Plant, Cell & Environment

WILEY

Plant, Cell & Environment

ORIGINAL ARTICLE

The Stomatal Response to Temperature Is Enhanced by High Evaporative Demand, Consistent With a Partially Hydraulic Mechanism

Colleen Mills[1] | Megan K. Bartlett[2] | Thomas N. Buckley[1]

[1]Department of Plant Sciences, University of California, Davis, California, USA | [2]Department of Viticulture and Enology, University of California, Davis, California, USA

Correspondence: Thomas N. Buckley (tnbuckley@ucdavis.edu)

Received: 21 September 2025 | **Revised:** 11 December 2025 | **Accepted:** 15 December 2025

Funding: National Science Foundation

Keywords: DRST | hydraulic conductance | stomata | temperature | VPD

ABSTRACT

The direct response of stomata to temperature (DRST, the response with the leaf-to-air vapor gradient, Δw, held constant) is poorly studied due to the difficulty of keeping Δw constant while changing leaf temperature. Most published data suggest a positive response, though the mechanisms behind such a response are unknown. We propose that a hydraulic mechanism should contribute to the DRST, wherein temperature decreases the viscosity of water, increasing hydraulic conductance and thereby increasing leaf water potential, which in turn drives stomatal opening. Because the sensitivity of leaf water potential to changes in hydraulic conductance should be proportional to transpiration rate and hence to Δw, this mechanism predicts a stronger positive DRST at higher Δw than at lower Δw. We tested this prediction by measuring the DRST at two different values of Δw, in six diverse angiosperm species. Our results are consistent with the hypothesis that a hydraulic mechanism contributes to the DRST, though the response varies widely across species, and in three of six species the effect of Δw was far stronger than predicted from theory, suggesting a role for other mechanisms in enhancing the effect of Δw on the DRST.

1 | Introduction

Stomata are the gateways between the leaf and the atmosphere, acting as valves to control the amount of water vapor transpired from the plant (Brodribb and McAdam 2011). These pores allow the entrance of CO_2 into the leaf for photosynthesis, but with the tradeoff that water vapor from the leaf interior diffuses out into the typically undersaturated atmosphere. Stomata are known to open in response to a number of environmental variables including light (Cowan 1977; Morison 1987) and low CO_2 (Messinger et al. 2006; Liang et al. 2023), and to close in response to other variables including high evaporative demand (Δw; the difference in water vapor mole fraction between the leaf airspaces and the atmosphere; equivalent to leaf-to-air vapor pressure difference [VPD_L] divided by atmospheric pressure) (López et al. 2021), which, assuming saturation of the leaf interior (Gaastra 1959), directly varies with both temperature and atmospheric water vapor mole fraction (w_a).

Stomata may also respond to temperature *per se*, even if Δw is held constant by adjusting ambient humidity. This "direct response of stomata to temperature" (DRST) is not well-studied, yet is integral to our understanding of stomatal behavior. The available data (Schulze et al. 1973; Schulze et al. 1974; Hall et al. 1975; Hall and Kaufmann 1975; Osonubi and Davies 1980; Teskey et al. 1986; Ball et al. 1987; Aphalo and Jarvis 1991; Lu and Zeiger 1994; Fredeen and Sage 1999; Day 2000; Eamus et al. 2008; Mott and Peak 2010; Urban et al. 2017; Diao et al. 2024) (reviewed by Mills et al. (2024)) suggest wide variation in the DRST, but with most species displaying a positive

https://doi.org/10.1111/pce.70347

response. Understanding the DRST is important because leaf temperature and w_a vary somewhat independently in nature, such that shifts in temperature are not always accompanied by commensurate shifts in Δw (e.g., Supporting Information Figure S1 in Mills et al. 2024). When a change in Δw is driven by a change in temperature, two distinct physiological responses may thus occur: a response to the effects of Δw on leaf water status via transpiration rate (Buckley 2005, 2019; McAdam and Brodribb 2016) and a distinct response to temperature *per se*. Most research on the physiology of stomatal responses to Δw has not attempted to disentangle the effects of Δw and temperature. An analysis by Mills et al. (2024) suggested that the DRST may contribute significantly to fluctuations in transpiration rate in nature.

There are several candidate mechanisms for the DRST (Mills et al. 2024). First, temperature-dependent changes in the binary diffusivity of water vapor in air and the molar volume of air should cause stomatal conductance to scale with the 0.81 power of absolute temperature (about 0.27% per degree C), independently of stomatal aperture. However, most available data suggest a much stronger response (averaging ~2% per degree C; Mills et al. 2024). Potential biological mechanisms include changes in photosynthetic biochemistry (Farquhar and Wong 1984; Buckley et al. 2003; Sibbernsen and Mott 2010; Busch 2014) or abscisic acid metabolism (Baron et al. 2012), or guard cell-endogenous processes (Rogers et al. 1979; Rogers et al. 1980; Ilan et al. 1995; Kostaki et al. 2020). Another likely mechanism is temperature-induced changes in plant hydraulic conductance. The viscosity of water has a strong temperature dependence – decreasing nearly 50% between 10°C and 40°C – which should cause xylem hydraulic conductance to increase by the same proportion. This increase in hydraulic conductance should increase leaf water potential (ψ_{leaf}), which in turn should cause stomatal opening, due to the well-established stomatal response to leaf water status (Buckley 2005, 2019; McAdam and Brodribb 2016). Temperature may also affect non-xylem components of plant hydraulic conductance by altering aquaporin function (Murai-Hatano et al. 2008b; Iglesias-Acosta et al. 2010; Ionenko et al. 2010; Zhao et al. 2019) and vapor transport proximal to stomata within leaves (Buckley et al. 2017).

Hydraulically-mediated effects of temperature on stomata should depend on the magnitude of Δw. For example, if $\Delta w = 0$, there is no transpiration and hence no steady-state water flow through the plant, in which case changes in hydraulic conductance should have no influence on leaf water potential, and nor, therefore, on stomatal conductance. This leads to a testable prediction: namely, the increase in water potential, and hence stomatal conductance, caused by a warming-induced increase in plant hydraulic conductance should be greater if Δw is large, than if Δw is small. Hence, the DRST should be more positive (or, if the response is negative, it should be less negative) at high Δw, compared to low Δw. To test this prediction, we measured the response of steady-state stomatal conductance to an increase in temperature of ~9°C in leaves of six angiosperm species, and repeated these experiments at two values of Δw (~13 and 22 mmol mol^{-1}). We compared our observations to theoretical predictions based on the known effect of temperature on viscosity and on literature reports for effects of temperature on other components of plant hydraulic conductance, in the context of a generic process-based model of stomatal responses to Δw.

2 | Materials and Methods

2.1 | Plant Material

Individuals of six species (*Vitis vinifera* cv. Pinot noir, *V. riparia, Hordeum vulgare, Zea mays, Eucalyptus polyanthemos, and Phaseolus vulgaris*) were grown in a greenhouse in Davis, CA during the spring and summer of 2024 and spring 2025. These species were selected for their diversity in habit and physiology (woody lianas, both cultivated [*V. vinifera*] and wild [*V. riparia*]; tree [*Eucalyptus*]; C3 forb [*Phaseolus*]; C3 grass [*Hordeum*]; C4 grass [*Zea*]). *H. vulgare*, *Z. mays*, and *P. vulgaris* were grown from seed in 7.6 L pots of soil (UC Agronomy mix), and fertigated with UNI-Old solution, a modified version of a Hoagland Solution (150:50:200 ppm N:P:K).

Vitis were grown from cuttings taken during June 2023 from the UC Davis teaching vineyard (38.530971 N, 121.754604 W) and the Hopkins vineyard (38.528811 N, 121.798641 W), rooted in vermiculite under a misting bench, and then planted in soil. The grapevines were moved to a greenhouse for the summer and fall and then to a lathhouse to allow leaf drop and dormancy over the winter. The following spring, they were repotted in 7.6 L pots and placed in a greenhouse with drip fertigation using the same solution as above.

Potted plants were brought into the lab the evening before measurements to allow the plants to acclimate to the lab environment. They were placed in trays with deionized water to keep the soil moist, and the laboratory lights were turned off overnight. *E. polyanthemos* measurements were performed on branches with juvenile foliage, excised from a single tree in the UC Davis Arboretum the evening before experiments and recut underwater in the lab. Each morning, plants were allowed to acclimate to light in the lab (PPFD ≈ 800–1600 μmol m^{-2} s^{-1}) for 1 to 2 h to facilitate photosynthetic induction and stomatal opening, before enclosing a leaf in a gas exchange cuvette as described below.

2.2 | Responses to Temperature and Δw

We measured steady-state stomatal conductance with a LI-COR LI-6800 (LI-COR, Lincoln, NE) in one leaf of each of 3–6 plants per species, at each of four conditions per leaf: low and high Δw (approx 13 and 22 mmol mol^{-1}) × low and high leaf temperature (approx 23°C and 32°C). These values were selected to maximize the Δw and T_{leaf} ranges that could be safely achieved without risking condensation, and without needing to use either excessively low or high chamber flow rates that would compromise the precision of gas exchange calculations. The order of conditions was randomized each day using a random number generator (RANDOM.ORG). Ambient CO_2 was kept at 421 ± 1 μmol mol^{-1} and flow rate was adjusted as needed to maintain the required Δw (typically 300–900 μmol s^{-1}). PPFD was measured using a GaAsp photodiode (Hamamatsu G1118, Hamamatsu, Tokyo, Japan) calibrated to the LI-6800s quantum sensor. PPFD at leaf level was kept constant throughout the experiment at ~671–1151 μmol m^{-2} s^{-1}, in most cases between 915 and 1151 μmol m^{-2} s^{-1}. After adjusting environmental conditions in the leaf cuvette at each point, stomata were allowed to acclimate until stomatal conductance was stable (defined as less than 5% change over 16 min; or if g_s was oscillating, less than 5% change in the mean of the oscillation). Plant pots were kept in a tray of deionized water throughout the experiment to minimize soil drying, except for excised branches of *E. polyanthemos*, for which the branch base was kept in water.

To improve leaf temperature control, we used custom leaf cuvettes in which water from a recirculating temperature-controlled water bath (Fisher Scientific Isotemp, Fisher Scientific, Hampton, NH, USA) circulated through channels in the aluminum cuvette body and chamber air was circulated with blowers to improve heat exchange and increase leaf boundary layer conductance. The cuvette interior was nickel plated to minimize water vapor adsorption. The upper leaf surface was illuminated with an LED light source (FECiDA Zinqolay) through a propafilm or glass window. The leaf cuvettes are further described in Supporting Information: Methods S1.

To prevent water vapor condensation in tubing downstream of the leaf cuvette, we controlled the temperature of the entire gas exchange system and plant by enclosing them in a larger 'outer chamber' (~1.04 × 0.55 × 1.04 m) constructed of polycarbonate twinwall and acrylic panels and containing a large circulation fan (JETFAN 6 inline fan, JETFAN, Hydrofarm, Shoemakersville, PA, USA), an air conditioner and space heater connected to a thermostat, and a baffle to ensure full circulation of air. This outer chamber is described further in Supporting Information: Methods S1 and a picture of both chambers is shown in Supporting Information: Figure S1. The thermostat was set to keep the temperature in the outer chamber close to that of the leaf cuvette; in most cases, the outer chamber temperature was within 2°C of the leaf temperature.

2.3 | Theoretical Predictions of the Effect of Δw on Hydraulically-Mediated Stomatal Responses to Temperature

The rationale for these experiments was that temperature-induced changes in plant hydraulic conductance (K) should lead to larger changes in leaf water potential (ψ_{leaf}) – and thus stronger stomatal responses – if Δw is large, because the sensitivity of water potential to K should scale with Δw. To see this, note that when transpiration rate (E) is in steady state with water flow through the plant, it follows that $E = K(\psi_{\text{soil}} - \psi_{\text{leaf}})$, where ψ_{soil} is soil water potential, and hence $\psi_{\text{leaf}} = \psi_{\text{soil}} - E/K$, so that the sensitivity of ψ_{leaf} to K is proportional to E, and therefore to Δw. In this section, we derive formal predictions of the strength of the resulting modulation of the stomatal response to temperature by Δw, with the aid of a process-based model of stomatal responses.

The relative sensitivity of stomatal conductance (g_s) to temperature (T), or $d\ln g_s/dT = (1/g_s)\cdot dg_s/dT$, is

$$\frac{d\ln g_s}{dT} = \frac{\partial \ln g_s}{\partial T} + \frac{\partial \ln g_s}{\partial \ln K}\cdot\frac{d\ln K}{dT}. \tag{1}$$

(The logarithm expresses stomatal sensitivity to T in relative rather than absolute terms, which allows comparison across leaves and species with widely varying absolute magnitudes of stomatal conductance.) The first term on the right hand side of Equation 1 ($\partial \ln g_s/\partial T$) encompasses all *non-hydraulic* contributions to the DRST, while the second accounts for the effect of temperature-dependent changes in K with T. The rationale for positing that g_s is sensitive to K arises from the well-established fact that angiosperm stomata regulate their apertures in response to some measure of leaf water status (Buckley 2005, 2019; McAdam and Brodribb 2016), which in turn is influenced by K. That response is well-described by the model of Buckley et al. (2003), and by adaptations thereof (Buckley et al. 2012; Rodriguez-Dominguez et al. 2016), which have the form

$$g_s = \frac{g_{smax}}{1 + b\frac{\Delta w}{K}}, \tag{2}$$

where g_{smax} is the value of g_s in the limit of zero Δw, and b is an empirical coefficient determined mainly by light intensity, all else being equal. Differentiating this model with respect to K to obtain an expression for $d\ln g_s/d\ln K$ to apply to Equation 1 gives

$$\frac{\partial g_s}{\partial K} = \frac{-\left(\frac{-b\Delta w}{K^2}\right)g_{smax}}{\left(1 + b\frac{\Delta w}{K}\right)^2} = \frac{g_s}{K}\cdot\frac{\Delta w}{K/b + \Delta w}, \tag{3}$$

so $\partial \ln g_s/\partial \ln K$ is $\Delta w/(K/b + \Delta w)$. Applied to Equation 1, this gives

$$\frac{d\ln g_s}{dT} = \frac{\partial \ln g_s}{\partial T} + \left(\frac{\Delta w}{K/b + \Delta w}\right)\cdot\frac{d\ln K}{dT}. \tag{4}$$

Assuming that the non-hydraulic component of the DRST ($\partial \ln g_s/\partial T$) is also not influenced by Δw, the difference in the strength of the DRST between experiments at two values of Δw (Δw_{low} and Δw_{high}) is then

$$\begin{aligned}&\frac{d\ln g_s}{dT}(\Delta w_{high}) - \frac{d\ln g_s}{dT}(\Delta w_{low})\\ &= \left(\frac{\Delta w_{high}}{K/b + \Delta w_{high}} - \frac{\Delta w_{low}}{K/b + \Delta w_{low}}\right)\cdot\frac{d\ln K}{dT}.\end{aligned} \tag{5}$$

Since the quantity in parentheses is positive, the response should be stronger at high Δw than at low Δw, and the size of this effect will depend on the relative magnitudes of Δw and K/b; namely, a small value of K/b represents a high stomatal sensitivity, making the quantity in parentheses larger. As shown in Figure 1, $d\ln g_s/dT$ is largest when stomatal sensitivity and $d\ln K/dT$ are large; moreover, it increases as Δw becomes larger, so that the difference in $d\ln g_s/dT$ between high and low Δw (the quantity given by Equation 5) is positive. The vertical distance between the red symbols in Figure 1 represents the prediction from Equation 5, assuming medium stomatal sensitivity and medium sensitivity of K to T.

We used bootstrap resampling of literature-based estimates of K/b and $d\ln K/dT$ to generate distributions of theoretical predictions. Oren et al. (1999) surveyed the sensitivity of g_s to Δw across species, and Buckley et al. (2012) later showed that K/b could be calculated from parameters in Oren's empirical model for g_s. We thus computed K/b from data in Table 1 in Oren et al. (1999). The resulting distribution of K/b is shown in Figure 2a. For $d\ln K/dT$, the sensitivity of K to T is often reported in the literature as Q_{10}, which is the number that, if raised to the power of $\delta T/10$ (a difference in temperature divided by 10°C), gives the proportional increase in K due to an increase in

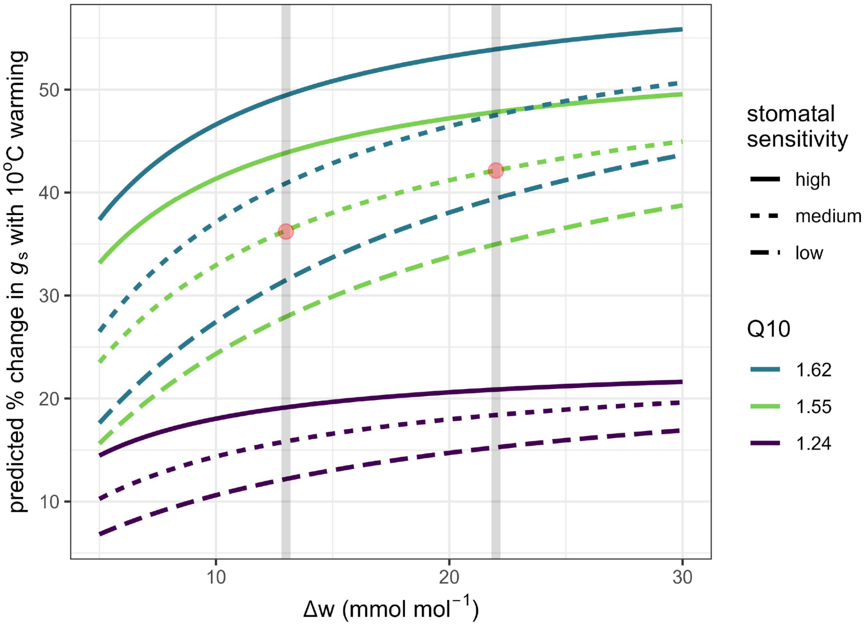

FIGURE 1 | Predictions from a process-based model of stomatal responses to water status, for the stomatal response to temperature at a given evaporative demand (Δw). "Sensitivity" refers to the stomatal sensitivity to Δw, gauged by the parameter "K/b" (low, medium and high sensitivity: $K/b = 12.6$, 6.7 and 3.3 mmol mol^{-1}, respectively); Q_{10} is the assumed temperature sensitivity of whole-plant hydraulic conductance (K), expressed as the natural logarithm of the ratio of K at 30°C to its value at 20°C. The ranges for K/b and Q_{10} are based on literature data: the K/b values are the 90th, 50th and 10th percentiles, respectively, calculated from data of Oren et al. (1999), and the Q_{10} values are the value for stems (1.24 [purple lines], representing the effect of temperature on viscosity) or the means of the distributions of published values for leaves (1.55 [green lines]) and roots (1.62 [blue lines]) listed in Table 1 and shown in Figure 2. The vertical shaded lines represent the Δw values used in this study (~13 [low] and 22 [high] mmol mol^{-1}); the red symbols are values for medium sensitivity and $Q_{10} = 1.55$ at those Δw values. Our hypothesis is that the response should be stronger at high Δw than that at low Δw, as illustrated here by the red point at high Δw being above that at low Δw. [Color figure can be viewed at wileyonlinelibrary.com]

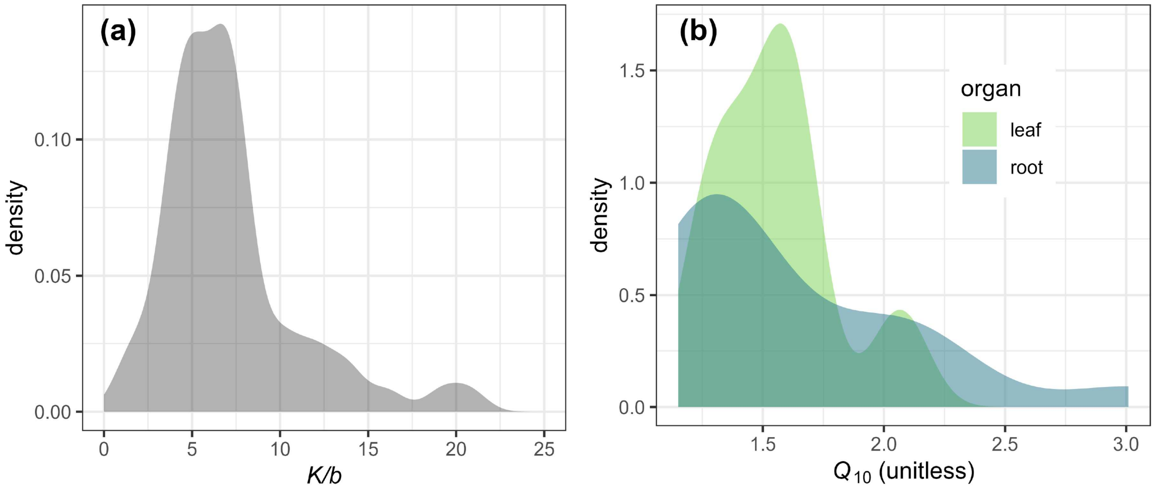

FIGURE 2 | Distributions of (a) the parameter describing stomatal sensitivity to Δw (K/b), and (b) Q_{10} values for leaf (green) and root (blue) hydraulic conductances found in the literature. [Color figure can be viewed at wileyonlinelibrary.com]

temperature of δT; i.e., $K(T_{\mathrm{low}} + \delta T)/K(T_{\mathrm{low}}) = Q_{10}^{\delta T/10}$. For example, if $Q_{10} = 1.5$, then 10°C of warming would cause K to increase by a factor of 1.5, or 50%. The Q_{10} for stem water transport is thought to be ~1.24, which corresponds to the effect of temperature on the viscosity of water for the temperature ranges used in this experiment. We extracted values for Q_{10} for root and leaf hydraulic conductance from the literature. In many cases, Q_{10} was given, or data were available from which we could calculate Q_{10}, for several different temperature steps or ranges; where possible, we chose the step or steps that most closely coincided with the temperature step used in our experiment (roughly 23°C–32°C). The resulting values of Q_{10} are listed in Table 1, and the corresponding distributions are shown in Figure 2b.

We generated predictions separately using distributions of Q_{10} for each organ (roots, leaves, and stems). In each case, we randomly sampled, with replacement, 10,000 pairs of values of

TABLE 1 | Values of the temperature response coefficient (Q_{10}) for leaf and root hydraulic conductances extracted from the literature and used in this study to constrain theoretical predictions. "T range" refers to the temperature range or shift (°C) for which Q_{10} was measured or calculated, or the range of such shifts over which we averaged Q_{10}.

Organ	Species	*T* range	Q_{10}	Ref
Leaf	*Oryza sativa*[17]	23–32	1.65	1
Leaf	*Phaseolus vulgaris*[18]	20–35	1.64	2
Leaf	*Juglans regia*	15–35	1.51	3
Leaf	*Panicum antidotale*	20–35	1.30	4
Leaf	*Panicum bisulcatum*	20–35	1.25	4
Leaf	*Tilia cordata*	20–35	2.07	5
Leaf	*Quercus rubra*	15–25	1.59	6
Leaf	*Acer saccharum*	15–25	1.41	6
Root	*Oryza sativa*[19]	> 15	1.45	7
Root	*Secale cereale*	10–20	1.8	8
Root	*Hordeum vulgare*	10–20	2.15	8
Root	*Phaseolus vulgaris*	> 18	1.32	9
Root	*Cucumis sativus*	23–32	1.36	10
Root	*Cucumis ficifolia*	23–32	3.01	10
Root	*Quercus robur*	22–32	1.26	11
Root	*Populus tremuloides*	15–20	1.94	12
Root	*Pinus halapensis*	20–30	1.25	13
Root	*Pinus pinea*	20–30	1.15	13
Root	*Pinus pinaster*	20–30	1.18	13
Root	*Pinus nigra*	20–30	1.34	13
Root	*Pinus uncinata*	20–30	1.25	13
Root	*Pinus sylvestris*	20–30	1.46	13
Root	*Gossypium hirsutum*	20–30	2.24	14
Root	*Ulmus americana*	15–20	1.25	15
Root	*Pinus* spp.[20]	18–23	2.08	16

[1] Yang et al. (2020).
[2] Matzner and Comstock (2001).
[3] Cochard et al. (2007).
[4] Sonawane et al. (2021).
[5] Sellin and Kupper (2007).
[6] Sack et al. (2004).
[7] Murai-Hatano et al. (2008a).
[8] Clarkson (1976).
[9] Kuiper (1964).
[10] Yoshida and Eguchi (1990).
[11] Cochard et al. (2000).
[12] Wan et al. (2001).
[13] Zuccarini et al. (2015).
[14] Bolger et al. (1992).
[15] Muhsin and Zwiazek (2002).
[16] Sayer et al. (2005).
[17] cultivar Shanyou 63.
[18] average of cultivars G4523 and Othello.
[19] cv. Akitakomachi.
[20] Average of *Pinus achinata, P. palustris* and *P. taeda*.

K/b and Q_{10}, from their respective distributions (in the case of stems, we simply set Q_{10} to 1.24, but still sampled K/b). We then applied the resulting values to Equation 5 using the mean experimental values of Δw_{high} and Δw_{low} for each species. This generated 18 distributions of theoretical predictions: three per species (for root-, leaf-, and stem-Q_{10}-based estimates of $d\ln K/dT$) × six species.

2.4 | Data Analysis

We calculated the average stomatal conductance, leaf temperature, and Δw during the last 16 min at each condition, when stomatal conductance had achieved steady state. To compare our results to the theoretical predictions from Equation 5, for each Δw value, we calculated the relative change in stomatal conductance with respect to temperature by dividing the difference in g_s between T_{high} and T_{low} by the value of g_s at T_{low}, and then dividing that quantity by the difference in T ($d\ln g_s/dT \approx \delta \ln g_s/\delta T \approx \{[g_s(T_{\text{high}}) - g_s(T_{\text{low}})]/[g_s(T_{\text{low}})]\}/\{T_{\text{high}} - T_{\text{low}}\}$). Results are presented below as means ± standard errors (SE) of this quantity within each species. To test whether $\delta \ln g_s/\delta T$ differed significantly between low and high Δw and between species, we used analysis of variance in linear models, using method *lm* in R. To test whether $\delta \ln g_s/\delta T$ differed significantly between low and high Δw overall (i.e., without concern for differences across species), we used a mixed-model analysis treating species as a random effect, using method *lmer* in R.

3 | Results

Stomatal conductance responded positively in most leaves to an increase in temperature from 23 ± 0.2°C to 32.2 ± 0.3°C (Figure 3). The overall average response was equivalent to an increase in g_s of 50.0 ± 8.3% for 10°C of warming; that is, the average Q_{10} for stomatal conductance was 1.50. We found the strongest positive responses to temperature in *P. vulgaris* at high Δw (126 ± 31% per 10°C, or $Q_{10} = 2.26$; Figure 3c), and the smallest in *V. riparia* at low Δw (12 ± 24% per 10°C, or $Q_{10} = 1.12$; Figure 3d).

Consistent with our hypothesis, the average stomatal response to temperature was stronger when measured at high Δw than when measured at low Δw, in all species (cf. red and blue lines in Figure 3); that is, $d\ln g_s/dT$ was larger at high Δw than it was at low Δw, as predicted by Equation 5. The effect of Δw on temperature responses was strongest in *P. vulgaris* and weakest in *V. riparia* (Figure 3). However, although the mean difference in $d\ln g_s/dT$ between high and low Δw was greater than zero in all species, these differences were not statistically significant; this result held whether species was included as a fixed effect in the statistical model (p value = 0.17508 for the effect of Δw on $d\ln g_s/dT$), or if instead species was treated as a random effect in a mixed-model analysis ($p = 0.17515$).

For the three woody species (*V. vinifera, V. riparia* and *E. polyanthemos*), the observed mean effect of Δw on in the % response of g_s to T was within the distributions of theoretical predictions based on published Q_{10} values for root and leaf hydraulic conductance (blue and green distributions, respectively, in Figure 4), but was much greater than predictions based on stem Q_{10} (purple distributions in Figure 4). For the three herbaceous species (*H. vulgare, Z. mays* and *P. vulgaris*), the mean observations exceeded the 95th percentiles of distributions of theoretical predictions, regardless of whether root-, leaf-, or stem-based Q_{10} values were used to generate those predictions.

On average, intercellular CO_2 concentration (c_i) increased in the steady-state following leaf warming (Figure 5), and this increase was substantially greater in experiments performed at high Δw (17.6 ± 6.8 μmol mol^{-1}) than at low Δw (5.9 ± 7.0 μmol mol^{-1}).

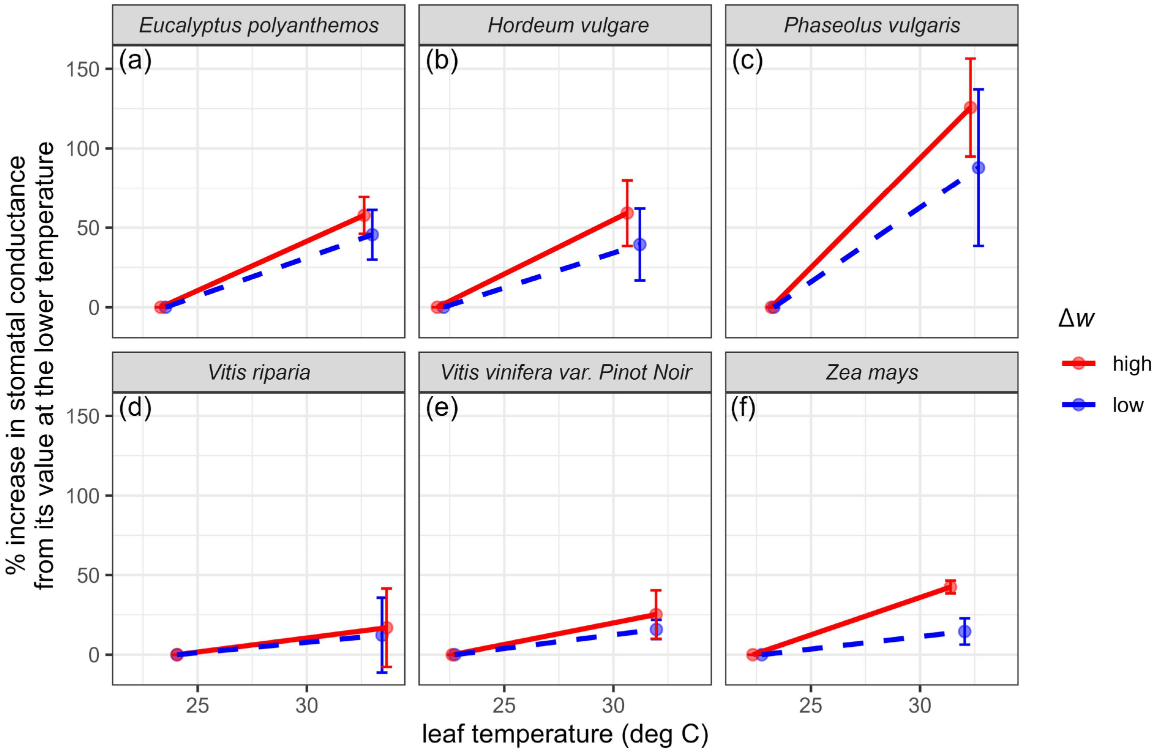

FIGURE 3 | Percent change in stomatal conductance (g_s) with increasing temperature across species (a–f). Symbols and error bars are means ± SE for the increase in g_s from low to high temperature in each species; lines simply connect the points for each Δw value (low $\Delta w \approx 13\,\text{mmol mol}^{-1}$ and high $\Delta w \approx 22\,\text{mmol mol}^{-1}$). [Color figure can be viewed at wileyonlinelibrary.com]

4 | Discussion

A meager but growing body of data suggest that stomata respond to temperature even when the leaf to air evaporative gradient (Δw) is held constant (Mills et al. 2024), and that in most cases the response is positive. However, the mechanism for this response is unknown. Based on a hypothesized hydraulic mechanism, we predicted that the stomatal response to temperature would be larger if Δw were held constant at a high value as temperature was varied, than if Δw were held constant at a low value. Our observations supported this prediction, qualitatively: in all six species examined, the average % change in stomatal conductance in response to a roughly 10°C increase in temperature was greater (more positive) at high Δw (~22 mmol mol^{-1}) than at lower Δw (~13 mmol mol^{-1}). For three of the six species that we examined, the mean observed magnitude of this effect exceeded the 95th percentile of our theoretical predictions, which we had generated by applying a process-based model of stomatal conductance to published data for the sensitivities of stomata to Δw, and of hydraulic conductance to temperature. Thus, our results support the hypothesis that temperature-induced changes in hydraulic conductance contribute to stomatal responses to temperature, but they also suggest that additional mechanisms likely enhance the effect of Δw on that response, at least in some species.

The rationale for our hypothesis was simple: warming should increase hydraulic conductance (due to the effect of temperature on viscosity, as well as biological effects such as enhancing aquaporin function), leading to higher leaf water potential, and stomata should respond positively to that increase in water potential. It is well established that stomata respond in the short term to changes in leaf water status by sensing rapid changes in abscisic acid concentration within leaves, and that those responses are similar whether water status is altered by shifts in evaporative demand, hydraulic conductance, or soil moisture (Buckley 2005, 2019; McAdam and Brodribb 2016; Sack et al. 2018). Thus, a straightforward and experimentally proven causal chain links temperature to stomatal conductance by way of hydraulic conductance. Moreover, the hydraulically mediated effect of temperature on stomata should depend on the magnitude of evaporative demand (Δw), because the sensitivity of leaf water potential to changes in hydraulic conductance is proportional to transpiration rate, and hence to Δw itself. For example, if Δw were zero, no steady-state water flow would occur through the plant, making leaf water potential and thus stomatal conductance independent of hydraulic conductance. Consistent with this notion, an earlier study by Mott and Peak (2010) found that the stomatal response to temperature disappeared in *Tradescantia pallida* when Δw was set to zero. However, the strength of this Δw effect should likewise depend on how sensitively stomatal conductance responds to water status. For example, if that response were very weak, then changes in hydraulic conductance or Δw would have little effect on stomata.

One might wonder why we did not directly test for changes in water potential (WP) with temperature, given that these changes are the putative driver of hydraulically mediated stomatal responses to temperature. There are several reasons. First, no current method can make repeated measurements of WP in a transpiring region of a leaf, so changes in leaf WP cannot be conclusively attributed to changes in temperature. In situ psychrometers prevent transpiration from the measured region, so the value they report is roughly in equilibrium with nearby

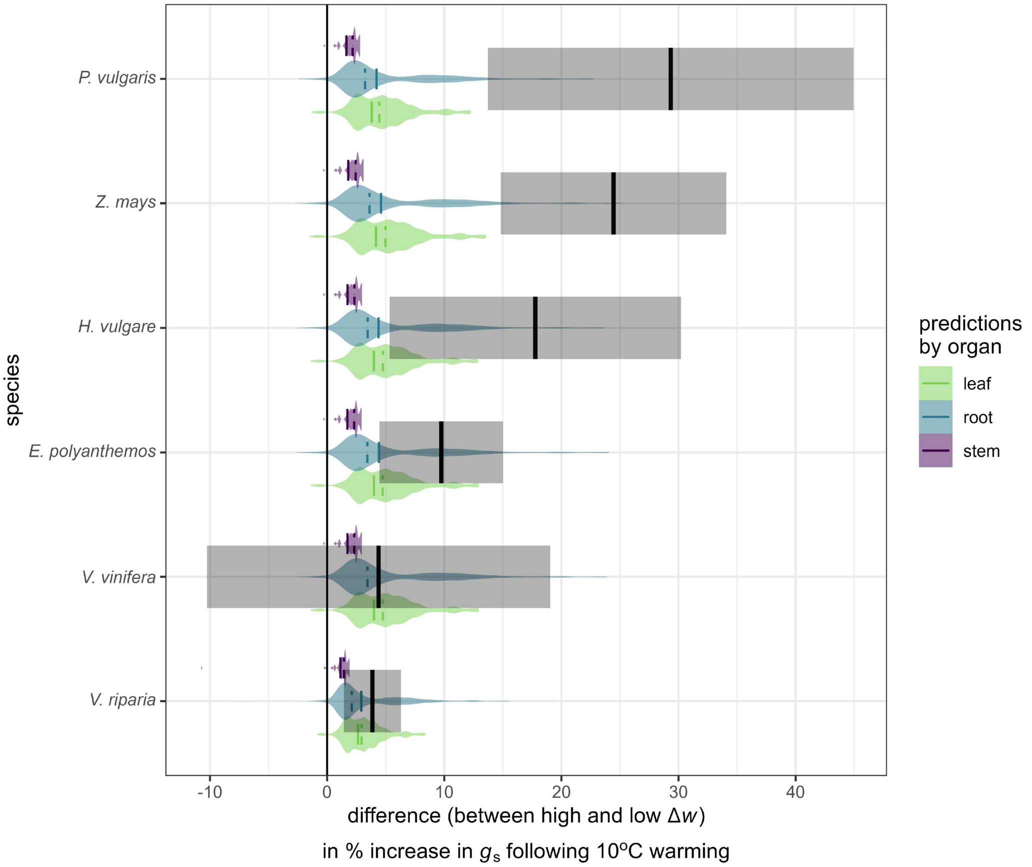

FIGURE 4 | Predictions (in color) and observations (in black and grey) for the percent difference in the relative stomatal response to temperature (% increase in stomatal conductance for a 10°C increase in temperature) between high and low Δ*w* (≈ 22 and 13 mmol mol^{-1}, respectively). Solid black lines are means of observations; bounds of shaded area = ± SE. Colored violin plots are distributions of theoretical predictions using bootstrap resampling ($n = 10{,}000$) of stomatal sensitivity to Δ*w* from data of Oren et al. (1999) and of Q_{10} for plant hydraulic conductance estimated based on published data for leaves (green) or roots (blue) (for stems [blue], Q_{10} was set to 1.24, based on the temperature response of viscosity). Solid and dashed colored lines are means and medians, respectively, of the distributions of theoretical predictions. [Color figure can be viewed at wileyonlinelibrary.com]

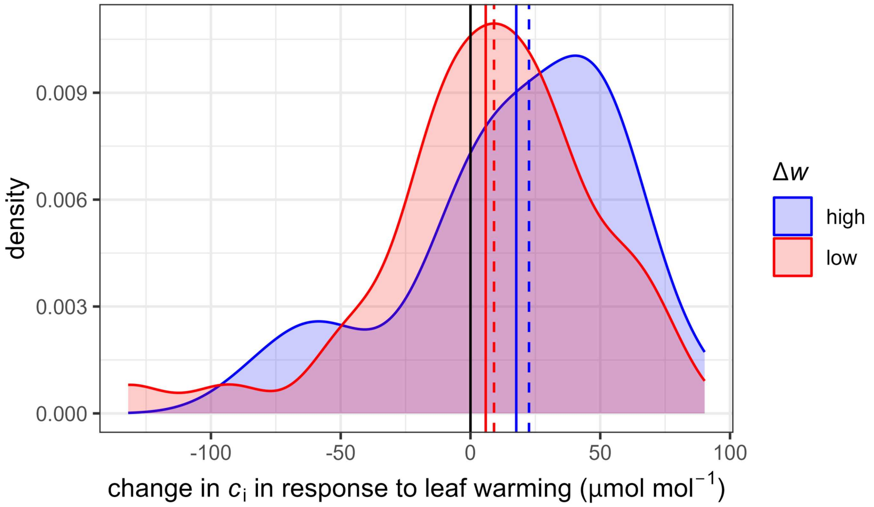

FIGURE 5 | Intercellular CO_2 concentration (c_i) increased in response to leaf warming of approximately 9°C, on average across species, and this increase was greater in experiments performed at high Δ*w* (blue shading and lines) than at low Δ*w* (red shading and lines). Solid vertical lines indicate mean values; dashed lines indicate medians. [Color figure can be viewed at wileyonlinelibrary.com]

xylem water, not the transpiring bulk leaf tissue. Optical dendrometers based on leaf width (e.g., Tonet et al. 2023) may not work reliably for a leaf enclosed in a gas exchange cuvette, which might mechanically constrain the leaf from expanding and contracting with WP. Second, changes in WP that drive stomatal responses may not manifest as measurable changes in bulk leaf WP at steady-state, both because the negative feedback response of stomata tends to mute any net change in WP at steady-state (Buckley 2005), and because the relevant change in WP could be highly localized, for example in guard or epidermal cells (Bauer et al. 2013; Kuromori et al. 2014; Buckley 2019; Scoffoni et al. 2023). Moreover, the decline in g_s in response to reduced WP is caused by production and/or release of abscisic acid (ABA) (Bauer et al. 2013; McAdam and Brodribb 2016; Sussmilch et al. 2017), but slow ABA catabolism can cause g_s to remain depressed for a time even after water status recovers (McAdam and Brodribb 2015); thus, there may not be a unique relationship between ABA levels (and hence g_s) and WP. Thus, measurements of bulk-leaf WP in relation to temperature could not conclusively test our hypothesis. That is precisely the dilemma that led us to examine the effect of Δw on the response as an alternative test.

To put reasonable constraints on the interacting influences of Δw and stomatal sensitivity on the experimental implications of our hypothesis, and to extend Mott and Peak's experiment to variation in Δw among nonzero values, we adapted a theoretical model of stomatal conductance (Buckley et al. 2003, 2012; Rodriguez-Dominguez et al. 2016). This model allows for a wide range of stomatal sensitivities of Δw and sensitivities of K to temperature. We constrained both parameters using data from the literature. The model predicted that the % increase in stomatal conductance in response to a 10°C increase in leaf temperature should be stronger when $\Delta w = 22\ \text{mmol mol}^{-1}$ than when $\Delta w = 13\ \text{mmol mol}^{-1}$ – by about 1.6%, 3.8%, or 4.2%, basing the Q_{10} for whole-plant hydraulic conductance on available data for stems, leaves, or roots, respectively.

In practice, we found mean enhancements ranging from 3.9% (*V. riparia*) to 29.3% (*P. vulgaris*) – exceeding the range of theoretical predictions in three of six species (*H. vulgare*, *Z. mays* and *P. vulgaris*), though the observed range of enhancement nonetheless overlapped with predictions. The finding of larger effects in herbaceous than in woody species is consistent with the fact that leaves and roots likely contribute a greater fraction of whole-plant resistance in herbs, given that Q_{10} is typically higher for roots and leaves than for stems. (On the other hand, thermal buffering by moist soil would likely have damped the temperature shifts actually experienced by the roots, making root Q_{10} less important in practice). However, our predictions accounted for those differences, and yet our observations nevertheless exceeded predictions for herbaceous species (Figure 4). The reasons for this divergence are not immediately obvious. One possibility is that our estimates of Q_{10} were too low, though this seems unlikely, given that the upper range of our theoretical estimates arose from root-based Q_{10} values, which have been measured for many species, including some of those used in this study. For example, for *Phaseolus vulgaris*, root and leaf Q_{10} have been reported in the literature as 1.32 and 1.64, respectively (Kuiper 1964; Matzner and Comstock 2001), and for *Hordeum vulgare*, Clarkson (1976) reported a root Q_{10} of 2.15. Those values are not atypical among published observations (Table 1). Notably, Kuiper (1964) did report $Q_{10} = 4$ for roots at temperatures below 18°C; we excluded that value from our distributions, because it applied well below the temperature ranges of our measurements. We also excluded other high values of Q_{10} that applied at lower temperatures (e.g., $Q_{10} = 3.8$ for 16°C–18°C in *Gossypium hirsutum* roots, Bolger et al. (1992); 2.9 for *Cucumis ficifolia* roots at 8°C–12°C, Yoshida and Eguchi (1990); 3.0 for *Juglans regia* leaves at 5°C–15°C, Cochard et al. (2007); 6.5 for *Tilia cordata* leaves at 16°C–17°C, Sellin and Kupper (2007)). These data show that Q_{10} can be large enough to produce as large an effect of Δw on the DRST as we observed in three species, though typically only at lower temperatures than we used here.

As discussed earlier, stomatal responses to WP may be driven by changes in water content of a small group of cells that are not representative of the whole leaf; if so, then Q_{10} values for whole-leaf hydraulic conductance (which are operationally based on bulk leaf WP measurements) would not be appropriate for our predictions. For example, if stomatal responses were primarily driven by WP changes in epidermal or guard cells, and if the resistance for water transport from the mesophyll to the epidermis were more temperature-sensitive than for the leaf as a whole, that could explain our results. Notably, simulations by Buckley et al. (2017) predicted that water transport from the mesophyll to the epidermis could be dominated by vapor transport, and the Q_{10} for vapor-phase hydraulic conductivity between 23°C and 32°C is about 1.79, which exceeds nearly all leaf-Q_{10} values we found in the literature (cf. Table 1). The possible role of vapor transport in stomatal function remains speculative (Buckley 2019).

Another possible explanation for the divergence between our predictions and observations is our assumption that the sensitivity of the non-hydraulic component of the DRST ($\partial \ln g_s/\partial T$ in Equation 5) would not be influenced by Δw itself. One way that this assumption could fail involves photosynthetic processes. It is well established that the stomatal responses to light and CO_2 are partly mediated by photosynthetic processes (Messinger et al. 2006; Mott 2009; Lawson et al. 2018), which in turn are affected by temperature, so temperature could influence g_s via changes in photosynthetic processes. The effect of photosynthesis on g_s is indirect: stomata do not "sense" the rate of photosynthesis itself (von Caemmerer et al. 2004), but rather, some measure of the balance between the supply of energy from the light reactions and its demand by the Calvin cycle (Dewar 2002; Messinger et al. 2006; Mott 2009; Busch 2014; Lawson et al. 2018). Stomata open when that balance shifts in favor of energy supply (e.g., when light intensity increases), and close when it shifts in favor of demand (e.g., when intercellular CO_2 concentration, c_i, increases). Suggested sensors linking photosynthetic energy balance to g_s include mesophyll ATP concentration (Farquhar and Wong 1984) and the redox state of plastoquinone (PQ) in the photosynthetic electron transport chain (Busch 2014). Light intensity was constant in our experiments, but c_i was not. Thus, if warming the leaf caused c_i to decrease more (or increase less) at high Δw than at low Δw, that would amplify the hydraulically-mediated enhancement of the DRST by high Δw, helping to explain our observations. However, we found the opposite: c_i increased in response to warming, on average, and that increase was greater at high Δw than at low Δw (Figure 5). This suggests photosynthetic processes cannot explain the divergence between our predictions and observations.

Yet another factor to consider is that we could not hold Δw constant for the rest of the plant (in the "outer chamber") when measuring the DRST for the leaf in the gas exchange cuvette. Although most of the rest of the plant was in relative darkness, it would nonetheless have been transpiring, due to residual stomatal opening and cuticular conductance. Warming would have increased Δw and hence transpiration in the outer chamber, reducing water potential for the leaf enclosed in the cuvette and potentially damping the DRST. The question, however, is whether that effect would differ between high and low cuvette-Δw; our analysis (Supporting Information: Methods S2) suggests that it would do so, but with negligible effect on our results.

4.1 | Implications of a Hydraulically-Mediated DRST

The evolutionary and ecological implications of the DRST – for example, enhancement of evaporative cooling to prevent heat damage (Schymanski et al. 2013; Huang et al. 2022; Moran et al. 2023) – may depend somewhat on its mechanism. Our novel evidence that the DRST is partly mediated by hydraulics suggests that the response may differ across species, in relation to differences in hydraulic function. For instance, theory predicts that a decline in hydraulic conductance – e.g., due to progressive drought – should make the DRST stronger (cf. Equation 4), so in species with high hydraulic vulnerability, the combination of warming and drought may exacerbate that vulnerability. We would tentatively predict this effect to be most important for leaf hydraulic conductance, because leaves likely experience stronger shifts in temperature than stems or roots, due to the high heat capacitance of stem and soil moisture. Conversely, species in which stomata are weakly sensitive or insensitive to leaf water status would have a weaker DRST. Validation of these predictions awaits better experimental knowledge about the phenomenology of the DRST, which remains poorly explored in comparison to most stomatal responses (Mills et al. 2024).

5 | Conclusion

The response of stomata to temperature with Δw held constant, and its potential mechanisms, have been long overlooked. We hypothesized that the positive effect of warming on plant hydraulic conductance should contribute to the stomatal response to temperature, and more specifically that this mechanism should be enhanced by high Δw, given the role of transpiration rate in mediating effects of hydraulic conductance on stomata via water potential. Our results were qualitatively consistent with the hypothesis, in that the relative sensitivity of stomatal conductance to temperature was greater at high Δw than at lower Δw in all species examined, though these differences were not statistically significant. Moreover, the magnitude of the difference greatly exceeded our theoretical predictions in two of six species. We recommend that future work should focus on quantifying the non-hydraulic component of the temperature response, as well as the temperature response of whole-plant hydraulic conductance, to better constrain the expected effect of temperature on stomata via hydraulic conductance.

Acknowledgements

Thanks to Avi Cannizzaro, Shaina Eagle, Rosie Gluck, Aaron McKee-Ota, and Eric Haddix for their assistance in growing and maintaining our plant collection for this experiment. This work was supported by the National Science Foundation (Awards #2036201 and 2307341) and the US Department of Agriculture National Institute of Food and Agriculture (Hatch award #CA-Dᴅ-PLS-2790-H to TNB). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s), and do not necessarily reflect the views of the National Science Foundation.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Aphalo, P. J., and P. G. Jarvis. 1991. "Do Stomata Respond to Relative Humidity?" *Plant, Cell & Environment* 14: 127–132.

Ball, J. T., I. E. Woodrow, and J. A. Berry. 1987. "A Model Predicting Stomatal Conductance and its Contribution to the Control of Photosynthesis Under Different Environmental Conditions." In *Progress in Photosynthesis Research*, edited by J. Biggens, 221–224. Martinus Nijhoff Publishers.

Baron, K. N., D. F. Schroeder, and C. Stasolla. 2012. "Transcriptional Response of Abscisic Acid (ABA) Metabolism and Transport to Cold and Heat Stress Applied at the Reproductive Stage of Development in Arabidopsis Thaliana." *Plant Science* 188–189: 48–59.

Bauer, H., P. Ache, S. Lautner, et al. 2013. "The Stomatal Response to Reduced Relative Humidity Requires Guard Cell-Autonomous ABA Synthesis." *Current Biology* 23: 53–57.

Bolger, T. P., D. R. Upchurch, and B. L. McMichael. 1992. "Temperature Effects on Cotton Root Hydraulic Conductance." *Environmental and Experimental Botany* 32: 49–54.

Brodribb, T. J., and S. A. M. McAdam. 2011. "Passive Origins of Stomatal Control in Vascular Plants." *Science* 331: 582–585.

Buckley, T. N. 2005. "The Control of Stomata by Water Balance." *New Phytologist* 168: 275–292.

Buckley, T. N. 2019. "How do Stomata Respond to Water Status?" *New Phytologist* 224: 21–36.

Buckley, T. N., G. P. John, C. Scoffoni, and L. Sack. 2017. "The Sites of Evaporation Within Leaves." *Plant Physiology* 173: 1763–1782.

Buckley, T. N., K. A. Mott, and G. D. Farquhar. 2003. "A Hydromechanical and Biochemical Model of Stomatal Conductance: Stomatal Model." *Plant, Cell and Environment* 26: 1767–1785.

Buckley, T. N., T. L. Turnbull, and M. A. Adams. 2012. "Simple Models for Stomatal Conductance Derived From a Process Model: Cross-Validation Against Sap Flux Data." *Plant, Cell and Environment* 35: 1647–1662.

Busch, F. A. 2014. "Opinion: The Red-Light Response of Stomatal Movement is Sensed by the Redox State of the Photosynthetic Electron Transport Chain." *Photosynthesis Research* 119: 131–140.

von Caemmerer, S., T. Lawson, K. Oxborough, N. R. Baker, T. J. Andrews, and C. A. Raines. 2004. "Stomatal Conductance Does Not Correlate With Photosynthetic Capacity in Transgenic Tobacco With Reduced Amounts of Rubisco." *Journal of Experimental Botany* 55: 1157–1166.

Clarkson, D. T. 1976. "The Influence of Temperature on the Exudation of Xylem Sap From Detached Root Systems of Rye (Secale Cereale) and Barley (Hordeum Vulgare)." *Planta* 132: 297–304.

Cochard, H., R. Martin, P. Gross, and M. Bogeat-Triboulot. 2000. "Temperature Effects on Hydraulic Conductance and Water Relations of Quercus robur L." *Journal of Experimental Botany* 51: 1255–1259.

Cochard, H., J. Venisse, T. Barigah, et al. 2007. "Putative Role of Aquaporins in Variable Hydraulic Conductance of Leaves in Response to Light." *Plant Physiology* 143: 122–133.

Cowan, I. R. 1977. "Stomatal Behaviour and Environment." *Advances in Botanical Research* 4: 117–228.

Day, M. E. 2000. "Influence of Temperature and Leaf-to-Air Vapor Pressure Deficit on Net Photosynthesis and Stomatal Conductance in Red Spruce (Picea Rubens)." *Tree Physiology* 20: 57–63.

Dewar, R. C. 2002. "The Ball-Berry-Leuning and Tardieu-Davies Stomatal Models: Synthesis and Extension Within a Spatially Aggregated Picture of Guard Cell Function." *Plant, Cell and Environment* 25: 1383–1398.

Diao, H., L. A. Cernusak, M. Saurer, A. Gessler, R. T. W. Siegwolf, and M. M. Lehmann. 2024. "Uncoupling of Stomatal Conductance and Photosynthesis at High Temperatures: Mechanistic Insights From Online Stable Isotope Techniques." *New Phytologist* 241: 2366–2378.

Eamus, D., D. T. Taylor, C. M. Macinnis-Ng, S. Shanahan, and L. De Silva. 2008. "Comparing Model Predictions and Experimental Data for the Response of Stomatal Conductance and Guard Cell Turgor to Manipulations of Cuticular Conductance, Leaf-to-Air Vapour Pressure Difference and Temperature: Feedback Mechanisms are Able to Account for All Observations." *Plant, Cell and Environment* 31: 269–277.

Farquhar, G., and S. Wong. 1984. "An Empirical Model of Stomatal Conductance." *Australian Journal of Plant Physiology* 11: 191–210.

Fredeen, A. L., and R. F. Sage. 1999. "Temperature and Humidity Effects on Branchlet Gas-Exchange in White Spruce: An Explanation for the Increase in Transpiration With Branchlet Temperature." *Trees* 14: 0161.

Gaastra, P. 1959. "Photosynthesis of Crop Plants as Influenced by Light, Carbon Dioxide, Temperature, and Stomatal Diffusion Resistance."

Hall, A. E., S. E. Camacho-b, and M. R. Kaufmann. 1975. "Regulation of Water Loss by Citrus Leaves." *Physiologia Plantarum* 33: 62–65.

Hall, A. E., and M. R. Kaufmann. 1975. "Stomatal Response to Environment With Sesamum Indicum. L." *Plant Physiology* 55: 455–459.

Huang, J., F. Kong, H. Yin, et al. 2022. "Transpirational Cooling and Physiological Responses of Trees to Heat." *Agricultural and Forest Meteorology* 320: 108940.

Iglesias-Acosta, M., M. C. Martínez-Ballesta, J. A. Teruel, and M. Carvajal. 2010. "The Response of Broccoli Plants to High Temperature and Possible Role of Root Aquaporins." *Environmental and Experimental Botany* 68: 83–90.

Ilan, N., N. Moran, and A. Schwartz. 1995. "The Role of Potassium Channels in the Temperature Control of Stomatal Aperture." *Plant Physiology* 108: 1161–1170.

Ionenko, I. F., A. V. Anisimov, and N. R. Dautova. 2010. "Effect of Temperature on Water Transport Through Aquaporins." *Biologia Plantarum* 54: 488–494.

Kostaki, K.-I., A. Coupel-Ledru, V. C. Bonnell, et al. 2020. "Guard Cells Integrate Light and Temperature Signals to Control Stomatal Aperture." *Plant Physiology* 182: 1404–1419.

Kuiper, P. J. C. 1964. "Water Uptake of Higher Plants as Affected by Root Temperature."

Kuromori, T., E. Sugimoto, and K. Shinozaki. 2014. "Inter-Tissue Signal Transfer of Abscisic Acid From Vascular Cells to Guard Cells." *Plant Physiology* 164: 1587–1592.

Lawson, T., I. Terashima, T. Fujita, and Y. Wang. 2018. "Coordination between photosynthesis and stomatal behavior." In *The Leaf: A Platform for Performing Photosynthesis. Advances in Photosynthesis and Respiration*, edited by I. T. W. W. Adams, III, 141–162. Springer.

Liang, X., D. Wang, Q. Ye, et al. 2023. "Stomatal Responses of Terrestrial Plants to Global Change." *Nature Communications* 14: 2188.

López, J., D. A. Way, and W. Sadok. 2021. "Systemic Effects of Rising Atmospheric Vapor Pressure Deficit on Plant Physiology and Productivity." *Global Change Biology* 27: 1704–1720.

Lu, Z., and E. Zeiger. 1994. "Selection for Higher Yields and Heat Resistance in Pima Cotton has Caused Genetically Determined Changes in Stomatal Conductances." *Physiologia Plantarum* 92: 273–278.

Matzner, S., and J. Comstock. 2001. "The Temperature Dependence of Shoot Hydraulic Resistance: Implications for Stomatal Behaviour and Hydraulic Limitation." *Plant, Cell and Environment* 24: 1299–1307.

McAdam, S. A. M., and T. J. Brodribb. 2015. "The Evolution of Mechanisms Driving the Stomatal Response to Vapor Pressure Deficit." *Plant Physiology* 167: 833–843.

McAdam, S. A. M., and T. J. Brodribb. 2016. "Linking Turgor With ABA Biosynthesis: Implications for Stomatal Responses to Vapor Pressure Deficit Across Land Plants." *Plant Physiology* 171: 2008–2016.

Messinger, S. M., T. N. Buckley, and K. A. Mott. 2006. "Evidence for Involvement of Photosynthetic Processes in the Stomatal Response to CO2." *Plant Physiology* 140: 771–778.

Mills, C., M. K. Bartlett, and T. N. Buckley. 2024. "The Poorly-Explored Stomatal Response to Temperature at Constant Evaporative Demand." *Plant, Cell and Environment* 47: 3428–3446.

Moran, M. E., L. M. T. Aparecido, D. F. Koepke, et al. 2023. "Limits of Thermal and Hydrological Tolerance in a Foundation Tree Species (*Populus fremontii*) in the Desert Southwestern United States." *New Phytologist* 240: 2298–2311.

Morison, J. I. L. 1987. "Intercellular CO2 Concentration and Stomatal Response to CO2." In *Stomatal function*, edited by E. Zeiger, G. D. Farquhar, and I. R. Cowan, 229–251. Stanford: Stanford University Press.

Mott, K. A. 2009. "Opinion: Stomatal Responses to Light and CO2 Depend on the Mesophyll." *Plant, Cell and Environment* 32: 1479–1486.

Mott, K. A., and D. Peak. 2010. "Stomatal Responses to Humidity and Temperature in Darkness." *Plant, Cell and Environment* 33: 1084–1090.

Muhsin, T. M., and J. J. Zwiazek. 2002. "Ectomycorrhizas Increase Apoplastic Water Transport and Root Hydraulic Conductivity in Ulmus Americana Seedlings." *New Phytologist* 153: 153–158.

Murai-Hatano, M., T. Kuwagata, J. Sakurai, et al. 2008a. "Effect of Low Root Temperature on Hydraulic Conductivity of Rice Plants and the Possible Role of Aquaporins." *Plant and Cell Physiology* 49: 1294–1305.

Murai-Hatano, M., T. Kuwagata, J. Sakurai, et al. 2008b. "Effect of Low Root Temperature on Hydraulic Conductivity of Rice Plants and the Possible Role of Aquaporins." *Plant and Cell Physiology* 49: 1294–1305.

Oren, R., J. S. Sperry, G. G. Katul, et al. 1999. "Survey and Synthesis of Intra- and Interspecific Variation in Stomatal Sensitivity to Vapour Pressure Deficit." *Plant, Cell and Environment* 22: 1515–1526.

Osonubi, O., and W. J. Davies. 1980. "The Influence of Water Stress on the Photosynthetic Performance and Stomatal Behaviour of Tree Seedlings Subjected to Variation in Temperature and Irradiance." *Oecologia* 45: 3–10.

Rodriguez-Dominguez, C. M., T. N. Buckley, G. Egea, et al. 2016. "Most Stomatal Closure in Woody Species under Moderate Drought can be Explained by Stomatal Responses to Leaf Turgor." *Plant, Cell and Environment* 39: 2014–2026.

Rogers, C., P. J. H. Sharpe, and R. D. Powell. 1980. "Dark Opening of Stomates of Vicia Faba in CO2-free Air: Effect of Temperature on

Stomatal Aperture and Potassium Accumulation." *Plant Physiology* 65: 1036–1038.

Rogers, C. A., R. D. Powell, and P. J. H. Sharpe. 1979. "Relationship of Temperature to Stomatal Aperture and Potassium Accumulation in Guard Cells of Vicia Faba." *Plant Physiology* 63: 388–391.

Sack, L., G. P. John, and T. N. Buckley. 2018. "ABA Accumulation in Dehydrating Leaves is Associated With Decline in Cell Volume, Not Turgor Pressure." *Plant Physiology* 176: 489–495.

Sack, L., C. M. Streeter, and N. M. Holbrook. 2004. "Hydraulic Analysis of Water Flow Through Leaves of Sugar Maple and Red Oak." *Plant Physiology* 134: 1824–1833.

Sayer, M. A. S., J. C. Brissette, and J. P. Barnett. 2005. "Root Growth and Hydraulic Conductivity of Southern Pine Seedlings in Response to Soil Temperature and Water Availability After Planting." *New Forests* 30: 253–272.

Schulze, E.-, O. L. Lange, M. Evenari, L. Kappen, and U. Buschbom. 1974. "The Role of Air Humidity and Leaf Temperature in Controlling Stomatal Resistance of Prunus Armeniaca L. Under Desert Conditions." *Oecologia* 17: 159–170.

Schulze, E.-D., O. L. Lange, L. Kappen, U. Buschbom, and M. Evenari. 1973. "Stomatal Responses to Changes in Temperature at Increasing Water Stress." *Planta* 110: 29–42.

Schymanski, S. J., D. Or, and M. Zwieniecki. 2013. "Stomatal Control and Leaf Thermal and Hydraulic Capacitances Under Rapid Environmental Fluctuations." *PLoS One* 8: e54231.

Scoffoni, C., C. Albuquerque, T. N. Buckley, and L. Sack. 2023. "The Dynamic Multi-Functionality of Leaf Water Transport Outside the Xylem." *New Phytologist* 239: 2099–2107.

Sellin, A., and P. Kupper. 2007. "Temperature, Light and Leaf Hydraulic Conductance of Little-Leaf Linden (Tilia Cordata) in a Mixed Forest Canopy." *Tree Physiology* 27: 679–688.

Sibbernsen, E., and K. A. Mott. 2010. "Stomatal Responses to Flooding of the Intercellular Air Spaces Suggest a Vapor-Phase Signal Between the Mesophyll and the Guard Cells." *Plant Physiology* 153: 1435–1442.

Sonawane, B. V., N. K. Koteyeva, D. M. Johnson, and A. B. Cousins. 2021. "Differences in Leaf Anatomy Determines Temperature Response of Leaf Hydraulic and Mesophyll Co_2 Conductance in Phylogenetically Related C_4 and C_3 Grass Species." *New Phytologist* 230: 1802–1814.

Sussmilch, F. C., T. J. Brodribb, and S. A. M. McAdam. 2017. "Up-Regulation of NCED3 and ABA Biosynthesis Occur Within Minutes of a Decrease in Leaf Turgor but AHK1 is not Required." *Journal of Experimental Botany* 68: 2913–2918.

Teskey, R. O., J. A. Fites, L. J. Samuelson, and B. C. Bongarten. 1986. "Stomatal and Nonstomatal Limitations to Net Photosynthesis in Pinus Taeda L. under Different Environmental Conditions." *Tree Physiology* 2: 131–142.

Tonet, V., M. Carins-Murphy, R. Deans, and T. J. Brodribb. 2023. "Deadly Acceleration in Dehydration of Eucalyptus Viminalis Leaves Coincides With High-Order Vein Cavitation." *Plant Physiology* 191: 1648–1661.

Urban, J., M. W. Ingwers, M. A. McGuire, and R. O. Teskey. 2017. "Increase in Leaf Temperature Opens Stomata and Decouples Net Photosynthesis From Stomatal Conductance in Pinus Taeda and Populus Deltoides X Nigra." *Journal of Experimental Botany* 68: 1757–1767.

Wan, X., J. J. Zwiazek, V. J. Lieffers, and S. M. Landhausser. 2001. "Hydraulic Conductance in Aspen (Populus Tremuloides) Seedlings Exposed to Low Root Temperatures." *Tree Physiology* 21: 691–696.

Yang, Y., Q. Zhang, G. Huang, S. Peng, and Y. Li. 2020. "Temperature Responses of Photosynthesis and Leaf Hydraulic Conductance in Rice and Wheat." *Plant, Cell and Environment* 43: 1437–1451.

Yoshida, S., and H. Eguchi 1990. "Root Temperature Effect on Root Hydraulic Resistance in Cucumber (Cucumis sativus L.) and Figleaf Gourd (Cucurbita ficifolia B.) Plants."

Zhao, C., Y. Wang, K. X. Chan, et al. 2019. "Evolution of Chloroplast Retrograde Signaling Facilitates Green Plant Adaptation to Land." *Proceedings of the National Academy of Sciences* 116: 5015–5020.

Zuccarini, P., E. Farieri, R. Vásquez, B. Grau, and R. Savé Monserrat. 2015. "Effects of Soil Water Temperature on Root Hydraulic Resistance of Six Species of Iberian Pines." *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology* 149: 914–921.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.
Methods S1: Description of custom cuvettes and outer chamber. **Methods S2:** Analysis of influence of changes in transpiration of leaves outside the cuvette. **Figure S1:** Photos of cuvette and outer chamber. **Figure S2:** Resistance diagram used in Methods S2. **Figure S3:** Distribution of plausible effect of outside-cuvette transpiration on results.