Risk Management Plan for *Fusarium circinatum*

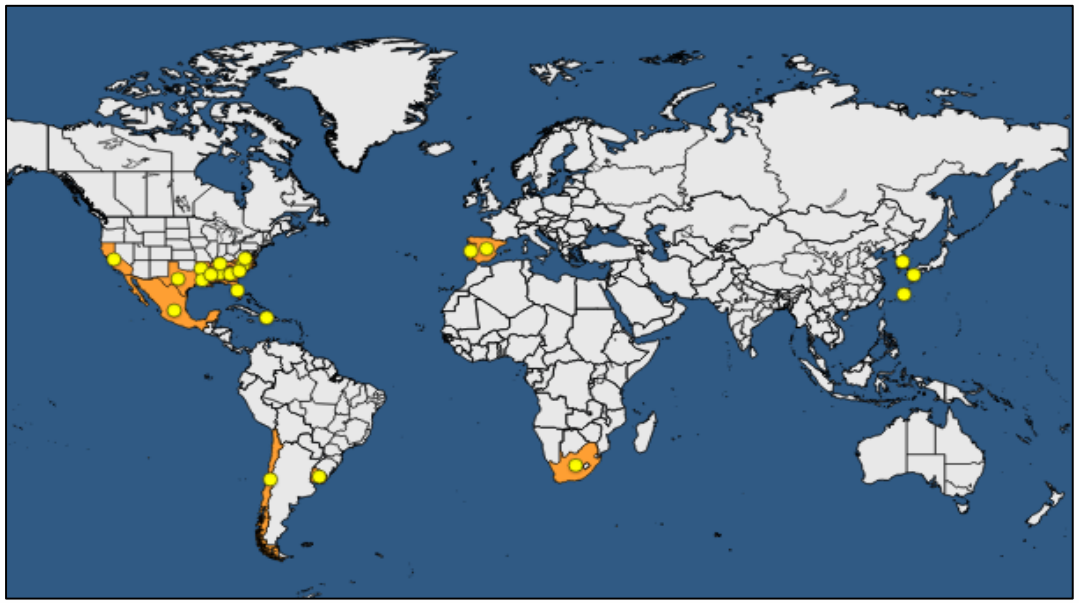
Julio Javier Diez, Carmen Romeralo, Pablo Martínez, Helena Bragança

General Information

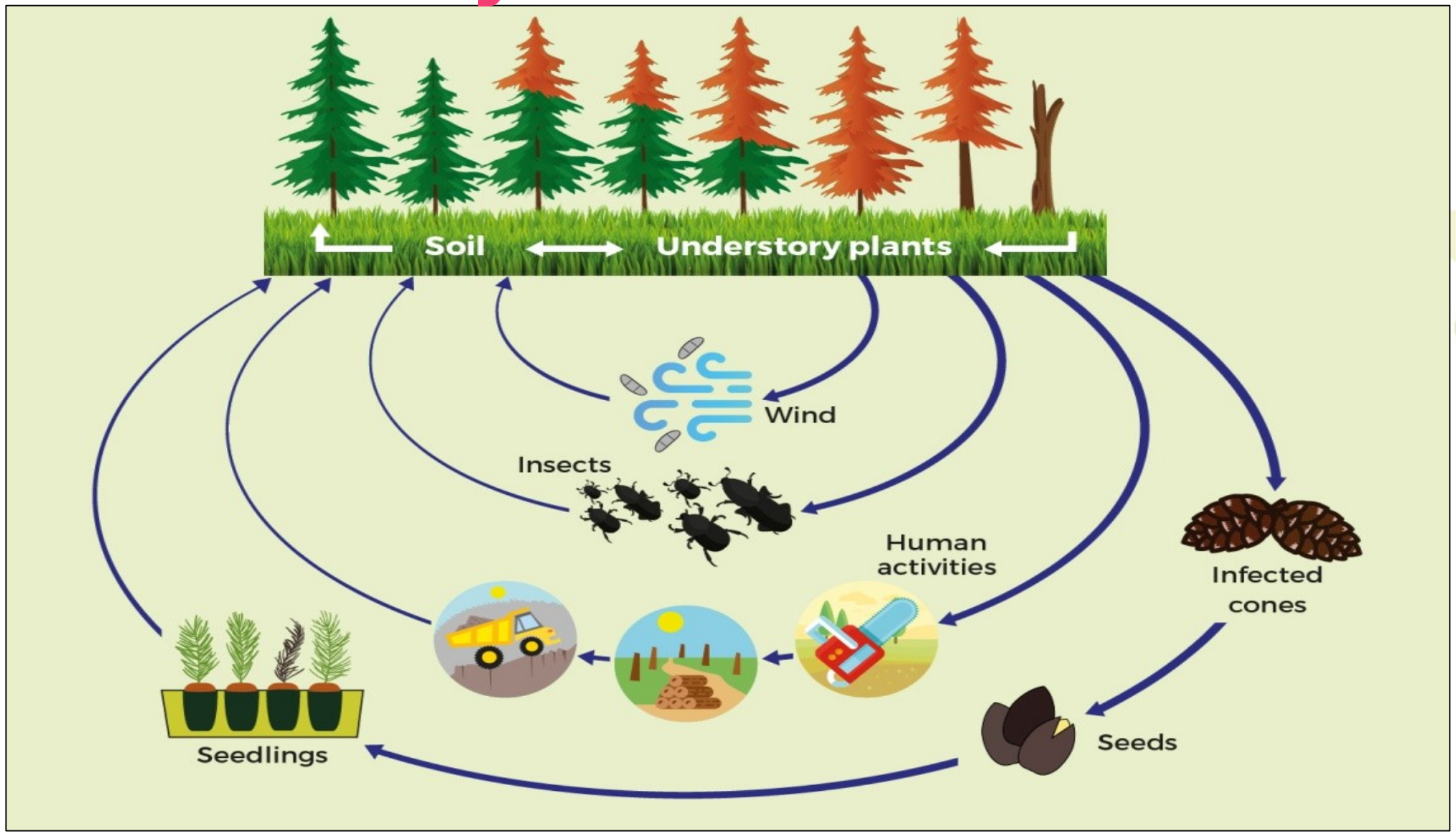
Pine Pitch Canker Disease

Global distribution

- Affects pines.
- *Pinus radiata.*
- Main symptoms; bleeding cankers.
- Deformation of trunks.
- Forests, plantations and nurseries.
- Seeds, seedlings and adult trees.



Disease cycle



Legislative framework

- **Region Castilla y León**

  Compensation for damages of the disease

- **Region Cantabria**

  BOC 82 28 Abril 2008 DES/32/2008

- **Region España**

  RD 637/2006 and modification 35/2010

- **Region Portugal**

  Portaria n.º 294/2013

- **European Union legislation**

  To prevent the introduction and spread inside the EU (Decision 2007/433/EC), quarantine organism (2014/78/EU) included in A2 EPPO list.

Current Contingency Plans

Portugal

ICNF
Instituto da Conservação da Natureza e das Florestas

POSF
AÇÃO

PLANO DE AÇÃO PARA PROSPEÇÃO E ERRADICAÇÃO DO FUNGO *GIBBERELLA CIRCINATA/FUSARIUM CIRCINATUM*
CANCRO-RESINOSO-DO-PINHEIRO

PARA O PERÍODO 2016-2020

Estabelece as ações para prospeção, controlo e erradicação do cancro-resinoso-do-pinheiro no território nacional, no sentido de evitar a dispersão da praga em Portugal.

Spain (No contingency Plan)

BOE núm. 137 Viernes 9 junio 2006 22069

MINISTERIO DE AGRICULTURA, PESCA Y ALIMENTACIÓN

10287 *REAL DECRETO 637/2006, de 26 de mayo, por el que se establece el programa nacional de erradicación y control del hongo Fusarium Circinatum Niremberg et O'donnell.*

BOE **BOLETÍN OFICIAL DEL ESTADO**
Núm. 44 Viernes 19 de febrero de 2010 Sec. I. Pág. 16157

I. DISPOSICIONES GENERALES

MINISTERIO DE MEDIO AMBIENTE, Y MEDIO RURAL Y MARINO

2695 *Real Decreto 65/2010, de 29 de enero, por el que se modifica el Real Decreto 637/2006, de 26 de mayo, por el que se establece el programa nacional de erradicación y control del hongo de las coníferas «Fusarium circinatum» Niremberg et O´Donnell.*

XUNTA DE GALICIA
CONSELLERÍA DO MEDIO RURAL
Edificio Administrativo San Caetano Santiago de Compostela
medio rural

PLANO DE CONTINXENCIA PARA A ERRADICACIÓN DO Fusarium circinatum Nirenberg & O´Donnel (*Gibberella circinata*) EN GALICIA

Junta de Castilla y León
Centro de Sanidad Forestal de Calabazanos

PLAN DE CONTINGENCIA Y MEDIDAS DESARROLLADAS PARA LA ERRADICACIÓN Y CONTROL DEL HONGO *Fusarium circinatum* Niremberg et O´Donnell EN CASTILLA Y LEÓN, hasta año 2008

Governance

Executive framework

Competencies

Strategical/Tactical Level

- **Region Castilla y León**

  Dirección General del Medio Natural de la Consejería de Medio Ambiente (Decreto 75/2007) de la Junta de Castilla y León.

- **Region Galicia**

  Consejería del Medio Rural (Decreto 562/2005) de la Xunta de Galicia.

- **Region Cantabria**

  a) La Dirección General de Biodiversidad (forest stands).

  b) La Dirección General de Desarrollo Rural (nurseries).

- **Region Portugal**

  Instituto da Conservação da Natureza e das Florestas, I.P. (ICNF, I.P.)

  Direção-Geral de Alimentação e Veterinária (DGAV)

  Instituto Nacional de Investigação Agrária e Veterinária, I.P. (INIAV, I.P.)

- **Transboundary coordination**

  There is a need of a transnational structure.

Expert advisory group

Main tasks

1.- Estimation of the risk outbreak

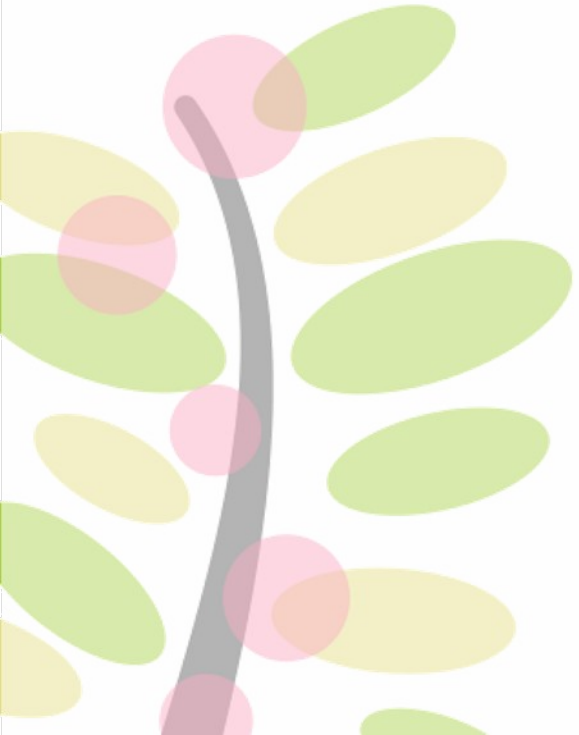
2.- Investigation of the extension of the outbreak, possibilities of eradication and costs.

3.- Mobilize and manage resources to carry out the eradication and control program.

4.- Authorization to carry out official measures.

5.- Establishment of communication with other organisms.

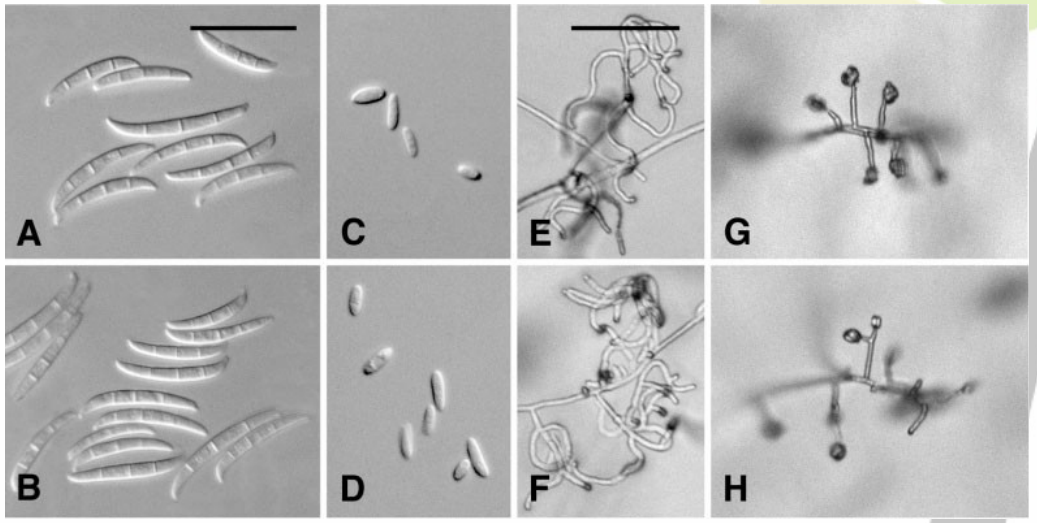
Risk assessment



Detection and Identification

- Detection: Periodic surveys at different levels are recommended to minimize the risks of installation and dispersion of the disease.

- Identification:
  a) Morphological (PDA, SNA)
  b) Molecular
     1. PCR-RFLP.
     2. Real time PCR with SyBr Green.
     3. Real time PCR with Ioos probe.



Contingency Plan

Prevention

Preventive measures

1. **Risk estimation of the pathways of entry of the pathogen;**
   a) High risk inoculum: wood, firewood, tissue cultures, cuttings, plants…
   b) Medium risk inoculum: footwear, camping equipment, packaging.
   c) Low risk inoculum: suitcases, goods, seeds for human consumption…
2. **Disinfection**.
3. **Information and awareness**;
   a) Updated information of the pathogen, prevention and control measures.
   b) Awareness campaigns.

Contingency Plan

Preparedness/prevision/surveillance/ control/monitoring

Control and monitoring

- Systematic surveys and controls:
  1. *Forest stands of the genus Pinus.*
  2. *Nurseries producing plants (pay attention to asymptomatic plants).*
  3. *Prospections of forest reproductive material (plants, seeds and parts of plants used in reforestation).*
  4. *Insect vector control.*
- At least one per year.
- Demarcated areas (presence organism verified + buffer zone).

Contingency Plan

Early warning and activation of the contingency plan

Activation of the Contingency Plan

If the presence of an initial focus of the organism is confirmed:

a) In forest stands,
   1. *The extent of the infested focus will be delimited.*
   2. *The sensitive material present in the affected area will be eliminated.*

b) Forest reproductive material,
   1. *The infested area will be delimited.*
   2. *The fields and facilities will probably be declared contaminated.*
   3. *All sensitive material affected will be disposed of.*

c) Seeds,
   1. *Destruction of the affected lot.*
   2. *The other lots, will probably be declared contaminated and immobilized.*

Contingency Plan

Crisis management/response/ eradication/control

First phase measures

Preventive measures in demarcated areas in forests

1. Cutting and elimination of the cut aerial part.
2. Preventive treatment against vector insects.
3. Prohibition of the exit of vegetables outside the demarcated area.
4. Prohibition of sowing, planting or replanting sensitive species.
5. Obligation to disinfect the tools.
6. Epidemiological investigation of the origin of the plant of sensitive species in the case of masses of artificial repopulation.

First phase measures

Control/eradication

1. Nurseries. Hygiene is the most useful practice.
2. Recreational areas. All affected trees should be cut.
3. Plantations All trees should be burned.
4. The equipment and machinery used in these operations should be properly washed and disinfected.

Circulation of vegetables and plant products.

5. Plant material has been peeled completely,
6. Heat treatment; minimum of 56 ° C for 30 minutes.

Contingency Plan

Rehabilitation/restoration/recovery

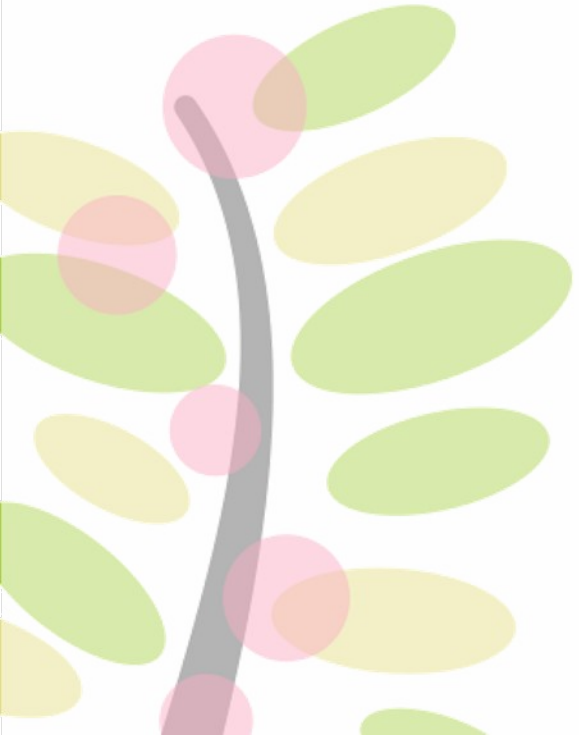
Rehabilitation/Restoration

The demarcated area will have a minimum validity of two years.

Long term–measures:

1. The use of individuals (genotypes) or provenances that are resistant to the disease.
2. The use of non-host plants (not *Pinus* or *Pseudotsuga*).
3. Periodic surveys and follow-ups. It is recommended to continue with the annual monitoring of all susceptible species.

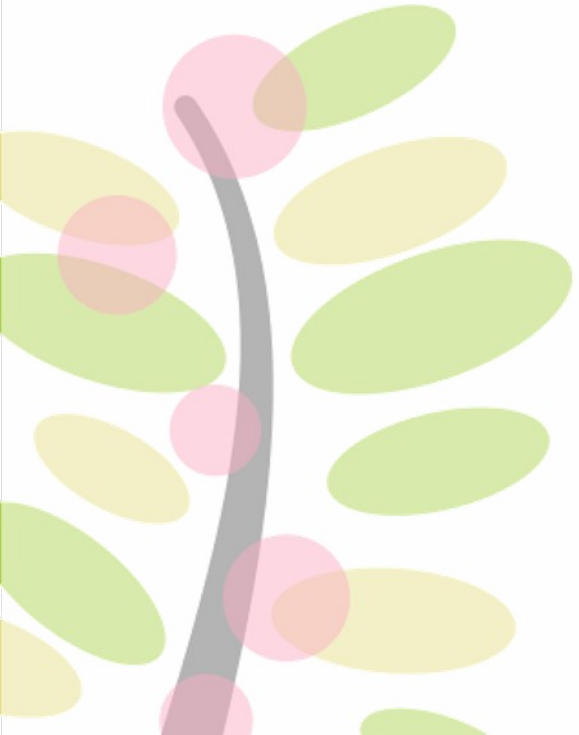
Communication strategy



Communication strategy

- **Internal**
  1. An communication network will be established to improve the response.
  2. Communication between authorities and the owners of the forest associations.
- **External**
  1. All pathogen information must be disseminated to ensure that citizens can help and participate.
  2. An application (SilvAlert) will be created to be able to communicate the presence of a focus immediately and effectively.

Improved tools for diagnosis



NGS technologies for detection of *F. circinatum*

Provides a new and powerful tool to speed up and cheapen the identification process and limit the expansion of the pathogen.

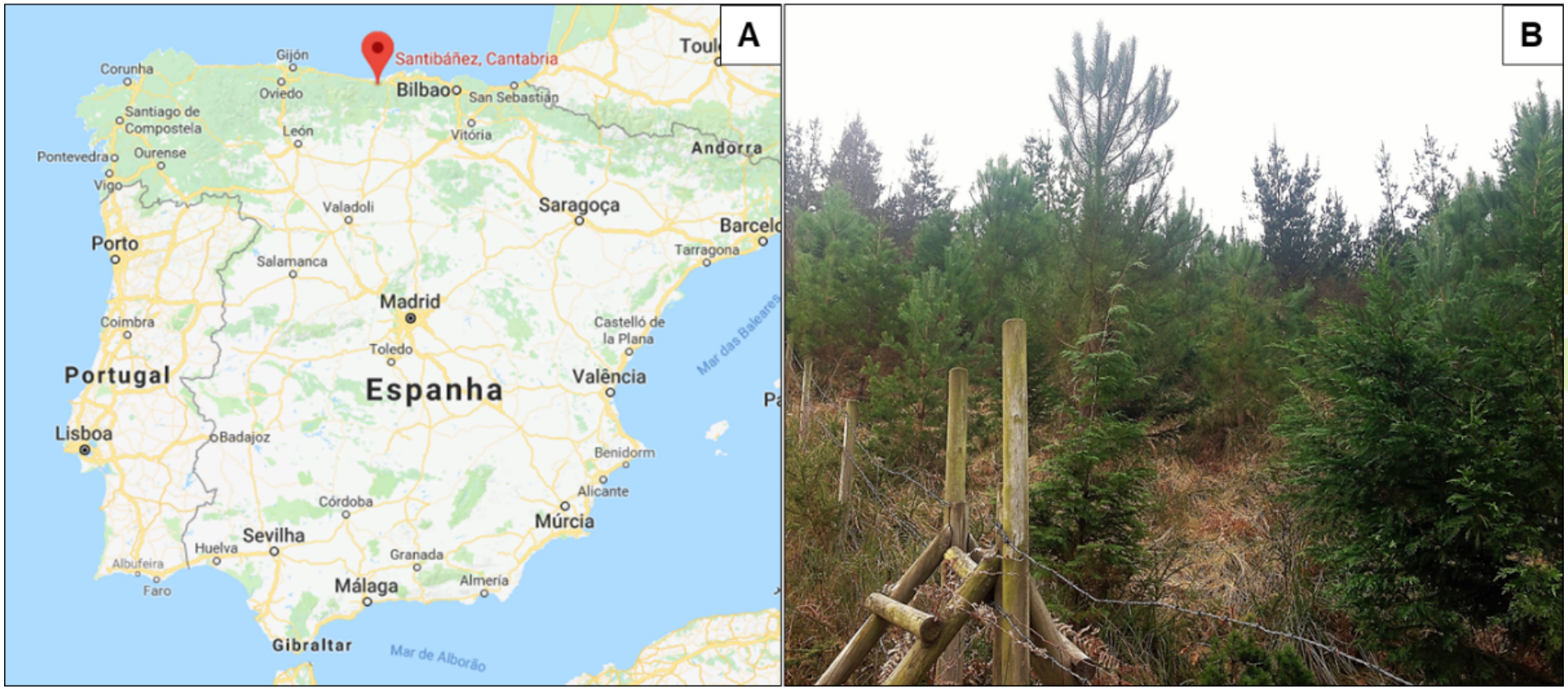
Based on the amplification of different genes or regions of the genome:

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

- **Description of the implementation steps**

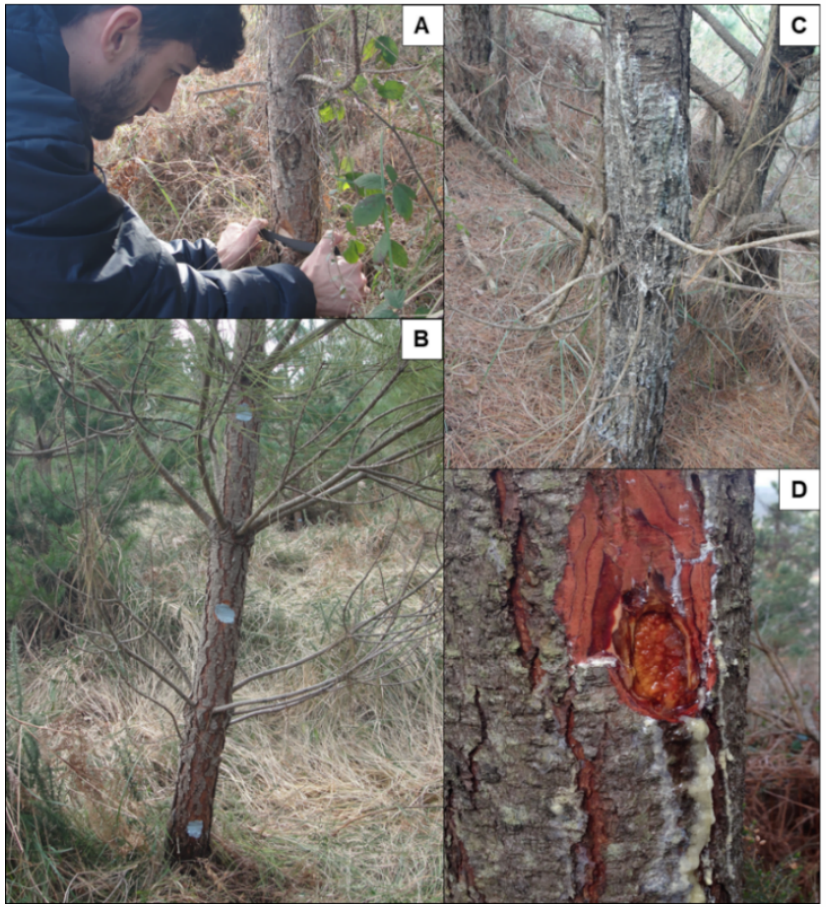
1. Genes or regions defined for identification.
2. Run of NGS analysis *(in progress).*
3. Revision of results *(in progress).*
4. Application of the tool *(not done)*.

NGS technologies for detection of *F. circinatum*



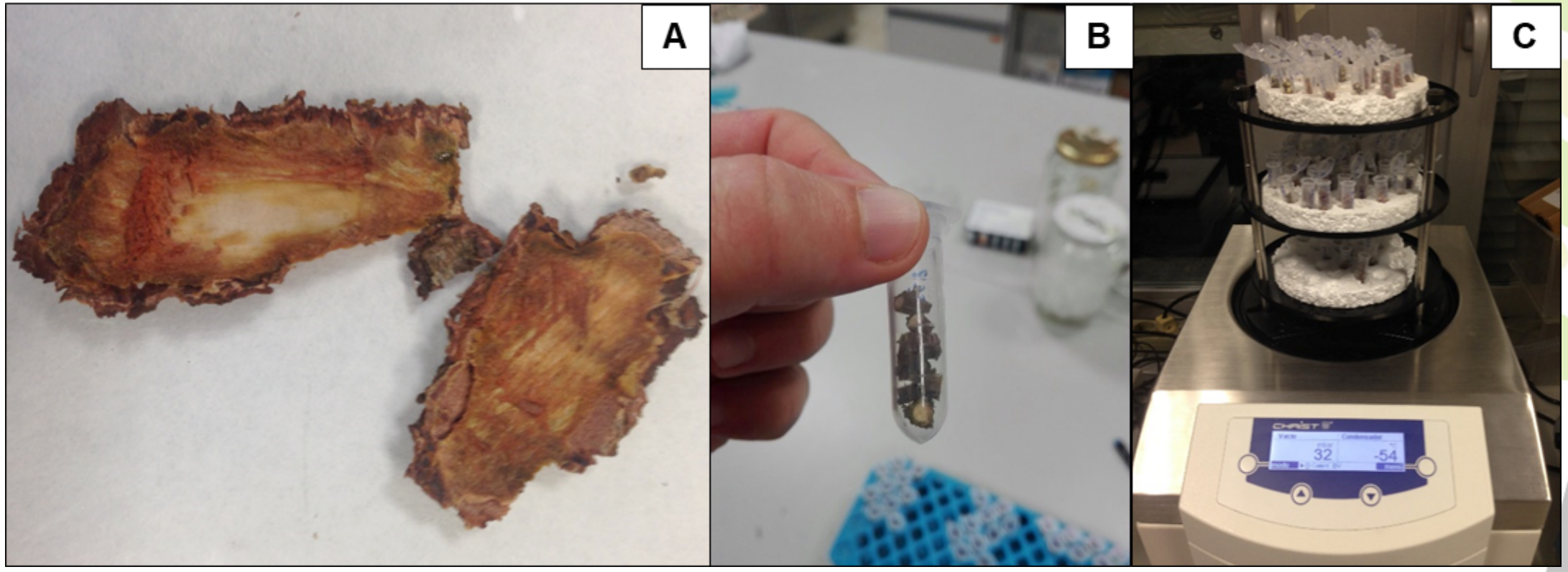
Location of the sampling site in the Province of Cantabria (red arrow) (A) and overview of the 9-years-old forest stand (B)

NGS technologies for detection of *F. circinatum*



Collection of samples at 15 cm using a sharp blade (A); *P. radiata* after sampling showing the wounds at different heights (B); canker in *P. radiata* exhibiting resinosis (C and D)

NGS technologies for detection of *F. circinatum*



Stem's samples (A); eppendorf tubes containing small pieces of stem prepared for lyophilisation (B) and freeze dryer used for lyophilisation (C).

NGS technologies for detection of *F. circinatum*

A38012	*P. pinaster*	15	*F. delphinoides*
A38013	*P. sylvestris*	15	*F. delphinoides, F. keratoplasticum*
A38014	*P. sylvestris*	15	-
A38015	*P. sylvestris*	15	*F. keratoplasticum*
A38016	*P. sylvestris*	15	-
A38017	*P. nigra*	15	*F. keratoplasticum, F. oxysporum*
A38018	*P. nigra*	15	*F. acutatum*
A38019	*P. nigra*	15	*F. acutatum, F. oxysporum*
A3801A	*P. nigra*	15	*F. acutatum, F. keratoplasticum*
A3801B	*P. uncinata*	15	*F. delphinoides, F. keratoplasticum, F. oxysporum, F. pseudensiforme*
A3801C	*P. uncinata*	15	*F. delphinoides, F. keratoplasticum, F. acutatum, F. oxysporum*
A3801D	*P. uncinata*	15	*F. acutatum, F. keratoplasticum*
A3801E	*P. uncinata*	15	*F. delphinoides, F. keratoplasticum, F. acutatum, F. oxysporum*

Fusarium **species detected through NGS using an ITS-based amplicon sequencing technology (Biome Makers®)**

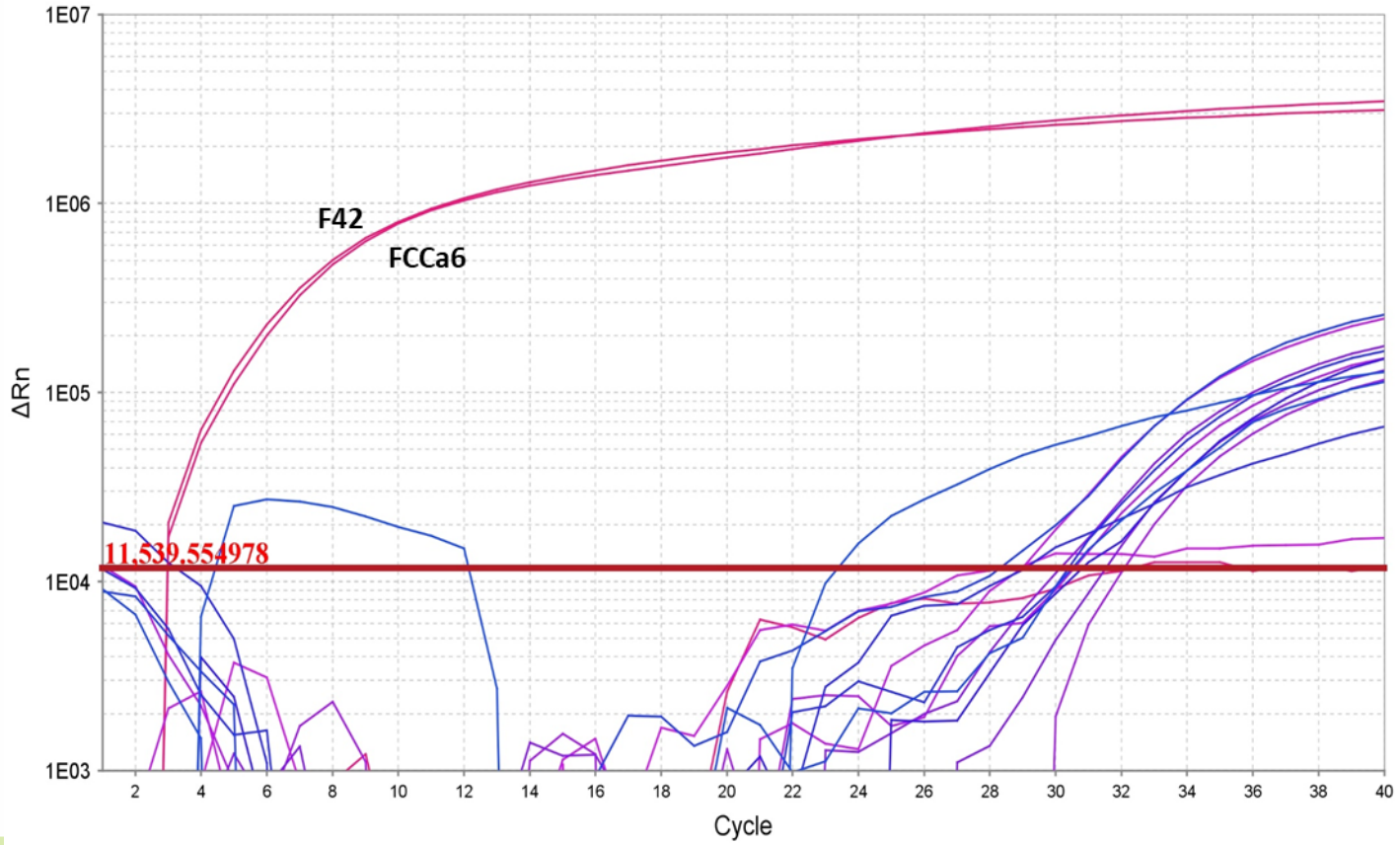
NGS technologies for detection of *F. circinatum*

Table 5. Cycle threshold values obtained in a quantitative polymerase chain reaction (qPCR) with samples of stem from pine trees located in Cantabria region, Spain.

Code	Species	Tree number	Sampling height (cm) or origin	Ct value				Presence of *F. circinatum*
				no dilution	1/100	1/1000	Nested PCR	
A3800B	*P. radiata*	1	15	undet	-	-	undet	absent
A3800C	*P. radiata*	1	65	undet	-	-	>20	absent
A3800D	*P. radiata*	1	115	undet	-	-	>20	absent
A3800E	*P. radiata*	1	165	undet	-	undet	4.265**	**present**
A3800F	*P. radiata*	1	200 (twig)	13.000	undet	-	>20	absent
A3800G	*P. radiata*	21	canker	11.009	undet	undet	5.504*	**present**
A3800H	*P. radiata*	2	15	>20	>20	-	29.633	absent
A3800I	*P. radiata*	2	65	>20	undet	-	11.410	**present**
A3800J	*P. radiata*	2	115	>20	undet	-	>20	absent
A3800K	*P. radiata*	2	165	>20	undet	-	>20	absent
A3800L	*P. radiata*	2	200 (twig)	undet	-	-	>20	absent
A3800M	*P. radiata*	22	canker	>20	>20	-	undet	absent
A3800N	*P. radiata*	3	15	undet	-	-	6.344	**present**
A3800O	*P. radiata*	3	65	>20	undet	-	4.215	**present**
A3800P	*P. radiata*	3	115	undet	-	-	>20	absent

***Fusarium* species detected through NGS using an ITS-based amplicon sequencing technology (Biome Makers®)**

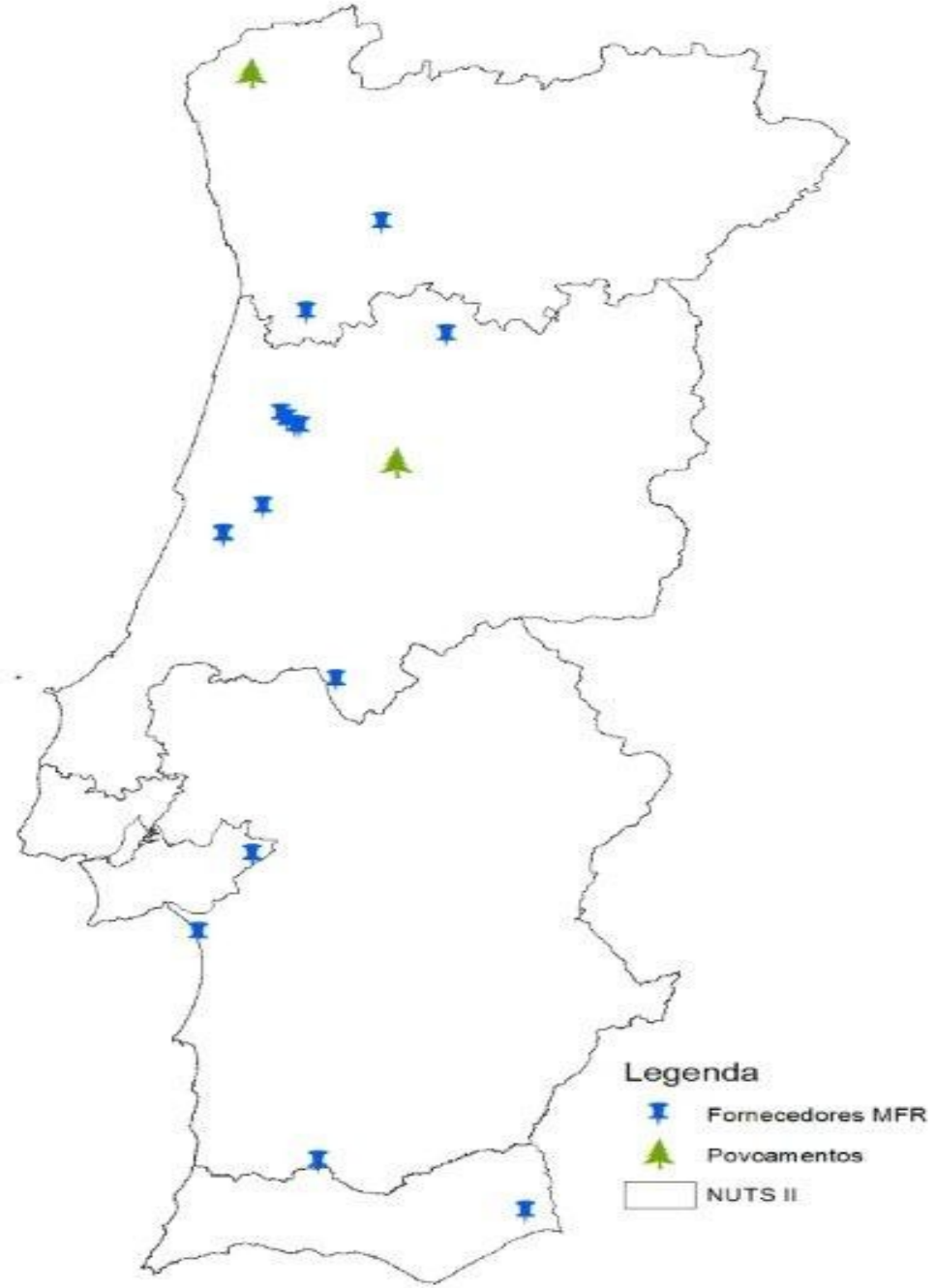
NGS technologies for detection of *F. circinatum*



Amplification curves (logarithmic representation) generated in a qPCR with PCR products from a conventional assay. Only curves from samples that resulted in a Ct value > 20 were included. FCCa6 and F42 are positive controls (DNA extracted from pure cultures of *F. circinatum*)

qPCR

Subtitle



Fusarium circinatum

TOOLS FOR FAST DISEASE DIAGNOSTIC

objectives: early detection in field

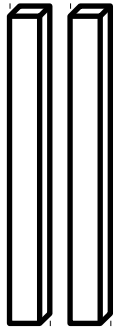
High priority to sites with recent positive detections

(INIAV/ICNF)

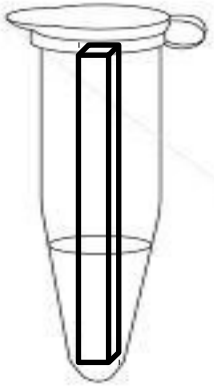
Spore Traps Combined With Real Time PCR

Rotor rod spore traps

Sticks with double side adhesive tape

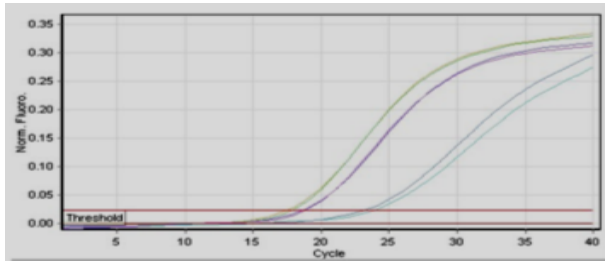


DNA extraction from traps



Real time PCR

Results

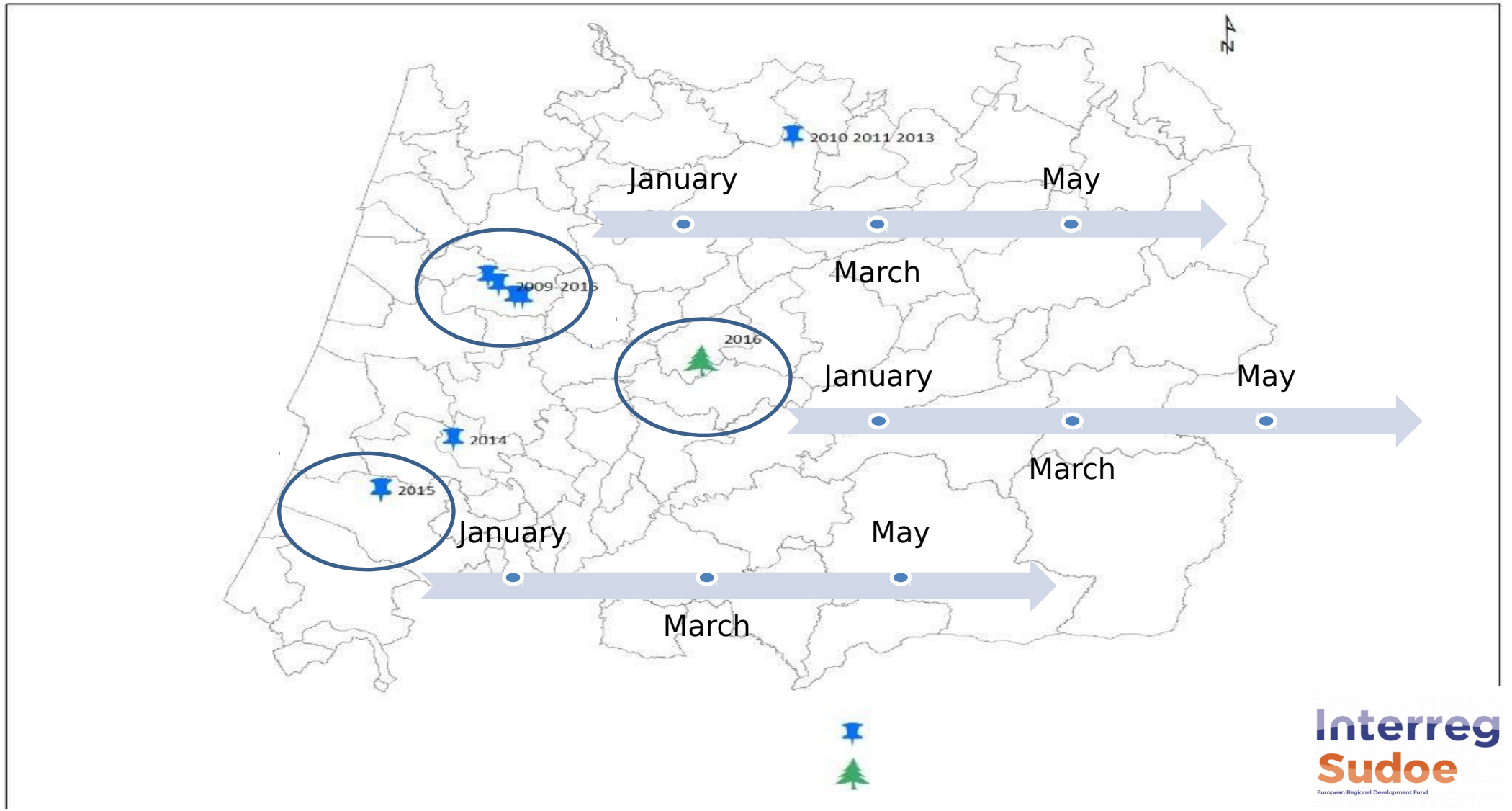


Interreg Sudoe
EUROPEAN UNION
European Regional Development Fund

Spore Traps
continuous rotation movement

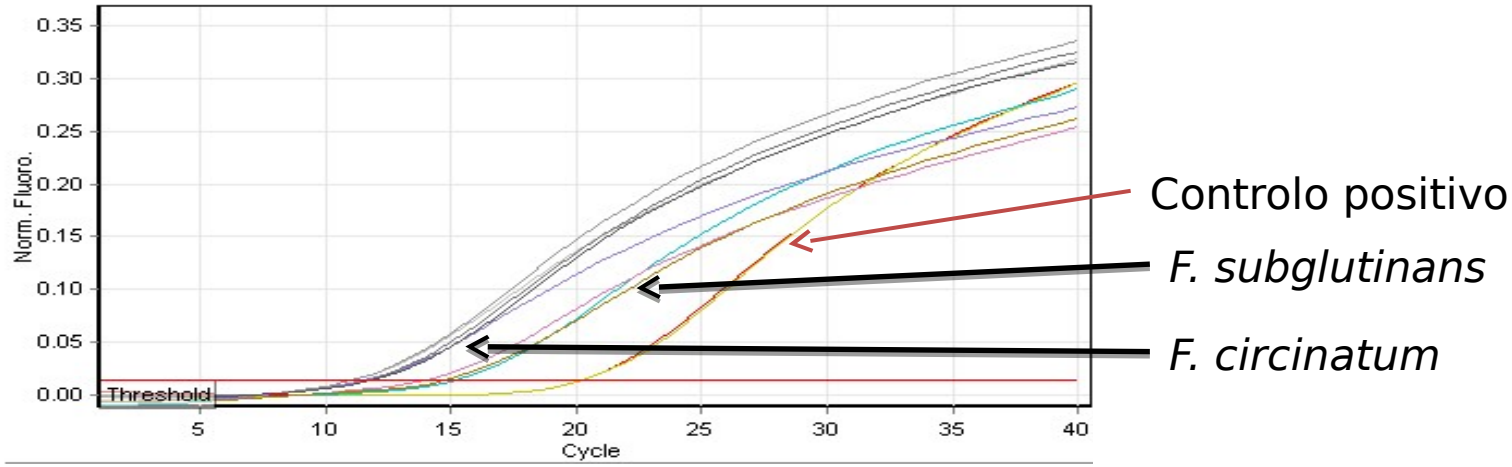
Field work planning ICNF/INIAV

2 spore traps during one week in each site



loos *et al.*, 2009 (PM7/91(1)-Appendix 6)

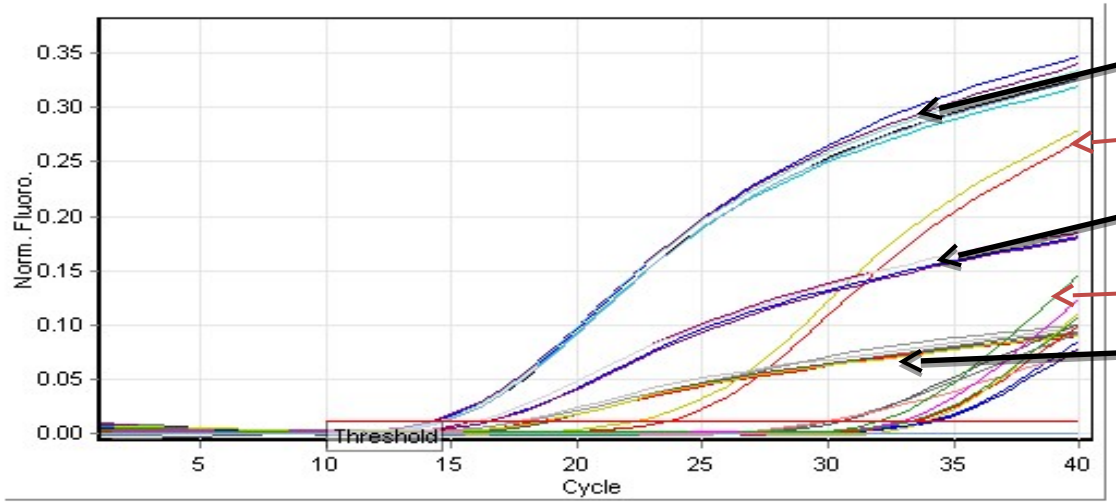
- Specificity – cross-reaction with *F. subglutinans*
  - no cross-reaction with *F. oxysporum* and *F. verticilioides*



Controlo positivo

F. subglutinans

F. circinatum



F. circinatum?

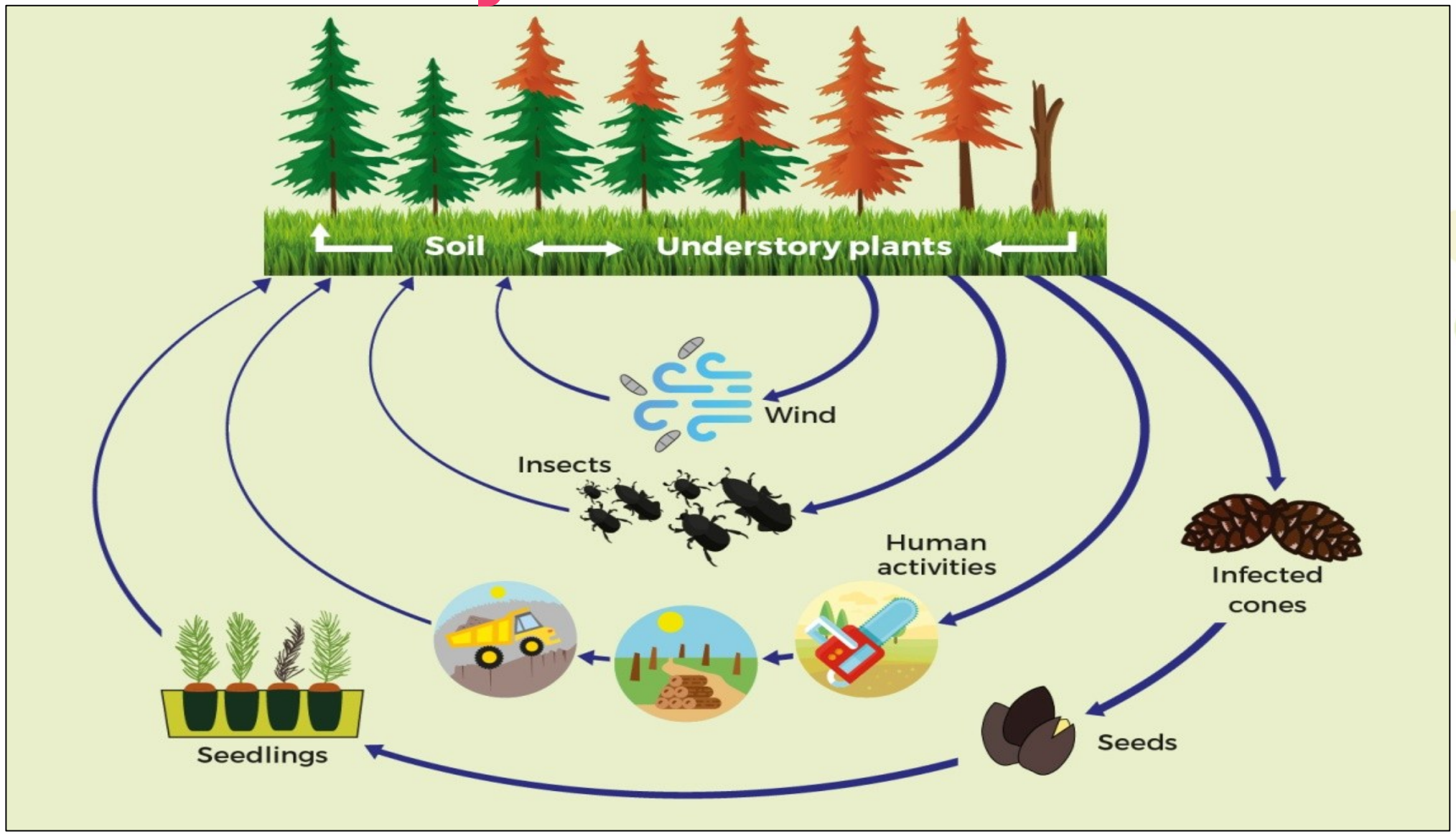
Controlo positivo

F. circinatum?

Negativo

F. circinatum?

Disease cycle



Acknowledgements

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- Danilo Reis Gonçalves (Universidad of Valladolid).
- Dina Ribeiro (ICNF)