Is Your Citrus Safe?: An Introduction to Huanglongbing Disease

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Oak Crest Institute of Science

Background

Citrus Huanglongbing (HLB) is one of the most destructive citrus diseases worldwide. The phloem limited bacteria, *Candidatus Liberibacter asiaticus* (*CLas*), is vectored from the Asian citrus psyllid (*Diaphorina citri Kuwayama*) (figure 1) during feedings [3]. Typical symptoms observed with HLB are blotchy mottle and chlorosis of the leaves, and yellow shoots. Symptomatic fruits are small, asymmetric, and lopsided, with a bitter taste, and often contain aborted seeds, with a bent fruit showing color inversion [2]. Knowledge of the bacteria being phloem limited opens the doors for potential screening, identification, and early diagnosis of the HLB disease in citrus plants which can allow us to better understand this disease. Utilizing different processing, embedding, sectioning, staining, and electron microscopy (EM) imaging methods [1][4][5] can be beneficial in understanding how the disease affects citrus trees.

Figure 1: Adult Asian citrus psyllid (Diaphorina citri Kuwayama). Photograph by The California Department of Food and Agriculture.

Methods

Sampling
- Calamansi Tree (*Citrus microcarpa)*, sterile sheers, Formalin

Conventional and Microwave sample processing [1][4][5]
- Pelco Biowave Pro, Glutaraldehyde, Cacodylate buffer, Osmium tetroxide, Acetone series, Spurr's resin

Embedding [1][4][5]
- SPURR's resin; ERL 4206, DER 736, NSA, DMAE

Sectioning [1]
- Leica EM UC6 Ultramicrotome, glass knives, Diatome Ultra 45° diamond knife

Staining [1]
- Uranyl Acetate & Lead Citrate

Imaging
- Zeiss EM10C (1985) Transmission Electron Microscope (TEM)

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Motivation

To inform others of the destructive Huanglongbing disease as the future of our citrus trees and fruit are at risk. A goal of a future career in food and agriculture has allowed for the learning and practice of standard Histology and Electron Microscopy methods from this research.

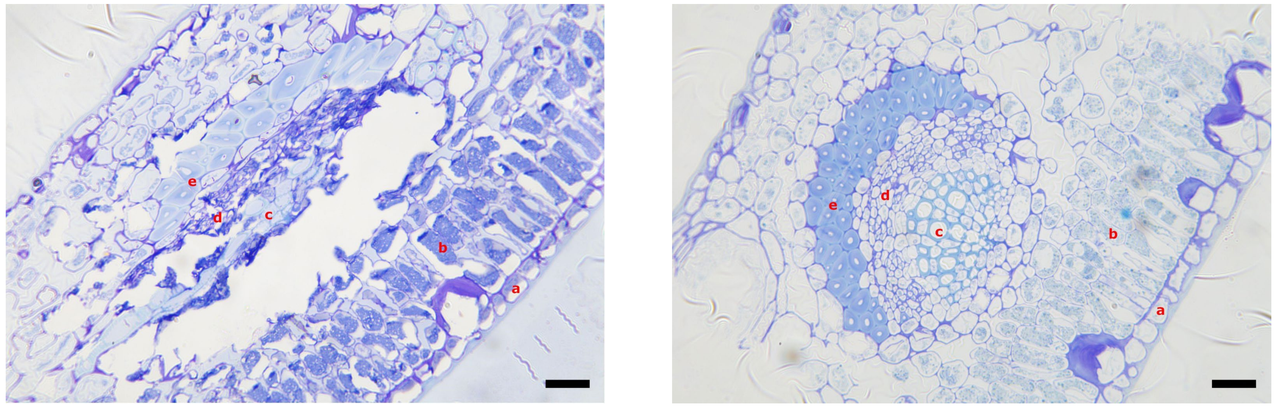

Figure 2: Light micrograph of the Upper Epidermis (a) and the Palisade Mesophyll (b), Xylem (c), Phloem (d) and Phloem Fibers Cap (e) of a Calamansi leaf. Sample 110_22 (left) was processed in the microwave. Sample 107_22 (right) was processed conventionally. Scale bar = 30 µm

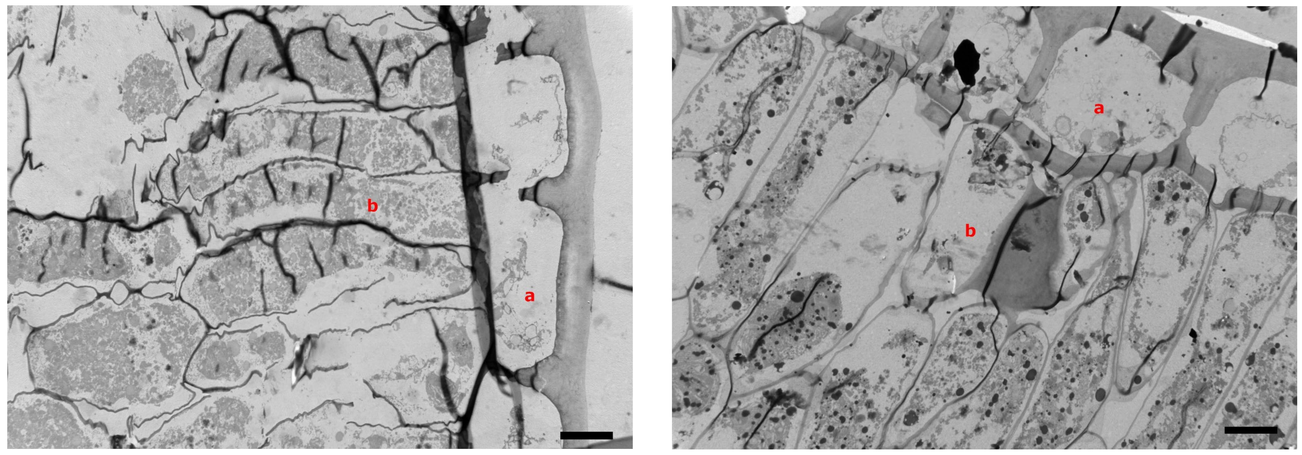

Figure 3: Transmission Electron micrograph of the Upper Epidermis (a) and the Palisade Mesophyll (b) of a Calamansi leaf. Sample 110_22 (left) was processed in the microwave. Sample 107_22 (right) was processed conventionally. Scale bar = 5 µm

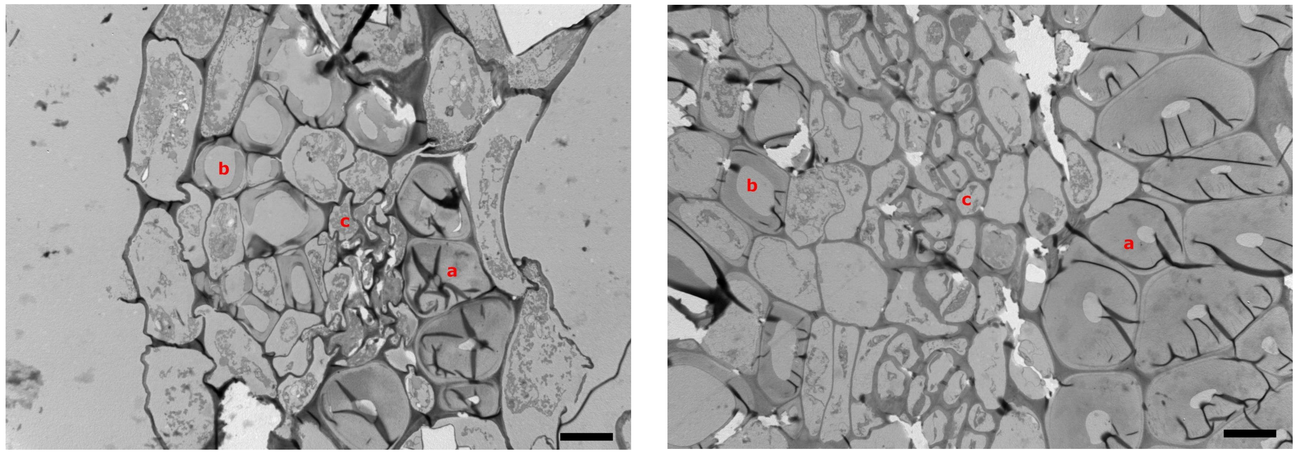

Figure 4: Transmission Electron micrograph of the Vascular System, Phloem Fibers Cap (a), Xylem (b), and Phloem (c), of a Calamansi leaf. Sample 110_22 (left) was processed in the microwave. Sample 107_22 (right) was processed conventionally. Scale bar = 5 µm

Results

Sampling
- Calamansi leaf obtained from a personal residence.
- 107_22 and 110_22 sampled from top portion of leaf near the tip utilizing a biopsy punch.

Processing
- Microwave processing saves time when compared to conventional processing (times excluding polymerization).
  - Microwave processing: ~ 2 h
  - Conventional processing: ≥ 71 h

Sectioning
- 350 nm sections were cut with glass knives for light microscopy to assess the quality of the processing methods then cut at 70 nm for EM.

Imaging
- Light microscopy micrograph of 110_22 shows a tear in the section where vascular bundle has collapsed while 107_22 looks whole (Figure 2). The upper epidermis and palisade mesophyll of 107_22 looks wrinkled and hollow while 110_22 looks to have a clear structure (Figure 3). The vascular system of 107_22 has collapsed in the phloem region and is also shrunk in comparison to 110_22 (Figure 4).

Discussion

Animal tissues benefit from microwave processing due to being a faster method with similar results to conventional processing. Although, plant tissues do not share this similarity and instead benefit from conventional processing. A speculation may be that this is due to the rigid cell walls plant cells have. The contrast from the staining and osmium tetroxide step seems to be uniform between the two methods.The addition of potassium ferrocyanide may bring additional contrast to the micrographs. Other processing methods, such as Freeze Substitution, may bring different and interesting results. My colleagues and I have reached out to city officials and the California Citrus Pest and Disease Prevention Program in hopes of locating infected trees to process and compare with healthy trees.

References

[1] Bozzola, J. J., & Russell, L. D. (1999). Electron Microscopy : Principles and Techniques for Biologists (2nd ed.). Jones and Bartlett Publishers.

[2] Liu, Y., Xue, A., Ding, L., Hao, Y., Liu, H., Cui, M,. Liu, L., Nie, Z., Luo, L. (2020). Direct Identification and Metabolomic Analysis of Huanglongbing Associated with Candidatus Liberibacter spp. in Navel Orange by MALDI-TOF-MS. Analytical and Bioanalytical Chemistry, 412(13), 3091–3101. doi:10.1007/s00216-020-02555-2

[3] Mead, F. W., & Fasulo, T. R. (2017) Asian Citrus Psyllid, Diaphorina citri Kuwayama (Insecta: Hemiptera: Psyllidae). University of Florida Institute of Food and Agricultural Sciences, Entomology and Nematology Department, 1-7

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[5] Wendt, K. D., Jensen, C. A., Tindall, R., & Katz, M. L. (2004). Comparison of Conventional and Microwave-Assisted Processing of Mouse Retinas for Transmission Electron Microscopy. Journal of microscopy, 214(Pt 1), 80–88. https://doi.org/10.1111/j.0022-2720.2004.01310.x

Acknowledgements

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Alternate text

Alexander Gomez

Oak Crest

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