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ORIGINAL ARTICLE

Quantifying differences in thermal dissipation probe calibrations for *Eucalyptus globulus* species and *E. nitens* × *globulus* hybrid

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Abstract

Key message **Calibration of sap flux density equations for Eucalytus is required when using sapflow thermal dissipation probes to avoid large underestimations of transpiration and water use.**

Abstract Eucalyptus plantations are expanding in response to global wood demand but are raising concerns about their impacts on water supplies. Sustainable plantation management in areas with water conflicts will require accurate assessments of tree and stand water use. Thermal dissipation probes have been used to estimate tree water use, but recent work suggests that species-specific calibrations may be required to obtain accurate results. In this study, we quantified sap flux density (SFD) in 2-year-old *Eucalyptus globulus* Labill (Eg) and *E. nitens* × *globulus* (Eng) species using the thermal dissipation method developed by Granier. For each species we compared the original Granier equation with species-specific calibrations using whole tree potometers over a 36-h period. Our results showed that on average, Granier's original equation significantly underestimated SFD in both species, and when scaled to the stand level, tree transpiration (Ec) was significantly lower compared to onsite calibrations. The Granier method also underestimated nocturnal transpiration for both genotypes. Measured calibration coefficients were similar and not statistically different between Eg and Eng. These results highlight the importance of species-specific calibrations using thermal dissipation probes for Eucalyptus species to improve stand water use estimates and inferences about ecological impacts.

Keywords Sapflow · Sap flux density · Granier · Transpiration · Nocturnal flow

Introduction

Eucalyptus species are some of the world's most important plantation species covering an estimated area of approximately 20.1 million ha (Albaugh et al. 2013; Iglesias and Wistermann 2009). Across regions, there is increasing concern over the effects of these plantations on watershed water yield and their impact on downstream communities (Albaugh et al. 2013; Almeida et al. 2007; Hoekstra and Mekonnen 2012). Furthermore, *Eucalyptus* plantations continue to expand worldwide because they are typically more productive relative to conifers and other hardwood species (Wullschleger et al. 1998), but this expansion is magnifying concerns over water use of forest plantations.

Although information on water use has been reported for several *Eucalyptus* species such as *E. grandis* × *E. urophylla* Blake (Hubbard et al. 2010; Otto et al. 2014), *E.urophylla* Blake (Morris et al. 2004), *E. globulus* (Forrester et al. 2010; Gurovich et al. 1996; Zang et al. 1996),

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E.saligna SM (Hubbard et al. 2004), *E. nitens* H.Deane & Maiden (Medhurst and Beadle 2002), *E. sieberi* Johnson (Roberts et al. 2001) and *E. grandis* W.Hill (Dye 1996; Olbrich et al. 1993) their impact on water resources is still strongly debated. For species such as *Eucalyptus globulus* and *Eucalyptus nitens*, extensively planted in Mediterranean regions, their potential effect on water resources have become a major social, environmental and economic concern in the face of climate change (Almeida et al. 2007; Calder et al. 1999; Dye 2000; Whitehead and Beadle 2004). Moreover, successful development of new highly productive *Eucalyptus* genotypes has increased uncertainty about plantations water sustainability and their ability to tolerate increased drought and temperature stress (Albaugh et al. 2013; Hubbard et al. 2010; Navarrete-Campos et al. 2012; Osorio and Pereira 1994; Watt et al. 2014).

The Granier thermal dissipation probe technique is based on continuous monitoring of the temperature differential of a heated vs. an unheated thermocouple pair inserted along the stem of an individual tree over time. This technique has been widely used to estimate sap flux density (SFD), defined as the amount of water moving through the tissues per unit of sapwood surface per unit of time (Yin et al. 2004). Once SFD has been quantified, whole tree transpiration (Ec) is calculated as:

$$\mathrm{Ec} = \mathrm{SFD} \times \mathrm{SA}, \tag{1}$$

where *SA* sapwood area (cm^2) is measured at the probe insertion point. In the original method, SFD ($\mathrm{g\ cm}^{-2}\ \mathrm{s}^{-1}$) is presented as a function applicable across species expressed as

$$\mathrm{SFD} = \ 11899 \times 10^{-6} K^{1.231}, \tag{2}$$

where K is the dimensionless flow index coefficient derived from the relative maximum differential temperature obtained under zero flow conditions ($\Delta T_{\ \mathrm{max}}$) and the instantaneous temperature differential (ΔT) being observed at any time (Granier 1987) and is calculated as:

$$K = \frac{\Delta T_{\mathrm{max}} - \Delta T}{\Delta T}. \tag{3}$$

Lu et al. (2004) reviewed the Granier technique and indicated that despite its robustness and based on evidence of work on other species calibration of the SFD equation, specific-species coefficients may be needed because of differences in wood structural characteristics that may affect the Granier SFD equation. Nadezhdina et al. (2002) suggested that differences in the model parametrization proposed by Granier may be due to abrupt changes in wood anatomy. In fact, Hubbard et al. (2010) using the Granier system on a stem section under hydraulic pressure showed that standard equations would underestimate by more than double SFD in *Eucalyptus urograndis* plantations. This evidence suggests that *Eucalyptus* species may require specific-species calibration equations, and additional efforts should be focused on its understanding to obtain reliable estimations of transpiration at the individual tree level.

The objective of this study was to assess the suitability of using the Granier equation for sap flux density (Granier 1987) for two *Eucalyptus* species. We hypothesized that (1) the Granier standard equation underestimates SFD for the *Eucalyptus* species tested and (2) *Eucalyptus nitens* and *E. nitens*×*globulus* fit a common equation for SFD estimation.

Methods

Site description

The study was conducted at Forestal Arauco Co. Quivolgo forest nursey in Constitución city, Maule region, Chile (35°18′ 49.14″S, 72°23′ 23.66″O). Land use history included *Eucalyptus sp.* seedling production and cuttings orchards. The site was located at 3 km from the coast approximately 20 m above sea level. Mean annual temperature and precipitation at the site were 13.1 °C and 798 mm, respectively (Source: Climate-data.org, 1982 to 2012 years). Weather information during the experiment was obtained from 15 min records from a weather station located nearby the site (<1 km). Soils, derived from granitic sediments, had a clayey–loamy surface and clayey texture in depth causing moderately well-drained conditions along the profile. Before establishment of the experiment, soil preparation included disking at 80 cm depth and mounding.

Trial establishment

The genotypes selected for this study were from a larger experiment examining the effects of irrigation on tree water use and wood growth. Trees were planted in July 2013, in a randomized complete factorial design with three replicates for each of two irrigation treatments (rainfed and irrigated). Improved operational plant genetic material consisting of cuttings of *Eucalyptus globulus* (Eg), *E. nitens* × *globulus* hybrids (Egn), and *E. nitens* (En) seedlings were planted at a 3×2 m spacing (1666 trees ha^{-1}), and divided into 5×5 trees experimental units (two tree width buffer strips) with 3×3 trees internal measurement plots. Trees were measured annually for ground line diameter (gld) at 10 cm above the ground at the base of each tree (±0.1 cm, gld), total height (±5 cm, ht), and diameter at breast height at 1.3 m (±0.1 cm, dbh) after the second year.

From October 2013 to January 2014, a sprinkler system irrigated the whole experimental area to ensure early survival during summer months. In February 2014, a drip irrigation system was installed to provide control of water supply on the experiment until April 2014, and from October to June (unusual extended dry season) in 2015. The irrigation treatment supplied water daily to maintain soil water availability above mid-range permanent wilting point and field capacity of the soil during all the years of evaluation. Each tree was fertilized in November 2014 using standard protocols to eliminate any nutrient deficiencies. Pre-planting and post-planting broadcast weed control was applied to the whole area (2.5 Kg ha^{-1} Glyphosate) during the first and second growing seasons to maintain weed-free conditions in the experiment.

Genotype and individual tree selection

We performed sap flux calibrations on three trees each for Eg and Eng from buffer areas of independent blocks representing the gld distribution of each genotype at the site after 2 years of stand development (Table 1).

Sap flux calibration

During the early morning of April 13th 2015, 2-cm long Granier heat dissipation probes (Hubbard et al. 2010) were installed at 80 cm above the ground on each selected tree, one day before sap flux monitoring to allow for probe/tissue acclimation after installation. At sunrise of April 14th, trees were cut and immediately put in a potometer which consisted of a 20-L-graduated bucket containing 7 L of water, and water uptake from each independent bucket was measured hourly. Review of daily patterns of SFD before and 1 day after cut suggested that one of the Eng trees exhibited symptoms of significant embolism from cutting and was excluded from the analysis. Probes were connected to a datalogger and multiplexer system (CR1000 and AM16/32B, Campbell Scientific Inc. Logan, UT). Water uptake was monitored every hour from 8:30 a.m. to 19:30 p.m. on April 14th and from 8:30 a.m. to 13:30 p.m. on April 15th. Hourly refilling of each bucket with known amount of water ensured a constant, measured water supply for each tree. The dimensionless coefficient K (Eq. 3) (Granier 1987) was calculated to estimate flow density by both the Granier equation and the fitted power function to the experimental data. Nocturnal sapflow estimates were made when photosynthetic active radiation (PAR) was <1 μmol m^{-2} s^{-1} (Phillips et al. 2010), which corresponded from 18:00 p.m. to 07:00 a.m., and direct assessments were obtained from the change in the volume of water in the potometer from the evening of April 14th through sunrise of April 15th.

Sapwood area and stem wood density

At the end of the experiment, trees were destructively sampled to assess individual tree sapwood area, wood density and foliar biomass (Table 1). Sapwood area (SA) was visually estimated from a 3-mm thickness disc cut above the probe insertion point, and the sapwood/heartwood boundaries at its major (D, Dh) and minor (d, dh) diameters were

Table 1 Sample tree characteristics for *Eucalyptus globulus* and *Eucalyptus nitens* × *globulus*

	dbh[a] (cm)	dmp[b] (cm)	ht[c] (m)	SA[d] (cm^2)	Wood density (kg m^{-3})	Foliar biomass (kg tree^{-1})
E. globulus ($n = 3$)						
Mean (se[e])	6.6 (0.9)	7.6 (1.0)	7.2 (0.5)	47.3 (12.6)	395 (27)	2.6 (0.8)
Max[f]	8.3	9.4	7.7	70.3	450	4.2
Min[g]	5.2	6.0	6.2	27.1	370	1.4
E. nitens × *globulus* ($n = 2$)						
Mean (se[e])	6.8 (0.6)	7.8 (0.2)	7.4 (0.1)	46.0 (5.8)	405 (6)	3.2 (0.5)
Max[f]	6.8	7.9	7.6	51.7	410	3.7
Min[g]	6.7	7.6	7.3	40.2	400	2.7

There were no significant differences ($p > 0.05$) between species for any of the variables listed

[a] Individual tree mean diameter at breast height (1.3 m)

[b] Individual tree height

[c] Individual tree mean diameter at thermal dissipation probe height of insertion

[d] Sapwood area

[e] Standard error

[f] Maximum

[g] Minimum

measured for each sampled tree. Sapwood area was calculated as:

$$\mathrm{SA} = \left[\left(\frac{D}{2} \times \frac{d}{2}\right) \times \pi\right] - \left[\left(\frac{\mathrm{Dh}}{2} \times \frac{\mathrm{dh}}{2}\right) \times \pi\right], \tag{4}$$

where SA is the Sapwood area (cm^2), D is the major disc diameter (cm), d is the minor disc diameter (cm), Dh is the heartwood diameter at major disc diameter (cm), dh is the heartwood diameter at disc minor diameter (cm). An additional stem disc sample, 5-cm thick, was obtained from each tree at dbh height to estimate wood density. Volume of the sample was obtained by water displacement on an electronic balance (±0.001 g) after saturation and oven dry weights by drying samples at 100 °C during 48 h. Foliar biomass was estimated by weighing all individual tree foliage after drying at 100 °C until constant weight.

Data and statistical analyses

The power function for SFD ($\mathrm{SFD} = \alpha K^{\beta}$) was parameterized using PROC NLIN of SAS 9.3 (SAS Institute, Cary, North Carolina, USA). Function parameter coefficients were tested for genotype differences using dummy variables. The thermal dissipation probe functions of this study were compared to the theoretical equation developed by (Granier 1987). Our analysis also compared practical differences among equations. Individual tree and stand level transpiration (Ec) estimates were obtained for each *Eucalyptus* genotype for a week in May and August 2015, from six instrumented trees at the site. Selected weeks were assumed to represent average transpiration conditions for the site during autumn and winter. Because of small fluctuations in maximum temperature differences between the heated and reference probe at night during these weeks, the correction method suggested by Lu et al. (2004) was applied to the data and the zero baseline (representing the zero flow at night) was set up once for each week. ANOVA analyses were used to evaluate differences among species in cumulative daily transpiration and individual sampled tree parameters. Linear regression analyses were used to evaluate nocturnal sapflow equation estimates against measured water uptake during night. Significant differences were assessed at $\alpha = 0.05$. Differences in tree characteristics between species were assessed using analysis of variance (SPSS, Inc.) after verifying normality and homogeneity of variance (Table 1).

Results and discussion

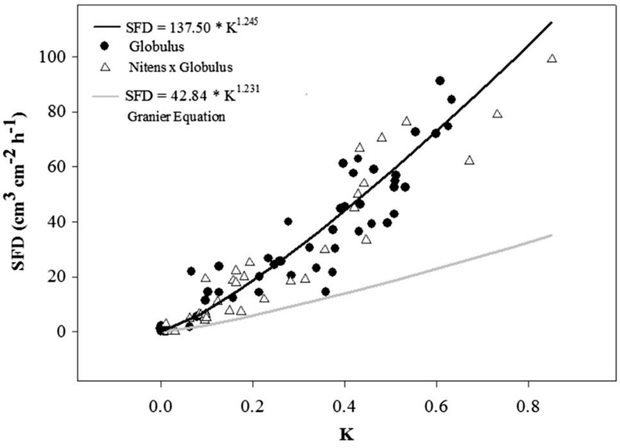


Fig. 1 Calibration equation for *Eucalyptus globulus* (Eg) (*closed symbols*) and *E. nitens*×*globulus* (Eng) (*open symbols*): $\mathrm{SFD} = 137.5 \times K^{1.245}$. K is the dimensionless coefficient defined by Granier (1987)

Our results support the hypotheses that standard coefficients of Granier's equation underestimate SFD for *Eucalyptus* species. The standard Granier equation underestimated SFD for both genotypes with larger discrepancies occurring at higher K values (Fig. 1). For instance, at high SFD ($K = 0.6$) the standard Granier's equation underestimated SFD by 219%. Although the Granier equation has been shown to work in several species (Braun and Schmid 1999; Clearwater et al. 1999; Granier 1985), other studies with hardwood species have shown similar results to ours (Hultine et al. 2010; Vellame et al. 2009). On average, our study suggests that the Granier equation underestimated SFD by threefold, which is less than the fivefold underestimation reported for *Eucalyptus grandis*×*urophylla* hybrid varieties in Brazil (Hubbard et al. 2010), and similar to the threefold underestimation found for *Eucalyptus benthamii* in the United States (Christopher Maier, personal communication, USDA Forest Service, January 2016).

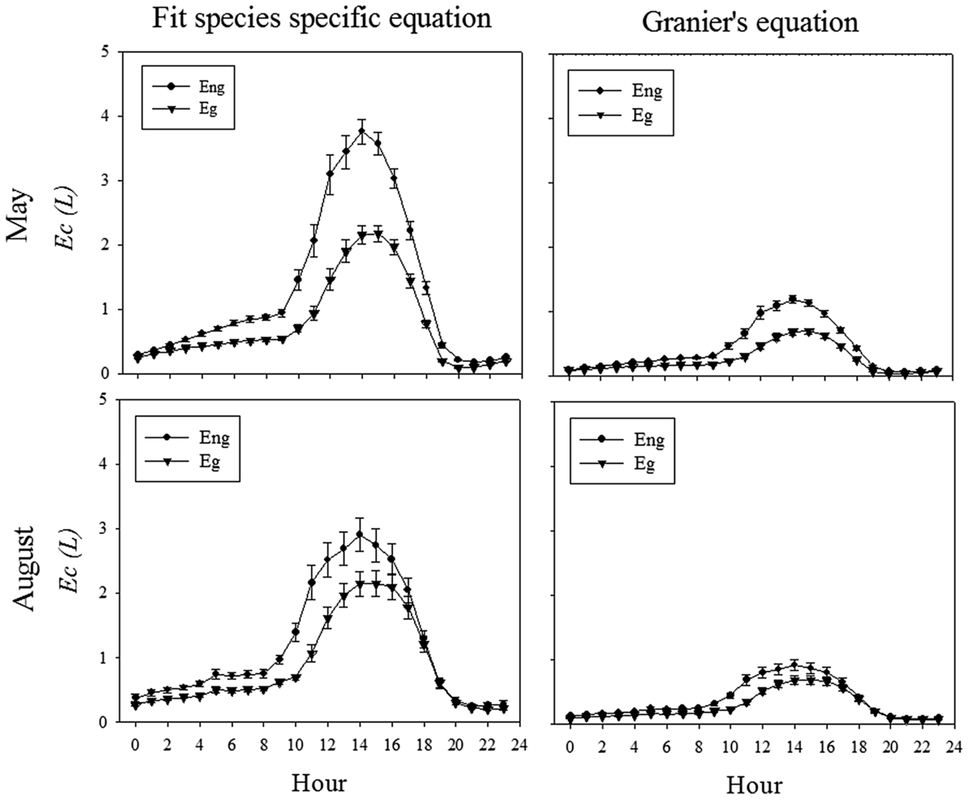
Differences in calibration coefficients from the original equation may be caused by abrupt changes in wood anatomy, radial variation in SFD across deep sapwood, sensor type, calibration set-up or some portion of the probes contacting non-functional sapwood (Clearwater et al. 1999; Lu et al. 2004; Nadezhdina et al. 2002). Although Granier's original equation has been considered to be species independent, our results and other empirical evidence (Hubbard et al. 2010; Lu et al. 2004) suggests that validation and specific coefficients may be required for *Eucalyptus* species.

The two Eucalyptus species evaluated did not differ in SFD equations as was hypothesized (Fig. 1). Given that no differences in SFD were observed among Eg and Eng ($p = 0.470$) a common function was fit for both species. No significant differences in dbh ($p = 0.894$), ht ($p = 0.687$), SA ($p = 0.942$) and foliar biomass ($p = 0.644$) between species also suggest a robust common SFD equation. However, logistical constraints limited our sample size and it is possible that SFD coefficients could diverge as these trees age so our results should be considered preliminary and highlight the need for further research.

Differences observed from the standard Granier equation with the evaluated species may be due to differences in wood density and the frequency and diameter of the vessels affecting hydraulic conductivity (Hoeber et al. 2014; Searson et al. 2004). Overall, higher wood density is associated with lower vessel diameters and hydraulic conductivity but higher vessel density (Hoeber et al. 2014). In our study wood density did not differ ($p = 0.819$) averaging 395 kg m^{-3} for Eg and 400 kg m^{-3} for Eng, respectively, suggesting the lack of difference in SFD may be related to the similar genetics of each genotype and maybe similar structural conducting tissue characteristics.

Deviations in SFD coefficients from those of Granier for these species may also be explained by abrupt changes in the SFD from the inner to the outer of the stem (Clearwater et al. 1999). Previous studies have reported that SFD in ring-porous species is underestimated by the standard Granier's equation compared to diffuse-porous species (Bush et al. 2010). For diffuse-porous species, other than *Eucalyptus*, significant radial variation differences in SFD have been previously reported (Gebauer et al. 2008). In the case of *Eucalyptus*, Forrester et al. (2010) found that SFD varied radially, being maximum in the middle sapwood and lower to the inner and outer sapwood in a 14-year-old *Eucalyptus globulus* plantation. Similar results were found by Medhurst and Beadle (2002) for 8-year-old *E. nitens*. However, small variation has been observed in young 3–4-year-old *E. urophylla* trees by Zhou et al. 2002 and Zhang et al. 2015. Results from Wullschleger and King (2000) also suggest that variation in radial sap flux increased for *Liriodendron tulipifera* L. after heartwood formation but not earlier. Given the young plants evaluated in our study, it is likely that both genotypes may have little radial variation as the presence of heartwood area was almost inexistent in these 2-year-old trees. Consequently, when scaling to tree and stand levels, we assumed that SFD did not vary across the sapwood profile and that our 2 cm probes accurately measured SFD for the entire sapwood area. However, we cannot dismiss the possibility that differences in radial variation in SFD may account for a portion of the discrepancy we find between our calibration coefficients of those of Granier.

Mean hourly transpiration (Ec) estimates for selected weeks in May (autumn) and August (winter) were compared for the evaluated species using our species-specific equation and the standard Granier equation (Fig. 2). On average, similar patterns were observed where Ec peaked at 14:00 h in autumn and at 15:00 during winter for both genotypes. However, differences were found during autumn and winter daily transpiration between Eg and Eng (Fig. 2). Average daily transpiration, assessed at individual tree


Fit species specific equation

Granier's equation

Fig. 2 Mean individual tree hourly transpiration in liters per hour (Ec) estimated using species specific equations (*left*) and Granier's original equation (*right*), for a representative day in May 2015 (autumn) (*upper panel*) and August 2015 (winter) (*lower panel*) level, ranged for August and May 28.3–31.7 L for Eng and 20.6–18.6 L for Eg. Granier's equation underestimated Ec by 213–215%. Despite the use of species-specific or the standard Granier equation, the Eng showed the largest Ec for autumn and winter. When scaled to the stand level, monthly water use was 317 and 313% higher for Eng and Eg, respectively, compared to estimates derived using the Granier equation (Fig. 3). Notably, water use was significantly higher for Eng compared to Eg using either equation but differences were greater using the species specific calibrations. Other studies with *Eucalyptus* genotypes have shown that hydraulic conductivity has been more affected by site water availability than genetic factors (Willigen and Pammenter 1998) but this does not apply to our study where all the genotypes were established in a common garden irrigation experiment and water was not a limiting factor.

Nocturnal sapflow has been recognized as an important component of plant water use and may also be underestimated using the original Granier equation. Mean individual tree estimates of nocturnal transpiration, using our specific calibrated equation, correlated well with overnight water withdrawn from each individual tree potometer ($R^2 = 0.85$, $p < 0.0079$) (Fig. 4). A good correlation was also obtained for Granier equation estimates; however, nocturnal flow was underestimated by more than threefold (3.2 times) compared to our species-specific equation. In our study, nocturnal transpiration accounted for 4.0% for Eg (2.2–6.8% range) and 9.7% for Eng (7.7–11.8% range). For Eg, our results are similar to the 5.3% reported by (Phillips et al. 2010) for *Eucalyptus globulus*, however, our Eng estimates of nocturnal transpiration were higher than those of Phillips et al. (2010) where summer nocturnal estimates ranged from 4.9 to 7.6% for a broad range of *Eucalyptus* species. In fact, our estimated nocturnal transpiration for Eng value agrees with the 10.27% mean nocturnal flow value reported by Forster (2014) for the genus *Eucalyptus*. These results highlight the importance of incorporating nocturnal estimates of flow to quantify tree water use in *Eucalyptus* plantations, and support the evidence for nocturnal canopy conductance considering the non-limited water availability conditions provided before and during the experiment (Forster 2014; Pfautsch et al. 2010; Resco de Dios et al. 2016).

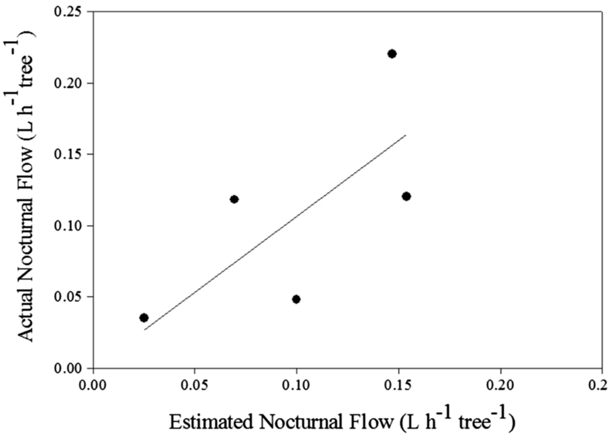


Fig. 4 Relationship between estimated individual mean tree nocturnal flow using species-specific equations vs. actual nocturnal flow measured on site (actual nocturnal flow = 1.0656 × estimated nocturnal flow, $R^2 = 0.85$ $p < 0.0079$). Given the small sample size a general linear relationship was fit for both genotypes

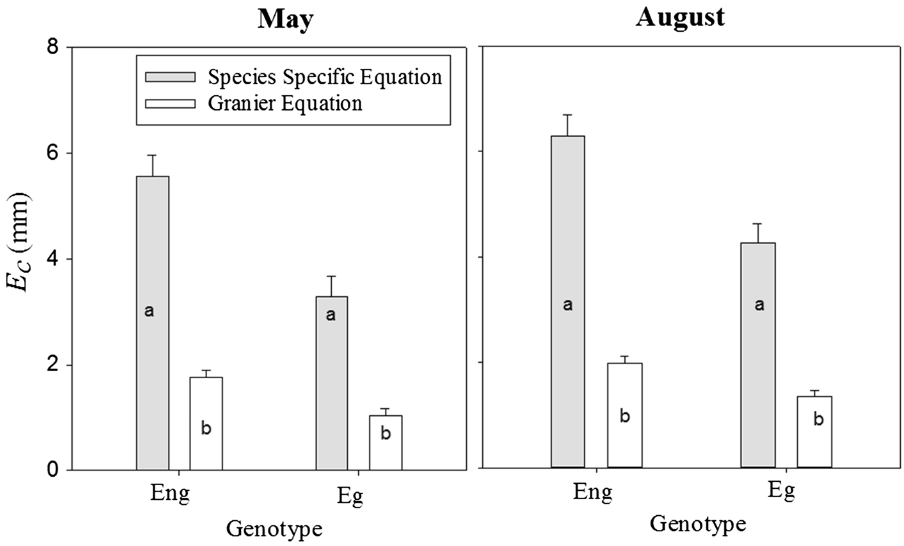


Fig. 3 Stand average daily transpiration (Ec) rates for *Eucalyptus nitens* × *globulus* and *Eucalyptus globulus* genotypes calculated with onsite species-specific calibrations (*gray bars*) and the standard Granier equation (*white bars*) for May (autumn) and August (winter). Different letters within bars represent significant differences between onsite and standard Granier equations ($p < 0.05$)

Conclusions

Our study emphasizes the importance of developing species-specific equation coefficients for SFD for *Eucalyptus* species. Large differences and biased results may be obtained if standard Granier equation coefficients are used for *Eucalyptus* species to estimate individual tree and stand level transpiration at different scales. A single equation may suffice for hybrids but caution is advised when using thermal dissipation probes to quantify water use in different genotypes. We also found significant nighttime transpiration rates in both of the *Eucalyptus* genotypes we evaluated in this study. Our species-specific calibration equations accurately captured nighttime fluxes of water while the standard Granier equation significantly underestimated this component of total tree water use.

Author contribution statement Dr. Rafael Rubilar contributed with scientific ideas for the development of this research, supported with funding, carried statistical analyses of all variables and writing of results and discussion. Dr. Robert Hubbard contributed scientific ideas for the development of this research, analyses of sap flux density, nocturnal flow, and collaborated on writing of the manuscript and edition. Dr. Marco Yañez contributed with execution of field work and writing of significant portion of the introduction, methods and portion of discussion of this manuscript. Mr. Alex Medina provided scientific support on genotypes selection, plant production of *Eucalytus nitens* × *globulus*, and field work and logistic support. Mr. Hector Valenzuela provided support on genotypes selection, plant production of *Eucalytus globulus*, establishment of trials, field work and logistic support.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest. Funding for this work was provided by the Chilean agency "Fondo de Fomento al Desarrollo Científico y Tecnológico" Grant FONDEF D11i1161 Granted to Dr. Rafael Rubilar.

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