# Preparation of plant tissue slices for investigation into fungal colonisation of roots.

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## Introduction

Interactions between plants and fungi play a crucial role in ecosystem functioning, influencing nutrient cycling, plant health, and resilience to environmental stresses. These interactions can be beneficial or detrimental to the plant host, depending on whether the fungi are mycorrhizal or pathogenic. Ectomycorrhizal fungi are a type of mycorrhizal fungi that enhance tree nutrient uptake by forming a protective 'mantle' around roots and a nutrient exchange matrix known as the 'Hartig net' between root cells. These structures are critical for facilitating the mutual exchange of nutrients. The thickness of the mantle and depth of the Hartig net can be used as indicators for how effectively ectomycorrhizal fungi colonise plant roots. In contrast, pathogenic fungi have deleterious effects on plant health by disrupting root structures, impairing nutrient acquisition and competing with mutualistic fungi. Therefore, establishing effective methods to visualise and measure fungal colonisation of plant tissues is essential for understanding both mutualistic and pathogenic relationships between plants and fungi and their broader ecological significance.

## Procedures

*Sample preparation:* Roots were selected by visual observation of fungal colonisation or, if not visible, predicted areas of fungal colonisation. Clean roots were removed from the plant and fixed to preserve cellular integrity in 4% (w/v) paraformaldehyde. After fixation, samples were washed thoroughly with phosphate-buffered saline to remove residual fixative. The roots were then embedded in 6% agarose, ensuring the roots were positioned parallel to the base to obtain uniform cross-sections.

*Sample sectioning:* A blade was used to cut out an acrylamide block containing a single root and the end of the root was sliced off to create parallel cross-section. With the root aligned vertically, the block was secured to the metal tissue mount with cyanoacrylate adhesive. The sample was then sliced at a frequency of 80 Hz, amplitude of 2.00 mm, speed of 0.25 mm/s, and a thickness of 25-30 µm. The cut sections were then placed into phosphate-buffered saline to maintain hydration and preserve tissue integrity.

## **Product Focus:** 7000smz-2 Vibrating microtome


Our top of the range high precision, vibrating microtome (vibratome for short) is the tissue slicer of choice for preparations for visual patch clamping or high-resolution imaging.

Using our vibrating microtomes, research detailing sectioning for visual patching of neurological tissue, heart, lung, and tissue scaffolds have all been published. The **7000smz-2** vibrating microtome represents significant advances with higher precision at a lower cost.

| Application Parameters | |
|---|---|
| Amplitude | 2.0mm |
| Frequency | 80Hz |
| Advance Speed | 0.25mm/s |

For more information click visit https://campdeninstruments.com/products/7000smz-2-vibrating microtome

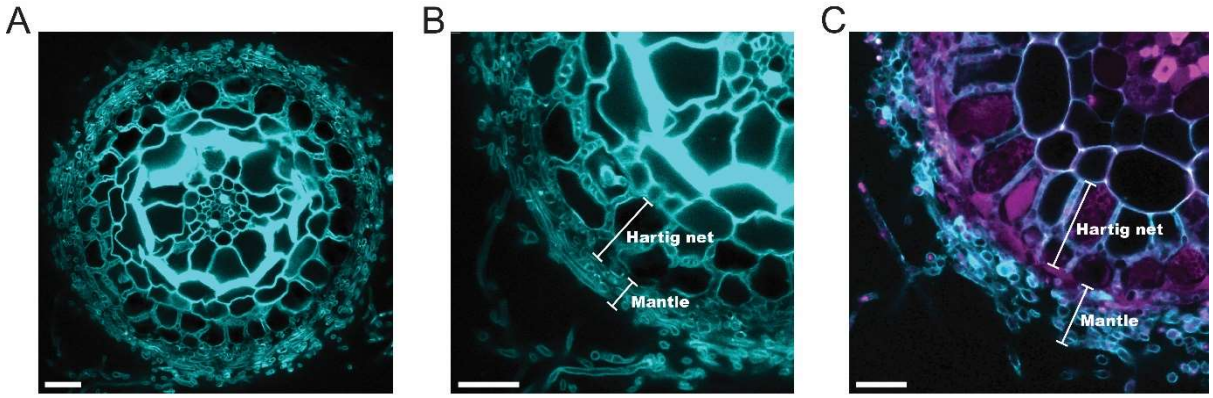


**Fig.1. Cross-sections of eucalypt root colonised with *Pisolithus microcarpus* to visualise the Hartig net and mantle.** **(A)** Full cross-section of a eucalypt root colonised with *P. microcarpus*. Stained with calcofluor white. **(B)** Quarter-section of euclaypt root colonised with *P. microcarpus* to visualise the Hartig net and mantle (as indicated). Stained with calcofluor white. **(C)** Quarter-section of euclaypt root colonised with *P. microcarpus* to visualise the Hartig net and mantle (as indicated). Stained with wheat germ agglutinin and propidium iodide. White scale bars indicate 20 µM.

## Specific examples

We have used samples prepared in this manner to visualise the mantle and Hartig net of *Pisolithus* species in the colonisation of eucalypt roots (Figure 1). More specifically, it has been used to elucidate the influence of environmental factors on colonisation of *Eucalyptus grandis* with various *Pisolithus* species (Plett, Kohler et al. 2015) and assisted in identifying genes that play a critical role in the establishment of symbiosis in these pairings (Plett, Miyauchi *et al.* 2023).

Additionally, we have also used this method to prepare samples of broccolini, broccoli and Gai-lan and visualise different stages of infection of *Plasmodiophora brassicae* (Liu, Quin *et al.*, 2020). The samples were collected at sixteen progressive time points, and identification through microscopy allowed us to determine early infection stages in each plant. This showed differential disease progression between the hybrid broccolini (Figure 2), and its parents broccoli and Gai-lan, providing a basis for further molecular work.

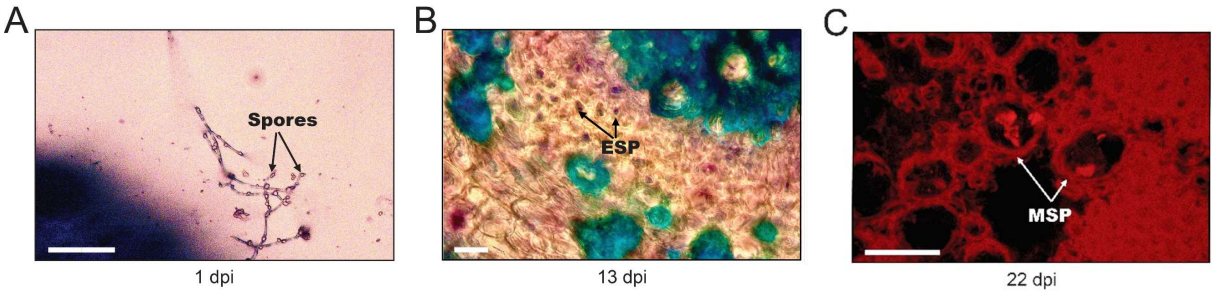


**Fig.2. Cross-section of broccolini root tissue at different time points showing *Plasmodiophora brassicae* infection.** **(A)** Spores attached to the surface of a root hair at 1-day post-inoculation (dpi), indicating the initial stage of infection. **(B)** Early secondary plasmodia (ESP) observed within a root cortical cell slice at 13 dpi, showing intracellular colonisation. **(C)** High-magnification view highlighting mature secondary plasmodia (MSP) at 22 dpi. The presence of these structures is indicative of advanced pathogen development and correlates with the formation of root galls characteristic of clubroot disease. White scale bars indicate 20 µM. All sections stained with methyl blue.

## References

Plett, J. M., et al. (2015). "The effect of elevated carbon dioxide on the interaction between *Eucalyptus grandis* and diverse isolates of *Pisolithus* sp. is associated with a complex shift in the root transcriptome." New Phytologist 206(4): 1423-1436. https://doi.org/10.1111/nph.13103

Plett, J. M., et al. (2023). "Speciation underpinned by unexpected molecular diversity in the mycorrhizal fungal genus *Pisolithus*." Molecular Biology and Evolution 40(3): msad045. https://doi.org/10.1093/molbev/msad045

Liu, L., et al. (2020) "Refining the life cycle of *Plasmodiophora brassicae*", Phytopathology, 110(10): 1704–1712. https://doi.org/10.1094/PHYTO-02-20-0029-R.