UNIVERSIDADE ESTADUAL “JÚLIO DE MESQUITA FILHO” - UNESP

FACULDADE DE CIÊNCIAS AGRONÔMICAS - FCA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA FLORESTAL

Relatório final de pós-doutoramento

Parallel heterochronic events in wood ontogeny resulting in habit and habitat shifts in Boraginaceae from two biodiversity hotspots

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Agência de Fomento: CAPES/PrInt

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Report – Kamil Frankiewicz - PD

PART I - Research

Parallel heterochronic events in wood ontogeny resulting in habit and habitat shifts in Boraginaceae from two biodiversity hotspots

Background

Heterochronies are any changes in the timing or rate of species' ontogeny relative to its ancestor. Even subtle heterochronies can significantly impact the ultimate size and shape of an organism. When these changes lead a descendant resembling ancestor's juvenile, the derived morphology is called paedomorphic; whereas morphologies transgressing the ancestral one are known as peramorphic. There are at least six distinct mechanisms leading to paedo- and peramorphosis (McNamara 2012).

Shifts between herbaceous and woody (shrubby, arborescent) habits – which are the key to angiosperm ecological adaptability – can be considered in terms of heterochronies. In the simplest scenario, woody species evolve from herbaceous ancestor through extension of cambial activity. In such case, a prolongation of patterns and processes present in the ancestral ontogeny are expected. On the other hand, it is feasible that woody and herbaceous life forms differ also qualitatively. If this is the case, one would expect deviation from patterns and processes of herbaceous ontogeny. For instance, in all plants early-formed vessels are narrower than those produced later. However, whether the rate of the widening with growing stem diameter is similar in herbs and woody plants is unknown.

The term 'paedomorphosis' was introduced to wood anatomy already in the 1960s by Sherwin Carlquist (1962). However, his understanding of heterochronies was rather idiosyncratic and far away from rigorous (Jones 2023). Carlquist also did not include the ancestral-derived comparison of ontogenies, which is a prerequisite to identify heterochronies and mechanisms they were caused by. Generally, studies directly comparing the wood development of a species with its putative ancestor are infrequent (Onyenedum and Pace 2021; Frankiewicz and Oskolski 2023). The primary objective of my project was to compare the development of selected quantitative wood anatomical traits between closely related herbaceous and woody species, and to assess if the putative differences correspond to habits or rather climatic niches. Additionally, I also delivered a lecture on the influence of climate on habit shifts and co-led an introductory course on wood and bark anatomy for students in the Graduate Program in Forest Sciences at UNESP.

Methods

Herbaceous *Echium plantagineum* (two specimens) and 12 species (16 specimens) of shrubby *Lobostemon* (Boraginaceae) collected in the Cape Floristic Region of South Africa served as models. These two genera form a monophyletic clade and their most-recent common ancestor was most likely a herb. Therefore, ontogeny of *Echium* can serve as a proxy for ancestral states.

In all cases, the basal-most, thickest part of the stem was sampled, its distance from the most-distant stem tip (hereafter 'height') and length of 10 leaves per specimen (hereafter 'leaf' or 'leaf size') were measured. Wood anatomical cross sections were obtained following standard procedures (IAWA Committee 1989).

Initially, five species were studied with respect to vessel area, vessel and fibre wall thickness, and vessel grouping. These traits were measured in a standard way, and for each measurement its distance from the stem pith was noted. As it was evident that fibre wall thickness and vessel grouping remain the same throughout ontogeny, the remaining species and specimens were examined only for vessel area and vessel wall thickness. In total, approximately 7500 vessel areas and 4500 vessel walls were measured; each with a corresponding distance from the pith. The data were analysed in three ways:

1. *Comparison of ontogenies between herbaceous and woody habits* – The first step was to assess if changes in vessel area and wall thickness that occur over time, differ to a significant degree between woody and herbaceous plants. To this end, the $\log_{10}$-transformed trait values were regressed against the $\log_{10}$-transformed distance from the pith, separately for woody and herbaceous species. The resulting slopes and intercepts with their 95% confidence intervals were compared to assess if putative differences were significant.
2. *Length of hydraulic route as a predictor of ontogenies* – The next step was to identify traits that explain the observed change in vessel area and wall thickness that occurs with growing distance from the pith. Thicker stems are also longer, so vessels deposited further from the pith can be safely assumed to have originated also further from the stem tip. It is well-known that vessel diameter (i.e., also area) scales predictably with distance from the stem tip (Rosell *et al.* 2017), and that leaf vasculature is a direct prolongation of vessels in the stem (Cao *et al.* 2022). Therefore, a number of progressively complex regression models were fitted with vessel area or wall thickness as dependent variable, and a selection of height, leaf, species, specimen, and habit as independent variables, with or without interaction terms. The best fitting model was selected based on the Akaike Information Criterion (AIC) from among those that did not encounter singularity issues. These models used specimen means of $\log_{10}$-transformed values, rather than all c. 12 000 measurements to mitigate the problem of autocorrelation. This solution is not perfect, as four species (*E. plantagineum*, *L. glaucophyllus*, *L. marlothii*, *L. stachydeus*) were represented by two or three specimens. However, mixed effect models which included random slope and/or intercept scored much higher with AIC (i.e., were of lower fit) and had singularity issues.
3. *Climatic niche as a correlate of ontogeny* – The final step was to assess whether the rate of vessel widening and wall thickening with distance from the pith correlates with species' climatic niche. To this end, all georeferenced occurrences of the studied species were downloaded from GBIF and cleaned using CoordinateCleaner to remove dubious

records. For each of the resulting data points, monthly temperature, precipitation, and aridity indices were downloaded from WorldClim and Global Aridity Index and Potential Evapotranspiration databases. Their values were averaged and medians extracted for each species to obtain species-specific estimates. Then, $\log_{10}$-transformed anatomical values were plotted against $\log_{10}$-transformed distance from the pith, similarly to procedures described in point 1., but this time measurements were grouped by species, not by specimen. The resulting slopes and intercepts with their 95% confidence intervals were extracted for each species, and plotted against corresponding climatic variables to assess presence and strength of potential correlation.

All analyses were carried out in R v. 4.3.2.

Results and interpretation

Comparison of ontogenies between herbaceous and woody habits – The results are summarised in figure 1. Both traits, vessel areas and vessel wall thickness, increase with the distance from the pith, irrespectively of the habit. The slopes for vessel area in both habits are virtually identical, meaning that vessel widening progresses at the same rate in herbaceous and woody plants. The intercepts are also very similar, suggesting that the first-formed vessel elements are of similar size in both habits. Also, the wall thickness increases in both groups at statistically identical rate.

These results indicate that woody plants simply prolongate the same developmental programme used by their herbaceous relatives and by their putative common ancestor. This indicates that other, universal factors affecting herbs and woody plants alike, necessitate vessel widening and vessel wall thickening.

Vessel diameter is known to scale positively with the distance from the stem tip, i.e., vessels at stem base are wider than those in twigs. This tapering allows to maintain a constant hydraulic resistance when stem grows. Although in theory, some differences in stem length-vessel diameter relationship could be possible between woody and herbaceous plants (e.g., due to different amounts of parenchyma which stores water), this apparently is not the case and both habits follow the same rule. Therefore, a follow-up expectation would be that distance from the tip and leaf size should be the best explanations for vessel area. This was tested in the next step.

Length of hydraulic route as predictor of ontogenies – In total, 27 models were fitted. All 10 models that included specimen as (one of) the independent variable yielded AIC scores of -Inf indicating a severe lack of fit, overfitting, and optimizing issues. The remaining models scored between –13 and –59. The less negative values (–13 to –18), indicating a poorer fit, were typical for models that used habit as the only categorical independent variable. Moreover, in all such cases the habit coefficient was close to zero and with high P-value. This emphasizes that the relationship between vessel area, height and leaf size is the same in woody and herbaceous plants.

The most negative AIC values (–51 to –59) were scored by models that included species as (one of) the independent variables. Surprisingly, the standardized coefficients for species variable were higher than those for height and leaf size. This hardly indicates that the relationship between height and leaf size in *Echium* and *Lobostemon* differs from that in other plants, but rather emphasises a limited sampling. Simultaneously, the fact that species variable is important for the model fit, points to species-specific traits that affect this relationship. As shown earlier, habit is not instrumental, therefore other factors, like climatic niche, should be considered and this was done in the next step.

Climatic niche as a correlate of ontogeny – Neither the slope, nor the intercept, of vessel area or wall thickness regressed against the distance from the pith, correlate with aridity or precipitation (Fig. 2–5). This means that the rate of change in both traits, as well as the qualities of the first-formed vessels, are statistically similar in all levels of aridity and precipitation. On the other hand, a considerable amount of variation (R^2 = 0.47, P = 0.01) in slope values is explained by temperature (Fig. 2 & 3). This means that the rate of vessel widening is related to temperature experienced by given species, with species occupying hotter climatic niches producing wider vessels faster than those from milder niches. On the other hand, the intercept correlation with temperature is negative, but its statistical significance is doubtful at the moment (R^2 = 0.25, P = 0.1, fig. 3). This may be due to limited sampling and weaker nature of correlation. If confirmed, this would suggest that in species from hotter niches the first-produced vessels tend to be narrower than those in plants from milder niches.

Also, in the case of vessel wall thickness the aridity and precipitation lack any explanatory power. The relationship with temperature is the same as in the case of vessel area – that is, the slope grows and the intercept diminishes with temperature (Fig. 4 & 5). However, temperature explains only c. 20–25% of variation in slope and intercept at P = 0.01. Therefore, the correlation, if any, is weaker than in the case of vessel area.

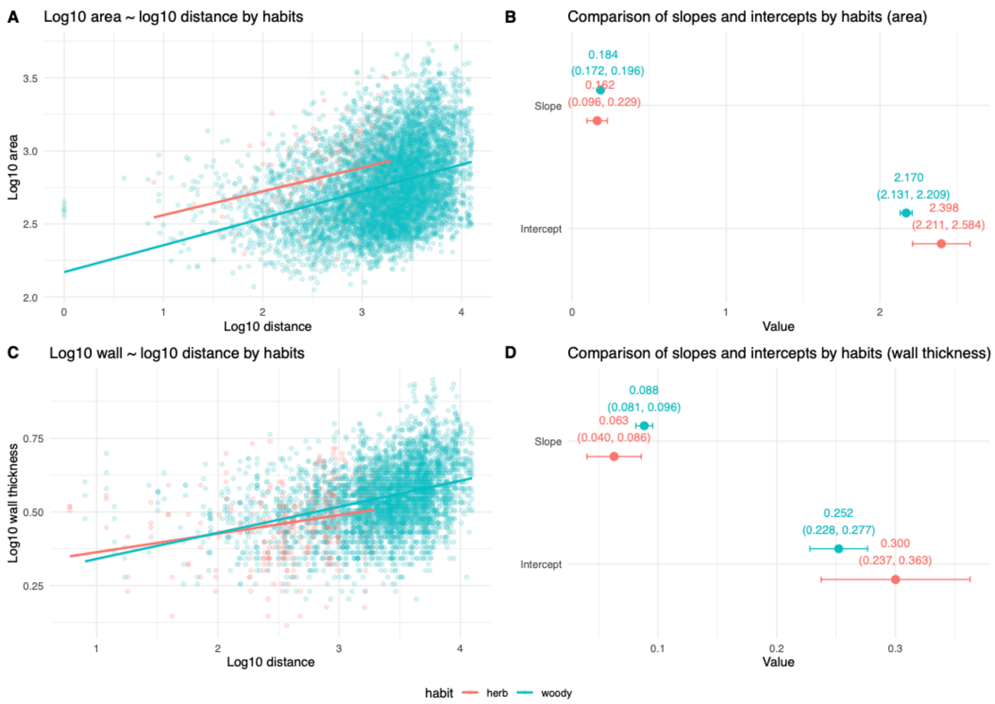

Figure 1 Regression analysis of log_{10}-transformed vessel area (A, B) and vessel wall thickness (C, D) against the log_{10}-transformed distance from the pith conducted for each level of habit (herb, woody) separately. In all cases, the 95% confidence intervals either overlap or are very close between groups (B, D) meaning that slopes and intercepts are statistically indistinguishable in woody and herbaceous plants.

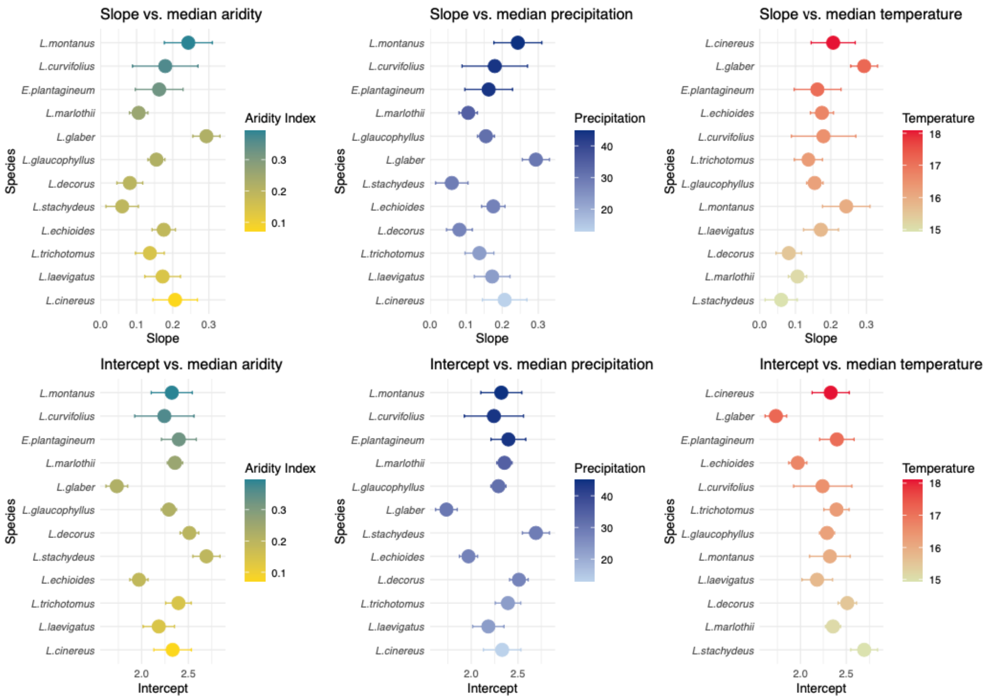

Figure 2 Plots showing the relationship between the slope (upper row) or intercept (bottom row) and the climatic variables for the species means of $\log_{10}$-transformed vessel area regressed against $\log_{10}$-transformed distance from the pith. A pattern is clearly seen in the slope vs. temperature, where higher slope (i.e., more rapid vessel widening with distance from the pith) correlates with higher temperature. Weak negative pattern is seen also in the intercept vs. temperature. Other climatic variables lack explanatory powers.

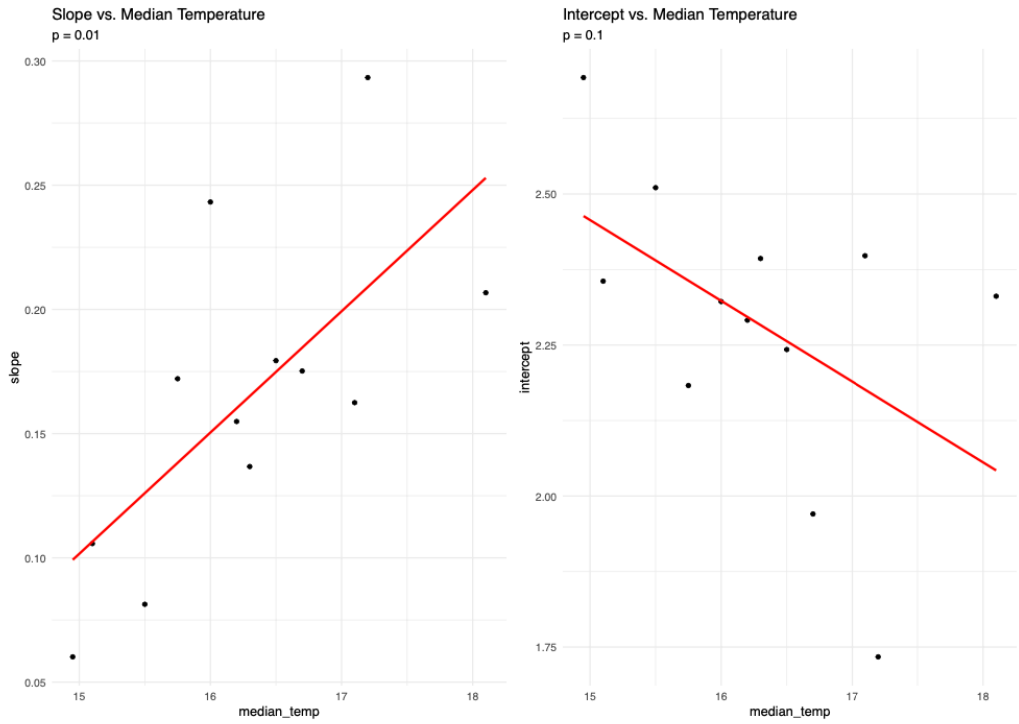

Figure 3 The regression analysis of slope and intercept against temperature supports previous conclusions, that slope correlates positively with temperature at statistically significant level, whereas the correlation between the intercept and temperature is negative and with higher P-value.

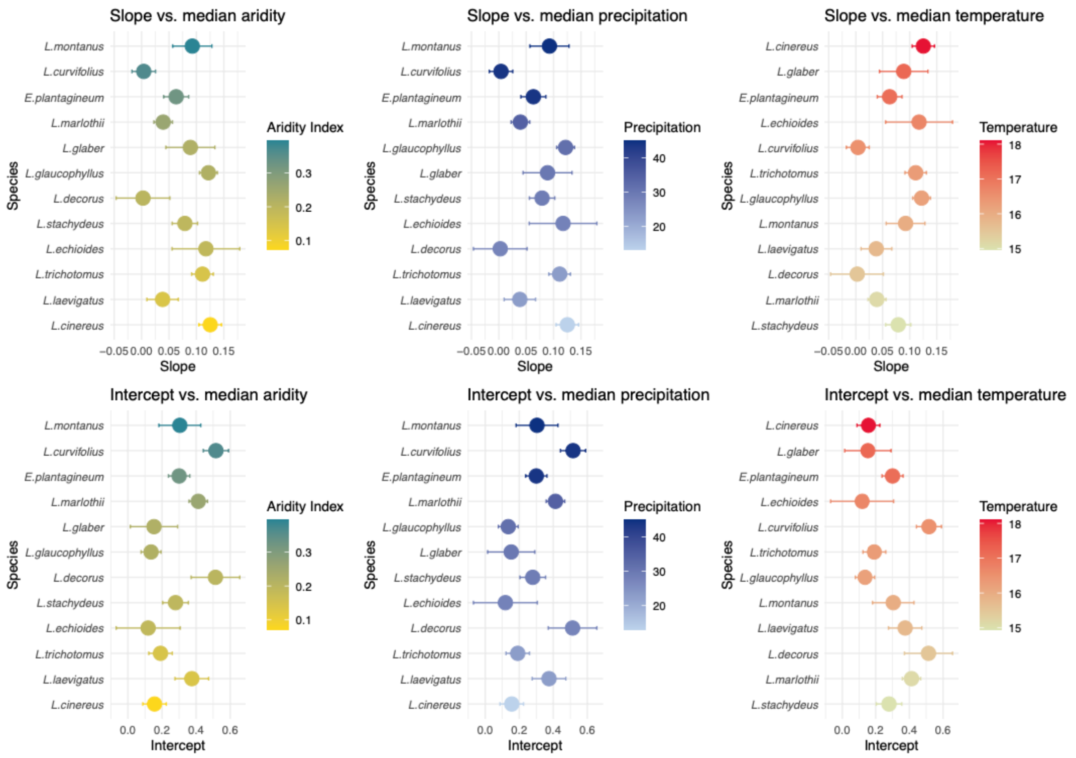

Figure 4 The plots are as in figure 2., but this time the slopes and intercepts were extracted from $\log_{10}$-transformed vessel wall thickness regressed against the distance from the pith. Aridity and precipitation again lack explanatory power, while the correlation with temperature is weaker (see fig. 5).

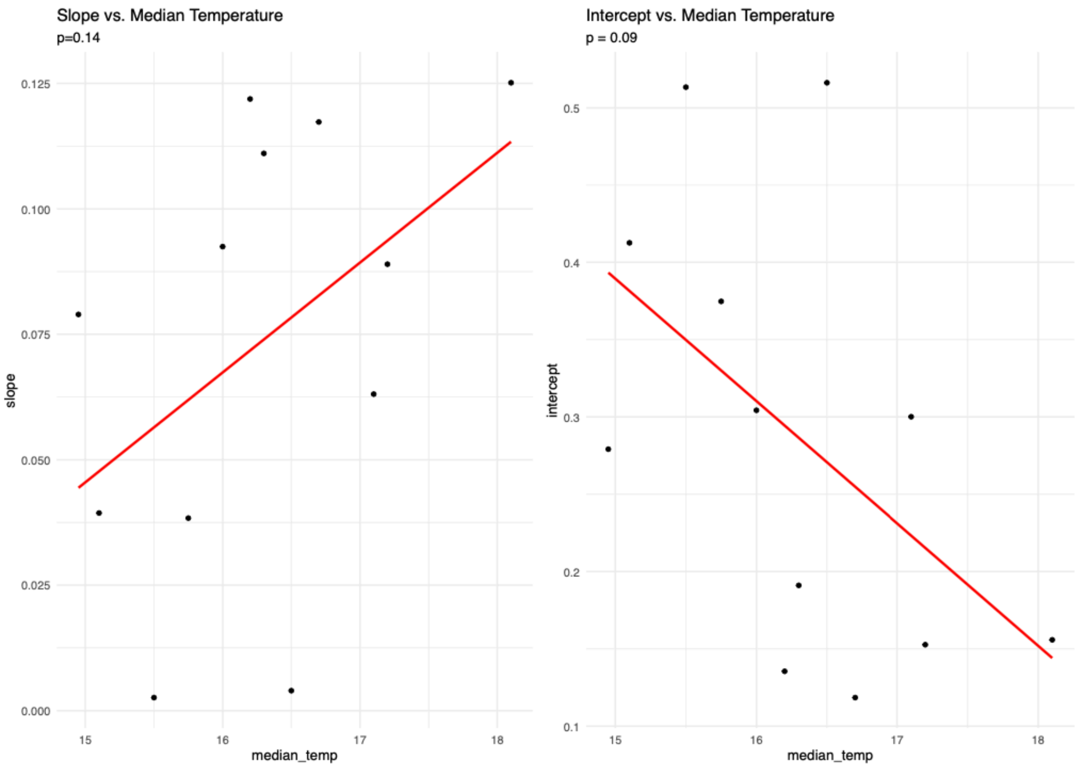

Figure 5 Regression of slope and intercept against species mean temperatures are on the verge of statistical significance, but follow the same pattern as recovered for vessel area.

Discussion

Out of the four wood anatomical traits – vessel area, vessel and fibre wall thickness, vessel grouping – only the vessel area and vessel wall thickness change considerably with the growing distance from the pith in *Echium* and *Lobostemon*. This is unexpected as vessel grouping patterns are known to change considerably with age, so that for descriptive purposes consultation of outer, mature wood is recommended (IAWA Committee 1989).

The fact that vessel diameter grows with the distance from the pith was observed already by Malphigi in the 17th century (Rosell *et al.* 2017). Due to the incremental nature of stem widening, the growing distance from the pith is commonly seen as an equivalent of age with thicker stems considered older than narrower ones. However, explicit comparison of stems of the same age but differing in width and those of similar sizes but of different ages, showed that absolute age lacks explanatory power with respect to anatomical or mechanical stem properties (Rosell and Olson 2007). Conversely, quantitative wood anatomical traits can be predicted well by stem size.

Stem length is closely and positively correlated with stem diameter (Rosell and Olson 2007; Olson *et al.* 2013; Olson and Rosell 2013; Rosell *et al.* 2017; M Olson *et al.* 2020). Therefore, any anatomical trait that correlates with either of stem dimensions, will necessarily correlate also with the other one. Our study showed that vessel area and wall thickness correlate closely with stem width (distance from the pith) and, simultaneously, with the stem length. Whereas there exists no explanation as to why vessel area or wall thickness should be greater with stem diameter *per se*, there is plenty of evidence that widening of vessels with growing stem length allows to maintain constant hydraulic resistance. Indeed, in our study the greater the distance from the stem tip, the greater the vessel area. Unexpectedly, adding leaf size to the models had only minor effect on the overall fit and coefficient for this variable were *negative* suggesting that the longer the leaves, the *narrower* the vessels. This is in contrast to finding of Cao et al. (2022), who showed that the total hydraulic route (i.e., the stem length *and* the leaf length) predicted vessel diameter well. One explanation for this incongruity is rather limited sampling in our study – with only 13 species represented by 18 specimens, possible measurement errors or specimen-specific idiosyncrasies would have higher effect than in wider study across hundreds of species. Therefore, for the time being we consider the negative effect of leaf size on vessel area an artefact.

Whereas our results on vessel area fit in the overall picture of stem size-hydraulic conductivity picture that has been emerging in the recent years, the correlation of vessel wall thickness is more novel. Only recently, a correlation between vessel diameter and wall thickness was reconstructed by Echeverría et al. (2022), who showed that above a threshold of 90 μm of vessel diameter, the wall thickness correlates moderately and positively with diameter. In our study, this observation is extended to vessels well-below the Echeverría's threshold as most of the measured vessels fall below 1000 μm^2, which corresponds to radius of only 18 μm. The mechanistic explanation could be that wider vessels require thicker walls to withstand implosion under negative pressure.

Most importantly, our study reconstructed a positive link between both the *rate* of change in both traits, vessel area and wall thickness, and temperature, whereas the aridity and precipitation lack explanatory power. Simply put, plants that occupy hotter climatic niches increase vessel area more per unit of distance from the pith, than those from milder niches. The same is true with vessel wall thickness, but this correlation is weaker and on the verge of statistical significance.

Higher temperatures increase vapour pressure deficit, i.e., the difference between the moisture in the air and the amount of moisture air could hold when saturated. Olson et al. (2020) showed that higher vapour pressure deficit is associated with wider vessels for plants of the same height across climates and species. This is because selection likely favours conductive systems that allow to meet the higher transpirational demand of warmer climates. It is possible that steeper slope of vessel area increase in higher temperatures is achieved by producing more slender stems, i.e., stems that produce greater number of length units for one unit of width. Since vessel area correlates with distance from the stem tip due to hydraulic requirements, slender stems would increase vessel size more rapidly than ‘plump’ ones. Of course, this relationship would necessarily fall within the boundaries of stem length-stem diameter scaling. The weak vessel wall thickness-temperature correlation is likely secondary, i.e., it results from the fact that wall thickness relates to vessel area that in turn correlates with temperature.

Conclusions

Woody and herbaceous species follow the same ontogenetic program with respect to vessel area and wall thickness increase with growing distance from the pith. The mechanistic explanation for this is most likely selection to maintain a constant hydraulic resistance in ever-elongating stems, while accounting for a constant stem length-stem diameter relationship. Vessel area and wall thickness increase with the distance from the pith more rapidly in plants from hotter climatic niches than those from milder ones. This may result from selection of conductive systems to meet higher transpirational demands and could possibly be achieved by producing more slender stems.

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PART II – Courses given

UNIVERSIDADE ESTADUAL PAULISTA

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ATESTADO

ATESTAMOS que o Prof. Dr. Kamil Eugeniusz Frankiewicz, PASSAPORTE EU7417962, ministrou/ministra aulas aos discentes do Programa de Pós-graduação desta Unidade Universitária, conforme abaixo descrito:

Programa: Ciência Florestal

Disciplina	Ano	Dt.Início	Dt.Término	Curso	Discentes	Hr/Aula
Tópicos Especiais Anatomia dos tecidos secundários das plantas	2023	11/07/2023	22/07/2023	MD	3	8

Botucatu, 21 de novembro de 2023

Edna Regina Prado
Supervisor Técnico de Seção
Pós-Graduação-FCA/UNESP

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UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Rio Claro

ATESTADO

ATESTAMOS que o Prof. Dr. Kamil Eugeniusz Frankiewicz, PASSAPORTE EU7417962, ministrou/ministra aulas aos discentes do Programa de Pós-graduação desta Unidade Universitária, conforme abaixo descrito:

Programa: Biologia Vegetal

Disciplina	Ano	Dt.Início	Dt.Término	Curso	Discentes	Hr/Aula
Tópicos Especiais Anatomia dos tecidos secundários das plantas	2023	11/07/2023	22/07/2023	MD	5	8

Rio Claro, 21 de novembro de 2023

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PART III – Supervisor comments

Kamil Frankiewicz brilliantly completed his research activities with great dedication and efficiency, independent study, and good coexistence with other work colleagues. Kamil also taught the course “Anatomy of secondary tissues in plants” for two graduate programs, Forest Science and Plant Biology, at Unesp.