# Preliminary Inoculation of Swampbay (*Persea palustris*) with a GFP transformant of *Raffaela* aguacate

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## INTRODUCTION

*Raffaelea lauricola* is a fungal pathogen that is causes Laurel wilt, a disease that causes the host to produce an abundance of tyloses that reduced the hydraulic conductivity of the tree until they wilt and die (S. Inch 2012).

The pathogen we used in this experiment is *Raffaelea aguacate* which is photogenically a close relative of *R. lauricola* (Simmons 2016). However, *R. aguacate* does not produce the same pathogenic results as *R. lauricola*. When inoculated into a host tree does not cause wilting, it is unknown why this is. *R. aguacate* does not affect its host and cause disease symptoms. Especially with how successful its close relative *R. lauricola* is as a pathogen.

This image shows an example of the symptoms cause by *Raffaelea lauricola*, this wilting is known as Laurel wilt as it is highly associated with this pathogen.

## RESEARCH QUESTION

-Why does *R. aguacate* not cause disease symptoms in host trees? Is the host recognizing it and destroying it, or is it simply unable to grow within the host and simply dies without causing issues?

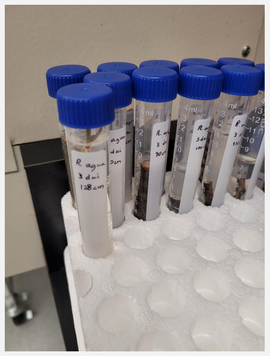
-With this experiment we hope to answer these questions and possibly learn more about *R. lauricola*, and what makes it such a successful pathogen, especially when compared to its relative *R. aguacate*.

-We will also be able to observe the movement, is any, of the *R. aguacate* after it is introduced into the host.

## METHODS

- Five tree were moved into a growth chamber and given one week to acclimate to their new environment (*image is of 3 dai tree in growth chambe*r)
- A 300,000 conidia solution in 100µl of sterile deionized water was prepared and used to inoculate the trees
- The five trees were then labeled 3,5,7,10 and 14 dai, indicating the number of days before each tree would be dissected.
- On the day of their dissection the trees were sampled in 10cm sections, starting below the inoculation point up until the apex of the main plant stem.
- The samples were then cut into smaller sections, that were surface sterilized using 70% EtoH, 10% bleach and sterile deionized water.
- The samples were then inserted into CSMA4+ media and then monitored for fungal growth
- The rest of the 10cm sections were then placed into 50 or 15mL tubes with paraformaldehyde until covered and then stored
- After 24hours the paraformaldehyde was changed with 1xpbs and stored again for a later date

## RESULTS

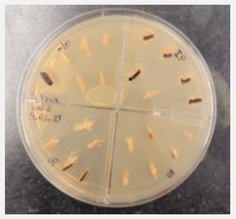


At this point in the experiment all of the trees have been dissected and plates have been made of their samples.

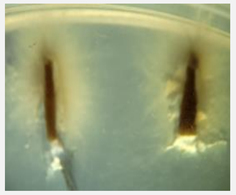
The plates are checked in frequently to collect data on any fungal growth. Data is collected when any pathogen grows on the plates, this is done using a microscope to detect growth at the earliest possible stages.

When fungal growth is detected on a plate it can be then be analyzed using the GFP as a guide to accurately identify the *R, aguacate.*

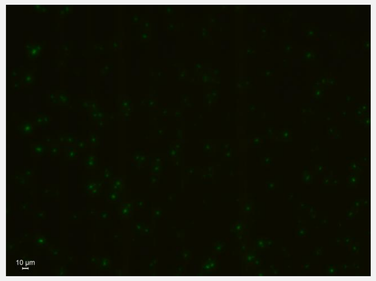
## RESULTS



The plate in this image (*right*) shows early detection of growth on the plates. This growth is marked off on the plate and recorded to narrow down the time it takes to start seeing growth.



The image (*right*) shows a closer look at the fungal growth on the plate, that is being seen at the inoculation points of the samples from the dissected trees.



The image (*left*) demonstrate the GFP positive *R. aguacate*. When present it will glow like in this picture, this makes it easy to correctly identify.

## CONCLUSION

The conclusion of the results so far, indicate that *R. aguacate* is not travelling past the inoculation point of the host tree. We are seeing it on some plates below the inoculation point but not in all cases. Further experimentation is need to discover what is preventing it from travel up the xylem and reproducing within the host.

## REFERENCES

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