



MY ENEMY'S ENEMY IS MY FRIEND



CES LOPE DE VEGA

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INTRODUCTION

Banana, *Musa acuminata* is one of the most important fruit and cash crops in terms of production volume and trade in the world. The global production of bananas is projected to grow at 1.5% per annum, to reach 135 million tonnes in 2028. However, a number of soil-borne pests and diseases are principal limiting factors for banana production worldwide. Among them, ***Fusarium oxysporum cubense*** is considered one of the most destructive soil-borne fungus affecting this crop producing a destructive disease called Fusarium Wilt of Banana. *Fusarium oxysporum* infects banana roots, progresses into the xylem of the rhizome, induces wilt, and may eventually kill susceptible cultivars.

Thus, research and crop management efforts are currently ongoing to prevent the propagation of the disease, which can seriously compromise the future of this staple food.

The manipulation and harnessing of the endophytic microbiome may help to increase crop production, reduce the incidence of diseases, improve plant resistance, and decrease agrochemical inputs.

On the other hand, cultivated olive (***Olea europaea L. subsp. europaea var. europaea***) is one of the most important oil crops in the world. It constitutes an agro-ecosystem of major relevance for the Mediterranean Basin since 90% of the global olive oil and table olive production is concentrated in this area. Severe losses, and even tree death, are caused by a range of olive pathogens. Among them, the soilborne fungus ***Verticillium dahliae* Kleb.**, causing Verticillium Wilt of Olive (VWO), represents a major threat in many regions where this tree is cultivated.

OBJECTIVES

- To compare the *in vitro* efficacy of different bacteria strains in the ***Fusarium oxysporum* f. sp. *cubense*** and ***Verticillium dahliae*** growth inhibition
- To observe different degrees of *Fusarium oxysporum* f. sp. *cubense* virulence
- To obtain monoconidial cultures

BASIC CONCEPTS

PLANT PATHOSYSTEM

Within an agro-ecosystem, the interaction between a plant host and a parasite, which feeds on it, causing a disease.



Olea europaea (plant, olive) and *Verticillium dahliae* (fungus).



Musa acuminata (plant, banana) and *Fusarium oxysporum* f. sp. *cubense* (fungus).



BASIC CONCEPTS

Verticillium Wilt of Olive (VWO) is caused by the soil-borne fungus *Verticillium dahliae* Kleb.



Verticillium Wilt of Olive (VWO), represents a major threat in many regions where this tree is cultivated.



Olea europaea L. subsp. *europaea* var. *europaea* constitutes an agro-ecosystem of major relevance for the Mediterranean Basin since 90% of the global olive oil and table olive production is concentrated in this area.

Fusarium Wilt of Banana (FWB) is caused by soil-borne fungus *Fusarium oxysporum* f. sp. *ubense*.



Fusarium oxysporum f. sp. *ubense* infects banana roots, progresses into the xylem of the rhizome, induces wilt, and may eventually kill susceptible cultivars.



Banana (*Musa acuminata*) is one of the most important fruit and cash crop in terms of production volume and trade in the world.

BASIC CONCEPTS

RHIZOSPHERE



The rhizosphere is the part of the soil close to the roots, which extends specifically between 1 and 3 mm from the surface of the roots to the interior of the soil. The composition of the rhizosphere is: soil, water, radical depositions (exudates and mucilage) and microbiota (bacteria, fungi, algae).



The root/rhizosphere of healthy olive plants is an important reservoir of microorganisms displaying biocontrol activity against VWO and FWB.



Different bacterial strains have been used *in vitro* and all strains displayed growth inhibition and biocontrol effectiveness against *Verticillium dahliae* and *Fusarium oxysporum* f. sp. *cupense*.

MATERIALS



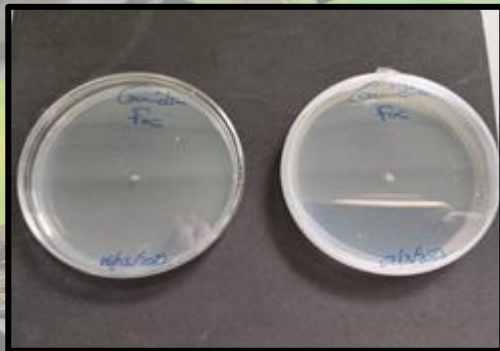
LAMINAR FLOW CHAMBER



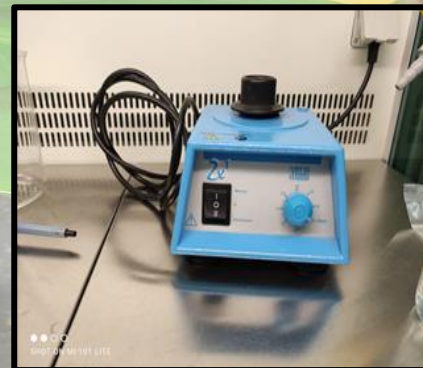
ISO JARS



STEREOMICROSCOPE



PETRI DISHES



VORTEX



AUTOCLAVE



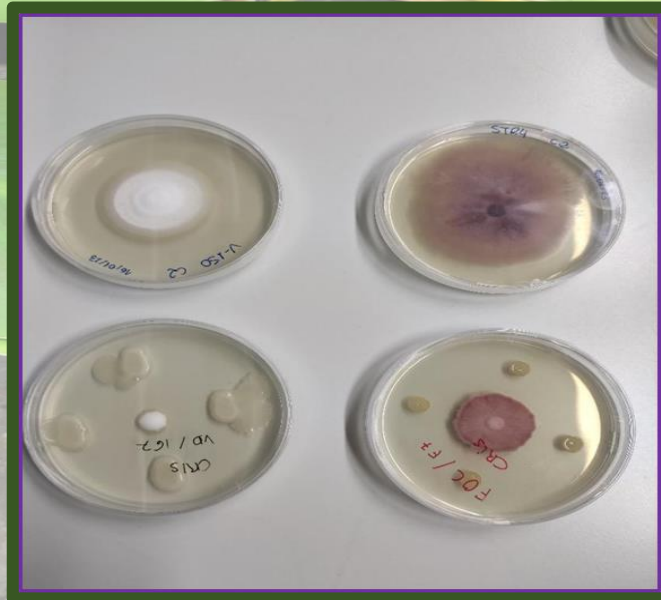
PLANTING HANDLES

METHODS

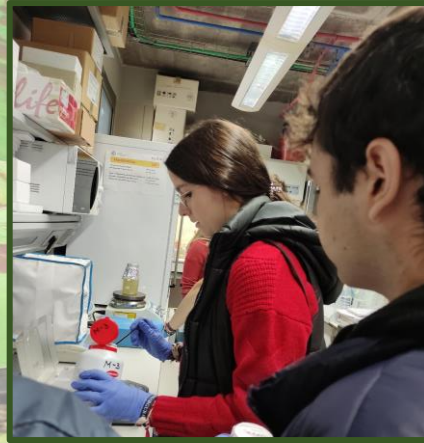
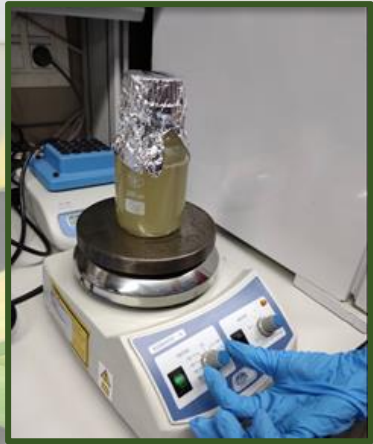
VARIABLES

Two types of variables were used in this research project:

- The independent variable is the diameter of the colony of the fungus
- The discontinuous dependent variable is the type of bacteria



METHODS



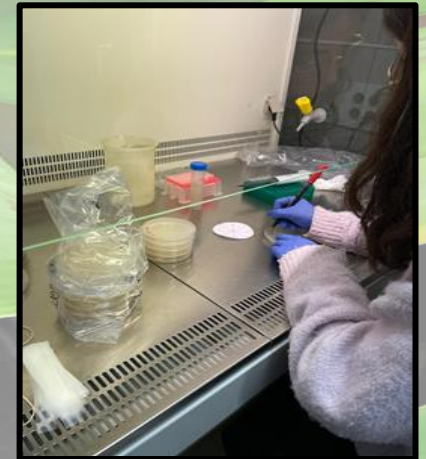
PREPARATION OF CULTURE MEDIA

POURING OF CULTURE MEDIA IN PETRI DISHES

METHODS



CONIDIA COUNT

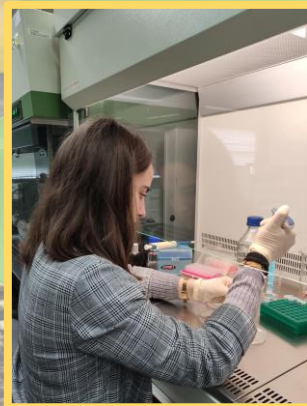
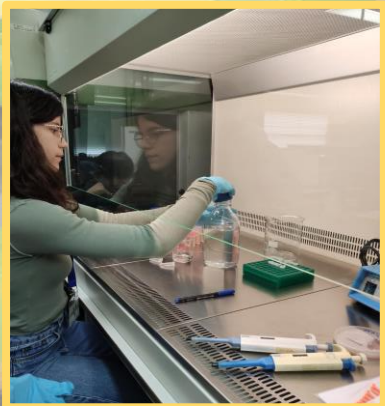


FUNGI AND BACTERIA INOCULATION IN PETRI DISHES

METHODS



OBSERVATION OF THE SIZES OF THE COLONIES AND
CALCULATION OF THE PERCENTAGE OF INHIBITION



PREPARATION OF THE DILUTIONS



SEEDING OF FUSARIUM TO OBTAIN
MONOCONIDICAL CULTURES



CONIDIA ISOLATION

RESULTS

Fungal colony radius measurement



Fusarium oxysporum
f.sp.cubense

Verticillium dahliae

Petri dishes with fungi and different bacteria used

The upper row corresponds to controls and from them and downwards each of the cultures with each bacterium and fungus ordered from the most to the least effective bacterium.

1- *Verticillium* on the left and *Fusarium* on the right . Control

2- Vd + IAS-B-102 / Foc + IAS-B-102

3- Vd + PIC F7 / Foc + PIC 167

4- Vd + IAS-B-103 / Foc + PIC 73

5- Vd + PIC 167 / Foc + PIC F7

6- Vd + PIC 73 / Foc + IAS-B-103

1.CONTROL

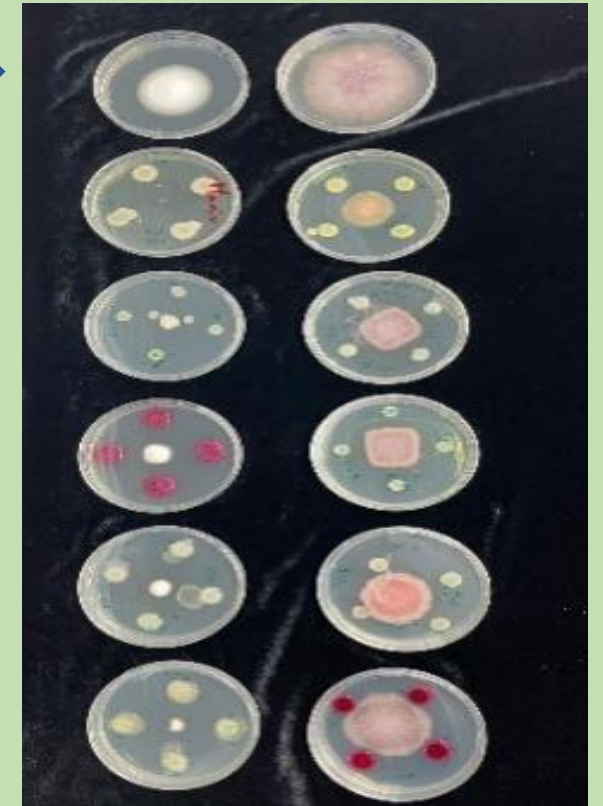
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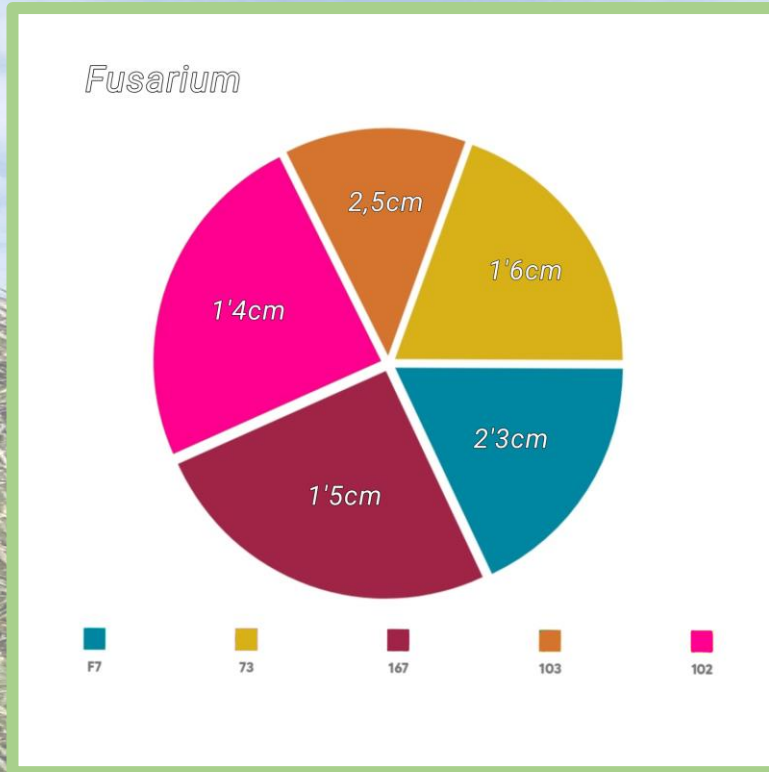
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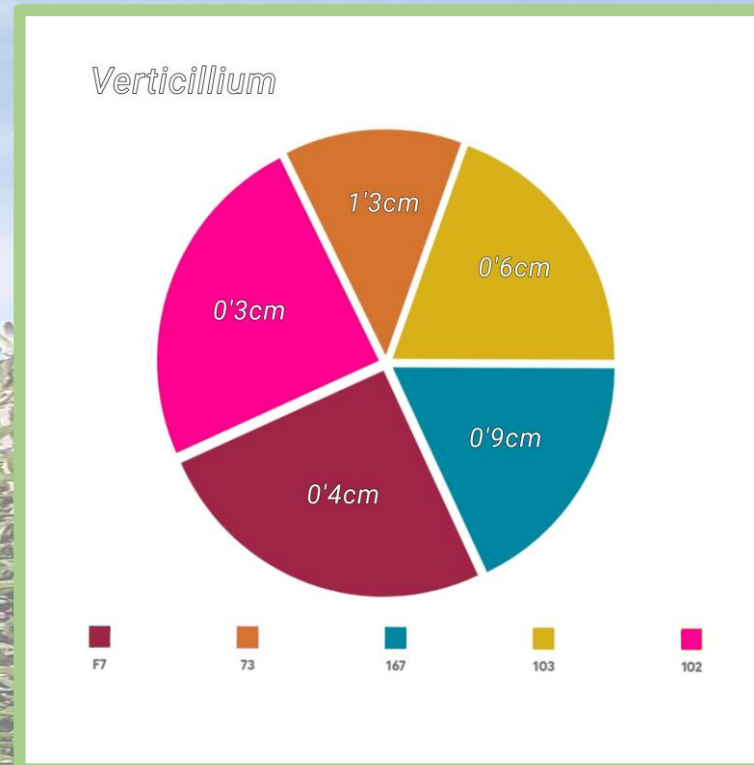
6



RESULTS



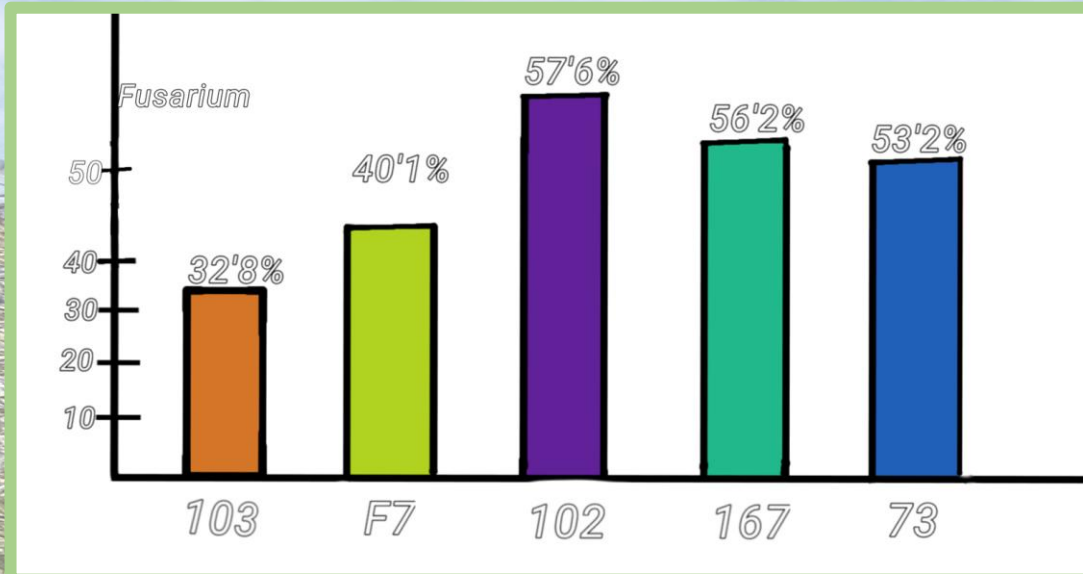
**Pie Chart 1. Graph with
Fusarium radius**



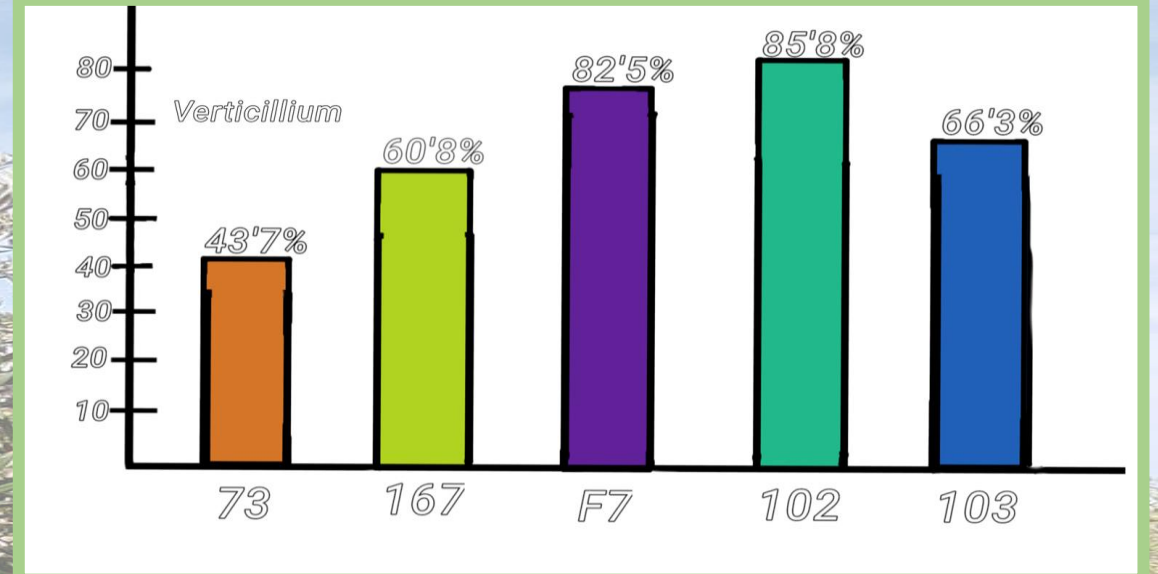
**Pie Chart 2. Graph with
Verticillium radius**

Pie charts 1 and 2 represent the radii of fungal colonies in the presence of each bacterium. Bacteria strains used were PICF7, PIC73, PIC167, IAS-B-103 and IAS-B-102.

RESULTS



Bar Graphic 1. Percentage of efficacy that each bacteria has had against *Fusarium oxysporum f.sp. cubense*



Bar Graphic 2. Percentage of efficacy that each bacteria has had against *Verticillium dahliae*

Of the bacteria tested, the most efficient against *Verticillium dahliae* and against *Fusarium oxysporum f. sp. cubense* has been *Pseudomonas chlororaphis IAS-B-102*.

CONCLUSIONS

- 1) Among the tested bacteria, the most efficient *in vitro* assay against *Verticillium dahliae* and against *Fusarium oxysporum* f. sp. *cubense* has been ***Pseudomonas chlororaphis* IAS-B-102**.
- 2) Among the tested bacteria, the least efficient *in vitro* inhibition assay of the fungus *Verticillium dahliae* was *Paenibacillus polymyxa* (PIC73) and the least effective against the fungus *Fusarium oxysporum* f. sp. *cubense* has been *Serratia marcescens* (IAS-B-103).

FINAL CONCLUSIONS

- According to these results, ***Pseudomonas chlororaphis* IAS-B-102** rhizobacteria would be the most promising candidate to combat Verticillium wilt of Olive and Fusarium Wilt of Banana.
- This approach of biological control of pathogens through the use of rhizobacteria can contribute substantially to the development of sustainable agriculture. Therefore, rhizobacteria offer an ecological alternative to control the pathogen attack and/or improve a certain crop.

ACKNOWLEDGEMENTS

We would like to thank:

- To the "Crop Protection" department and the "Plant-microorganism interaction" laboratory for providing us with the instruments and the place to carry out the project.
- Especially to our researchers Leire Molinero Ruiz, Carmen Gómez-Lama Cabanás, Antonio Valverde Corredor and Ana Domínguez Carmona for having taught us and explained everything we needed to get this project off the ground.
- To the tutor of the IES Fidiana M^a del Mar Moreda Moreno for all the help we have received from her at all times.
- To the research centers, IAS and CSIC for their collaboration
- To the Fidiciencia 2.0 and Erasmus+ projects for one more year helping students to expand their field of learning and to the Junta de Andalucía for the grant of the Fidiciencia 2.0 Educational Innovation Project.
- To CES Lope de Vega and IES Fidiana for facilitating our approach to a research project.
- To our parents and families for their unconditional support.

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The Banana Root Endophytome: Differences between Mother Plants and Suckers and Evaluation of Selected Bacteria to Control Fusarium oxysporum f. sp. cubense.

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Published: 23 February 2018

Indigenous Pseudomonas spp. Strains from the Olive (Olea europaea L.) Rhizosphere as Effective Biocontrol Agents against Verticillium dahliae: From the Host Roots to the Bacterial Genomes.

This article was submitted to Plant Microbe Interactions, a section of the journal Frontiers in Microbiology

Agriculture. Carmen Gómez-Lama Cabanás, David Ruano-Rosa Garikoitz Legarda, Paloma Pizarro-Tobías, Antonio Valverde-Corredor, Juan Carlos Triviño, Amalia Roca and Jesús Mercado-Blanco

Bacillales Members from the Olive Rhizosphere Are Effective Biological Control Agents against the Defoliating Pathotype of Verticillium dahliae

Published: 23 June 2018



THANKS FOR YOUR ATTENTION