Intraspecific variation in xylem anatomical traits related to drought resistance of Cypress Pine

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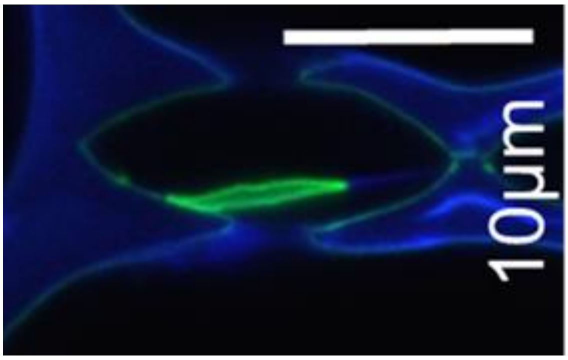
One of the biggest limitations to research on xylem anatomy is the time consuming preparation, imaging and analysis. The stigma surrounding biological specimen preparation for electron microscopy is palpable. TEM samples require several concentrated days delicately guiding samples through fixatives and solvents, resin infiltration, polymerisation only to be met with an ultra-microtome. Whilst the resolution is incomparable, is it always necessary?

In tree species, xylem is a highly specialised network of compartmentalised conduits responsible for the transport of water from the tree roots to the leaves. In many tree species, drought conditions can generate tension within the water column of the xylem, that can become sufficiently strong to cavitate and cause emboli ('air bubbles') within the conduits, progressively leading to complete hydraulic failure and plant death. Cavitation resistance in trees, is most often linked to anatomical data, such as conduit size, density and wall thickness derived from high-throughput sample preparation and imaging techniques, primarily optical microscopy. However, one of the primary determinants of drought resistance in trees, bordered pits, remains relatively unexplored due to their size. Bordered pits permit the free flow of water between tracheids, while preventing the spread of air between embolised and water filled tracheids. In effect, they act as safety valves in the plant hydraulic system.

The aim of this PhD project is to determine the intra-specific variability of hydraulic traits in several populations of Australian native, *Callitris glaucophylla* ("White Cypress") growing across an environmental gradient. Several microscopy techniques are employed to acquire 'big data' for quantitative measurement and comparison of hydraulically-linked anatomical traits responsible for drought resistance at a plant, population and species level. One such technique takes advantage of confocal laser scanning microscopy (CLSM) as a quantitative and high-throughput technology that requires minimal sample preparation. A novel method is developed to observe and measure the depth of the bordered pit chamber and the thickness of its membrane, in fresh tissue. Only previously observed using TEM, this study doubled the previously reported sample size of pits in the branches and roots in several tree populations. The number of samples observed and the increased certainty of spatial location within the membranes was prioritised over the higher resolution attainable by TEM. Observations are correlated with their vulnerability to cavitation and environment and the statistical significance of the trade-off between lower resolution, and larger sample size is evaluated.



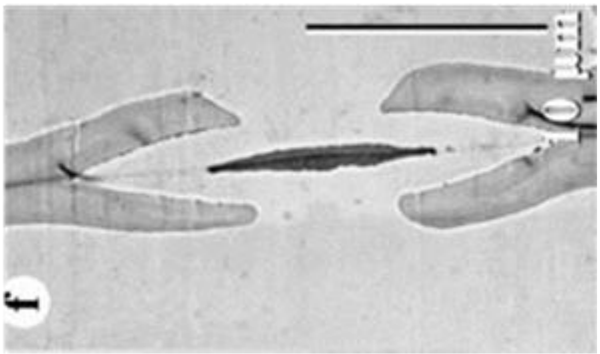


Figure 1. Comparison between CLSM (produced from this study) and TEM (Hacke & Jansen, 2009) images of pits in the roots of conifer species.