RESEARCH PAPER

Constant hydraulic supply enables optical monitoring of transpiration in a grass, a herb, and a conifer

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Abstract

Plant transpiration is an inevitable consequence of photosynthesis and has a huge impact on the terrestrial carbon and water cycle, yet accurate and continuous monitoring of its dynamics is still challenging. Under well-watered conditions, canopy transpiration (E_c) could potentially be continuously calculated from stem water potential (Ψ_stem), but only if the root to stem hydraulic conductance (K_r-s) remains constant and plant capacitance is relatively small. We tested whether such an approach is viable by investigating whether K_r-s remains constant under a wide range of daytime transpiration rates in non-water-stressed plants. Optical dendrometers were used to continuously monitor tissue shrinkage, an accurate proxy of Ψ_stem, while E_c was manipulated in three species with contrasting morphological, anatomical, and phylogenetic identities: Tanacetum cinerariifolium, Zea mays, and Callitris rhomboidea. In all species, we found K_r-s to remain constant across a wide range of E_c, meaning that the dynamics of Ψ_stem could be used to monitor E_c. This was evidenced by the close agreement between measured E_c and that predicted from optically measured Ψ_stem. These results suggest that optical dendrometers enable both plant hydration and E_c to be monitored non-invasively and continuously in a range of woody and herbaceous species. This technique presents new opportunities to monitor transpiration under laboratory and field conditions in a diversity of woody, herbaceous, and grassy species.

Keywords: Canopy transpiration monitoring, optical dendrometer, root to stem hydraulic conductance, water potential.

Introduction

Plant transpiration is an unavoidable consequence of biomass production and a key component of the terrestrial water cycle (Schlesinger and Jasechko, 2014). Maximum transpiration generally occurs in moist soil where stomata are not forced to close by leaf water deficit generated directly by drying soil, or by reductions in soil-leaf hydraulic conductivity (Scoffoni and Sack, 2017; Rodriguez-Domínguez and Brodribb, 2019; Carminati and Javaux, 2020; Abdalla et al., 2021; Bourbia et al., 2021). Quantifying the dynamic behaviour of transpiration under non-stressed conditions is necessary to explain the atmospheric cycling as well as temporal dynamics of carbon assimilation and plant water use under varying atmospheric conditions (Brodribb et al., 2020). However, continuous in situ estimation of plant transpiration is still a challenging task due to technical limitations.

Few techniques have been developed to continuously quantify transpiration at the plant level. Sap flow measurement is perhaps the most commonly applied method, but this has limited temporal resolution and is typically restricted to measuring woody plants (Granier, 1987; Steppe et al., 2015;
Tfwala et al., 2018). Gravimetric methods can provide precise information, but are really only suitable for small (potted) plants (Herbst et al., 1996). Other methods relying on meteorological variables, such as the Bowen ratio energy balance (Spittlehouse and Black, 1980; Malek and Bingham, 1993) or eddy covariance systems (Baldocchi et al., 2001; Williams et al., 2004), and satellite-based remote sensing combined with complex modelling (Allen et al., 2007; Senay et al., 2013; Reyes-González et al., 2018) have been also employed for larger scale measurements. However, these techniques are unable to partition soil and plant water loss and differentiate transpiration between species in spatially heterogenous environments (Tang et al., 2006; Schlesinger and Jasechko, 2014; Bai et al., 2019; Nelson et al., 2020). Therefore, they are unsuitable for studying temporal dynamics of a species-specific water budget or competition for water between different species.

Canopy transpiration (E c) is strongly influenced by plant hydraulic conductance, mainly root–stem conductance (K r-s; here denoting the flow path from the root surface to the stem xylem) which is known to be the lowest within the liquid component of the soil–plant–atmosphere continuum and the most sensitive to water stress (Brodrribb and Cochard, 2009; Rodriguez-Dominguez and Brodrribb, 2019; Bourbia et al., 2020, 2021). The relationship between K r-s and E c can be described using Darcy’s law in the form E c = K r-s×ΔΨ (Equation 1) (Sperry et al., 1998), where ΔΨ is the water potential difference between the root surface (Ψ soil) and the stem xylem (Ψ stem). Based on Equation 1, if K r-s remains constant when E c changes, clearly ΔΨ should be directly proportional to the E c at equilibrium. In this case ΔΨ could be used as a sensitive proxy for E c. Clearly this approach would not be valid under conditions of drought because K r-s has been shown to decline drastically during the early stages of water stress (around −1 MPa; Ψ soil), triggering stomatal closure in different species (Blizard and Boyer, 1980; North and Nobel, 1991; Cuneo et al., 2016, 2021; Rodriguez-Dominguez and Brodrribb, 2019; Bourbia et al., 2021). However, under relatively wet soils (0≥ Ψ soil≥ −1 MPa), where most transpiration typically occurs, K r-s may be sufficiently stable to allow monitoring by ΔΨ proxy. Yet this assumption of constant K r-s in unstressed plants is still uncertain due to various reports that root K changes in response to E c (Boyer, 1985). Some studies argue that, in well-watered plants, ΔΨ remains constant over a wide range of E c rates, suggesting that K r-s is dynamic (i.e. K r-s increases and decreases with diurnally changing E c) (Macklon and Weatherley, 1965; Tinklin and Weatherley, 1966; Aston and Lawlor, 1979; Black, 1979). A similar number of papers present data indicating, on the contrary, that ΔΨ varies in a linear manner with E c in other species, indicating constant K r-s with changing E c (Hailey et al., 1973; Neumann et al., 1974; Dubé et al., 1975; Bunce, 1978; Ike et al., 1978; Hiratsawa and Ishihara, 1991).

In species displaying constant K r-s under non-limiting water conditions where Ψ soil was close to zero, Ψ stem could be used to monitor E c, potentially providing a new window into the dynamics of plant water use. However, the main hurdle for employing such an approach is to accurately resolve the dynamics of Ψ stem under variable field conditions. Psychrometers are the most widely used instruments for continuous monitoring of the in situ Ψ stem. However, their application is limited by installation difficulties, particularly on soft herbaceous plants, and the low degree of stability under fluctuating temperatures (Dainese et al., 2022). Alternatively, several studies have shown that plant Ψ stem can be readily estimated, indirectly but accurately, using tissue width variation (e.g. petioles and branchlets) because of the strong linear relationship found between these two parameters across a wide range of Ψ stem (Bourbia et al., 2021).

Stem width variation can be recorded continuously using automated dendrometers (Klepper et al., 1971; Fereres and Goldhamer, 2003; De Swaef et al., 2009, 2015), but a newly described optical dendrometer provides sufficient resolution to measure changes in tissue width in determinate (non-growing) structures such that changes in petiole or leaf width over extended periods can be used to monitor plant water status (Bourbia et al., 2021). Optical dendrometers thus have the potential to continuously track daily fluctuations in plant transpiration at high temporal resolution using tissue width changes as a proxy for transpiration.

With the aim of assessing the potential for E c to be monitored optically using dendrometers attached to leaves, we first investigated whether K r-s is constant or a function of changing E c in well-watered individuals of three divergent species. We use three phylogenetically, morphologically, and ecologically divergent species—Tanacetum cinerariifolium is a perennial herb with herbaceous roots, Zea mays is a grassy monocot which also has herbaceous roots, and Callitris rhomboidea is a hardy conifer with woody roots—in an attempt to test the generality of our findings. Establishing that K r-s remains static in each species, we test the accuracy of predicting dynamic E c from variation in petiole or leaf shrinkage.

Materials and methods

Plant material and growth conditions

Plants of Tanacetum cinerariifolium (Trevir.) Sch. Bip, Zea mays L, and Callitris rhomboidea R. Br. ex A. Rich. & Rich. were grown from seeds or rootstock (in the case of T. cinerariifolium) in glasshouse facilities at the University of Tasmania. Due to the different growth characteristics of the species, plants were potted in the optimum growth medium which was different for the different species. Tanacetum cinerariifolium plants were grown in 5 litre pots containing natural soil (day) obtained from north-west Tasmania where it is grown commercially. The woody C. rhomboidea seedlings ranged from 30 cm to 40 cm in height and were potted in 2 litre pots using potting mix (medium 7:4 mix of composted fine pine bark and coarse washed river sand). Zea mays plants were grown in 2 litre pots filled with loamy soil. All plants were grown under unfiltered natural light in a controlled glasshouse cell at 25/15 °C day/night temperature and 40/80% day/night relative humidity (RH), and were watered daily to field capacity. Plants of Z. mays, T. cinerariifolium, and C. rhomboidea used in this experiment were 2–6, and 14 months old, respectively.
K_c response to changes in E_t

K_c was determined at high and low rates of steady-state whole plant transpiration (E_t, mmol m^{-2} s^{-1}) to verify whether it is static or dependent on E_t or \( \Psi_{stem} \). K_c was calculated based on the normal application of Darcy's law standardized to viscosity of water at 20 °C:

\[
K_{c\rightarrow s} = \frac{E_c}{\Psi_{stem} - \Psi_{soil}}
\]

This was done by simultaneously and continuously measuring E_t and \( \Psi_{stem} \) in well-watered plants subjected to different transpirational demands by manipulating RH in a controlled chamber as described below. \( \Psi_{soil} \) was assumed to be 0 MPa because pots were watered before and throughout measurements. Steady-state conditions refer here to conditions where both E_t and corresponding \( \Psi_{stem} \) are at steady state under stable RH, meaning that the plant capacitance effect is negligible.

Continuous measurements of E_t and \( \Psi_{stem} \)

On the evening preceding measurements, pots of four plants per species were watered and allowed to drain excess water then covered with a plastic bag to prevent evaporation from the soil. Plants were then transferred to a well-ventilated controlled-environment chamber, placed on computer-interfaced balances, and weighed continuously (every 5 min) to an accuracy of ±0.01 g (model PGS002-S; Mettler Toledo, Columbus, OH, USA). In each individual plant, a high-resolution automated optical dendrometer (Cavicam Co, Hobart, Australia) (for details see www.cavicam.co and Bourba et al., 2021) was attached to a mature (non-growing) petiole (T. cinerariifolium), leaf blade (Z. mays), and terminal branchlet (C. rhomboidea). The optical dendrometer was used to monitor width variation continuously (at 1–5 min intervals) from which the temporal dynamics of \( \Psi_{stem} \) could be inferred (see calibration details below) during E_t measurements.

Prior to the transpiration treatments, plants were left in the dark during the night at 20 °C (for T. cinerariifolium and C. rhomboidea) and 25 °C (for Z. mays), and high RH (~90%). During the next morning, plants were illuminated at a photosynthetic photon flux density (PPFD) of 450 μmol quanta m^{-2} s^{-1} (at the canopy level) and RH was decreased to ~70% [vapour pressure deficit (VPD)=0.6 kPa for T. cinerariifolium and C. rhomboidea] and sustained using a commercial humidifier (SeccoUltra 00563, Olimpia-Splendid, Gualtieri, Italy) until ~70% [vapour pressure deficit (VPD)=2.4 kPa for Z. mays] and sustained using a commercial humidifier (SeccoUltra 00563, Olimpia-Splendid, Gualtieri, Italy) until RH was decreased to ~70% (VPD=0.6 kPa) for T. cinerariifolium and C. rhomboidea [VPD=1 kPa for Z. mays] and sustained using a commercial humidifier (SeccoUltra 00563, Olimpia-Splendid, Gualtieri, Italy) until RH was decreased to ~40–60% (VPD=1.6 kPa for T. cinerariifolium and C. rhomboidea; VPD=2.4 kPa for Z. mays) and maintained at this level using a dehumidifier until E_t and width reached a new steady state and remained stable for at least 1–2 h, then RH was decreased to ~40–60% (VPD=1.6 kPa for T. cinerariifolium and C. rhomboidea; VPD=2.4 kPa for Z. mays) and maintained at this level using a dehumidifier until E_t and width reached a new steady state and remained stable for at least 1–2 h (Fig. 1). RH levels in the chamber were always modified in a regular sequence; from high RH in the morning to low RH at mid-day as described above. Temperature was held constant throughout measurement irrespective of RH change and was maintained at the same level as night temperature.

Leaf width was calibrated against \( \Psi_{stem} \) measured with a Scholander chamber (PMS, Albany, OR, USA) in each individual plant across a range of 2–3 \( \Psi_{stem} \) values encompassing the maximum range observed during transpiration treatments. These measurements were performed on non-transpiring mature leaves in T. cinerariifolium, branchlets in C. rhomboidea, and the tips of the uppermost fully expanded leaves in Z. mays (15 cm in length=one-third of the total leaf length) that were enclosed in a plastic bag covered with aluminium foil the night before measurements. One measurement was made in the dark before switching on the lights and the others were made at the two levels of RH to which the plants were exposed after width had reached a steady state.

Soil temperature in the centre of the pot was monitored using a thermocouple connected to a datalogger (CR850, Campbell Scientific, Logan, UT, USA). Soil temperature reached air temperature during the night, and both remained constant during measurements throughout the day (within 0.5 °C) independent of RH change.

Air temperature and RH in the growth chamber were monitored at 30 s interval with a temperature/humidity probe (HMP45AC; Vaisala Inc., Helsinki, Finland) placed close to the measured plants, and logged on the same datalogger.

During measurements, water lost by transpiration was added at the top of the pot periodically to avoid any drop in soil water potential (or soil hydraulic conductance) especially at high transpiration after decreasing RH. The water added into the soil was at the same temperature as the soil. E_t was normalized by the projected canopy leaf area measured at the end of the experiment with a flatbed scanner in all species.

Predicting E_t from optically monitored \( \Psi_{stem} \) dynamics using a constant K_c

According to Equation 2, if K_c remains constant, E_t can be estimated from \( \Psi_{stem} \) after correcting for viscosity. In each individual plant, we used plant-specific K_c values (corrected for changes in viscosity due to soil temperature) measured at high RH (and less negative \( \Psi_{stem} \)) to estimate E_t in the same individual at low RH from \( \Psi_{stem} \) inferred from petiole/leaf width measured with the optical dendrometer. This predicted E_t was compared with E_t measured gravimetrically under these conditions. To determine the error associated with using species mean K_c rather than individual K_c, mean measured K_c of each species was also used to predict E_t in individual plants of each species at varying measured \( \Psi_{stem} \) levels, and the values were compared with the measured E_t values.

Statistical analysis

Differences between species mean values of K_c were tested with Student’s t-tests after testing for normality and homogeneity of variances. We used linear regressions to quantify the correlation between tissue width variation and \( \Psi_{stem} \) in each plant individual used for K_c measurements. Results are presented as mean values ±SE. Differences were considered to be significant when P<0.05. The accuracy of predicted E_t relative to the observed E_t was computed using the mean absolute percentage error metric: MAEP = \( \frac{100}{n} \sum_{i=1}^{n} \left| \frac{B_i - A_i}{\bar{B}} \right| \), where \( A_i \) is the actual value, \( B_i \) is the predicted value, and \( n \) is the total number of observations. All analyses were performed using R version 3.5.3 (R Core Team, 2019). Figures were created using SigmaPlot version 12.5 (Systat Software Inc., San Jose, CA, USA).

Results

Leaf width monitored continuously with optical dendrometers was highly linearly correlated with measured \( \Psi_{stem} \) in each individual plant (\( r^2=0.99, P<0.001 \)) (Supplementary Fig. S1), allowing the \( \Psi_{stem} \) dynamics to be predicted at high temporal resolution and in situ from leaf/petiole width variation. Changes in \( \Psi_{stem} \) inferred from petiole/petiole width changes, followed E_t changes closely in all species (Fig. 1). Based on the calibrated optical dendrometers, \( \Psi_{stem} \) was found to fall and reach a new steady state quickly (within 30 min) once E_t increased in all species. There was no lag between E_t and a resultant change in \( \Psi_{stem} \) under non-steady-state conditions either in the morning after turning on the lights or at mid-day after changing RH (i.e. both E_t and \( \Psi_{stem} \) reached steady state at the same time).
Mean $K_{rs}$ varied considerably among species ($P<0.05$) and was significantly higher in the monocot $Z.\ mays$ ($6.94 \pm 0.75$ mmol m$^{-2}$ s$^{-1}$ MPa$^{-1}$) and the herbaceous $T.\ cinerariifolium$ ($5.48 \pm 0.16$ mmol m$^{-2}$ s$^{-1}$ MPa) compared with the woody $C.\ rhomboidea$ ($3.84 \pm 0.37$ mmol m$^{-2}$ s$^{-1}$ MPa; Fig. 2). However, due to the variability in $K_{rs}$ among $Z.\ mays$ plants (SD=22%), differences in mean $K_{rs}$ between this species and $T.\ cinerariifolium$ were not significant. Significant variability in $K_{rs}$ was also observed between plants in $C.\ rhomboidea$ (SD=19%) but it was very small in $T.\ cinerariifolium$ (SD=6%).

Fig. 1. The response of stem water potential ($\Psi_{stem}$; black line), inferred from foliar width variation measured continuously with optical dendrometers, to changes in whole-plant transpiration ($E_c$; red line) measured gravimetrically in one representative individual of $Z.\ mays$, $T.\ cinerariifolium$, and $C.\ rhomboidea$ subjected to two RH levels under well-watered conditions. Temporal dynamics of $\Psi_{stem}$ were monitored at 1 min intervals in $Z.\ mays$ and at 5 min intervals in $T.\ cinerariifolium$ and $C.\ rhomboidea$. The vertical dashed lines represent the time at which RH was decreased from 70% to 40–30%. Grey background, PPFD=0 $\mu$mol m$^{-2}$ s$^{-1}$; white background, PPFD=450 $\mu$mol m$^{-2}$ s$^{-1}$. Arrows indicate when pots were watered.
**Constancy in** $K_{r-s}$

Across a range of RH designed to simulate typical daytime conditions, the steady-state $E_c$ and $\Psi_{stem}$ remained in constant proportion in each individual plant of the three species (Fig. 3), revealing a constant $K_{r-s}$ with changing $E_c$ in all species (Fig. 4). As a result, $K_{r-s}$ also remained constant alongside changing $\Psi_{stem}$ in all species (Fig. 5).

**Predicted versus observed $E_c$**

According to Equation 2 applied during steady-state, the constancy of $K_{r-s}$ should enable $E_c$ to be predicted from monitored values of $\Psi_{stem}$ (as measured by the optical dendrometer). In each individual plant, the predicted values for $E_c$ at low RH calculated from individual $K_{r-s}$ measured at high RH closely followed a 1:1 line ($R^2=0.92$) and were within 5% (MAEP) of the measured values (Fig. 6A).

High predictive accuracy in $E_c$ was also achieved when the species average $K_{r-s}$ was used to estimate $E_c$ at the individual level in the herbaceous $T. cinerarifolium$ ($R^2=0.97$, MAEP=6%) (Fig. 6B). However, this accuracy was limited by the substantial variation in the species mean $K_{r-s}$ measured in $Z. mays$ ($R^2=0.69$, MAEP=14%) and $C. rhomboidea$ ($R^2=0.48$, MAEP=16%).

**Discussion**

$K_{r-s}$ remains constant with changing $E_c$

Our results demonstrated conclusively that, under steady-state conditions in hydrated soil, $\Psi_{stem}$ varies in a proportional manner with $E_c$, indicating that $K_{r-s}$ remains constant with changing $E_c$ in the three distinctly different species studied here (Figs 3, 4). Our results are consistent with those observed in several other species such as cotton, pea, cassava, rice, and sunflower (Hailey et al., 1973; Ike et al., 1978; Passioura, 1980; McBurney and Costigan, 1982; Hirasawa and Ishihara, 1991), and extend those presented by Neumann et al. (1974) and Dubé et al. (1975) for $Z. mays$. The consistent lack of variation in $K_{r-s}$ in response to changes in $E_c$ observed across our experimental species suggests that this is a consistent pattern across seed plants. Nevertheless, some studies reported $K_{r-s}$ to vary with $E_c$ in species grown in hydroponics (Macklon and Weatherley, 1965; Tinklin and Weatherley, 1966; Aston and Lawlor, 1979; Black, 1979). Macklon and Weatherley (1965) suggested that the decrease in $\Psi_{stem}$ with increasing $E_c$ usually observed in soil-rooted plants under field conditions is due to the steady drop in the hydraulic conductance of the rhizosphere (at the soil-root interface), occurring when water is absorbed more quickly at the root surface than it is replaced by that moving...
from untapped soils, even in well-watered conditions. They also argued that this does not occur in hydroponically grown plants because roots are constantly surrounded with water. Local dehydration around the root would violate the assumption in Equation 2 that soil $\Psi_{soil}=0$ MPa. Here we avoided this possible error in $K_{rs}$ calculation by carefully adding water to the pot to ensure the water content remained saturated throughout the experiment. Furthermore, we did not observe any change in steady-state $E_c$ or $\Psi_{stem}$ after the addition of water to high transpirational demands (i.e. at RH=30%), suggesting that rhizosphere water potential remained close to zero (Fig. 1) and that our calculation of constant $K_{rs}$ was correct. Water flows radially across the root cylinder (from the root surface to the stele/xylem) along two parallel apoplastic and symplastic pathways, the latter being mediated by aquaporins. The discrepancy between our results and those discussed above regarding the constancy of $K_{rs}$ with changing $E_c$ may be attributed to differences in the relative contribution of the apoplastic and cell to cell pathways to the whole $K_{rs}$ between different species during transpiration due to differences in root morphological and anatomical features (Kim et al., 2018). A cell to cell transport of water has been shown to dominate in roots of some species such as barely (Steudle and Jeschke, 1983; Knipfer and Fricke, 2010) and bean (Steudle and Brinckmann, 1989), but to play a minor role in roots of maize (Steudle et al., 1987), cotton (Radin and Matthews, 1989), and grapevine (Gambetta et al., 2013) (i.e. water flows mostly through the apoplastic pathway). If water flows predominantly through the cell to cell pathway during transpiration, then $K_{rs}$ may change with $E_c$ due to changes in aquaporin expression or activity (Maurel, 1997; Javot and Maurel, 2002; Knipfer and Fricke, 2011; Laur and Hacke, 2013). In the three species measured here, the constancy of $K_{rs}$ with changing $E_c$ suggests that water flow in the roots follows a largely apoplastic pathway during transpiration under natural conditions.

In this study, $K_{rs}$ measured using the gravimetric method remained constant alongside decreasing $\Psi_{stem}$ induced by increasing $E_c$ in all species. This behaviour has also been demonstrated by Bourbia et al. (2021) in roots of T. cinerarifolium and C. rhomboidea measured with a non-steady-state rehydration technique over a wide range of $\Psi_{soil}$ induced by drought (between $-0.35$ MPa and $-1$ MPa). This observation contrasts with that of a recent study in cotton (Wang et al., 2020) which reported $K_{rs}$ to decline by $>50\%$ as $\Psi_{stem}$ fell marginally from 0 MPa to $-0.16$ MPa. This surprisingly high sensitivity of $K_{rs}$ to water potential was measured on small segments of roots using the centrifuge technique which is known to produce contrasting results in roots depending on the pressure gradient protocols used (Bouche et al., 2015). Our data support the conclusions of Bouria et al. (2015) that very high sensitivity of $K_{rs}$ to water deficit seen in centrifuge studies may be artefactual. However, it remains a possibility that a high sensitivity of
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$K_{\text{r-s}}$ to water stress observed in cotton compared with other species could also be attributed to physiological and morphological differences between these species. Testing $K_{\text{r-s}}$ in intact cotton plants would be a valuable step to confirm these results.

**Constancy of $K_{\text{r-s}}$ allows in situ estimation of $E_c$ from optically measured $\Psi_{\text{stem}}$.**

The constancy of $K_{\text{r-s}}$ observed here indicates that the dynamics of $E_c$ can be calculated from $\Psi_{\text{stem}}$ when it is at steady state according to Equation 2. The validity of using $\Psi_{\text{stem}}$ as a proxy for $E_c$ was evidenced by the strong agreement between gravimetrically measured $E_c$ and that calculated from steady-state $\Psi_{\text{stem}}$ at varying transpirational demands in all species (Fig. 6). The ability to continuously monitor $\Psi_{\text{stem}}$ in situ using optical dendrometers, as demonstrated in this study (Fig. 1), provides a promising approach for tracking $E_c$ changes on a fine temporal scale in both woody and soft herbaceous species.

Unlike other plant-based methods, such as sap flux methods, optical dendrometers are very simple and easy to install, insensitive to external temperature variation, and very responsive to rapid changes in $\Psi_{\text{stem}}$ (Fig. 1). Compared with microclimatological techniques (Bowen ratio and eddy covariance) which provide estimates of evapotranspiration, incorporating both plant and soil water loss (Williams et al., 2004; Tang et al., 2006; Schlesinger and Jasechko, 2014; Perez-Priego et al., 2018), the optical technique measures plant transpiration alone, thus making it suitable for studying spatial and temporal dynamics of species-specific water use and carbon assimilation in mixed stands, and the responses to changes in climate in both natural and agricultural systems. This method can also be used, if scaled up to stand or regional level, as an independent ground-based method to validate models partitioning evaporation and vegetation transpiration (Lawrence et al., 2007; Sutanto et al., 2012), and as a tool to quantify irrigation demands in agricultural systems.

**Optical estimation of $E_c$ under non-steady state conditions**

The highly accurate estimations of $E_c$ from optically derived $\Psi_{\text{stem}}$ observed in the studied species were restricted to periods of steady-state conditions with no influence of plant capacitance (internal stored water). However, under field conditions, $E_c$ may fluctuate substantially and rapidly over the course of the day in response to variations in climatic conditions, and seldom reaches a steady state (Jones et al., 1982). In this case, the use of a steady-state model to predict instantaneous and fast changes of $E_c$ from optically measured $\Psi_{\text{stem}}$ can be valid only if the plant capacitance is negligible or its contribution to $E_c$ is accounted for.

**Conclusion**

The constancy of $K_{\text{r-s}}$ under varying transpirational demands observed in this study was a common feature among different species. This means that the optical technique presented here can probably be used to estimate $E_c$ in real-time in diverse plant species under steady-state conditions. Yet, further work is necessary to elucidate the applicability of this technique in monitoring instantaneous non-steady changes of transpiration under non-steady atmospheric conditions over the long term.

**Supplementary data**

The following supplementary data are available at JXB online. Fig. S1: Relationship between foliar tissue width and $\Psi_{\text{stem}}$.
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Author contributions

IB and TB: conceptualization; IB and CL: design of the experiment; IB: data collection and analysis; IB: writing, with revisions by TB.

Conflict of interest

The authors declare no conflicts of interest.

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Data availability

The data supporting the findings of this study are available from the corresponding author, Timothy Brodribb, upon request.

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