Instituto Universitario de Investigación iuFOR GESTIÓN FORESTAL SOSTENIBLE

GOBIERNO de CANTABRIA
CONSEJERÍA DE GANADERÍA, PESCA Y DESARROLLO RURAL
DIRECCIÓN GENERAL DE MONTES Y CONSERVACIÓN DE LA NATURALEZA

Interreg Sudoe
Plans transnationaux pour la gestion des risques forestiers
PLURIFOR
European Regional Development Fund

New tools for diagnosing *Fusarium circinatum*:

Presentation of a new tool using Next Generation Sequencing for the monitoring of *Fusarium circinatum* in the REINFFORCE arboreta

Julio Javier Diez Casero, UVa

Pine Pitch Canker Workshop
Aveiro, Portugal. October 3th, 2017

Risks

Risk management plan	Coordinator	Regions directly involved	Region participating in the workshops
Abiotic			
Storm	Barry Gardiner-EFIATLANTIC	Aquitaine, Basque Country	Asturias
Fire	Francisco Rego - ISA	Basque Country, Asturias, Portugal, Galicia	Aquitaine
Soil degradation	Ander Gonzalez-NEIKER	Basque Country, Asturias, Galicia, Portugal	
Biotic			
Pinewood nematode (*Bursaphelenchus xylophilus*)	Hervé Jactel-INRA and Edmundo Sousa-INIAV	Aquitaine, Castille y Léon, Portugal	Basque-Country
Chestnut gall wasp (*Dryocosmus kuriphilus*)	Edmundo Sousa-INIAV	Estremadura, Portugal	Basque-Country, Aquitaine
Eucalypt weevil (*Gonipterus platensis*)	Manuela Branco-ISA	Asturias, Cantabria, Portugal	Basque-Country
Pine pitch canker (*Fusarium circinatum*)	Julio Diez-UVA	Cantabria, Portugal	
Emerging pests and pathogens	Hervé Jactel-INRA	Aquitaine, Basque-Country, Portugal	Asturias

Project GT2

Coordinator: ISA

Goal: Improving risk management plans

T1: Transnational workshop gathering organisations involved in risk management and labs in charge of new studies

→ One workshop per hazard with exchanges: feedback on successful methods and sharing needs for tools

Deliverables: 1/3

Minutes of workshops, report on conclusions

Project GT2

T2: Improving tools for risk management
→according to conclusion of GT1 and of workshops from GT2.T1, development of new, improved tools : examples...

- New diagnostic tools for *Fusarium circinatum* detection
- defoliation protocol for *G. Platensis*
- tool to prioritize fuel reduction to decrease fire risk
- traps to detect pinewood nematode in vector beetles
- methods for early detection of exotic pests and pathogens
- ...any other tool identified

→Tools developed transnationally: for one risk, one tool should be developed and useful in several regions

Deliverables: 2/3
Synthesis sheet on each developed tool (available on the website)

Project GT2

Goal: Improving risk management plans

T3: Writing risk management plans

→ Integration of developed tools

→ Considering several steps in RMP: prevention, detection, crisis management and rehabilitation

- One plan per region per risk
- Showing cooperation between SUDOE regions
- Reviewed by forest owners (USSE)

Deliverables: 3/3

First draft of each plan: one report per hazard, with synthesis of main common steps to several regional plans

Project GT2

Pine Pitch Canker caused by *Fusarium circinatum*

Problems to be solved

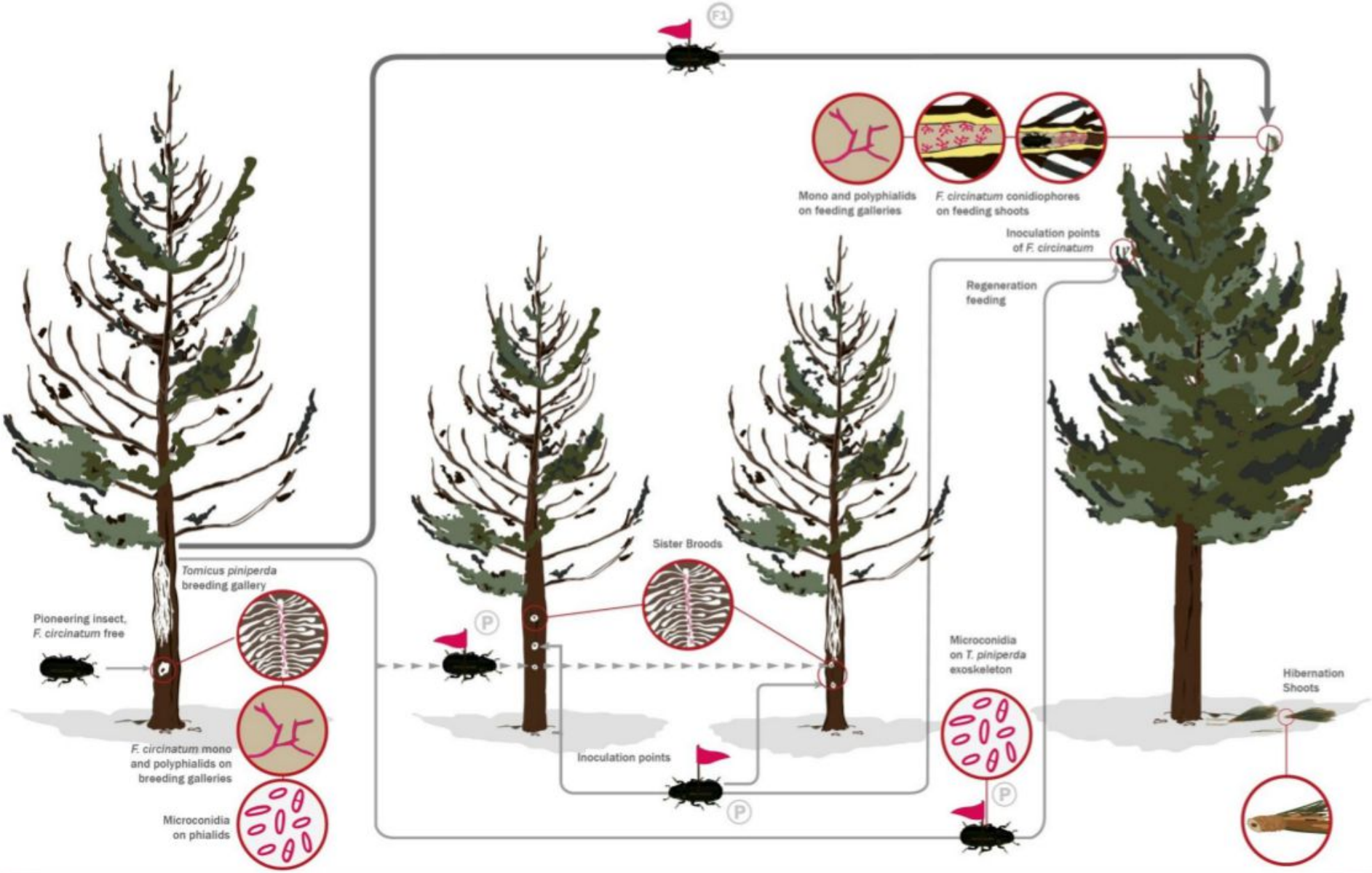
1. Pathways of Disease Spread

→ Endophytic State of the Fungus
→ Healthy Plants Harvouring PPC
→ Other Plants in the Understory as Reservoir of FC

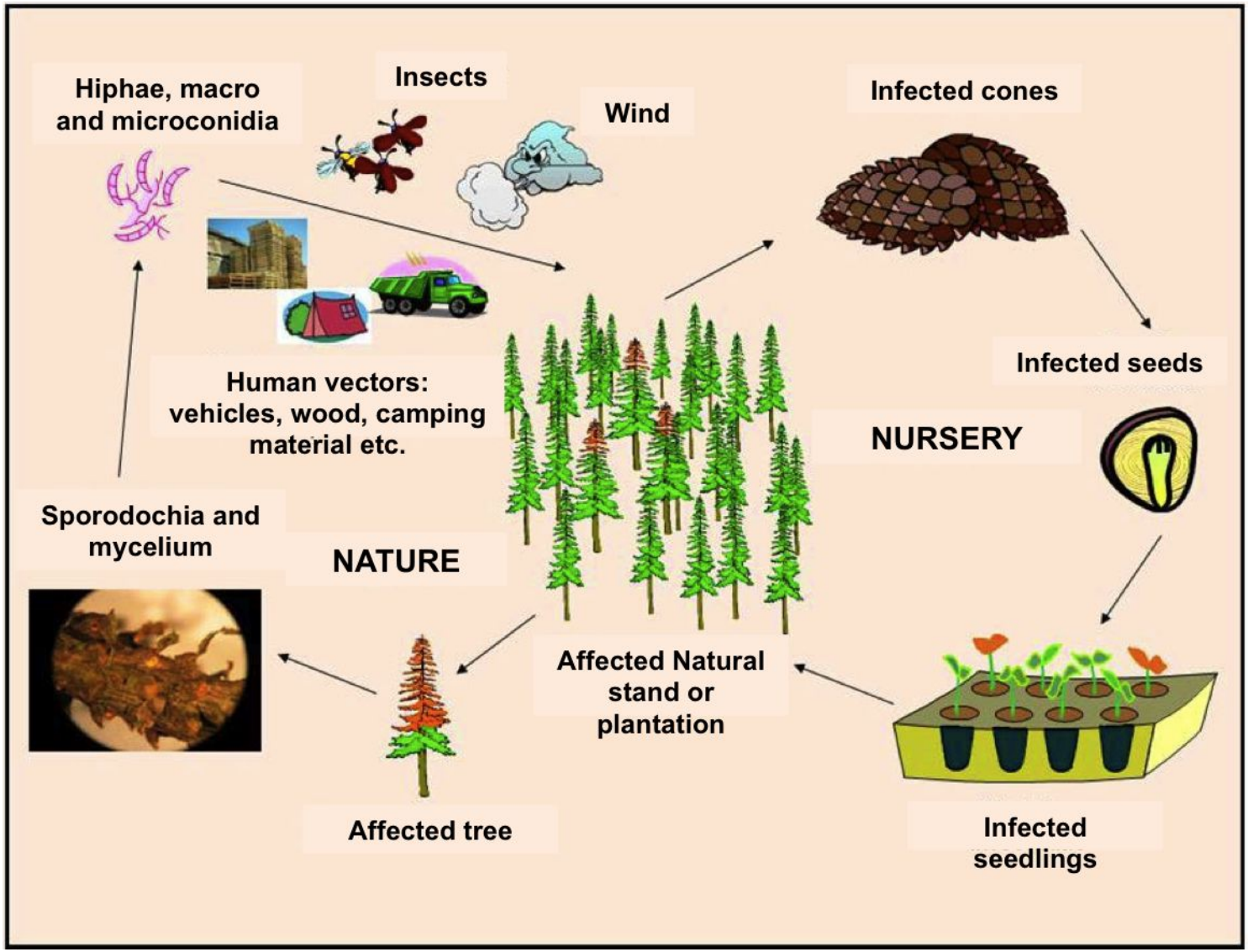
2. Accuracy in Fungal Identificacion

- qPCR
- NGS Analyses

Cycle of the PPC



Cycle of the PPC



Cycle of the PPC

Forest Pathology
WILEY-BLACKWELL
Explore this journal >

ORIGINAL ARTICLE

Exposure to a pine pathogen enhances growth and disease resistance in *Pinus radiata* seedlings

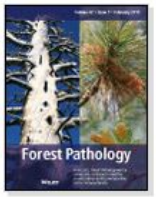
C. L. Swett, T. R. Gordon

First published: 7 December 2016 Full publication history

DOI: 10.1111/efp.12298 View/save citation

Cited by (CrossRef): 0 articles Check for updates Citation tools ▾

Am score



View issue TOC
Volume 47, Issue 1
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e12298

Summary

Most studies of *Fusarium circinatum*, the cause of pitch canker in pines, have focused on its activity as a pathogen. However, recent findings indicate that this fungus can colonize roots of *Pinus radiata* without inducing symptoms. Contrary to expectations, this study revealed that seedlings grown in infested sand grew more rapidly than seedlings not exposed to *F. circinatum*, based on root and shoot biomass, with modifications to root system architecture, including increased mycorrhizal root development. These effects were dependent on inoculum density and duration following growth in infested rooting medium. Plants exposed to *F. circinatum* expressed elevated resistance to stem infections, which significantly decreased the incidence of mortality; as above, effects were dependent on inoculum density. Resistance to stem infections was also enhanced in seedlings that emerged through infested litter, as occurs in native stands. Beneficial to neutral interactions of *F. circinatum* with its host suggest that the life history of this fungus may be more complex than previously recognized, with activities similar to non-pathogenic endophytes. The potential for non-lethal infections by *F. circinatum* to induce resistance in seedlings may influence dynamics of stand establishment. Overall, these results

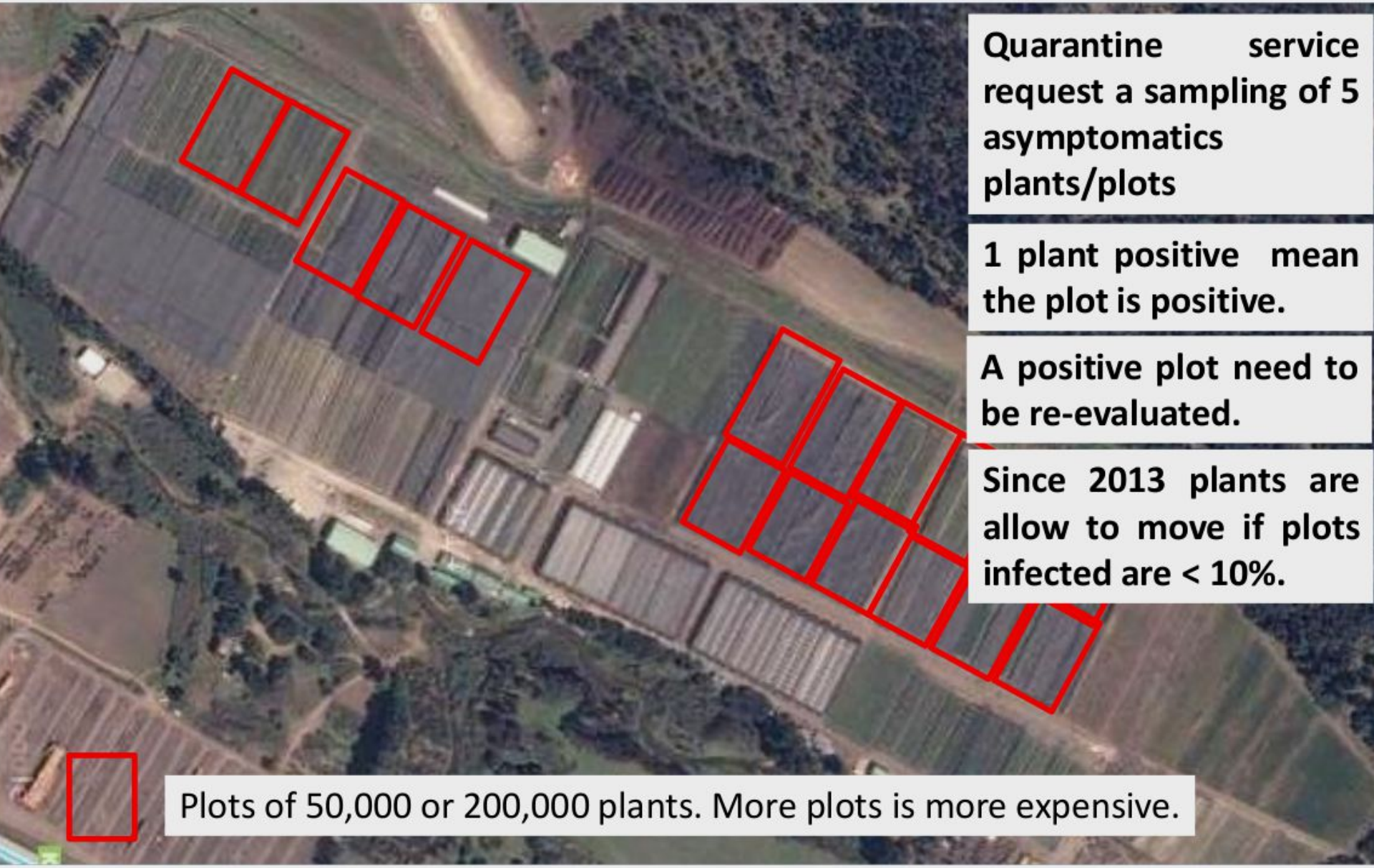
ARAUCO | Bioforest

Fusarium circinatum in Chile:
Regulations and Implications

Rodrigo Ahumada

Viterbo, Italy - November 2016

Sampling at the nursery



Fusarium circinatum
arrived to stay forever

Project GT2

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→ Endophytic State of the Fungus
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2. Accuracy in Fungal Identificacion

- qPCR
- NGS Analyses

Next Generation Sequencing (NGS)

With its unprecedented throughput, scalability, and speed, next-generation sequencing (NGS) enables researchers to study biological systems at a level never before possible.

Today's complex genomic research questions demand a depth of information beyond the capacity of traditional DNA sequencing technologies. Next-generation sequencing has filled that gap and become an everyday research tool to address these questions.

https://www.youtube.com/watch?v=-7GK1HXwCtE

ILLUMINA MiSeq

- Fast
- Flexible
- Simple

- Output: 15 Gb
- Number of reads: 25 M
- Read length: 2 X 300 bp

- Illumina MiSeq used mainly in metagenomics, RNA sequencing, amplicon sequencing, de novo sequencing small genomes

Material and Methods

- **10** plots of *Pinus pinaster* were sampled. The plots were selected according to Prieto et al., (2015)

- All the studied plots showed dieback symptoms.

- Ten soil subsamples were collected and mixed to get a representative mycobiota from the soil.

Material and Methods

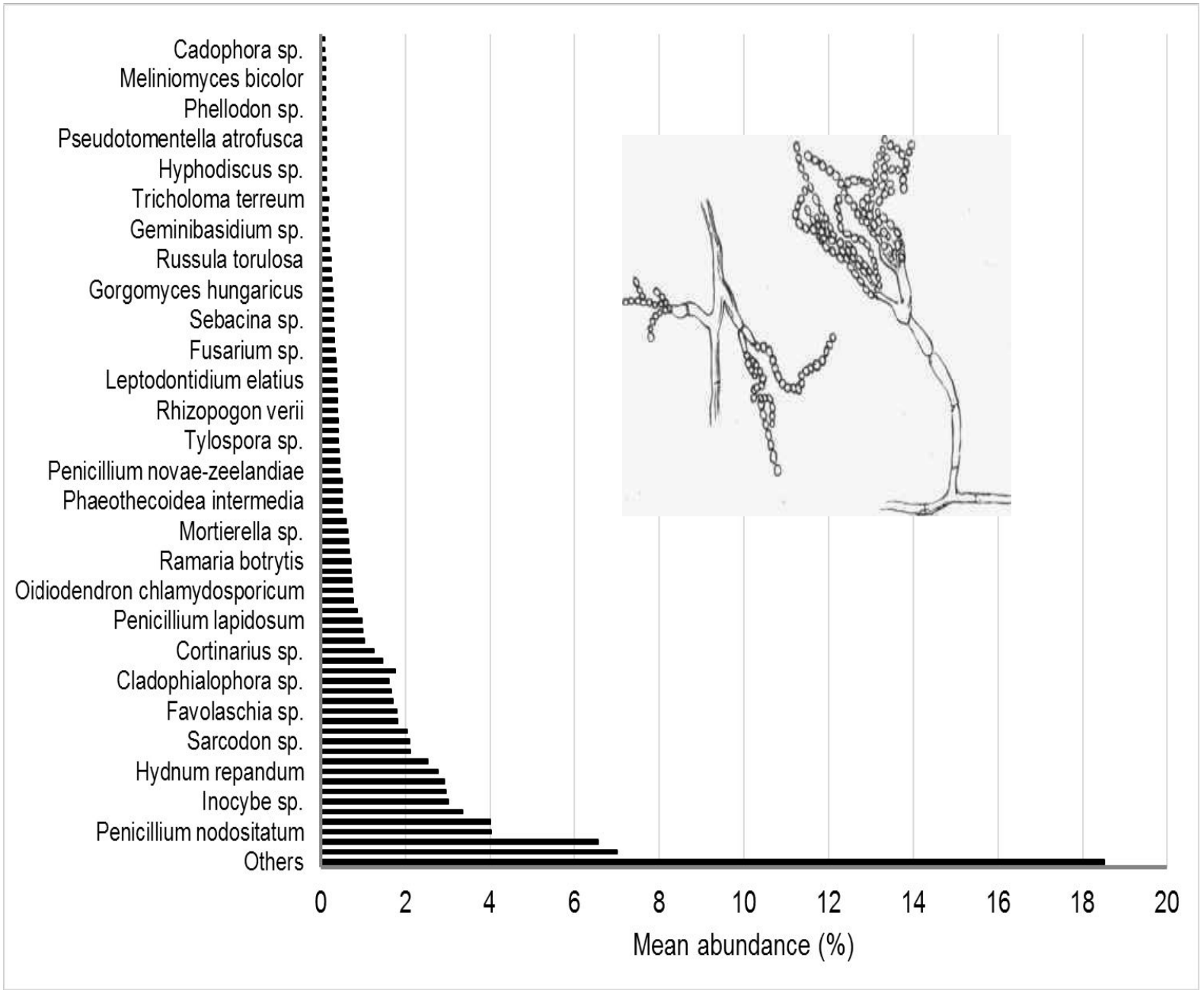
- Total DNA was extracted from the soil sample
- Next Generation Sequencing (NGS) was applied using Illumina sequencing.
- The molecular marker were ITS (fungi) and 16s (bacteria).
- The resulting reads were clustered by OTUs (operational taxonomic units) in order to manage the millions of reads.
- OTUs were compared with a specific database of soil microbiome.

Plot	Bacteria		Fungi	
	Number of bacterial species	Shannon index	Number of fungal species	Shannon index
A3800A	562	6.28	47	2.52
A38001	535	6.38	158	2.08
A38002	663	6.79	106	2.16
A38003	663	6.59	277	4.55
A38004	629	6.77	316	5.34
A38005	463	6.40	241	5.03
A38006	599	6.66	372	5.58
A38007	595	6.48	423	5.97
A38008	526	6.60	358	5.47
A38009	561	6.35	187	3.54

Results (Fungi)

- High detection rate (>100 fungal taxa per plot; 47 to 423)

- Strong dominance by a few taxa:
  - *Tricholoma portentosum*
  - *Epicoccum nigrum*
  - *Penicillium nodositatum*
  - *Mycena plumipes*


Cadophora sp.
Meliniomyces bicolor
Phellodon sp.
Pseudotomentella atrofusca
Hyphodiscus sp.
Tricholoma terreum
Geminibasidium sp.
Russula torulosa
Gorgomyces hungaricus
Sebacina sp.
Fusarium sp.
Leptodontidium elatius
Rhizopogon verii
Tylospora sp.
Penicillium novae-zeelandiae
Phaeothecoidea intermedia
Mortierella sp.
Ramaria botrytis
Oidiodendron chlamydosporicum
Penicillium lapidosum
Cortinarius sp.
Cladophialophora sp.
Favolaschia sp.
Sarcodon sp.
Hydnum repandum
Inocybe sp.
Penicillium nodositatum
Others
0
2
4
6
8
10
12
14
16
18
20
Mean abundance (%)

Results (Fungi)

- Some pathogenic genera / species were identified by ITS:

-*Botrytis cinerea*

-*Fusarium* spp.

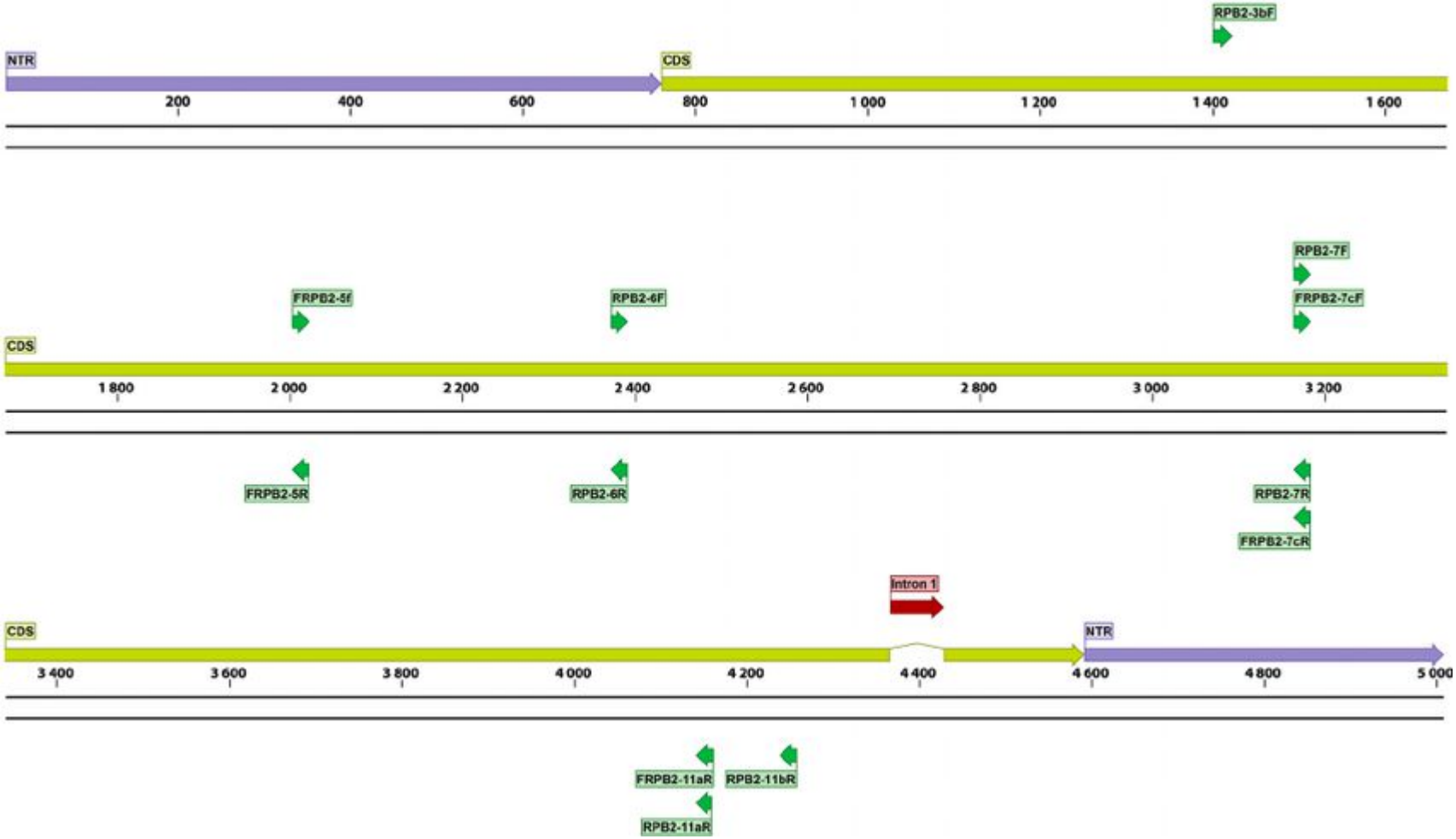
-*Chalara* spp.

-*Venturia* spp.

New specific searches using more accurate algorithms (i.e. Blastn, Megablast and Blastx) are required to ensure the detection of *Fusarium circinatum.*

Genes useful to ***Fusarium*** spp identification:

(1) ITS, (2) LSU, (3) Elongation Factor



Genes useful to ***Fusarium*** spp identification:

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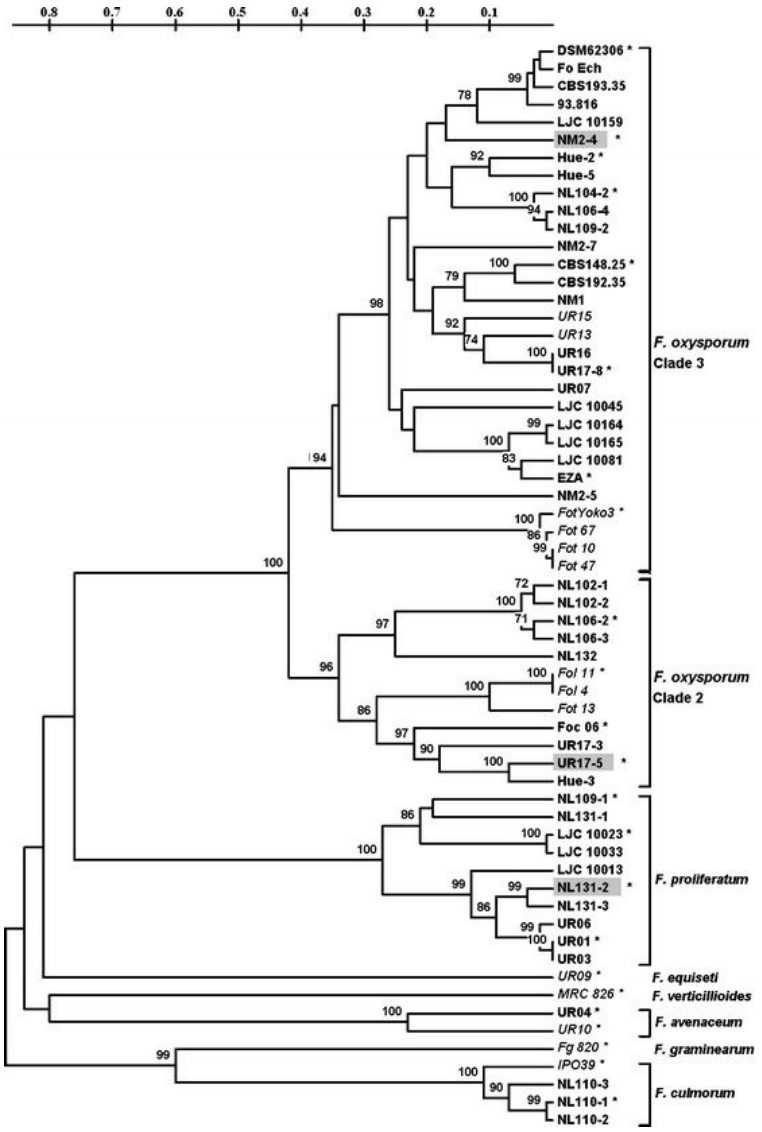


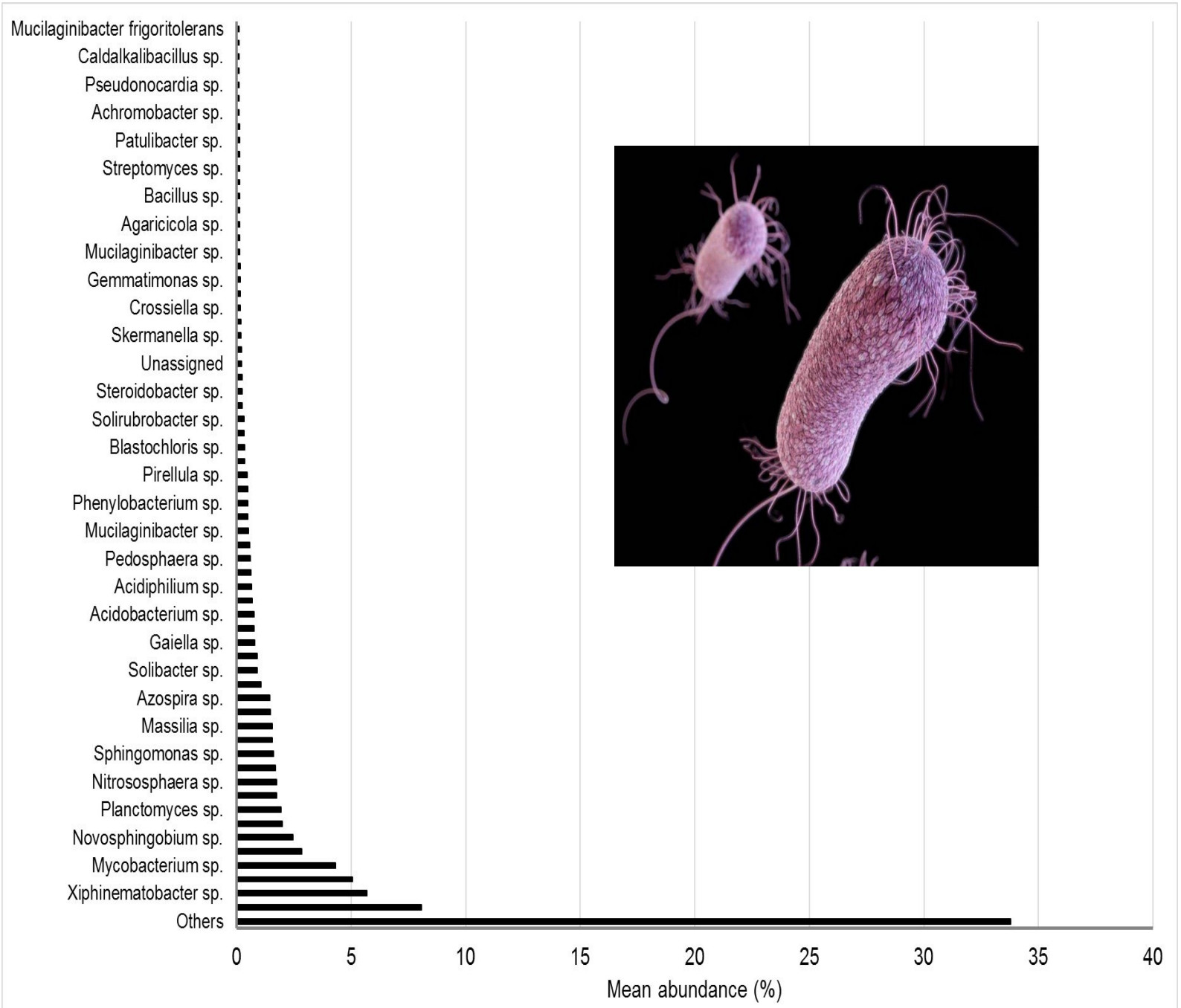
Results (Bacteria)

- High detection rate (>200 fungal taxa per plot; 463 to 663)

- Some interesting genera:

→*Pseudomonas* sp.

- Some of these genera could be interesting in biocontrol (plant health promoters or fungal antagonist)


Mucilaginibacter frigoritolerans
Caldalkalibacillus sp.
Pseudonocardia sp.
Achromobacter sp.
Patulibacter sp.
Streptomyces sp.
Bacillus sp.
Agaricicola sp.
Mucilaginibacter sp.
Gemmatimonas sp.
Crossiella sp.
Skermanella sp.
Unassigned
Steroidobacter sp.
Solirubrobacter sp.
Blastochloris sp.
Pirellula sp.
Phenylobacterium sp.
Mucilaginibacter sp.
Pedosphaera sp.
Acidiphilium sp.
Acidobacterium sp.
Gaiella sp.
Solibacter sp.
Azospira sp.
Massilia sp.
Sphingomonas sp.
Nitrososphaera sp.
Planctomyces sp.
Novosphingobium sp.
Mycobacterium sp.
Xiphinematobacter sp.
Others
0
5
10
15
20
25
30
35
40
Mean abundance (%)

REINFFORCE

(**RE**source **INF**rastructure for monitoring and adapting European Atlantic **FOR**ests under **C**hanging climat**E**)

"ESTABLECIMIENTO DE UNA RED DE ARBORETUMS EN LA ZONA ATLÁNTICA EUROPEA, PARA EL SEGUIMIENTO DE LA ADAPTACIÓN DE LOS BOSQUES AL CAMBIO CLIMÁTICO"

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CRISTINA PRIETO RECIO
FELIPE BRAVO OVIEDO
JULIO JAVIER DIEZ CASERO

REINFFORCE *-REsource INFrastructure for monitoring and adapting European Atlantic FORests under Changing climatE*

- El proyecto, para la monitorización del cambio climático a través de su impacto en los bosques del área occidental de Europa, está integrado por **11 instituciones** de diferentes países de la Unión Europea:

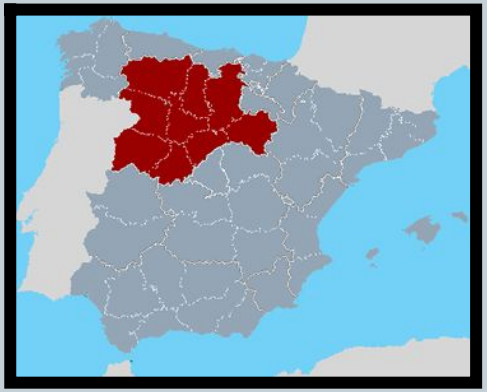
- FOREST RESEARCH. **Reino Unido.**

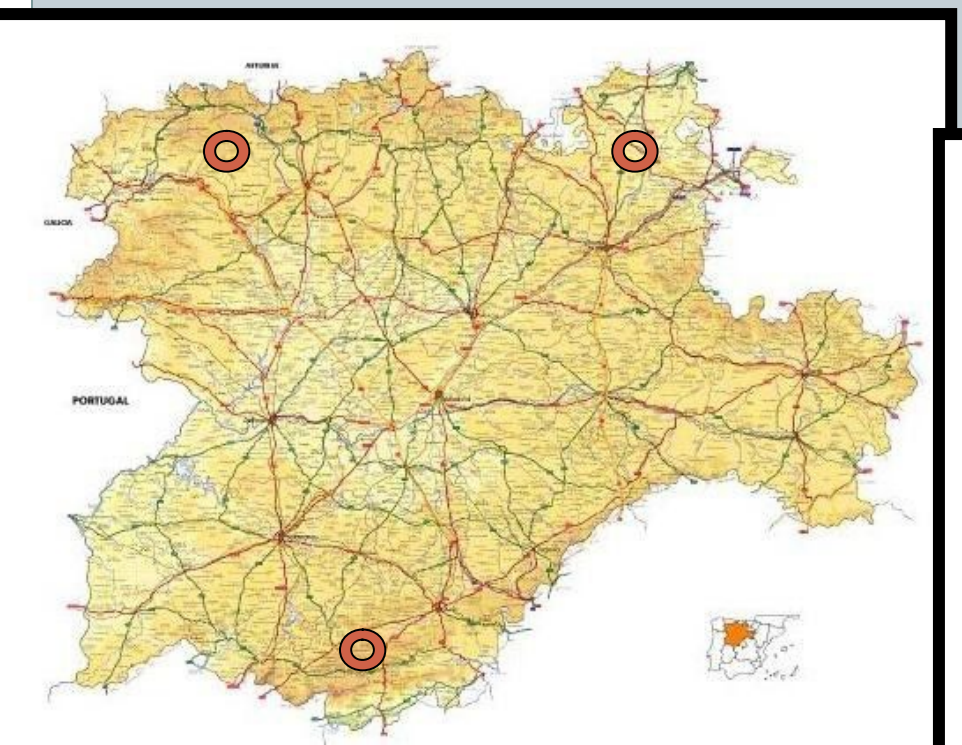
- INSTITUT EUROPÉEN DE LA FORêT CULTIVÉE (IEFC). Francia.
- CENTRE RÉGIONAL DE LA PROPRIÉTÉ FORESTIÉRE (CRPF). Francia.
- INSTITUT NATIONAL DE RECHERCHE AGRONOMIQUE (INRA). Francia
- CENTRE NATIONAL PROFESSIONNEL DE LA PROPRIÉTÉ FORESTIÉRE. **Francia.**

- XUNTA DE GALICIA. Centro de Investigación e Información Ambiental. (CINAM)
- VIVEROS Y REPOBLACIONES DE NAVARRA S.A. (GAVRN)
- NEIKER. Bizkaia. País Vasco.
- NEKAZAL IKERKETA ETA TEKNOLOGÍA (IKT). Alava. Pais Vasco.
- FUNDACIÓN GENERAL DE LA UNIVERSIDAD DE VALLADOLID (FGUVA). Castilla y León. **España.**

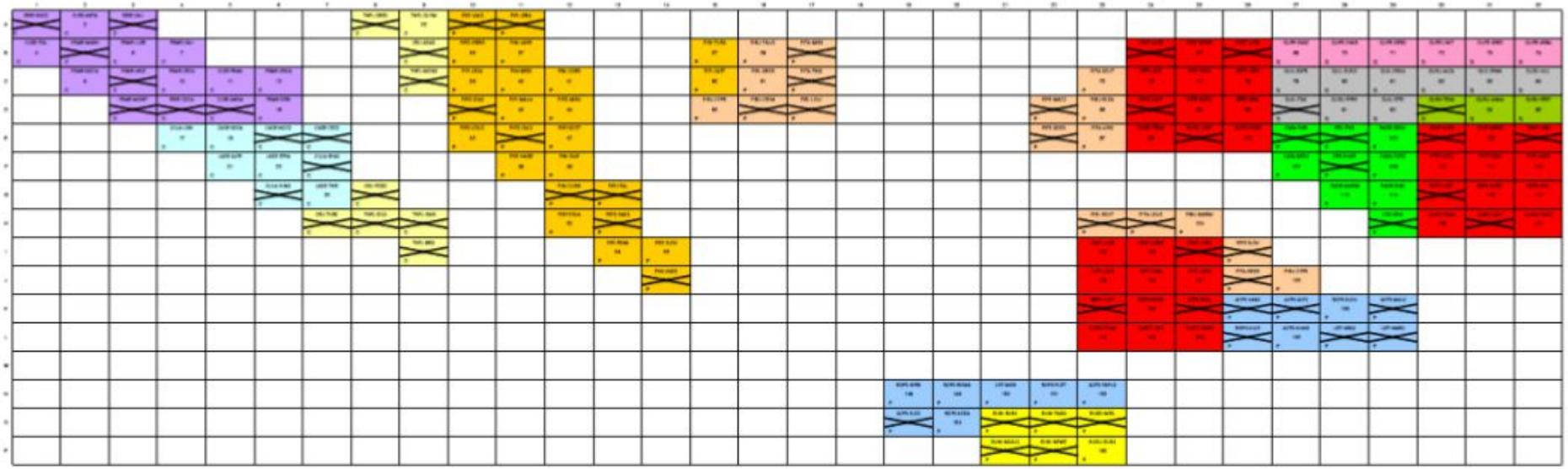
- INSTITUTO SUPERIOR DE AGRONOMÍA. (ISA). **Portugal.**

REINFFORCE - Castilla y León - Cantabria

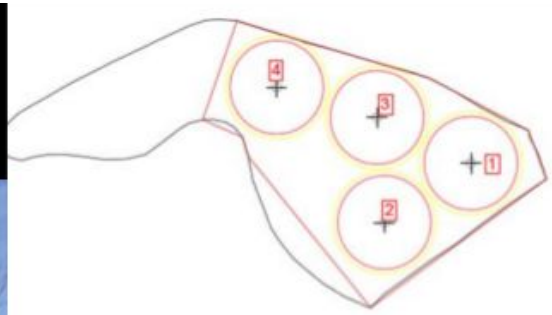


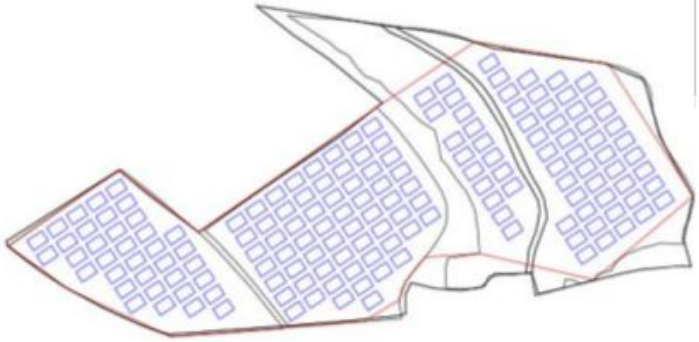


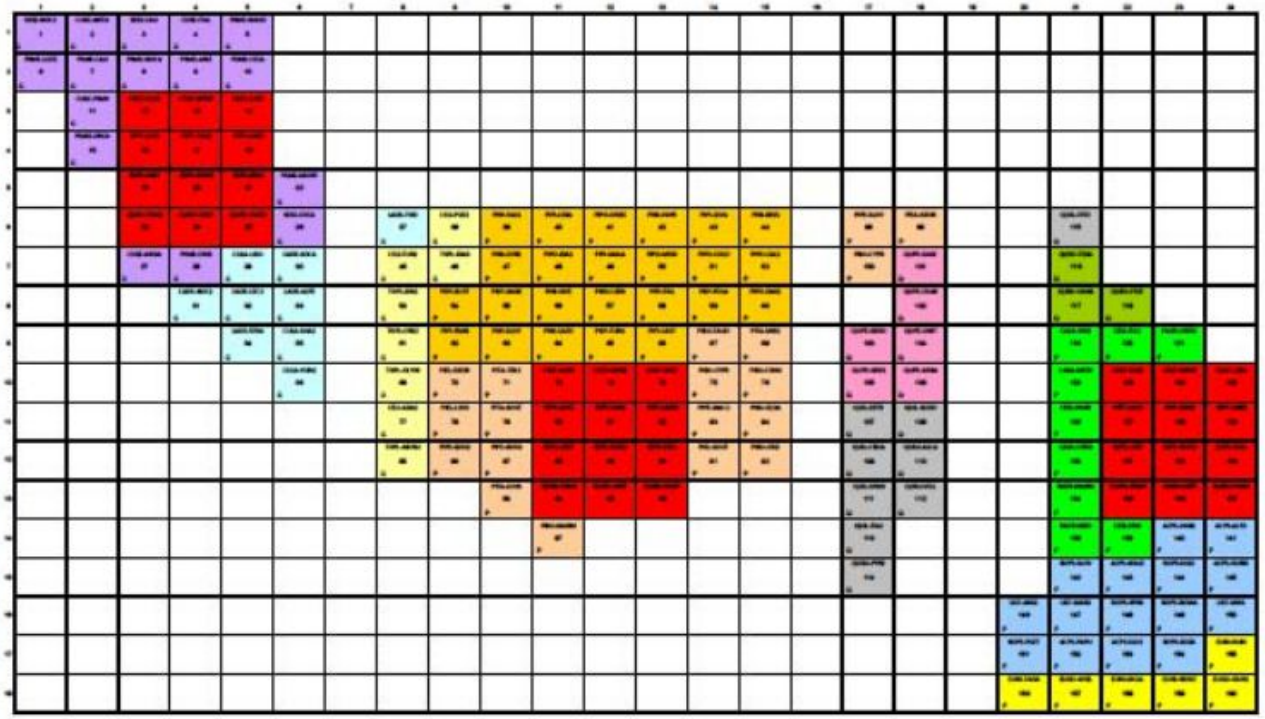
Potes



Monte Corona







REINFFORCE - Selección de especies

- ***Betula pendula***
- ***Cedrus atlantica***
- *Calocedrus decurrens*
- *Cedrus libani*
- *Cupressus sempervirens*
- *Ceratonia siliqua*
- *Fagus orientalis*
- *Larix decidua*
- *Liquidambar styraciflua*
- *Pinus brutia*
- *Pinus elliottii*
- *Pseudotsuga menzienesii*
- *Pinus nigra subspecie laricio and subspecie salzmanii*
- *Pinus peuce*
- ***Pinus pinaster***
- *Pinus pinea*
- *Pinus ponderosa*
- *Pinus sylvestris*
- *Pinus taeda*
- *Quercus ilex subspecie rotundifolia*
- *Quercus petraea*
- ***Quercus robur***
- *Quercus rubra and Q. phellos*
- *Quercus suber*
- *Robinia pseudoacacia*
- *Sequoia sempervirens*
- *Thuja plicata*
- *Eucalyptus nitens, E. gundal and E. globulus*
- *Abies cephalonica*
- *Acer pseudoplatanus*
- *Castanea Sativa*
- *Cunninghamia lanceolata*

2 Replicas → 36 árboles

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Thank you!