

Further Observations on the Nature of the Lemon Oil Glands by Confocal Microscopy and Allied Techniques

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Previous work revealed distinctive oil bodies situated within glandular sacs of the outer exocarp, the flavedo and numerous smaller oil bodies occurring in the cells of the interglandular regions [1]. Employing the stain FM 4-64, *in vitro* oil bodies retained the stain around the periphery suggesting the presence of a physical boundary [2]. A closer examination of the oil bodies *in situ* suggested they contain additional intriguing substructures. Further examination of the lemon rind was undertaken in an attempt to elucidate the substructures of the intra- and interglandular oil bodies.

A lipophilic stain, Nile red, in 0.67 M sucrose in 0.2M phosphate buffer at pH 7.0 was used to enhance the resolution of the oil bodies and the intensity of their fluorescence (Figs. 1-3). Further evidence of a physical boundary can be seen in the fluorescent and confocal images (Figs. 2-3). A heat shock of 5min in 55C water immersion of the flavedo tissue caused the oil bodies in the cell layers immediately below the epidermis to appear less distended than the untreated control (Figs.4-5).

Flavedo tissue was fixed in 4% glutaraldehyde in 0.67 M sucrose in 0.2M phosphate buffer at pH 7.0 and post-fixed in 1% osmium tetroxide in 0.67 M sucrose in 0.2M phosphate buffer and examined by SEM. Preliminary SEM examination of the gland-borne oil bodies provided further evidence of a boundary and the presence of substructure within the boundary (Figs.6-7). Work is continuing to confirm these early observations.

[1] D.A. Margosan et al., *Phyton* 69 (2001) 107

[2] D.A. Margosan and L.A. Aung, *Microsc. Microanal.* 8 (Suppl. 2), 2002

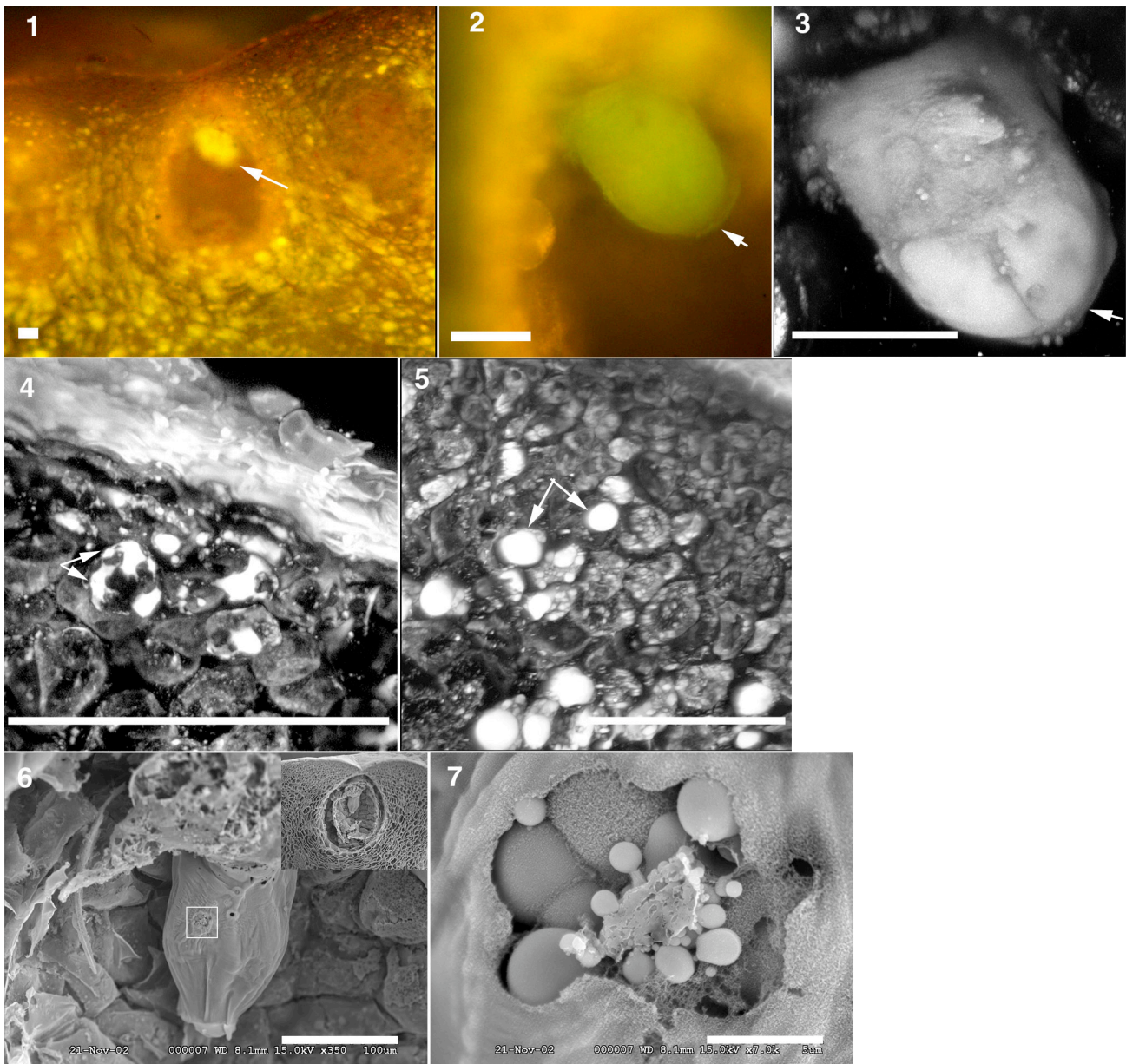


Fig. 1. Fluorescent image of lemon oil gland with oil body (arrow) stained with Nile red. Scale bar = 100 μ m.

Fig. 2. Magnification of oil body in Fig. 1. Outer boundary indicated by arrow. Scale bar = 100 μ m.

Fig. 3. Maximum projection of confocal images of oil body in Fig. 2. Outer boundary indicated by arrow. Scale bar = 100 μ m.

Figs. 4,5. Maximum projection of confocal images of oil bodies (arrows) in the sub-epidermal cells of heat-treated and control lemon flavedo, respectively. Scale bar = 100 μ m.

Fig. 6. SEM image of oil body in a lemon oil gland. Inset, lemon oil gland. Scale bar = 100 μ m.

Fig. 7. Magnification of area delimited by outline in Fig. 6. Numerous structures are visible in the oil body. Scale bar = 5 μ m.