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Supplementary Material

Thermogenesis and developmental progression of *Macrozamia macleayi* pollen cones

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Text S1. We consider three questions concerning the nature of the air flow within the chamber and of the water mass loss versus dry mass loss for the constant ambient temperature tests (see Fig. 10 and Table 2 in main text). Is the flow laminar or turbulent? Is the flow dominated by natural convection (with the air flow driven by buoyant forces) or by forced convection? And, what are the relative losses of dry and water mass during the spontaneous thermogenic events?

Laminar or turbulent flow? We first consider whether the forced convection flow in the respiratory chamber is laminar. The volumetric air flow rate through the cylindrical respirometry chamber was ~ 300 mL/min giving for the chamber diameter (D) of 12 cm, an average upward air velocity (V) in the cylinder of ~3 cm/min, which is quite slow, so we might expect laminar flow. The transition from laminar to turbulent flow occurs at a Reynolds number of Re ~2300 for forced convection in a cylinder (Incropera and DeWitt 1996), where $Re = VD/\gamma$, V is the average air velocity, and γ is the kinematic viscosity of air. In comparison, the actual forced convection Reynolds number for the respiratory chamber is Re ~ 4 so flow through the chamber under our experimental conditions is clearly laminar.

Next, when considering whether natural convection could induce turbulence, the critical Rayleigh number at which that transition occurs is $Ra_{transition} = 10^9$ for a vertical surface (Incropera and DeWitt 1996), where $Ra = g\beta(T_{cone} - T_{air})L^3/\gamma\alpha$, g is the gravitational constant, β is the volumetric thermal expansion coefficient of air, α is the thermal diffusivity of air, and L is the height of the surface. For the cone in Fig. 10, L = 11 cm and the largest cone temperature elevation above the air temperature was ~10 °C, so the largest Rayleigh number reached by the cone was $Ra_{cone} = 1.2 * 10^6$. This is almost three orders of magnitude below the value needed for the onset of turbulence, so the free convection flow is also highly likely to be laminar.

In summary, both forced and natural convection flows are expected to be laminar, and although the effects of surface roughness, transient conditions during events, off-vertical tipping of the cones, and recirculation within the respiratory chamber due to the additive effects of forced and free convection that could change the flow patterns may all be present, it is unlikely that those factors would result in a three order of magnitude change, so the flow inside the chamber was most likely laminar, and not turbulent. The lack of turbulent mixing implies that the RH of the air in the respiratory chamber is thus best estimated by the average of the entering and exiting air values—as opposed to the case when “perfect” mixing of the air in respiratory chamber occurs in which case the RH of the air in the chamber would be best estimated by the RH of the exiting air.

Combined free and forced convection: To determine the combined effects of the natural and free convection laminar flows on the convective conductance term, it is common to determine the Nusselt number (Incropera and DeWitt 1996) for the two flows as $Nu_{combined}^3 = Nu_{forced}^3 + Nu_{free}^3$, where Nu = hL/k, h is the cone heat transfer coefficient, and k is the thermal conductivity of air. Using this equation and those for the Nusselt numbers for laminar free and forced convection flow over a vertical surface (Incropera and DeWitt 1996), the ratio of the combined to forced convection heat transfer coefficients can be calculated at different temperature differences between the cone and the air to estimate how the combined effects would vary during a thermogenic event. Doing so for the conditions of the cone in Fig. 10 gives the results in Table S1. Those results show that the effects of free convection are dominant, and so the convective conductance ($G_{ambient,\ conv}$) of the cones should increase during the initial part of a thermogenic event, reach a peak value at the time of the maximum temperature difference, and then decline as that temperature difference decreases. Since this maximization is not seen in the results, it appears that the major resistance to water loss is due to that of the cone (with parallel losses through the stomata and the cuticle) rather than that of the air flow.

Table S1. Estimated ratio of the combined (forced and free) convection heat transfer coefficient to the forced convection coefficient for different temperature differences during a thermogenic event. The forced and free convection heat transfer coefficients were calculated for laminar flow over a vertical flat plate using the conditions of the test for the cone in Fig. 10.

Temperature difference between cone and air	$h_{combined}/h_{forced}$
0	1.0
2	13
4	15.5
6	17.2
8	18.4
10	19.5

Loss of water and dry mass during thermogenic events: To maintain a constant percentage of water in the sporophylls across thermogenic events, i.e. ~ 63% in the sporophyll tests, the sporophylls must, since they start with more water than dry mass, lose more water mass per event than dry mass. To see if that actually occurred we analyzed the events of Table 2 in the main text. Because the aerobic respiratory processes of the cones utilizes one mole glucose (dry mass) for each six moles of O_2 taken in, the loss of dry mass can be determined from the total intake of O_2 per event. This dry mass loss was evaluated for each event by integrating $\dot{V}_{O_2}$ between the inter-event minimal points bracketing each event, and converting the resulting total uptake of moles of O_2 into grams of glucose used by the cone. Similarly, for each mole of O_2 taken in, one mole of H_2O is produced by plant respiration, so the total production of water by respiration could be determined for each event. The net water loss from the cone during each event was then determined as the difference between the total water mass leaving the respiratory cylinder (obtained by integrating $\dot{m}_{H2O}$ over each event) and the total production of water through respiration in the cone. Doing so, on average over all events, the ratio of the net water mass loss to total water production was ~ 3.9 (± 1.2), and the ratio of the net water mass loss to net dry mass loss was 1.8 ($\pm$ 0.54). The latter number is very similar to the average value of that ratio (1.7) needed to maintain the constant water percentage of 63% seen in the sporophyll experiments.

Reference

Incropera F, DeWitt D (1996) 'Fundamentals of Heat and Mass Transfer.' (John Wiley: New York).