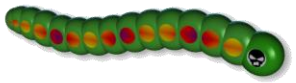
Modulation of Intracellular Reactive Oxygen Species and Calcium in Tomato Protoplasts by Hemolymph from Tobacco hornworm (*Manduca sexta*) Caterpillar



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MS biology

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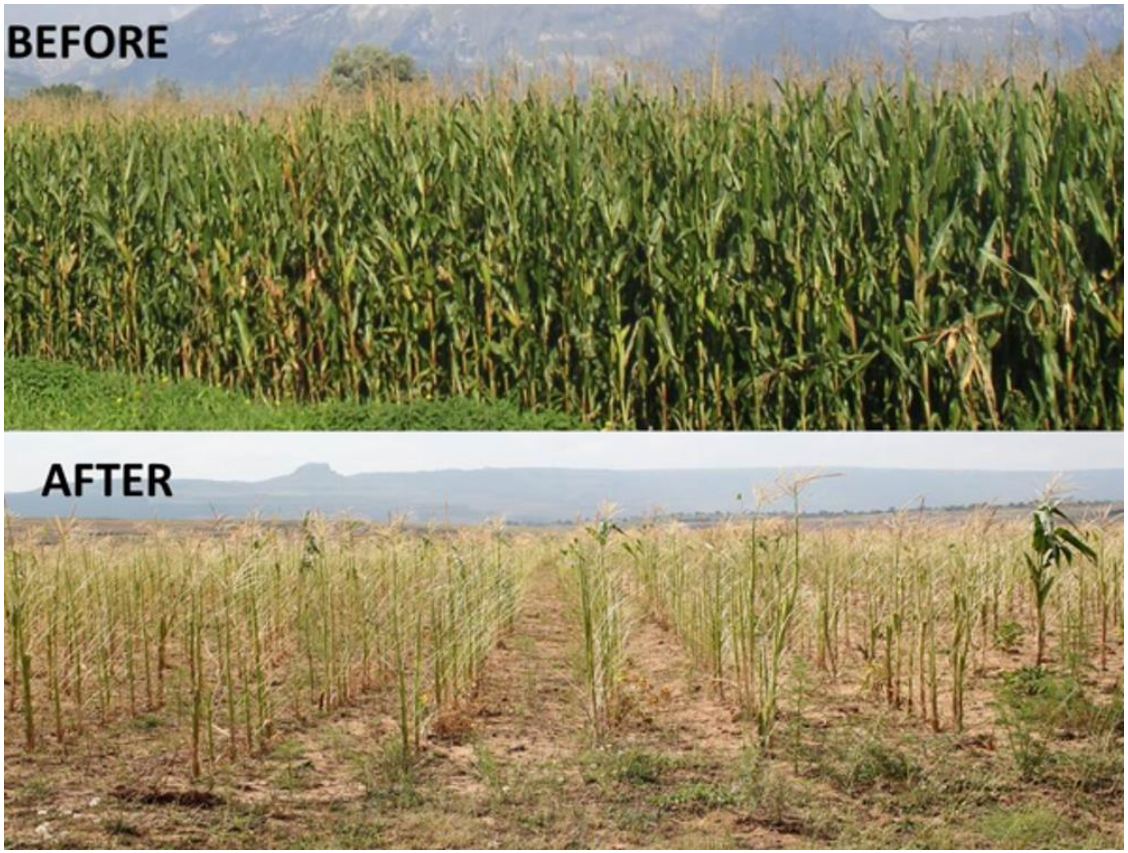
University of Texas Rio Grande Valley

Edinburg

Introduction: Herbivory



https://theconversation.com/armyworms-are-wreaking-havoc-in-southern-africa-why-its-a-big-deal-72822

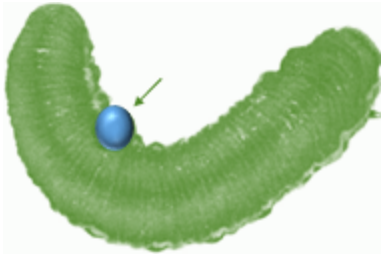
Introduction : Perception of insect attack

Plant-Insect Interactions

Mechanical Damage

Herbivore Associated Elicitors (HAEs)

Oral Secretions

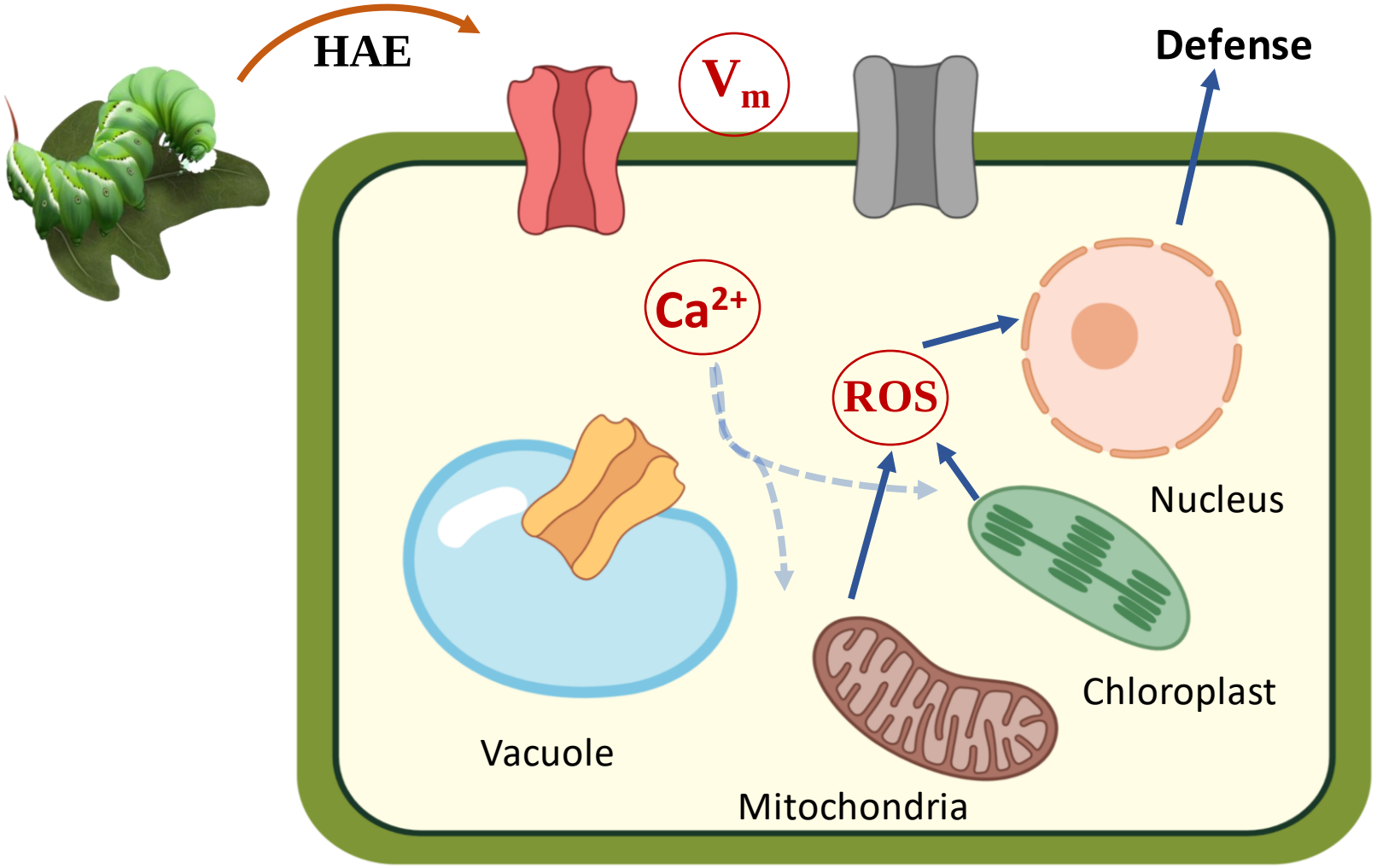


Hemolymph

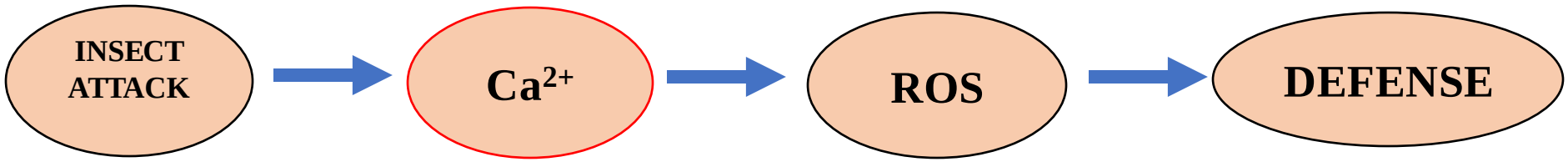
Eggs

Frass

Introduction: Early signaling events in plant-insect interactions

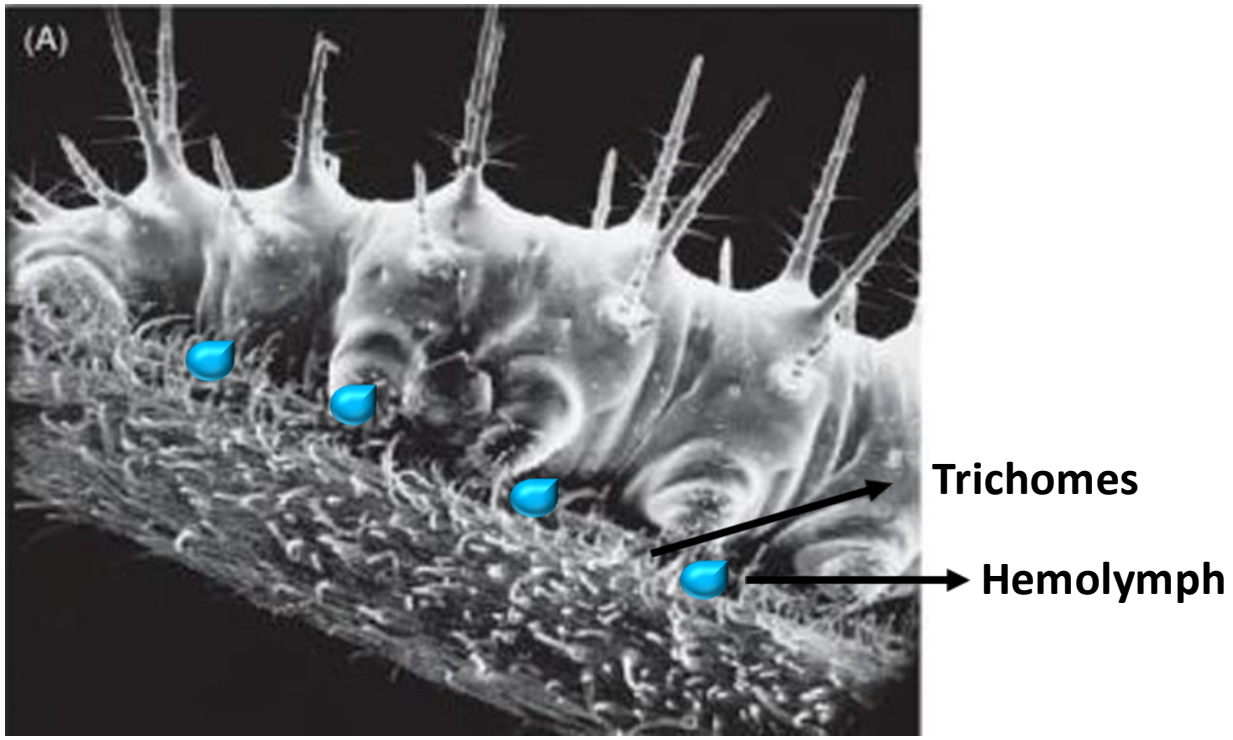


Ca^{2+} as an intracellular signaling molecule in plant defense



Toyota, Masatsugu, Dirk Spencer, Satoe Sawai-Toyota, Wang Jiaqi, Tong Zhang, Abraham J. Koo, Gregg A. Howe, and Simon Gilroy. 2018. "Glutamate Triggers Long-Distance, Calcium-Based Plant Defense Signaling." Science 361 (6407): 1112–15. https://doi.org/10.1126/science.aat7744

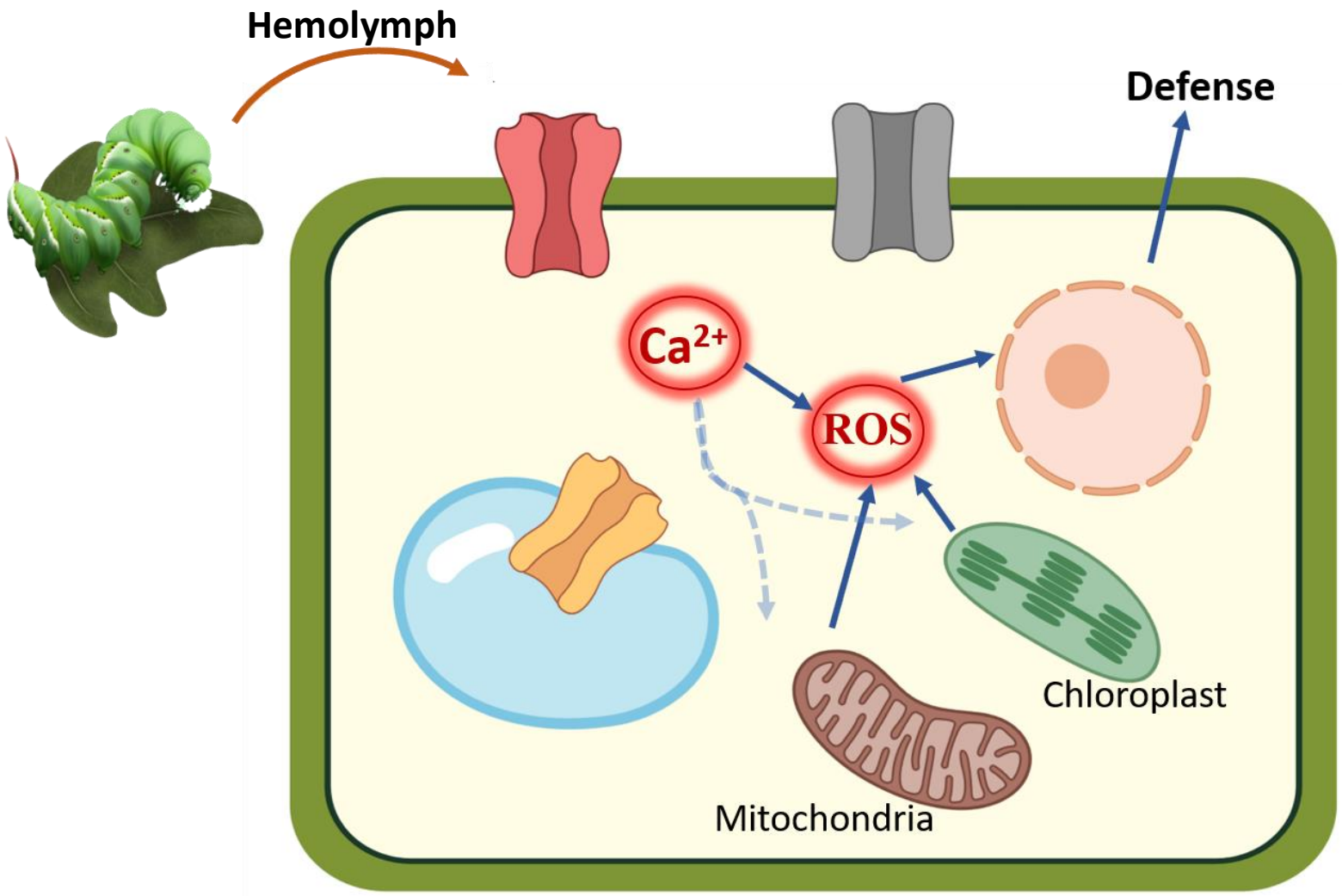
Hemolymph as an intracellular signaling molecule



https://livingwithinsects.wordpress.com/2013/02/01/friday-cat-erpillar-blogging-trichomes/

Hypothesis

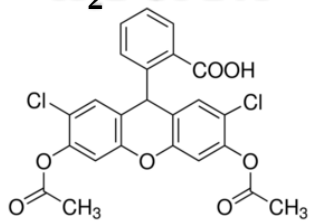
We hypothesized that *M. sexta* hemolymph will lead to ROS and Ca^{2+} generation.

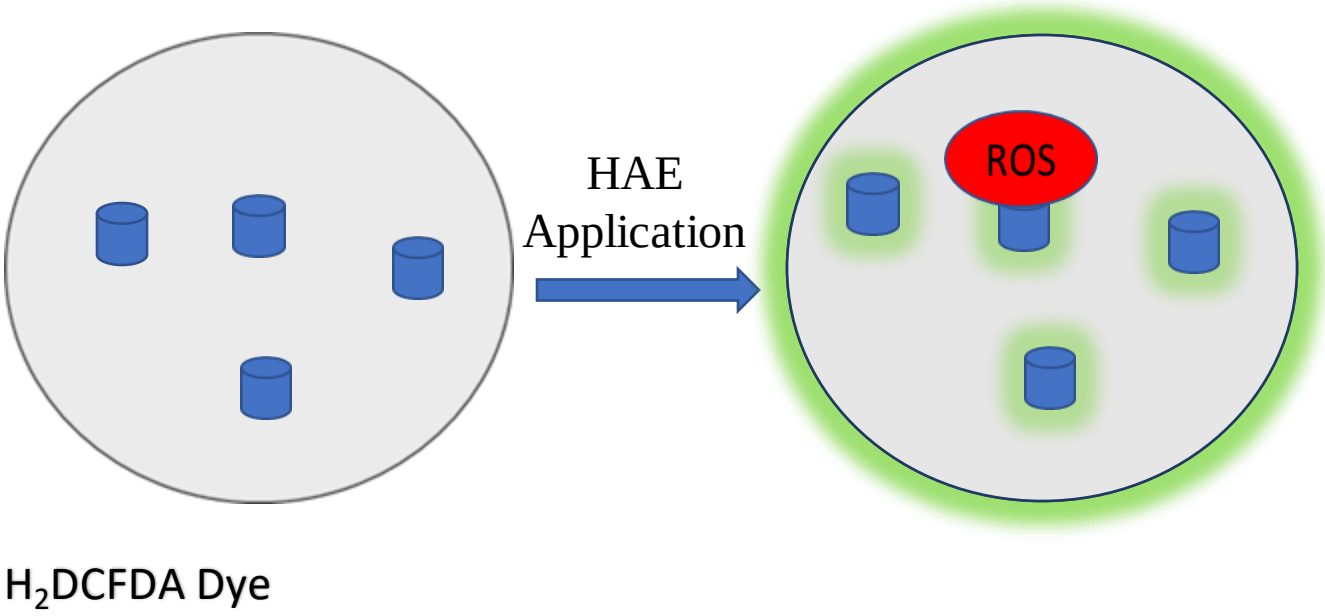


Methodology: Technique used for ROS detection

ROS Detection

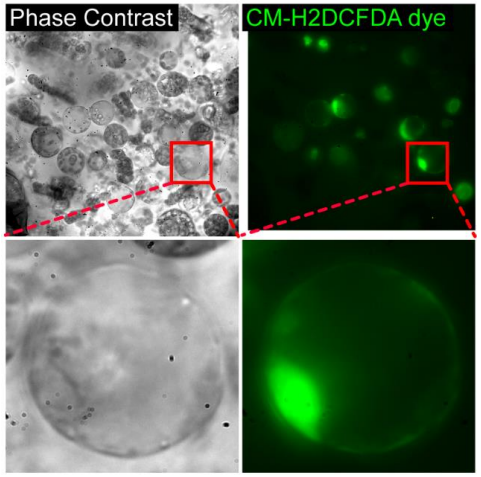
ROS sensing dye
H_2DCFDA





M. sexta hemolymph induced ROS generation in tomato protoplasts

A



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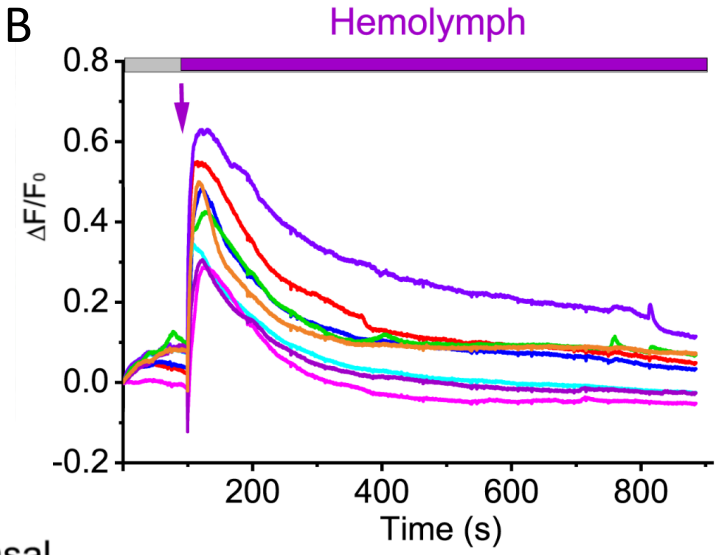

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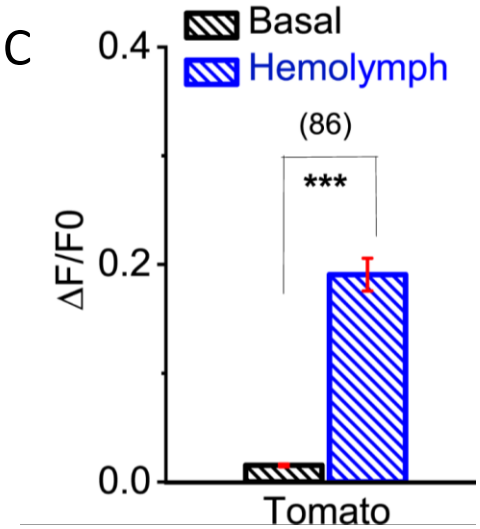
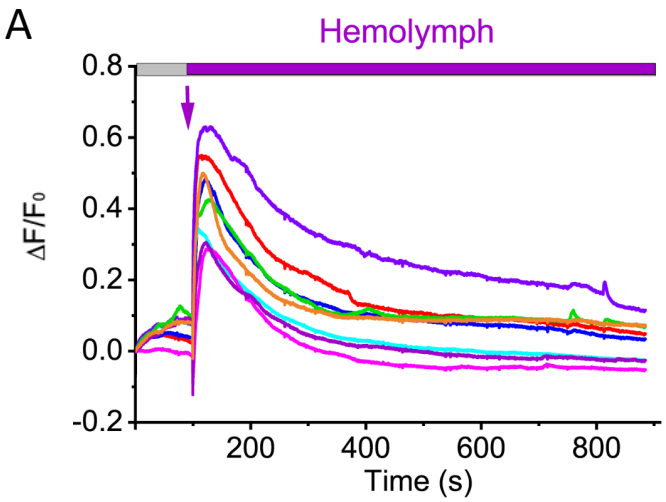
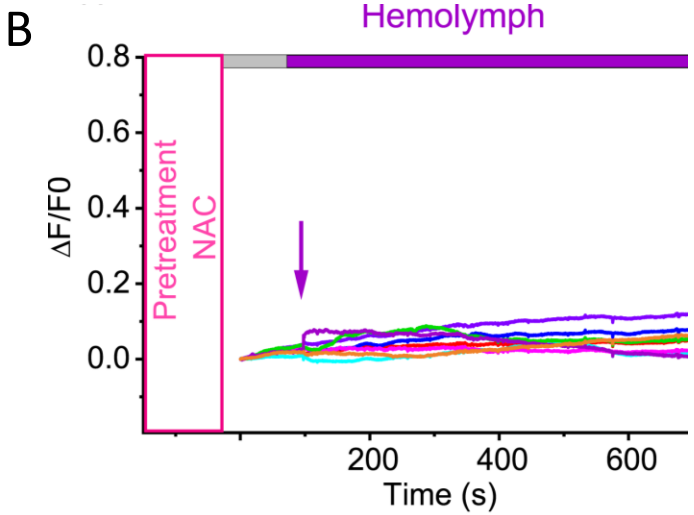


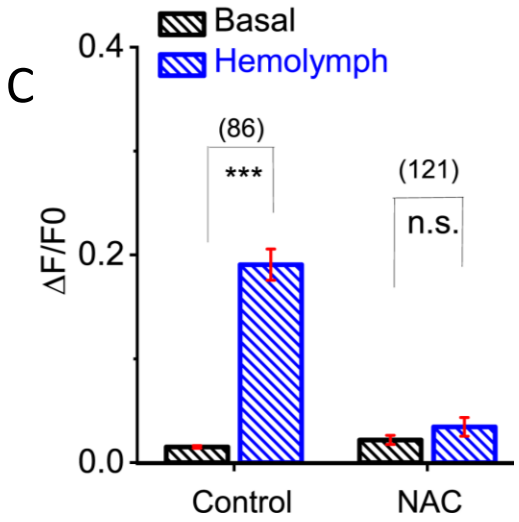
Statistics is from 3-5 independent experiments

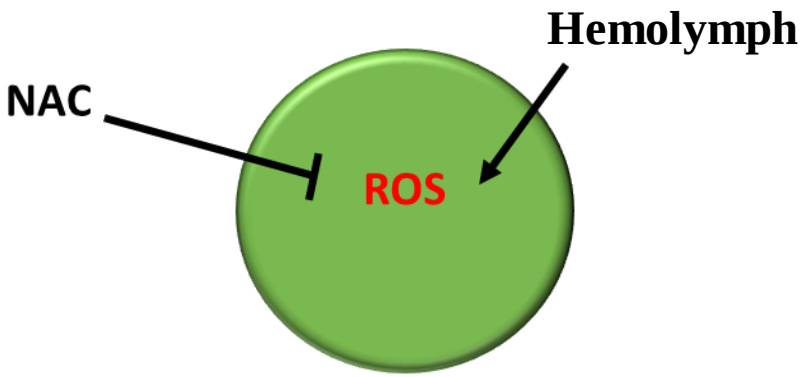
Protoplasts were incubated with ROS sensing dye, H_2DCFDA

M. sexta hemolymph-mediated ROS generation was abolished in the presence of antioxidant, NAC in tomato protoplasts

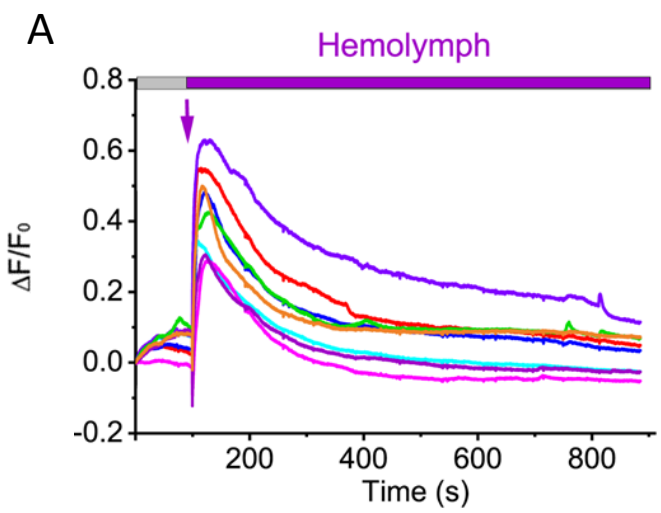


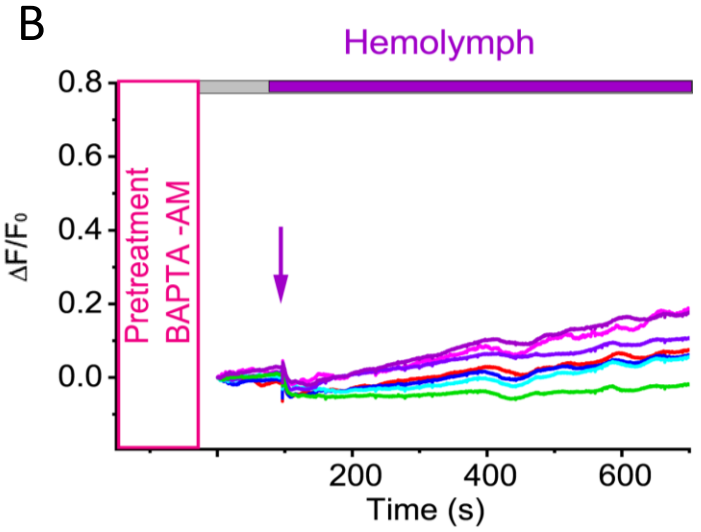


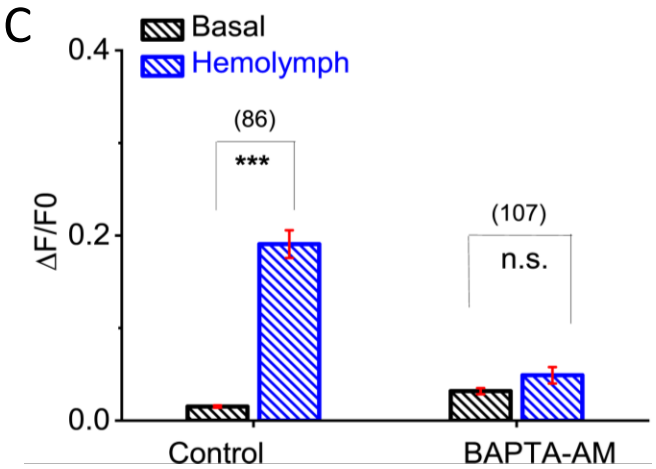


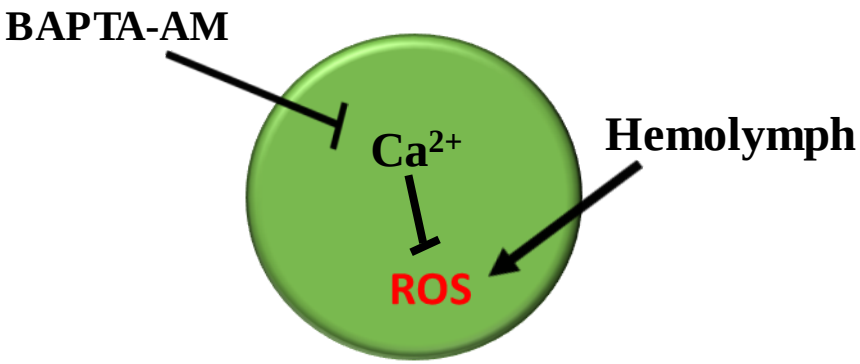


M. sexta hemolymph-mediated ROS generation was abolished in the presence of BAPTA-AM in tomato protoplasts





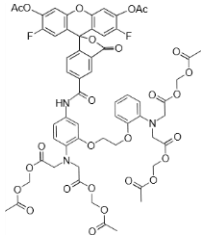


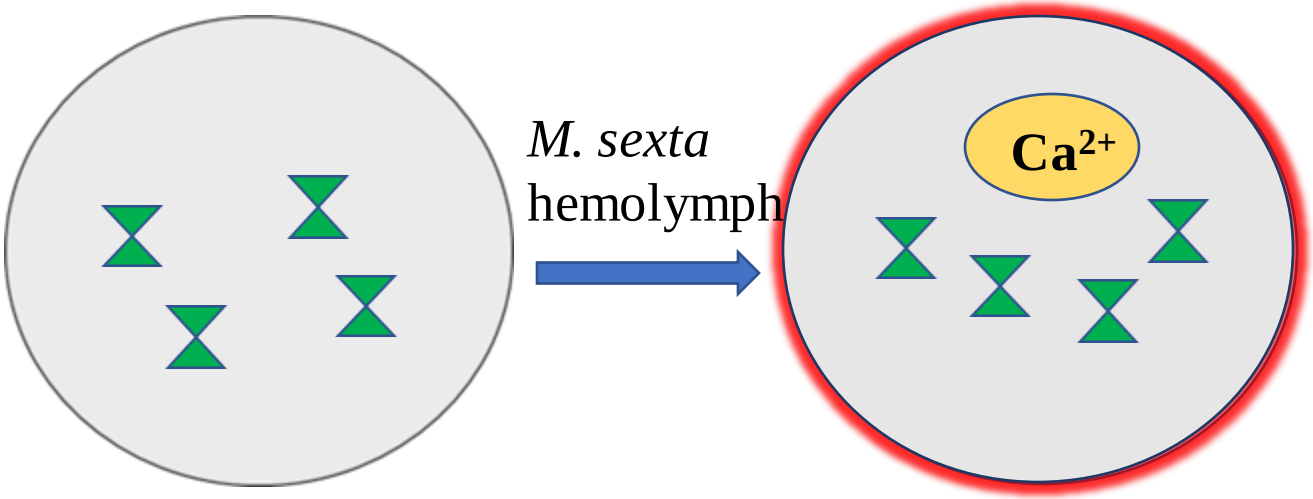


Methodology: Ca^{2+} detection with Oregon Green 488 BAPTA-AM 1 dye

Ca^{2+} Detection

Ca^{2+}sensing dye Oregon green

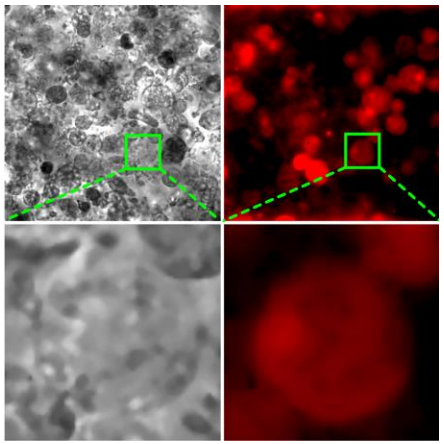




Oregon Green 488 BAPTA-1 AM Dye
(Binding Constant- 170 nM)

M. sexta hemolymph induced Ca^{2+} elevation in tomato protoplasts

A



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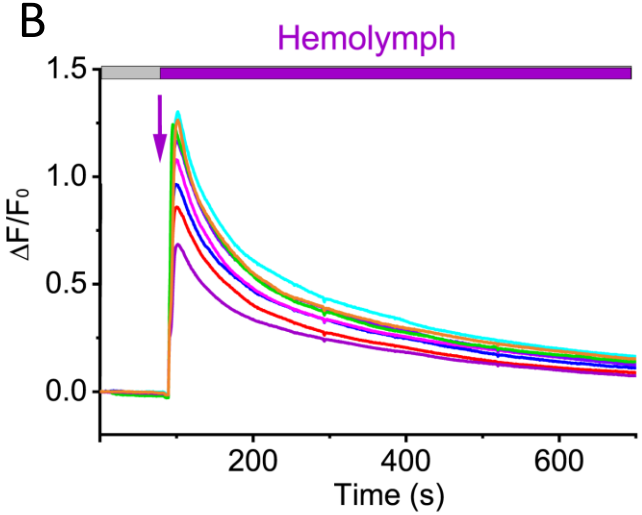

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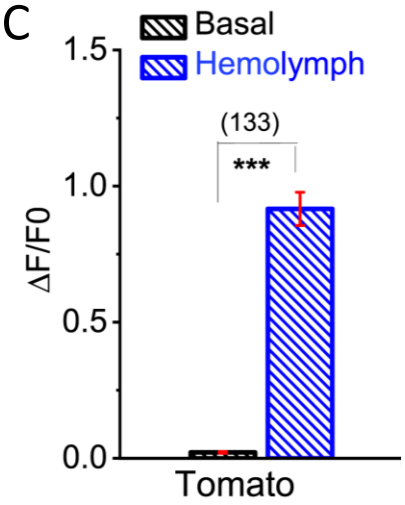
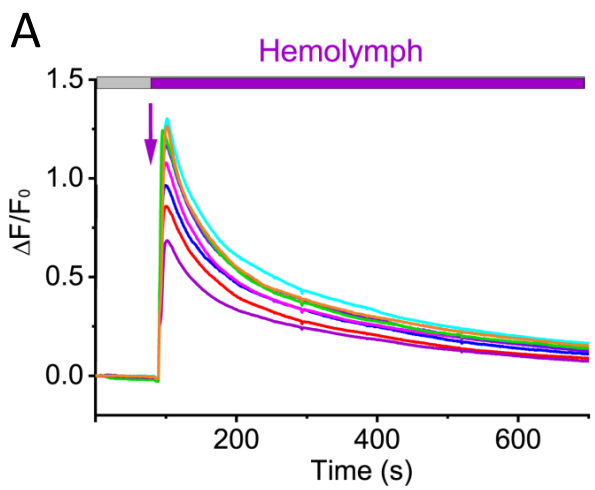


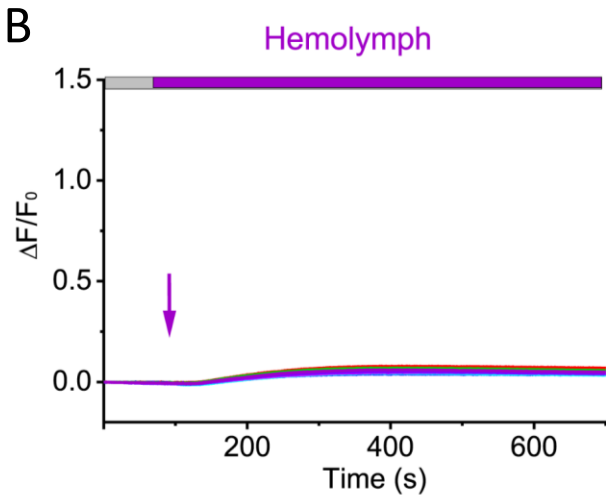
Statistics is from 3-5 independent experiments

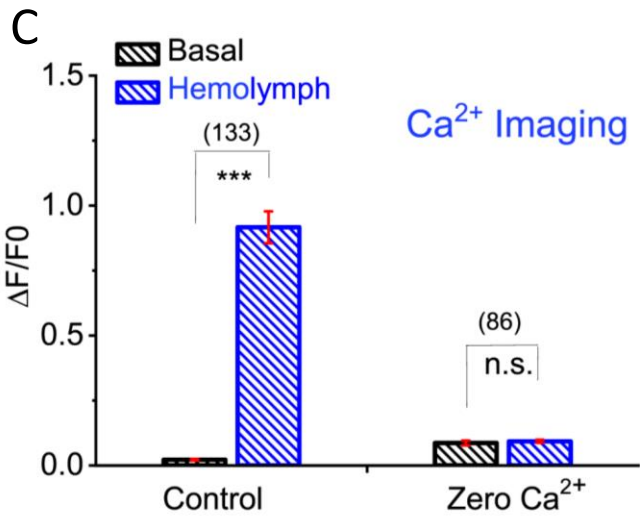
Protoplasts were incubated with Ca^{2+} sensing dye, Oregon green

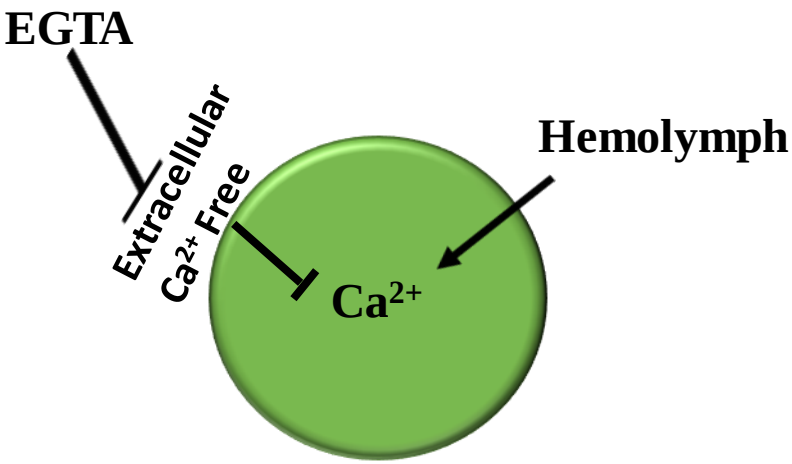
Extracellular Ca^{2+} is essential for inducing hemolymph-mediated Ca^{2+} generation

Ca^{2+} Imaging





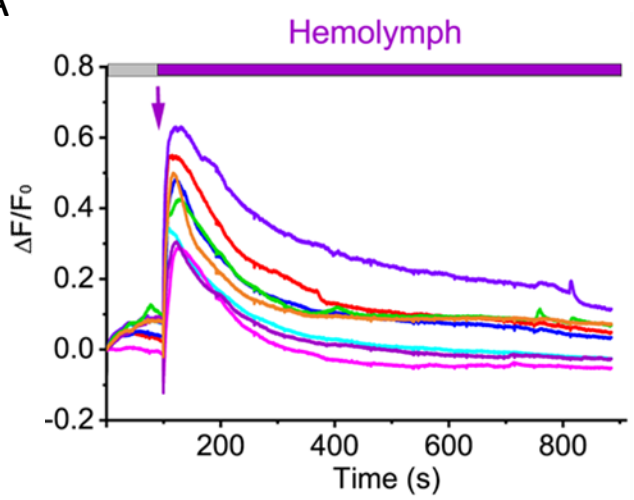




Extracellular Ca^{2+} is essential for inducing hemolymph-mediated ROS generation

ROS Imaging

A



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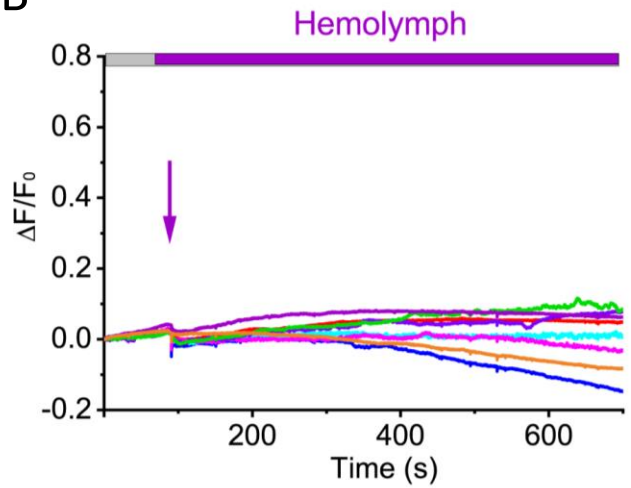

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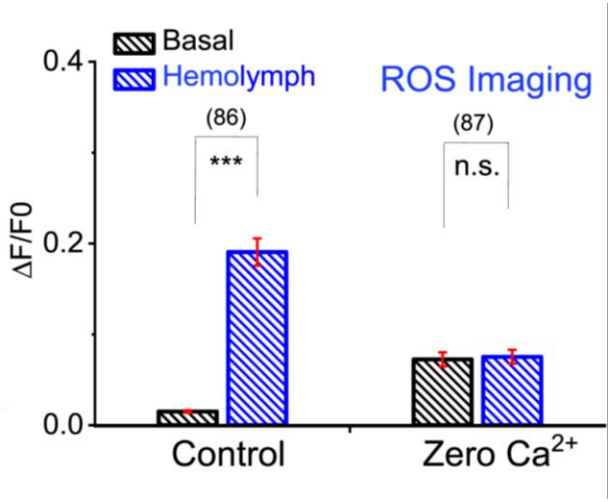
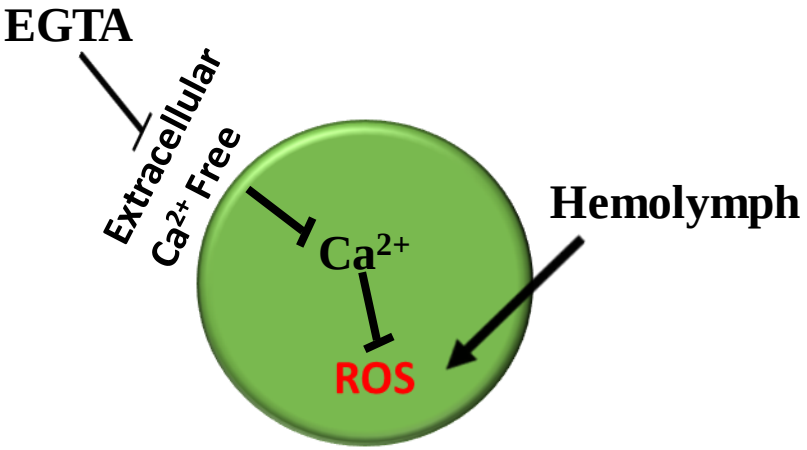
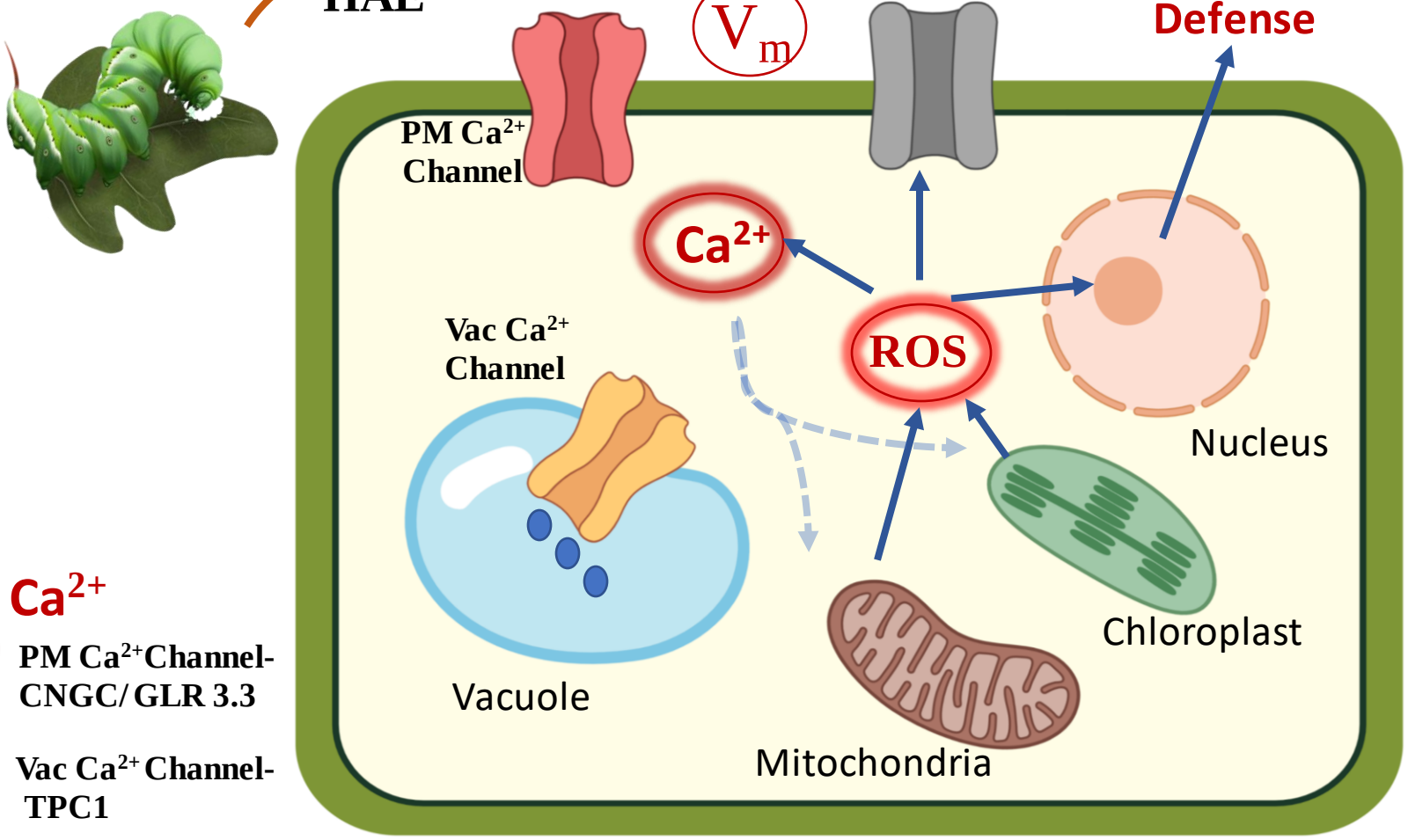




Conclusion/ Proposed model



Acknowledgement

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- Tripti Saini
- Sarah Gonzalez
- Leon Vargas

Kariyat Lab Members

- Mandeep Tayal
- Sukhman Singh

Thank you for your attention

Hemolymph