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Nighttime transpiration represents a negligible part of water loss and does not increase the risk of water stress in grapevine

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Abstract

Nighttime transpiration has been previously reported as a significant source of water loss in many species; however, there is a need to determine if this trait plays a key role in the response to drought. This study aimed to determine the magnitude, regulation and relative contribution to whole plant water-use, of nighttime stomatal conductance (g_{night}) and transpiration (E_{night}) in grapevine (*Vitis vinifera* L.). Our results showed that nighttime water loss was relatively low compared to daytime transpiration, and that decreases in soil and plant water potentials were mainly explained by daytime stomatal conductance (g_{day}) and transpiration (E_{day}). Contrary to E_{day} , E_{night} did not respond to VPD and possible effects of an innate circadian regulation were observed. Plants with higher g_{night} also exhibited higher daytime transpiration and carbon assimilation at midday, and total leaf area, suggesting that increased g_{night} may be linked with daytime behaviors that promote productivity. Modeling simulations indicated that g_{night} was not a significant factor in reaching critical hydraulic thresholds under scenarios of either extreme drought, or time to 20% of soil relative water content. Overall, this study suggests that g_{night} is not significant in exacerbating the risk of water stress and hydraulic failure in grapevine.

KEYWORDS

circadian regulation, grapevine, nighttime transpiration, stomata, stomatal conductance, water loss

1 | INTRODUCTION

The opening of the stomata at night is observed in many species and challenges some assumptions regarding plant function, namely that plants constantly strive to maximize carbon gain while minimizing water loss (Cowan & Farquar, 1977; Farquhar, 1973). Stomata are theorized to be closed overnight when no net photosynthetic carbon fixation is possible, however, this contrasts with most observations

that nighttime stomatal opening is commonplace (Costa et al., 2015; Dawson et al., 2007; Fuentes, Mahadevan, Bonada, Skewes, & Cox, 2013; Schoppach, Claverie, & Sadok, 2014; Zeppel, Tissue, Taylor, Macinnis-Ng, & Eamus, 2010). Reports across different species indicate that water loss due to nighttime stomatal opening represents around 12% of daily transpiration (Forster, 2014) and in some cases it can be as high as 30% (Caird, Richards, & Donovan, 2007). However, the relative importance and impact of this nighttime water

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loss to overall plant performance and water use is still not well understood.

Several hypotheses have been raised to explain the significance or potential functions of nighttime stomatal conductance (g_{night}). The most common explanation is that plants simply lack complete stomatal control at night and/or nighttime transpiration (E_{night}) is due to water leakage through the cuticle (Barbour et al., 2005). Nighttime fluxes could, however, serve a purpose of enhanced nutrient uptake or nutrient distribution to distal parts of the plant (Scholz et al., 2007), the delivery of dissolved oxygen to woody tissues (Daley & Phillips, 2006), and the prevention of excessive leaf turgor (Donovan, Linton, & Richards, 2001). A recent meta-analysis including published datasets from 176 different species found that the variation of g_{night} across plants was not consistent with the hypotheses mentioned above (i.e., simple leakage, nutrient uptake enhancement, or delivery of dissolved oxygen) and that changes in g_{night} could be partially explained by the circadian clock (Resco de Dios, Chowdhury, Granda, Yao, & Tissue, 2019).

Quantifying and understanding water losses at night is still challenging because some methods are not sensitive enough to accurately measure the low nighttime fluxes of water (e.g., sap flows, lysimeters; Tolk, Howell, & Evett, 2006; Zeppel et al., 2010; Fuentes et al., 2013) and direct measurements of g_{night} through porometry or gas-analyzers do not always respond to environmental drivers as during the daytime (Ogle et al., 2012). Drawbacks for each method must be recognized, particularly when comparing species or environments. For instance, vapor pressure deficit (VPD) has been well documented as the main factor driving daytime transpiration and, after light, stomatal conductance (Monteith, 1995; Oren et al., 1999). However, the VPD at night is much lower than during the day and responses of g_{night} to VPD have been observed to be variable among species: it can be invariable (Barbour et al., 2005), or decrease with increasing VPD (Bucci et al., 2004; Cirelli, Equiza, Lieffers, & Tyree, 2016). Despite being intimately associated with each other, E_{night} and g_{night} are different processes and may respond differently to environmental conditions (Caird, Richards, & Donovan, 2007).

Although g_{night} and E_{night} are increasingly the subject of study many gaps in our knowledge remain, in particular an understanding of the costs and/or benefits of high g_{night} and E_{night} . Many studies have emphasized that g_{night} represents a significant source of water loss for the plant with reductions in water use efficiency (WUE) under drought conditions (Caird, Richards, & Hsiao, 2007; Coupel-Ledru et al., 2016; Kavanagh, Pangle, & Schotzko, 2007; Rogiers, Greer, Hutton, & Landsberg, 2009). Furthermore, common quantitative trait loci (QTLs) underlying genetic variability in both growth and E_{night} have been reported (Coupel-Ledru et al., 2016). However, drought studies focused on E_{night} and g_{night} need to be carefully interpreted because they generally attribute water loss to the stomata only. When stomata are mostly closed, water loss continues at a very low rate through the cuticle (Duursma et al., 2019). This residual rate of water loss is referred to as the minimum conductance (g_{min}) and is generally not directly measured.

Different mechanisms have been postulated to explain the significance of nighttime transpiration, and there is a need to better integrate the physiological traits that determine its contribution to whole plant water use. One limitation in understanding the significance of E_{night} and g_{night} for whole plant water balance is that we still do not know if this trait plays a key role in exacerbating water stress and the risk of hydraulic failure. In a recent study in grapevine (Dayer et al., 2020), we observed that the maximum water use given by maximum daytime transpiration (E_{max}) was strongly correlated with other drought-related traits such as the water potential at stomatal closure (P_{g50}), the leaf turgor loss point (π_{TLP}) and the leaf water potential inducing 50% of loss of hydraulic conductance (P_{50}), highlighting the importance of integrating multiple traits in characterizing drought tolerance. In the current study, we used the same three cultivars to explore E_{night} and g_{night} in natural conditions (i.e., outdoors, to avoid any artefactual VPD effects brought about by a greenhouse environment) to address the following question: Is nighttime water loss a key trait in exacerbating water stress? Accordingly, we examined here (a) the relative importance of water loss at night relative to daytime transpiration (E_{day}), (b) the drivers of transpiration at night and if they are the same as during the day, (c) the potential association between E_{night} and g_{night} with daytime variables related to productivity (g_{max} or Pn_{max}), and (d) the modelling of the contribution of different conductance's (i.e., g_{night} , g_{min} and g_{day}) to the time necessary to reach 20% of soil relative water content (representing impacts on productivity and yield in an agronomic context) and the time to hydraulic failure (representing the risk of drought induced mortality) under different experimental conditions.

2 | MATERIALS AND METHODS

2.1 | Plant material

The experiment was conducted in 2019 in one-year-old plants of own rooted *Vitis vinifera* L. "Grenache," "Syrah" and "Semillon" from INRAE nursery (Villenave d'Ornon, France) planted in 7 L pots containing 1 kg of gravel and 5.5 kg of commercial potting media (70% of horticultural substrate and 30% sand). Plants were grown outside in a drip irrigated platform, well-watered (without stress) with nutritive solution ($\text{NH}_4\text{H}_2\text{PO}_4$ 0.1 mmol L⁻¹; NH_4NO_3 0.187 mmol L⁻¹; KNO_3 0.255 mmol L⁻¹; MgSO_4 0.025 mmol L⁻¹; 0.002 mmol L⁻¹ Fe, oligo-element [B, Zn, Mn, Cu, Mo]) to avoid any deficiency during their development. The surface of the pots was covered with a plastic bag to prevent water loss by soil evaporation. The cultivars were distributed following a completely randomized design.

2.2 | Gas-exchange dynamics

Dynamics of leaf gas exchange measurements were conducted periodically at different days of the season, from July fourth to August 23rd (DOY 185, 186, 193, 204, 205, 206, 215, 216, 221, 234 and 235) in four plants per cultivar ($n = 4$). Stomatal conductance (g) and

transpiration (E) were measured at different times during the day (g_{day} and E_{day}) and night (g_{night} and E_{night}). In addition, maximum stomatal conductance (g_{max}) and photosynthesis (Pn_{max}) were registered on 5 days (DOY 185, 193, 205, 216 and 235). At each DOY, different time points were registered every 2 hr, starting and finishing at different times. For example, on DOYs 185–186 we started at 06:00 (solar time) and finished at 04:00 the following day, and in DOY 206 we started at 02:00 and finished at 07:00 the same day. In this way, we could cover the range of most of all times during the day and night. Measurements were performed on mature, well-exposed leaves using a portable open-system including an infrared gas analyzer (GFS-3000, 180 Heinz Walz GmbH, Effeltrich, Germany) equipped with CO_2 , humidity, temperature and light control modules. During the daytime measurements, conditions inside a 3 cm^2 cuvette were controlled and set to conditions easily reproducible all along the experimental period (i.e., PPFD = 1,500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, temperature = 20°C, vapor pressure deficit ~ 1.0 – 1.3 kPa, relative humidity $\sim 50\%$, impeller speed = 7 and CO_2 = 400 ppm).

During the night, we allowed the cuvette to follow the ambient conditions, except for the CO_2 that was set at 400 ppm. Impeller speed was set to standard value, corresponding to a boundary layer conductance close to 4,200 $\text{mmol.m}^{-2}.\text{s}^{-1}$ for our cuvette (Burllett, personal communication). To maximize the differential of water vapour mole fraction (and therefore increase the resolution), the instrument flow rate was set to 650 $\mu\text{mol s}^{-1}$. Leaves were enclosed in the cuvette, and the instantaneous gas exchange was logged following stability in cuvette conditions (after approximately 1.5 min). All the plants were kept well-watered (no stress from soil moisture).

2.3 | Environmental conditions

Air temperature, relative humidity and radiation were obtained hourly from a meteorological station (Climatik meteo station 33550003) located in very close proximity to the experimental site (Figure S1). Leaf temperature was recorded every 20 min using TinyTag Talk 2 data loggers (Gemini Data Loggers, Ltd., UK) associated with temperature probes that were carefully attached to the abaxial side of the leaf by adhesive tape. This type of probe records temperature from -40 to 125°C with an accuracy of 0.4°C and a resolution of 0.05°C . The values of leaf temperature obtained with these probes were used to calculate the leaf-air vapor pressure deficit ($\text{VPD}_{\text{leaf-air}}$).

2.4 | Total leaf area

Total leaf area (TLA) was estimated through the relationship obtained between the leaf midrib length and the leaf area (measured with a leaf area meter Model LI-3000, LI-COR, Lincoln, NE) prior the experiment for each cultivar using approximately 100 leaves per cultivar. The leaf midrib length was measured once every 6–7 days on all the leaves of each plant.

2.5 | Minimum conductance (g_{min})

In the same set of plants used to measure nighttime g_{night} , minimum conductance (g_{min} ; minimum water loss after stomatal closure) was determined in eight leaves from each of the three cultivars by using the mass loss of detached leaves (MLD) technique (Billon, Ruiz, Sleiman, Hitmi, & Cochard, 2019; Duursma et al., 2019). The technique consists in measuring the leaf mass loss monitored over time as the leaf dries out. Leaves from well-watered plants were detached and suspended by their petiole (to allow the lamina to transpire from both sides) in a controlled chamber (Fitoclima 1200, Aralab, Portugal) set to a constant temperature of 25°C and relative humidity of 45%. The petioles were sealed with parafilm immediately after the cutting to avoid water losses. Photosynthetic Photon Flux Density (PPFD) at the position of the samples was around $400 \pm 50 \mu\text{mol m}^{-2} \text{s}^{-1}$. The mass loss of the leaves was measured every 5–10 min for the first hour and then every 15–20 min as long as the leaves dehydrated with a 0.0001 g resolution balance (Sartorius LE5201 Expert, Goettingen, Germany). The evaporation (E) of each sample was computed from the relationship between leaf mass and time, once water loss rates reached a steady state. The minimum conductance (g_{min}) was calculated by using the measured vapor pressure deficit (VPD) according to the Equation $E = g_{\text{min}} \cdot \text{VPD} / P$ where D is the VPD in kPa and P is the atmospheric pressure expressed in $\text{mmol m}^{-2} \text{s}^{-1}$ (Duursma et al., 2019).

2.6 | Scanning electronic microscopy (SEM) for stomatal observation

Stomata cryo-SEM observations were performed in three plants per cultivar that were kept in a dark chamber overnight. Leaf samples were collected in dark conditions using a red headlight lamp in a very low intensity (0 – $23 \mu\text{mol m}^{-2} \text{s}^{-1}$) and for no more than 3–5 s (the time needed to punch and drop the leaf sample in N2), avoiding any response of the stomata (Raven 2014). Three leaf discs (9 mm diameter) from different leaves were sampled with a punch holder ("Biopsy Punch") from the middle zone of the lamina between major veins. Leaf samples were immediately mounted in copper specific supports with cryo-glue, freshly made up as a 50/50 mixture of colloidal graphite and Tissue-Tek mounting medium, and frozen in slush nitrogen (mix of solid and liquid nitrogen). Frozen samples were transferred under nitrogen to the preparation chamber (maintained at -140°C) in which sublimation at -95°C for 5 min and metallization with Platinum at 10 μA for 15–20 s was performed. Finally, samples were transferred under vacuum to the cold stage of the microscope chamber in which stomata were observed and photographed in high vacuum mode at 3kV acceleration voltage. Cryo-SEM observations were carried out in a Gemini SEM 300 FESEM (ZEISS, Germany) microscope coupled with a CRYO-SEM PP3010T module (Quorum Technologies, England).

2.7 | The impact of decreasing E_{night} on leaf water potential

Eight well-watered plants of approx. 1.0 m height from each cultivar were chosen for a bagging experiment. On DOY 182 after sunset (radiation $<50 \mu\text{mol m}^{-2} \text{s}^{-1}$) four plants ($n = 4$) were randomly selected and entirely bagged with a transparent polyethylene film that was hermetically sealed with tape. The plants were left bagged overnight and the pre-dawn (Ψ_{PD}) and midday (Ψ_{leaf}) leaf water potentials were measured the next day prior any light exposure and at midday respectively. A set of four more plants were left un-bagged and kept as controls. The Ψ_{PD} and Ψ_{leaf} were measured in basal fully expanded leaves from all plants (bagged and unbagged) using a "Hammel-Scholander" pressure chamber (DG Meca, Gradignan, France).

2.8 | Model simulations of hydraulic traits

A soil-plant water transport model (SurEau; Cochard, Martin-StPaul, Pimont, & Ruffault, 2020) was used to determine the predicted time it would take under drought to reach a particular threshold (thresholds are described below). A detailed explanation of the model is given in Martin-StPaul, Delzon, and Cochard (2017). Briefly, the plant is described as a series of variable symplasmic and apoplasmic hydraulic conductances and capacitances that determine water flows and water potential along the soil-plant-atmosphere continuum. The model computes the leaf transpiration, driven by leaf-air VPD, its regulation by stomatal closure and thus the variation in soil water content. Beyond the point of stomatal closure, residual leaf transpiration is maintained, leading to plant dehydration and hydraulic failure under extreme water stress. Environmental conditions were either constant ($T_{\text{air}} = 25^\circ\text{C}$, $\text{RH}_{\text{air}} = 50\%$, $\text{PPFD} = 400 \mu\text{mol m}^{-2} \text{s}^{-1}$) or variable with day/night cycles (day as above; night: $T_{\text{air}} = 20^\circ\text{C}$, $\text{RH}_{\text{air}} = 90\%$, $\text{PPFD} = 0 \mu\text{mol m}^{-2} \text{s}^{-1}$; day as above). In each simulation, the stomatal conductance's, g_{night} , g_{day} and g_{min} were varied one at a time and the total leaf area was maintained constant to avoid any effect of this variable on dehydration. The modeling takes boundary layer effects into consideration (Cochard et al., 2020). The simulations were performed at a time step of 0.01 s and stopped when two different thresholds of water stress were reached in the leaf apoplasm: (a) the time needed to reach hydraulic failure (THF) corresponding to a total loss of conductivity (100% PLC), and (b) the time needed to reach a soil relative water content (RWC) of 20%.

2.9 | Statistical analyses

Data were analyzed by a one-way ANOVA using the general linear model procedure for completely randomized design. Means were compared by Fisher's LSD test ($p \leq .05$), and significant interactions between treatments are indicated by letters and described in the text. Time series data, such as gas exchange values, were fitted using linear

mixed-effect models and Hotelling's multiple test ($p \leq .05$) for comparison of means between treatments. Statistical analyses and fit were performed using R software (<http://www.R-project.org>) and Infostat version 2017 for Windows (Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. URL <http://www.infostat.com.ar>).

3 | RESULTS

3.1 | Dynamics of stomatal conductance and transpiration

Stomatal conductance (g_{night}) and transpiration (E_{night}) measured at night showed constant, minimal rates from 20:00 to 00:00 (solar time) with a significant increase at 02:00 for the three cultivars (Figure 1b, e). Mean g_{night} ranged from 8 to 17 $\text{mmol m}^{-2} \text{s}^{-1}$ in Grenache and Syrah and from 15 to 25 $\text{mmol m}^{-2} \text{s}^{-1}$ in Semillon (Figure 1 inset B') while E_{night} mean values ranged from 0.1 to 0.22 $\text{mmol m}^{-2} \text{s}^{-1}$ in Grenache and Syrah and 0.25 to 0.3 $\text{mmol m}^{-2} \text{s}^{-1}$ in Semillon (Figure 1 inset E'). For both variables, Semillon had significantly higher nighttime rates that were about two times higher than Grenache and Syrah (Figure 1 inset B', E').

Since g_{day} and E_{day} values are constantly changing across each day, the choice of these values in calculating a night/day ratio is somewhat subjective. As an alternative approach, we calculated multiple night/day ratios using maximum daytime values across the whole day (Figure S2). When plotting all the data estimated from these ratios we observed that the overall variation of $g_{\text{night}}/g_{\text{day}}$ ranged from 8 to 15% and that of $E_{\text{night}}/E_{\text{day}}$ from 5 to 13% with no significant differences between cultivars (Figure 2).

3.2 | Potential drivers of E_{night}

A significant positive relationship between transpiration and $\text{VPD}_{\text{leaf-air}}$ was observed for the three cultivars (Figure 3). This correlation was significant ($p < .0001$) when plotting all E data during the day and night at different dates during the season. When correlating only E_{night} values to $\text{VPD}_{\text{leaf-air}}$ no significant relationship was obtained for any cultivar (space delimited by dotted lines in Figure 3).

To further explore possible drivers of E_{night} we examined the variation of E_{night} and g_{night} along different times during the night (from 20:00 to 02:00) and we observed that not all the values were the same in magnitude despite radiation being $0 \mu\text{mol m}^{-2} \text{s}^{-1}$. For example, when comparing "early night" (20:00) and "late night or predawn" (02:00) stomatal conductance, pre-dawn g_{night} was significantly higher than early night g_{night} for the three cultivars (Figure 4). Differences between pre-dawn and early night g_{night} was significantly higher in Semillon followed by Syrah and finally Grenache (Figure 4). This increase at 02:00 was not associated with increases in $\text{VPD}_{\text{leaf-air}}$ which decreased progressively across the nighttime hours (Figure S3).

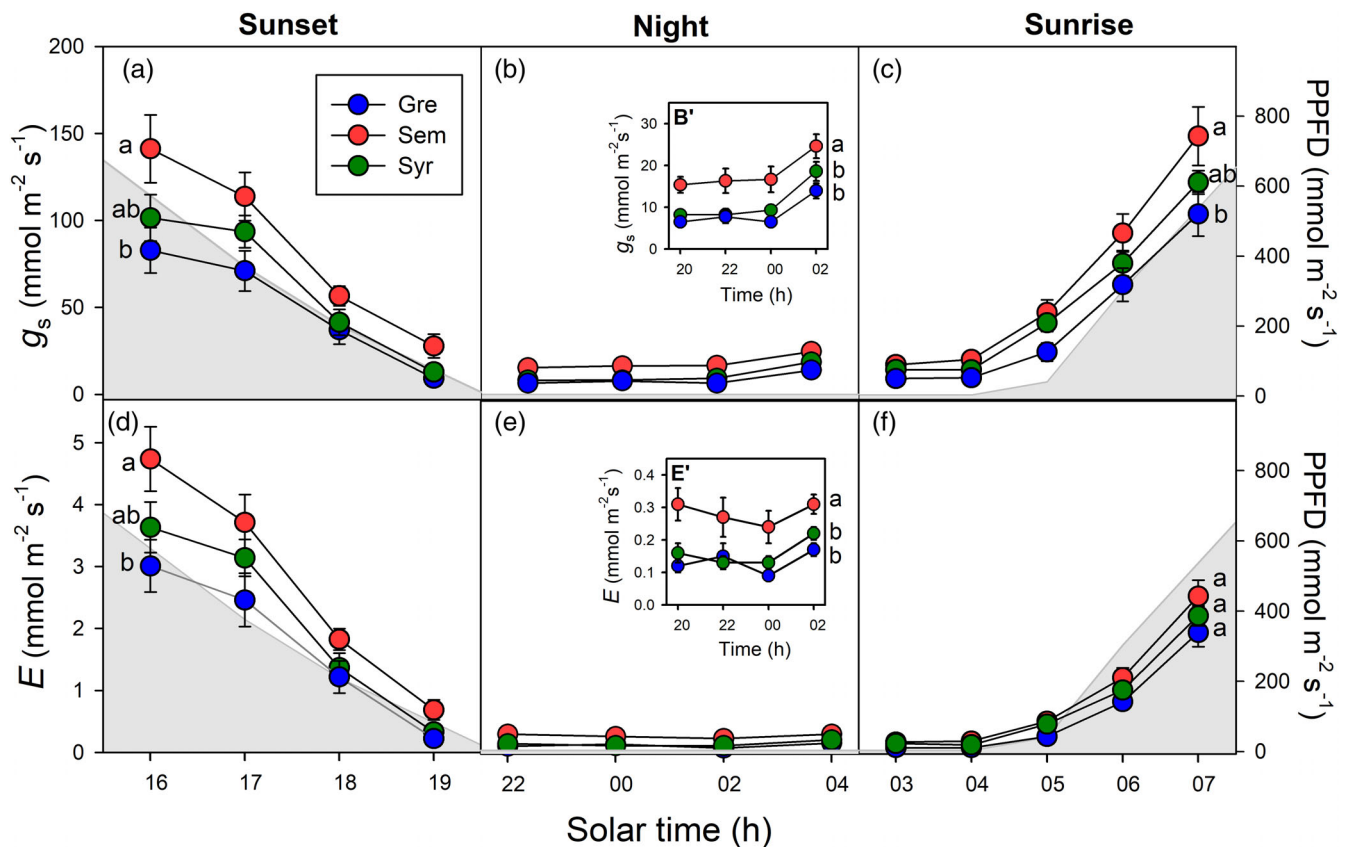


FIGURE 1 Leaf stomatal conductance (g_s) and transpiration (E) before and during sunset (a, d) at night (b, e) and before and after sunrise (c, f) in potted grapevines of Grenache (Gre), Semillon (Sem) and Syrah (Syr). $n = 4$. Inset panels (B') and (E') correspond to the same data than in panels (b and e) respectively, but with a zoomed range on y axis (0 to $32 \text{ mmol m}^{-2} \text{ s}^{-1}$) for better visualization. Values are means of six different dates (DOYs 186, 193, 204, 206, 215 and 221). Different lower-case letters indicate statistically significant differences between cultivars at $p \leq .05$ by Hotelling's test. Light grey area represents the light intensity (PPFD; $\mu\text{mol m}^{-2} \text{ s}^{-1}$) [Colour figure can be viewed at wileyonlinelibrary.com]

3.3 | Relationship between g_{night} and daytime productivity variables

Significant positive relationships between maximum stomatal conductance, photosynthesis and total leaf area ($\text{Max } g_s$, $\text{Max } P_n$ and TLA) were observed with g_{night} at pre-dawn (Figure 5). However, no correlations were observed when correlating other values of g_{night} (i.e., measured at 20:00, 22:00 or 00:00) with any of these daytime variables (data not shown). Semillon showed the highest values relative to Grenache and Syrah in all correlations.

We also investigated the reduction of nighttime transpiration on plant water status by enclosing the plants overnight and measuring the pre-dawn (Ψ_{PD}) and midday (Ψ_{leaf}) water potentials the following day. Two of the three cultivars evaluated, Semillon and Syrah, showed significantly more negative Ψ_{PD} values than Grenache (Figure 6a, unbagged plants). When E_{night} was stopped (via bagging the plants overnight) bagged plants all exhibited identical average Ψ_{PD} (Figure 6a, bagged plants) suggesting that the unbagged differences in Ψ_{PD} reflected differences in E_{night} . Bagging also reduced daytime leaf water potential for all cultivars but differences in midday Ψ_{leaf} between bagged and unbagged plants were not significant (Figure 6b).

3.4 | Minimum conductance and overnight stomata observations

The leaf minimum conductance (g_{min}) presented mean values of around $9.5 \mu\text{mol m}^{-2} \text{ s}^{-1}$ with no differences among cultivars as previously reported (Dayer et al., 2020; Table S1).

When nighttime stomatal opening was assessed visually by cryo-SEM we observed variability in the extent to which the stomata were closed, and additionally, the extent to which the leaf cuticle covers the stomatal aperture (Figure 7). For some stomata, the guard cells appeared slightly open (Figure 7a), while for others they were clearly closed (Figure 7b). However, there was an additional state commonly observed where the cuticle completely covers the stomatal opening (Figure 7c).

3.5 | Modelling time to 20% of soil relative water content and to hydraulic failure

Using the "SurEau model" and the data collected in this study allowed us to compare the contribution of different conductance's (g_{night} , g_{min} ,

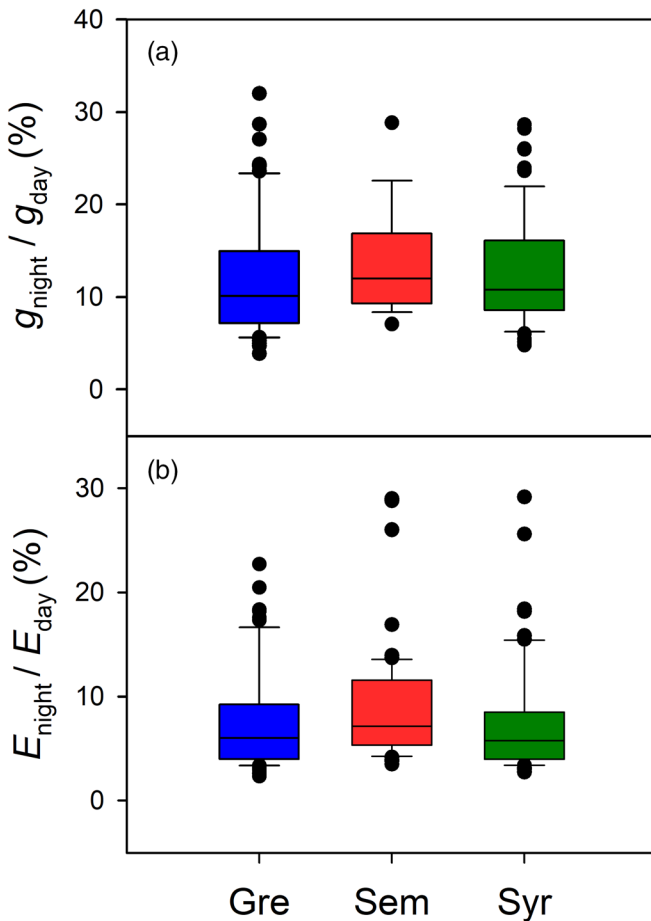


FIGURE 2 Variability of the night to day stomatal conductance ratio (a) and of the night to day transpiration ratio (b) expressed in percentage in potted Grenache (Gre), Semillon (Sem) and Syrah (Syr) grapevines. ($n = 4$). Night values are the mean of measurements taken at 22:00, 00:00 and 02:00 whereas day values correspond to measurements taken during from 07:00 to 16:00 with saturating levels of radiation ($>800 \mu\text{mol m}^{-2} \text{s}^{-1}$). Data include values from all DOYs measured during the season (11 dates) [Colour figure can be viewed at wileyonlinelibrary.com]

or g_{day}) in controlling water loss and changing the time to reach particular thresholds during a simulated drought. We observed that when considering a small rooting volume g_{min} had a stronger influence on the THF than g_{night} or g_{day} (Figure 8a). For example, a decrease in g_{min} by 60% increases the THF by nearly 100%, when reductions of similar magnitude in g_{night} or g_{day} have almost no impact (Figure 8a). In terms of days, when keeping g_{day} and g_{night} constant, decreasing g_{min} increased the simulated THF from 5 to 30 days (Figure S4A). In contrast, when keeping g_{day} and g_{min} constant, the impact of changing g_{night} was not significant, and THF remained at 7 days at any value of g_{night} ($0\text{--}50 \text{ mmol m}^{-2} \text{s}^{-1}$). These results changed when using a larger rooting volume, where g_{day} became much more important than g_{min} in determining the THF (Figure 8b; Figure S4B). When looking at the time to reach 20% of soil RWC we observed that g_{day} was more important in determining the time to reach this threshold, independent of the rooting volume (Figure 8c,d; Figure S4C,D). In any

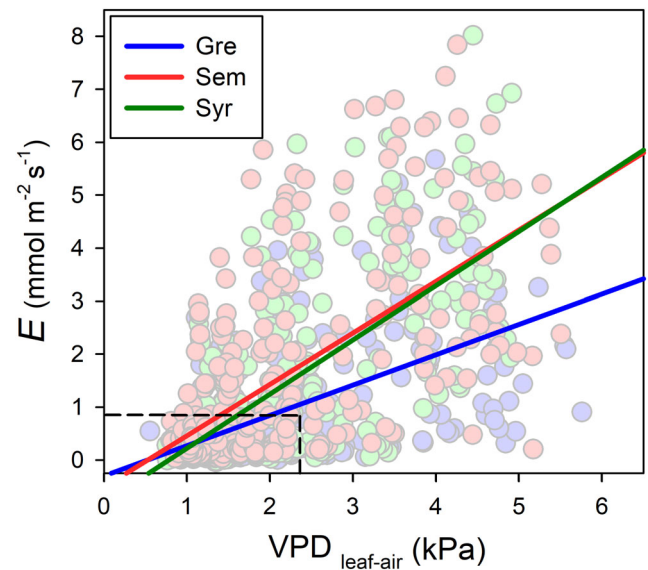


FIGURE 3 Response of leaf transpiration (E) to increasing leaf-air vapour pressure deficit ($\text{VPD}_{\text{leaf-air}}$) in three grapevine cultivars (Grenache, Semillon and Syrah). Each dot is a single value registered with an InfraRed Gas Analyzer at different dates and hours (day and night) during the season. The $\text{VPD}_{\text{leaf-air}}$ was calculated using the leaf temperature registered by TinyTag probes. Significant linear relationships were observed for each cultivar $E(\text{Gre}) = 0.572 \text{ VPD}_{\text{leaf-air}} - 0.301$; $E(\text{Sem}) = 0.964 \text{ VPD}_{\text{leaf-air}} - 0.503$; $E(\text{Syr}) = 1.024 \text{ VPD}_{\text{leaf-air}} - 0.808$. Nighttime transpiration (from 20:00 to 02:00 solar time) corresponds to the values delimited by the dotted black lines [Colour figure can be viewed at wileyonlinelibrary.com]

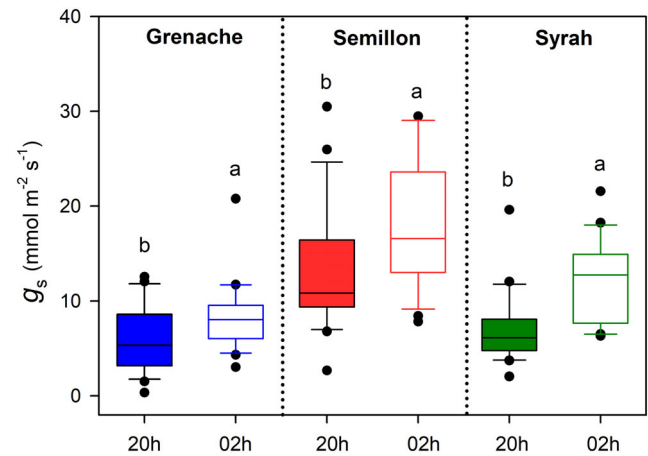


FIGURE 4 Nighttime stomatal conductance measured at 20:00 (early night) and at 02:00 (pre-dawn) the previous night, in three grapevine cultivars (Grenache, Semillon and Syrah). The data include different nights of the season, that is, DOYs 185, 186, 193, 205, 206, 215, 216, 234, 235. Different lower-case letters indicate statistically significant differences between g_s measured at 20:00 and 02:00 solar time for each cultivar at $p \leq .05$ by Fisher's LSD [Colour figure can be viewed at wileyonlinelibrary.com]

situation considered by the model, varying g_{night} did not have a significant impact on the time to reach the THF or the 20% of soil RWC under water stress.

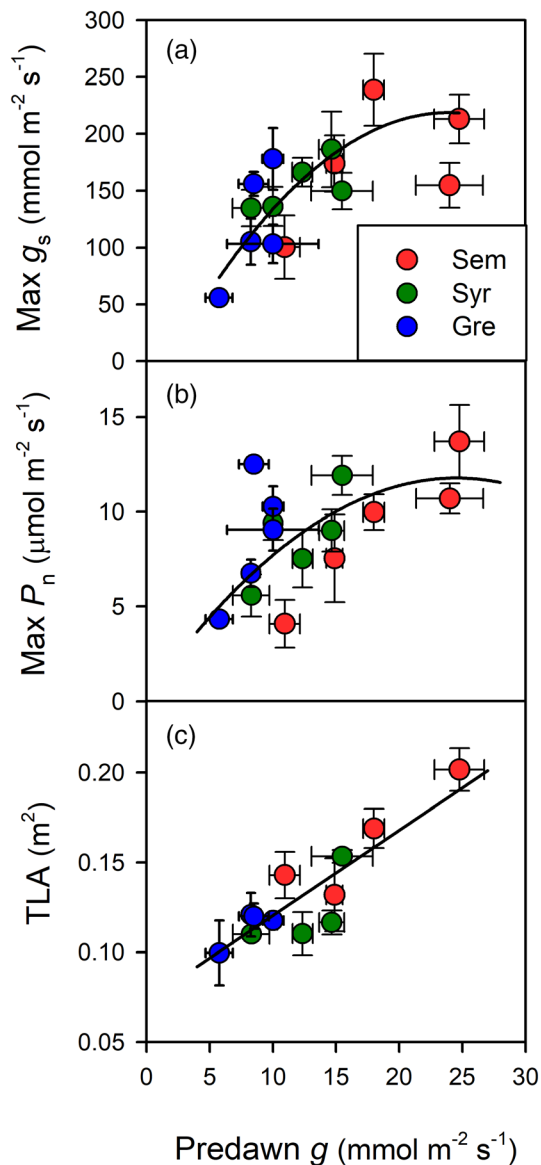


FIGURE 5 Correlations of maximal conductance ($\text{Max } g_s$; a) maximal photosynthesis ($\text{Max } P_n$; b) and Total Leaf Area (TLA; c) to the stomatal conductance measured before dawn at 02:00 solar time (Pre-dawn g) in three grapevine cultivars (Grenache, Semillon and Syrah). Values are means \pm SE ($n = 4$). A single relationship was fitted in each panel for all the cultivars since no significant differences were obtained among them: In (a) [$\text{Max } g_s = -0.438 \text{ Pre-dawn } g^2 + 20.993 \text{ Pre-dawn } g - 32.765$; $r^2 = 0.73$], in (b) [$\text{Max } P_n = -0.006 \text{ Pre-dawn } g^2 + 0.511 \text{ Pre-dawn } g + 3.391$; $r^2 = 0.40$] in (c) [$\text{TLA} = 0.005 \text{ Pre-dawn } g + 0.068$; $r^2 = 0.80$] [Colour figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

In this study, we investigated the regulation of nighttime transpiration, its contribution to whole plant water use, and its potential benefits and/or consequences. This is the first work to assess the relative importance of different conductances (g_{day} , g_{night} , and g_{min}) in exacerbating water stress and the risk of hydraulic failure. Our results

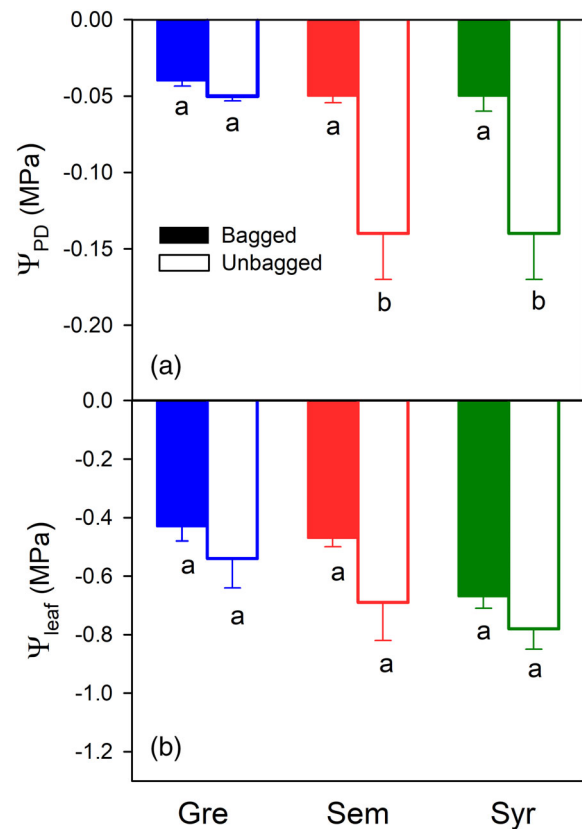


FIGURE 6 Effect of nighttime bagging of potted Grenache (Gre), Semillon (Sem) and Syrah (Syr) vines on pre-dawn (Ψ_{PD} ; a) and midday (Ψ_{leaf} ; b) leaf water potentials. The plants were bagged (filled symbols) after sun-set and left overnight until the Ψ_{PD} was measured the next day at pre-dawn. Unbagged plants (empty symbols) were used as controls. Values are means \pm SE ($n = 4$). Different lower-case letters indicate statistically significant differences between bagged and unbagged plants for each cultivar at $p \leq .05$ by Fisher's LSD [Colour figure can be viewed at wileyonlinelibrary.com]

suggested that nighttime water loss was relatively low compared to daytime transpiration and reductions in soil and plant water potentials were mainly explained by g_{day} and E_{day} . The drivers of E_{night} were not the same climate variables as previously reported for E_{day} (i.e., VPD) and possible effects of circadian regulation were observed. Pre-dawn g_{night} was correlated with maximum gas exchange and total leaf area in the three cultivars suggesting a potential benefit of increased g_{night} on growth. Overall, g_{night} did not represent a key trait in exacerbating water stress and further hypotheses need to be explored to test its potential benefit for the plant.

4.1 | Quantification of E_{night} and g_{night}

Daily dynamics of transpiration and stomatal conductance revealed that the loss of water at night and the proportion of this loss relative to daytime values were rather low in grapevine for the three cultivars examined. The night/day ratios of E and g_s were calculated considering different values of E_{day} as the rate of transpiration is a dynamic

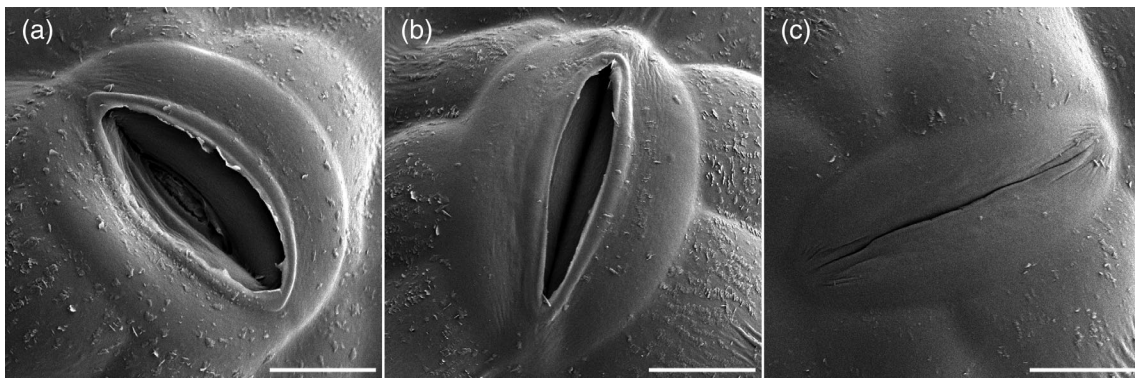


FIGURE 7 Cryo-SEM microscopy images of stomata slightly open (a), closed with cuticles open (b) and completely closed (c) on the abaxial side of leaf vines after 12 hr spent under dark conditions. Bar scale = 10 μm

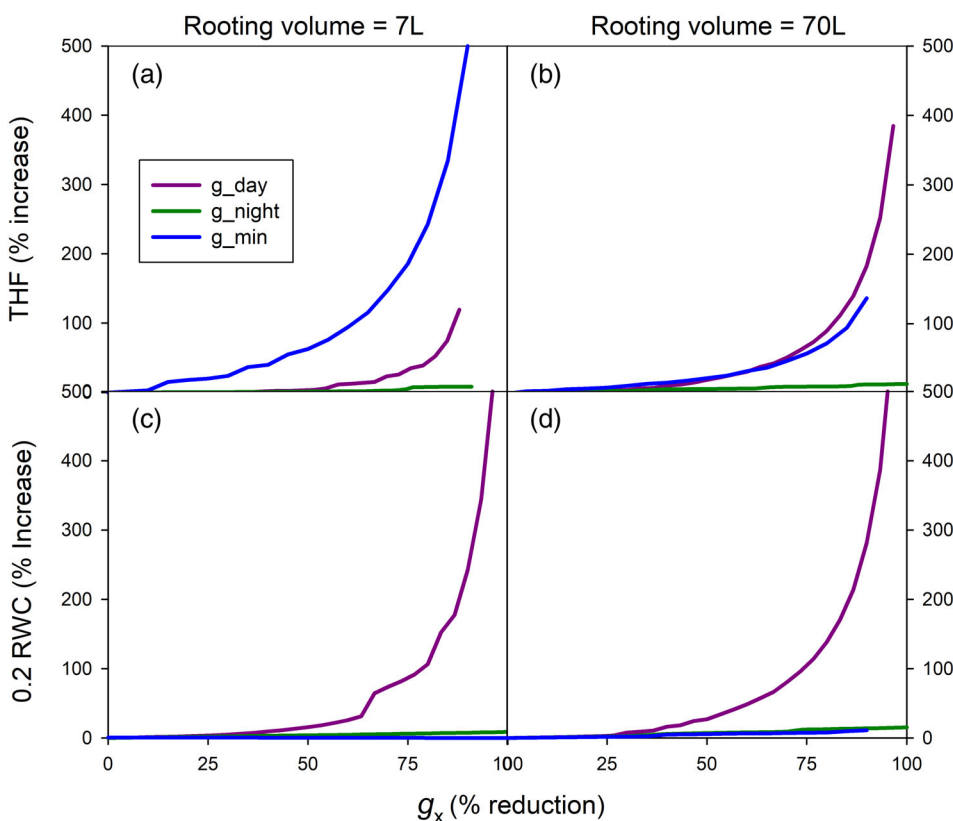


FIGURE 8 Simulated reduction of stomatal conductance's ($g_x = g_{\text{day}}$, g_{night} or g_{min}) and the corresponding increase of the time to hydraulic failure (THF; a, b) and to 20% soil relative water content (0.2 RWC; c, d) in 7 L and 70 L rooting volumes under the progression of a drought. Data correspond to a "mean" grapevine cultivar constructed by pooling the data of Grenache, Semillon and Syrah [Colour figure can be viewed at wileyonlinelibrary.com]

variable that changes significantly across each day according to the environmental conditions (e.g., light, VPD, etc.). The vast majority of the time these ratios never exceeded 15% in any cultivar (Figure 2) which falls within the lower end of the range reported in the literature from 5 to 15%, and up to 30% in some species (Caird, Richards, & Donovan, 2007; Daley & Phillips, 2006). For grapevine specifically, a range, across different grapevine cultivars and studies, from 0.05 to 0.46 $\text{mmol m}^{-2} \text{s}^{-1}$ for E_{night} , and 5 to 40 $\text{mmol m}^{-2} \text{s}^{-1}$ for g_{night} , have been reported (Table S2). Only three of these studies measured E_{day} and the reported $E_{\text{night}}/E_{\text{day}}$ ratios were no higher than 15% in any case (Coupel-Ledru et al., 2016; Rogiers et al., 2009; Rogiers & Clarke, 2013). In addition, in most of these studies, experiments were

performed in greenhouses where the VPD is difficult to control at night, generally remaining higher relative to natural conditions.

Regarding differences between cultivars, Semillon exhibited higher E_{night} and g_{night} values than Grenache and Syrah, similar to previous observations under field conditions (Rogiers et al., 2009). Despite these differences, the dynamics performed before/during sunset, at night and before/after sunrise in this study (Figure 1) showed that in addition to the absolute nighttime values it is also relevant to observe the timing and speed at which stomata open and close, which can vary significantly across species (Lawson & Vialet-Chabrand, 2019). In this study, we observed a higher rate of g_s in Semillon relative to Grenache after sunrise (Figure 1c), although we

cannot make conclusions about the mechanisms leading to these differences. It is important to note that the stomatal density (number per mm of leaf and per leaf area) was not significantly different across the three cultivars studied (data not shown).

4.2 | Drivers of E_{night} : VPD Or circadian regulation?

In a recent study, we reported that E_{day} was mainly driven by VPD in the same three grapevine cultivars (Dayer et al., 2020). While daytime $\text{VPD}_{\text{leaf-air}}$ can be as high as 4–5 kPa or even more under extreme events such as heat waves, nighttime $\text{VPD}_{\text{leaf-air}}$ is typically much lower. In the current study, most of nighttime $\text{VPD}_{\text{leaf-air}}$ values ranged from 0.8 to 1.8 kPa, with the exception of the night of 24th July (DOY 205) where an episode of extreme heat led to $\text{VPD}_{\text{leaf-air}}$ values as high as 2.5 kPa registered at 20:00 solar time (leaf temperature was 32°C and air relative humidity 48%). It is important to highlight that July 2019 has been the hottest month in this region (Bordeaux) ever recorded, with a daytime temperature of 41.6°C on the 23rd, meaning that our nighttime VPD values were not underestimated. Despite the positive correlation between leaf E and $\text{VPD}_{\text{leaf-air}}$ for all data (including daytime and nighttime values) we did not observe any significant relationship between E_{night} and $\text{VPD}_{\text{leaf-air}}$ (Figure 3). An absence of any relationship between temperature and VPD, and nighttime transpiration or stomatal conductance has been reported for other species (Barbour et al., 2005; Resco de Dios et al., 2015).

Examining the regulation of g_{night} as a dynamic across the nighttime hours we observed an increase during pre-dawn for most nights and cultivars (Figure 4) and this increase was not associated with increases in $\text{VPD}_{\text{leaf-air}}$. In fact, $\text{VPD}_{\text{leaf-air}}$ decreased progressively until pre-dawn. According to these observations, it seems unlikely that the increase of g_{night} from early night to pre-dawn was driven by $\text{VPD}_{\text{leaf-air}}$. A possible explanation is that temporal changes in g_{night} appear to be driven partly or perhaps largely by the circadian clock (Bucci et al., 2004; Caird, Richards, & Donovan, 2007; Resco de Dios et al., 2013, 2015). It is well known that the circadian clock regulates other key traits for plant fitness, including seed germination, gas exchange, growth, and flowering among others (Dodd et al., 2005). However, given that there is no photosynthesis at night; water loss would be detrimental, unless there is an unknown benefit underlying this mechanism and/or if it is necessarily linked to other advantageous traits.

4.3 | Benefits and/or consequences of g_{night}

To gain insight into the potential benefits and/or consequences of nighttime water loss by open stomata we correlated early night and pre-dawn g_{night} against daytime variables related to productivity. In the current study, Semillon exhibited the highest E_{night} and g_{night} , consistent with previous studies (Rogiers et al., 2009; current study), and

also showed the highest values in these correlations relative to Grenache and Syrah. Thus, high E_{night} and g_{night} may be necessarily linked to mechanisms by which intraspecific variation in plant carbon gain occur. However, Semillon (and also Syrah) also showed a more negative pre-dawn water potential than Grenache which could be associated with a higher nighttime water loss, and these differences were translated to some extent into more negative daytime Ψ_{leaf} as well (Figure 6). In addition, the correlations between g_{night} at pre-dawn and daytime max P_n and g_s appeared to level off. Thus, we can hypothesize that higher pre-dawn g_{night} values displayed by some genotypes involve a benefit and a consequence; this is higher daytime gas exchange (and productivity) at the expense of more negative pre-dawn and midday water potentials (and soil-plant Ψ_{PD} disequilibrium).

Potential effects of g_{night} in enhancing growth could be mediated indirectly by priming stomatal opening and photosynthesis. Genetic variation in the capacity for anticipating sunrise translates into differences in carbon uptake and growth in some species (Resco de Dios, Loik, Smith, Aspinwall, & Tissue, 2016). A faster stomatal opening before dawn would accelerate the plant response to radiation, shortening the time needed to reach optimum conductance and carbon gain by photosynthesis (Bucci et al., 2005; Caird, Richards, & Donovan, 2007; Dawson et al., 2007). Consistent with this, we observed an earlier stomatal opening in Semillon relative to Grenache which may support its higher rates of maximum g_{day} and $P_{n\text{day}}$ (Figure 1c).

Alternative hypotheses regarding the benefits of g_{night} have been proposed in the literature such as enhanced nutrient uptake or nutrient distribution to distal parts of the plant (Scholz et al., 2007), the delivery of dissolved oxygen to woody tissues (Daley & Phillips, 2006), and/or the prevention of an excess of turgor in species with very negative leaf osmotic potentials (Donovan et al., 2001). Nevertheless, a recent study across numerous species including grapevine did not support any of these hypotheses, but instead offered more support for the priming hypothesis discussed in the previous paragraph (Resco de Dios et al., 2019).

4.4 | The significance of minimum leaf conductance

The minimum leaf conductance includes two pathways; one across the cuticle and the other through the incompletely closed stomata (Duursma et al., 2019). While the cuticular component has received much more attention in the literature, g_{min} is in general not directly quantified. In this study, mean values of g_{min} measured by the MLD technique were around $10 \text{ mmol m}^{-2} \text{ s}^{-1}$ for the three cultivars. This value was very close to the values measured with the IRGA at night for Grenache and Syrah (Figure 1b) indicating that g_{min} can be as high as g_{night} . A similar finding has been reported for oaks where g_{night} and g_{min} had comparable rates (Cavender-Bares, Sack, & Savage, 2007). In that study, nighttime transpiration was reduced to a minimum under

drought through stomata closure, however, water loss through the leaf cuticle continued (Cavender-Bares et al., 2007).

In other studies, a wide range of variation in g_{\min} across genotypes has suggested that this trait cannot be easily explained by other leaf traits or by the cuticle structure (Duursma et al., 2019). Genetic variation in nighttime transpiration has been considered a relevant drought tolerance trait in grapevine, but without considering the contribution of g_{\min} in explaining these differences (Coupel-Ledru et al., 2016). However, the reported genetic differences in transpiration in that study were assessed under well-watered and extremely mild water deficit conditions (soil water potentials of -0.15 MPa; Coupel-Ledru et al., 2016), rather than under the more negative water potentials that grapevines regularly encounter in the field. Our study suggests that during drought g_{\min} is significantly more important. Additional studies that include a larger range of stress levels are therefore needed to explore the contribution of g_{\min} to drought tolerance differences across genotypes.

4.5 | Time to 20% of soil RWC and THF: Contribution of different conductances

Simulations of the contribution of different conductances to the time to reach 20% of soil RWC or hydraulic failure (THF) varied according to the threshold and soil volume considered. The finding that g_{\min} had a significantly higher contribution than g_{night} and g_{day} in determining THF in small soil volumes (Figure 8a) indicated that g_{\min} is a cornerstone trait that should be assessed in drought stress experiments and included in plant models (e.g., Zhu et al., 2018). Other studies have also emphasized the importance of g_{\min} in controlling plant water potential decline after the stomata close and in determining thresholds of hydraulic failure (Brodrigg, Powers, Cochard, & Choat, 2020; Duursma et al., 2019; Martin-StPaul et al., 2017). When considering the THF under higher soil volumes (e.g., in a vineyard), g_{day} becomes more important than g_{\min} , although both have a much more significant impact than g_{night} (Figure 8b). This result reinforces the observation that g_{night} has a very small impact in defining the time to reach mortality under extreme drought.

When looking at a more agronomic context, time to 20% soil RWC, g_{day} is the most important variable and both g_{night} and g_{\min} are insignificant in any soil volume (Figure 8c,d). This is a relevant finding since a reduction to 20% of the soil RWC is a frequent situation in most of vineyards suggesting that in a production setting it is g_{day} that should be considered in estimating the overall plant water use. Our results challenge many of previous studies in grapevine that highly emphasize g_{night} as an important source of water loss affecting whole plant water balance and water use efficiency (Coupel-Ledru et al., 2016; Fuentes et al., 2013; Fuentes et al., 2014). In our study, simulations suggested that g_{night} was not as significant of a factor when compared to g_{day} and g_{\min} . Furthermore, eliminating E_{night} by bagging the plants did not significantly attenuate subsequent midday values of Ψ_{leaf} (Figure 6b). These observations suggested that g_{day} and E_{day} were the predominant factors affecting daytime Ψ_{leaf} .

5 | CONCLUSIONS

This study evaluated the relative significance and potential benefits and/or consequences of nighttime water loss in different grapevine cultivars. To our knowledge, this is the first study that evaluates the relative importance between different conductances (g_{day} , g_{night} and g_{\min}) to whole plant water-use. Overall, the results of this study indicated that g_{night} cannot be considered a trait that exacerbates water stress within the cultivars examined here, and a much more important role was observed for g_{day} and g_{\min} . The potential benefits of higher nighttime stomatal conductance need to be explored further to test whether g_{night} increases growth and if so, whether this is driven by stomatal priming or other mechanisms. Finally, an important contribution of g_{\min} was observed in determining the magnitude of hydraulic failure, stressing the importance of including this variable in drought experiments and water relations models.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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