



Effects of Oryzalin and Latrunculin-B on *Arabidopsis thaliana* Calcium Wave Production and the Improvement of Treatment Uptake via Adjuvants

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Background

Light has many effects on plant cells other than aiding in photosynthesis, it can help the plant sense its environment. One particular example is the wave of calcium that is released from the endoplasmic reticulum (ER) when the chloroplast-ER contact site is photostimulated by a high-fluence 405nm laser (1). While the physiological function of this response remains unknown, there are still many cellular processes of interest currently being studied. The focus of this project is the interplay, if any, between the cytoskeleton and calcium wave. This was tested by incubating the plants in oryzalin and latrunculin-b, which depolymerize the microtubules and actin, respectively. It was found that there was no effect on the calcium wave's duration or magnitude. It is likely that there was uneven permeation throughout the hypocotyl (see Figure 1) due to the waxy cuticle, possibly resulting in a reduced effect.

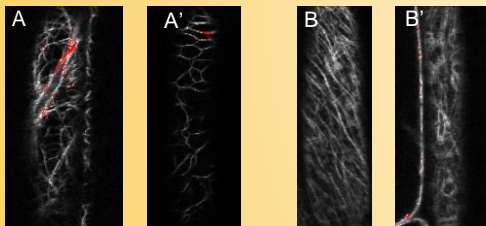


Figure 1: All images are of surface hypocotyl cells of 7-day old *Arabidopsis thaliana* seedlings acquired using fluorescent confocal microscopy. A- untreated Actin-YFP cell; A'- Latrunculin-B treated Actin-YFP cell; B- untreated Tubulin-GFP cell; B'- Oryzalin treated Tubulin-GFP cell. Both show some, yet incomplete, depolymerization.

So ways of making a more consistent and effective treatment were researched, while ensuring the plant is kept alive and stressed as little as possible. One method that was found comes from agriculture: adjuvants. These are oils that aid the uptake of herbicides in weeds by providing a hydrophobic shield around the herbicide to allow it to pass easily through the weed's waxy cuticle and membrane (2). Tween20 was tested with fluorescein diacetate (FDA) (a cytosolic fluorescent stain) to see if it could improve the uptake of FDA without harming the plant.

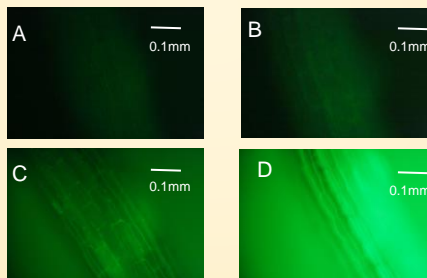
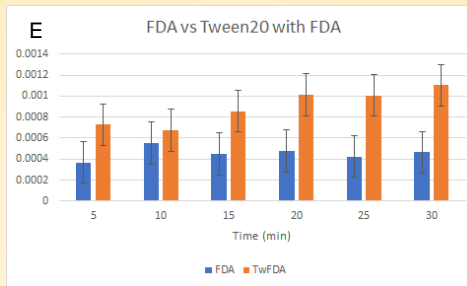
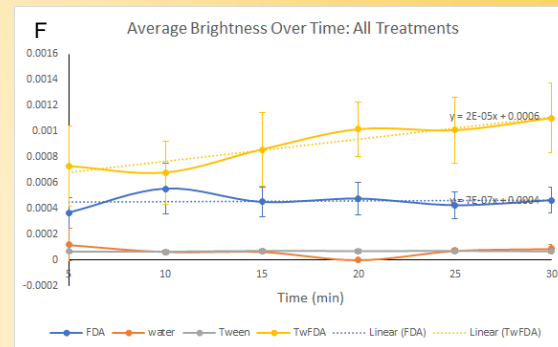


Figure 2: All images are of surface hypocotyl cells of 7-day old *Arabidopsis thaliana* seedlings acquired using an Olympus BH-2. All images were processed in ImageJ and graphed in Microsoft Excel. A- *Arabidopsis* seedling in water after 30 mins (brightened for visibility); B- *Arabidopsis* seedling in 0.01% Tween20 after 30 mins (brightened for visibility); C- *Arabidopsis* seedling in 20µm FDA after 30 mins; D- *Arabidopsis* seedling in 0.01% Tween20 with 20µm FDA after 30 mins; E- graph of average hypocotyl fluorescence at each time interval for FDA and Tween20 with FDA treated seedlings; F- graph of average hypocotyl fluorescence over time for all treatments with trendlines for FDA and Tween20 with FDA treatments

References

- Maynard, S and Griffing L, 2021: in process
- Gillickson, Gus. *All About Adjuvants*. Successful Farming, 6 November, 2020.



Discussion

The data from the Tween20 and water treatments show that the Tween20 appears to have no effect on plant autofluorescence and the plant's autofluorescence changes little over time (see Figure 2F). The FDA data shows that there is some uptake, but it does not increase over time (see Figure 2E, 2F). The Tween20 with FDA, however, shows a consistent uptake of more and more FDA over time (see Figure 2E, 2F), showing that it appears to be an effective method of increasing treatment uptake and making it more consistent. This can be applied to many projects that depend on treatments consistently and effectively entering the hypocotyl of *Arabidopsis thaliana* seedlings.

Next Steps

Previous experiments with oryzalin and latrunculin-B will be performed again with Tween20 to increase treatment uptake. This may give a better idea of how the cytoskeleton interacts with the ER since the treatment can more effectively spread to the desired areas of the hypocotyl. Additionally, other current projects in Griffing lab may begin to use this procedure.