Variance in plant functional traits in *Eucalyptus baxteri* and *E. obliqua* – a pilot study to assess utility for inferring climate vulnerability

Introduction

Dieback is affecting forests of the stringybark eucalypt species, *Eucalyptus obliqua* and *E. baxteri*, in the Mount Lofty Ranges region of South Australia. This pilot project examined variation in plant functional traits across eight sites spanning a gradient of mean annual rainfall of approximately 700–1100 mm. The aim was to test the utility of functional traits for inferring relative climate vulnerability and to inform the level of replication needed in sampling within and between individuals and sites to capture variation.

Methods

We measured key plant functional traits (relating to leaf temperature balance, water use efficiency, maximum photosynthetic rate and climatic tolerances) in stringybarks sampled across climatic gradients. Traits included were:

1. Stem specific density (SSD; negatively correlated with lumen fraction and linked to hydraulic capacity, tolerance to low xylem water potential, and resistance to cavitation; Preston et al. 2006)

2. Leaf area (linked to leaf temperature and therefore photosynthetic rates and need for evaporative cooling; Wright et al. 2017)

3. Leaf length:width ratio (linked to boundary layer resistance and therefore rates of convective and evaporative heat loss; Pérez-Harguindeguy et al. 2016)

4. Specific Leaf Area (SLA; fresh leaf area divided by dry mass; linked to resource investment and maximum photosynthetic rate; Lowry and Smith 2018).

We sampled three–five individual trees per site and collected 1x stem samples and 5x leaf samples per individual for analysis. Stem samples were taken from young wood sections approximately 1.5 cm diameter (Pérez-Harguindeguy *et al.* 2016), selected straight sections with no branching where possible. Young but fully mature sun leaves with minimal insect damage were selected as leaf samples.

To calculate SSD, we calculated fresh volume as the cross-sectional area of the stem segment multiplied by its length and also, for comparison, we measured the volume by placing each sample into a measuring cylinder full of water. The displacement of water when the sample is added (final volume- initial volume) gave the volume of the sample. Samples were then air dried and weighed on a 0.1 mg balance after oven drying at 100°C. SSD is equal to oven dry mass divided by fresh volume.

To calculate leaf area, length:width ratio and SLA, we pressed and dried leaf samples and scanned them with a scale bar. ImageJ was used to measure one-sided leaf area, length and width. A recent pilot analysis in a set of eucalypt species showed that that amount of eucalypt leaf shrinkage is small (~10%) and consistent across samples (Morgan et al. 2021). Therefore, the use of dry leaf area for SLA is valid as it is not biased by shrinkage, and can be converted to fresh leaf area with minimal error. Leaves were subsequently oven dried at 70°C for 72 hours before being weighed on a 0.1 mg balance. SLA was calculated as dry leaf area divided by oven dry mass.

Results

Significant variation was observed across all four traits measured (leaf area, leaf length:width ratio, SLA and SSD). Variation among species and sites across a rainfall gradient would be useful to explore across a wider sampling of sites and individuals to infer drought vulnerability. Variance calculations based on sums of squares show important variation split among species, sites, and individual trees, with high within-individual variance in leaf traits suggesting that sampling of multiple individuals and leaves is required to compare differences at site and species level.

SLA and L:W ratio were higher in *E. obliqua* than *E. baxteri*, while SSD was lower. While site-to-site variation was high and somewhat site-specific, there was an overall increase in leaf area and SLA along the rainfall gradient, whereas L:W ratio and SSD did not show any overall pattern with rainfall across these sites. Site-level characteristics other than macro-rainfall are likely to explain some of the variation, for example, the relatively low SLA values for both species at the Bradwood site, which is high rainfall (>1 m MAP) but has soil consisting of shallow sand over rock, thus limited in both nutrients and dry season moisture availability, both of which may limit leaf size (Wright et al. 2017). It is likely that site-specific influences would be less prominent in assessing trends across a wider range of sites. SSD shows promising variation but needs to be measured for more individuals and sites to better assess climatic patterns.

References

Morgan, R., Martín‐Forés, I., Leitch, E. & Guerin, G. (2021) Assessment of protocols and variance for specific leaf area (SLA) in 10 *Eucalyptus* species to inform functional trait sampling strategies for TERN Ausplots. The University of Adelaide. Dataset. https://doi.org/10.25909/14197298

Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., et al. (2016) New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany* 61, 167-234.

Preston, K.A., Cornwell, W. K. & DeNoyer, J.L. (2006) Wood density and vessel traits as distinct correlates of ecological strategy in 51 California coast range angiosperms. *New Phytologist* 170, 807-818.

Wright, I.J., Dong, N., Maire, V., Prentice, I. C., Westoby, M., Díaz, S., et al. (2017) Global climatic drivers of leaf size. *Science* 357, 917-921.

Lowry, C.J. & Smith, R.G. (2018) ‘Chapter 5 - Weed Control Through Crop Plant Manipulations, in Non-Chemical Weed Control’, Elsevier Inc, pp. 73–96.