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

<table>
<thead>
<tr>
<th>Risk management plan</th>
<th>Coordinator</th>
<th>Regions directly involved</th>
<th>Region participating in the workshops</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Abiotic</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Storm</td>
<td>Barry Gardiner-EFIATLANTIC</td>
<td>Aquitaine, Basque Country</td>
<td>Asturias</td>
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<tr>
<td>Fire</td>
<td>Francisco Rego - ISA</td>
<td>Basque Country, Asturias, Portugal, Galicia</td>
<td>Aquitaine</td>
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<tr>
<td>Soil degradation</td>
<td>Ander Gonzalez-NEIKER</td>
<td>Basque Country, Asturias, Galicia, Portugal</td>
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<tr>
<td><strong>Biotic</strong></td>
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<tr>
<td>Pinewood nematode <em>(Bursaphelenchus xylophilus)</em></td>
<td>Hervé Jactel-INRA and Edmundo Sousa-INIAV</td>
<td>Aquitaine, Castille y Léon, Portugal</td>
<td>Basque-Country</td>
</tr>
<tr>
<td>Chestnut gall wasp <em>(Dryocosmus kuriphilus)</em></td>
<td>Edmundo Sousa-INIAV</td>
<td>Estremadura, Portugal</td>
<td>Basque-Country, Aquitaine</td>
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<td>Eucalypt weevil <em>(Goniapterus platensis)</em></td>
<td>Manuela Branco-ISA</td>
<td>Asturias, Cantabria, Portugal</td>
<td>Basque-Country</td>
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<tr>
<td>Pine pitch canker <em>(Fusarium circinatum)</em></td>
<td>Julio Diez-UVA</td>
<td>Cantabria, Portugal</td>
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</tr>
<tr>
<td>Emerging pests and pathogens</td>
<td>Hervé Jactel-INRA</td>
<td>Aquitaine, Basque-Country, Portugal</td>
<td>Asturias</td>
</tr>
</tbody>
</table>
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
2. Accuracy in Fungal Identification

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

- Hiphae, macro and microconidia
- Insects
- Wind
- Infected cones
- Infected seeds
- NURSERY
- Human vectors: vehicles, wood, camping material etc.
- Sporodochia and mycelium
- Affected tree
- Affected Natural stand or plantation
- Infected seedlings
Cycle of the PPC

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

C. L. Swett, T. R. Gordon

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DOI: 10.1111/efp.12298  View/save citation
Cited by (CrossRef): 0 articles  Check for updates  Citation tools

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...
Fusarium circinatum in Chile: Regulations and Implications

Rodrigo Ahumada

Viterbo, Italy - November 2016
Sampling at the nursery

- Quarantine service request a sampling of 5 asymptomatics plants/plots
- 1 plant positive mean the plot is positive.
- A positive plot need to be re-evaluated.
- Since 2013 plants are allow to move if plots infected are < 10%.

Plots of 50,000 or 200,000 plants. More plots is more expensive.
Fusarium circinatum arrived to stay forever
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
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.
<table>
<thead>
<tr>
<th>Plot</th>
<th>Bacteria</th>
<th>Shannon index</th>
<th>Fungi</th>
<th>Number of fungal species</th>
<th>Shannon index</th>
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<tr>
<td></td>
<td>Number of bacterial species</td>
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<tr>
<td>A3800A</td>
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<td>187</td>
<td>3.54</td>
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</tr>
</tbody>
</table>
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*
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:

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

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

• Some interesting genera:
  \( \rightarrow Pseudomonas \) sp.

• Some of these genera could be interesting in biocontrol (plant health promoters or fungal antagonist)
"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
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 FORESTIÈRE (CRPF). Francia.**
- **INSTITUT NATIONAL DE RECHERCHE AGRONOMIQUE (INRA). Francia.**
- **CENTRE NATIONAL PROFESSIONNEL DE LA PROPRIÉTÉ FORESTIÈRE. 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. País Vasco.**
- **FUNDACIÓN GENERAL DE LA UNIVERSIDAD DE VALLADOLID (FGUVA). Castilla y León. España.**
- **INSTITUTO SUPERIOR DE AGRONOMÍA. (ISA). Portugal.**
REINFFORCE - Castilla y León - Cantabria
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 menziesii
- Pinus nigra subspecie laricio and subspecie salzmania
- 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
Visit http://www.efiatlantic.efi.int/portal/research/plurifor/

Thank you!