

Stem photosynthetic efficiency across woody angiosperms and gymnosperms with contrasting drought tolerance

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Abstract

Key message Stem photosynthesis seems to play an adaptive role for woody plants that prosper in hot and dry ecosystems.

Abstract Stem photosynthesis is thought to be involved in tree resistance/resilience to water shortage. Recent studies have focused on the coordination between stem photosynthesis and hydraulics, but the generality of association of stem photosynthetic efficiency with species-specific adaptation to drought is still unclear. We quantified bark and wood chlorophyll *a* fluorescence (in terms of F_v/F_m) in current-year, 1-year and 2-year-old stems of several woody species harvested in diverse habitats. We ranked species in terms of relative drought tolerance on the basis of their vulnerability to xylem embolism (P_{50}), and compared stem photosynthetic efficiency of drought-tolerant vs drought-sensitive species. F_v/F_m values decreased with increasing stem age, and were generally higher for Angiosperms than Gymnosperms. F_v/F_m both at the bark and wood level was higher for drought-tolerant Angiosperms compared to drought-sensitive ones. Our results highlight the potential adaptive role of stem photosynthesis in drought-tolerant species, thriving under arid conditions likely leading to prolonged stomatal closure and halt of leaf-level carbon gain.

Keywords Bark · P_{50} · Stem photosynthesis · Drought · Wood · F_v/F_m

Introduction

Leaves are the most important photosynthetic organs in most woody plants, but nearly all of them can also perform this fundamental physiological process at stem level. Early observations of ‘green stems’ date back to the early twentieth century (e.g., Cannon 1908). Since then, photosynthetically competent stems have been detected in a wide range of woody plants typical of Mediterranean ecosystems, subtropical warm deserts, and tropical arid lands worldwide (e.g., Dima et al. 2006; Ávila et al. 2014). Indeed, photosynthetic stems are widely spread throughout different species and biomes (Teskey et al. 2008; Ávila et al. 2014). Stem photosynthesis is obviously common in succulent stems of CAM plants, but it occurs also in bark, wood, and pith of C_3 plants (Pfan et al. 2002; Lüttge 2008; Kocurek et al. 2015; Wittmann et al. 2016). Stem photosynthetic efficiency is apparently species-specific and varies on a seasonal scale, thus suggesting that it might be very important from a functional and ecological point of view (Rentzou and Psaras 2008).

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Although stems are not specialized for photosynthesis, due to their unfavorable surface-to-volume ratio and low light transmittance, it is known that chlorophyll is synthesized in functional chloroplasts even in deep wood layers of aged stems (Pfanz et al. 2002). Most woody species show a greenish, photosynthetically active inner bark (e.g., Pfanz et al. 2002; Wittmann and Pfanz 2008, 2014; Rosell et al. 2017), but chloroplasts can also be found along the xylem rays and in the pith, suggesting that they are not restricted to the relatively more illuminated outer cell layers of the stem (Dima et al. 2006; Berveiller et al. 2007; Yiotis et al. 2009). Among these stem compartments, bark exhibits higher chlorophyll concentration and photochemical efficiency (e.g., Yiotis et al. 2009; Wittmann and Pfanz 2016; Liu et al. 2018).

The amount and quality of light transmitted through the stem might be a key factor governing stem photosynthetic capacity (Manetas et al. 2005; Cernusak and Cheesman 2015; Wittmann and Pfanz 2016). Typically, about 15% of incident PAR can penetrate the periderm (Cernusak and Marshall 2000), but light transmittance changes according to stem anatomy (bark thickness, cell-wall lignification), age, and environment (Cernusak and Marshall 2000; Pfanz et al. 2002; Wittmann and Pfanz 2008). Blue light photons are largely absorbed by the outer bark, while red and far-red light penetrate more in depth, reaching the underlying xylem and pith. In addition, it is also possible that the optical properties of vascular tissue allow some axial light conduction to the pith cells, e.g., from roots (Sun et al. 2003).

Stems covered by an epidermis with stomata and/or periderm with lenticels can rely on net uptake of atmospheric CO₂ (Ávila et al. 2014). Otherwise, internal re-assimilation of CO₂ released by respiration of underlying heterotrophic tissues is also possible (e.g., Cernusak and Marshall 2000; Pfanz et al. 2002; Wittmann and Pfanz 2008; Berveiller et al. 2007). Internal CO₂ might be available at much higher concentrations within the stem (Teskey et al. 2008) compared to leaves, up to levels inhibiting photorespiration, thus increasing quantum yield of photosynthesis (Berveiller et al. 2007).

Previous studies have shown that net photosynthetic rates of stems can be up to 60% of those recorded for leaves (Ávila et al. 2014), and these rates are maintained even throughout the dry season when stomatal closure reduces leaf-level net carbon uptake (Ávila-Lovera et al. 2017). Hence, stem photosynthesis might provide an extra carbon gain under conditions imposing stomatal closure and limiting leaf photosynthesis. The carbon gain assured by stem photosynthesis might also contribute to bud development, flowering, and recovery after herbivores' attack (Saveyn et al. 2010; Kocurek et al. 2020). Stem photosynthesis has also been suggested to contribute to the maintenance and recovery of xylem hydraulic function (Saveyn et al. 2010; Schmitz et al.

2012; Bloemen et al. 2016; De Baerdemaeker et al. 2017; Trifilò et al. 2017, 2021; Tomasella et al. 2021).

Based on the above, stem photosynthesis might be particularly useful to maintain carbon uptake and physiological activities of plants during leafless periods, or under environmental conditions limiting leaf gas exchange (Ávila et al. 2014), with specific reference to drought. In particular, locally produced photosynthates might be used to sustain stem non-structural carbohydrates (NSC) pools to buffer carbon starvation under drought stress (De Roo and Salomón 2020), and refill embolized xylem upon drought relief (Schmitz et al. 2012; Liu et al. 2019).

In this study, we investigated possible differences in stem photosynthetic efficiency across a set of woody plants from different habitats. Our analysis was based on 39 woody species (both Angiosperms and Gymnosperms) from a broad phylogenetic range that were categorized in terms of relative drought tolerance on the basis of species-specific vulnerability to xylem embolism retrieved from the literature (see below). We specifically addressed the following questions: (i) are there differences in maximum quantum yield of photosystem II (F_v/F_m) of bark and wood in stems of different ages, and between various Angiosperm and Gymnosperm species? (ii) Are there any significant differences in stem F_v/F_m in drought-tolerant vs drought-sensitive species?

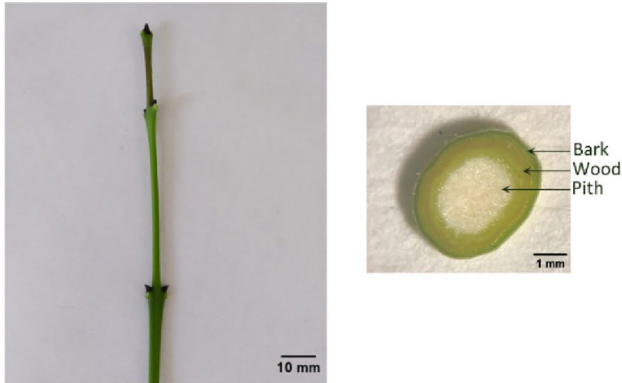
Material and methods

Plant material and experimental design

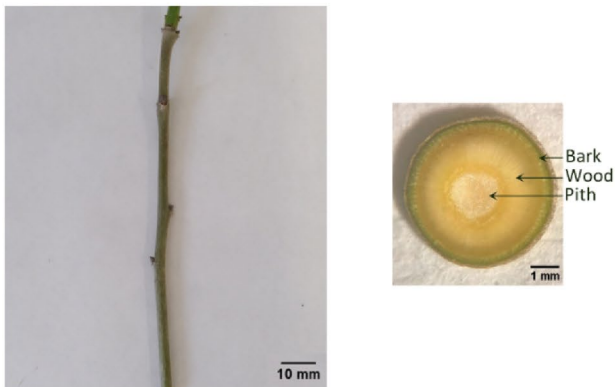
All experiments and measurements were carried out at the University of Trieste, Italy. Sampling of plant material was done in three different geographical areas to obtain stems from species from diverse families and/or adapted to different environmental conditions: (i) the botanical garden at the University of Trieste; (ii) the Classical Karst, a limestone plateau extending ~500 km², nearby Trieste; and (iii) montane forests nearby the village of Forni di Sopra, located in the Friulian Dolomites. Sampling was done between 6 and 8 a.m. in June 2021 for sites (i) and (ii), and in mid-July for site (iii). Three individuals per species were randomly selected, and two sun-exposed branches (about 3–4 years old) were sampled from each tree, for a total of six branches per species. Branches were immediately re-cut for 2–5 cm underwater and stored in a bucket with the cut section immersed in water to avoid dehydration until processing in the laboratory. Three branches (one per individual) were used for chlorophyll fluorescence measurements, while the other three branches were used to measure wood density, as described below. The complete list of species is reported in Fig. 2 and Table S1. Analyses

of photosynthetic efficiency were made on current-year, 1-year and 2-year-old stem segments, both at bark and wood level (Fig. 1).

Current-year stem segment



1-year stem segment



2-year stem segment



Fig. 1 Example of a stem sample (*Fraxinus ornus*) selected for the measurements

Chlorophyll fluorescence

Measurement of chlorophyll *a* fluorescence is a powerful technique for in vivo analysis of different photosynthetic parameters (Maxwell and Johnson 2000). One of the most relevant parameters to estimate the effective yield (or maximal photochemical efficiency) of PSII is the F_v/F_m ratio (Maxwell and Johnson 2000; Schreiber 2004), which indicates the intrinsic efficiency of PSII photochemistry of dark-adapted samples (Aschan et al. 2005). Chlorophyll fluorescence was measured in the laboratory using an imaging PAM chlorophyll fluorometer equipped with a high-resolution camera ($6.45 \mu\text{m} \times 6.45 \mu\text{m}$, resolution 1360×1024 px) (Open FluorCam SN-FC800-398, Photon Systems Instruments, Brno, Czech Republic) to quantify F_v/F_m for bark, wood [outer xylem and xylem longitudinal section (sapwood + pith)] (Fig. 1, S1), and leaves (Tomasella et al. 2022; Natale et al. 2023). Specifically, each sample was placed at a distance of c.18 cm below the camera, and the LED panels were adjusted to an angle of 45° to the center of the measurement area to maintain a homogeneous illumination of samples. The standard protocol “ F_v/F_m ” was selected from the wizard and the appropriate triggering of the shutter opening of the CCD camera was adjusted. First, the basal fluorescence (F_o) was measured, and then, a saturating pulse of actinic light ($\sim 4000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, 0.8 s) was turned on to induce maximum fluorescence (F_m), and F_v/F_m was calculated according to Maxwell and Johnson (2000) as: $F_v/F_m = (F_m - F_o)/F_m$. Each measurement took about 4 s.

Branches, still maintained with their cut end in water, were dark-adapted for 2 h before measurements to ensure relaxation of photosystems. Three to five mature leaves were detached from each branch and stem segments of different age were sectioned both longitudinally and radially, and bark and wood were manually and carefully separated. Samples were carefully placed on a petri dish with a bottom layer of moisten paper towels, and kept hydrated via partial immersion in a water film during the analysis with the PAM fluorometer.

Vulnerability to xylem embolism and wood density

Data of xylem pressure values inducing 50% loss of xylem hydraulic conductivity (P_{50}) for the study species were derived both from the Xylem Functional Traits (XFT) database (Choat et al. 2012), and integrated with data derived from more recent studies not included in the XFT. In accordance with previous studies (e.g., Petruzzellis et al. 2022), we considered only P_{50} data obtained for stems (excluding those measured on trunk, root, petiole, and leaf) and we discarded values based on r-shaped vulnerability curves (Cochard et al. 2013). When more than one value was available for a given species, we considered the average value from different

studies for subsequent analysis. Mean values of traits for each species included in the study, along with the relative references, are reported in Tables S1 and S2.

Based on global relationships between P_{50} and environmental water availability (Maherali et al. 2004; Choat et al. 2012), we set a threshold of $P_{50} = -3.0$ MPa to separate relatively drought-tolerant ($P_{50} < -3.0$ MPa) from relatively drought-sensitive ($P_{50} > -3.0$ MPa) species. We were thus able to separate two groups of Angiosperms based on their P_{50} values. The same approach was not possible for Gymnosperms, due to the relatively low number of species included in the study.

Wood density (WD) is a key functional trait indicating variation in wood structure/carbon investment, and often used as a proxy for vulnerability to embolism due to its general correlation with P_{50} (Hacke and Sperry 2001; Kiorapostolou et al. 2019). Basic WD was measured on 3 cm-long segments from 2-year-old stems (one stem per individual), as

WD = Wood dry weight / Wood fresh volume (g cm^{-3}).

Bark was removed and samples were rehydrated overnight by immersion in vials filled with tap water. The following morning, samples were weighed, and their fresh volume was measured using a water displacement method (Hughes 2005; Petruzzellis et al. 2019). Samples were then oven-dried at 70 °C for 24 h and weighed again to obtain their dry weight (Petruzzellis et al. 2022).

Statistical and phylogenetic analysis

Statistical analyses were performed with R (version 3.4.4) software (R Core Team 2019).

Boxplot panels were made using “ggplot2” package in R. Bar charts were prepared with Origin 9.0 software (Northampton, MA, USA). We set the F_v/F_m as the response variable and the drought sensitiveness (drought-tolerant or drought-sensitive species) and each sample (e.g., bark of current-year stem) as the first and second explanatory variables, respectively. Since homogeneity of variance assumption was violated, generalized least square (GLS) models were run, using the ‘nlme’ R package (Pinheiro et al. 2019), specifying a ‘varPower’ variance structure. Differences between groups (drought-tolerant vs drought-sensitive) were tested post hoc with Holm contrasts using the emmeans function (“emmeans” package). For GLS models, the pseudo R^2 was calculated using the Nagelkerke method (“rcompanion” R package; Nagelkerke 1991).

To account for eventual phylogenetic signals from general trends of variation in stem photosynthesis and drought-tolerance traits, we constructed a phylogenetic tree according to Fletcher et al. (2018) and used “pgls” function in the “caper”

R package to analyze data using a phylogenetic generalized least-squares (PGLS) approach. The PGLS approach enabled calculations of relationships between variables controlling for possible phylogenetic signals (Garland et al., 2005), by taking into account the phylogenetic autocorrelation when testing the relationship between the considered traits. Covariance analysis was based on the Brownian evolution model. Branch length transformation was optimized by calculating Pagel’s lambda (λ , Pagel 1999) using the «maximum likelihood» method (Lambda = 1: phylogenetic co-variation is equal to that estimated by the Brownian evolution model, Lambda = 0: traits’ evolution is independent of phylogeny).

We also used the “phylobase” R package to combine the phylogenetic data with the dataset containing the P_{50} values. Then, we used “ggtree” R package to create a phylogenetic tree in which each species is color-coded according the corresponding P_{50} value, to highlight the evolutionary differences between species in terms of resistance to drought stress (Maherali et al. 2004).

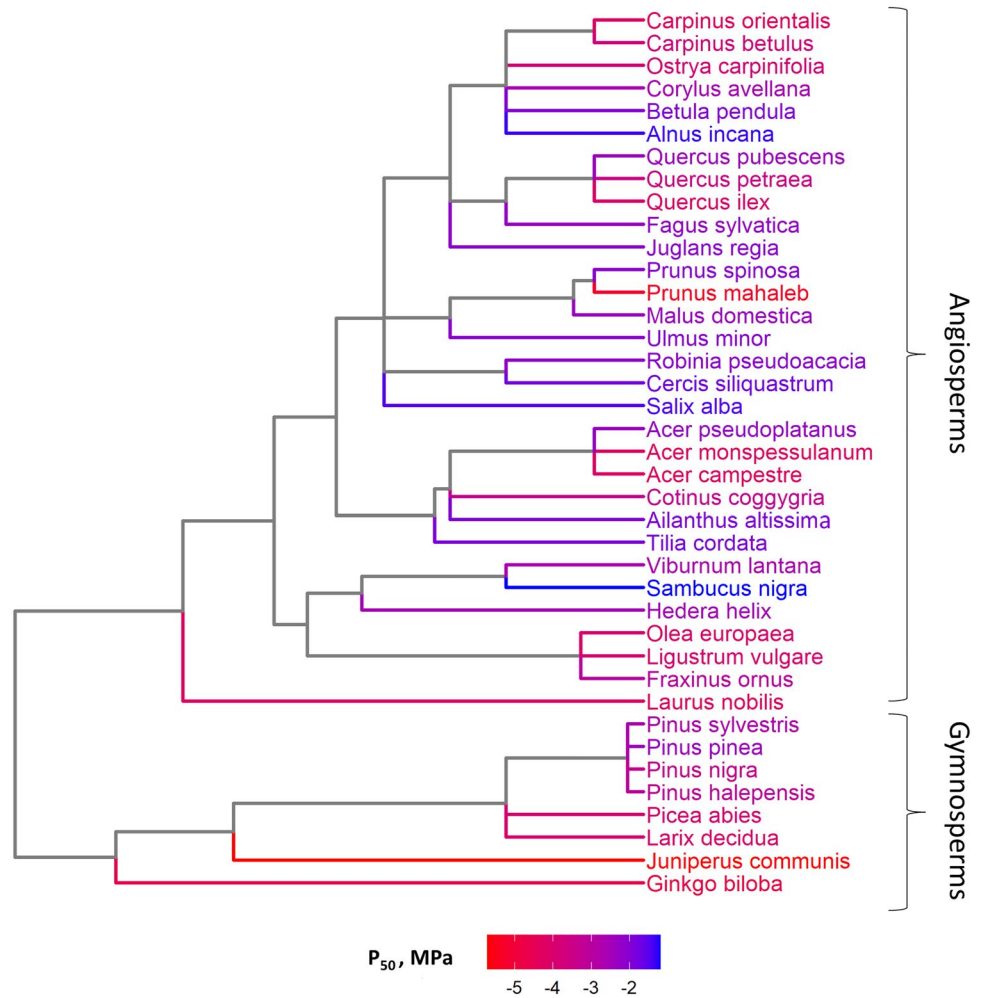
Results

We retrieved data of P_{50} for all the species under study (Table S1, references in Table S2). Since our starting hypothesis was that stem photosynthesis might be linked to tree resistance/resilience to drought stress, we mapped P_{50} to the phylogenetic tree to better/visually show that the species “drought-tolerant” or “sensitive” were not phylogenetically close to each other, so that their drought tolerance level was most likely linked to a convergent evolution. Data showed in Fig. 2 exclude the possibility of strong phylogenetic bases for the correlation between P_{50} and PSII function. Indeed, high resistance to embolism (more negative P_{50}) appeared in several species, both Angiosperms and Gymnosperms (Fig. 2), very distant from each other from a phylogenetic point of view. The highest value was found in *Sambucus nigra* (-1.2 MPa), while *Juniperus communis* had the lowest value (-5.7 MPa).

Mean values of WD for each species are reported in Table S1. The lowest WD was measured in *Tilia cordata* (0.30 ± 0.01 g cm^{-3}), while *Quercus ilex* had the highest value (0.68 ± 0.07 g cm^{-3}). A significant negative relationship was found between P_{50} and WD for all the years investigated (c-yr stem segments: $P = 0.03$, $R^2 = 0.11$, Lambda = 0.38; 1-yr stem segments: $P = 0.02$, $R^2 = 0.13$, Lambda = 0.28). We decided to show only the data for the 2-yr stem segments, with lower P value and higher R^2 (Fig. 3).

F_v/F_m of leaves, bark, and wood for each species and each growth year are also reported in Table S3. Values of F_v/F_m for leaves were above 0.80 for all the study species. The lowest F_v/F_m value for wood was recorded in *Pinus halepensis*

Fig. 2 Phylogenetic tree showing species selected for this study, color-coded according to their values of xylem water potential inducing 50% loss of hydraulic conductivity (P_{50}). Note that more negative values characterize drought-tolerant species. Data are retrieved from literature



(0.42 ± 0.5), with *Cercis siliquastrum* scoring the highest one (0.81 ± 0.02). Instead, the lowest F_v/F_m value at bark level was measured in *Prunus spinosa* (0.62 ± 0.08), while *Laurus nobilis* had the highest value (0.83 ± 0.004). In both Angiosperms and Gymnosperms, average F_v/F_m values for leaves were similar across different species, but consistently higher than those measured at bark and wood level in each species. When considering the wood, Angiosperms showed higher values of F_v/F_m than Gymnosperms, especially when considering the wood (Fig. 4). We found a decreasing trend of photosynthetic efficiency with increasing stem age. This trend was more evident for Gymnosperms than for Angiosperms, and more marked when considering the wood, compared to the bark (Fig. 4).

When exploring possible differences in F_v/F_m according to the relative drought tolerance of different species based on the P_{50} threshold, we found that F_v/F_m values for the leaves were similar for drought-tolerant and drought-sensitive species. However, significant differences emerged between the two groups when considering bark of 1-year-old ($p=0.02$) and 2-year-old stems ($p=0.02$) (Fig. 5a, Table S4A).

When considering wood, we found significant differences between the two groups in the current-year ($p=0.007$) and 1-year-old stems ($p=0.023$) (Fig. 5b, Table S4B). The trend toward lower F_v/F_m values in drought-sensitive compared to drought-tolerant species was maintained also in the 2-year-old wood, although no significant differences were found in this case due to high variability of data.

Values of F_v/F_m in the bark did not change over different stem ages for drought-tolerant species. On the contrary, in the drought-sensitive species, bark F_v/F_m showed a decreasing trend with age (Table S4A). F_v/F_m values in the wood tended to decrease with age in both drought-tolerant and drought-sensitive species (Table S4B).

Discussion

Stem photosynthesis is thought to play different roles in tree resistance/resilience to water shortage, but the actual correlation of species-specific stem photosynthetic efficiency with adaptation to drought has never been clearly demonstrated.

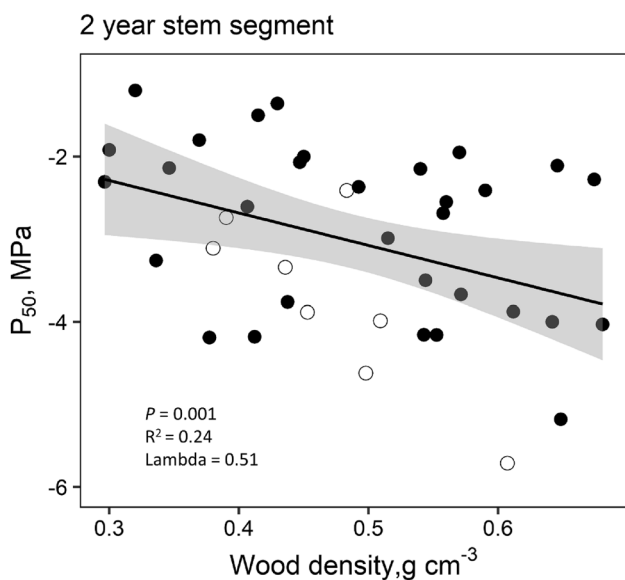


Fig. 3 Relationship between xylem water potential inducing 50% loss of hydraulic conductivity (P_{50}) and wood density (WD) obtained from “pgls”. P_{50} data are retrieved from literature; WD was measured in the species selected for this study. Solid and open circles represent Angiosperm and Gymnosperm species, respectively. Solid line represents the overall regression, while shaded area represents 95% confidence intervals ($r^2=0.24$)

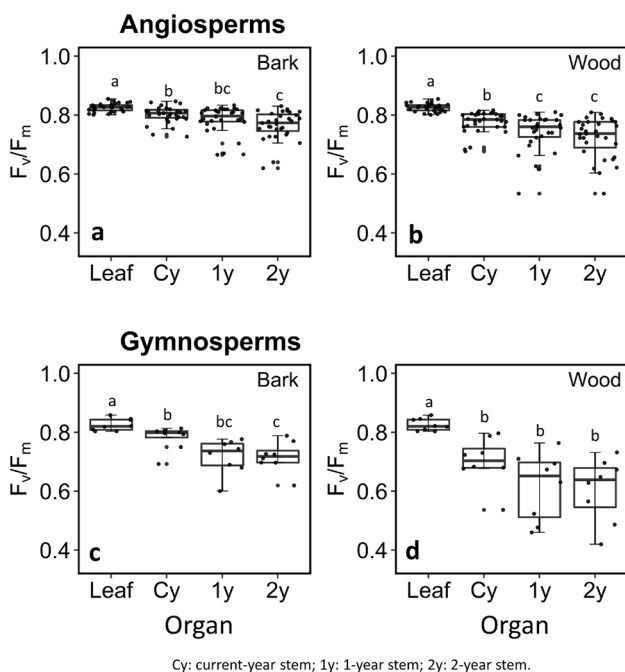


Fig. 4 Median values, 25th and 75th percentiles of F_v/F_m values measured in (a, b) Angiosperms and (c, d) Gymnosperms in leaf (same data for both left and right panels) as well as bark and wood of current-year, 1-year-old and 2-year-old stem segments. Each point represents one species. Different letters indicate significant differences between samples ($P < 0.05$) obtained by gls model

Our data reveal significant differences in F_v/F_m for both bark and wood of species with contrasting levels of drought tolerance, highlighting possible functional roles of stem photosynthesis for woody plants thriving in water-limited habitats. Phylogenetic analysis suggested that such differences were not arising by evolutionary co-variation of stem hydraulic and photosynthetic properties, but rather provide evidence for convergent adaptation to drought involving both functional traits.

Chlorophyll *a* fluorescence is considered an excellent tool to quantify the physiological status of photosynthetic tissues (Govindjee 2004), and F_v/F_m is the parameter most commonly used to this aim. The average F_v/F_m for photosynthetically active and healthy leaves is about 0.83 (Harbinson et al. 2018). Lower values indicate reduced maximum quantum efficiency, due to intrinsic features of the photosynthetic apparatus or to damage resulting from environmental stress (Maxwell and Johnson 2000). Hence, F_v/F_m emerges as a convenient parameter to assess stem photosynthetic efficiency. F_v/F_m for leaves of the study species was above 0.80, and similar among Angiosperms and Gymnosperms. Interestingly, the F_v/F_m of bark for both current-year stems and 1-year-old stems was very close to values measured in leaves (although significantly lower based on statistical analysis), suggesting that the bark of young stems can perform photosynthesis with an efficiency very similar to that of leaves. The current-year wood displayed F_v/F_m particularly high in Angiosperms compared to Gymnosperms, indicating that even this compartment might significantly contribute to carbon uptake in this group of plants.

We found F_v/F_m to be stem age dependent, with relatively high values in current-year stems, but then decreasing in bark and especially wood of older stems. This finding is consistent with the observation that light penetration and CO_2 diffusion generally decrease from the outer surface inside the stem, and as stems age and grow thicker (Saveyn et al. 2010; Wittmann et al. 2006; Wittman and Pfanz 2018). In accordance, blue light is mainly absorbed by the bark, while the wood can be reached by far-red enriched light (Wittmann and Pfanz 2016; Natale et al. 2023) that is far from optimal for the photosynthetic process. However, it is interesting to note that even 2-year-old wood displayed appreciable levels of F_v/F_m (especially in Angiosperms), consistent with possible residual CO_2 fixation even in this compartment. This finding would be in agreement with the role proposed for wood chloroplast as ‘recyclers’ of CO_2 released by respiration of wood parenchyma (Ávila et al. 2014). This process might help plants to buffer NSC depletion during periods of stress leading to stomatal closure (see below). Another factor possibly influencing the recorded age-dependent trend might be the progressive chloroplast senescence induced in the inner stem portions due to the reduction of incident light, and this hypothesis deserves further studies.

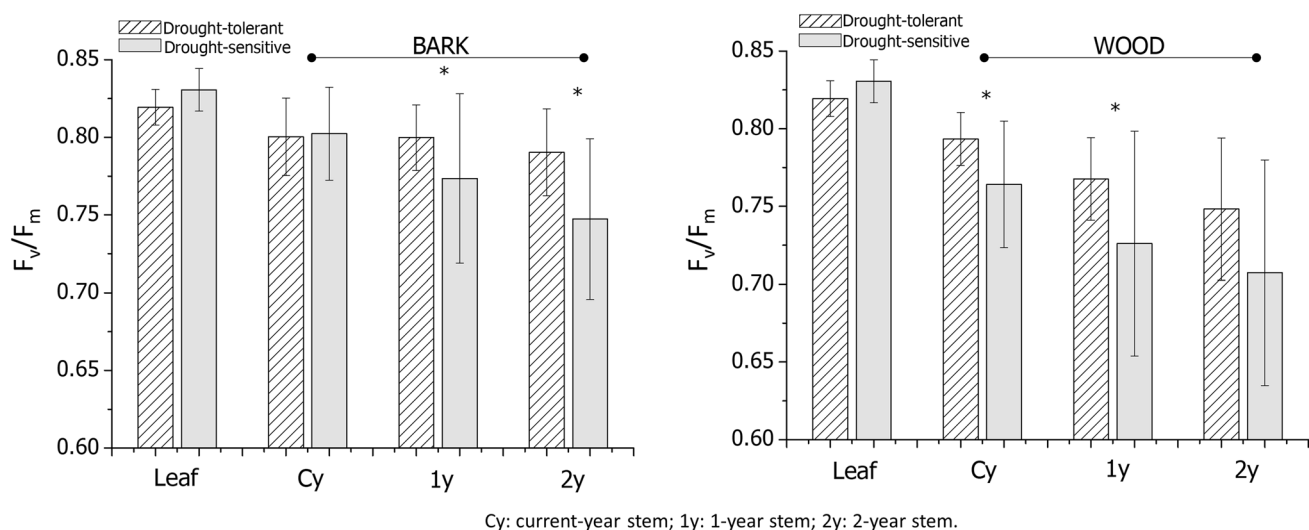


Fig. 5 Mean maximum quantum yield of PSII (F_v/F_m) in drought-tolerant and drought-sensitive Angiosperm species, according to values of xylem water potential inducing 50% loss of hydraulic conductivity (P_{50}). Values are reported for leaves (same data for both left and right

panels), as well as for bark and wood samples of different age. Vertical bars indicate standard deviation. Asterisks indicate significant differences between groups ($P < 0.05$)

As expected, statistical analyses revealed that F_v/F_m values of bark and wood differed between Angiosperm and Gymnosperm species. Specifically, Angiosperms had higher F_v/F_m values, especially in the wood, compared to Gymnosperms. These differences are consistent with the xylem anatomical features in the two groups, with specific reference to the percentage wood volume occupied by parenchymatic cells. In fact, Gymnosperms have typically low amounts of parenchyma in their wood (in the range of 5–10% of the total volume), in sharp contrast with Angiosperms where 25–50% of the wood volume can be occupied by living cells (Morris et al. 2016; Kiorapostolou et al. 2019). Hence, Gymnosperm wood can accommodate relatively fewer chloroplasts and is likely less efficient in light transmission below the bark, compared to that of Angiosperms.

The vulnerability of xylem to embolism (P_{50}) is considered one of the most reliable proxies to quantify plant tolerance to drought stress (e.g., Choat et al. 2012; Petruzzellis et al. 2022). P_{50} is a measure of the apoplastic vulnerability to dehydration, since drought stress can affect the soil-to-leaf pathway by causing dysfunction of water columns in xylem conduits (e.g., Nardini and Luglio 2014), potentially inducing embolism formation and further desiccation (e.g., Nardini et al. 2018; McDowell et al. 2008; Adams et al. 2009). Indeed, P_{50} values show large inter-specific variation, and are generally coordinated with climatic conditions of the species' natural range with specific reference to precipitation, evapotranspiration, and temperature (Maherali et al. 2004; Choat et al. 2012). Values of P_{50} in our dataset spanned a twofold range for both Angiosperms and Gymnosperms, and allowed us to separate two groups of relatively

drought-tolerant (11 species) and drought-sensitive (20 species) Angiosperms. The same classification was not possible for Gymnosperms, due to the relatively small number of species analyzed. Drought-tolerant and drought-sensitive species were distributed along independent phylogenetic trajectories; therefore, their level of drought adaptation is not due to phylogenetic proximity (Fig. 2). A possible pitfall of our analysis lies in the fact that P_{50} values were retrieved from the literature, and not measured on the same individuals used for F_v/F_m measurements. Although species-specific vulnerability to xylem embolism displays limited genotypic variability (Wortemann et al. 2011; Unterholzner et al. 2020), we decided to measure wood density in the same plants used for analysis of stem photosynthetic efficiency to further test the overall reliability of the P_{50} dataset as based on literature data. WD is recognized to be positively linked to hydraulic safety in some species, and negatively related to hydraulic efficiency in most species (Chave et al. 2009; Markesteijn et al. 2011; Scholz et al. 2013; Nardini et al. 2013; Kiorapostolou et al. 2019), and this trait was correlated with P_{50} values across our dataset, confirming the overall validity of our classification of Angiosperms according to their relative drought tolerance. We found that woody species with more negative P_{50} values were also characterized by higher F_v/F_m values in bark and wood, although not for all age ranges. This finding might suggest that drought-tolerant species have greater stem photosynthetic efficiency, as a possible adaptation to environmental conditions imposing prolonged leaf stomatal closure.

It is also noteworthy that drought-tolerant species have slightly lower leaf F_v/F_m values than drought-sensitive

species, but the former have generally greater stem F_v/F_m than the latter. This finding might indicate a possible trade-off between leaf and stem photosynthetic efficiencies. These conclusions would be in accordance with the previous studies, suggesting that photosynthetic stems could have evolved as an adaptation to life in tropical-dry and Mediterranean-like ecosystems (Ávila-Lovera and Ezcurra 2016; Ávila-Lovera and Garcillán 2020). In fact, under prolonged drought, leaf stomata close to prevent excessive tissue dehydration (Brodrribb and Holbrook 2003; Bartlett et al. 2016; Choat et al. 2018). Stomatal closure cannot fully prevent residual water loss and progressive drop of xylem pressure, so that these species gain an adaptive advantage from developing xylem conduits highly resistant to xylem embolism that can assure the integrity of the root-to-leaf water pathway. Under such conditions, plants face a reduction of leaf-level photosynthetic carbon gain (Meinzer et al. 2009; Choat et al. 2012; Manzoni et al. 2013; Nardini et al. 2018), potentially leading to depletion of stored non-structural carbohydrates (NSC) to maintain primary and secondary metabolism (McDowell et al. 2022). Prolonged drought stress might provoke exhaustion of NSC pools (McDowell et al. 2008; Adams et al. 2009), so that additional carbon gain at the stem level might represent a significant adaptive advantage for such species.

In this study, we used P_{50} values to disentangle drought-sensitive from drought-tolerant species, and to compare stem photosynthetic efficiency in these two groups. Hence, our data cannot provide any insight into possible mechanistic relationships between vulnerability to xylem embolism and stem photosynthesis. However, some observations lead to interesting speculations. In particular, it is worth noting that wood photosynthetic efficiency was higher in Angiosperms than in Gymnosperms. Locally produced non-structural carbohydrates have been suggested to be involved in the post-drought recovery of stem hydraulics, pending refilling of embolized conduits (Schmitz et al. 2012; Bloemen et al. 2016; Nardini et al. 2018). In this light, it is important to remember that embolism repair has been observed more frequently in Angiosperms compared to Gymnosperms (Johnson et al. 2012; Klein et al. 2018). Thus, it is tempting to speculate that the limited ability of Gymnosperms to recover their hydraulic functions after drought partly depends on their low photosynthetic efficiency at wood level, that would prevent adequate production of non-structural carbohydrates to be invested in the generation of osmotic forces which are thought to be required for embolism reversal (Nardini et al. 2011; Secchi and Zwieniecki 2016; Secchi et al. 2021). It is also possible to speculate that PSII function in drought-tolerant species is important for some oxygen production at wood level, thus favoring respiration and ATP production based on consumption of starch reserves under conditions of

impaired photosynthesis, but further studies are required to support or discard such speculative conclusions.

To conclude, our data suggest an adaptive role for stem photosynthesis for woody plants thriving in hot and dry ecosystems. Considering that this is the first report showing the connection between stem photosynthesis and drought-related functional traits, we call for more studies aimed at verifying these findings at larger geographical, ecological, and taxonomic scales, to better understand the ecophysiological importance of photosynthetic stems for plants facing ongoing climate changes.

Author contributions AN, SN, and FP planned and designed the research. SN performed experimental measurements. SN, FP, and AN analyzed the data. TM, NLR, and AA contributed to the interpretation of the data. SN and AN wrote the manuscript, with contribution by all co-authors.

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Data availability The data supporting the findings of this study are either reported in Supplementary Information files, or available from the corresponding author upon reasonable request.

Declarations

Conflict of interest We declare the absence of any competing interests at the time of submission.

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