Microsc. Microanal. 8 (Suppl. 2), 2002
DOI 10.1017.S1431927602106945

Application of Stain FM4-64 and Confocal Microscopy to Investigate The Nature of The Lemon, *Citrus limon* (L.) Burm. F. Oil Glands

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The rind of citrus fruit is composed of two distinct morphological tissues, the outer exocarp and the inner mesocarp. Lemon oil glands in the exocarp consist of several layers of radially flattened cells which surround thin-walled cells, in the early gland development. As the gland development progresses, the thin-wall cells break down leaving a cavity (sac) and becomes filled with oil bodies [1]. The liberation of the oils and their spread into tissues of the exocarp has been implicated in the development of the citrus disorder called oleocellosis. Furthermore, the lemon oil gland is known to be under positive pressure [2]. The relationships of the oils in the glandular oil bodies and pressure is not fully understood, nor is the nature of the oil bodies.

To test the hypothesis that the oils confined in oil bodies of the lemon glands are confined/encased by a boundary or membrane, FM4-64 stain and confocal microscopy were used. The stain will differentially dissolve in lipophilic portion of the outer membrane component, but will not be taken up by the hydrophilic portion of the membrane. FM4-64 was used to stain oil bodies in the glands of the exocarp, and oil bodies freed by freezing the rind tissues in liquid nitrogen and grinding the tissues into a fine powder. The powder was mounted in 1M sucrose solution and stained with FM4-64. Nile red, a lipophilic stain, also was used to stain lemon exocarp sections to determine the presence of lemon oil bodies in the glands and surrounding tissues (Fig. 1).

Optical sectioning by confocal microscopy of oil bodies from the ground tissues revealed that the stain was delimited to the outer margin of the bodies (Figs.2-14), evidence that the oils are contained by a boundary. The disruption of this boundary would release the oils into the gland or diffuse into the surrounding tissues of the exocarp to cause oleocellosis.

References

[1] D.A. Margosan et al., *Phyton* 69 (2001) 107.
[2] L.H. Aung et al., *Phyton* 69 (2001) 121.

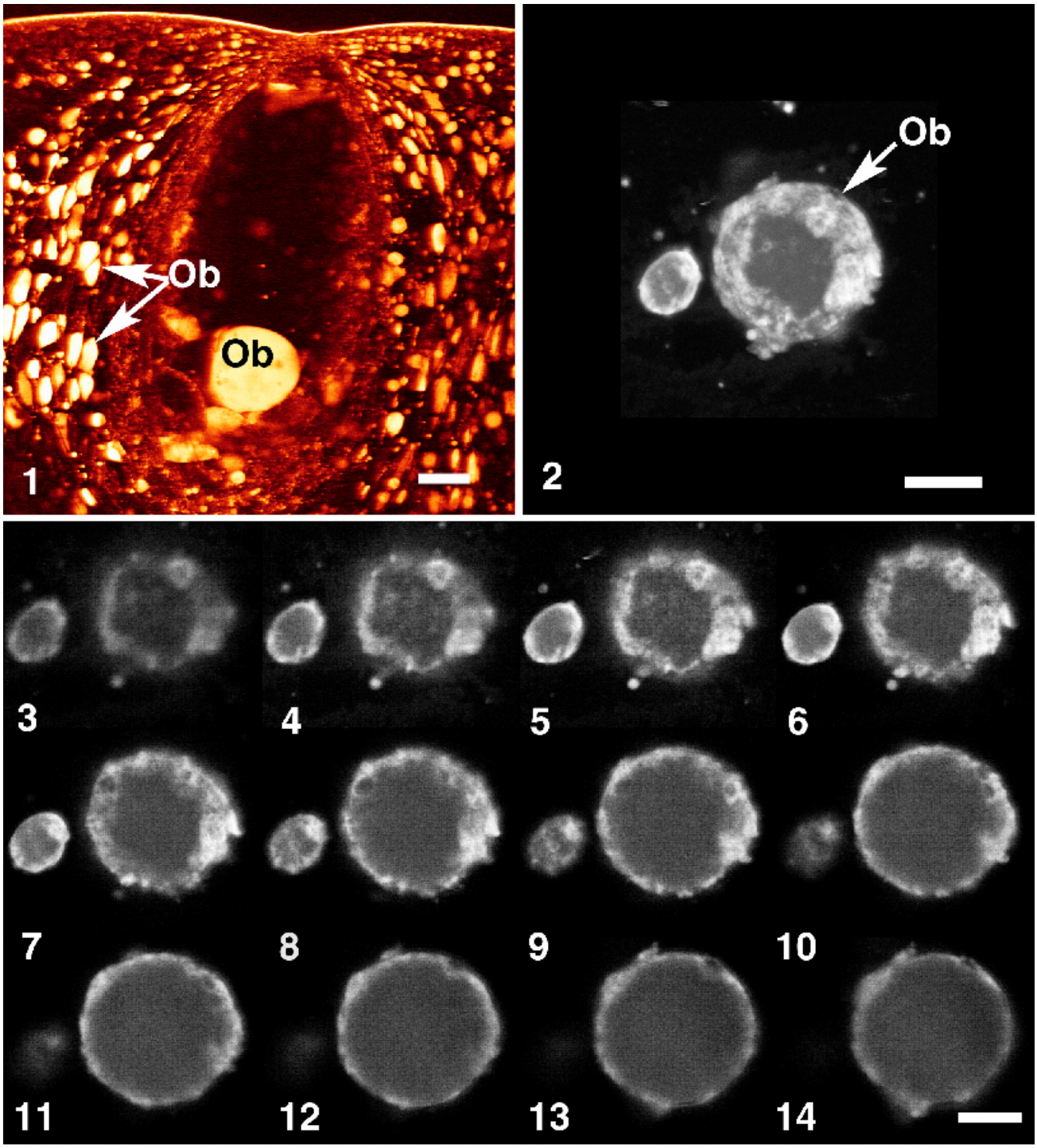

Fig. 1. Confocal image of lemon oil gland stained with Nile red. Ob white, oil bodies outside of lemon oil gland; Ob black, oil body in gland. Scale bar = 100µm.
Fig. 2. Maximum projection lemon oil body freed by freezing and grinding, and then stained with FM4-64. Ob, oil body. Scale bar = 10µm.
Figs. 3-14. Confocal series at 2µm intervals through the oil body in Fig. 2 The FM4-64 has not penetrated into the body but has remained in the boundary/membrane. Scale bar = 10µm.

https://doi.org/10.1017/S1431927602106945 Published online by Cambridge University Press