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A Multiscale Approach to Understanding Calcium Toxicity in Australian Proteaceae

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The Proteaceae are a family of plants predominantly distributed within the Southern hemisphere, with >600 species in West Australia alone. They display staggering diversity and endemism but are highly restricted in their distibution by soil quality and type. In order to understand the role of calcium in influencing distribution patterns, we are sampling plant species that are soil-indifferent (few, grow across all environments) and calcifuge (common, grow in acidic, nutrient poor soils). From this, the distribution, form, and amount of calcium in leaves is being investigated at the cellular level using a variety of correlative techniques, including optical-based microscopies, Raman spectroscopy, X-ray microscopy, and quantitative EDS X-ray microanalysis.

For optical based imaging and analysis, chemically fixed samples are either sectioned (100 um thickness) using a vibratome or embedded in ultra low viscosity resin and microtomed (1 um thickness). Samples are subsequently imaged using brightfield and ultraviolet techniques, and analysed via Raman spectroscopy (WITec alpha 300RA+). For X-ray microscopy (Xradia Versa XRM-520), chemically fixed samples are incrementally scanned over 360 degrees to produce 3-dimensional data sets, which are then reconstructured and quantitatively analysed using a variety of software packages. For quantitative EDS X-ray microanalytical mapping, samples are rapid frozen on pins and cryoplaned to produce a flat, transverse cross section. Element distributions are mapped using a cryoSEM (Zeiss Supra FESEM) fitted with an 80 mm EDS detector and quantified by AZtec Software (Oxford Instruments).

Paired optical and ultraviolet imaging allows for the determination of the overall structure of the leaf, including visualizing distinct tissue layers, stomates, and crypts (Figs. 1 and 2). Imaging of resin embedded sections reveals a higher level of structural detail at the cellular level, including observation of intracellular crystals within mesophyll cells (Fig. 3), which can then be characterized using Raman spectroscopy. X-ray microscopy allows for 3-dimensional visualization and quantification of these crystals within a large area of the leaf (Fig. 4). The presence, abundance, size, shape, and composition (calcium or silicon based) of these crystals varies extensively between plant species and with regard to their location within the leaf tissues. Elemental mapping (Figs. 5 and 6) and quantitation is providing further insight into the cellular distribution of calcium within leaves. The arrangement and concentration of calcium within different cell layers also varies immensely between plant species. These data suggest that calcium regulation, storage, and toxicity in the Proteaceae is not a conserved, family trait.

References:
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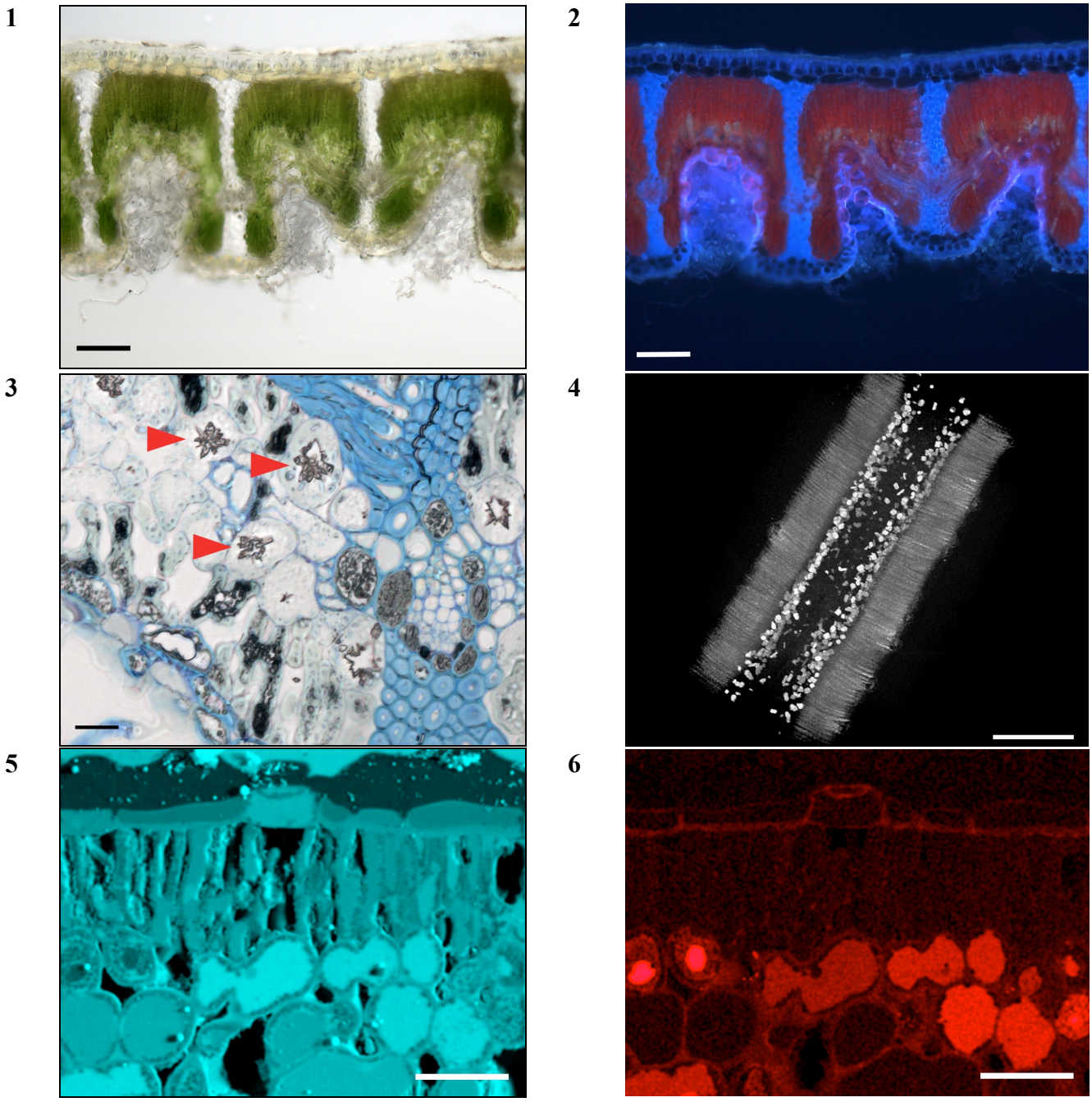

Figures 1, 2. Paired optical micrographs of a vibratome section from a *Banksia prionotes* leaf, imaged using 1) brightfield, and 2) ultraviolet techniques. Scale bars = 100 um.

Figure 3. Optical micrograph of a resin-embedded section from a *Banksia menziesii* leaf, highlighting cellular structure and the presence of calcium oxalate crystals (red arrows). Scale bar = 20 um.

Figure 4. Single image from a 3-D tomogram, showing an array of crystals distributed throughout the inner tissues of a *Petrophile macrostachya* leaf. Scale bar = 250 um.

Figures 5, 6. Paired qualitative EDS X-ray maps from a *Persoonia comata* leaf, showing 5) oxygen, and 6) calcium distributions. Scale bars = 100 um.

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