

# The poorly-explored stomatal response to temperature at constant evaporative demand

Colleen Mills<sup>1</sup>  | Megan K. Bartlett<sup>2</sup> | Thomas N. Buckley<sup>1</sup> 

<sup>1</sup>Department of Plant Sciences, University of California, Davis, USA

<sup>2</sup>Department of Viticulture and Enology, University of California, Davis, USA

## Correspondence

Thomas N. Buckley, Department of Plant Sciences, University of California, Davis, USA.  
Email: [tnbuckley@ucdavis.edu](mailto:tnbuckley@ucdavis.edu)

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## Abstract

Changes in leaf temperature are known to drive stomatal responses, because the leaf-to-air water vapour gradient ( $\Delta w$ ) increases with temperature if ambient vapour pressure is held constant, and stomata respond to changes in  $\Delta w$ . However, the direct response of stomata to temperature (DRST; the response when  $\Delta w$  is held constant by adjusting ambient humidity) has been examined far less extensively. Though the meagre available data suggest the response is usually positive, results differ widely and defy broad generalisation. As a result, little is known about the DRST. This review discusses the current state of knowledge about the DRST, including numerous hypothesised biophysical mechanisms, potential implications of the response for plant adaptation, and possible impacts of the DRST on plant-atmosphere carbon and water exchange in a changing climate.

## KEYWORDS

climate change, modelling, stomata, VPD

## 1 | OVERVIEW

Few physiological parameters are as important for plant growth and survival and plant-atmosphere interactions as stomatal conductance ( $g_s$ ) and its responses to the environment (Berry et al., 2010; Hetherington & Woodward, 2003). Stomatal responses are key determinants of photosynthesis (Farquhar & Sharkey, 1982), transpiration (Jarvis & McNaughton, 1986), crop yield (Fischer et al., 1998) and plant survival (Martin-StPaul et al., 2017). Stomatal opening enables  $\text{CO}_2$  uptake for photosynthesis in the light, and stomatal closure conserves water in darkness and prevents fatal desiccation in drought. Since most water that falls on the Earth's land surface evaporates through stomata, the signature of stomatal responses can even be seen in global hydrologic cycles (Betts et al., 2007; Fowler et al., 2019; Gedney et al., 2006). Because of their importance, stomata have been studied intensively for over a century (Darwin, 1898; Lawson & Matthews, 2020). Most stomatal responses are well known—notably, the responses to light,  $\text{CO}_2$ , soil moisture, and evaporative demand (Cowan, 1977; Liang et al., 2023; López et al., 2021)—as are relationships between  $g_s$  and key parameters like photosynthetic capacity (Wong et al., 1979) and hydraulic

conductance (Brodribb et al., 2017) among leaves and within and across species. We know much about the mechanisms of most stomatal responses, at cellular and whole-leaf scales (Assmann & Jegla, 2016; Buckley, 2019; Lawson et al., 2018; Schroeder et al., 2001; Wang et al., 2017).

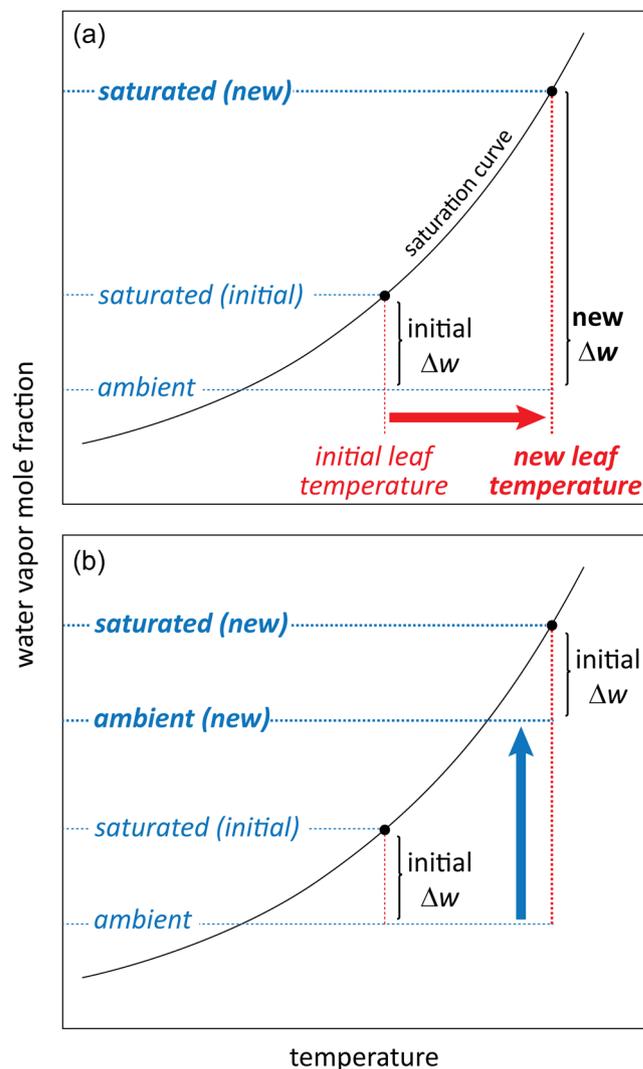
Like stomatal conductance, temperature is among the most important determinants of plant function, yet stunningly, we know little about how temperature directly affects stomata. We know temperature affects stomata indirectly via the leaf-to-air vapour mole fraction difference ( $\Delta w$ ), which increases as leaves warm due to rising saturation vapour pressure in the nearly-saturated leaf intercellular airspaces. Although many studies have reported stomatal conductance under different temperatures (e.g., Cerasoli et al., 2014; Drake & Salisbury, 1972; Drake et al., 1970; Hall & Kaufmann, 1975; Heath & Meidner, 1957; Heath & Orchard, 1957; Lahr et al., 2015; Nielson & Jarvis, 1975; Raschke, 1970; Sage & Sharkey, 1987; Schulze et al., 1974; von Caemmerer & Evans, 2015; Wuenscher & Kozłowski, 1971), very few have held  $\Delta w$  constant as temperature varies. Thus, the direct response of stomata to temperature (DRST)—the response when  $\Delta w$  is held constant by adjusting ambient vapour pressure—has only been reported in the literature a few times for intact leaves (Aphalo & Jarvis, 1991;

Ball et al., 1987; Day, 2000; Eamus et al., 2008; Fredeen & Sage, 1999; Hall & Kaufmann, 1975; Hall et al., 1975; Lu & Zeiger, 1994b; Mott & Peak, 2010; Osonubi & Davies, 1980a; Schulze et al., 1973, 1974; Teskey et al., 1986; Urban et al., 2017). As a result, little is known about the DRST. We know little about its shape (is it positive or negative; is it linear, convex, or concave), and whether it acclimates to growth conditions, or varies across taxa and environments. We have only hypotheses, largely untested, for temperature-dependent processes that may cause the DRST (e.g., membrane transport, photosynthesis, and water transport). Current models do not explicitly include the DRST, which may skew projections of carbon and water flux. And due to its obscurity, the DRST's adaptive significance has not been thoroughly examined. This article aims to shed light on this poorly understood, yet potentially quite important response.

### 1.1 | Why is the DRST so poorly studied, and why does it matter?

The main reason for the paucity of knowledge about the DRST is rather banal and technical. For some physiological parameters, the response to temperature can be straightforward to measure and interpret. For example, temperature affects the maximum velocity of RuBP carboxylation catalysed by Rubisco ( $V_{\text{cmax}}$ ), and although temperature affects other processes linked to photosynthesis, biochemical models can isolate the effect of temperature on  $V_{\text{cmax}}$  per se, given measurements of photosynthetic rate and intercellular  $\text{CO}_2$  concentration (Diaz-Espejo, 2013). With temperature and stomata, however, it is not so simple. This is because the water vapour mole fraction in the leaf airspaces ( $w_i$ ) is largely determined by the saturated value ( $w_{\text{sat}}$ ), which in turn is determined by temperature ( $T$ ), so any rise in leaf temperature leads immediately to an increase in the vapour concentration in the leaf intercellular airspaces (Figure 1a). This in turn increases the evaporative gradient that drives transpiration (the leaf-to-air vapour-pressure difference, expressed as a mole fraction,  $\Delta w \approx w_i - w_{\text{air}}$ , where  $w_{\text{air}}$  is the ambient water vapour mole fraction). Thus, in testing for a stomatal response to temperature, one immediately causes an *additional* response: namely, to  $\Delta w$ . To prevent the change in  $\Delta w$  and isolate the DRST per se (which we call the DRST), temperature and ambient vapour pressure must be adjusted simultaneously in precise fashion to keep  $\Delta w$  constant (Figure 1b). This can be a tricky balancing act.

One might question whether the DRST really matters: after all, it can't be very common in nature for temperature and vapour pressure to change simultaneously so that  $\Delta w$  remains constant, right? This logic overlooks a key point, which is that temperature and  $\Delta w$  differentially modulate two distinct physiological conditions—temperature and water status—which may in turn influence stomatal conductance by distinct mechanisms. When  $\Delta w$  changes because of a shift in vapour pressure, only the water status response is engaged, but when  $\Delta w$  changes because of a shift in temperature, both the water status *and* temperature responses are engaged. In effect, stomatal responses to temperature-driven shifts in  $\Delta w$  are superpositions of two different responses. Moreover, although shifts in atmospheric vapour pressure deficit (VPD)

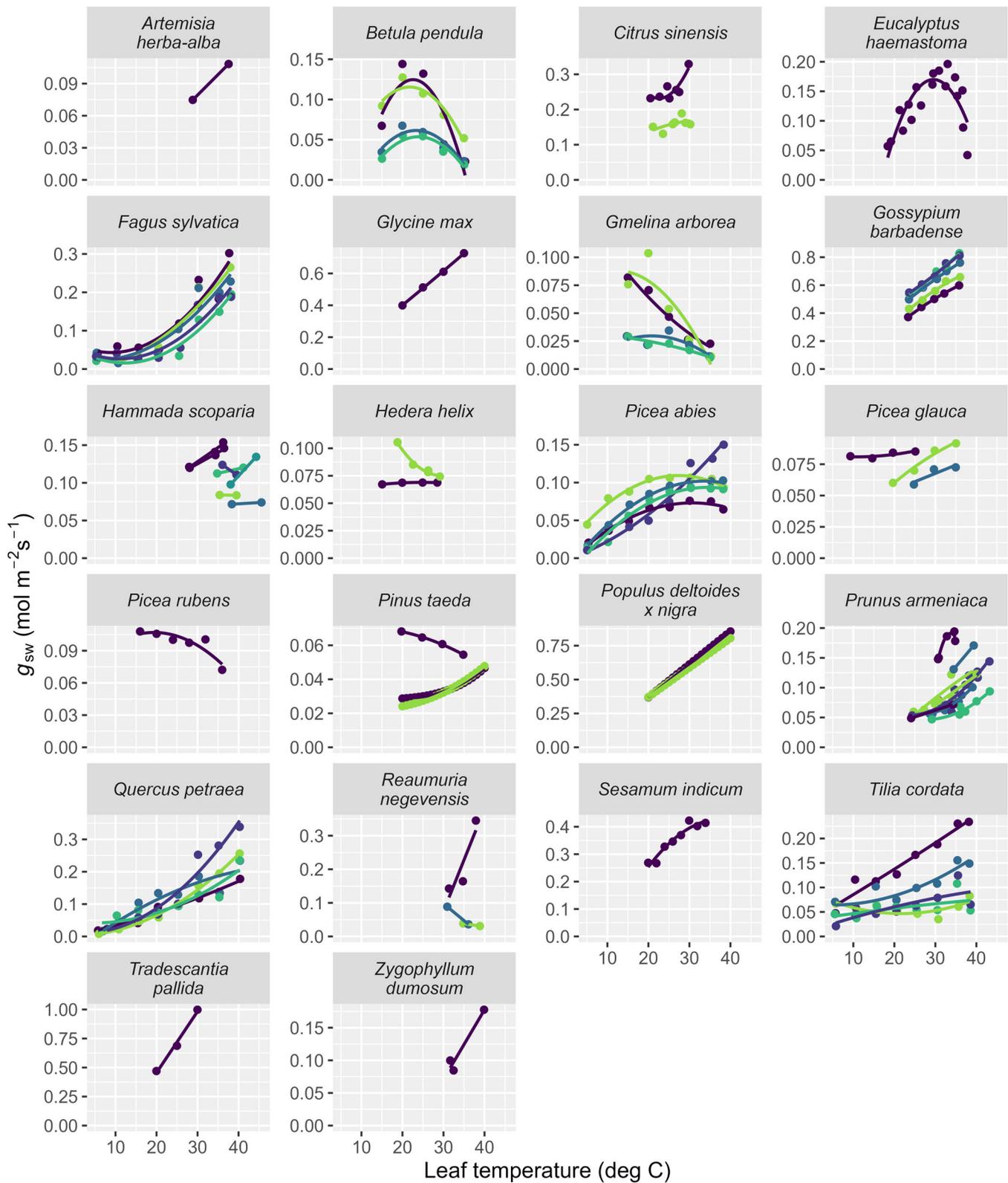


**FIGURE 1** Diagram illustrating how a change in leaf temperature (a, red arrow) causes a change in the leaf-to-air water vapour mole fraction difference ( $\Delta w$ ), due to an increase in the saturated water vapour mole fraction (solid black line and circles), and how this change in  $\Delta w$  can be prevented by imposing a simultaneous increase in the ambient water vapour mole fraction (b, blue arrow). [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

are most often driven by changes in temperature, vapour pressure can and does change independent of temperature, and these changes contribute substantially to temporal variation in VPD (Supporting Information: Figure 2; Supporting Information: Methods 1). Thus, responses to  $\Delta w$  or VPD in nature cannot be understood, nor predicted reliably, without understanding the overlapping but distinct underlying responses to water status and temperature.

### 1.2 | What is known about the direction, magnitude and shape of the DRST?

Few published studies to date have quantified the DRST in intact leaves (Figure 2 and Table 1). In most cases, the DRST has been isolated by



**FIGURE 2** Published measurements of the direct response of stomatal conductance to leaf temperature in intact leaves. In each plot, separate lines and colors represent responses measured on different leaves. Lines were fitted to responses with  $\leq 3$ -points; 2nd-order polynomials were fitted to all other responses. References and additional information are given in Table 1. Note: values shown for *Tradescantia pallida* were given by the original authors as  $g_{sw}$  relative to its value at 30°C, and are thus unitless. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

**TABLE 1** Studies reporting measurements of the response of stomatal conductance ( $g_s$ ) to temperature, in which it was unambiguous that the leaf to air water vapour mole fraction ( $\Delta w$ ) was held constant as temperature varied, and  $g_s$  was measured by gas exchange.

Study	Species	Response	$\Delta w$	Other notes
Schulze et al. (1973)	<i>Prunus armeniaca</i>	+	unk.	
	<i>Hammada scoparia</i>	+, -, 0	unk.	
	<i>Zygophyllum dumosum</i>	+	35	T was first increased then decreased
	<i>Artemisia herba-alba</i>	+	25	
	<i>Reaumuria negevensis</i>	+, -	24–30	Water Stress changed + response to -
Schulze et al. (1974)	<i>Prunus armeniaca</i>	+	15–40	Responses @ $\Delta w = 15, 30, 30, 40$
Hall et al. (1975)	<i>Citrus sinensis</i>	+	5	
Hall and Kaufmann (1975)	<i>Sesamum indicum</i>	+	15	
Osonubi and Davies (1980a, 1980b)	<i>Betula pendula</i>	p	15	Four Responses/species: Moderate/high light $\times$ well-watered/ water stressed
	<i>Gmelina arborea</i>	-	15	
Teskey et al. (1986)	<i>Pinus taeda</i>	-	15	
Ball et al. (1987)	<i>Glycine max</i>	+	10	Responses Inferred By Interpolation
Aphalo and Jarvis (1991)	<i>Hedera helix</i>	0, -	10	
Lu and Zeiger (1994a, 1994b)	<i>Gossypium barbadense</i>	+	5	Five genotypes examined
Fredeen and Sage (1999)	<i>Picea glauca</i>	+, 0	10–30	Responses @ $\Delta w = 10, 20, 30$
Day (2000)	<i>Picea rubens</i>	0/-	20	
Eamus et al. (2008)	<i>Eucalyptus haemastoma</i>	p	21	
Mott and Peak (2010)	<i>Tradescantia pallida</i>	+	17	Response in darkness
Urban et al. (2017)	<i>Pinus taeda</i>	+	10	Responses inferred by interpolation
	<i>Populus deltoides</i> $\times$ <i>nigra</i>	+	10	
Diao et al. (2024)	<i>Fagus sylvatica</i>	+	8	
	<i>Quercus petraea</i>	+	8	
	<i>Tilia cordata</i>	+, 0	8	
	<i>Picea abies</i>	p, +	8	

Note: Column 'response' describes the character of the response observed: + (positive), - (negative), 0 (little discernible response), p (peaked:  $g_s$  first rises and then falls as temperature increases); responses are illustrated graphically in Figure 1. Column ' $\Delta w$ ' refers to the value(s) of  $\Delta w$  during measurements ( $\text{mmol mol}^{-1}$ ).

changing leaf temperature while also adjusting vapour pressure to hold  $\Delta w$  constant (Day, 2000; Eamus et al., 2008; Fredeen & Sage, 1999; Hall & Kaufmann, 1975; Hall et al., 1975; Lu & Zeiger, 1994b; Mott & Peak, 2010; Osonubi & Davies, 1980b; Schulze et al., 1972; Schulze et al., 1974; Teskey et al., 1986). A few studies have instead measured  $\Delta w$  responses at different temperatures, and inferred the DRST by interpolating between the resulting curves (Ball et al., 1987; Urban et al., 2017). Several others have examined the temperature response in isolated epidermes and guard cells (Ilan et al., 1995; Kostaki et al., 2020; Rogers et al., 1979, 1980; Spence et al., 1984).

Among the 22 species for which the DRST was examined in intact leaves in the studies listed above, the response was consistently positive in 11 species, consistently negative in one, peaked in three (positive at low temperature and negative at high temperature), and inconsistent or weak in seven (Figure 2 and Table 1). Among studies reporting a

consistently positive DRST, the magnitude of the response varied widely; on average stomatal conductance changed by about 2% per degree C (relative to its value at 25°C), roughly equivalent to a doubling of stomatal conductance between 10 and 40°C. Two studies reported that the DRST depended on conditions experienced by the plant, such as soil water stress or evaporative demand: Schulze et al. (1973) found the response switched from positive to negative under extreme water stress, and Mott and Peak (2010) found that the DRST disappeared at zero  $\Delta w$ .

No consistent differences are yet known between angiosperms and gymnosperms. To our knowledge, it is unknown whether the DRST occurs in seedless plants, nor whether it acclimates to sustained shifts in growth temperature over weeks or longer. One report found that whole-plant transpiration rate in *Arabidopsis* increases following sustained growth at higher temperatures, despite new leaves produced in warmer conditions having reduced stomatal

density and area (Crawford et al., 2012), which suggests  $g_s$  was greater in leaves developed at high temperatures.

Many questions thus remain concerning the DRST. First, as the DRST has only been published for a few species, its generality across taxa and its possible evolutionary relationships remain entirely unknown. Second, few data are available to suggest, let alone to test, hypotheses regarding either the mechanism(s) of the DRST or its adaptive significance. Third, due to the paucity of data and the lack of awareness of the DRST in the plant ecophysiology and modelling communities, existing models of plant-atmosphere gas exchange may not properly represent the DRST, and nor, therefore, the distinct influences of two of the most important environmental drivers of leaf and vegetation function: temperature and evaporative demand.

## 2 | WHAT ARE THE POTENTIAL MECHANISMS OF THE DRST?

Stomatal behaviour involves a web of processes, each of which may be affected by temperature and may therefore mediate a direct temperature response. We focus here on six major categories of potential mechanisms: a purely physical effect caused by changes in gas properties with temperature, guard cell-endogenous processes such as membrane ion transport or osmolyte metabolism, modulation of leaf water status by temperature-dependent changes in water transport, metabolism of abscisic acid (ABA), and temperature-driven shifts in photosynthetic processes that influence stomata.

### 2.1 | The nonbiological ('baseline') DRST

Stomatal conductance should increase with temperature even if stomatal *aperture* remains constant, due to the temperature dependencies of molecular diffusivities and of the density of air. By Fick's Law, the diffusive flux of water vapour in air,  $J_{wa}$  (mol m<sup>-2</sup> s<sup>-1</sup>), in the direction of decreasing vapour concentration, is

$$J_{wa} = D_{wa} \frac{\partial c_w}{\partial x} \approx \frac{D_{wa}}{\Delta x} \Delta c_w = \frac{P}{RT} \cdot \frac{D_{wa}}{\Delta x} \Delta \chi_w, \quad (1)$$

where  $D_{wa}$  is the binary diffusivity (m<sup>2</sup> s<sup>-1</sup>) of water vapour in air,  $c_w$  is the volumetric concentration of water vapour (mol m<sup>-3</sup>),  $x$  represents position along the diffusion path (m),  $\partial$  denotes a partial derivative,  $\Delta$  denotes a finite difference,  $P$  is atmospheric pressure (Pa),  $R$  is the gas constant (8.31446 Pa m<sup>3</sup> mol<sup>-1</sup> K<sup>-1</sup>),  $T$  is temperature (K), and  $\chi_w$  is the water vapour mole fraction (mol mol<sup>-1</sup>) (Nobel, 1991). The third step in Eqn 1 arises because, by the ideal gas law and Dalton's Law,  $\Delta c_w = \Delta \chi_w (P/RT)$ . (Note that this expression describes diffusion generally, and does not account for features specific to stomatal diffusion, such as stomatal density and size or the three-dimensional structure of stomatal diffusion pathways (Brown & Escombe, 1990); however, accounting for those features should not introduce any other temperature effects).

Molar conductance is proportional to everything on the right side of Eqn 1 except  $\Delta \chi_w$ ; considering  $P$ ,  $R$  and  $\Delta x$  as constants, it follows that

molar conductances have an intrinsic temperature sensitivity equal to that of  $D_{wa}/T$ . Massman (1998) surveyed the literature and found that the temperature sensitivities of the binary diffusion coefficients of both water vapour and CO<sub>2</sub> in air are best described by a 1.81 power of absolute temperature, giving  $g_s$  a net intrinsic temperature sensitivity  $\propto T^{0.81}$ . Between 0 and 50°C, this sensitivity is essentially linear ( $r^2 = 0.999998$ ), with a relative slope of 0.00272; that is, an increase of 0.27% per K (Supporting Information: Figure 1). We consider this intrinsic sensitivity to be part of the DRST, because it contributes to observed shifts in stomatal conductance and gas exchange rates; it can be considered a 'baseline' DRST on top of which other (biological) mechanisms may operate. Given that the observed DRST is often on the order of four to twelve times larger than this baseline (~1–3% per K, albeit varying widely; Figure 2), one or more biological mechanisms is clearly required to explain most of the phenomenon.

### 2.2 | Guard cell-endogenous processes

Most steady-state stomatal movements in seed plants involve actively-mediated changes in the osmotic pressure of stomatal guard cells, driven by modulation of membrane ion transport and/or endogenous metabolism of organic osmolytes (Buckley, 2005; Hetherington & Woodward, 2003; Lawson & Matthews, 2020; Zeiger et al., 2002). Any of these processes might be influenced by temperature, causing a DRST. There is evidence, albeit meagre, of such influences. In *Vicia faba*, warming increases the conductance of inward-rectifying guard cell potassium channels, and decreases that of outward-rectifying channels above 20°C (Ilan et al., 1995). Stomata in isolated epidermes of *V. faba* (Rogers et al., 1979, 1980) and *Arabidopsis* (Kostaki et al., 2020) open in response to warming, with varying sensitivity, often stronger above 30°C. The response is smaller in mutants deficient in H<sup>+</sup>-ATPases, but persists in mutants with loss-of-function mutations in phototropins (which mediate the stomatal response to blue light) (Kostaki et al., 2020).

The role of these phenomena in the DRST in intact leaves and across taxa has not been shown experimentally. Doing so is particularly challenging, because it is difficult to isolate contributions of guard cell processes per se from influences arising outside of guard cells, without physically isolating the guard cells from their native milieu in the intact leaf. Yet, it is also known that stomata often behave quite differently in epidermal peels versus intact leaves (Mott, 2009; Mott et al., 2008). Notably, one report (Mott & Peak, 2010) found that the DRST is absent in non-transpiring intact leaves, as discussed in II(c) below; that finding is not consistent with an entirely guard-cell endogenous response mechanism.

### 2.3 | Water transport

Stomatal guard cells sense changes in leaf water content (Sack et al., 2018) and respond by releasing osmotic solutes, which leads to a decline in stomatal aperture. Water potential decreases in

proportion to the product of stomatal conductance,  $\Delta w$  and hydraulic resistance; therefore, if warming decreases hydraulic resistance, it should increase water potential, and hence stomatal conductance (provided  $\Delta w$  is held constant).

It is certain that one major component of whole-plant hydraulic resistance, namely the xylem resistance, decreases with warming, because viscosity declines sharply with temperature, corresponding to a  $Q_{10}$  at 25°C of roughly 1.25 for hydraulic conductance (the inverse of resistance), or equivalently a relative increase of 2.5% per K. This is solidly in the range of DRST observations reported to date (Figure 2). However, water transport into the stele in roots, and distal to the xylem in leaves, likely involves transport across cell membranes, and the effect of temperature on membrane water transport is less clear. Some data suggest positive enhancement of membrane aquaporin permeability by temperature (e.g., Murai-Hatano et al., 2008) while other data suggest a peak at around 20°C (Ionenko et al., 2010). Iglesias-Acosta et al. (2010) found that AQP abundance decreased with  $T$  in broccoli roots, yet membrane permeability increased, suggesting a role for changes in lipid bilayer structure. Supporting this, Zhao et al. (2017) found little  $T$  response for permeability in membrane vesicles, but when AQP function was suppressed with chemical inhibitors, the % response to  $T$  was much greater, yet permeability itself was 2–3 orders of magnitude lower. Murai-Hatano et al. (2008) surveyed  $Q_{10}$  values for root water transport from the literature, and found most values were between 1.2 and 2.2, with a few over 4. In intact leaves, hydraulic conductance ( $K_{leaf}$ ) has variously been reported to increase with  $T$  (e.g.,  $Q_{10}$  at 25°C  $\approx$  1.3 in *Oryza sativa* cv. Shanyou 63 [Yang et al., 2020], 1.4 in *Juglans regia* [Cochard et al., 2007], and 1.7 in *Tilia cordata* [Sellin & Kupper, 2007]) or to decrease ( $Q_{10} \approx$  0.75 in *Triticum aestivum* cv. Yannong 19 [Yang et al., 2020]). Experimental data differ regarding the effect of temperature on  $K_{leaf}$ , ranging from increases greater than predicted from viscosity effects (Sellin & Kupper, 2007), similar to or smaller than viscosity effects (Sonawane et al., 2021), or even negative responses (Yang et al., 2020). These results suggest that the potential role of root and leaf hydraulics in the DRST may differ across species.

Vapour phase transport can also contribute substantially to water transport distal to the leaf xylem (Buckley, 2015; Buckley et al., 2015, 2017; Rockwell et al., 2014). Although it may seem counterintuitive to think of vapour transport as part of 'hydraulic conductance', vapour flux does influence water potential of tissues distal to the leaf xylem, so the temperature dependence of intra-leaf vapour transport must be taken into account when considering how water transport might drive the DRST. Expressed as a hydraulic conductance (i.e., water flux per unit water potential gradient), vapour-phase transport in the leaf is highly sensitive to temperature, increasing by about 6% per K at 25°C (2.6%  $K^{-1}$  at 10°C and 12.3%  $K^{-1}$  at 40°C; Supporting Information: Figure 3). Moreover, vapour can also flow without any water potential gradient, due to the strong temperature dependence of saturation vapour pressure. Simulations suggest that in illuminated leaves, even small transdermal temperature gradients on the order of a few tenths of a kelvin can strongly

drive vapour flux towards the transpiring leaf surface, supporting a large fraction of total water flux through porous leaf tissues (Buckley et al., 2017; Rockwell et al., 2014). For flux driven by temperature gradients, the 'conductance' (flux per unit temperature difference) also increases strongly with temperature itself, by  $\sim$ 5.5%  $K^{-1}$  at 25°C (2.6% at 10°C and 10.5% at 40°C; Supporting Information: Figure 3).

More generally, the resistance for water transport downstream from (distal to) the leaf xylem—whether in vapour-phase or liquid-phase pathways—can be a substantial fraction of whole-plant resistance (Sack & Holbrook, 2006). However, whether and how those pathways, and their response to temperature, will influence stomatal conductance depends on exactly where water potential is 'sensed' in the leaf and transduced into a guard cell response (Buckley, 2019). For example, if sensing occurs primarily in the epidermis, vapour transport could be quite important, because the sensor is downstream of those pathways; by contrast, if sensing occurs primarily in the bulk mesophyll, vapour transport could actually drive stomatal closure as the leaf warms (by hydrating the epidermis and increasing epidermal backpressure, without also driving the active guard cell solute accumulation needed to overcome such backpressure) (Buckley, 2019; Scoffoni et al., 2023). In leaves with stomata on both surfaces, stomata on one surface respond little or not at all when  $\Delta w$  is changed at the opposite surface (Mott, 2007; Richardson et al., 2017). This suggests stomata do not sense the water status of the bulk leaf, but rather, that of some cells very close to the guard cells—likely the guard cells themselves or nearby epidermal or mesophyll cells—implying that the sensing site is situated near the end of the transpiration stream, downstream of many hydraulic 'resistors'.

Note that these considerations regarding water movement outside the xylem in leaves, and particularly vapour flux, assume that liquid water and water vapour are locally in near-equilibrium throughout the leaf; that is, that symplastic water exchanges freely with the adjacent intercellular airspaces. Two recent studies have brought this assumption into question (Cernusak et al., 2018; Wong et al., 2022), with potentially far-reaching implications, including for our understanding of water movement within leaves (Buckley & Sack, 2019; Rockwell et al., 2022). If the findings of Cernusak et al. and Wong et al. are broadly validated, the preceding discussion would need to be extensively reconsidered. Namely, if steep gradients in water potential occur locally on very small scales (such as across mesophyll cell membranes, from the symplast to the adjacent apoplastic water), as suggested by the results of Cernusak et al. (2018) and Wong et al. (2022), it would no longer be valid to consider the various water transport pathways to the epidermis to be approximately in parallel—which was the basis of the modelling by Rockwell et al. (2014) and Buckley et al. (2017), and conventional interpretations of experimental data by Mott (2007) and others. It would then be impossible to discern how water potential varies among leaf tissues based simply on their locations, and progress on these questions would await a far more detailed understanding of the precise pathways for water movement outside the leaf xylem and their resistances.

We suggest several types of experiments to test the hypothesis that water transport is involved in the DRST. First, verifying the core premise that hydraulic conductance increases with temperature, both for the plant as a whole and for each major organ (roots, stems and leaves); the viscosity effect nearly ensures this to be true for stems, but the likely involvement of aquaporins and possibly vapour transport leaves the question more open for roots and leaves. Second, verifying the corollary that leaf water potential increases (for a given transpiration rate) if temperature is increased, and furthermore comparing the effects of varying temperature by different amounts for different parts of the plant to verify that the increase in water potential matches predictions based on changes in whole-plant resistance. For example, the strength of the DRST should depend on whether temperature is changed for the entire plant (all the 'resistors'), or for only a portion of the plant (only a subset of resistors). Third, replicating across species the finding by Mott and Peak (2010) that the DRST is absent at zero  $\Delta w$  (using microscopic observation of stomatal apertures—gas exchange is not possible at zero  $\Delta w$ ), and more generally confirming the inference that the strength of the DRST scales with  $\Delta w$ , would lend credence to the possibility that temperature effects on water transport contribute to the DRST.

## 2.4 | ABA metabolism

Guard cell responses to declining leaf water status, discussed above, are mediated in part by ABA signals in leaves. ABA initiates a cascade of events leading to efflux of solutes and thus water from guard cells, reducing turgor and thus stomatal aperture (Bauer et al., 2013; McAdam & Brodribb, 2016; Sussmilch et al., 2017; Xie et al., 2006; Zhang et al., 2018). ABA may also increase leaf hydraulic resistance, which could amplify water status responses (Pantin et al., 2013). Any temperature-dependent process that modulates ABA synthesis (Jalakas et al., 2017), catabolism, or sensitivity (Dittrich et al., 2019) could cause a DRST. For example, if the rate of ABA catabolism were enhanced by temperature, steady-state ABA levels would be lower under warm conditions (all else equal)—amplifying temperature-induced stomatal opening caused by any other process. The outcome would be similar if warming reduced guard cell sensitivity to ABA—for example, by reducing the efficacy of ABA receptors, altering activity further downstream in the ABA signalling pathway, or upregulating ABA-independent mechanisms that counteract ABA signalling. These mechanisms have all been implicated in regulating guard cell responses to humidity (Dittrich et al., 2019; Hsu et al., 2021; Jalakas et al., 2021; Merilo et al., 2018).

Notably, water potential and ABA may mediate stomatal responses without any appreciable change in *bulk leaf* water potential or ABA levels. The cells that sense water status may be near the end of the transpiration stream, such as in the epidermis or guard cells themselves, so they may experience larger shifts in water potential

than most of the leaf. A sensitive feedback response to water status could therefore arise from highly localised changes in water potential and ABA, which may not be experimentally detectable at the bulk-leaf level (Sperry, 2000). Thus, the absence of leaf-level changes in [ABA] or water potential cannot by themselves be taken as conclusive proof against their involvement in a stomatal response.

The possible roles of ABA, and more generally of localised changes in water potential, in driving the DRST, could be tested experimentally using modern tools that enable the underlying physiology to be quantified with microscopic spatial precision. For example, laser micro-dissection could be used to isolate epidermal, guard and mesophyll cells to assess temperature-induced changes in expression of genes involved in ABA metabolism, and novel nanoparticle sensors like AquaDust (Jain et al., 2021) could be used to quantify changes in water potential among leaf tissues.

## 2.5 | Photosynthesis

Stomatal responses to red light and CO<sub>2</sub> in intact leaves are partly mediated by properties of photosynthesis that are affected by temperature (Lawson et al., 2014, 2018). Evidence suggests that stomata do not sense the rate of photosynthesis per se (Baroli et al., 2008; von Caemmerer et al., 2004; Price et al., 1998), but instead some measure of the balance or redox poise between the Calvin cycle and the light reactions (Busch, 2014; Farquhar & Wong, 1984; Messinger et al., 2006). The sensor is unknown. Hypotheses include the redox state of plastoquinone in the photosynthetic electron transport chain (Busch, 2014), ATP concentration in mesophyll cells (Buckley et al., 2003; Farquhar & Wong, 1984), and a vapour-phase ion that travels from mesophyll to guard cells (Sibbersen & Mott, 2010). Any of these putative sensors could be modulated by temperature. Some evidence also suggests the DRST is unrelated to photosynthesis: in *V. faba*, the response persists in darkness in leaf pieces floated on water and in epidermal peels (Rogers et al., 1980; Spence et al., 1984), and the DRST in *Populus deltoides x nigra* and *Pinus taeda* was unaffected by large changes in [CO<sub>2</sub>], which alter the redox poise of photosynthesis (Urban et al., 2017). These results await verification in intact leaves and/or across species.

Given that stomata open in response to decreased inter-cellular CO<sub>2</sub> concentration ( $c_i$ ) (Mott, 1988), one might hypothesise that a DRST would result from depression of  $c_i$  caused by rising photosynthetic demand for CO<sub>2</sub> as temperature increases. However, that hypothesis is strongly contradicted by the available evidence. Among the studies reported earlier on the DRST,  $c_i$  generally changed in the same direction as stomatal conductance itself during the DRST, with a median increase of +1.2  $\mu\text{mol mol}^{-1} \text{K}^{-1}$  (Ball et al., 1987; Day, 2000; Eamus et al., 2008; Fredeen & Sage, 1999; Hall & Kaufmann, 1975; Lu & Zeiger, 1994a; Teskey et al., 1986; Urban et al., 2017) (the change in  $c_i$  ranged from -3.8 to +4.5  $\mu\text{mol mol}^{-1} \text{K}^{-1}$ ; negative values generally correspond to the few reported cases of

negative DRSTs). These results imply that any changes in photosynthetic CO<sub>2</sub> demand with temperature are typically smaller than the changes in CO<sub>2</sub> supply caused by the DRST, and more importantly, that the change in stomatal conductance could not have been caused by the change in  $c_i$ .

Potential experiments to assess the role of photosynthetic processes in the DRST would need to modulate or suppress photosynthesis independent of factors that affect stomata more directly, like CO<sub>2</sub> and light; possibilities include using photosynthetic inhibitors such as DCMU to suppress electron transport (Messinger et al., 2006), or examining the character of the DRST in nocturnally open stomata, or in stomata in achlorophyllous leaves (Assmann & Zeiger, 1985) or regions of variegated leaves.

## 2.6 | Guard cell vapour exchange with air in the stomatal pore channel

Peak & Mott (Peak & Mott, 2011; 'PM') suggested another mechanism for a DRST. They posited that guard cell water potential is decoupled from leaf water status, and instead equilibrates with the relative humidity of air in the stomatal pore channel. That humidity should increase with temperature if leaf-to-air  $\Delta w$  is constant, which would hydrate guard cells, opening stomata as the leaf warms. However, there is strong evidence that guard cells are in fact strongly hydraulically coupled to the rest of the leaf, not hydraulically isolated as Peak and Mott assumed. Moreover, several of PM's predictions and assumptions contradict other evidence, and the model also contains internal contradictions. We discuss these issues in detail in Supporting Information: Methods 2, but will not consider PM's DRST mechanism further here.

## 2.7 | Predictions from a leaf-level process model

Process-based models are useful tools for guiding research, because they tie together multiple mechanistic constraints and can translate qualitative hypotheses into quantitative predictions. One such model of stomatal conductance (Buckley et al., 2003; 'BMF') combines putative influences of water transport and photosynthetic processes. The model's core assumptions are that guard cell osmotic pressure is regulated in proportion to leaf turgor pressure, and in proportion to the concentration of ATP in photosynthesising mesophyll cells. As modified by Rodriguez-Dominguez et al. (2016), the model is

$$g_{sw} = \frac{\chi\alpha(\psi_{soil} + \pi)K_{plant}}{K_{plant} + \chi\alpha\Delta w}, \quad (2)$$

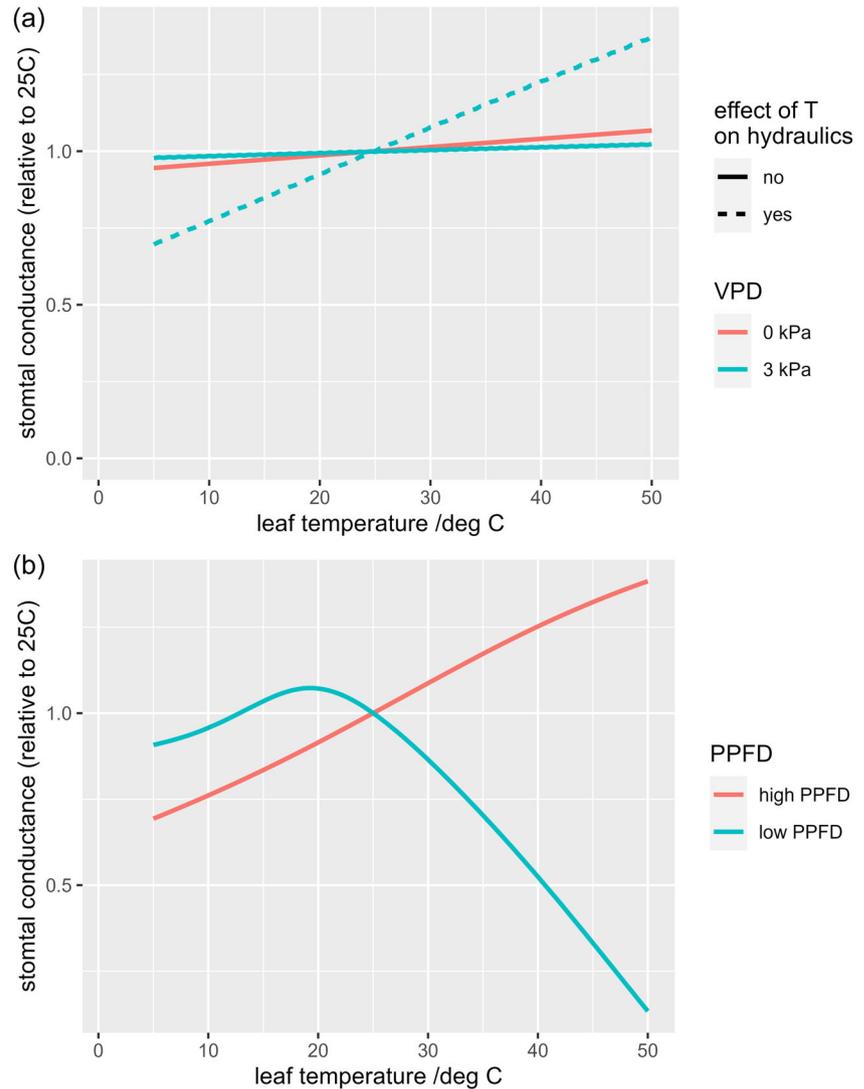
where  $\chi$  is an empirical parameter,  $\psi_{soil}$  is soil water potential,  $\pi$  is leaf osmotic pressure, and  $K_{plant}$  is plant hydraulic conductance. The term  $\alpha$  is a dimensionless factor that captures the responses to light and CO<sub>2</sub> through an assumed proportionality to the concentration of ATP in photosynthesising cells, and is predicted using the model of Farquhar and Wong (1984) (which in turn is

based on the Farquhar et al. (1980) photosynthesis model). A DRST could arise from several terms in this model. Firstly, the proportionality factor  $\chi$  implicitly captures the effect of variations in the diffusion coefficient and molar volume of air, which as discussed earlier cause a purely physical DRST of about  $0.0027 \text{ K}^{-1}$ . Secondly, temperature effects on photosynthetic processes can influence  $\alpha$  via [ATP]. Thirdly, temperature affects hydraulic conductance, as discussed earlier.

Figure 3a shows DRSTs predicted by the BMF model for different values of  $\Delta w$  (if the parameter  $\alpha$ , which is mainly affected by light and intercellular CO<sub>2</sub> concentration, is held constant to isolate hydraulic effects); Figure 3b shows how  $g_{sw}$  varies with temperature in the model for different light levels (if both  $\Delta w$  and hydraulic conductance are held constant but  $\alpha$  is allowed to vary with temperature, to isolate photosynthetic effects). At high  $\Delta w$ , the DRST is quite strong (Figure 3a, dashed line). However, if the effects of temperature on water potential (via hydraulic conductance) are eliminated, either by setting  $\Delta w$  to zero (thus eliminating transpiration), or by excluding the effects of temperature on hydraulic conductance, the DRST is very weak. These predictions are consistent with the hypothesis that  $T$  effects on water transport could cause a DRST. It is important to note that these simulations assumed that all components of  $K_{plant}$  respond to temperature identically, namely in proportion to  $1/\text{viscosity}$ . This is probably correct for xylem transport, but at least partly incorrect for transport across the root cortex and into the stele, and distal to the xylem in leaves; water likely has to cross membranes in those regions, and is thus subject to effects of  $T$  on aquaporins as discussed earlier in II(b).

The model's predictions regarding photosynthesis-mediated effects (via the parameter  $\alpha$ ) depend on light intensity. At high light, the model predicts a strong positive DRST, whereas at low light, the model predicts a response that peaks at around 20°C and then becomes negative at higher temperatures (Figure 3b). This reversal in low light results from details of the Farquhar and Wong (1984) model: in low light [ATP] declines as the leaf warms, but in high light [ATP] increases very weakly. These effects on [ATP] translate directly into qualitatively similar changes in  $g_{sw}$ , because the effect of [ATP] on  $g_{sw}$  (via  $\alpha$ ) is always positive (cf. Eqn 2). To understand these trends in predicted [ATP] with temperature, first note that [ATP] depends positively on the ratio of potential electron transport rate ( $J$ ) to carboxylation capacity ( $V_{cmax}$ ) (Supporting Information: Figure 4), and that  $J$  peaks at moderate temperatures while  $V_{cmax}$  continues increasing. However, the peak in  $J$  occurs at higher  $T$  in high light than in low light (~40°C vs. ~30°C), which is because electron transport capacity ( $J_{max}$ ) dominates control of  $J$  at high light while quantum yield ( $\Phi_{PSII}$ ) dominates at low light, and the  $T$  response of  $\Phi_{PSII}$  peaks well earlier than that of  $J_{max}$  (Supporting Information: Figure 4). These predictions depend on the assumed temperature responses of each parameter involved in photosynthesis, which may differ across species. Curiously, a negative DRST under minimally evaporative conditions was also reported in the CAM plant *Kalanchoe daigremontiana*, but in darkness (Jewer et al., 1981).

**FIGURE 3** Predicted responses of stomatal conductance to temperature, with (a) the photosynthesis-related parameter,  $\alpha$ , held constant at a value representing saturating light, or (b)  $\alpha$  allowed to vary with temperature and PPFD, but with VPD and hydraulic conductance held constant. In (a), simulations are shown both with (dashed lines) and without (solid lines) the effect of temperature on hydraulic conductance included; for VPD = 0, the result when that effect is included is hidden beneath the result when the effect is excluded, because both results are identical. Simulations were based on the process-based BMF model (Buckley et al., 2003). PPFD, photosynthetic photon flux density; VPD, vapour pressure deficit. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



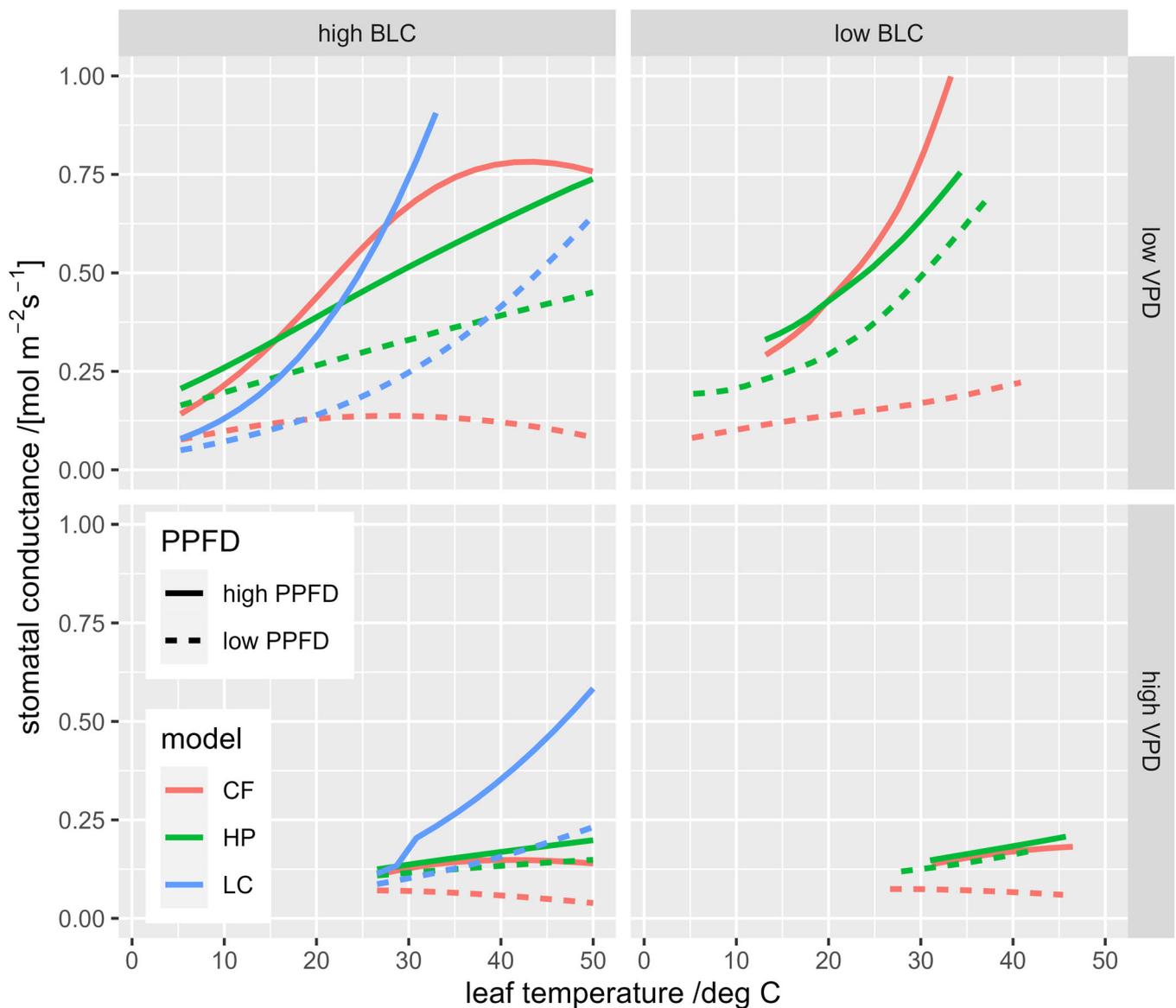
### 3 | WHAT ARE THE POTENTIAL IMPLICATIONS OF THE DRST?

A strong DRST would have many implications, from influencing land-atmosphere carbon and water fluxes to altering the adaptive landscape of gas exchange traits and responses.

#### 3.1 | Adaptive significance of the DRST

The adaptive value of any given stomatal response depends on how it affects the costs and benefits of stomatal opening. For example, if warming at constant  $\Delta w$  increased photosynthesis (a benefit) more than transpiration (a cost), then it may be beneficial for stomata to open as temperature increases (i.e., a positive DRST may be 'optimal'). More generally, the optimal DRST depends on exactly how 'optimal' is defined. Competing theories about optimal stomatal behaviour predict different DRSTs (Figure 4).

1. *Cowan-Farquhar (CF)*. The classical Cowan-Farquhar (1977) theory, which treats the total amount of water transpired over a given period as an imposed constraint, predicts a DRST with a peak (positive at lower  $T$ , negative at higher  $T$ ) when boundary layer conductance is large, with the peak occurring at higher temperatures under high irradiance, and at lower temperatures under low irradiance (red lines in Figure 4). This prediction can be traced to the fact that, in the CF theory, optimal stomatal conductance is approximately proportional to the slope of the biochemical  $\text{CO}_2$  demand curve (the 'A vs.  $c_i$ ' curve) with other conditions such as  $\text{CO}_2$ ,  $\Delta w$  and irradiance held constant. That slope, and its increase with temperature, is much larger when photosynthesis is limited by carboxylation, such as at high irradiance. When boundary layer conductance is low, however, CF predicts a positive DRST with no peak. This is because low boundary layer conductance makes it possible for leaf temperature to depart substantially from air temperature, which in turn makes it possible for evaporative cooling to lower the leaf temperature substantially; as a result, it can be economically



**FIGURE 4** Direct responses of stomata to temperature (DRST) predicted by three optimisation-based models (Cowan and Farquhar (1977), CF; Eller et al. (2020), HP [hydraulic penalty]; Prentice et al. (2014), LC [least-cost]), for (a, c) high and (b, d) low boundary layer conductance (BLC:  $g_{bw} = 2000$  and  $2 \text{ mol m}^{-2} \text{ s}^{-1}$ , respectively), (c, d) high and (a, b) low VPD (3.4 kPa and 0.86 kPa respectively;  $\Delta w = 0.034$  or  $0.0086 \text{ mol mol}^{-1}$ , respectively), and high and low PPFD 1786 and  $179 \mu\text{mol m}^{-2} \text{ s}^{-1}$  (solid and dashed lines, respectively). Results are omitted for LC at low BLC because that model does not yield an analytical solution if BLC is not assumed infinite. PPFD, photosynthetic photon flux density; VPD, vapour pressure deficit. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/terms-and-conditions)]

beneficial for the leaf to increase its stomatal conductance as the leaf warms, to promote evaporative cooling. Such cooling would not eliminate leaf warming caused by some other factor, but it could act as a 'brake' on leaf warming, perhaps limiting the maximum temperature experienced by a leaf. The exception to these generalisations is that CF predicts a negative DRST at low photosynthetic photon flux density (PPFD) and high  $\Delta w$ .

2. *Hydraulic penalty models (HP)*. Several recent optimisation-based models (Eller et al., 2018, 2020; Sperry et al., 2016; Wang et al., 2020; Wolf et al., 2016) assume that stomatal responses maximise the difference between net  $\text{CO}_2$  assimilation rate and a 'hydraulic penalty' that captures the risks posed by hydraulic

failure at low water potentials. If warming (at constant  $\Delta w$ ) enhances hydraulic conductance by decreasing viscosity, it increases water potential for a given stomatal conductance, reducing hydraulic risk and driving a positive DRST under all conditions that we examined (green lines in Figure 4 show predictions using the model of Eller et al. [2018]).

3. *Least-cost theory (LC)*. An alternative theory assumes stomatal responses adjust the ratio of intercellular to ambient  $\text{CO}_2$  concentrations ( $c_i/c_a$ ) to minimise the respiratory costs of photosynthesis and water transport, per unit of photosynthetic rate. As in HP models, warming enhances water transport, driving a positive DRST (Lavergne et al., 2020). The LC model was

originally derived assuming leaf respiration rate is negligible and photosynthesis is always light-saturated; relaxing these assumptions and re-deriving the model leads to DRST predictions that depend on irradiance and  $\Delta w$ . As with the CF theory, the response predicted by LC is weaker under light-limited conditions, but unlike CF, the response remains positive across all conditions examined (Figure 4).

Other factors may also shape the pattern and adaptive significance of the DRST:

1. *Plant traits.* Stomatal behaviour should become more conservative when the costs of stomatal opening, such as the risk of hydraulic failure or turgor loss, are heightened. During mild soil drought, species with turgor loss points (TLP; the magnitude of the water potential at which turgor is lost) closer to zero will be at greater risk of losing turgor than species with TLP farther from zero, suggesting selection may favour a weaker DRST in species with higher TLP. Likewise, the DRST may be larger in leaves with a short lifespan (because hydraulic risks are amortised over a shorter time period), and in species with very fast stomatal responses, such as grasses (because they may be able to close their stomata more rapidly to avoid exceeding critical water potential thresholds, allowing them to more closely approach such thresholds).
2. *Irreversible heat damage.* Potentially irreversible thermal damage can occur at high temperatures—for example, oxidative damage, protein destabilization, or flower abortion. The risk of irreversible damage during episodes of extreme temperature could be ameliorated by enhanced evaporative cooling. Thus, we would hypothesise a stronger positive DRST might evolve in environments where dangerously high temperatures occur more frequently. Optimisation models based on instantaneous cost-benefit assessments may be unable to account for such benefits, which extend well beyond the current instant, though some theoretical treatments do account for mortality risk, either explicitly (e.g., Cowan, 1982) or tacitly (as in the HP models).
3. *Evolutionary constraints.* Mechanistic constraints may drive divergence of the DRST across major plant groups. For example, ferns and other seedless vascular plants appear to respond to changes in leaf water status mainly via a rapid, passive mechanism, with little role for the slower mechanism of active regulation of guard cell osmotic pressure that governs steady-state responses in angiosperms (Brodribb & McAdam, 2011; Cardoso et al., 2019; Martins et al., 2016). However, fern stomatal responses to light are actively mediated and yet also generally faster than light responses in angiosperms (Cai et al., 2021). This predicts that the DRST should be faster in ferns, regardless of the extent to which the response is caused by temperature effects on hydraulics or on active guard cell processes.
4. *Climate and biogeography.* Species that have evolved in thermally stable environments, with low diurnal or annual temperature ranges, may lack a strong DRST, due to the lack of selective

pressure for such a response. Species from environments where water is abundant during the hottest time of the year may have adapted to use water for evaporative cooling—that is, to open stomata at high temperatures (a strong positive DRST)—whereas species for which water is scarce during hot periods may display the opposite behaviour. The benefit of a strong DRST would be enhanced by leaf traits that promote effective evaporative cooling, such as dense leaf hairs (trichomes) or large leaf size, both of which increase boundary layer resistance, decoupling leaf temperature from air temperature and thereby making leaf temperature more sensitive to transpiration rate.

### 3.2 | Representation of the DRST in existing empirical models of stomatal conductance

Empirical  $g_s$  models are parameterised from a variety of data sources, including both leaf-level gas exchange measurements and field flux data, so the influences of  $\Delta w$  and temperature in these models may reflect any combination of the responses to water status and temperature. The Ball-Berry model (Ball et al., 1987) and the related Ball-Berry-Leuning (BBL, Leuning, 1995) and Unified Stomatal Optimisation (USO, Medlyn et al., 2011) models all predict changes in stomatal conductance with temperature, because they contain temperature-dependent terms such as net  $\text{CO}_2$  assimilation rate ( $A$ ), but they predict very different  $T$  responses if  $\Delta w$  is held constant. The Ball-Berry model is

$$g_s = \frac{mhA}{c_a} + g_o \quad (\text{BB}), \quad (3)$$

where  $h$  is ambient relative humidity ( $0 \leq h \leq 1$ ),  $c_a$  is ambient  $\text{CO}_2$  mole fraction ( $\mu\text{mol mol}^{-3}$ ), and  $m$  and  $g_o$  are fitted parameters. To visualise this model in relation to the DRST, in which  $\Delta w$  is constant by definition, it helps to express relative humidity in terms of  $\Delta w$  and the saturation water vapour mole fraction:

$$g_s = \frac{mA}{c_a} \left( 1 - \frac{\Delta w}{w_{\text{sat}}} \right) + g_o. \quad (4)$$

Equation 4 predicts a DRST in two ways. First, the increase of  $w_{\text{sat}}$  with temperature drives a positive DRST that scales with the magnitude of  $\Delta w$ . Second, it predicts a DRST that tracks the effect of temperature on assimilation rate; that effect can be positive or negative depending on the temperature range, and on whether photosynthesis is limited by RuBP carboxylation or regeneration at any given moment.

The BBL and USO models are given by Eqns 5 and 6, respectively:

$$g_s = \frac{mA}{(c_i - \Gamma) \left( 1 + \frac{D}{D_o} \right)} + g_o \quad (\text{BBL}), \quad \text{and} \quad (5)$$

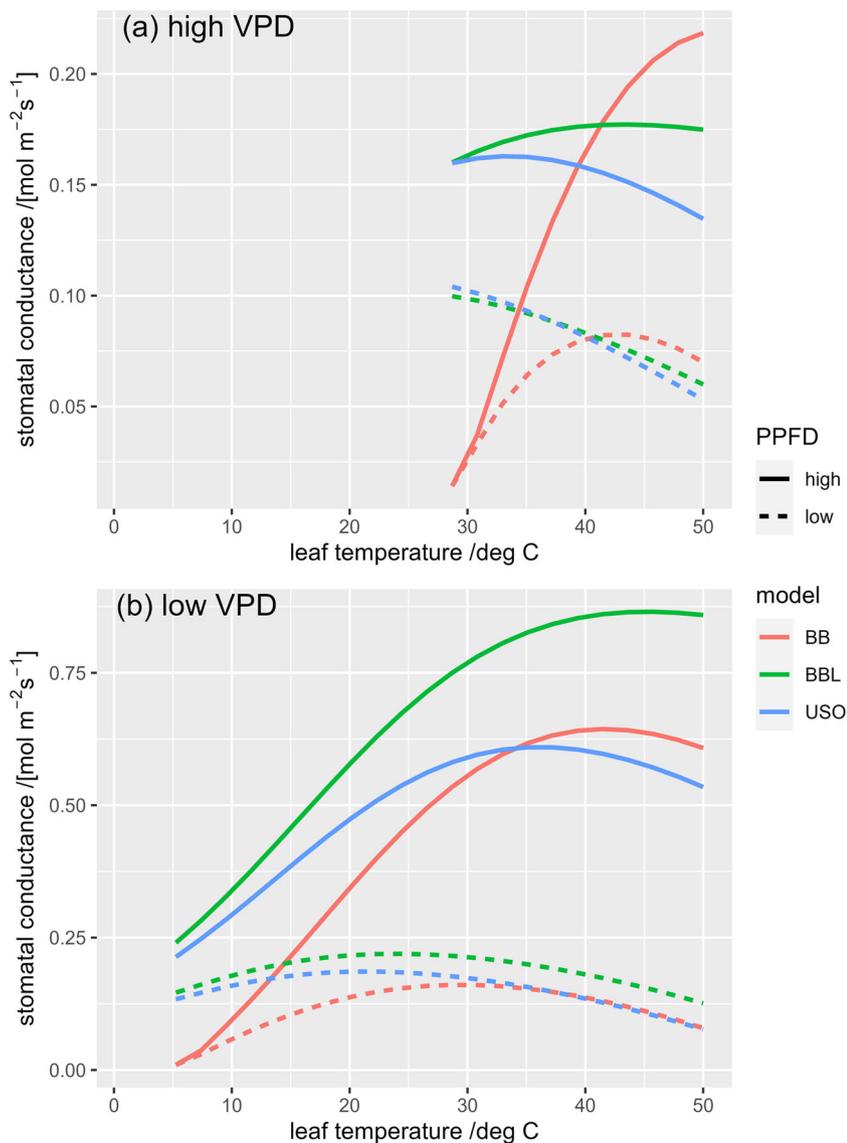
$$g_s = \frac{A}{c_a} \left( \frac{g_1}{\sqrt{D}} + 1 \right) + g_o \quad (\text{USO}), \quad (6)$$

in which  $D$  is vapour pressure deficit (VPD, the product of  $\Delta w$  and atmospheric pressure),  $m$ ,  $D_0$ ,  $g_0$  and  $g_1$  are empirical parameters,  $c_i$  is intercellular  $\text{CO}_2$  concentration, and  $\Gamma$  is the (total, not photorespiratory)  $\text{CO}_2$  compensation point. Temperature affects  $A$  in these models as in BB, but since BBL and USO use  $\Delta w$  (as  $D$ ) rather than relative humidity, the terms involving  $D$  in these models do not contribute to any DRST (because  $D$  is by definition constant during the DRST). Thus, USO's DRST is simply the response of assimilation rate, and BBL's DRST is that of the ratio of  $A$  to  $c_i - \Gamma$ . As a consequence, USO and BBL both predict weaker DRSTs than BB under most conditions.

Figure 5 shows the DRSTs predicted by all three models, using BB parameters reported by Xu and Baldocchi (2004) for *Quercus douglasii* at the Tonzi Ranch AmeriFlux site, for low and high PPFD (179 and 1790  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and low and high  $\Delta w$  (0.82 and 3.41 kPa) (corresponding to 10% and 100% of the maximum PPFD, and the mean diurnal VPD on a mild spring day [24 May 2023] and a hot

summer day [23 July 2023], respectively, at Tonzi). We adjusted the empirical parameters in BBL and USO to produce the best match to BB's predictions for the actual diurnal conditions on the 2 days mentioned above. At high light and high  $\Delta w$ , BB predicts a very strong positive DRST that weakens as temperatures reach extremes (near 50°C), whereas BBL predicts a very weak DRST, and USO predicts a moderate, negative DRST across most of this range. All models predict less-positive DRSTs under low PPFD, but across a meaningful range of temperature at high  $\Delta w$ , BB predicts a positive response and BBL and USO both predict substantial negative responses. The three models' predictions are more similar at low  $\Delta w$ , though again USO and BBL predict somewhat less positive DRSTs than BB.

It is unsurprising that these three models should diverge in their predictions of the DRST, given that neither BBL nor USO were formulated with this response in mind. BB, however, was formulated using data in which a DRST was implicit: the original study of



**FIGURE 5** Direct responses of stomata to temperature (DRST) predicted by three empirical models of stomatal conductance: Ball-Berry (BB, Ball et al., 1987), Ball-Berry-Leuning (BBL, Leuning, 1995) and Unified Stomatal Optimisation (USO, Medlyn et al., 2011), for (a) high VPD (3.4 kPa) and (b) low VPD (0.86 kPa), and for high and low PPFD (1786 and 179  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; solid and dashed lines, respectively). The parameter values used for BB model predictions are those given by Xu and Baldocchi (2004) for *Quercus douglasii* at the Tonzi Ranch AmeriFlux site; the two VPD values are mean diurnal VPDs on a mild spring day [24 May 2023] and a hot summer day [23 July 2023], respectively, at Tonzi, and the PPFD values correspond to 10% and 100% of the maximum values across those two days. The empirical parameters in BBL and USO were adjusted to maximise their fit to BB model predictions for both days combined, given actual hourly meteorological conditions reported by the local CIMIS station. PPFD, photosynthetic photon flux density; VPD, vapour pressure deficit. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/terms-and-conditions)]

Ball et al. (1987) is one of very few that documented this response (like Urban et al. [2017], Ball et al. measured  $\Delta w$  responses at numerous different temperatures), and those data partly drove the discovery and validation of the linear correlation between  $g_s$  and  $hA/c_a$  that we now know as the BB model.

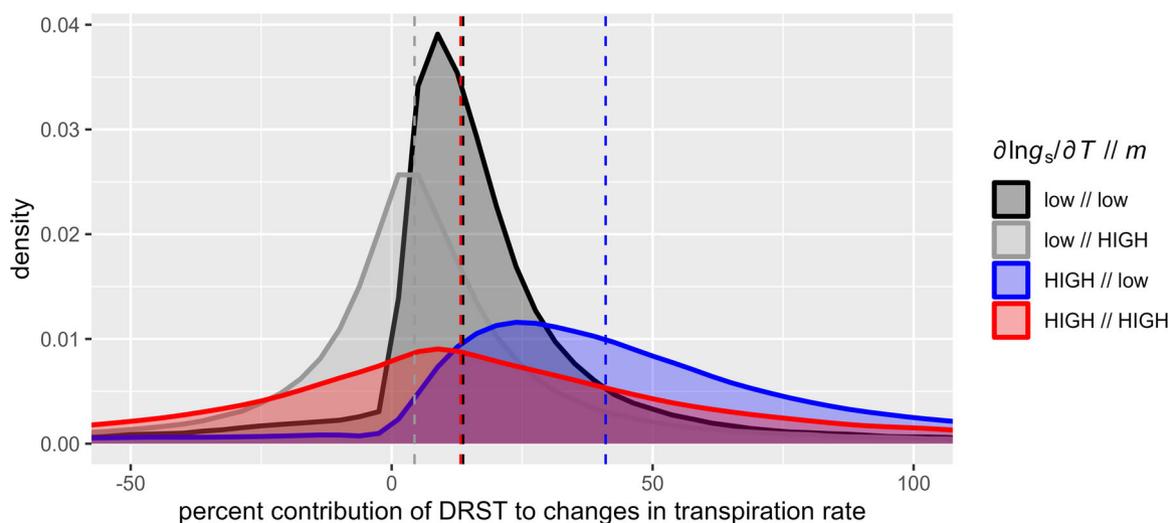
The significance of these models' different representations of the DRST will lead to different predictions depending on the extent to which variation in  $\Delta w$  in nature is driven by shifts in temperature versus vapour pressure. Although it is most common for temperature, not vapour pressure, to drive shifts in  $\Delta w$ , vapour pressure also contributes in many cases (Supporting Information: Figure 2). Supporting Information: Figure 5 shows that the models' predicted responses of  $g_s$  to an increase in  $\Delta w$  diverge depending on the contribution of vapour pressure to shifts in  $\Delta w$ ; in general, the BB model predicts a wider range of  $\Delta w$  sensitivities than the USO or BBL models, including many conditions in which  $g_s$  increases even as  $\Delta w$  increases, particularly when initial relative humidity is low.

### 3.3 | Impact of the DRST on water loss, temperature regulation, and photosynthesis

The DRST may substantially affect gas exchange and related processes. However, it is not immediately obvious how important it is to understand the DRST per se—as distinct from the stomatal response to  $\Delta w$ —given that  $\Delta w$  is so strongly determined by

temperature in nature. Indeed, if  $\Delta w$  always varied in lockstep with temperature, then the relationship between stomatal conductance and  $\Delta w$  observed in the field would encompass the effects of both temperature and  $\Delta w$ , and there would be no need to distinguish these responses for the sake of predicting plant function in the real environment. However, although  $T$  and  $\Delta w$  do often strongly co-vary in the environment, they can also vary independently, at least to some degree (Supporting Information: Figure 2; Supporting Information: Methods 1). To quantify the potential importance of the DRST in modulating transpiration rate ( $E$ ) and leaf temperature ( $T_l$ ), we conducted a formal analysis of their sensitivities to changes in air temperature and  $\Delta w$  (Supporting Information: Methods 3 and 4). In this analysis, we also quantified the actual covariation (and lack thereof, that is, independence) of temperature and  $\Delta w$  in the environment, using 1 year of hourly meteorological data from 145 stations in California's CIMIS network, and applied those data to our mathematical analysis.

First, we considered the sensitivity of transpiration to air temperature (assuming aerodynamically coupled conditions, so that leaf and air temperatures are equal), under four scenarios, representing a weak versus strong DRST and a weak versus strong stomatal response to  $\Delta w$  (Supporting Information: Methods 3). The results were striking: our analysis predicted that the DRST contributes on the order of 4% to 40% of variation in transpiration rate in response to changes in  $T$  and  $\Delta w$  (Figure 6). As one might expect, the DRST is more important in controlling transpiration if the response is strong,

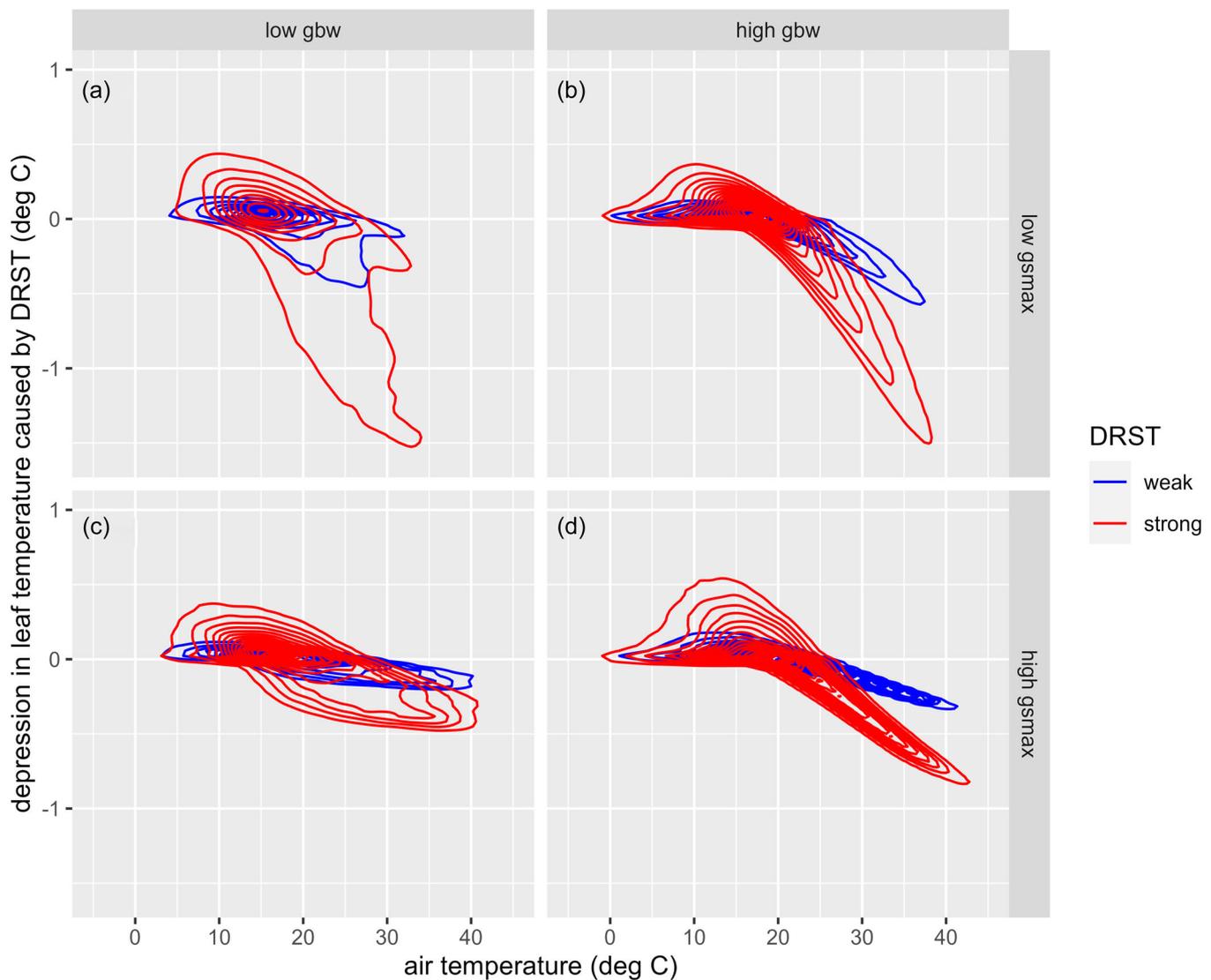


**FIGURE 6** The DRST contributes substantially, but variably, to changes in leaf transpiration rate, given observed variation in the degree to which air temperature and evaporative demand ( $\Delta w$ ) covary over time in the environment. The derivative of transpiration rate with respect to either temperature or  $\Delta w$  has three components, one of which represents the DRST (Supporting Information: Eqns 2–5); values shown on the x-axis here are the DRST component expressed as a percentage of the sum of all three components (derivations are given in Supporting Information: Methods 3). We calculated this percentage across a distribution of values for environmental variables (relative changes in  $\Delta w$  and  $T$ , and values of  $\Delta w$ ), obtained using hourly diurnal data from 145 CIMIS stations in California for calendar year 2022; calculations assumed leaf and air temperatures were equal. Colours represent four scenarios: weak versus strong DRST (low vs. high  $\partial \ln g_s / \partial T$ ) and weak versus strong stomatal response to  $\Delta w$  (low vs. high  $m$ ,  $m$  being the sensitivity parameter in the Oren et al. (1999) model of stomatal responses to  $\Delta w$ ). Dashed vertical lines are medians of each distribution (4.4, 13.2, 13.7 and 41.0%, respectively, for the scenarios shown in the legend). Negative % contributions represent scenarios where an increase in  $T$  or  $\Delta w$  would decrease transpiration in the absence of the DRST, as might commonly occur, for example, if  $T$  increased at constant vapour pressure, driving stomatal closure. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

and less important if the stomatal response to  $\Delta w$  is itself strong. Equally important is the prediction that the role of the DRST should vary widely. For example, the mid-50th percentile range for the percent contribution of the DRST to shifts in transpiration rate was from  $-0.7\%$  to  $+44.4\%$  (across all scenarios combined). This suggests that it may not be reasonable to expect that the DRST is typically subsumed into the  $\Delta w$  response due to strong covariation of  $T$  and  $\Delta w$ .

Second, we considered the influence of the DRST on leaf temperature using simulations based on energy balance and driven by the CIMIS data set described earlier, with  $g_s$  predicted

from an empirical model that included a linear DRST (Supporting Information: Eqn 15). We isolated the effect of the DRST by comparing the results with a parallel set of simulations in which the DRST was absent. The results (Figure 7) predict that a strong positive DRST could reduce leaf temperatures by up to  $1.5^\circ\text{C}$ , relative to its value in the absence of a DRST. Notably, the effect was greatest at high air temperatures, and was smaller by about half in leaves with natively higher  $g_s$ , independent of the DRST. These results suggest that the DRST may be most effective precisely when it is needed most: at high temperatures in water-stressed leaves.



**FIGURE 7** The DRST can substantially cool the leaf, particularly at high temperatures and in leaves with low maximum stomatal conductance. Values on the y axis are the difference in leaf temperature between simulations with and without a DRST; lines are contours of two-dimensional density distributions. To generate this figure, we simulated leaf temperature using hourly diurnal measurements of meteorological variables for all 145 active CIMIS stations in California in 2022, using a biophysical model of energy balance and an empirical model of stomatal conductance that included a linear DRST with weak ( $0.01\text{ K}^{-1}$ , blue lines) or strong ( $0.03\text{ K}^{-1}$ , red lines) relative sensitivity. We considered four scenarios: low (a, c) and high (b, d) leaf boundary layer conductance ( $g_{bw} = 0.5$  and  $3.0\text{ mol m}^{-2}\text{ s}^{-1}$ , respectively), and low (a, b) and high (c, d) values of stomatal conductance under saturating light, at reference  $\Delta w = 0.01\text{ mol mol}^{-1}$ , and reference  $T = 18.8^\circ\text{C}$  (the median in the meteorological data set) ( $g_s = 0.1$  or  $0.5\text{ mol m}^{-2}\text{ s}^{-1}$ , respectively). Additional details are provided in Supporting Information: Methods 4. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1111/pce.14911)]

It is also important to consider the influence of the DRST on photosynthesis. A positive DRST would enhance photosynthesis at high temperature by reducing the diffusional limitation posed by stomata, which would increase the temperature optimum of photosynthesis\*. As a result, the DRST could have disproportionate importance in extreme heat conditions, with a strong positive DRST partly ameliorating negative effects of high temperatures on photosynthetic biochemistry per se. (\*A formal proof of this point is given in Supporting Information: Methods 5, but the logic can be understood as follows. When temperature is at its optimum for photosynthesis, the effects of temperature on all the factors that determine photosynthesis cancel each other out. For an imaginary leaf with no DRST, the effect of temperature per se on  $g_s$  is by definition not among those factors. Therefore, all the non-DRST effects of temperature on photosynthesis exactly cancel out at the temperature that maximises photosynthesis in the absence of a DRST (we will call this temperature  $T_{OPT(no\ DRST)}$ ). Now suppose this imaginary leaf experiences a mutation that causes a positive DRST. Since the effect of  $g_s$  on photosynthesis is positive, a positive DRST also increases photosynthesis. But as we just noted, all the other  $T$  effects cancel out when  $T = T_{OPT(no\ DRST)}$ . Thus, in our mutated leaf, photosynthesis increases with  $T$  at  $T = T_{OPT(no\ DRST)}$ . If photosynthesis is still increasing with  $T$  at this point, it means the optimal temperature has not yet been reached, and is therefore higher in the presence of the DRST. Note that this argument does not require that  $g_s$  is greater at  $T = T_{OPT(no\ DRST)}$  in the presence of the DRST, but merely that it should be increasing with temperature at that point).

#### 4 | CONCLUSION: WHAT IS THE WAY FORWARD IN UNDERSTANDING THE DRST?

Given how little is known about the DRST, progress is needed on multiple fronts:

1. We should recognise that data remain scarce on the DRST partly because it is difficult to hold  $\Delta w$  constant while varying leaf temperature in a gas exchange chamber. This is largely due to several technical challenges, including the limited ability of commercial systems to control leaf temperature, the risk of condensation, delays in thermal adjustment of the dewpoint in humidifier columns, and instability caused by interactions between the system's regulatory feedback algorithms and transpiration rate itself. Table 2 describes these challenges in greater detail and suggests solutions for each; the most important consideration is to prevent condensation by enclosing the entire gas exchange system in a temperature-controlled chamber, and warming the system along with the leaf.
2. It is essential to test hypotheses about the DRST and update models to reflect the knowledge that results. Section II discussed experimentally testable hypotheses for several putative mechanisms for the DRST. As the scope of available data increases, it will also become possible to begin to ask questions about the evolution of the DRST across phylogenetically diverse taxa, and its variation in relation to climate of origin.

**TABLE 2** Technical challenges in holding  $\Delta w$  constant while varying leaf temperature, as needed to quantify the DRST in intact leaves.

Challenge	Solution
1. Controlling temperature across a wide range. Most commercial gas exchange systems have quite limited capacity to control leaf temperature, partly due to technical constraints – heat exchangers must not cool below the dewpoint of chamber air – and partly to design limitations – commercial chambers mostly rely on convective exchange to regulate air temperature.	Place the temperature-regulated exchange surface directly beneath the leaf, enabling both radiative and convective exchange with the leaf.
2. Sample gas will be very humid for a warm leaf at a moderate or low $\Delta w$ , and will condense if it encounters a cooler environment. Condensation is fatal to gas exchange calculations and can seriously damage gas analysers.	Keep the entire gas exchange system and the leaf at similar temperatures, for example by using a temperature controlled growth chamber (Urban et al., 2017). This also helps with Challenge #1.
3. When temperature is increased, the humidifier column in a modern gas exchange system may take a long time to warm to the new temperature, limiting the reference stream vapour pressure and thus possibly preventing achievement of a given target $\Delta w$ .	When preparing to shift to a higher temperature, pre-warm a spare humidifier column to the target temperature and insert it when chamber temperature is near the target.
4. The feedback control algorithms that regulate humidity and temperature can interact with transpiration in potentially unstable ways, causing oscillations that confound inference of steady-state stomatal conductance. For example, if reference stream humidity is above its target, the algorithm decreases reference vapour pressure, which increases $\Delta w$ and transpiration, cooling the leaf. That causes the heat exchanger to warm up, which further increases $\Delta w$ . The humidity-control algorithm then increases incoming vapour pressure in response, reversing the cycle.	Set the heat exchanger to a given target temperature, rather than targeting leaf temperature per se. This removes temperature regulation (though not leaf temperature itself) from the feedback loop. Minimise leaf-to-air temperature difference, and thus the potential amplitude of temperature oscillations, by maximising fan speed and using radiative temperature control.

3. Once enough data are available to allow some degree of generalisation, existing models of stomatal conductance should be modified as needed to reconcile their predictions with the divergent effects of changes in  $\Delta w$  caused by shifts in temperature, versus changes in  $\Delta w$  caused by shifts in vapour pressure. Given the central role of both warming per se and changes in evaporative demand in the effects of global climate change on plant-atmosphere carbon and water exchange (e.g., Ficklin & Novick, 2017; Grossiord et al., 2020; Kumarathunge et al., 2019; Moore et al., 2021), it is critical that our models accurately represent the influence of these factors on leaf stomata.

We call for plant biologists across subdisciplines, from molecular biology to physiological ecology, to pursue research on this topic. Compared with the state of knowledge and the history of intensive research on all the other known effectors of stomatal conductance, the response to temperature remains a frontier, ripe for discovery.

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#### DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no data sets were generated or analysed during the current study.

#### ORCID

Colleen Mills  <http://orcid.org/0009-0006-6698-397X>

Thomas N. Buckley  <http://orcid.org/0000-0001-7610-7136>

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## SUPPORTING INFORMATION

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

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