Survey on the persistence of *Plenodomus tracheiphilus* inoculum in lemon groves during summer

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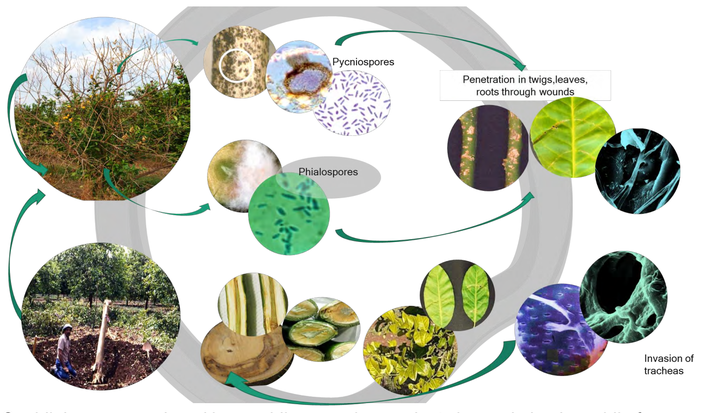
P.tracheiphilus is the causal agent of "mal secco", the most destructive lemon disease, spread in the EU-Med region (EPPO list A2), and is of quarantine concern to most regional plant-protection organizations (APPPC, CPPC, COSAVE, IAPSC, NAPPO).



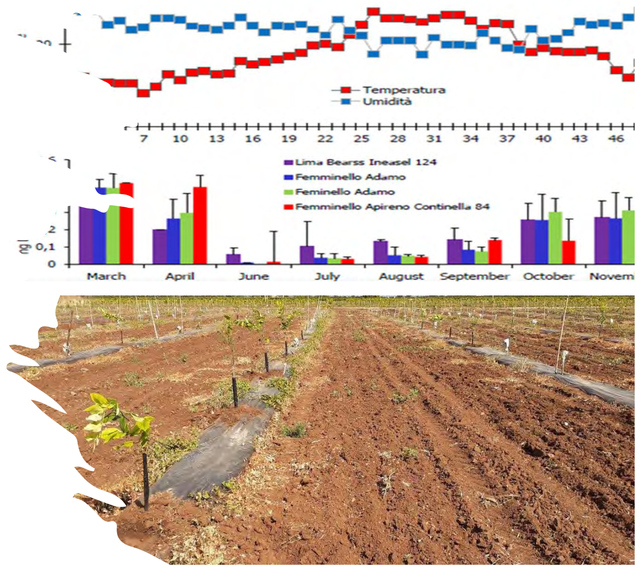
Conidial spores produced in pycnidia emerging on dry twigs and phyaloconidia from mycelial hyphae are dispersed by wind and rain. The hyphae grown after spore germination move toward wounded tissues and penetrate in leaves, twigs and roots and moves through the tracheas. When the infection occurs through large roots a sudden death may occur. Infected plant parts lying on the ground serve as a source of inoculum for root infection as well as results of leaf shedding and pruning.

IS THE SOIL A RESERVOIR OF Pt PROPAGULES ?

Replanting of lemon groves after uprooting of infected trees is frequently associated to dramatic outcomes of infection.

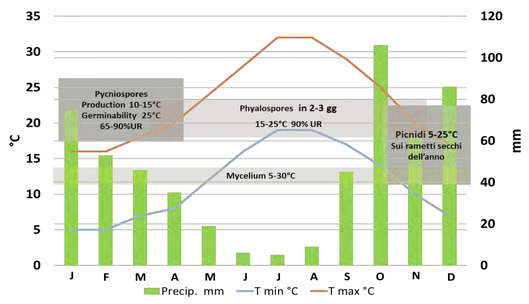
Under controlled conditions the survival period of the spores is about 30 days in sandy soil, 120 days in clay. In the orchard they may stay alive for up to 1 year in heavy soil (De Cicco et al., 1988).

Quantitative real-time PCR protocol has shown the highest fungal DNA concentrations in March-April, nearly undetectable values in July and August, and an increase in September-November (Russo et al.,2010).

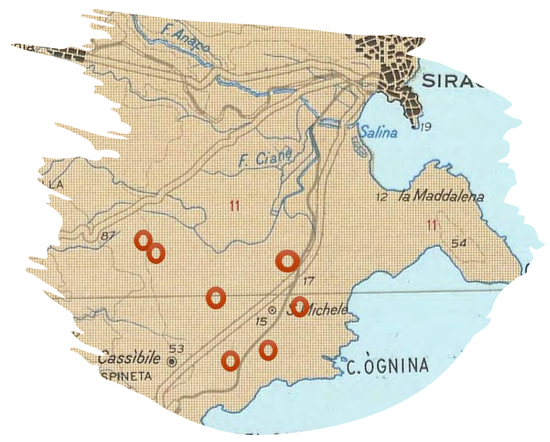


AIMS AND SCOPE OF THE STUDY

- Investigate the summer survival of the propagules
- Understand factors potentially affecting their persistence
- Study the soil microbiomes
- Evaluate protocols to monitor the risk



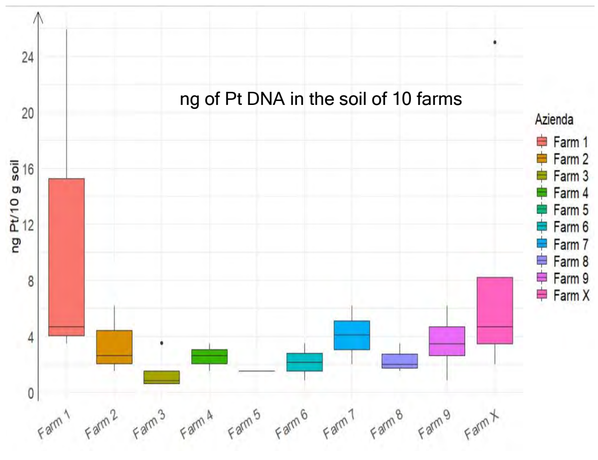
MATERIALS AND METHODS



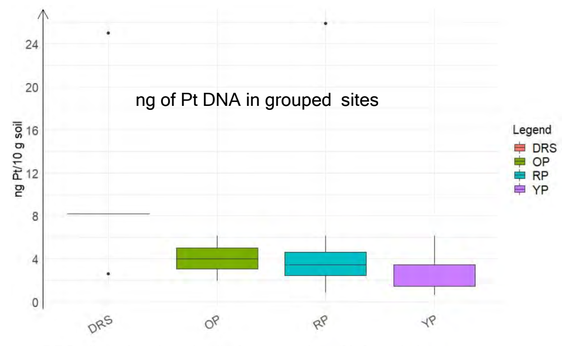
The survey was carried out from the end of June to the end of july in Syracuse area

- 10 orchards, growing three lemon varieties grafted on three rootstocks were evaluated
- 48 soil samples were obtained from single trees
- 5 samples were collected from ripped soil after uprooting infected trees
- Total fungal DNA was extracted by Kit DNeasy PowerSoil (QIAGEN) and processed by real time PCR
- Microbiome components of three samples were investigated
- To confirm the viable of the propagules, ten samples were cultured on a selective media

DETECTION OF P. TRACHEIPHILUS DNA IN SOIL SAMPLES

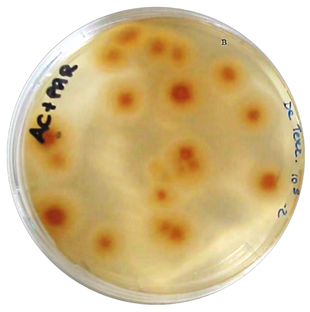


A) Boxplot shows the median values were less than 0,8 ng per g of soil, but single samples showed values extremely high, likely related to the presence of host plant residues in the soil

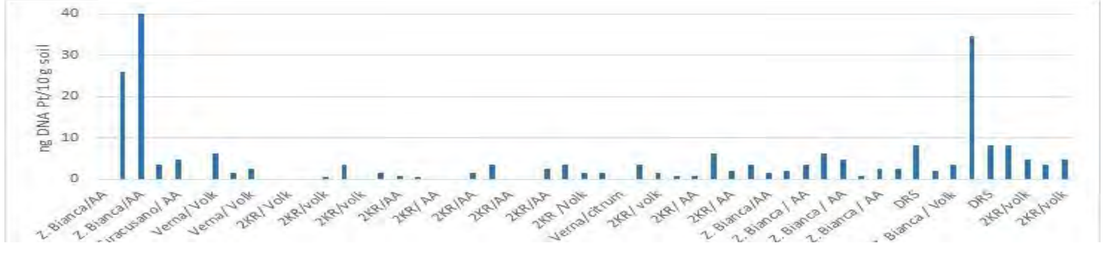


- DRS, digged or ripped soil after uprooting of infected plants
- OP, old plantings (>25 ys)
- RP, regrafted young plantings
- YP, young plantings (up to 5 ys)
- n= number of sites tested

B) OP and RP sites showed very similar level of the fungal DNA, while YP showed a lower level, likely because of the increased sun exposure. Surprisingly, DRS sites reached the highest median value and one was extremely high.

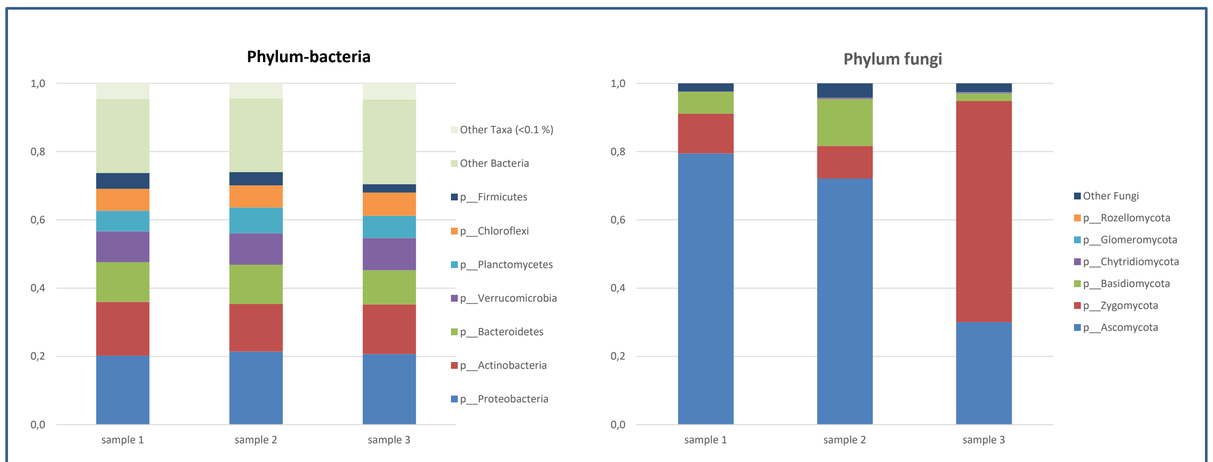


Farm	Locality	ID	Scion/stock	Year of planting	ng DNA Pt / 10 g soil	CFU/g^{-1}
1	Spinagallo	A1.4	Femminello SR / SO	1982	3.4	21
		A1.5	Femminello SR / SO	1982	4.64	13
2	S. Michele	A2.8	Verna/ Volk	2018	1.48	9
3	S. Elia	A3.6	Femminello 2KR/SO	2017	1.48	12
		A3.8	Femminello 2KR/SO	2017	0.6	10
4	S. Michele	A4.11	Femminello 2KR/SO.	2013	1.48	7
5	Fontane Bianche	A5.4	Femminello 2KR /Volk	2017	1.48	15
9	Bonavia	A9.8	Femm. Zagara bianca/SO	R2019	2.6	17
10	Cuba	A10.1	Ripped soil	2016	1.48	16
		A10.8	2KR/volk R	2019	4.64	11

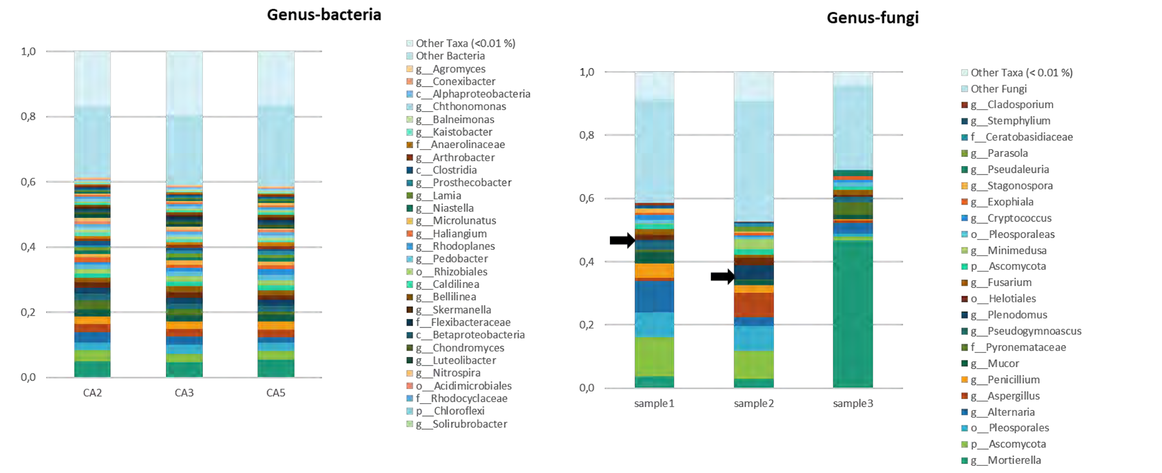


Real time PCR and culturing in semiselective media showed scion/rootstock combinations did not affect the soil value of Pt DNA

METAGENOMIC ANALYSIS OF FUNGAL AND BACTERIAL COMMUNITIES



Relative abundance of major taxonomic groups at the phylum level for bacterial (A) and fungal (B) communities in 3 samples of farm 1 obtained by amplicon based metagenomics using the 16S rRNA gene and ITS region, respectively



Differently from fungi, at genus level, bacterial communities were quite similar. Arrows show Plenodomus band in samples 1 and 2, that were positive in real-time PCR test, whereas is absent in the negative sample 3.

CONCLUSIONS

- *Plenodomus tracheiphilus* was detected in 77% of tested samples of soil
- A variable level of DNA was detected in 10 farms, not related to scion/rootstock combination
- > 95% of tested aliquots showed less than 0,8 ng of Pt DNA/g of soil
- Few samples showed over 2 ng of Pt DNA/g of soil, likely related to fractions of plant
- Soil microbiome analysis confirmed the pathogen in soil is associated to several fungi and bacteria, which could play a role on its persistence
- Colonies obtained on selective media confirmed a variable level of viability

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