

Root phenotyping strategies for screening the susceptibility of chestnut progenies to *Phytophthora cinnamomi*



2nd APPN/EPPN 2020 Meeting - 17th of April 2018 - Vienna

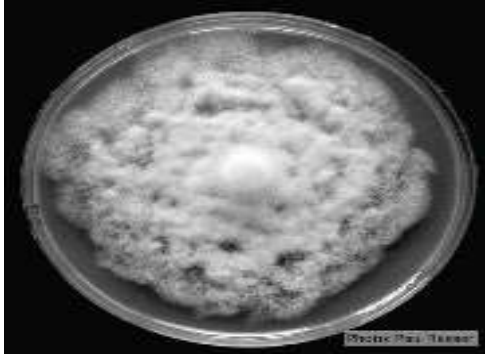


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Root rot – The main threat for chestnut in Europe

Phytophthora cinnamomi (Phyto= Plant; phthora= destroyer)



Micelium



Zoospore production



Zoospore penetration



Root rot

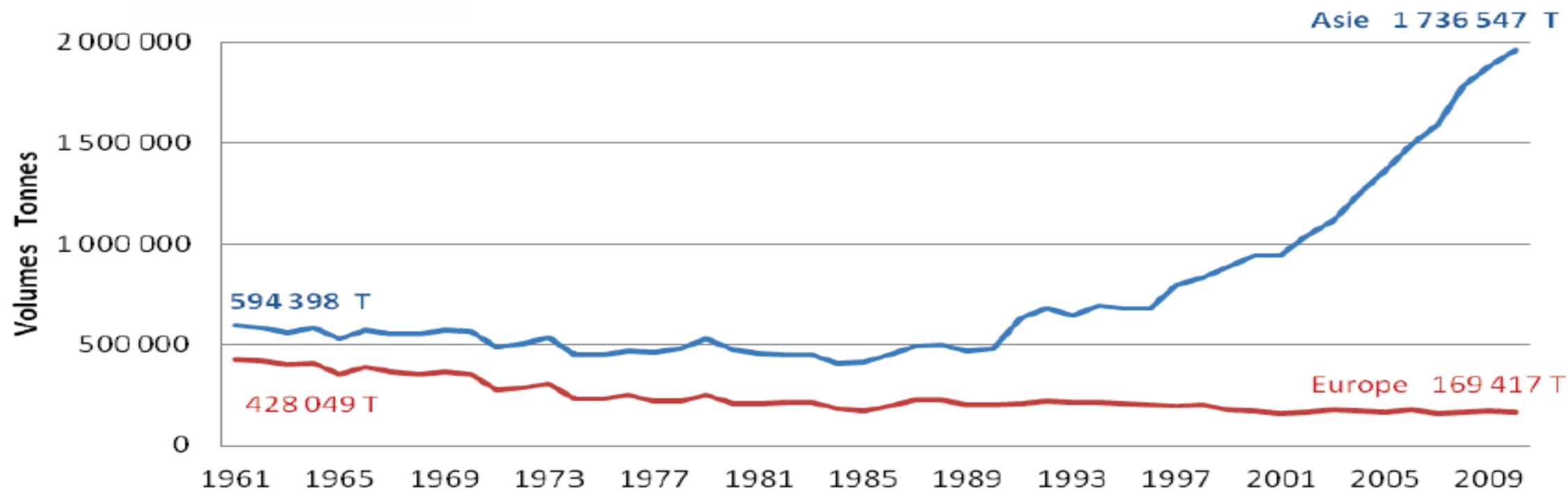


Collar rot



Death

Evolution of chestnut production in Asia and Europe (1961-2009)



Castanea mollissima na região de Pequim



Susceptibility of different species of *Castanea* to *P. cinnamomi*

Susceptible

European chestnut- *C. sativa*



American chestnut- *C. dentata*



The American Chestnut Foundation

Japanese chestnut- *C. crenata*



Chinese chestnut- *C. mollissima*



RESISTANT

The Breeding Program

RESISTANT

Japanese chestnut
(*C. crenata*) – donor of resistance



X

SUSCEPTIBLE

European chestnut
(*C. sativa*)



=

F1 Population: 155
progenies genotyped
and phenotyped for
the trait –
susceptibility to *P.
cinnamomi*



The Research Program

Mapping Approach

Castanea sativa x *Castanea crenata*

Phenotyping

Genotyping

Resistance level

Genetic
map

QTLs for *P. cinnamomi* resistance

Transcriptomic Approach

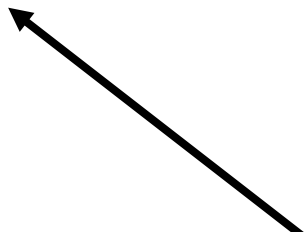
C. sativa and *C. crenata* inoculated and
non-inoculated

candidate genes for
Resistance

New markers
development

Gene expression
analysis

Hypothetical mechanism of
resistance



Phenotyping: two inoculation tests

Inoculation with a hypervirulent strain of *P. cinnamomi* (IMI 340340), Vila Real

Root Inoculation



- Biological replicates produced by micropropagation

Cuttings Inoculation



- Cuttings from mother stock plants (progenies)

Phenotyping: Root inoculation

Confined lesions

- Resistant and intermediate genotypes (Level 1 and 2)



Invasive lesions

- Susceptible genotypes (Level 6)



Phenotypic and genetic correlations

- **Level of Root Rot** : less important symptom associated to Survival;
- **Shoot Internal Lesion**: the main symptom negatively associated to Survival.

Phenotyping: Cuttings inoculation

- **139 progenies; cuttings of 15 cm**
- **2 seasons** (Spring and Autumn)
- **5 time-points** after inoculation (5, 7, 9, 12, 14 dai)
- **Lesion Progression rate** cm/day



Lesion length



Confined lesions

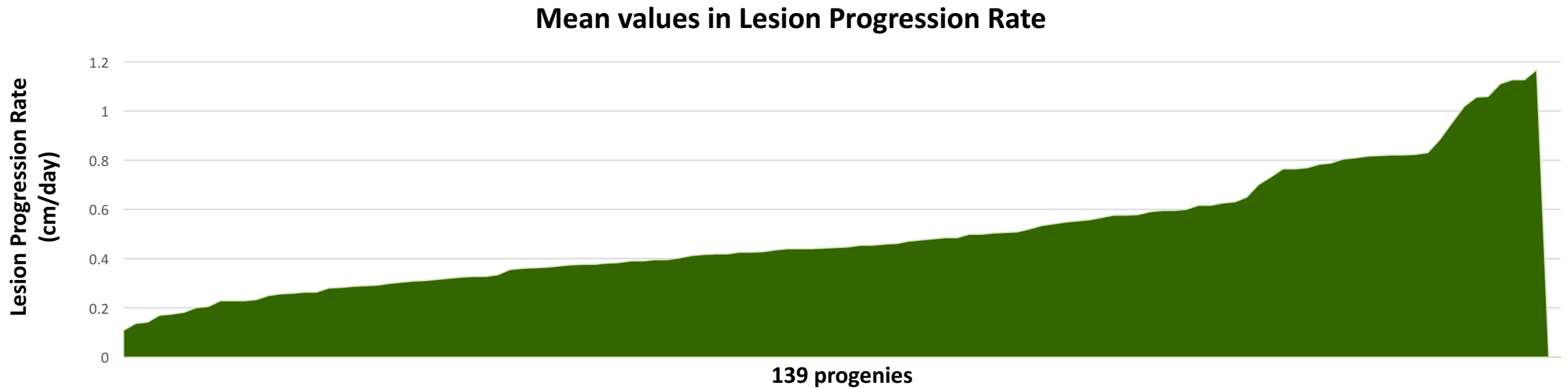


Invasive lesions



Phenotyping: cuttings inoculation

● Lesion Progression Rate (cm/day)



Quantitative phenotyping: continuous range from resistance - susceptibility

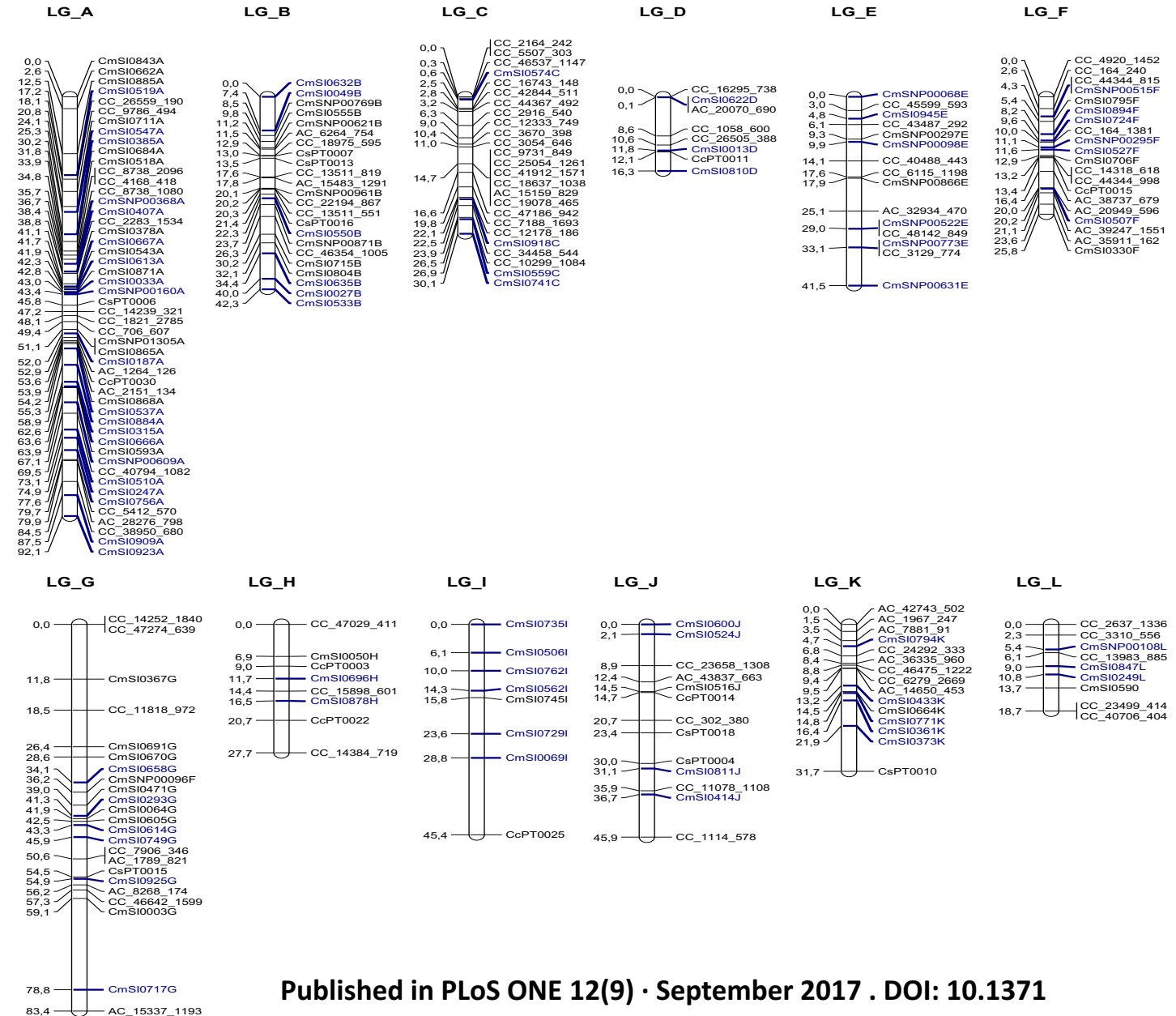


QTL analysis

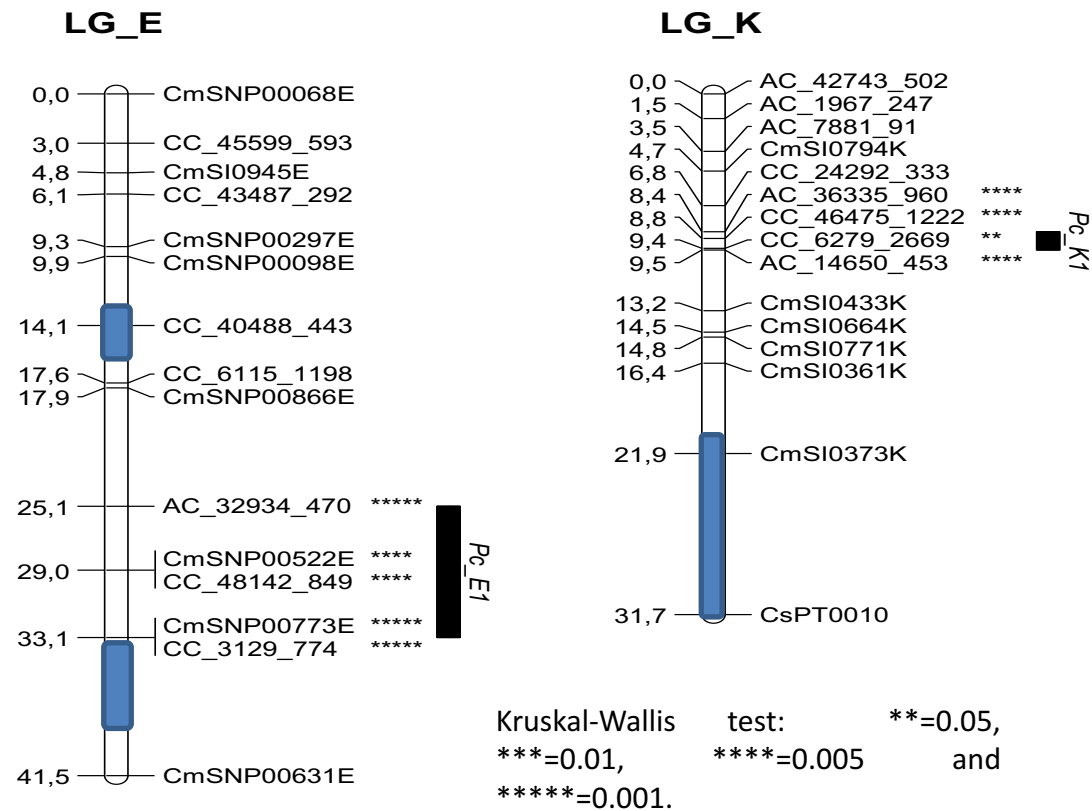
First interspecific genetic linkage map for *C. sativa* x *C. crenata* *

Parents and 133 progenies were genotyped to construct the first interspecific (*C. sativa* x *C. crenata* population) genetic map

- 217 markers (105 SSRs and 112 SNPs)
- 12 Linkage Groups (LG_A to LG_L)
- JoinMap software (LOD=4)
- Total genetic distance of 498,9 cM
- Marker density: 1/2.3 cM
- 34.3% of markers were also mapped on the consensus *C. mollissima* reference map (in blue)



P. cinnamomi resistance QTLs



- Trait: Lesion Progression Length in cuttings
- 2 QTLs were identified on 2 Linkage Groups
- Each QTL explains 9-13% of phenotypic variance
- 9 SNP markers

The presence of the QTL located in **LGE**, is coincident with QTL identified for blight resistance, in a highly saturated genetic linkage map produced with GBS markers for a cross between *C. dentata* x *C. mollissima* (USA) - **Tatyana Zhebentyayeva**

QTLs related with *P. cinnamomi* resistance were identified for the first time in trees

Transcriptomic approach: gene expression analysis

Plant Material



Gene selection

- 8 candidate genes for resistance selected from differential expressed genes identified in *Cci/Ccn* transcriptomes:
 - $\text{Log}_2(\text{Cci/Ccn}) > 1.5$
 - Not** involved in **general** biological processes
 - Involved** in different layers of defense response

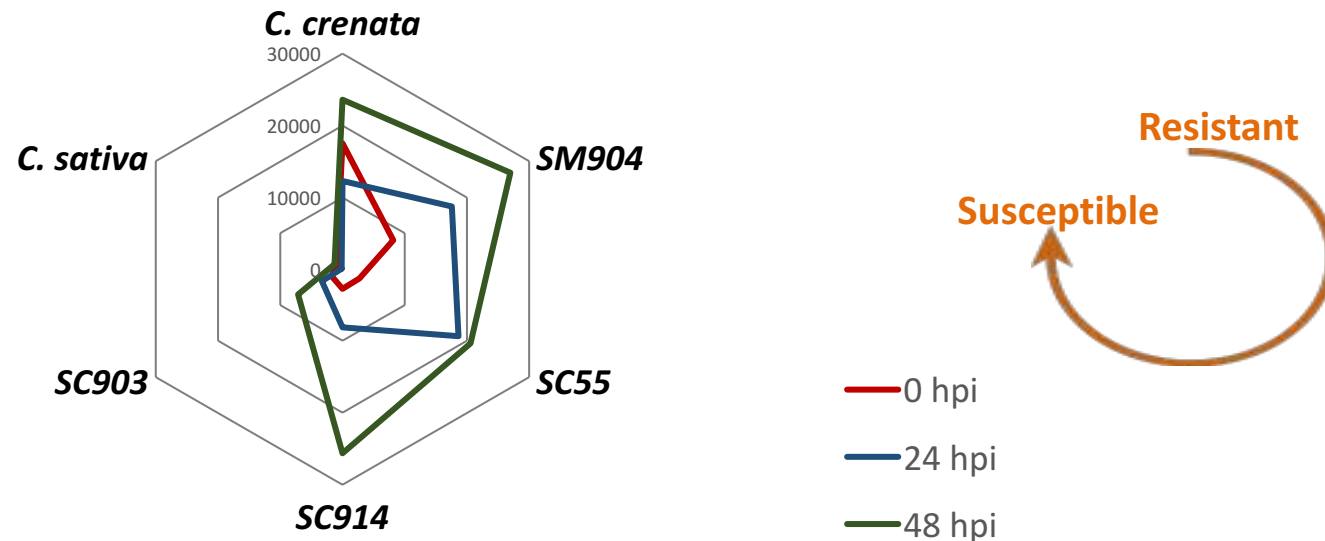
- 6 genotypes (biological triplicates) with different phenotypes;
- Extraction of RNA from roots at 0h (non-inoculated), 24h and 48h after inoculation with *Phytophthora cinnamomi* ;

Gene acronyms	BLAST best hit/Function
<i>Cast_Gnk2-like</i>	Ginkobilobin 2/ Antifungal secretory protein
<i>Cast_PE-2</i>	Pectinesterase 2/ Cell wall reinforcement
<i>Cast_LRR-RLK</i>	LRR receptor-like kinase/ pathogen recognition
<i>Cast_C2CD</i>	C2 calcium-dependent membrane / pathogen recognition
<i>Cast_ABR1</i>	PR transcriptional factor, ABR1/ ABA regulator
<i>Cast_Myb4</i>	DNA binding, Myb4/ C4H regulator
<i>Cast_WRKY 31</i>	WRKY transcription factor 31/ PR gene regulation
<i>Cast_RNF5</i>	RING finger protein 5/ Ubiquitination regulator

Transcriptomic approach: gene expression analysis

Gene expression variation assessed by **3D Digital PCR** (chip-based)

- ***Cast_Gnk2-like***: Antifungal secretory protein

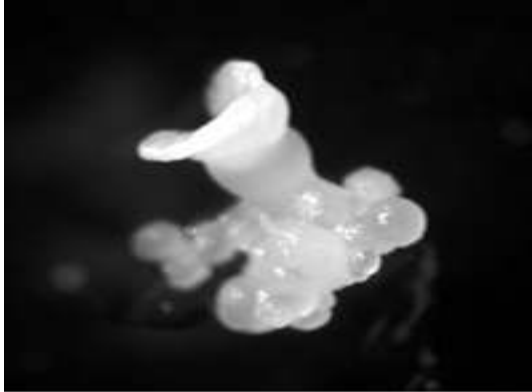


- *Gnk2-like* may inhibit pathogen penetration or growth in resistant genotypes (hostile environment)

AMERICAN CHESTNUT

RESEARCH AND RESTORATION PROJECT

Establishment



Multiplication



Regeneration



Rooting



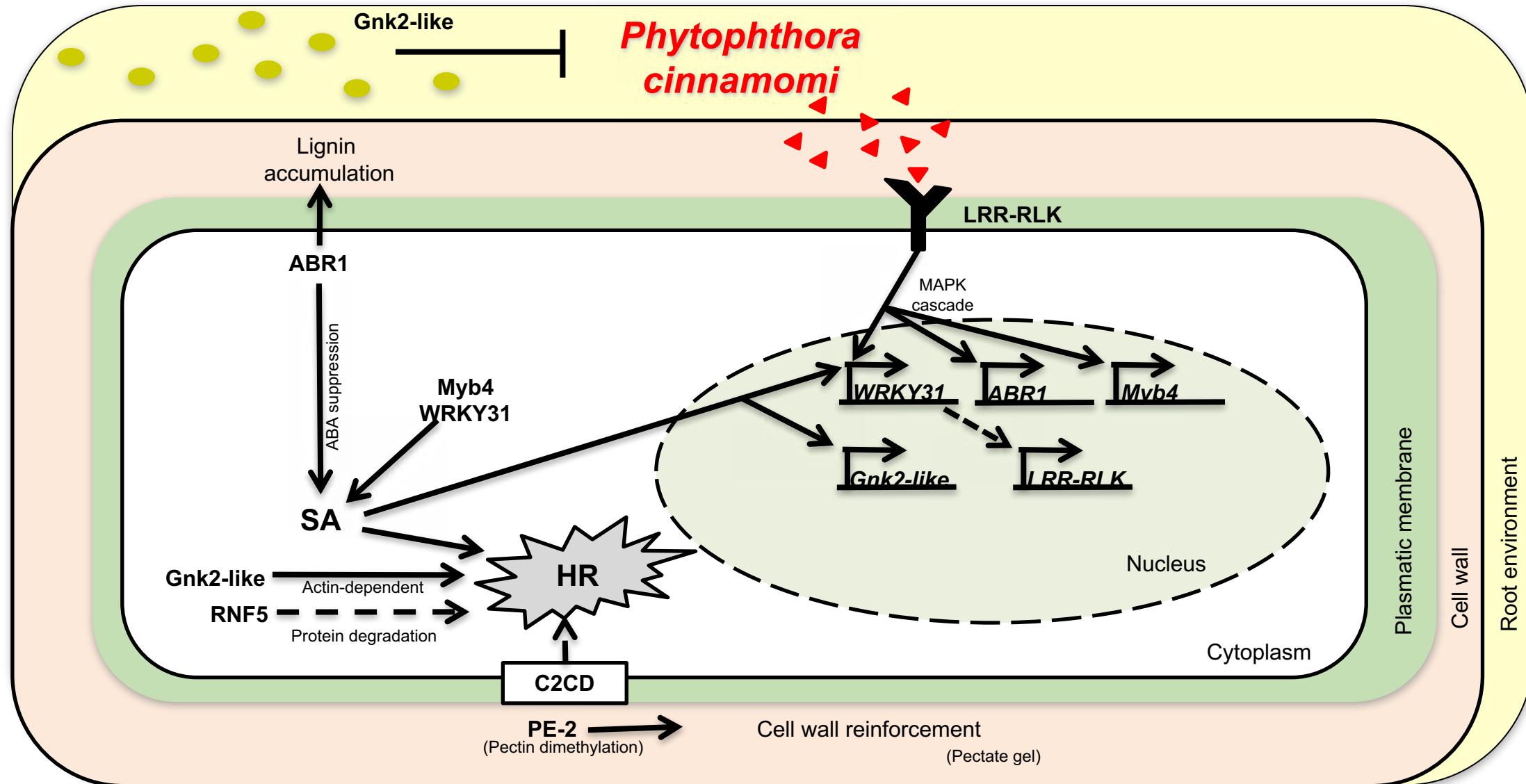
Acclimatization



Functional Validation

Abordagem transcriptômica: modelo hipotético

SA, Salicylic Acid; HR, Hypersensitive Response;



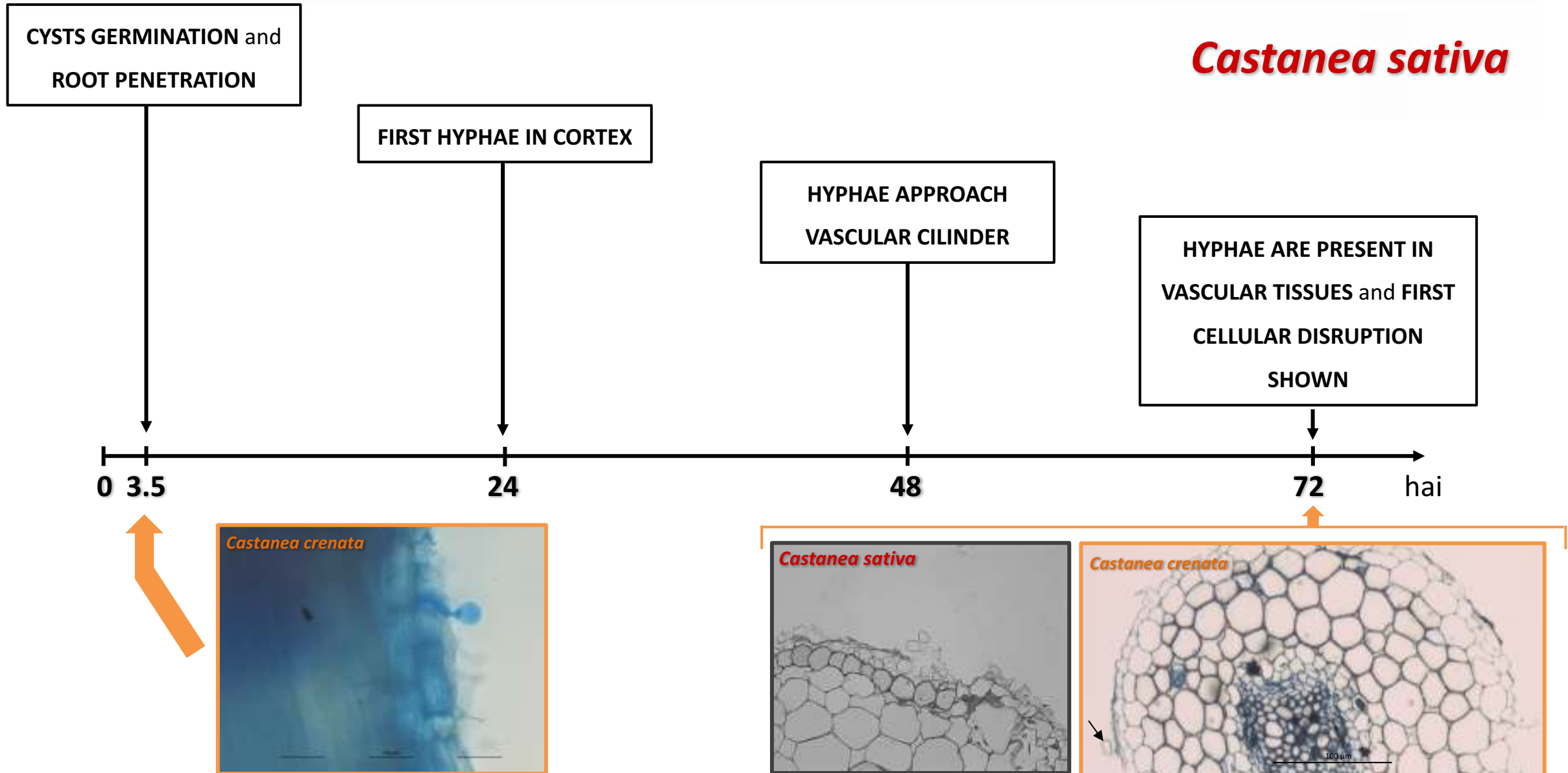
Histopathology - Experimental procedure

CLARIFY THE PATTERN OF INFECTION AND HISTOLOGICAL CHANGES INDUCED BY *P. cinnamomi*



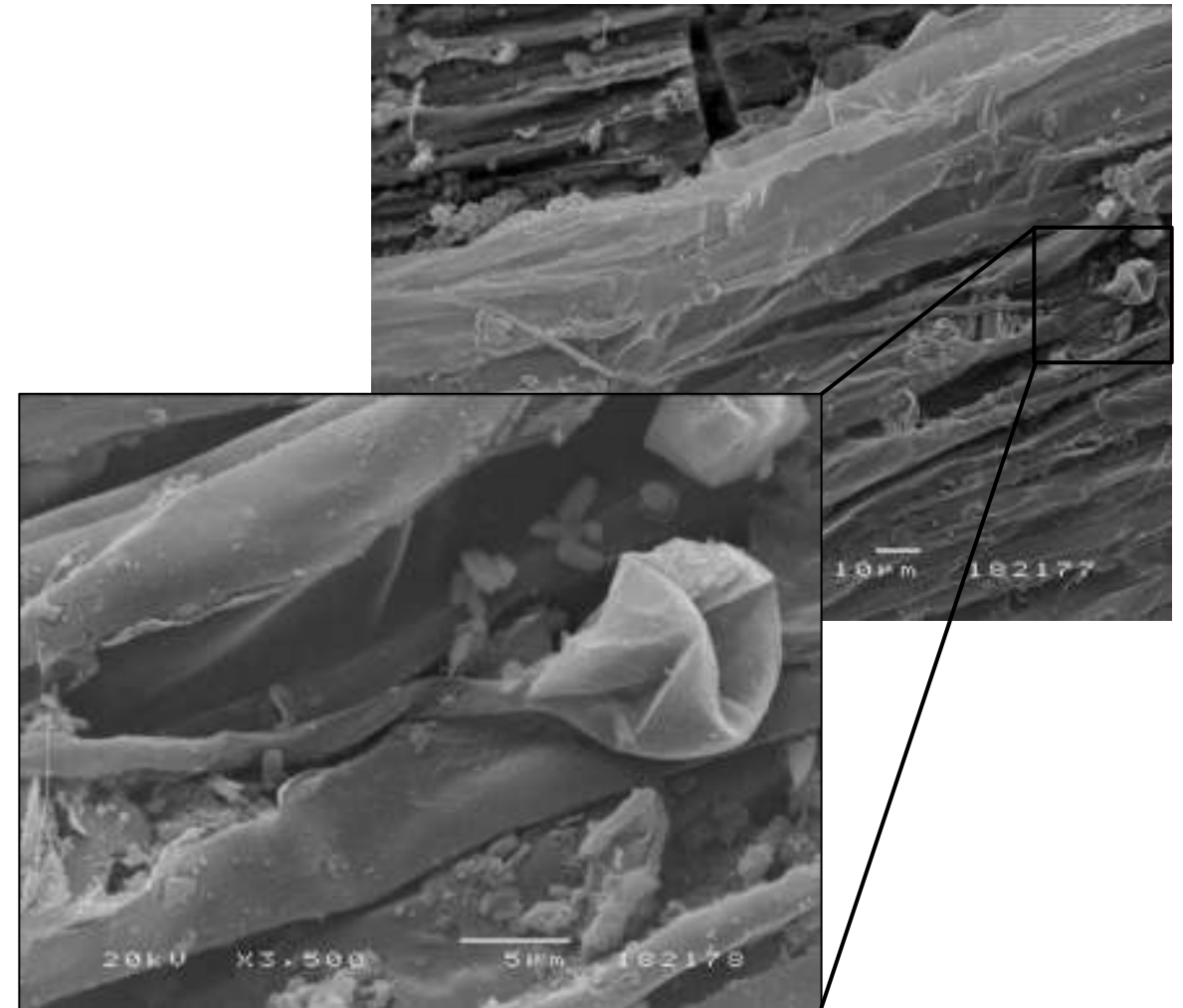
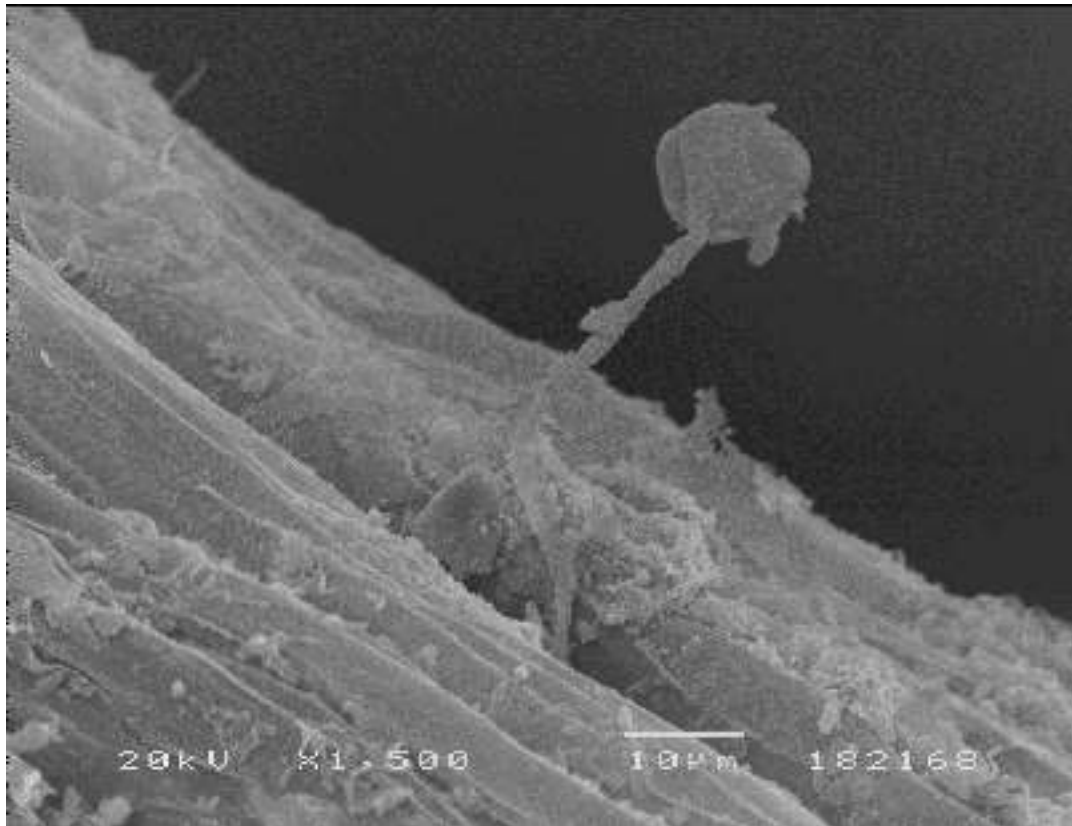
- Contrasting genotypes: *Castanea sativa* and *Castanea crenata*
 - Inoculation with a *P. cinnamomi* zoospore suspension
 - Roots of six months-old plantlets, obtained by micropropagation
 - Samples were collected at 3.5, 24, 48 and 72 hours after inoculation
 - Control plantlets were also collected (non-inoculated)
- } Light microscopy

First results



Zoósporos - *Castanea sativa* 3h30

SEM
10000 zoósporos/ml



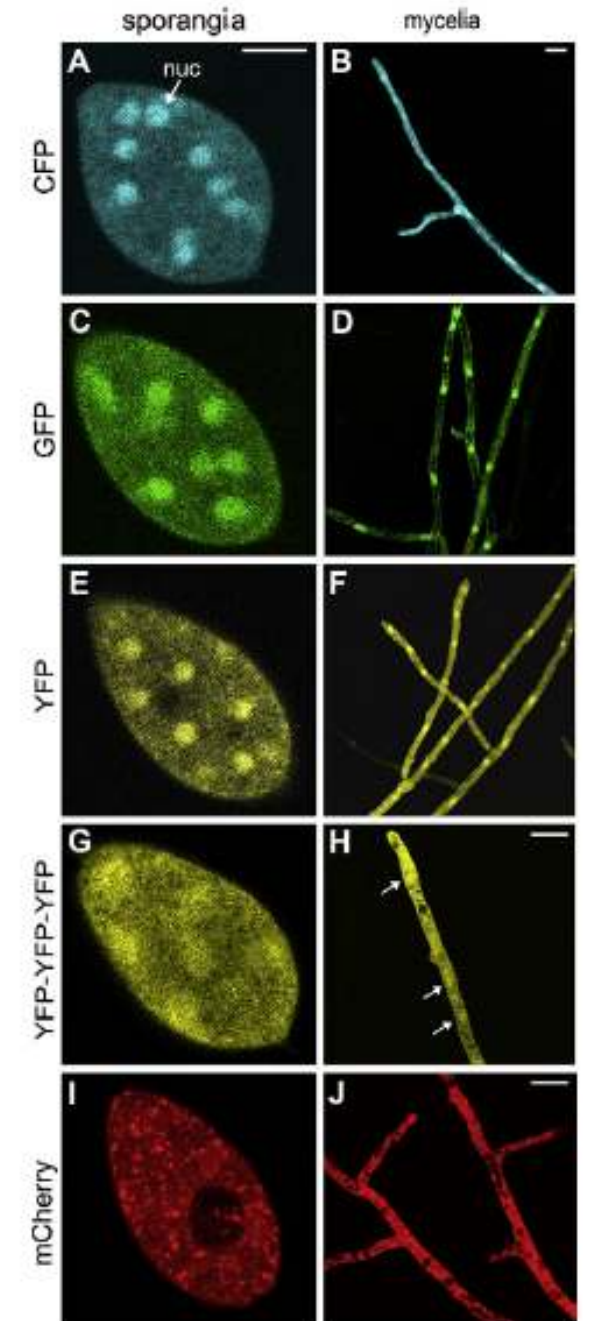
In Progress:

Transformation of *P. cinnamomi* with vectors for protein tagging*

SignalSCREEN - EPPN2020 automated high-throughput confocal imaging platform
Leader: Dr. Corina Vlot-Schuster - Helmholtz Zentrum Muenchen, Institute of Biochemical Plant Pathology - Germany



*Ah-Fong AMV, Judelson HS.(2011) - Vectors for fluorescent protein tagging in Phytophthora: tools for functional genomics and cell biology [Fungal Biology](#). Volume 115, Issue 9, September 2011, Pages 882-890.
doi:10.1016/j.funbio.2011.07.001



Future perspectives

Mapping Approach

Castanea sativa x *Castanea crenata*

303 progenies

Phenotyping

Histopathology

Genotyping

Genotyping by
sequencing

Resistance level

High Density Genetic
map

QTLs for *P. cinnamomi* resistance

Genes underlying QTLs

Marker Assisted Selection

Key issue for phenotyping

High throughput platform

Transcriptomic Approach

C. sativa and *C. crenata* replicates
inoculated and not-inoculated with *P.*
cinnamomi

Resistance candidate genes

New markers
development

Gene expression
analysis

Functional analysis

Thank You

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EUROPEAN UNION



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