly correlated between the two water regimes (R and τ in Fig. 2c).

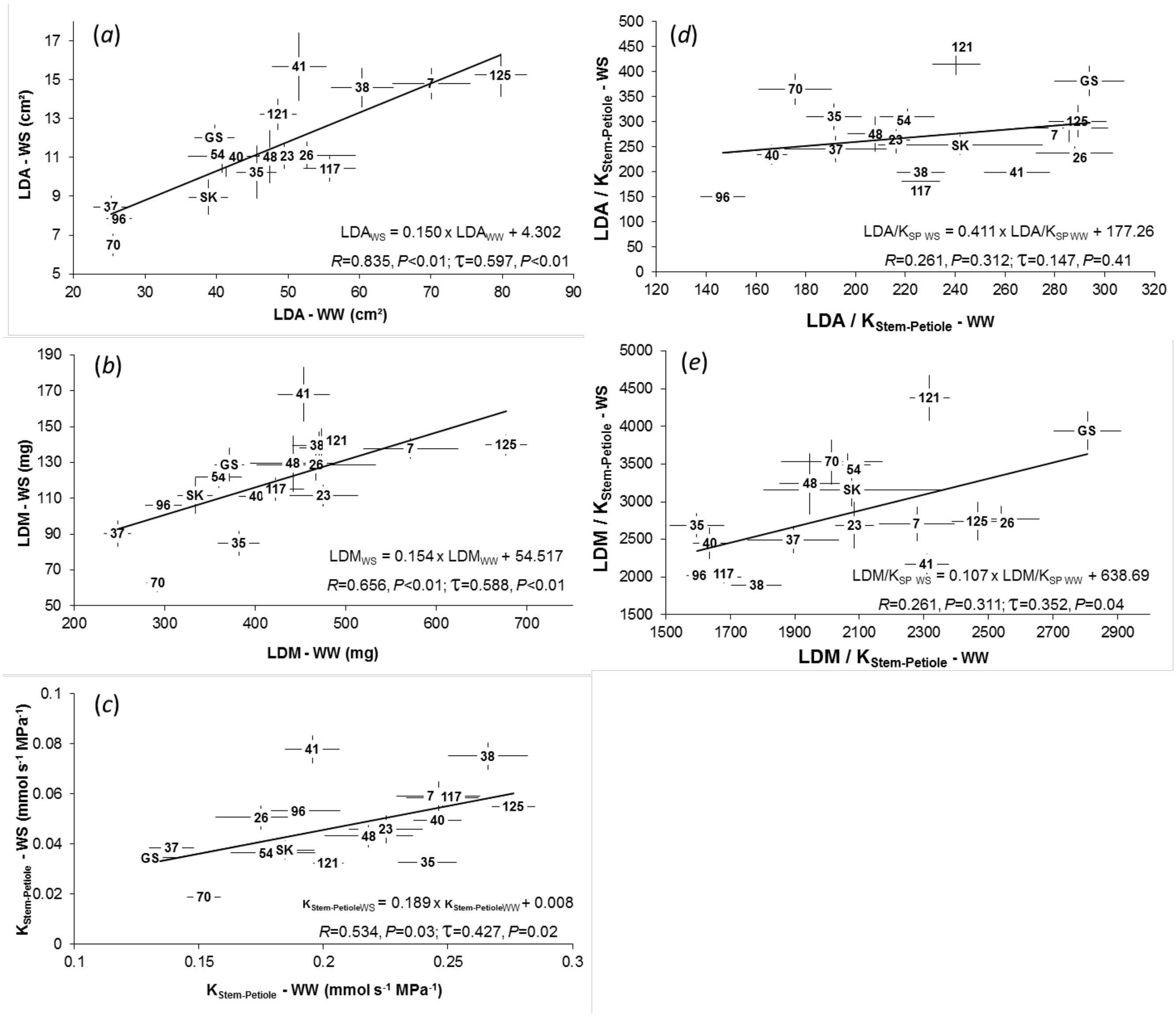

Figure 2: Ordinary least square (OLS) regressions of individual leaf and stem-to-petiole hydraulic traits between well-watered (WW) and water-stressed (WS) shoots: leaf dry area (LDA) (a), leaf dry mass (LDM) (b), $K_{Stem\text{-}Petiole}$ (c), and the two ratios LDA/$K_{Stem\text{-}Petiole}$ (d) and LDM/$K_{Stem\text{-}Petiole}$ (e). Data (mean±SE) are for 17 apple genotypes including the two parent cultivars, 'Starkrimson' (SK) and 'Granny Smith' (GS), and 15 of their progenies identified by their number. Correlations are quantified through the Pearson (R) and the Kendall (τ) coefficients across genotype means and are figured at the bottom of each graph. For a better legibility, $K_{Stem\text{-}Petiole}$ in line equations in (d) and (e) are abbreviated as K_{SP}.

Relationships between leaf area and mass

Across all genotypes, LDA and LDM were highly and positively correlated with a positive allometry in both WW and WS conditions (Table 1a). This indicated a more than proportional increase in LDA compared to LDM in the two water regimes, with however a slight but significantly higher slope for WW than for WS leaves (Fig. 3a; Table 1a). At the genotype level, most genotypes exhibited an isometric relationship (13 and 12 out of 17 relationships for WW and WS, respectively) with however four and two positive allometries for WW and WS, respectively, and a negative allometry for only one genotype in the WS condition (Table 1b).

Table 1. Standardized major axis (SMA) regressions between leaf dry area (LDA) and leaf dry mass (LDM), and between leaf dry mass (LDM) or leaf dry area (LDA) and the stem-to-petiole maximal xylem conductance ($K_{\text{Stem-Petiole}}$). By convention, relationships are in the form Y vs. X. All analyses are on $\log_{10}$-transformed data and are for 17 apple genotypes including the two parent cultivars 'Starkrimson' and 'Granny Smith' and 15 of their progenies, cultivated in two water regimes, well-watered (WW) and water-stressed (WS). Results are shown for (a) all genotypes, and (b) individual genotypes considering the number of genotypes in each type of allometric relationships.

In a) n: sample size; Slope: slope of the relationship; R^2 and P_{R^2}: coefficient of determination of the SMA regression and associated probability; $P_{\text{slope=1}}$: probability associated to isometry; $P_{\text{Common slope}}$: probability associated to the test of common slope for the regressions for WW and WS leaves; Type of allometry: +, positive allometry (slope>1); -, negative allometry (slope<1). Differences for Y-intercept and for shift along the common slope were not tested because $P_{\text{Common slope}}$ indicated that the two slopes were significantly different.
In b) ns: slope of the relationship not significantly different from 0; Isometry (slope=1); positive allometry (slope>1); negative allometry (slope<1). Threshold for significant relationships is P=0.01. Covariations of traits and fitting lines are illustrated in Figure 3.

Relationship	LDA vs. LDM		LDM vs. $K_{\text{Stem-Petiole}}$		LDA vs. $K_{\text{Stem-Petiole}}$	
Water regime	WW	WS	WW	WS	WW	WS
a) Across all genotypes						
n	339	324	339	324	339	324
Slope	1.207	1.083	1.107	0.773	1.336	0.838
R^2	0.837	0.741	0.574	0.460	0.552	0.371
P_{R^2}	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
$P_{\text{slope = 1}}$	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
$P_{\text{Common slope}}$	<0.01		<0.01		<0.01	
Type of allometry	+	+	+	-	+	-
b) Individual genotypes: nb of genotypes in each type of allometry						
ns	0	2	3	6	4	7
Isometric	13	12	10	10	7	6
Positive allometry	4	2	4	0	6	1
Negative allometry	0	1	0	1	0	3

Leaf traits and maximum stem-to-petiole hydraulic conductance

There were significant effects of the genotype and the water regime on the ratios LDA/$K_{\text{Stem-Petiole}}$ (genotype: Df=16, F=11.2, P<0.001; water regime: Df=1, F=37.7, P<0.001) and LDM/$K_{\text{Stem-Petiole}}$ (genotype: Df=16, F=13.7, P<0.001; water regime: Df=1, F=156.8, P<0.001). However, these significant effects were mostly due to some genotypes (eg, 70 and 121 for both LDA/$K_{\text{Stem-Petiole}}$ and LDM/$K_{\text{Stem-Petiole}}$) where these ratios were significantly higher in WS than in WW shoots (Figure 1d,e). Moreover, there were no significant correlations between these ratios in the two water regimes (Figure 2d,e).

The relationships between LDA and $K_{\text{Stem-Petiole}}$ for WW and WS shoots were similar to those between LDM and $K_{\text{Stem-Petiole}}$, with however, slightly better correlations in the latter case (Fig. 3 b,c; Table 1a). Results are thus presented for LDM only.

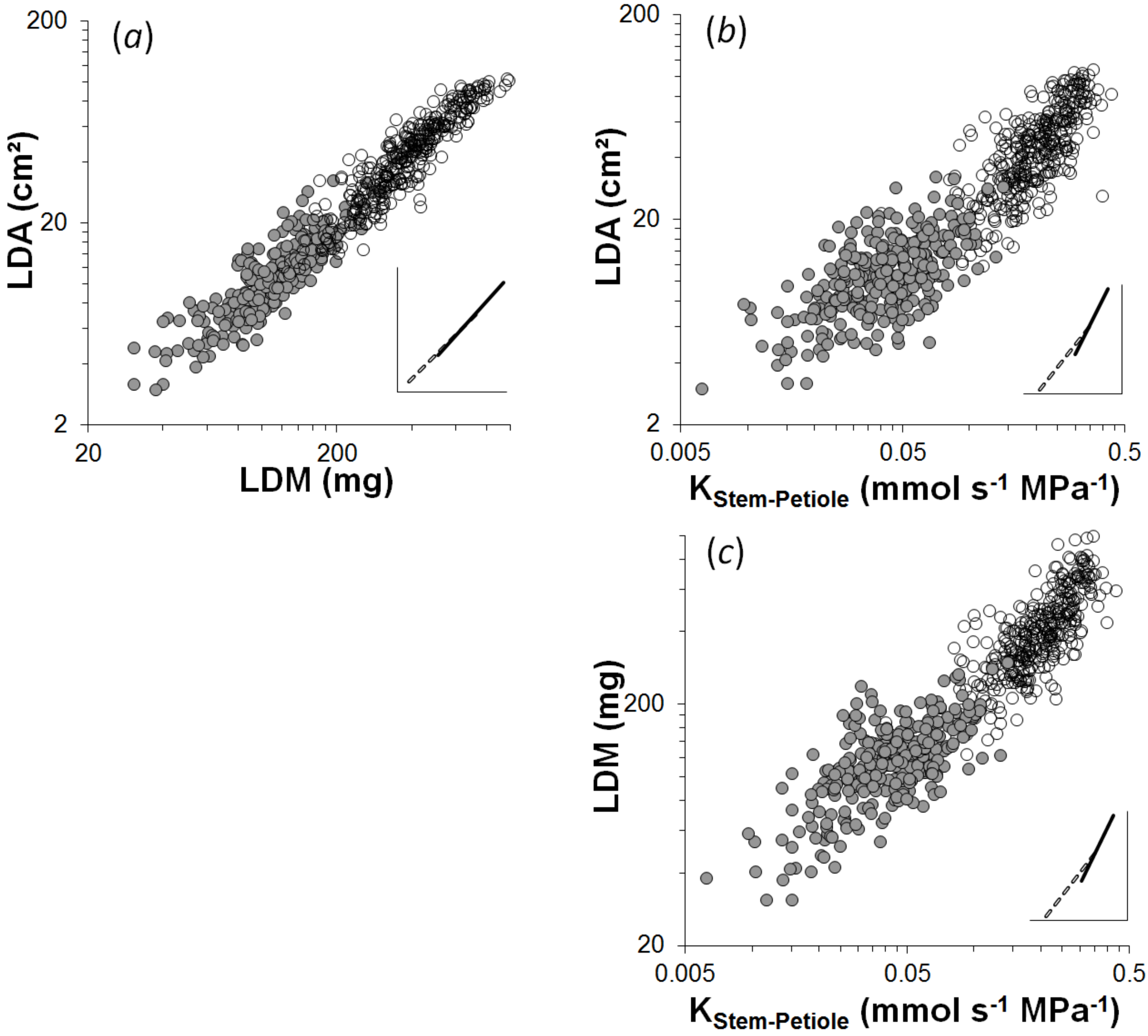

Figure 3: Covariation between leaf traits (a), and between each leaf trait and the stem-to-petiole continuum maximal xylem conductance, $K_{Stem\text{-}Petiole}$ (b-c) in Log_{10} axes. Bivariate line fitting with standardized major axis method (SMA) for log_{10}–log_{10} relationships between leaf dry area (LDA) and leaf dry mass (LDM) (a), leaf dry area (LDA) and $K_{Stem\text{-}Petiole}$ (b), and leaf dry mass (LDM) and $K_{Stem\text{-}Petiole}$ (c). Data are for 17 apple genotypes including the two parent cultivars, 'Starkrimson' and 'Granny Smith', and 15 of their progenies, cultivated in two water regimes, well-watered (WW, open circle) and water-stressed (WS, grey circle). Each symbol represents one leaf. For each relationship, fitting lines are figured in inset. Fitting lines parameters are detailed in Table 1.

Across genotypes, and in both WW and WS conditions, LDM and $K_{Stem\text{-}Petiole}$ were positively and significantly correlated (Table 1a). The slopes of the relationships were significantly different from 1 for the two water regimes, indicating that increasing $K_{Stem\text{-}Petiole}$ was related to a non-proportional increase in LDM (Table 1a). However, the type of allometry between the two variables depended on the water regime, with a positive allometry for WW shoots (Slope=1.107; Table 1a) vs. a negative allometry for WS shoots (Slope=0.773; Table 1a). At the genotype level, the relationships were isometric for 10 out of 17 genotypes in either WW or WS condition. Four genotypes had a positive allometry in the WW condition, whereas one genotype had a negative allometry in the WS condition (Table 1b).

Discussion

The leaf is a major hydraulic bottleneck in the whole-plant hydraulic system and the way water moves through the stem-to-petiole pathway and then the lamina is critical to the plant functioning under a changing environment (Sack and Scoffoni 2013). As shown here in the apple, drought not only decreased leaf area and mass (Fig. 1a,b) but also altered the relationships between these two morphological leaf traits, and between these two traits and the efficiency of the sap pathway to the lamina (Fig. 3b,c). Our discussion will focus on general trends across genotypes where relationships were allometric, either positive or negative, although these relationships were more variable at the intra-genotypic scale, varying from isometry to positive or negative allometry (Table 1).

Drought differentially affected leaf area and mass

Drought-induced changes in leaf area and mass have consequences on how the plant copes with its environment, since both traits are means by which the plant matches environmental cues. The reduction of leaf area, and more generally shoot biomass, in water-stressed shoots is interpreted as an avoidance mechanism preserving water resource in the soil through a reduction of leaf transpiration, with the adverse effect of reducing photosynthetic rate (Tardieu 2013). Our results agreed with this general statement and indicated, depending on the genotype and considering dried leaves, a 3- to 5-fold reduction of LDA and LDM in WS compared to WW shoots (Fig. 1a,b). We also showed a rather similar ranking of the genotypes in the two water regimes (Fig. 2a,b), which indicated that phenotyping for leaf area and mass in well-watered conditions would give a rather good approximation of the ranking of the same genotypes in drought conditions. We showed here significant and positive relationships between LDA and LDM (Table 1a; Fig. 3a), and a positive allometry (slope>1) between these two traits in the two water regimes, indicating that the drought-related reduction of leaf size was more related to a reduction of LDA than to a reduction of LDM (Table 1a; Fig. 3a). The slightly higher but significantly different slope for leaves in the two water regimes (Table 1a; Fig. 3a) also indicated that, for a same decrease of LDM, LDA decreased less for WS shoots than for WW shoots. However, due to almost no overlapping of LDA and LDM data between the two water regimes, it was not clear from our study if the decrease of the allometric coefficient in WS shoots compared to WW shoots was due to a drought-mediated effect or to a leaf size effect (Fig. 3a).

Allometric relationships between leaf size and stem-to-petiole xylem conductance were affected by drought

It is often assumed that the leaf conductance, K_{leaf}, can be normalized by leaf area or leaf mass ($K_{leaf,area}$ or $K_{leaf,mass}$; e.g. Simonin et al. 2012). Calculating such a ratio is based on the assumption that there is an isometric relationship between the two traits, i.e. a proportional variation of one trait relative to the other without any size effect. At the genotype level, isometry was observed between LDA or LDM and $K_{Stem\text{-}Petiole}$ in about half of the cases, depending on the genotype and the water regime (Table 1b). It is noticeable that the few positive allometries observed at the genotype level were on WW shoots (four and six for LDM and LDA, respectively). There was no (for LDM) or only one (for LDA) positive allometry in WS shoots (Table 1b). There were no negative allometries at the genotype level on WW shoots whereas there were one and three negative allometries for LDA and LDM, respectively, in WS shoots (Table 1b). These differences between WW and WS shoots at the genotype level could in part explain the fact that, across genotypes, there were positive or negative allometric relationships between LDA or LDM and $K_{Stem\text{-}Petiole}$ depending on the water regime.

In our study, LDA and LDM were both positively and allometrically related with $K_{Stem\text{-}Petiole}$. These results a posteriori confirmed that working with water perfused at high pressure at the base of the shoot ensured that the $K_{Stem\text{-}Petiole}$, investigated here instead of the xylem conductance of the petiole only, was a relevant variable to analyze the relationships between xylem conductance through the stem-to-petiole pathway and the leaf area supplied distally. Across genotypes, we showed a positive allometry between LDA or LDM and $K_{Stem\text{-}Petiole}$ for leaves of WW shoots, i.e. an increase of the ratio LDA/$K_{Stem\text{-}Petiole}$ or LDM/$K_{Stem\text{-}Petiole}$ when $K_{Stem\text{-}Petiole}$ increased. We also showed a negative allometry for leaves of WS shoots, i.e. a decrease of the ratio LDA/$K_{Stem\text{-}Petiole}$ or LDM/$K_{Stem\text{-}Petiole}$ when $K_{Stem\text{-}Petiole}$ increased. These results indicated, across genotypes, an increase in hydraulic efficiency in WW shoots compared to WS shoots. Therefore, our study indicated that beyond the general decrease in biomass production, drought also affected the stem-to-petiole hydraulics of the WS shoots compared to the WW shoots. Whether the drought-induced variations in leaf mass and hydraulics were related to leaf thickness and/or to leaf density or to local hydraulic demand during the expansion phase (Zwieniecki et al. 2004), and how these traits vary with the genotype, should be further studied. As suggested from studies on sun and shade leaves of *Quercus rubra*, the adaptation to local evaporative demand starts relatively early in leaf growth and partly determines the distribution of vein density across the lamina (Zwieniecki et al. 2004). We may therefore hypothesize that drought not only reduces in absolute value the efficiency of the water pathway to each individual lamina (reduction of $K_{Stem\text{-}Petiole}$), in relation to the decrease of leaf area and mass as observed here, but also entails a more opportunistic lamina growth leading to a variability of lamina size in order to tightly and dynamically adjust itself to the micro-environment. The smaller leaf area but also the shorter internodes (data not shown) of WS shoots would also likely alter light interception and leaf temperature compared to the WW shoots as shown at the whole-tree scale (Padhi et al. 2012).

The effects of drought on the relationships between leaf size and hydraulics could be interpreted through a water transport efficiency perspective

There was a high variability of the ratios LDA/$K_{Stem\text{-}Petiole}$ and LDM/$K_{Stem\text{-}Petiole}$ depending on the genotype, with however a tendency towards a higher biomass allocation to the lamina per unit of maximal water transport through the stem-to-petiole xylem pathway in WS shoots (Figure 1d,e). This first interpretation based on the ratios is however misleading because it only gives a mean value of the ratio over the whole values for a given water regime, for a genotype or across genotypes. The negative and positive allometries for WS and WW shoots, respectively, evidenced that both LDA/$K_{Stem\text{-}Petiole}$ and LDM/$K_{Stem\text{-}Petiole}$ change with leaf size and/or with $K_{Stem\text{-}Petiole}$ in a different way depending on the water regime: a same increase in $K_{Stem\text{-}Petiole}$ entailed a lower gain in LDA or LDM in WS shoots than in WW shoots (Figure 3b,c). This suggested a higher xylem transport efficiency in WW shoots compared to the WS ones. As drought tolerance induces changes in several morpho-anatomical features such as an increase of wood density in stems (Hacke et al. 2001) and/or of lignification in leaves (Claeys and Inzé, 2013), this results in the higher cost of the vascular system of a drought-tolerant species compared to the vascular system of a drought-sensitive one (Nardini et al. 2012; Hacke et al. 2001). Our results would support these findings at the intra-specific scale, i.e. when two plants of a same genotype are submitted to either a well-watered or a drought condition, and suggested that the changes of allometries evidenced here between WW and WS shoots were related to morpho-anatomical responses to drought. Moreover, in our study, the measurement of $K_{Stem\text{-}Petiole}$ was done at high pressure and thus likely overestimated the actual $K_{Stem\text{-}Petiole}$ that could be measured at a low pressure close to physiological pressure in the living shoot. This was especially true for WS shoots which were presumably affected by a higher proportion of cavitated conduits than WW shoots. It is likely that the mechanisms underlying morpho-anatomical responses to drought are related to the coordination between the development of the petiole and the lamina vasculature (Nardini et al. 2012), and the genotype-dependent functioning of stomata (Cruiziat et al. 2002). By analogy to Simonin et al. (2012) taking the ratio of K_{leaf} to leaf mass as a measure of the cost of water transport, we suggest that WS shoots could also be characterized by a higher cost efficiency for leaf construction than WW shoots. The matching between the maximum water transport to the leaf ($K_{Stem\text{-}Petiole}$ here) and the maximum water loss (positively related to stomatal conductance), is usually stated for leaves (Cruiziat et al. 2002). How our results can be connected to the lower stomatal conductance typical of leaves on water-stressed, less transpiring, plants (data not shown; Chaves et al. 2010), needs further investigations. Furthermore, how these short-term adaptations, over one month here, at the hydraulic and morphological levels may change or not over a longer term opens new research perspectives.

Our study evidenced that drought changed the allometric relationships between stem-to-petiole xylem conductance and leaf area or mass. A possible interpretation could be that during the transition from sink to source during leaf growth, which occurs concurrently with the progressive hydraulic limitation whatever the water regime (Pantin et al. 2011), the petiole diameter is likely designed with low hydraulic limitation. As the leaf grows, the progressive hydraulic constraint would increase more in the water-stressed condition compared to the well-watered condition. In the first case, drought would entail a higher discrepancy between the actual $K_{Stem\text{-}Petiole}$ and leaf size for the large leaf compared to the small one eventually leading to the negative allometry between $K_{Stem\text{-}Petiole}$ and leaf area and mass shown here (Table 1a). On the contrary, the non-limitation of water supply in the WW shoot would increase more than proportionally the lamina area and mass built per unit of $K_{Stem\text{-}Petiole}$ as leaf size increases, leading to the positive allometry between these traits. In this sense our results support the idea that leaf ontogeny is crucial to correctly interpret the relationships between leaf traits and petiole hydraulics (Pantin et al., 2011), especially in the context of how the plant adapts to drought.

Transpiration was not measured in our experiment. However, our results suggested that the postulated balance between water transport capacity and leaf transpiring area, maintaining a constant leaf-specific hydraulic conductance in response to drought (Limousin et al. 2009), can be upheld as a trend at the whole-tree level but does not appear to be so tightly adjusted at the level of the individual leaf. It is likely that, in response to the environment, a great plasticity is effective at the leaf scale since early ontogenic stage, permitting a tight coordination of leaf water gain and loss to determine actual leaf area and mass.

Leaf lifespan is also an important trait positively related to the plant's return on investment (Wright et al. 2004). Although they are not presented here, companion studies developed on the same apple genotypes showed that WS shoots were also prone to speed up leaf senescence and to shed their leaves more rapidly in the autumn than WW shoots, confirming previous results (Tuberosa 2012). Therefore, the detrimental effects drought has on leaves of WS shoots could result from two interacting factors: (i) the negative allometry between LDM or LDA and the stem-to-petiole xylem conductance, i.e. the lower xylem conductance efficiency, as opposed to the positive allometry for leaves on WW shoots (Table 1a), and (ii) a shorter leaf lifespan compared to leaves on WW shoots.

Conclusions

The analysis of the relationships between stem-to-petiole xylem conductance and final leaf area and mass across several apple genotypes showed that drought not only decreased leaf size but also altered the allometric relations between these traits. Our study demonstrated that the water regime did not strongly affect the ranking of genotypes for leaf area and mass. However, across genotypes, drought significantly, although slightly, decreased the positive allometry between the two traits, meaning that for a same decrease of leaf dry mass the WS shoot had a lower decrease of leaf dry area than WW shoot. Conversely, for a same decrease of leaf dry area the WS shoot had a higher decrease of leaf dry mass than the WW shoot. Nevertheless, the postulated balance between water transport capacity to the leaf and leaf transpiring area cannot be upheld at the scale of the individual leaf. Over a large range of leaf size, i.e. across genotypes, changes in allometries between leaf area or mass and maximal xylem conductance through the stem-to-petiole xylem pathway demonstrated here that a same amount of additional water entering a lamina led to a higher final leaf area or mass in the WW shoot compared to the WS shoot. This result can be interpreted as a higher xylem transport efficiency to the leaf in the WW shoot compared to the WS shoot.

Acknowledgements

The authors thank Gilbert Garcia for his help in taking care of the trees. This work has been partly funded under the EU seventh Framework Programme by the FruitBreedomics project N°265582: Integrated Approach for increasing breeding efficiency in fruit tree crop. The views expressed in this work are the sole responsibility of the authors and do not necessary reflect the views of the European Commission.

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