# A preliminary investigation into the relationship between petiole and water-related traits in tropical flowering trees

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## Abstract

Water deficit impacts photosynthetic productivity and whole plant energy budgets, and generally leads to stress and decreased growth. Severe water deficit can lead to embolism formation and plant death. The petiole plays a key role in mitigating against water stress, as the petiole is the terminal region of water transport before the leaf. Little is known about the role of the petiole in water relations in tropical trees. This study aimed to provide a preliminary investigation into the relationship between petiole traits, and water-related traits: leaf area and plant relative growth rate. We hypothesised species tropical flowering trees with larger petiole volume would have larger leaf area and higher relative growth rate. We measured petiole volume, a proxy for hydraulic conductance and leaf area in 10 species of tropical angiosperms.

We found petiole volume scaled with internal structures and leaf area. Petiole volume increased with increasing size of the largest xylem vessels. Large vessels have larger hydraulic conductance than small vessels. Petiole volume also increased with leaf size, which may be related to hydraulic vulnerability segmentation. Within families, RGR was correlated with petiole volume, which suggests the potential for a relationship between plant lifespan and hydraulic conservatism. More research is needed into the relationship between lifespan, petiole and hydraulic strategy. We also found that petiole volume could be used as a proxy for hydraulic conductance. Measuring petioles from outer dimensions is non-destructive and could be used in future research as a proxy for conduit conductivity of living plants. Improved understanding of the physiology that underpins water stress response is particularly important considering the predictions for reduced rainfall availability in many regions under projected climate change scenarios.

**Key Words:** ecology, ecophysiology, water relations, hydraulic conductance

## Introduction

Water is vital for plant survival and function. Water is involved in key plant processes, such as photosynthesis, growth and repair, turgidity for organ structure, and evapotranspiration. When water becomes limited plants can become adversely impacted, as reduced gas exchange has negative implications for growth and survival (Bray 1997; Hsiao 1973). Water deficits occur when soil water availability is reduced in the

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environment and when evapotranspiration demand is high. Plants are periodically subjected to water deficit during natural cycles of water availability, and therefore have a range of strategies for coping with water stress (Chaves *et al.* 2002). These mechanisms include specialised leaf anatomy, root and leaf developmental changes, stomatal regulation, and leaf conformational changes (Schultz and Matthews 1988). However, the frequency and severity of water deficit are increasing due to climate change (New *et al.* 2001; Oreskes 2004), and is predicted to increase further (Dai *et al.* 2018). Tropical rainforest systems are particularly vulnerable because variability of water availability is predicted to increase (Corlett 2011). Typically, rainforests plants do not experience water deficits due to high rainfall. Rainforest plants might, therefore, be less adapted to a climate future with variable rainfall. In light of this uncertain future, it is pertinent to investigate the ecophysiology of tropical plants concerning water stress.

One of the major effects of water stress on plant physiology is embolism formation, which occurs when the water column in the xylem is broken. The resulting air bubble blocks the flow of water through the xylem and renders the effected conduits incapable of transporting water (Zufferey *et al.* 2011). Continued vessel failure can lead to hydraulic failure and ultimately plant death. Embolism formation is more likely to occur in large vessels because there is less surface area of the xylem lumen to provide adhesion for the water column (Knipfer *et al.* 2015). As such, a conservative hydraulic strategy is to have many small vessels. The trade-off to this approach is that water flows slower through many small vessels. When water availability is high, the best strategy can be to have fewer larger vessels to maximise movement of water for photosynthesis. Trees in tropical rainforests have a larger mean vessel size compared to trees from other biomes (Hacke *et al*, 2017). When water availability is low, avoiding embolism formation is a larger selective pressure than maximising photosynthesis. For example, trees from the Mediterranean have smaller vessel sizes because they are constrained by avoiding embolism formation in the dry summer months (Hacke *et al.* 2017).

Petioles play an important role in regulating water use under water stress. The petiole is the terminal stem region before the leaf, which is the site of transpiration. Petioles are the limiting factor of water transport, as their smaller size and resulting smaller vessel lumen leads to water transport capacity generally several-fold smaller than that of the internodes on the main stem (Schultz and Matthews 1993). Additionally, embolism formation in the petioles prevents cavitation in the leaves; this is known as hydraulic vulnerability segmentation (Hochberg *et al.* 2016). Previous studies have investigated the role of the petiole in water stress, mainly in terms of that hydraulic vulnerability segmentation. However, the petiole has multiple functions, including supporting the leaf and maintaining its aspect and orientation. As such, we expect that petiole xylem characteristics will be related to other key plant traits. Currently, the role of petioles in tropical trees has been studied in terms of architecture. For example, petioles help support leaf display for maximising light interception (Poorter and Danaë 2008). However, little is known about the role of petioles in tropical trees when it comes to water-related traits.

The aim of this study is to provide a preliminary investigation of petioles in tropical evergreen angiosperms. We investigated the fundamental links between water-related traits and petiole volume. Our hypotheses were:

1) Petiole volume will be correlated with potential conduit conductance (and thereby the potential for use as a proxy).

2) Species with greater leaf area (LA) will have greater petiole volume. Larger leaves have a greater need for transpiration due to higher absorption of irradiance causing higher leaf

temperatures (Okajima *et al.* 2012). Additionally, larger leaves provide a larger area for stomata, thereby increasing the amount of water that can be transported out of the leaf.
3) Species with greater relative growth rate (RGR) will have greater petiole volume. Plant lifespan is correlated with hydraulic safety mechanisms because long-lived plants have a higher likelihood of encountering hydraulic stress. Long-lived plants often are slower growing and therefore have a lower RGR (Black *et al.* 2008). RGR was used instead of lifespan because RGR was known for the species studied.

## Methods

### Field site and sample collection

Leaves were collected in Bukit Timah Nature Reserve (BTNR), Singapore, in July 2018. BTNR is a 164 ha national park located in the centre of Singapore's main island, Pulau Ujong. The floristic composition of BTNR includes primary and secondary tropical lowland evergreen rainforest. The region of BTNR on the upper slopes above 80 m above sea level (asl) contains most of the remnant intact primary rainforest in Singapore.
Leaves were collected from the BTNR summit track areas above 80 m asl (around 1.353270°E, 103.778674°S). The leaves were stored in sealed plastic bags containing wet paper towel to maintain humidity and freshness of the samples. Leaves from 10 species were collected (Table 1). One branch from one individual was collected for each species, from which five of the youngest fully expanded healthy leaves were selected for testing.
The relative growth rates (RGR) were obtained from an ongoing Tropical Forest Science plot network study in BTNR, and the data provided by Dr Shawn Lum (Nanyang Technological University). RGR was used in this study instead of plant lifespan because lifespan is not known for these species.

**Table 1: The 10 species from three families of tropical flowering plants studied in Bukit Timah Nature Reserve.**

| Family | Species | RGR |
|---|---|---|
| Dipterocarpaceae | *Shorea curtisii* * | 1.679 |
| | *Shorea pauciflora* | - |
| | *Dipterocarpus caudatus* | 1.016 |
| | *Hopea mengarawan* * | 2.200 |
| Calophyllaceae | *Calophyllum ferrugineum* | 0.540 |
| | *Calophyllum pulcherrimum* | 0.503 |
| | *Calophyllum wallichianum* | 0.997 |
| Myristicaceae | *Knema cinerea* | 0.985 |
| | *Knema latericia* | 0.756 |
| | *Knema laurina* | 0.827 |

Note: * indicates that the species was not included in the microscopy portion of this study.

### Petiole volume

Calipers were used to measure the length and diameter of the petiole. The diameter was measured at the proximal and distal ends across both the horizontal and vertical axes (Figure 1). If the petiole was curved, the petiole was straightened to measure the unbent length. While petioles are best characterised as a truncated cone, we found that there was no significant difference between approximating the petiole volume to a truncated cone or a cylinder; subsequently, we used the cylinder formula, as this was the simpler option (Equation 1).

Equation 1: petiole volume

$$V = \pi \bar{r}^2 h$$

$$\bar{r} = \frac{A + B + C + D}{8}$$

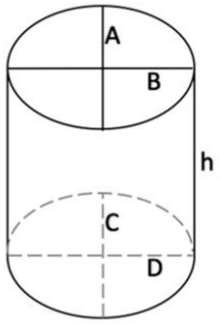

**Figure 1: Locations at which caliper measurements were taken on the petiole. Measurements A, B, C and D are diameters. Measurement h is the length of the petiole.**

### Microscopy

Petioles from 7 individual leaves were hand sectioned and dyed with Methylene blue. Petioles were transversely cross-sectioned close to the leaf. Species included in microscopy are indicated in Table 1; others were excluded due to time constraints. Wet mount slides were prepared with each of the sections and viewed with a light microscope. Photographs were taken of each slide at 400× magnification, using an iPhone5 (Apple Inc.) camera application, pressed directly against the ocular lens. Images were processed in the measurement tool of ImageJ (version 1.6; NHI and LOCI, University of Wisconsin).

### Petiole capacity

To investigate the hydraulic capacity of the petiole we developed a proxy for conductance. Hydraulic conductance ($K_{pp}$) is a measure of the efficiency of bulk flow through a material. In plants, this flow is water through vessels. Larger vessels carry exponentially more volume than smaller vessels, because $K_{pp}$ is the diameter of the vessel of to the fourth power, as per the Hagen–Poiseuille law. For our conductance proxy, we applied the Hagen-Poiseuille law to the 10 largest vessels. This did not give us the full conductance of the petiole but provided a rapidly assessable proxy. Our proxy is henceforth referred to as ‘approximate petiole conductance’.

We used photographs of the petiole transverse sections to measure the xylem vessels. We measured the diameter of the largest xylem vessels for approximate petiole conductance. The diameters were standardised according to the field of view. The true size of the vessels in SI units could not be determined, as we did not have access to a stage micrometer and reticule for calibration.

### Statistical analysis

*Shorea pauciflora* was excluded from analysis involving RGR as no RGR data was available for this species. All scatter plots and linear regressions were created using ggplot2 (version 3.3) (Wickham 2016) in R-studio (version 3.2.1). The SMATR package (version

3.4) (Warton *et al.* 2012) in R-studio was used to compare the slope and elevations of different families for linear regression of RGR against petiole volume.

## Results

Petiole volume scaled with both internal structures and leaf area. There was a strong correlation between petiole volume and approximate petiole conductance ($R^2 = 0.992$; $n = 7$; Figure 2a). Note that this relationship holds even without the highest point ($R^2 = 0.961$; $n = 6$). Across all species, 85.9 per cent of the variation in leaf area was explained by petiole volume (Figure 2b; $R^2 = 0.859$; $n = 48$). This trend persisted across all families, and no significant difference was detected between families in the slope of the correlation between leaf area and petiole volume ($p = 0.499$). However, the families differed in the elevations of the linear regression, and this effect was significant ($p = 0.001$).

There was no cross-family correlation between petiole volume and RGR (Figure 2c; $R^2 = 0.0048$; $n = 43$). Within families, petiole volume and RGR were weakly correlated in both the Dipterocarpaceae (negatively, $R^2 = 0.546$; $n = 13$) and Calophyllaceae (positively, $R^2 = 0.478$; $n = 15$) and more strongly correlated in Myristicaceae where petiole volume and RGR increased in concert, and 72.7 per cent of the variation in RGR was explained by petiole volume ($R^2 = 0.727$; $n = 15$).

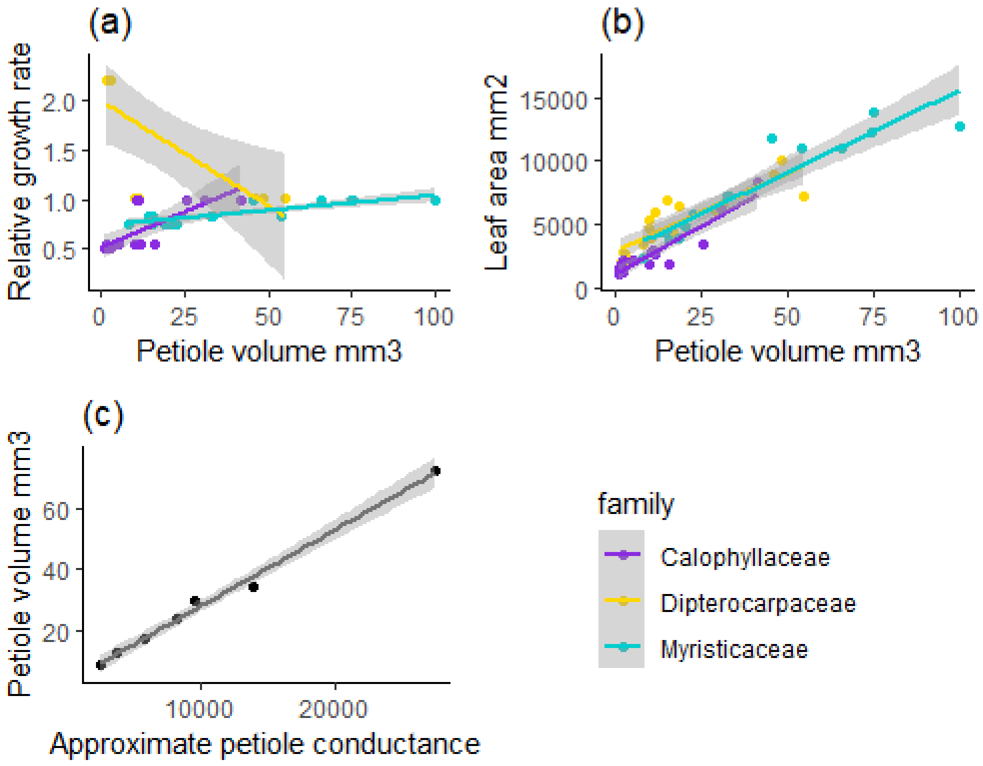

**Figure 2: (a) Relative growth rate (RGR) against petiole volume. Linear trendlines are given for each family: Dipterocarpaceae ($R^2 = 0.546$; $n = 13$), Calophyllaceae ($R^2 = 0.478$; $n = 15$) and Myristicaceae ($R^2 = 0.727$; $n = 15$); (b) Leaf area by petiole volume. Linear trendlines are shown for each family. For all**

**datapoints $R^2 = 0.859$; $n = 48$. (c) Petiole volume in mm$^3$ and approximate petiole conductance in arbitrary units. Linear regression is shown ($R^2 = 0.991$; $n = 7$).**

## Discussion

We tested the relationship between petiole volume and two plant traits related to water use and hydraulic safety: leaf area and relative growth rate (RGR). We found that petiole volume was an excellent predictor of leaf area. We did not find a relationship across families between petiole volume and RGR. However, there was a relationship between petiole volume and RGR within families.

### Relationship between petiole volume and conductance

Hydraulic conductance ($K_{pp}$) is a measure of the efficiency of bulk flow through a material. In plants, this flow is water through vessels. The Hagen-Poiseuille equation describes the relationship between vessel diameter and water conductance and can be used to predict the potential conduit hydraulic conductance (McCulloh *et al.* 2009). Conductance is calculated as vessel diameter to the fourth power; therefore, small changes in diameter result in major changes to flow. Accurately measuring real $K_{pp}$ requires sophisticated equipment such as psychrometers or a pressure bomb. We did not have the ability to measure true $K_{pp}$. Using the Hagen-Pouseuille equation to calculate potential hydraulic conductance requires measuring all the vessels in the system. Time limitations prevented us from measuring all the vessels in each petiole, which is why we used a subset of the 10 largest vessels. Opting for this proxy enabled us to explore water and traits under restricted field settings.

Approximating potential hydraulic conductance from the 10 largest vessels does not consider the many smaller vessels present in the petiole. Ray and Jones (2018) found most of the petiole vessel area was found in a central bundle of large vessels. However, there are many more small vessels in the peripheral bundles, which still account for water transport. In some plants, there are no central petiole bundles (Ray and Jones 2018), in which case, smaller bundles explain all the hydraulic conductance. Therefore, measuring the largest 10 vessels is not a good proxy for hydraulic conductance in all species.

We found a strong relationship between approximate petiole conductance and petiole volume. Despite all the above caveats regarding the precision of this metric, this strong relationship ($R^2 = 0.991$) merits consideration here and in future investigation. Easy-to-measure traits are valuable for time and cost-saving measures in a wide range of applications, such as forestry breeding programs and ecological monitoring. For example, Gebauer *et al.* (2016) found that leaf dry mass predicted hard-to-measure petiole traits such as xylem area, lumen area, and number of vessels in poplars (Salicaceae); this then could be applied to poplar early selection criteria. Our study shows petiole volume potentially predicts hard-to-measure traits such as vessel size and potential conductance. Measuring petioles from outer dimensions is non-destructive and could be used in future research as a proxy for $K_{pp}$ of living plants.

It has not yet been tested whether petiole volume predicts specific conductance. Specific conductance is the water potential of plant tissues at a specific time, such as pre-dawn or midday. Potential conductance is the maximum possible conductance according to the vessel dimensions; however, specific conductance can be much lower, and varies on both a diurnal and seasonal timescale with water stress (Zwieniecki *et al.* 2000). Specific conductance is typically tested by measuring water potential with a pressure bomb, which is a destructive mode of testing. Investigation of the relationship between easy-to-measure petiole traits and specific conductance could provide another method for simple and non-invasive hydraulic measurements.

### Petiole traits as predictors of plant strategies: Leaf area

We found a strong positive correlation between leaf area and petiole volume. This relationship has not been previously documented. The slope of this relationship held between families of tropical flowering trees.

We selected leaf area as a water-related trait that was easy to measure for this study. Leaf area is an important component of the leaf economic spectrum: a large leaf surface area enables larger light interception and greater surface area for gas exchange (Onoda *et al.* 2017). Because of our first finding, that petiole volume is strongly correlated with approximate hydraulic conductance, it is possible that leaf area is related to petiole hydraulic conductance. However, this link is a second degree of separation and not a direct correlation, so future research is required. Nonetheless, an argument can be made for the logical connection between leaf size and hydraulic conductance: larger leaves have greater rates of gas exchange, which requires greater water transport (Buckley 2005), hence necessitating the need for greater hydraulic conductance in the petiole, because the petiole is the bottleneck of water supply to the leaf.

However, utilising the petiole as a bottleneck may be beneficial in preventing cavitation of the stems. The hydraulic vulnerability segmentation hypothesis predicts that distal organs, such as petioles, are more susceptible to embolism; distal organs are more expendable or readily repaired (Hochberg *et al.* 2016). Petiole cavitation acts as a 'hydraulic fuse', where petiole embolisms limit leaf transpiration under water stress, and thereby limits the propagation of embolisms in other organs such as the roots and shoots (Zufferey *et al.* 2011). This 'fuse' behaviour prevents the cavitation of the stem at the expense of the expendable distal organs of the plant (Hochberg *et al.* 2016).

Perhaps the relationship between petiole hydraulic conductance and leaf area depends on the climatic conditions in which a species lives. We tested tropical angiosperms, which have evolved under conditions of high rainfall and little water deficit. The risk of embolism formation and stem cavitation for these trees is low, and therefore there is no need for the petioles to act as a hydraulic fuse. Rather, maximising hydraulic conductance to the leaves assists in maximising gas exchange for photosynthesis (Buckley 2005). In other biomes, where water deficit limits the size of vessels (Hacke *et al.* 2017), limiting petiole size may be the conservative hydraulic strategy. It would be instructive to compare our results with areas of varying water deficit.

Likewise, the hydraulic strategy at the petiole level may depend on leaf-lifespan. The petiole acts as a hydraulic fuse at the expense of the leaves, but in species with long-lived leaves this may be a detrimental sacrifice of invested resources. Therefore, a long-lived leaf requires a conservative hydraulic strategy, in order to avoid embolism, which is mediated by smaller $K_{pp}$. It would be beneficial to determine the leaf lifespan of our study species, in order to investigate if this is the case. Alternatively, perhaps long-lived leaves invest instead in the mechanism of embolism repair. Embolism formation and repair in the petiole occurs over the diurnal variation in water stress, peaking in the midday, and then reducing in the late afternoon, as embolism repair takes place and conduit conductance is restored (Bucci *et al.* 2003).

Alternatively, the correlation between leaf area and petiole volume may be due to the architectural role of the petiole in supporting the leaf. Previous studies have found that biomass distribution in structural components of the plant are modified to support leaf area, including biomass distribution to the petiole (Niinemets *et al.* 2006; Niklas 1993; Poorter and Danaë 2008).

### Petiole traits as predictors of plant strategies: RGR

Plant lifespan is correlated with hydraulic safety mechanisms because long-lived plants have a higher likelihood of encountering hydraulic stress. Long-lived plants often are slower growing and therefore have a lower RGR (Black *et al.* 2008). RGR was used instead of lifespan because RGR was known for the species studied. It is worth noting, however, the relationship between RGR and plant lifespan is somewhat tenuous, with little experimental evidence (Lee *et al.* 2013). Furthermore, the connection between RGR and leaf-level gas exchange is also moderated by many other factors, such as stomatal density. We did not find a relationship across families between petiole volume and RGR. Instead, Calophyllaceae and Myristicaceae RGR increased slightly with increasing petiole volume, whereas Dipterocarpaceae RGR decreased with increasing petiole volume. The assumption driving our hypothesis was that low RGR species were long-lived and that long-lived plants have a higher likelihood of encountering hydraulic stress and would subsequently exhibit more conservative hydraulic architecture. As such, it was surprising to see a negative correlation between RGR and petiole volume in Dipterocarpaceae; this was contradictory to our hypothesis. The highest RGR species was *Hopea mengarawan*, which also exhibited the lowest petiole volume (Figure 2). Dent and Burslem (2016) found that Dipterocarpaceae species that specialised on nutrient-poor sandstone exhibited conservative strategies, including constrained growth rates, even when grown on alluvial soils. *Hopea mengarawan* is known in agroforestry to grow on poor sandy and clay soils (Fern 2014); however, this distribution could not be confirmed in the primary literature as the species is poorly known and critically endangered (Ashton 2018). Perhaps the relationship between petiole volume and RGR depends on the general strategy of Dipterocarp species, according to their natural distribution. This may be beneficial for Dipterocarps in the future under water-stress if, like *H. mengarawan*, they can maintain high growth rates with conservative hydraulic strategies. *H. mengarawan* may be able to resist embolism formation and vessel cavitation, while maintaining a high growth rate. It would be informative to investigate the acclimation of *H. mengarawan* growth rates under water-stressed growth conditions, in order to determine whether their petiole-xylem strategy varies. Schultz and Matthews (1993) found that acclimation to light intensity affected water conductance; shade-acclimated plants had fewer petiole xylem conduits. As such, environmental factors such as water and light could affect the development of petiole xylem architecture.

## Conclusions

We found a positive correlation between petiole volume and leaf area. The link between petiole volume and RGR is more tenuous and exists only within families. While this study observed relationships between petiole volume, leaf area and RGR, it did not have the power nor experimental framework to explain them. Further research may elucidate the fundamental links between the petiole and other water-related traits. It would be informative to study the link between a conservative hydraulic strategy in the petiole and plant lifespan. Knowing which plants are susceptible to the impacts of prolonged and seasonal droughts may help identify areas particularly at risk due to climate change.

While rainforest communities as a whole are resilient to change, individual species may not be, and these species could become extinct without intervention.

Further research is also needed into the possibility of using petiole volume as a proxy for hydraulic conductance. If the strong relationship we documented between petiole volume and the largest 10 vessels holds true for all vessels, then petiole volume could be a useful non-invasive way to measure potential hydraulic conductance. This would aid field experiments into the effect of climate change on rainforest trees.

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