Microscopy methods

Both fresh and dry pollen has been successfully employed in TEM studies of pollen wall ultrastructure by various researchers. Fresh material will be used whenever possible, and herbarium material will be used if no fresh pollen is available. Fresh pollen will be taken from mature anthers and immediately fixed in 70% ethanol for later use.

Herbarium pollen will be cleaned and rehydrated using the enzyme-based method of Schols et al., (2004). Enzymatic treatment of dry pollen provides sufficient cleaning and rehydration of grains intended for use in microscopy, without the risk of collapse incurred with standard acetolysis (Martin, 1969). Enzymatic treatment uses a 1:200 dilution of Agepon in distilled water to gently rehydrate whole anthers, followed by grinding through fine phosphor-bronze mesh into small test tubes. The solution is then centrifuged at 2800 rpm for 3 minutes, decanted, and the tubes refilled with a solution of distilled water, citrate buffer, cellulase, and pectinase. This mixture is shaken periodically over 24 hours, then centrifuged and decanted. The clean and rehydrated pollen grains are stored in 70% ethanol for later use. Pollen prepared in this manner is appropriate for SEM, TEM, and LM.

Pollen for SEM and TEM will be further prepared at Iowa State University’s Roy J. Carver High Resolution Microscopy Facility following their preferred protocols. One graduate student will receive training in SEM preparation methods and assist microscopy facility staff in processing and imaging pollen. The same graduate student will assist in capturing appropriate TEM images, but all preparations for TEM will be completed by microscopy facility staff.

Pollen intended for LM will be stained with safranin and mounted on slides in silicone oil (Dow Corning Corporation). Silicone oil is preferred because pollen grains are less prone to swelling in this medium, and grains can be easily rotated under the cover slip to capture images from multiple angles. Measurements will be made at 1000x magnification using oil immersion on a Nikon Ni-U upright microscope, and high-resolution images will be captured with a microscope-mounted Nikon camera.

References

Martin, P.S. 1969. Pollen analysis and the scanning electron microscope. *In* Proceedings of the 2nd Annual Scanning Electron Microscope Symposium, 89–103. IIT Research Institute, Chicago.

Schols, P., K. Es, C. D’Hondt, V. Merckx, E. Smets, and S. Huysmans. 2004. A new enzyme-based method for the treatment of fragile pollen grains collected from herbarium material. *Taxon* 53: 777–782.