How we measured drought and heat tolerance in Which Plant Where

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In the Which Plant Where plant selection tool, there are a number of species and cultivars for which we have additional information on drought and heat tolerance. This information is based on rigorous testing conducted in *state-of-the-art* climate-controlled glasshouses, both at Macquarie University and Western Sydney University.

WHAT WE DID

A total of 113 perennial plant species and cultivars, including both native and exotic to Australia and across five different growth forms (trees, shrubs, climbers, herbs, graminoids), were selected for the experiments. These species were a mixture of species commonly available in the Australian horticultural market, as well as uncommon species less readily available commercially and infrequently planted in urban areas.

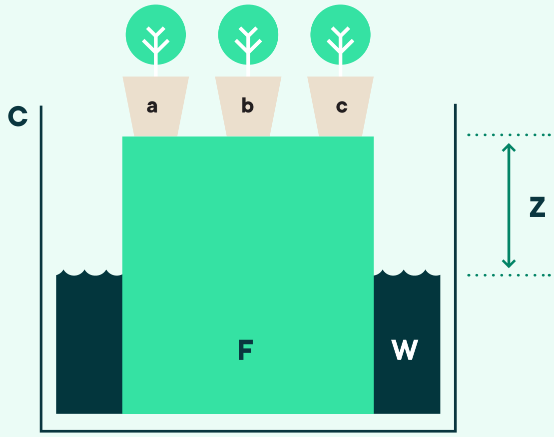
All plants used in the glasshouse experiments were obtained from commercial Australian nurseries as either tubestock, 140 mm or 200 mm pot size. The original soil/media from around the roots of each plant was carefully removed and plants were re-potted into 6 L pots (or 9 L pots for larger individuals) containing native potting mix (<30% sand/ coir, >70% screened composted pine bark; Australian Growing Solutions, Tyabb, VIC, Australia), 38 g of controlled release native plant fertiliser (Scotts Australia Osmocote Slow Release, Bella Vista, NSW, Australia), and a 1.25 g systemic insecticide and fertiliser tablet (Yates Confidor, Padstow, NSW, Australia). All plants were well watered using drip irrigation, and grown for 6-7 weeks in the glasshouses to allow for acclimation and the formation of new leaves. This reduced the chance that cultural practises in source nurseries would cause unexplained variation in the results. Plants were maintained at an average temperature of 27 °C, with a day time range of 21 – 34 °C. These temperatures were chosen to mimic warm, summer urban conditions that could occur in the major centres of Australia, but were not excessive or stressful temperatures for plant species in this study.

After approximately two months of acclimation, plants were assigned to one of four treatments; control (plants remained well-watered and grown at average summer temperatures), drought (plants were subjected to a controlled drought while growing at average summer temperatures), heat wave (plants were subjected to elevated temperatures while being well watered) and a combination of drought and heatwave (plants were subjected to drought conditions and elevated temperatures).

DROUGHT TREATMENT

For the plants in the drought treatment, a gradual drought was imposed to slowly dry the soil, simulating natural drought events in the field. Pots were placed in large plastic containers on top of a block of commercial florist foam (Fig. 1). The large container was then filled with water to just below the top of the florist foam. The gradual drought was then imposed by slowly decreasing the level of the water (z in Fig. 1) over a period of a month. Water was drawn down from the large container until volumetric water content (VWC) of the soil in pots reached ~10%, after which period this VWC level was maintained for three weeks to ensure that plants experienced an adequate level of stress from the drought.

FIGURE 1 Diagram of the drought imposition method, where *C* plastic container, *F* floral foam, *W* water, a,b,c pots with nylon mesh at the bottom and filled with potting mix, and z water table depth. Figure from Marchin *et al.* 2020.



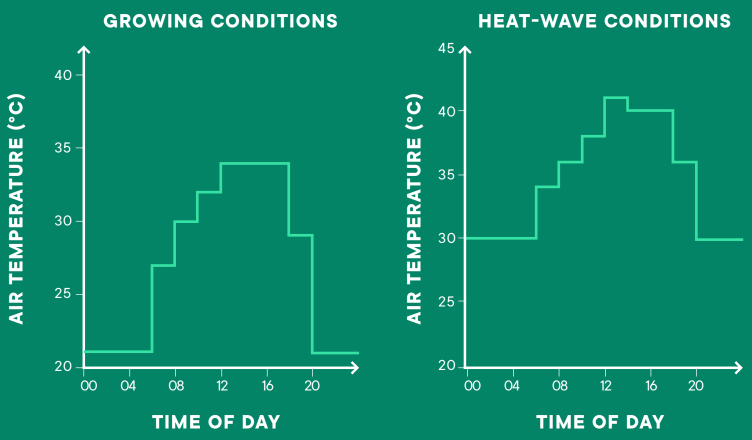


FIGURE 2 Daily temperature profile of the growing conditions (left panel) and heat wave conditions (right panel) for the glasshouse experiments.

HEATWAVE TREATMENT

Plants in the heatwave treatment were exposed to a six day heatwave after the drought period. During the heatwave, the average temperature of the glasshouse was 35 °C (8 °C warmer on average than the control temperature, Fig. 2), reaching a maximum of 41 °C. Air humidity was kept high during the heatwave regime to avoid the confounding effects of vapour pressure deficit and heat on plants.

WHAT WE MEASURED

In order to formulate drought and heat tolerance information, the Which Plant Where team measured a range of traits on the experimental plants.

DROUGHT TOLERANCE TRAITS

LEAF AREA: Plants with smaller sized leaves have been shown to have lower vulnerability to drought due to more efficient water transport. In general, large leaved species are most common in wet environments whereas small leaved species are most common in hot, arid environments. To measure leaf area, we collected 1-5 mature leaves from each plant and scanned them using a flatbed scanner. We then used image analysis software to calculate individual leaf area.

LEAF MASS PER AREA (LMA): Leaves with high LMA are often associated with stiffer cell walls that are more physically resilient to water loss during dry periods. LMA is calculated as the dry mass of a leaf divided by its area. To measure LMA, we collected 1-5 mature leaves for each plant and scanned them using a flatbed scanner. We used image analysis software to calculate individual leaf area. These leaves were then oven dried at 70 °C for at least 72 hours before they were weighed to obtain dry mass, using a digital scale.

LEAF THICKNESS: Thick leaves are often associated with stiffer cell walls that are more physically resilient to water loss during dry periods. Leaf thickness was measured on 1-5 mature leaves for each plant. Thickness measurements were taken at three points along each leaf using digital callipers, near the tip, middle and bottom, being careful to avoid any major veins, and averaged for each leaf.

LEAF DRY MATTER CONTENT (LDMC): LDMC is the ratio between leaf dry mass divided by its fresh mass. Leaves with high LDMC are often associated with stiffer cell walls that are more physically resilient to water loss during dry periods. LDMC was measured on 1-5 mature leaves per plant.

TURGOR LOSS POINT (TLP): Leaf water potential at turgor loss point (TLP, or wilting point) is the water potential value at which a leaf loses turgor, and is strongly related to drought tolerance (Fig. 3). Species with more negative TLP have higher leaf-level drought tolerance. Up until now, TLP measurements have typically been conducted using pressure-volume curves, which often take several hours to several days to complete for a single species. However, for the Which Plant Where experiments, we used a relatively new technique which measures osmotic potential (a trait strongly related to TLP) and gives results within 30 minutes. Briefly, 1-5 leaves were removed from each plant and hydrated in water for three hours. A 40 mm diameter disk was then cut out of each leaf and frozen in liquid nitrogen for two minutes. Leaf discs were then allowed to thaw at room temperature in a humidified bag for 10 minutes before being punctured 50-70 times with fine tipped forceps. Osmotic potential values were then measured on the leaf discs using a dewpoint hydrometer, with these values being converted to TLP values, using established equations (Bartlett et al., 2012a).

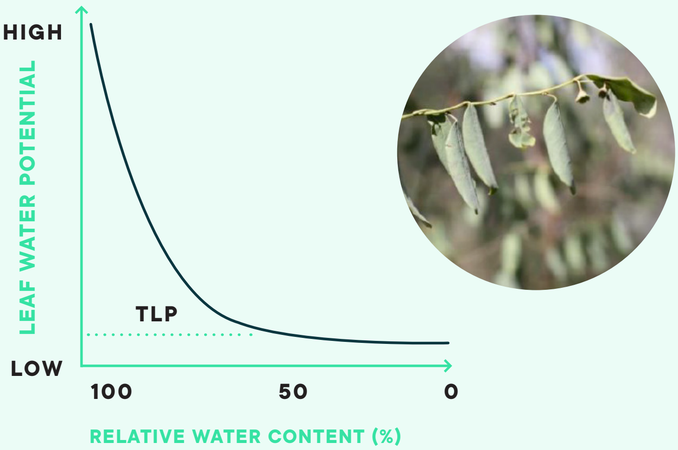


FIGURE 3 Graph showing the point where TLP is reached and (insert) leaves that have reached their TLP. Graph modified from Bartlett *et al.*, 2012b.

HEAT TOLERANCE TRAITS VISUAL ASSESSMENT OF DAMAGE: One of the most noticeable signs that a plant is suffering from heat stress is the visible browning and desiccation of leaves as cells begin to die. We assessed the effect of the heatwave on plants by visually estimating the percentage of leaf desiccation at the whole plant level after the final day of the heatwave.

DARK-ADAPTED CHLOROPHYLL FLUORESCENCE (Fv/Fm): Fv/Fm represents a measure of how 'healthy' green leaves of a plant are. During photosynthesis, light enters the chloroplasts in leaves, but not all of the light energy can be used by the plant. This unused light energy is released back as red light and this is known as fluorescence. This fluorescence can be measured using a Pulse Amplitude Modulated (PAM) fluorometer, which emits a high intensity beam of light onto a leaf surface and measures the amount of light reflected back (fluorescence). In order to take this measurement, plants were first dark adapted for 30 minutes to allow all of the reaction centres in the chloroplasts to be ready to receive light. 'Fo' is the background level of fluorescence when the plant has been kept in the dark (Fig. 4). A strong pulse of light is then fired onto the leaf by the PAM machine, saturating all the reaction centres. 'Fm', or maximal fluorescence, is then measured when all the reaction centres have been saturated and the excess light energy is emitted. Fv/Fm is then calculated as variable fluorescence (which is Fm – Fo) divided by Fm (Fig. 4). High values of Fv/Fm indicate that the plant is healthy with healthy photosynthetic machinery. Low values of Fv/Fm, however, indicate that a plant may be stressed as the photosynthetic machinery may be damaged.

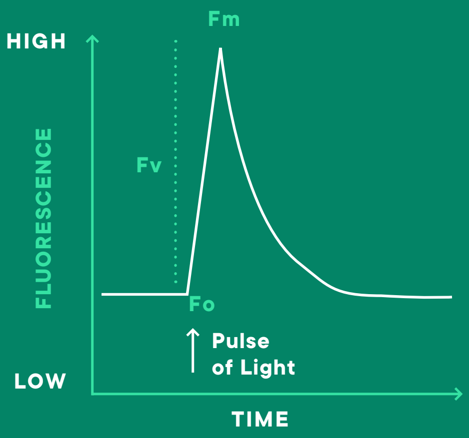


FIGURE 4 Diagram showing a typical fluorescence curve and the relationship between Fm, Fo and Fv.

LEAF CRITICAL TEMPERATURE (Tcrit):

Tcrit marks the temperature where the photosynthetic machinery of a leaf starts to fail and cell death occurs. We measured Tcrit on five individuals of all species by placing a mature leaf on top of a thermoelectric heater and raising the leaf temperatures from 30 °C to 60 °C at the rate of 1 °C per minute. Fluorescence of the leaf was measured with the PAM machine, with the exact temperature at which leaves began to die marked by the sharp increase in fluorescence as photosynthetic machinery is damaged (Fig. 5). Tcrit can be used to rank species based on thermal tolerance, with plants from warmer regions found to have higher Tcrit values.

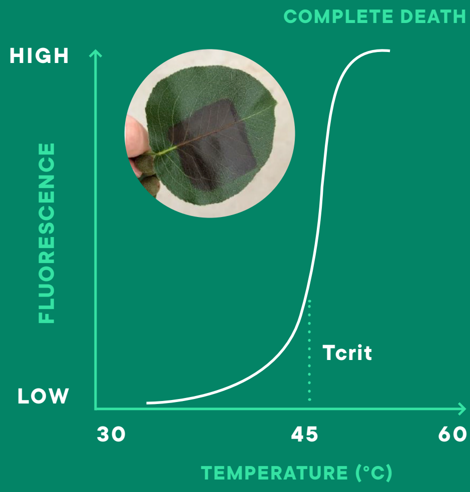


FIGURE 5 Graph showing the point where Tcrit is reached and (insert) the aftermath of Tcrit testing on a leaf. Graph modified from Knight & Ackerly 2002.

DROUGHT TOLERANCE CLASSES

Drought tolerance classes were calculated by conducting a principle components analysis (PCA) on the drought tolerance traits (LMA, leaf area, leaf thickness, LDMC, TLP). A principle components analysis essentially takes data that would ordinarily be multi-dimensional (in this case our data would be in five dimensions because we measured five traits) and represents this in two dimensions, preserving as much information as possible. In this analysis, each species is represented by a single point, with points clustering together in space if species have similar traits. PCA analysis provides a powerful and impartial means to understand how species cluster together based on similarities and dissimilarities between their traits.

After conducting a PCA analysis on our data, we found that our study species clustered into three distinct groups, meaning the species in each cluster were more similar to each other than species in other clusters, based on their drought tolerance traits (Fig. 6). Further analysis revealed which traits each cluster of species shared with each other. As it turns out, our computer-generated clustering matched perfectly with our knowledge of drought strategies from the scientific literature. This allowed us to define three distinct groups of 'drought strategy' based on particular combinations of traits:

DROUGHT TOLERATOR: Species in this cluster had high LMA and LDMC, thick leaves and low (more negative) TLP, characteristic of a drought tolerator strategy.

DROUGHT AVOIDER: Species in this cluster had low LMA and LDMC and high (less negative) TLP, characteristic of a drought avoider strategy. Many of these species lost leaves during drought and can be considered drought deciduous.

INTERMEDIATE (TOLERATOR/AVOIDER): Species in this cluster had a combination of tolerator and avoider traits, namely, high LDMC, large, thin leaves, low LMA and high (less negative) TLP.

For more information on the difference between drought tolerators and drought avoiders, please refer to the blog post titled 'drought tolerators vs drought avoiders'.

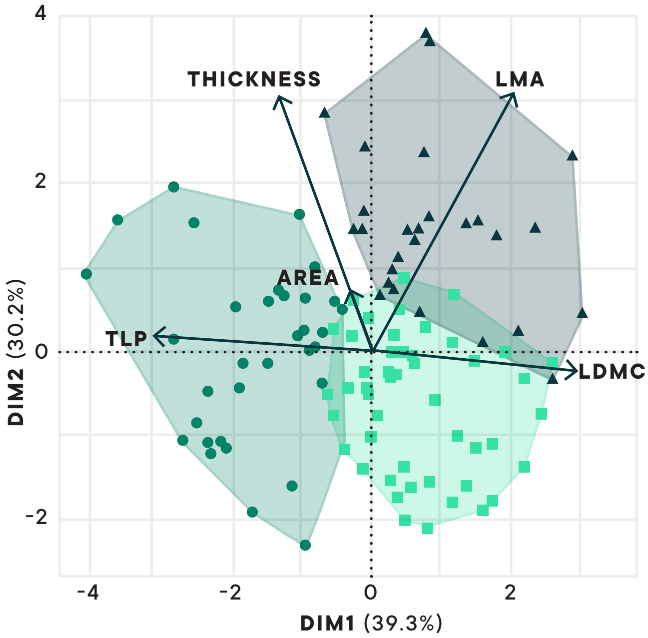


FIGURE 6 Visual representation of the PCA results for the drought tolerance traits, with species colour coded into their respective groups. The directions of the arrows represent increasing values for that trait. Modified from Tabassum *el at.*, 2021.

HEAT TOLERANCE RANKINGS

Heat tolerance rankings were calculated by weighting the heat tolerance traits (60% visual damage, 20% Tcrit, 20% Fv/Fm), which were then used to classify species into four categories (sensitive, intermediate, intermediate/tolerant, tolerant). These categories were determined based on the distribution of values for the heat tolerance traits.

CAVEATS

It is important to note that the heat rankings on the Which Plant Where selection tool are based on relative comparisons between the study species. As more and more species are tested experimentally in the glasshouse, it is likely that these rankings may change as we gather more data. Furthermore, plants were only subjected to one heatwave treatment, with additional testing underway to assess species' responses to multiple heatwaves. Nonetheless, rankings like these may be used to validate existing knowledge about tolerances of horticultural species and also identify up and coming opportunity species that may be well suited to urban planting.

REFERENCES

Bartlett, M.K., Scoffoni, C., Ardy, R., Zhang, Y., Sun, S., Cao, K., Sack, L., 2012a. Rapid determination of comparative drought tolerance traits: using an osmometer to predict turgor loss point. Methods in Ecology and Evolution, 3, 880-888. https://doi.org/10.1111/j.2041-210X.2012.00230.x

Bartlett, M.K., Scoffoni, C., Sack, L., 2012b. The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. Ecology Letters, 15, 393-405. https://doi.org/10.1111/j.1461-0248.2012.01751.x

Knight, C.A., Ackerly, D.D., 2002. An ecological and evolutionary analysis of photosynthetic thermotolerance using the temperature-dependant increase in fluorescence. Oecologia, 130, 505-514. https://doi.org/10.1007/s00442-001-0841-0

Marchin, R.M., Ossola, A., Leishman, M.R., Ellsworth, D.S., 2020. A simple method for simulating drought effects on plants. Frontiers in Plant Science, 10, 1715. https://doi.org/10.3389/fpls.2019.01715.

Tabassum, S., Ossola, A., Marchin, R.M., Ellsworth, D.S., Leishman, M.R., 2021. Assessing the relationship between trait-based and horticultural classifications of plant responses to drought. Urban Forestry & Urban Greening, 61, 127109. https://doi.org/10.1016/j.ufug.2021.127109.