

# Methods matter for assessing global variation in plant thermal tolerance

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Lancaster and Humphreys (1) attempted to identify drivers of plant thermal tolerances by analyzing a newly compiled database of heat and cold tolerances. Lancaster and Humphreys conclude that variation in thermal tolerances is attributable to a combination of phylogeny, geography, and local environment, and that the observed patterns “are not an artifact of measurement method” used to estimate tolerances. We applaud Lancaster and Humphreys’ efforts to compile and analyze global patterns in thermal tolerances, but we feel that it is necessary to highlight additional sources of methodological variation that could alter the authors’ analyses and conclusions about plant thermal tolerance macrophysiology.

Here, we focus on heat tolerances included within Lancaster and Humphreys’ database, which are generally estimated by 1) subjecting plant tissues to heat, 2) allowing tissues to recover, 3) quantifying damage, and 4) using damage to calculate some metric of heat tolerance. Methodological variation in any of these steps is common among studies and leads to differences in heat tolerance estimates that often render direct comparisons inappropriate. For example, increasing the duration of heat treatment from 5 to 60 min can decrease heat tolerance estimates by 5 °C (2), and increasing recovery time from 15 min to 24 h can increase heat tolerance estimates by 2 °C (3). Lancaster and Humphreys’ database combined heat tolerance estimates based on heat treatments ranging from minutes to many hours, and recovery times ranging from 0 to 11 d. Furthermore, heat tolerance metrics are typically calculated as the temperature causing a predetermined level of physiological damage. There is no standard, but heat tolerances are generally estimated as the temperatures causing 0–25% damage

(often termed  $T_{crit}$ ), 50% damage ( $T_{50}$ ), or ~100% damage (often termed  $T_{max}$ ).

Lancaster and Humphreys did not account for differences in steps 1–3 when analyzing their data and may not have adequately disentangled the effects of methodology and biogeography on plant heat tolerance. Lancaster and Humphreys did account for step 4, but surprisingly they found no differences between the metrics and combined them in their final analyses. This is despite the fact that these damage responses are inherently ordinal—e.g., the temperature causing 50% damage is always less than the temperature causing 100% damage.

There are several other potential problems that may complicate—or even invalidate—Lancaster and Humphreys’ analyses. Namely, Lancaster and Humphreys were inconsistent in choosing which metric to include (e.g., they included  $T_{max}$  from ref. 4 despite  $T_{crit}$  estimates being available), they did not include all available data from their sources (e.g., Cyperaceae records from ref. 5 were omitted), they disregarded factors known to influence thermal tolerances (e.g., sun vs. shade leaves in ref. 6), and they combined measures reflecting different physiological responses (e.g., carbon assimilation, respiration, photosystem II photochemistry) to heat.

Given these methodological issues, we suggest cautious interpretation of Lancaster and Humphreys’ results. Addressing these methodological issues may reveal that methods explain more than the reported 25% and 10% of variation in heat and cold tolerances, respectively. To advance our understanding of the “processes structuring global variation in heat and ... cold tolerances of plants,” methodological differences in estimating thermal tolerances need to be carefully considered.

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