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

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**Abstract**

Changes in leaf temperature are known to drive stomatal responses, because the leaf-to-air water vapor gradient (*w*) increases with temperature if ambient vapor pressure is held constant, and stomata respond to changes in *w*. However, the direct response of stomata to temperature (DRST; the response when *w* is held constant by adjusting ambient humidity) has been examined far less extensively. Though the meager available data suggest the response is usually positive, results differ widely and defy broad generalization. As a result, little is known about the DRST. This review discusses the current state of knowledge about the DRST, including numerous hypothesized 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**

Stomata, temperature, modeling, climate change, VPD

**I. 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 (Hetherington & Woodward 2003; Berry, Beerling & Franks 2010). 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, Delzon & Cochard 2017). Stomatal opening enables CO2 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 (Gedney *et al.* 2006; Betts *et al.* 2007; Fowler, Kooperman, Randerson & Pritchard 2019). Because of their importance, stomata have been studied intensively for over a century (Darwin 1898). Most stomatal responses are well known (Cowan 1977) – notably, the responses to light, CO2, soil moisture, and evaporative demand – as are relationships between *g*s and key parameters like photosynthetic capacity (Wong, Cowan & Farquhar 1979) and hydraulic conductance (Brodribb, McAdam & Carins Murphy 2017) among leaves and within and across species. We know much about the mechanisms of most stomatal responses, at cellular and whole-leaf scales (Schroeder, Kwak & Allen 2001; Assmann & Jegla 2016; Wang *et al.* 2017; Lawson, Terashima, Fujita & Wang 2018; Buckley 2019).

Like stomatal conductance, temperature is among the most important environmental controls on life, yet stunningly, we know little about how temperature directly affects stomata. We know temperature affects stomata indirectly via the leaf-to-air vapor mole fraction difference (*w*), which increases as leaves warm due to rising saturation vapor pressure in the nearly-saturated leaf airspaces. Although many studies have reported stomatal conductance under different temperatures (e.g., Heath & Meidner 1957; Heath & Orchard 1957; Drake, Raschke & Salisbury 1970; Raschke 1970; WUENSCHER & KOZLOWSKI 1971; Drake & Salisbury 1972; Schulze, Lange, Evenari, Kappen & Buschbom 1974; Hall & Kaufmann 1975; Nielson & Jarvis 1975; Sage & Sharkey 1987; Cerasoli *et al.* 2014; Lahr, Schade, Crossett & Watson 2015; von Caemmerer & Evans 2015), very few have held *w* constant as temperature varies. Thus, the **direct response of stomata to temperature** (**DRST**) – the response when *w* is held constant by adjusting ambient vapor pressure – has only been reported in the literature a few times for intact leaves (Schulze, Lange, Kappen, Buschbom & Evenari 1973; Schulze *et al.* 1974; Hall, Camacho-B & Kaufmann 1975; Hall & Kaufmann 1975; Osonubi & Davies 1980a; Teskey, Fites, Samuelson & Bongarten 1986b; Ball, Woodrow & Berry 1987; Aphalo & Jarvis 1991; Lu & Zeiger 1994b; Fredeen & Sage 1999; Day 2000; Eamus, Taylor, MACINNIS‐NG, Shanahan & De Silva 2008; Mott & Peak 2010; Urban, Ingwers, McGuire & Teskey 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.

***I(a). 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 catalyzed by Rubisco (*V*cmax), and although temperature affects other processes linked to photosynthesis, biochemical models can isolate the effect of temperature on *V*cmax *per se*, given measurements of photosynthetic rate and intercellular CO2 concentration (Diaz-Espejo 2013). With temperature and stomata, however, it is not so simple. This is because the water vapor mole fraction at the sites of evaporation in the leaf (*w*i) is largely determined by the saturated value (*w*sat), which in turn is determined by temperature (*T*), so any rise in leaf temperature leads immediately to an increase in the vapor concentration in the leaf airspaces (Fig 1a). This in turn increases the evaporative gradient that drives transpiration (the leaf-to-air vapor-pressure difference, expressed as a mole fraction, *w* ≈ *w*i – *w*air, where *w*air is the ambient water vapor mole fraction). Thus, in testing for a stomatal response to temperature, one immediately causes an *additional* response: namely, to *w*. To prevent the change in *w* and isolate the direct response of stomata to temperature *per se* (which we call the DRST), temperature and ambient vapor pressure must be adjusted simultaneously in precise fashion to keep *w* constant (Fig 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 vapor pressure to change simultaneously so that *w* remains constant, right? This logic overlooks a key point, which is that temperature and *w* differentially modulate two distinct physiological conditions – temperature and water status – which may in turn influence stomatal conductance by distinct mechanisms. When *w* changes because of a shift in vapor pressure, only the water status response is engaged, but when *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 *w* are superpositions of two different responses. Moreover, although shifts in atmospheric vapor pressure deficit (VPD) are most often driven by changes in temperature, vapor pressure can and does change independent of temperature, and these changes contribute substantially to temporal variation in VPD (Fig S1; Methods S1). Thus, responses to *w* or VPD in nature cannot be understood, nor predicted reliably, without understanding the overlapping but distinct underlying responses to water status and temperature.

***I(b). 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, Table 1). In most cases, the DRST has been isolated by changing leaf temperature while also adjusting vapor pressure to hold *w* constant (Schulze, Lange, Buschbom, Kappen & Evenari 1972; Schulze *et al.* 1974; Hall *et al.* 1975; Hall & Kaufmann 1975; Osonubi & Davies 1980b; Teskey *et al.* 1986b; Lu & Zeiger 1994b; Fredeen & Sage 1999; Day 2000; Eamus *et al.* 2008; Mott & Peak 2010). A few studies have instead measured *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 (Rogers, Powell & Sharpe 1979; Rogers, Sharpe & Powell 1980; Spence, Sharpe, Powell & Wu 1984; Ilan, Moran & Schwartz 1995; Kostaki *et al.* 2020).

Among the 18 species for which the DRST was examined in intact leaves in the studies listed above, the response was consistently positive in nine species, consistently negative in one, peaked in two (positive at low temperature and negative at high temperature), and inconsistent or weak in six (Figure 2, 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 25oC), roughly equivalent to a doubling of stomatal conductance between 10 and 40oC. Two studies reported that the DRST depended on conditions: 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 *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, McLachlan, Hetherington & Franklin 2012), which suggests *g*s was greater in leaves developed at high temperatures.

Many questions thus remain concerning the DRST. Firstly, as the DRST has only been published for a few species, its generality across taxa and its possible evolutionary relationships remain entirely unknown. Secondly, few data are available to suggest, let alone to test, hypotheses regarding either the mechanism(s) of the DRST or its adaptive significance. Thirdly, due to the paucity of data and the lack of awareness of the DRST in the plant ecophysiology and modeling 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.

**II. What are the potential mechanisms of the DRST?**

Stomatal behavior 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.

***II(a). The non-biological ("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 vapor in air, *J*wa (mol m-2 s-1), in the direction of decreasing vapor concentration, is

where *D*wa is the binary diffusivity (m2 s-1) of water vapor in air, *c*w is the volumetric concentration of water vapor (mol m-3), *x* represents position along the diffusion path (m),  denotes a finite difference, *P* is atmospheric pressure (Pa), *R* is the gas constant (8.31446 Pa m3 mol-1 K-1), *T* is temperature (K), and w is the water vapor mole fraction (mol mol-1) (Nobel 1991). The third step in Eqn 1 arises because, by the ideal gas law and Dalton's Law, *c*w = 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 w; considering *P*, *R* and *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 vapor and CO2 in air are best described by a 1.81 power of absolute temperature, giving *g*s a net intrinsic temperature sensitivity ∝ *T*0.81. Between 0 and 50oC, this sensitivity is essentially linear (*r*2 = 0.999998), with a relative slope of 0.00272; i.e., an increase of 0.27% per K. 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; Fig 2), one or more biological mechanisms is clearly required to explain most of the phenomenon.

***II(b). 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 (Zeiger, Talbott, Frechilla, Srivastava & Zhu 2002; Hetherington & Woodward 2003; Buckley 2005). Any of these processes might be influenced by temperature, causing a DRST. There is evidence, albeit meager, 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 20oC (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 30oC. The response is smaller in mutants deficient in H+-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 vs intact leaves (Mott, Sibbernsen & Shope 2008; Mott 2009). 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.

***II(c). Water transport***

Stomatal guard cells sense changes in leaf water content (Sack, John & Buckley 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, *w* and hydraulic resistance; therefore, if warming decreases hydraulic resistance, it should increase water potential, and hence stomatal conductance (provided *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 Q10 at 25oC 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 (Fig 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 20oC (Ionenko, Anisimov & Dautova 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 Q10 values for root water transport from the literature, and found most values were between 1.2 to 2.2, with a few over 4. In intact leaves, hydraulic conductance (*K*leaf) has variously been reported to increase with *T* (e.g., Q10 at 25oC ≈ 1.3 in *Oryza sativa* cv. Shanyou 63 (Yang, Zhang, Huang, Peng & Li 2020), 1.4 in *Juglans regia* (Cochard *et al.* 2007), and 1.7 in *Tilia cordata* (Sellin & Kupper 2007)) or to decrease (Q10 ≈ 0.75 in *Triticum aestivum* cv. Yannong 19 (Yang *et al.* 2020)). Experimental data differ regarding the effect of temperature on leaf hydraulic conductance (*K*leaf), ranging from increases greater than predicted from viscosity effects (Sellin & Kupper 2007), similar to or smaller than viscosity effects (Sonawane, Koteyeva, Johnson & Cousins 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.

Vapor phase transport can also contribute substantially to water transport distal to the leaf xylem (Rockwell, Holbrook & Stroock 2014; Buckley 2015; Buckley, John, Scoffoni & Sack 2015, 2017). Although it may seem counterintuitive to think of vapor transport as part of "hydraulic conductance", vapor flux does influence water potential of tissues distal to the leaf xylem, so the temperature dependence of intra-leaf vapor 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), vapor-phase transport in the leaf is highly sensitive to temperature, increasing by about 6% per K at 25oC (2.6% K-1 at 10oC and 12.3% K-1 at 40oC; Fig S2). Moreover, vapor can also flow without any water potential gradient, due to the strong temperature dependence of saturation vapor pressure; simulations suggest that in illuminated leaves, even small transdermal temperature gradients on the order of a few tenths of a degree C can strongly drive vapor flux towards the transpiring leaf surface, supporting a large fraction of total water flux through porous leaf tissues (Rockwell *et al.* 2014; Buckley *et al.* 2017). For flux driven by temperature gradients, the "conductance" (flux per unit temperature difference) also increases strongly with temperature itself, by ~5.5% K-1 at 25oC (2.6% at 10oC and 10.5% at 40oC; Fig S2).

More generally, the resistance for water transport downstream from (distal to) the leaf xylem – whether in vapor-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, vapor transport could be quite important, because the sensor is downstream of those pathways; by contrast, if sensing occurs primarily in the bulk mesophyll, vapor 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, Albuquerque, Buckley & Sack 2023). In leaves with stomata on both surfaces, stomata on one surface respond little or not at all when *w* is changed at the opposite surface (Mott 2007; Richardson, Brodribb & Jordan 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 vapor flux, assume that liquid water and water vapor are locally in near-equilibrium throughout the leaf; i.e., 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.

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, leaves); the viscosity effect nearly ensures this to be true for stems, but the likely involvement of aquaporins and possibly vapor 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 *w* (using microscopic observation of stomatal apertures – gas exchange is not possible at zero *w*), and more generally confirming the inference that the strength of the DRST scales with *w*, would lend credence to the possibility that temperature effects on water transport contribute to the DRST.

***II(d). 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 (Xie *et al.* 2006; Bauer *et al.* 2013; McAdam & Brodribb 2016; Sussmilch, Brodribb & McAdam 2017b; 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 signaling pathway, or upregulating ABA-independent mechanisms that counteract ABA signaling. These mechanisms have all been implicated in regulating guard cell responses to humidity (Merilo *et al.* 2018; Dittrich *et al.* 2019; Hsu, Dubeaux, Takahashi & Schroeder 2021; Jalakas, Takahashi, Waadt, Schroeder & Merilo 2021).

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 localized 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 localized 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.

***II(e). Photosynthesis***

Stomatal responses to red light and CO2 in intact leaves are partly mediated by properties of photosynthesis that are affected by temperature (Lawson, Simkin, Kelly & Granot 2014; Lawson *et al.* 2018). Evidence suggests that stomata do not sense the rate of photosynthesis per se (Price *et al.* 1998; Caemmerer *et al.* 2004; Baroli, Price, Badger & von Caemmerer 2008), but instead some measure of the balance or redox poise between the Calvin cycle and the light reactions (Farquhar & Wong 1984; Messinger, Buckley & Mott 2006; Busch 2014). The sensor is unknown. Hypotheses include the redox state of plastoquinone in the photosynthetic electron transport chain (Busch 2014), ATP concentration in mesophyll cells (Farquhar & Wong 1984; Buckley, Mott & Farquhar 2003), and a vapor-phase ion that travels from mesophyll to guard cells (Sibbernsen & 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 [CO2], 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 intercellular CO2 concentration (*c*i) (Mott 1988), one might hypothesize that a DRST would result from depression of *c*i caused by rising photosynthetic demand for CO2 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 mol mol-1 K-1 (Hall & Kaufmann 1975; Teskey, Fites, Samuelson & Bongarten 1986a; Ball *et al.* 1987; Lu & Zeiger 1994a; Fredeen & Sage 1999; Day 2000; Eamus *et al.* 2008; Urban *et al.* 2017) (the change in *c*i ranged from -3.8 to +4.5 mol mol-1 K-1; negative values generally correspond to the few reported cases of negative DRSTs). These results imply that any changes in photosynthetic CO2 demand with temperature are typically smaller than the changes in CO2 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 CO2 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.

***II(f). Guard cell vapor 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 *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 Methods S2, but will not consider PM's DRST mechanism further here.

***II(g). 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 photosynthesizing mesophyll cells. As modified by Rodriguez-Dominguez et al (2016), the model is

where ** is an empirical parameter, **soil is soil water potential, ** is leaf osmotic pressure, and *K*plant is plant hydraulic conductance. The term ** is a dimensionless factor that captures the responses to light and CO2 through an assumed proportionality to the concentration of ATP in photosynthesizing 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 ** 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 K-1. Secondly, temperature effects on photosynthetic processes can influence ** via [ATP]. Thirdly, temperature affects hydraulic conductance, as discussed earlier.

Figure 3a shows DRSTs predicted by the BMF model under various conditions and assumptions, and Fig 3b shows how [ATP] itself varies with temperature in the model. In high light and high *w*, the model predicts a DRST broadly similar to the available data (cf. Fig 2), with an average sensitivity of ~+0.014 K-1 between 5oC and 50oC. At low light the predicted DRST becomes strongly negative above 25oC, and its direction below 25oC depends on *w* (dashed lines in Fig 3a). 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. 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) (Fig S3), 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 (~40oC vs. ~30oC), which is because electron transport capacity (*J*max) dominates control of *J* at high light while quantum yield (PSII) dominates at low light, and the *T* response of PSII peaks well earlier than that of *J*max (Fig S3). These predictions depend on the assumed temperature responses of each parameter involved in photosynthesis, which may differ across species.

Notably, if the effect of *T* on water transport is excluded from the model (blue lines in Fig 3a), the predicted DRST is nearly absent in high light and negative in low light. Similarly, reducing *w* to zero also mostly eliminates the predicted response in high light. 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/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).

**III. What are the potential implications of the DRST?**

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

***III(a). 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 *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 behavior predict different DRSTs (Fig 4).

* 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 Fig 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 CO2 demand curve (the "*A* vs *c*i" curve) with other conditions such as CO2, *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, because low boundary layer conductance facilitates leaf cooling via increased transpiration rate. The exception to these generalizations is that CF predicts a negative DRST at low PPFD and high *w*.
* Hydraulic penalty models (HP). Several recent optimization-based models (Sperry *et al.* 2016; Wolf, Anderegg & Pacala 2016; Eller *et al.* 2018, 2020; Wang, Sperry, Anderegg, Venturas & Trugman 2020) assume that stomatal responses maximize the difference between net CO2 assimilation rate and a "hydraulic penalty" that captures the risks posed by hydraulic failure at low water potentials. If warming (at constant *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 Fig 4 show predictions using the model of Eller et al (2018)).
* Least-cost theory (LC). An alternative theory assumes stomatal responses adjust the ratio of intercellular to ambient CO2 concentrations (*c*i/*c*a) to minimize 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 *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 (Fig 4).

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

* Plant traits. Stomatal behavior 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 favor a weaker DRST in species with higher TLP. Likewise, the DRST may be larger in leaves with a short lifespan (because hydraulic risks are amortized 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).
* 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 hypothesize a stronger positive DRST might evolve in environments where dangerously high temperatures occur more frequently. Optimization 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).
* 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; Martins, McAdam, Deans, DaMatta & Brodribb 2016; Cardoso, Randall & McAdam 2019). 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.
* 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 – i.e., to open stomata at high temperatures (a strong positive DRST) – whereas species for which water is scarce during hot periods may display the opposite behavior. 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.

***III(b). Representation of the DRST in existing empirical models of stomatal conductance***

Empirical *g*s models are parameterized from a variety of data sources, including both leaf-level gas exchange measurements and field flux data, so the influences of *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 Optimization (USO, Medlyn *et al.* 2011) models all predict changes in stomatal conductance with temperature, because they contain temperature-dependent terms such as net CO2 assimilation rate (*A*), but they predict very different *T* responses if *w* is held constant. The Ball-Berry model is

where *h* is ambient relative humidity (0 ≤ *h* ≤ 1), *c*a is ambient CO2 mole fraction (mol mol-1), and *m* and *g*o are fitted parameters. To visualize this model in relation to the DRST, in which *w* is constant by definition, it helps to express relative humidity in terms of *w* and the saturation vapor pressure:

Equation 4 predicts a DRST in two ways. First, the increase of *w*sat with temperature drives a positive DRST that scales with the magnitude of *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:

in which *D* is vapor pressure deficit (VPD, the product of *w* and atmospheric pressure), *m*, *D*o, *g*o and *g*1 are empirical parameters, *c*i is intercellular CO2 concentration, and  is the (total, not photorespiratory) CO2 compensation point. Temperature affects *A* in these models as in BB, but since BBL and USO use *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 – . 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 mol m-2 s-1) and low and high *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 two days mentioned above. At high light and high *w*, BB predicts a very strong positive DRST that weakens as temperatures reach extremes (near 50oC), 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 *w*, BB predicts a positive response and BBL and USO both predict substantial negative responses. The three models' predictions are more similar at low *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 direct response of stomata to temperature, 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 Ball *et al*. (1987) is one of very few that documented this response (like Urban *et al*. (2017), Ball *et al*. measured *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 *w* in nature is driven by shifts in temperature vs vapor pressure. Although it is most common for temperature, not vapor pressure, to drive shifts in *w*, vapor pressure also contributes in many cases (Fig S1). Figure S4 shows that the models' predicted responses of *g*s to an increase in *w* diverge depending on the contribution of vapor pressure to shifts in *w*; in general, the BB model predicts a wider range of *w* sensitivities than the USO or BBL models, including many conditions in which *g*s increases even as *w* increases, particularly when initial relative humidity is low.

***III(c). 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 *w* – given that *w* is so strongly determined by temperature in nature. Indeed, if *w* always varied in lockstep with temperature, then the relationship between stomatal conductance and *w* observed in the field would encompass the effects of both temperature and *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 *w* do often strongly co-vary in the environment, they can also vary independently, at least to some degree (Fig S1; Methods S1). 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 *w* (Methods S3 and S4). In this analysis, we also quantified the actual covariation (and lack thereof, i.e., independence) of temperature and *w* in the environment, using one 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 vs strong DRST and a weak vs strong stomatal response to *w* (Methods S3). 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 *w* (Fig 6). As one might expect, the DRST is more important in controlling transpiration if the response is strong, and less important if the stomatal response to *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 *w* response due to strong covariation of *T* and *w*.

Second, we considered the influence of the DRST on leaf temperature using simulations based on energy balance and driven by the CIMIS dataset described earlier, with *gs* predicted from an empirical model that included a linear DRST (Eqn S15). 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 (Fig 7) predict that a strong positive DRST could reduce leaf temperatures by up to 1.5oC. 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.

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 Methods S5, 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 maximizes 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.)

**IV. Conclusion: what is the way forward in understanding the DRST?**

Given how little is known about the direct response of stomata to temperature, progress is needed on multiple fronts:

* (1) We should recognize that data remain scarce on the DRST partly because it is difficult to hold *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.
* (3) Once enough data are available to allow some degree of generalization, existing models of stomatal conductance should be modified as needed to reconcile their predictions with the divergent effects of changes in *w* caused by shifts in temperature, vs changes in *w* caused by shifts in vapor 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; Kumarathunge *et al.* 2019; Grossiord *et al.* 2020; 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.

**Acknowledgements**

All authors were supported by the National Science Foundation (Award 2307341); TNB was also supported by the National Institute of Food and Agriculture (NIFA, Hatch award 1016439).

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**Tables**

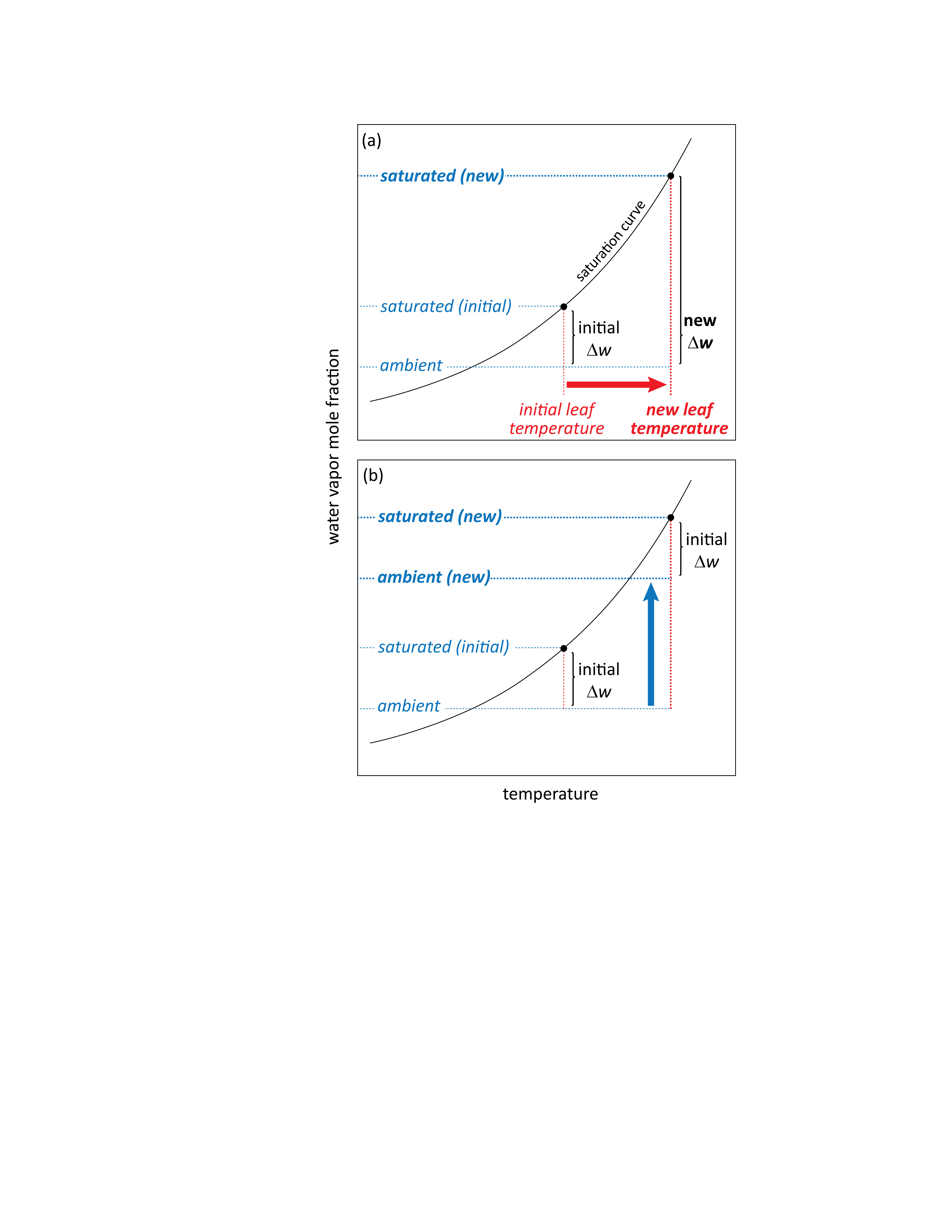
**Table 1**. Studies reporting measurements of the response of stomatal conductance (*g*s) to temperature, in which it was unambiguous that the leaf to air water vapor mole fraction (*w*) was held constant as temperature varied, and *g*s was measured by gas exchange. Column "response" describes the 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 "*w*" refers to the value(s) of *w* during measurements (mmol mol-1).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **study** | **species** | **response** | ***w*** | **Other notes** |
| Schulze *et al.* (1973) | *Prunus armeniaca* | + | *unk.* |  |
| *Hammada scoparia* | +, -, 0 | *unk.* |  |
| *Zygophyllum dumosum* | + | 35 | *T* was first increased then decreased |
| *Artemisia herba-alba* | + | 25 |  |
| *Reaumuria negevensis* | +, - | 24-30 | water stress changed + response to - |
| Schulze *et al.* (1974) | *Prunus armeniaca* | + | 15-40 | responses @ *w* = 15, 30, 30, 40 |
| Hall *et al*. (1975) | *Citrus sinensis* | + | 5 |  |
| Hall & Kaufmann (1975) | *Sesamum indicum* | + | 15 |  |
| Osonubi & Davies (1980) | *Betula pendula* | p | 15 | four responses/species: moderate/high light × well-watered/water stressed |
| *Gmelina arborea* | - | 15 |
| Teskey *et al*. (1986) | *Pinus taeda* | - | 15 |  |
| Ball *et al*. (1987) | *Glycine max* | + | 10 | responses inferred by interpolation |
| Aphalo & Jarvis (1991) | *Hedera helix* | 0, - | 10 |  |
| Lu & Zeiger (1994) | *Gossypium barbadense* | + | 5 | five genotypes examined |
| Fredeen & Sage (1999) | *Picea glauca* | +, 0 | 10-30 | responses @ *w* = 10, 20, 30 |
| Day (2000) | *Picea rubens* | 0/- | 20 |  |
| Eamus *et al*. (2008) | *Eucalyptus haemastoma* | p | 21 |  |
| Mott & Peak (2010) | *Tradescantia pallida* | + | 17 | response in darkness |
| Urban *et al*. (2017) | *Pinus taeda* | + | 10 | responses inferred by interpolation |
| *Populus deltoides × nigra* | + | 10 |

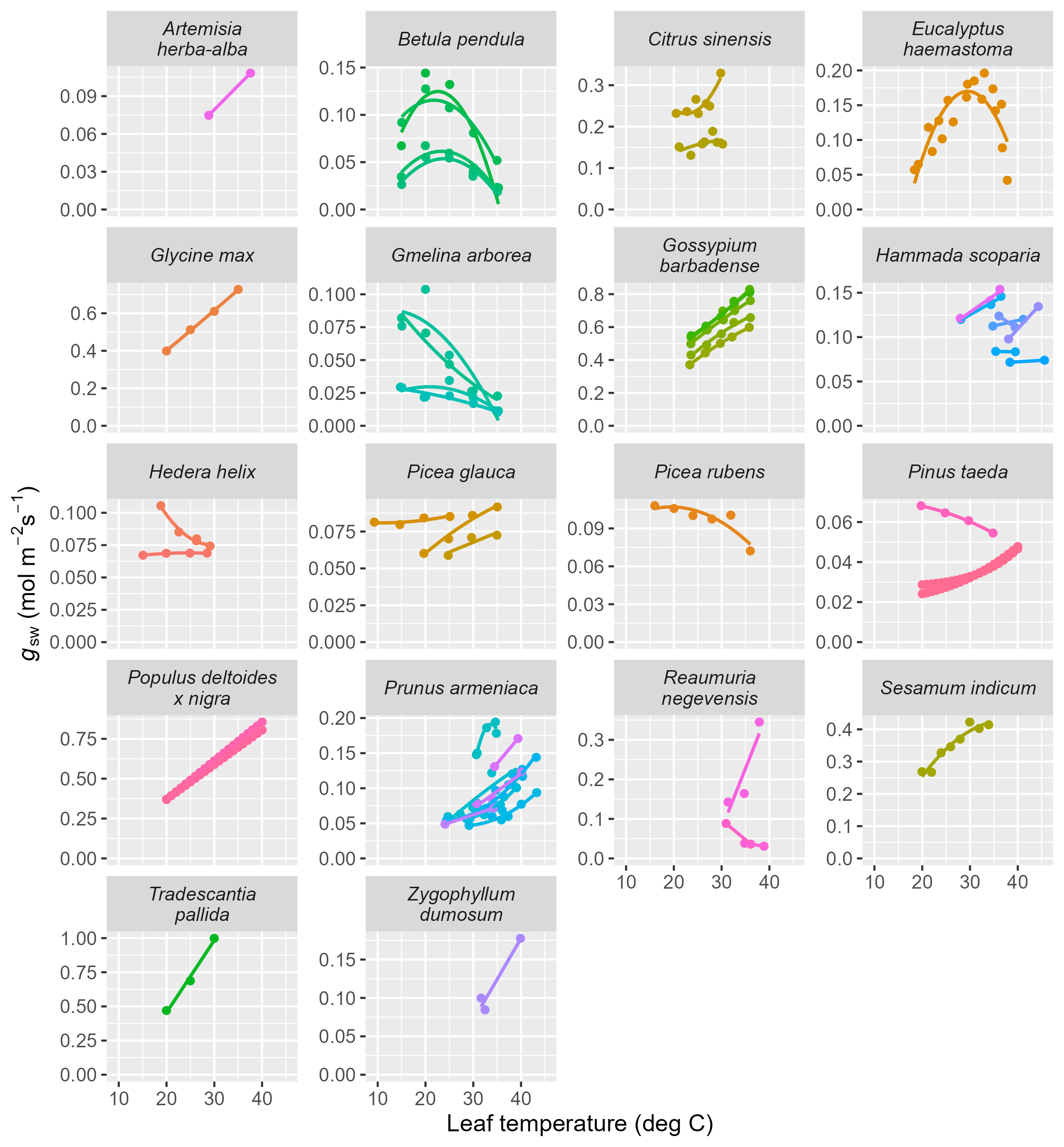
**Table 2**. Technical challenges in holding *w* constant while varying leaf temperature, as needed to quantify the DRST in intact leaves.

|  |  |
| --- | --- |
| **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 *w*, and will condense if it encounters a cooler environment. Condensation is fatal to gas exchange calculations and can seriously damage gas analyzers. | 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 vapor pressure and thus possibly preventing achievement of a given target *w*. | When preparing to shift to a higher temperature, pre-warm a spare humidifer 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 vapor pressure, which increases *w* and transpiration, cooling the leaf. That causes the heat exchanger to warm up, which further increases *w*. The humidity-control algorithm then increases incoming vapor 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.  Minimize leaf-to-air temperature difference, and thus the potential amplitude of temperature oscillations, by maximizing fan speed and using radiative temperature control. |

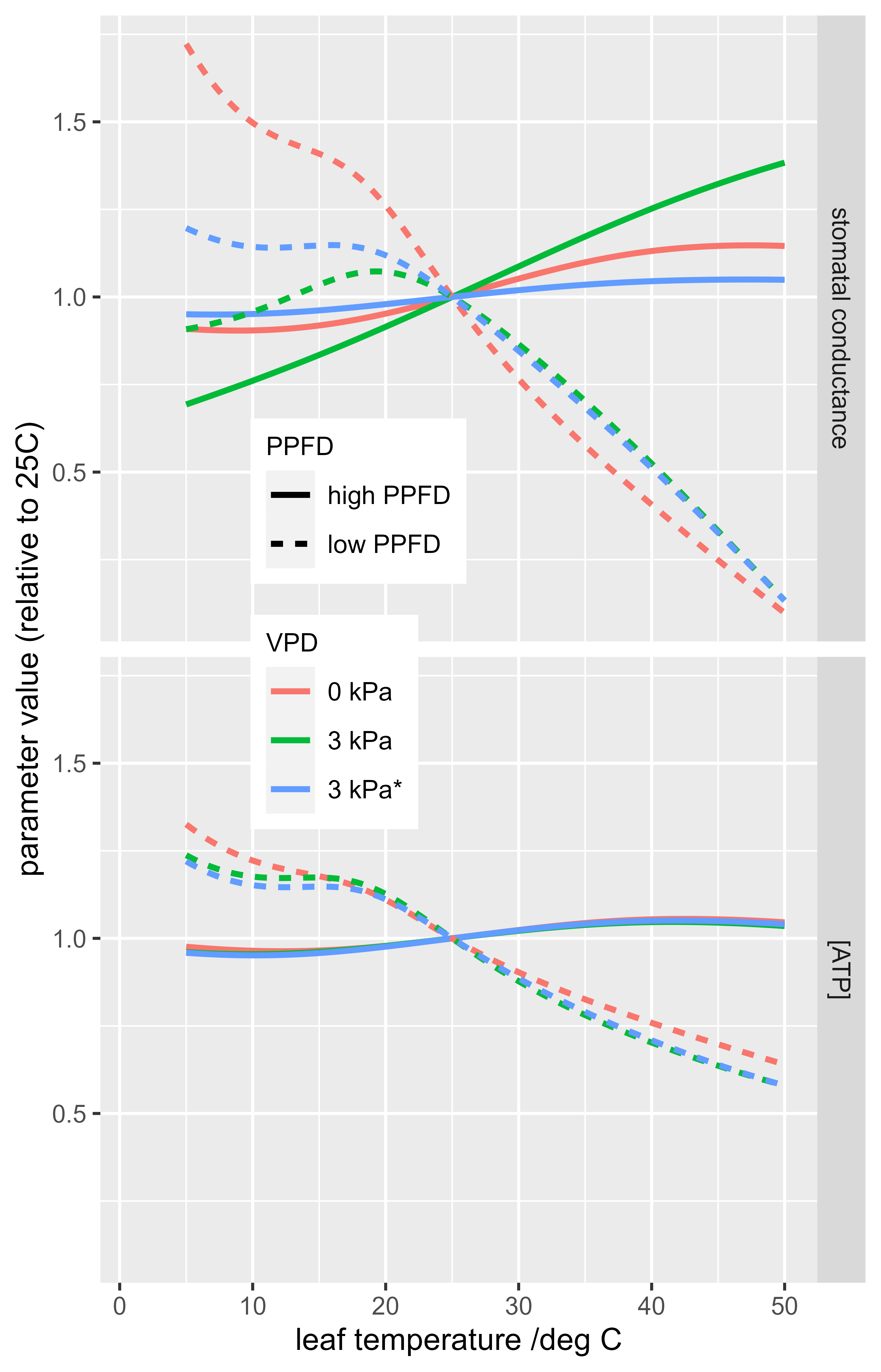
**Figures**



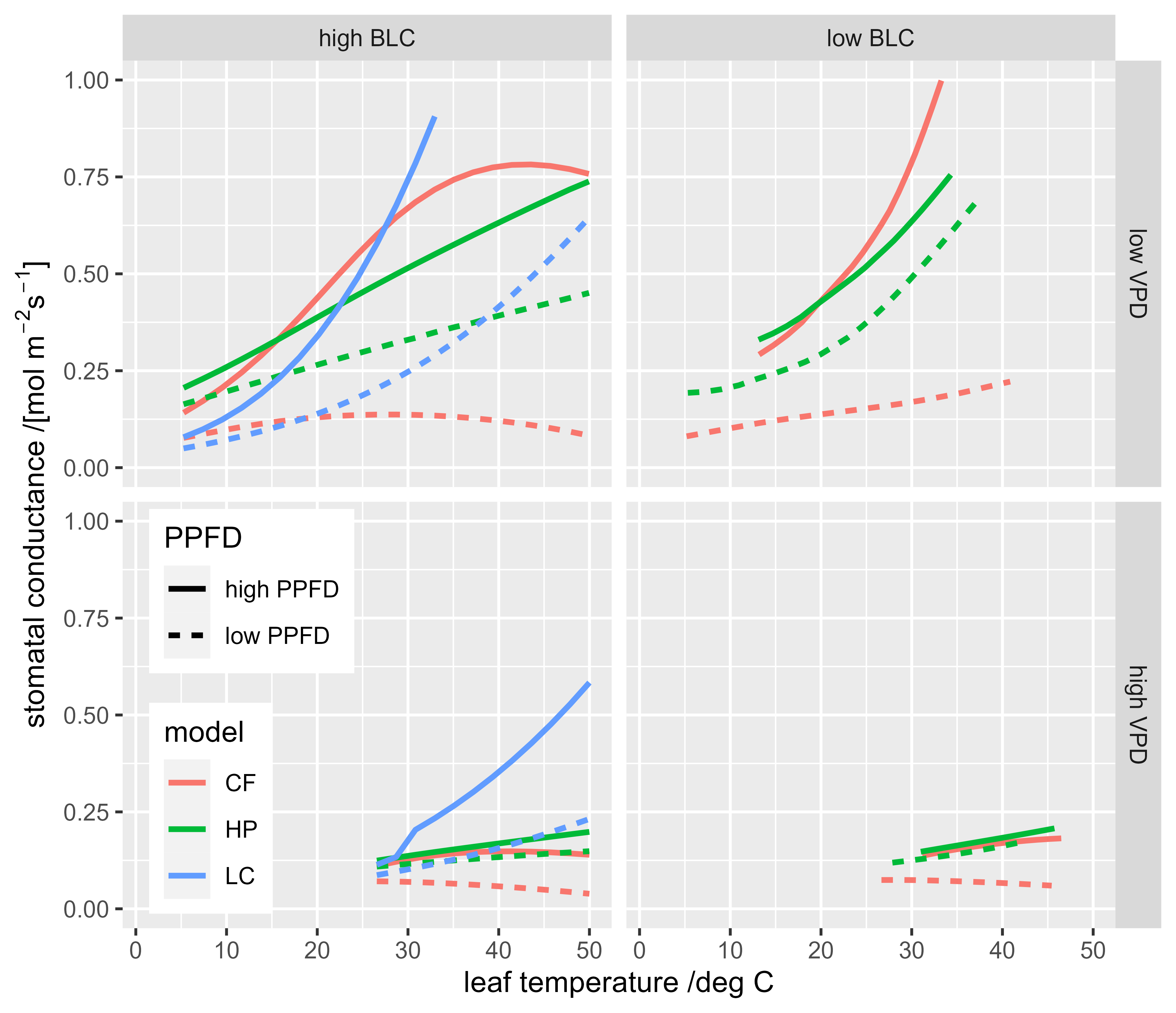
**Figure 1**. Diagram illustrating how a change in leaf temperature (a, red arrow) causes a change in the leaf-to-air water vapor mole fraction difference (*w*), due to an increase in the saturated water vapor mole fraction (solid black line and circles), and how this change in *w* can be prevented by imposing a simultaneous increase in the ambient water vapor mole fraction (b, blue arrow).



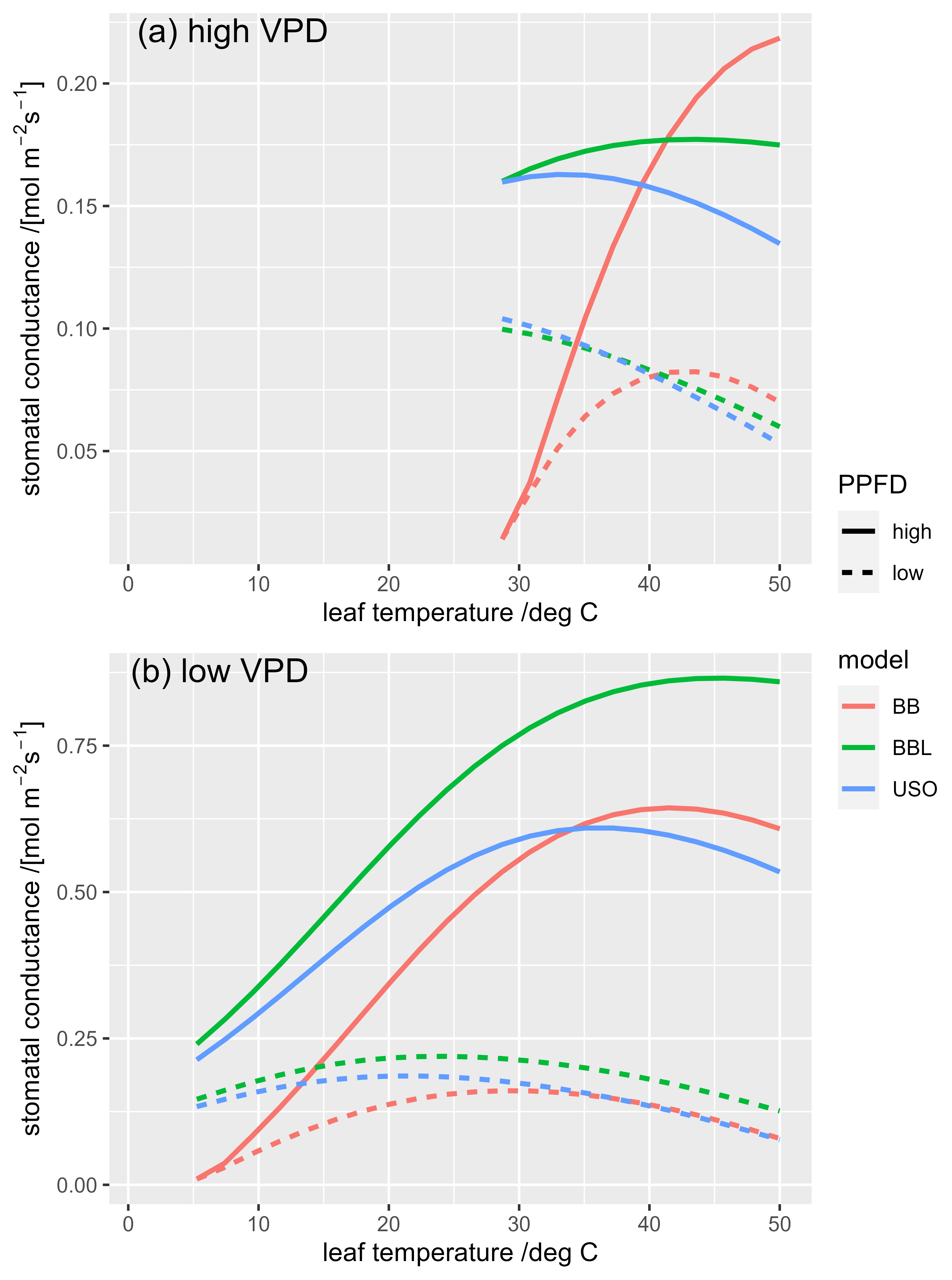
**Figure 2**. Published measurements of the direct response of stomatal conductance to leaf temperature in intact leaves. In each plot, separate lines represent responses measured on different leaves. Lines were fitted to responses with ≤ three points; 2nd-order polynomials were fitted to all other responses. References and additional information are given in Table 1. Note: values shown for *T. pallida* were given by the original authors as *g*sw relative to its value at 30oC, and are thus unitless.



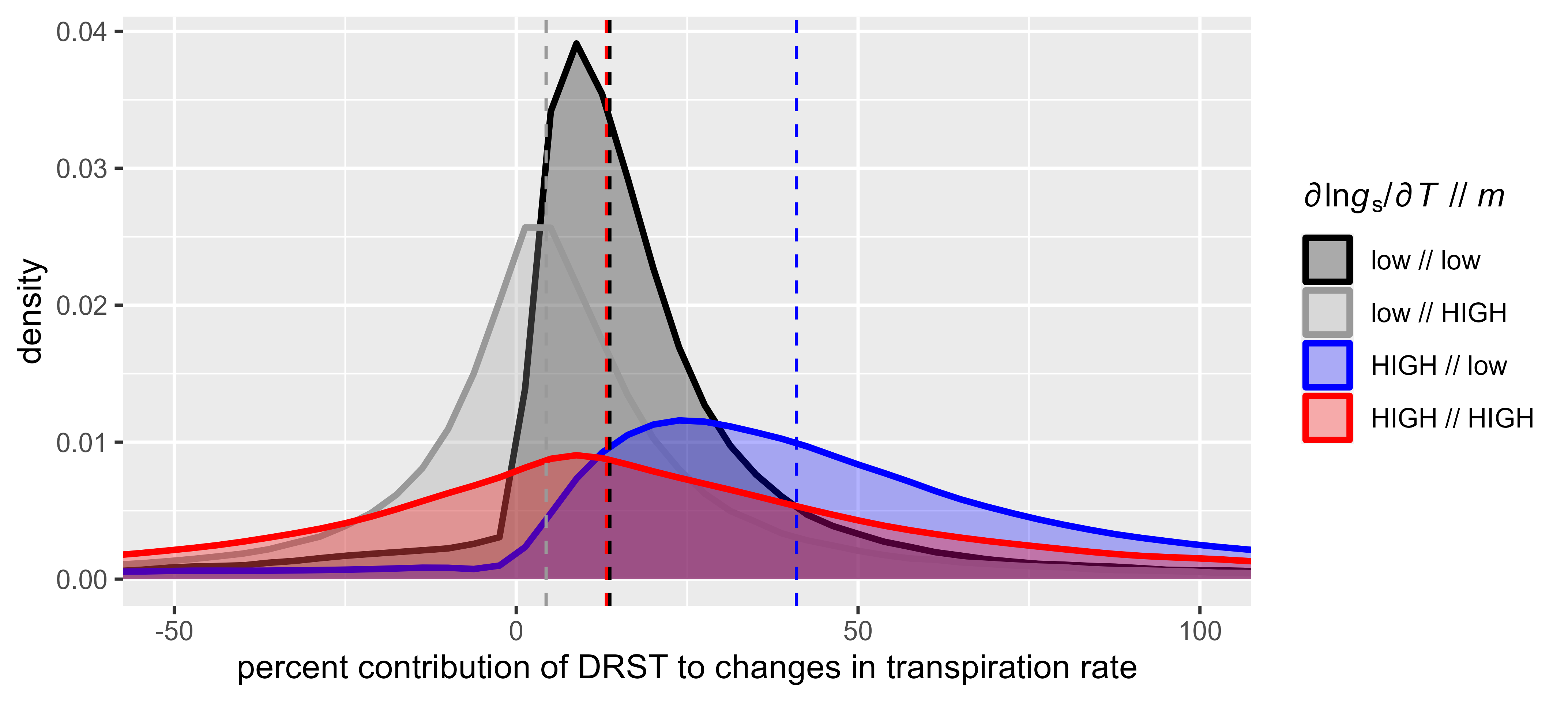
**Figure 3**. Predicted responses of (a) stomatal conductance, and (b) ATP concentration, from the process-based BMF model (Buckley *et al.* 2003). \*Blue lines show results at VPD = 3 kPa (*w* = 0.03 mol mol-1) in which the effect of temperature on viscosity, and hence on plant hydraulic conductance, was excluded from the model by fixing hydraulic conductance at its 25oC value.



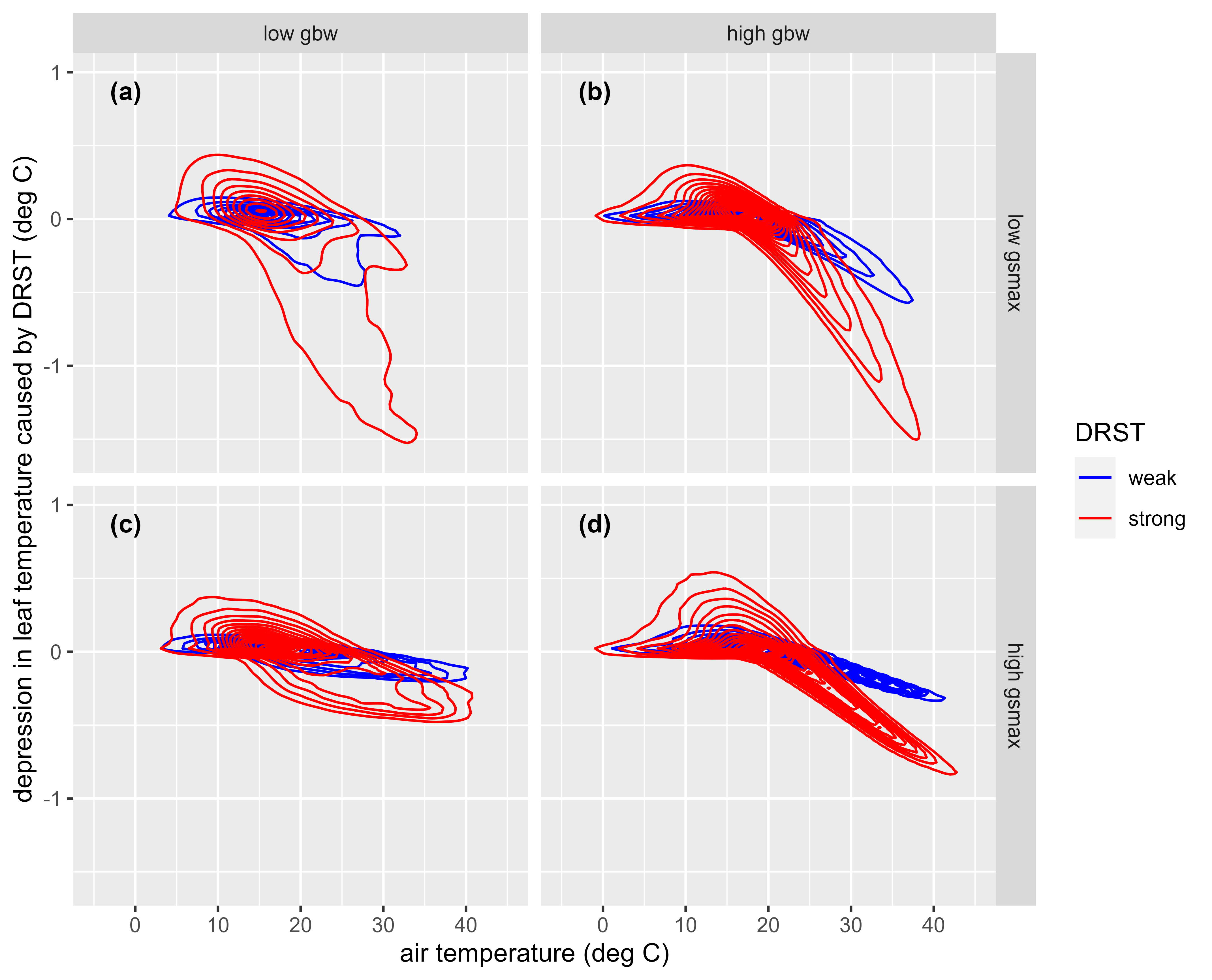
**Figure 4**. Direct responses of stomata to temperature (DRST) predicted by three optimization-based models (Cowan and Farquhar (1977), CF; Eller et al (2020), HP [hydraulic penalty]; Prentice et al. (2014), LC [least-cost]), for (a, c) high and (b, d) low boundary layer conductance (BLC: *g*bw = 2000 and 2 mol m-2 s-1, respectively), (c, d) high and (a, b) low VPD (3.4 kPa and 0.86 kPa respectively; *w* = 0.034 or 0.0086 mol mol-1, respectively)), and high and low PPFD (1786 and 179 mol m-2 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.



**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 Optimization (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 mol m-2 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 maximize their fit to BB model predictions for both days combined, given actual hourly meteorological conditions reported by the local CIMIS station.



**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 (*w*) covary over time in the environment. The derivative of transpiration rate with respect to either temperature or *w* has three components, one of which represents the DRST (Eqns S2-S5); 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 Methods S3). We calculated this percentage across a distribution of values for environmental variables (relative changes in *w* and *T*, and values of *w*), obtained using hourly diurnal data from 145 CIMIS stations in California for calendar year 2022; calculations assumed leaf and air temperatures were equal. Colors represent four scenarios: weak vs strong DRST (low vs high ∂ln*g*s/∂*T*) and weak vs strong stomatal response to *w* (low vs high *m*, *m* being the sensitivity parameter in the Oren et al (1999) model of stomatal responses to *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 *w* would *decrease* transpiration in the absence of the DRST, as might commonly occur, for example, if *T* increased at constant vapor pressure, driving stomatal closure.



**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 K-1, blue lines) or strong (0.03 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 mol m-2 s-1, respectively), and low (a,b) and high (c,d) values of stomatal conductance under saturating light, at reference *w* = 0.01 mol mol-1, and reference *T* = 18.8oC (the median in the meteorological dataset) (*g*s = 0.1 or 0.5 mol m-2 s-1, respectively). Additional details are provided in Methods S4.