Title

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

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Supplemental Materials

Methods S1. Description of custom cuvettes and outer chamber

As described briefly under *Methods* in the main text, we constructed custom leaf gas-exchange cuvettes to improve leaf temperature control and isolate this control from the feedback control algorithm used by the LI-6800 to control Δw itself. Earlier attempts at measuring the DRST using the LI-6800's standard cuvette were sometimes unsuccessful, because feedback occurred between shifts in Δw and T induced by the system in order to regulate these variables, and stomatal responses to these regulatory shifts, leading to large oscillations in calculated stomatal conductance.

To prevent these oscillations and also better control leaf temperature, we constructed leaf cuvettes in which water from a temperature-controlled recirculating water bath (Fisher Scientific Isotemp 250 LCU, Fisher Scientific, Hampton, NH, USA) flowed through channels in the aluminum block from which the leaf cuvette was milled. The cuvettes were nickel-plated to decrease water vapor adsorption. These cuvettes were connected to the LI-6800 head through the 6800-19 (LI-COR Biosystems, Lincoln, NE, USA) via Bev-A-Line (Thermoplastic Processes, Georgetown, DE, USA) tubing with a 1/4 inch outer diameter and a 1/8 inch inner diameter. This design shifts temperature control from the LI-6800 to the recirculating water bath, and also adds radiative exchange (between the cuvette's aluminum block and the leaf) to convective exchange, thereby improving temperature control.

We constructed two different leaf cuvettes . Both included a window above the leaf, with an adjacent space milled out to include a set of fins for heat exchange, and a DC blower placed below the fins, circulating air through the fins and across the leaf. Gas inlets and outlets were milled into the fin area, separated by enough distance to ensure that incoming air would be drawn into the blower and thus circulate over the leaf at least once before exiting the chamber. One cuvettes enclosed a leaf area of up to

8.96 cm^2 and had a square window consisting of propafilm, square 3 x 3 cm advanced polymer gaskets from LI-COR, and a small blower (AGB304H, Pelonis Technologies, Exton, PA). The other cuvettes enclosed a leaf area of up to 5.6 cm^2 and had a rectangular glass window (28 x 20 mm), custom gaskets 6 mm wide and 3.5 mm thick made from platinum-catalyzed silicone rubber (Ecoflex 00-30, Smooth-On, Inc., Macungie, PA), and a somewhat larger blower (BFB0412HHA-A, Delta Electronics, Taiwan). In both cuvettes , a type-E thermocouple was appressed to the lower leaf surface. Both cuvettes were closed using handle nuts on threaded rods that passed through either a portion of the cuvette's block that did not contact the cuvette interior, or plates mounted above and below the cuvette to apply closing pressure. Almost all experiments used the 8.97-cm^2 cuvette, except for a few measurements on some *V. vinifera* cv 'Pinot Noir', and *P. vulgaris* replicates.

The plant, the LI-6800, and the leaf cuvette were all enclosed in a larger temperature-controlled 'outer chamber' to facilitate leaf-cuvette temperature control and prevent temperature condensation that might otherwise occur when air with a high dewpoint, generated by the warmed leaf, exits the leaf cuvette and encounters cooler surfaces. This outer chamber measured 1 x 1 x 0.6 m, and was constructed from aluminum T-slot rails, covered with twin-wall polycarbonate sheets, and included a clear acrylic door for access. Windows were cut in one chamber wall to allow for temperature control via an air conditioner (FHWC054WB1, Frigidaire, Charlotte, NC, USA) and a ceramic space heater (Chikit PTC-SH008), both of which were controlled with a digital thermostat (Bayite BTC211, Shenzhen Bayite Technology Co., Inc., Shenzhen Guang Dong, China), with a temperature setpoint within 2 degrees C of the target leaf temperature in most cases. Chamber air was mixed and circulated using a 6 inch fan (JETFAN 6 inline fan, JETFAN, Hydrofarm, Shoemakersville, PA, USA). The air conditioner and heater inlets and the fan were separated from the main volume of the chamber by an internal twin-wall polycarbonate baffle with gaps at the bottom and top, which helped to ensure full circulation of temperature-controlled air through the chamber volume. Figure S1 shows photos of the larger chamber and the leaf cuvette.

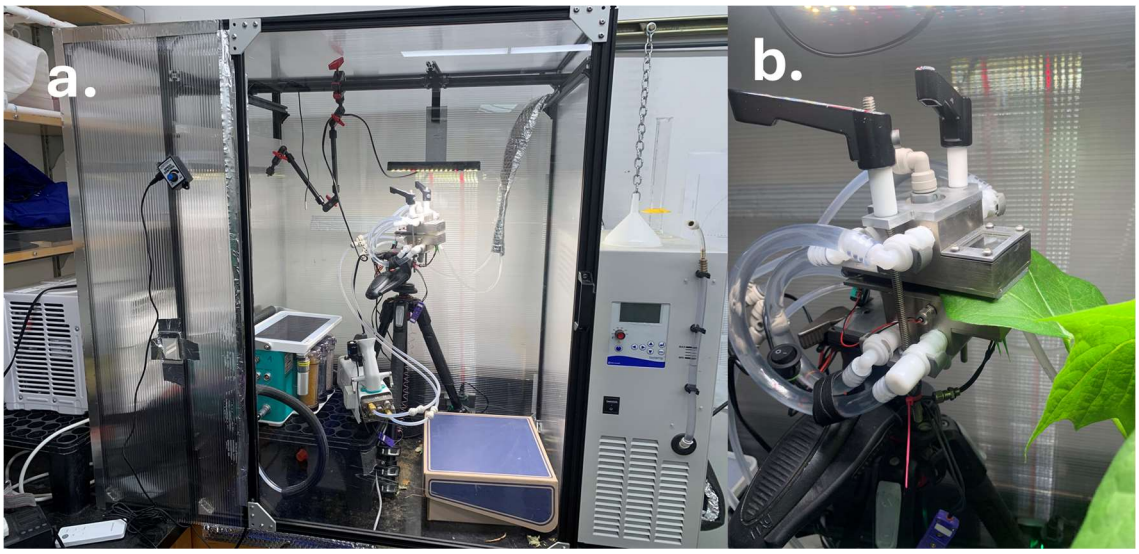

Figure S1. (a) The entirety of the gas-exchange system used in this experiment. The LI-6800, the plant (not shown), and the leaf cuvette are enclosed in a large chamber covered with clear acrylic and twin-wall polycarbonate. The temperature of the larger chamber is controlled via an air conditioner (white box seen on the left, mostly outside the larger chamber but with its air outlet placed in a window in the outer chamber) and a small ceramic heater (placed atop the air conditioner, obscured in this view), with a powerful fan for circulation (also obscured in this view, but located behind the baffle on the left side of the large chamber, above the AC and heater). Leaf cuvette temperature is controlled by water flowing through channels in the aluminum cuvette body from a recirculating water bath (seen to the right of the large chamber). (b) A close-up of the leaf cuvette; the nylon tubes conduct temperature-controlled water through channels in the cuvette body, and the two black handles at the top rotate along threaded rods on either side of the cuvette body to apply pressure to close the cuvette onto the leaf.

Methods S2. Analysis of influence of changes in transpiration of leaves outside the cuvette

We were able to regulate Δw in the leaf cuvette, but not in the outer chamber that enclosed the rest of the plant. Thus, when temperature was increased in both the outer chamber and in the leaf cuvette to measure the DRST, the "outer" portion of the plant would have experienced an increase in Δw, and thus in transpiration rate. We did not attempt to measure that effect, and we suspect it was small, given that most of the outer portion of the plant was typically under low levels of illumination (typical of the laboratory; a PPFD of ~ 15-30 μmol m^{-2} s^{-1}). Nonetheless, here we evaluate how any change in transpiration in the outer portion of the plant would likely have affected our results – more precisely, not how it would have affected the DRST itself, but the influence of Δw on the strength of the DRST, which was the quantity most directly relevant to our hypothesis.

The process-based model of stomatal conductance that we used to derive our theoretical predictions can also be expressed as

$$g_s = b(\psi + \pi) \quad \text{(S1)}$$

where b is a factor that depends on light, CO_2 and possibly temperature, but is not directly affected by hydraulic factors; ψ is the water potential of the leaf in question; and π is the leaf osmotic pressure. (To arrive at Eqn S1, replace g_{smax} in Eqn 2 in the main text with $b\pi$, calculate ψ as $0 - E/K = -g_s\Delta w/K$, and apply the result to Eqn S1; the result is identical to Eqn 2.) ψ can be modeled using the resistance network shown in Figure S2, in which the leaf in the cuvette (with transpiration rate E and area a) and the rest of the plant's leaves (each having a transpiration rate E_o and a total leaf area $n_o \cdot a$ [equivalent to a number of leaves n_o equal in size to the leaf in the cuvette]) share a water transport pathway from the soil to the base of the petiole of the cuvette leaf, and that shared pathway has a resistance of r_s (s for shared). There is also

a resistance of r between the end of the shared pathway and the cuvette leaf's lamina, and another resistance of r from the end of the shared pathway to each of the other leaves. Other more realistic structures are possible, but this structure maximizes the possible hydraulic interactions among leaves, because in this structure they share the largest possible portion of the water supply pathway.

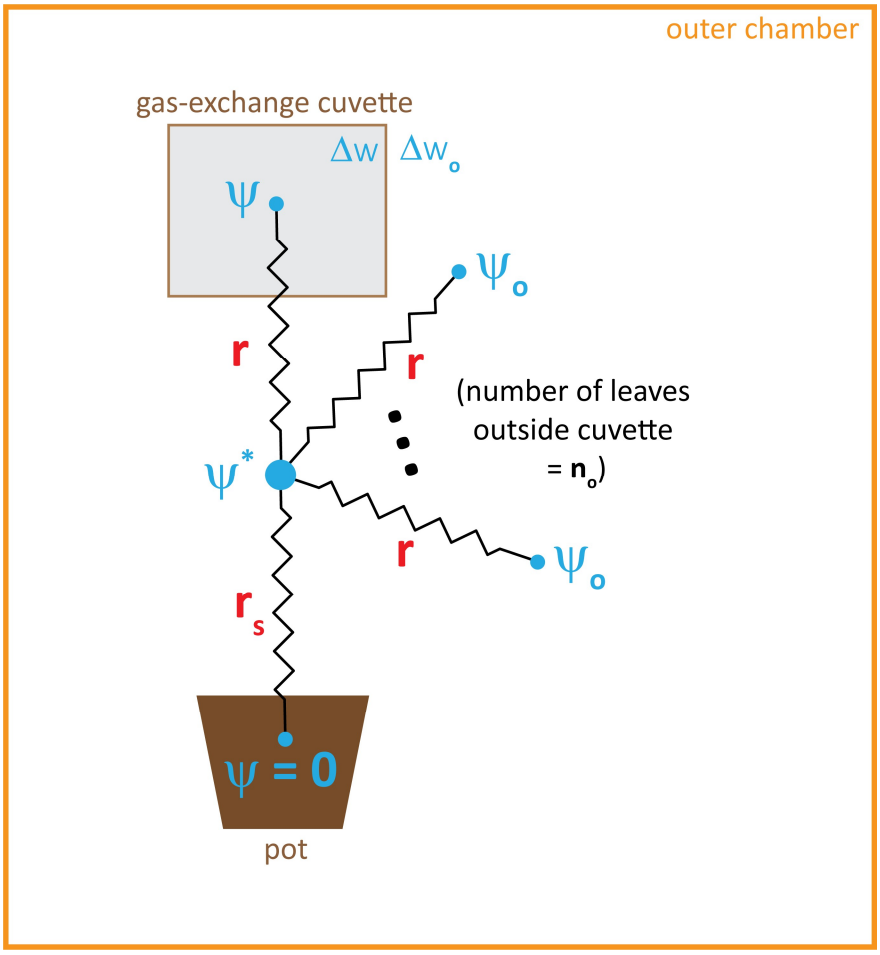

Figure S2. Diagram of resistance network used in this analysis. The "junction" point is indicated by the large blue circle. The total resistance upstream of that point (from the soil to the junction) is r_s; the resistance of each pathway downstream of that point is r. There are a total of n_o + 1 leaves: 1 in the cuvette and n_o outside the cuvette. The leaf in the cuvette has a water potential of ψ; those outside the cuvette have water potentials of ψ_o. The water potential is ψ^* at the junction point and 0 in the soil. The evaporative demand in the cuvette is Δw; that outside the cuvette is Δw_o.

Denote the water potential at the end of the shared pathway (i.e., the "junction" point where the pathways to the each leaf diverge; the large blue circle in Fig S2) as ψ^*. At steady state, ψ^* is equal to the soil water potential (assumed zero in this experiment, because the pot was kept in a tray of water) minus the product of whole-plant transpiration ($aE + n_o aE_o$) and r_s:

$$\psi^* = 0 - (aE + an_o E_o)r_s \quad \text{(S2)}$$

The cuvette leaf's water potential, in turn is, ψ^* minus the product of r and aE:

$$\psi = \psi^* - raE = -(r + r_s)aE - an_o r_s E_o \quad \text{(S3)}$$

Writing cuvette-leaf transpiration rate as the product of stomatal conductance and cuvette Δw gives

$$\psi = -(r + r_s)ag_s \Delta w - an_o E_o r_s \quad \text{(S4)}$$

and applying this to Eqn S1 and solving for g_s gives

$$g_s = \frac{b(\pi - an_o r_s E_o)}{1 + ba(r + r_s)\Delta w} \quad \text{(S5)}$$

From this equation, it is clear that transpiration of leaves outside the cuvette (E_o) will affect the stomatal conductance of the leaf in the cuvette. However, the question at hand is more specifically whether a change in E_o with temperature will affect the *difference in the stomatal response to temperature between low and high* Δw; or equivalently, whether the effect of changing E_o on the stomatal response to temperature will depend on the value of Δw in the cuvette. The temperature response, expressed in relative terms as in the main text, is

$$\frac{d \ln g_s}{dT} = \frac{\partial \ln g_s}{\partial b}\frac{db}{dT} + \frac{\partial \ln g_s}{\partial r_s}\frac{dr_s}{dT} + \frac{\partial \ln g_s}{\partial r}\frac{dr}{dT} + \frac{\partial \ln g_s}{\partial E_o}\frac{dE_o}{dT} \quad \text{(S6)}$$

The question at hand is equivalent to asking whether the last term in Eqn S6 is a function of cuvette-Δw (as distinct from the value of Δw outside the cuvette, Δw_o), and if so, how much does it influence the overall difference in $d\ln g_s/dT$ between two different values of Δw. The partial derivative in the last term in Eqn is found from Eqn S5 as

$$\frac{\partial \ln g_s}{\partial E_o} = -\frac{a n_o r_s}{\pi - a n_o r_s E_o} \quad \text{(S7)}$$

and the total derivative is

$$\frac{dE_o}{dT} = \frac{\partial E_o}{\partial \Delta w_o}\frac{d\Delta w_o}{dT} \quad \text{(S8)}$$

making the last term in Eqn S6

$$\frac{\partial \ln g_s}{\partial E_o}\frac{dE_o}{dT} = -\left(\frac{a n_o r_s}{\pi - a n_o r_s E_o}\right)\frac{\partial E_o}{\partial \Delta w_o}\frac{d\Delta w_o}{dT} \quad \text{(S9)}$$

The value of Δw in the leaf cuvette will affect this quantity indirectly: cuvette transpiration will affect the "shared" water potential ψ^*, which in turn will affect the stomatal conductance and transpiration rate of leaves outside the cuvette (g_{so} and E_o, respectively). To quantify the magnitude of the resulting feedback on the stomatal response to temperature in the cuvette (Eqn S9), we first need to quantify the effect of ψ^* on g_{so} and E_o. We assume that stomata of leaves outside the cuvette obey the same model as the cuvette

leaf (Eqn S1), but with a different (likely lower) value of b, due to the lower irradiance outside the cuvette:

$$g_{so} = b_o(\psi_o + \pi) \quad \text{(S10)}$$

where ψ_o is the water potential of leaves outside the cuvette, which equals ψ^* minus the drawdown to those leaves, which is the product of the corresponding resistance and flow (transpiration rate). The flow to a given leaf will be aE_o, and the corresponding resistance will be r; equivalently, the total flow to the portion of the canopy outside the cuvette will be an_oE_o, but the resistance – having n_o paths in parallel – will be r/n_o, which gives the same result. Thus

$$\psi_o = \psi^* - arE_o = -ar_sE - a(r_s + r)E_o \quad \text{(S11)}$$

Expanding E_o as the product of g_{so} and Δw_o gives

$$\psi_o = -ar_sE - a(r_s + r)g_{so}\Delta w_o \quad \text{(S12)}$$

and applying this to Eqn S10 and solving for g_{so} gives

$$g_{so} = \frac{b_o(\pi - ar_sg_s\Delta w)}{1 + b_oa(r_s + r)\Delta w_o} \quad \text{(S13)}$$

and E_o as

$$E_o = \frac{b_o(\pi - ar_sg_s\Delta w)\Delta w_o}{1 + b_oa(r_s + r)\Delta w_o} \quad \text{(S14)}$$

The sensitivity of transpiration outside the chamber (E_o) to Δw outside the chamber (Δw_o) is thus

$$\frac{\partial E_o}{\partial \Delta w_o} = \frac{E_o}{\Delta w_o}\left(\frac{1}{1 + b_o a(r_s + r)\Delta w_o}\right) \quad \text{(S15)}$$

which modifies Eqn S9 to

$$\frac{\partial \ln g_s}{\partial E_o}\frac{dE_o}{dT} = -\left(\frac{a n_o r_s E_o}{\pi - a n_o r_s E_o}\right)\left(\frac{1}{1 + b_o a(r_s + r)\Delta w_o}\right)\frac{1}{\Delta w_o}\frac{d\Delta w_o}{dT} \quad \text{(S16)}$$

Note from Eqn S2 that the quantities $-ar_s n_o E_o$ and $-ar_s E$ sum to ψ^*; i.e., they are the two additive contributions (from transpiration outside and inside the cuvette, respectively) to the water potential drawdown from the soil to the "junction" point where the paths to the leaves diverge. Define f_o as fraction of that total drawdown that is attributable to transpiration outside the cuvette (equivalently, the fraction of total transpiration that occurs outside the cuvette):

$$f_o \equiv \frac{a r_s n_o E_o}{a r_s n_o E_o + a r_s E} = \frac{a n_o E_o}{a n_o E_o + a E} \quad \text{(S17)}$$

so that $ar_s n_o E_o = -f_o \psi^*$ and $ar_s E = -(1 - f_o)\psi^*$. Then

$$\frac{\partial \ln g_s}{\partial E_o}\frac{dE_o}{dT} = -\left(\frac{-f_o \psi^*}{\pi + f_o \psi^*}\right)\left(\frac{1}{1 + b_o a(r_s + r)\Delta w_o}\right)\frac{1}{\Delta w_o}\frac{d\Delta w_o}{dT} \quad \text{(S18)}$$

or

199 $$\frac{\partial \ln g_s}{\partial E_o}\frac{dE_o}{dT} = -\left(\frac{-f_o\psi^*/\pi}{1+f_o\psi^*/\pi}\right)\left(\frac{1}{1+b_o a(r_s+r)\Delta w_o}\right)\frac{1}{\Delta w_o}\frac{d\Delta w_o}{dT} \quad \text{(S19)}$$

200

201 To apply this equation, we need to constrain the ratio of ψ^* to π, and the quantity $b_o a(r_s + r)$. We can

202 address the first question in two steps: first, by asking how ψ^* compares to ψ, and then how ψ compares

203 to π. The ratio of ψ^* to ψ is found from Eqns S2 and S4 as

204

205 $$\frac{\psi^*}{\psi} = \frac{r_s(aE + an_oE_o)}{raE + r_s(aE + an_oE_o)} = \frac{\frac{r_s}{r}\left(\frac{aE + an_oE_o}{aE}\right)}{1+\frac{r_s}{r}\left(\frac{aE + an_oE_o}{aE}\right)} = \frac{\frac{r_s}{r}\left(\frac{1}{1-f_o}\right)}{1+\frac{r_s}{r}\left(\frac{1}{1-f_o}\right)} = \frac{\frac{r_s}{r}}{1-f_o+\frac{r_s}{r}} \quad \text{(S20)}$$

206

207 For how ψ compares to π, apply $-an_o r_s E_o = f_o\psi^*$ to the expression for g_s in the cuvette (Eqn S5) and

208 combine the result with Eqn S1 to give

209

210 $$\frac{b(\pi + f_o\psi^*)}{1+ba(r+r_s)\Delta w} = b(\pi+\psi) \quad \text{(S21)}$$

211

212 and then apply Eqn S20 to express ψ^* in terms of ψ, giving

213

214 $$\frac{b\left(\pi + f_o\psi\dfrac{\frac{r_s}{r}}{1-f_o+\frac{r_s}{r}}\right)}{1+ba(r+r_s)\Delta w} = b(\pi+\psi) \quad \text{(S22)}$$

215

216 Rearranging gives

217

$$\frac{\pi + f_o\psi \dfrac{\dfrac{r_s}{r}}{1 - f_o + \dfrac{r_s}{r}}}{\pi + \psi} = 1 + ba(r + r_s)\Delta w \quad \text{(S23)}$$

The quantity $a(r + r_s)$ appears in both Eqns S23 and S19. This quantity is related to b/K, which is constrained by data of Oren et al. (1999) as discussed in the main text. However, K is not simply $1/(a(r + r_s))$. In natural conditions, when all leaves in the plant are transpiring equally, the resistance diagram simplifies to a single series pathway in which the terminal resistance is $r/(1 + n_o)$, which represents the leaf component of whole-plant resistance ($1/K$) in that case. Thus, the leaf fraction of whole plant resistance under normal conditions (i.e., as relevant to comparison with published values of K) is

$$f_L \equiv \frac{r/(1 + n_o)}{r_t} = \frac{r/(1 + n_o)}{r_s + r/(1 + n_o)} = \frac{r}{(1 + n_o)r_s + r} \quad \text{(S24)}$$

where r_t is whole pant resistance. Solving for r gives

$$r = \frac{f_L(1 + n_o)r_s}{1 - f_L} \quad \text{(S25)}$$

so $r + r_s$ is

$$r + r_s = \left[\frac{f_L(1 + n_o)}{1 - f_L} + 1\right] r_s = \left[\frac{1 + n_o f_L}{1 - f_L}\right] r_s \quad \text{(S26)}$$

but noting that $r_s = (1 - f_L)r_t$, we have

$$r + r_s = [1 + n_o f_L] r_t \quad \text{(S27)}$$

240

241 Since $ar_t = 1/K$, $r_t = 1/aK$, giving

242

243 $$r + r_s = \frac{[1 + n_o f_L]}{aK} \quad \text{(S28)}$$

244

245 This makes Eqn S23

246

247 $$\frac{\pi + f_o\psi\dfrac{\frac{r_s}{r}}{1 - f_o + \frac{r_s}{r}}}{\pi + \psi} = 1 + [1 + n_o f_L]\frac{b}{K}\Delta w \quad \text{(S29)}$$

248

249 From Eqn S25, note that $r_s/r = (1 - f_L)/[f_L(1 + n_o)]$, giving

250

251 $$\frac{\pi + f_o\psi\dfrac{\frac{1 - f_L}{f_L(1 + n_o)}}{1 - f_o + \frac{1 - f_L}{f_L(1 + n_o)}}}{\pi + \psi} = 1 + [1 + n_o f_L]\frac{b}{K}\Delta w \quad \text{(S30)}$$

252

253 and

254 $$\frac{\pi + \psi\dfrac{f_o(1 - f_L)}{(1 - f_o)f_L(1 + n_o) + 1 - f_L}}{\pi + \psi} = 1 + [1 + n_o f_L]\frac{b}{K}\Delta w \quad \text{(S31)}$$

255

256 Solving for ψ/π gives

257

258 $$\frac{\psi}{\pi} = \frac{-[1 + n_o f_L]\frac{b}{K}\Delta w}{1 + [1 + n_o f_L]\frac{b}{K}\Delta w - \dfrac{f_o(1 - f_L)}{(1 - f_o)f_L(1 + n_o) + 1 - f_L}} \quad \text{(S32)}$$

Finally, ψ^*/π is then given by

$$\frac{\psi^*}{\pi} = \frac{\psi^*}{\psi} \cdot \frac{\psi}{\pi} = \left(\frac{\frac{r_s}{r}}{1 - f_o + \frac{r_s}{r}} \right) \left(\frac{-[1 + n_o f_L] \frac{b}{K} \Delta w}{1 + [1 + n_o f_L] \frac{b}{K} \Delta w - \frac{f_o (1 - f_L)}{(1 - f_o) f_L (1 + n_o) + 1 - f_L}} \right) \quad \text{(S33)}$$

Replacing r_s/r with $(1 - f_L)/[f_L(1 + n_o)]$ as before gives

$$\frac{\psi^*}{\pi} = \frac{-[1 + n_o f_L] \frac{b}{K} \Delta w \frac{1 - f_L}{(1 - f_o) f_L (1 + n_o) + 1 - f_L}}{1 + [1 + n_o f_L] \frac{b}{K} \Delta w - \frac{f_o (1 - f_L)}{(1 - f_o) f_L (1 + n_o) + 1 - f_L}} \quad \text{(S34)}$$

Eqn S19 also requires $r_s + r$ to be expressed in terms of K; we apply Eqn S28 and expand b_o/K as $(b_o/b)\cdot(b/K)$, giving

$$\frac{\partial \ln g_s}{\partial E_o} \frac{dE_o}{dT} = -\left(\frac{-f_o \psi^*/\pi}{1 + f_o \psi^*/\pi} \right) \left(\frac{1}{1 + [1 + n_o f_L] \frac{b_o}{b} \frac{b}{K} \Delta w_o} \right) \frac{1}{\Delta w_o} \frac{d\Delta w_o}{dT} \quad \text{(S35)}$$

This expression can be applied at low and high cuvette-Δw values to calculate feasible ranges for the effect of changes in non-cuvette transpiration on differences in $d\ln g_s/dT$ between low and high Δw, given reasonable constraints on the values of n_o, f_L, f_o, K/b, b_o/b, and known values for $d\Delta w_o/dT$ and Δw_o. $d\Delta w_o/dT$ is defined by temperature, for which we used the intermediate value in our experiments (27.5°C); for Δw_o itself, we also used that temperature, and assumed ambient vapor pressure was 13 mmol mol^{-1} in the outer chamber (the value typical for our laboratory and building). We assumed that n_o (the ratio of leaf area outside the cuvette to that inside the cuvette; equivalently, the number of leaves outside the cuvette)

could vary from 1 to 500; that f_L could vary between 0.1 and 0.9, and that b_o/b could vary between 0 and 1. We assumed the fraction of total transpiration that occurs outside the cuvette is the product of the fraction of leaf area outside the cuvette ($n_o/(n_o + 1)$) and the ratio b_o/b. We randomly sampled each of these quantities uniformly within those respective ranges. For K/b, we randomly resampled, with replacement, from the distribution inferred from the Oren data, as described in the main text. We repeated this procedure for 100,000 samples to bootstrap the distribution of possible contributions of the last term in Eqn S6 to the difference in $d\ln g_s/dT$ between low and high Δw. As in the main text, we predicted finite changes by multiplying the derivative in Eqn S35 by a finite temperature difference of 10ºC, and then by 100 to convert relative changes to percent changes, for comparison with Figure 4 in the main text.

The results are shown in Fig S3 below. The mean and median effects were 0.031% and 0.0006%, respectively, and the 99th percentile was 0.61%. These values are very small in comparison to the total observed differences in the DRST between high and low Δw, which were on the order of 4-30%. Thus, we conclude that uncontrolled changes in Δw outside the cuvette during warming would not have significantly affected our results.

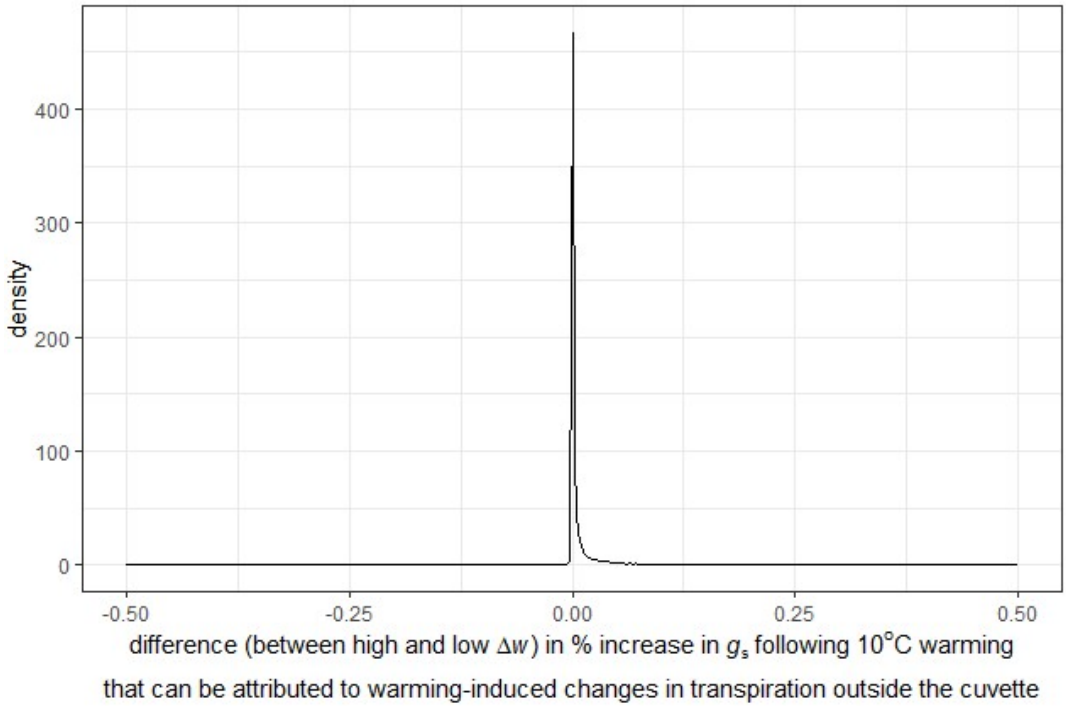

Fig S3. Density distribution of predictions for the component of our results (the difference in the stomatal response to temperature between high and low Δ*w*) that could be attributed to changes in transpiration in the portion of the leaf outside the gas-exchange cuvette, as a result of uncontrolled increases in Δ*w* in that region during warming.