Webinar Final do Grupo Operacional Desenvolvimento de estratégias integradas para a prevenção do Cancro-resinoso-do-pinheiro (+PrevCRP)

Enquadramento da situação do fungo *Fusarium circinatum* em Espanha

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UVa

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PLURIFOR

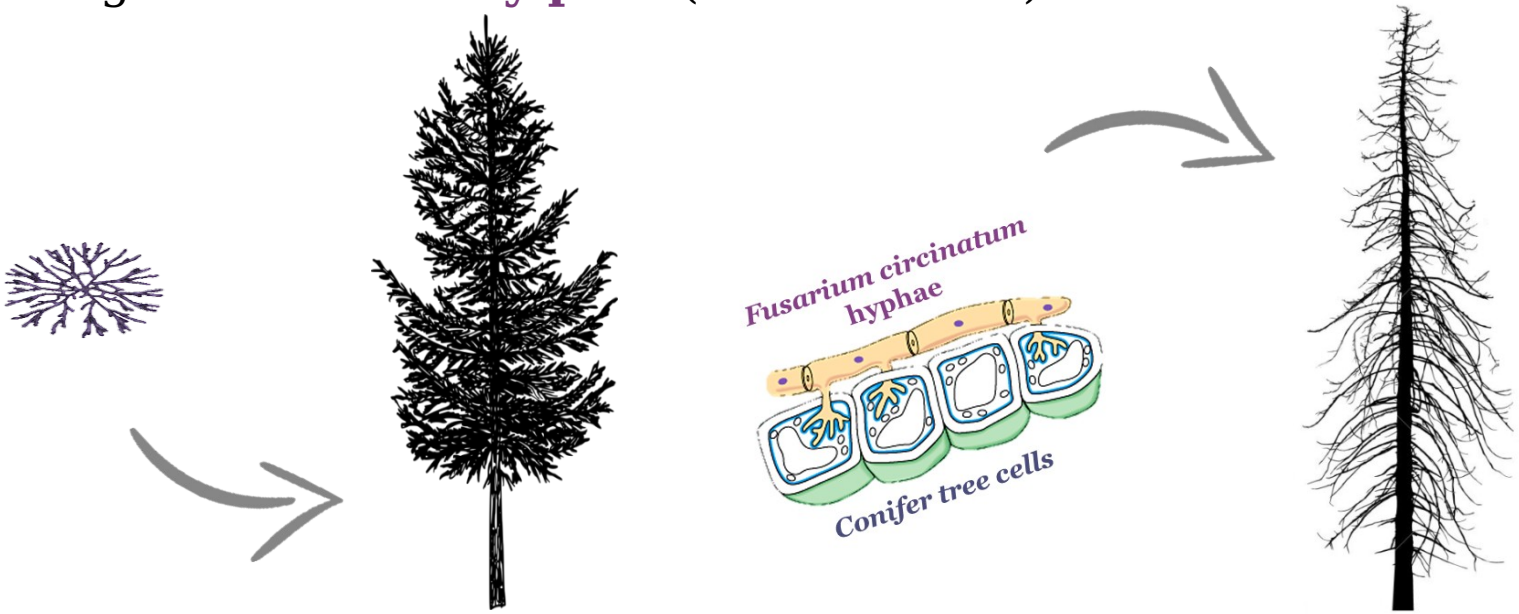
What will we talk about today?

- ***Fusarium circinatum*: the pathogen**
- **Symptoms of PPC disease**
- **First report in Spain and EU**
- **Quarantine measures in EU and Spain**
- **Situation in the Nurseries**
- **Damages in the Forests**
- **Environmental Friendly Methods for *F. circinatum* Management**
- **Future of *F. circinatum* and hosts**

The Pathogen

Fusarium circinatum is the causal agent of **Pine Pitch Canker (PPC)** disease, one of the most devastating forest diseases worldwide.

It is an ascomycete that affects at least 57 species of the **genus *Pinus***, as well as *Pseudotsuga menziesii*, being the most important damage on **Monterrey pine** (*Pinus radiata*).



The Pathogen

Since **2002** the **EPPO** (European Plant Protection Organisation) has considered it as a **quarantine organism** due to its high potential for damage in case of introduction into European territory, and has included it in the **A2 list**.

Fungi
Botryosphaeria laricina
Ceratocystis platani
Ciborinia camelliae
Cronartium kamtschaticum
Cryphonectria parasitica
Diaporthe vaccinii
Fusarium circinatum
Fusarium foetens

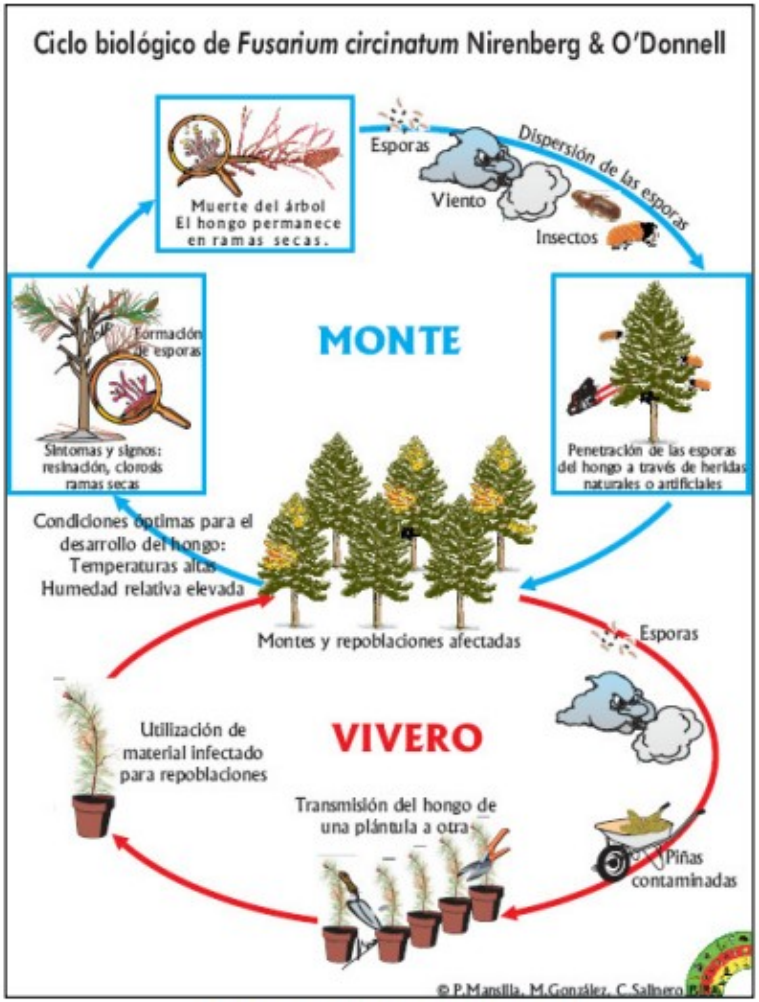
Dispersal

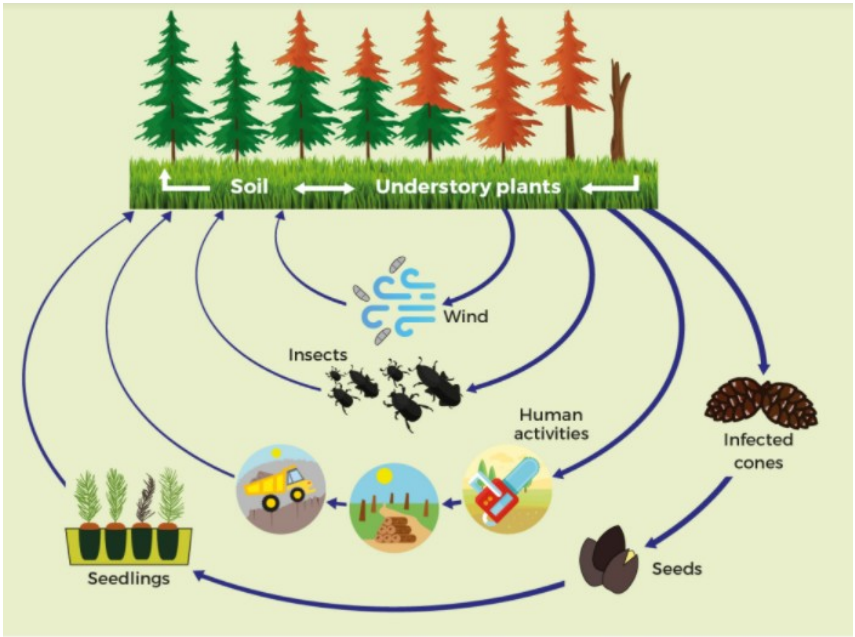
The dispersal of the disease may occur naturally or may be mediated by human assisted means.

There are numerous **insect species** that commonly occur in pine nurseries and forests throughout Europe and elsewhere which have the potential to spread PPC as either vectors.

Long-distance spread occurs mainly though the **movement of infected seeds**.

Dispersal


Ciclo biológico de Fusarium circinatum Nirenberg & O'Donnell
Esporas
Dispersión de las esporas
Viento
Insectos
Muerte del árbol
El hongo permanece
en ramas secas.
Formación de esporas
Síntomas y signos:
resinación, clorosis
ramas secas
MONTE
Penetración de las esporas
del hongo a través de heridas
naturales o artificiales
Condiciones óptimas para el
desarrollo del hongo:
Temperaturas altas
Humedad relativa elevada
Montes y repoblaciones afectadas
Esporas
VIVERO
Utilización de
material infectado
para repoblaciones
Transmisión del hongo de
una plántula a otra
Piñas
contaminadas
© P.Mansilla, M.González, C.Salinero


Soil
Understory plants
Wind
Insects
Human
activities
Infected
cones
Seedlings
Seeds

Symptoms of PPC disease

SYMPTOMS

Seedlings and young plants (in nurseries)

Infected seedlings usually show symptoms of **damping off**:
needles turn red, brown or chlorotic and die from the base upwards. The affected seedling usually dies.

However, seedling infection can also be **asymptomatic.**

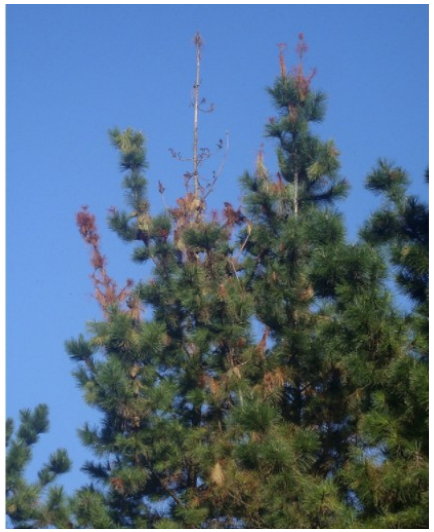
SYMPTOMS

Mature trees (in forests)

* Yellowing of the needles, which turn red in time and finally drop, and dieback of the shoots from the tip, occasionally producing **'shepherd's crook' symptoms**.

* **Crown dieback**.

* **Cankers** might appear on the shoots, on the main branches and even on the trunk, associated with conspicuous resin exudate (pitch) in response to fungal infection.




Symptoms: Browning and Dieback

Symptoms: **Brown flagging of twigs**

Symptoms: Cankers with resin ooze

Symptoms: Resin ooze


Symptoms: Resin ooze

Symptoms: **Bark necrosis**

Symptoms: **Resin inside the wood**

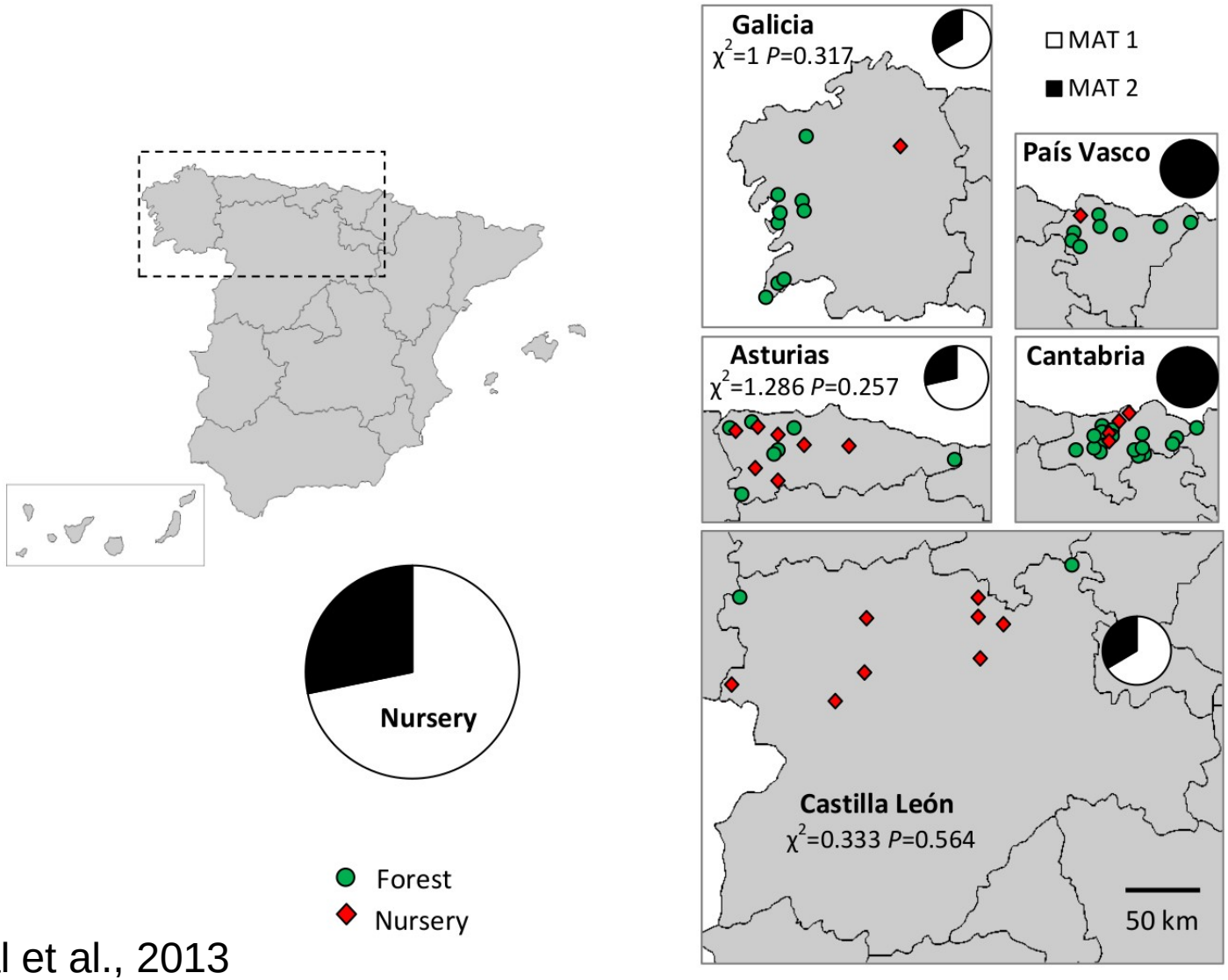
Symptoms: Stem deformation

Symptoms: **Broken trees after strong winds and Tree dead**

Symptoms: **Dead Seedlings**

First report in Spain and EU

First report in Spain and EU



Berbegal et al., 2013

DISPERSION

The presence *of Fusarium circinatum* was officially detected in **Spain for the first time in 2004** and it is mainly found in the north of the country.

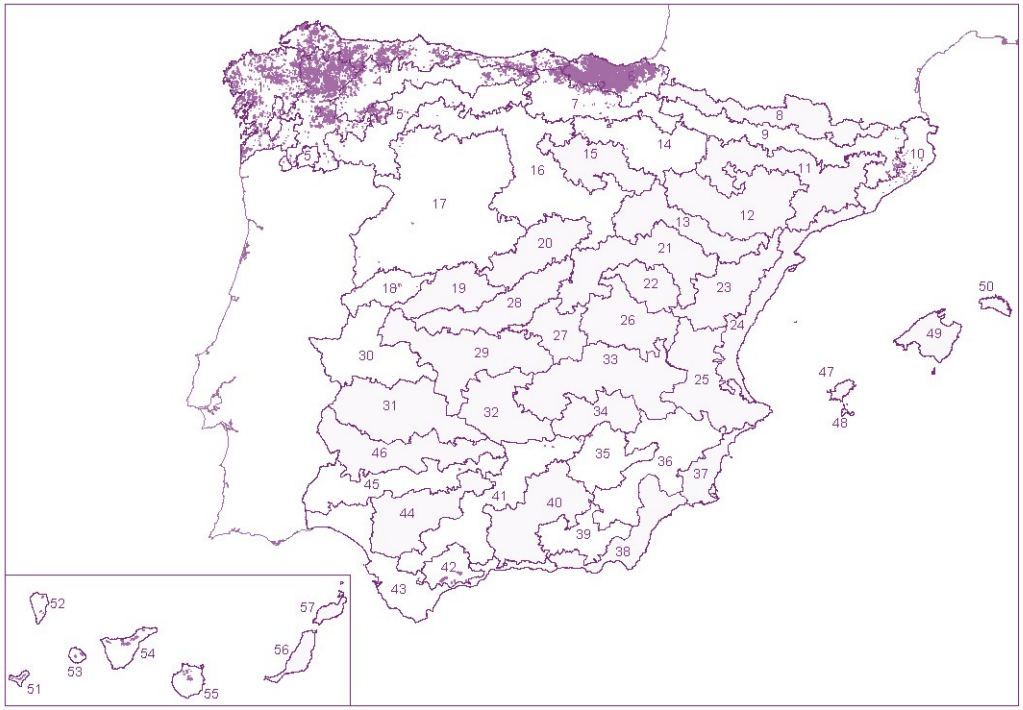


Figura 2. Regiones infectadas con F. circinatum en España. Fuente: Martínez-Álvarez, 2015.

PPC : The Pathogen – increase in distribution of PPC in Europe

Reports of occurrence of *G. circinata* in the European Union (nurseries, plantations or native forests) according to data retrieved until end 2009 (Source: EFSA)

DISPERSION

Since then the pathogen has spread into Central and South America, South Africa, Asia and, more recently, Europe (2004).

First detected in North America


France 2006
Spain 2005
Italy 2007
Portugal 2009
Korea 2000
U.S.A. & Mexico 1945
Haiti 1953
Japan 2001
Brazil 2014
Colombia 2012
Chile 2002
Uruguay 2009
South Africa 1994

CURRENT SITUATION

Present in 14 countries (Review July 2020)

- Uruguay, Brazil and Chile only in nurseries
- Colombia, France, Haiti, Italy, Japan, South Korea, Mexico, Portugal, Spain, South Africa, USA in nurseries and also found on mature trees in the wider environment.

The fungus is considered absent in 28 countries

- 24 European countries, Australia, New Zealand, Turkey and Israel

Officially eradicated

- Italy and France

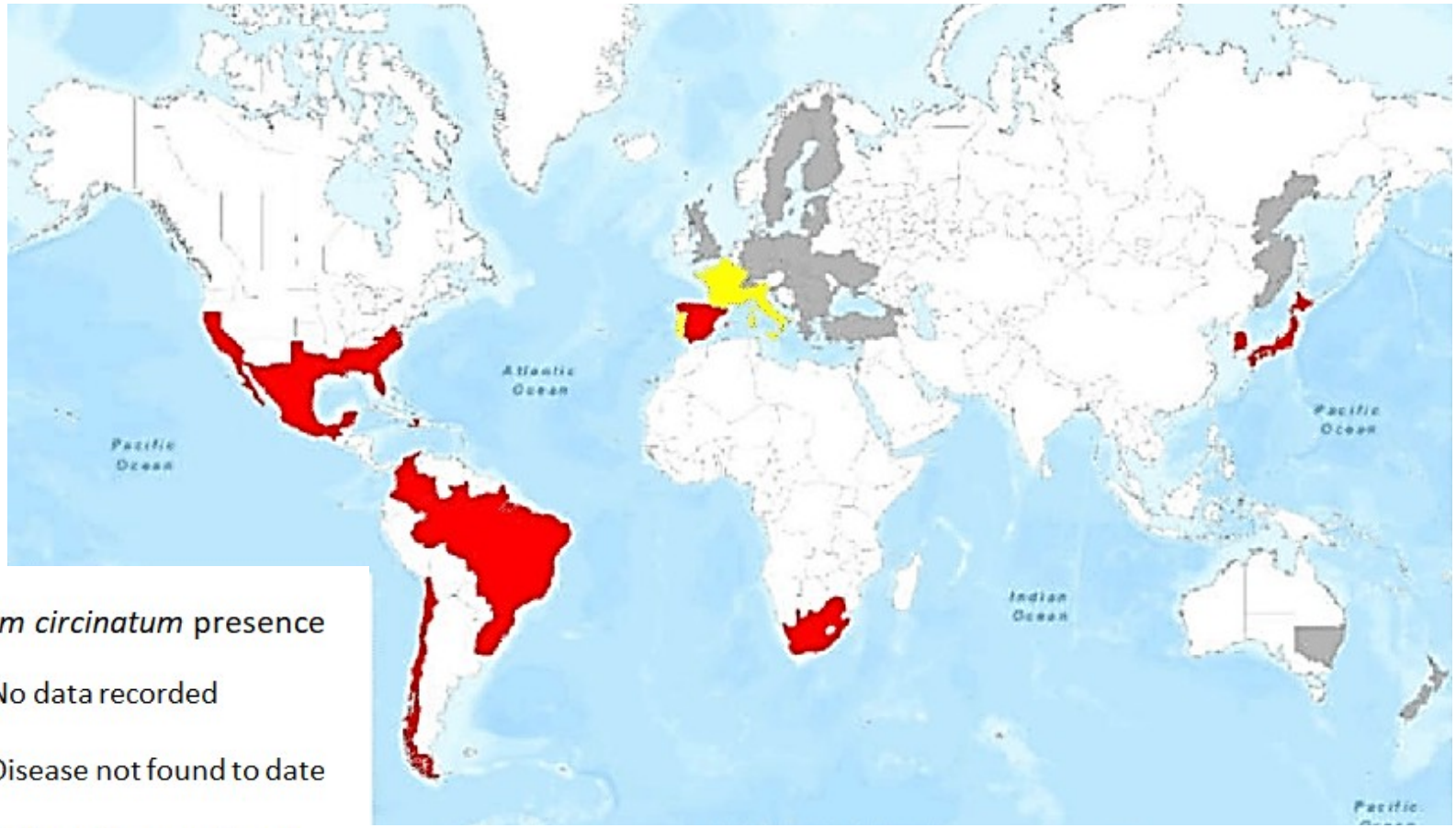
forests

MDPI

Review

Global Geographic Distribution and Host Range of *Fusarium circinatum*, the Causal Agent of Pine Pitch Canker

CURRENT SITUATION



Fusarium circinatum presence

- No data recorded
- Disease not found to date
- Fc found but eradicated
- Fc present

July 2020

Damages in the Forests

Table 2. Sampling approaches in production sites for planting material—demarcated *Pinus* spp. and Douglas-fir (*Pseudotsuga menziesii*) forest sites based on article 1 of the Commission Decision 2007/433/EC (separate, demarcated part of forest where propagating material as seeds and cones were collected). The data is based on the annual reports submitted to the European Commission by the NPPOs.

Country and Years	Total Hectares/Number in the Member State	Visually Surveyed Locations Hectares/Number	Number of Laboratory Samples Taken	Results for Visually Surveyed Locations +/−	Results for Laboratory Analyses+/−	In case of Positive Findings Size (Ha) and Location of Demarcated Area (Infected Zone + Buffer Zone)
Spain, Castilla y León, 2009–2017	3–10 sites per year	50 sites per year (129 sites in total)	23–2329 samples per year (3560 in total)	1–8 sites with symptoms	11 positives (between 2009 and 2011)	2535.7 ha in total
Spain, Cantabria, 2009–2017	data not available	data not available	data not available	data not available	data not available	data not available
Portugal, 2009–2017	183 sites; about 12,000 ha (per year)	161 sites; about 201,802 ha (per year)	1–30 per year (110 in total)	1–26 sites with symptoms per year (79 in total)	2 positive (2016); other years negative	600 ha located in 1 region (2016); other years n.a.
Italy, 2010–2017	17,920–25,279 ha + 0–4 sites (2011–2017) 68 sites (2010) per year	27–1693 + 0–4 sites (2011–2017) 41 sites (2010) per year (total 5120 ha + 53 sites)	0–46 per year (108 in total)	all negative	all negative	n.a.
France, 2013–2017	21,894 ha per year (109,470 ha in total)	included in forest survey	see nurseries and forests	see nurseries and forests	see nurseries and forests	see nurseries and forests
Bulgaria, 2015–2016	3134.52 ha *Pinus* spp. and *P. menziesii* forestry stand and seed orchard	6.05–6.50 ha	0–11 (seed samples)	negative	all negative	not applicable
Northern Macedonia, 2007–2017	not available	*Pinus* spp. 3 ha and *P. menziesii* 0.5 ha	0	n.a.	all negative	n.a.
Slovenia, 2007–2017	not available	included in forest surveys	included in forest surveys	all negative	all negative	n.a.
Great Britain, 2007–2017	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Sweden, 2014–2017	for *Pinus* spp. a 628 ha forestry stand and 1080 ha seed orchard; and for *P. menziesii* a 2 ha forestry stand	0	n.a.	n.a.	n.a.	n.a.
Finland, 2007–2017	1782–2434.2 ha (2007–2010); 0 ha (2011–2018)	0	10 places, 500 seed/place (2008); 0 (other years)	n.a.	all negative	n.a.

n.a. = not applicable.

Vainio et al., 2019

Eradication in the forest: **Cut and burn**

Eradication in the forest: **Cut and burn**

Eradication in the forest: **Cut and burn**

Cost: 6000 €/ha

Situation in the Nurseries

Table 1. Sampling approaches in production sites (nurseries) for planting material. The data is based on the annual reports submitted to the European Commission by the NPPOs.

Country and Years	Number in the Member State (Total Hectares)	Visually Surveyed Locations (Number/Hectares)	Number of Laboratory Samples Taken	Results for Visually Surveyed Locations +/−	Results for Laboratory Analyses+/−	In case of Positive Findings Size (ha) and Location of Demarcated Area (Infected Zone + Buffer Zone) *
Spain, Castilla y León, 2009–2017	13–121 nurseries of forest reproductive material (*Pinus* and/or *P. menziesii*) per year (658 in total)	21–52 per year (304 in total)	106–822 seedlings per year (3499 in total), and 1–165 seed lots per year (371 in total)	1 without symptoms	3 positive/3496 negative	Data not available
Spain, Cantabria 2009–2017	1–3 nurseries of forest reproductive material (*Pinus* and *P. menziesii*) per year	1–3 nurseries per year (17 in total)	102 samples in total	all negative	4 positive in total	n.a.
Portugal, 2009–2017	100–224 nurseries of forest reproductive material (*Pinus* and *P. menziesii*) per year (1593 in total)	64–209 nurseries of forest reproductive material (*Pinus* and *P. menziesii*) per year (1245 in total)	154–259 per year (1908 in total)	4–17 with symptoms per year (79 in total)	0–13 positive, 154–257 negative per year (in total 40 positive, 1869 negative)	500–6269 ha located in 1–3 different regions per year (25,935 ha in total)
Italy, 2010–2017	509–966 nurseries and garden centers of forest and ornamental trees per year	309–481 nurseries and garden centers of forest and ornamental trees per year (3240 in total)	43–309 per year (1111 in total)	all negative	all negative	n.a.
France, 2013–2017	1650–2554 nurseries per year (8187 in total; some of them are inspected every year)	1062–1331 per year (4868 in total)	17–47 per year (0–9 plants, 14–47 seed lots; 152 in total)	all negative	one positive seedlot from USA in 2016; all other years negative	n.a.
Bulgaria, 2015–2016	109 registered nurseries/1583.58 ha (authorized to issue plant passports)	84–89 nurseries (plants for planting) 226–334 ha	3–9	all negative	all negative	n.a.
Northern Macedonia, 2007–2017	25–26 nurseries (total 80 ha) of forest propagation material (*Pinus* spp. and *P. menziesii*) + 5 ha of nurseries of ornamental shrubs and trees	25–26 nurseries; 50–52 checks per year (*Pinus* spp. 30,337,150 seedlings and *P. menziesii* 1,612,400 seedlings in total)	58–80	all negative	all negative or n.a.	n.a.
Slovenia, 2007–2017	19–45 places of production of plants for planting (per year)	19–45 visually surveyed locations (per year)	0–10 (per year)	all negative	all negative	n.a.
Great Britain, 2007–2017	206–313 Nurseries registered to issue plant passports where plants were inspected	163–323 sites inspected each year	0–84 (per year)	all negative	all negative or n.a.	n.a.
Sweden, 2014–2017	98 registered nurseries for forest propagation material	26–90 nurseries of forest reproductive material + 100 garden centers	0	no occurrence detected in surveys; no further sampling	n.a.	n.a.
Finland, 2007–2017	33–76 nurseries of forest propagation material (*Pinus* and *P. menziesii*) + ca. 400 ha of nurseries of ornamental shrubs and trees	0–21 nurseries of forest reproductive material (up to ca. 22 million plants) + 9–77 nurseries of ornamental shrubs and trees	0–11 per year	samples taken from up to 4 locations; all negative	all negative or n.a.	n.a.

* As described in article 6 of the Commission Decision 2007/433/EC. n.a. = not applicable.

Vainio et al., 2019

Eradication in the nurseries: **Burnt Seedlings**

Eradication in the nurseries: **Burnt Seedlings**

Quarantine measures in EU and Spain

Regulation (EU) 2016/2031 of the European Parliament of the Council of 26 October 2016 on protective measures against pests of plants, amending Regulations (EU) No 228/2013, (EU) No 652/2014 and (EU) No 1143/2014 of the European Parliament and of the Council and repealing Council Directives 69/464/EEC, 74/647/EEC, 93/85/EEC, 98/57/EC, 2000/29/EC, 2006/91/EC and 2007/33/EC

Key aspects of this Regulation:

- New pest classification
- Preventive import strategy
- Responsability of professional operators
- Strengthening and extension of the plant passport issuance system

LEGISLATION

In addition to the above legislation, the Commission Decisions on emergency measures for **specific pests** are maintained

COMMISSION IMPLEMENTING DECISION (EU) 2019/2032 of 26 November 2019

establishing measures to prevent the introduction into and the spread within the Union of ***Fusarium circinatum*** Nirenberg & O'Donnell (formerly Gibberella circinata) and repealing Decision 2007/433/EC

LEGISLATION

COMMISSION IMPLEMENTING DECISION (EU) 2019/2032 (specific for *Fusarium circinatum*)	
Article 1	Definitions
Article 2	Action in case of detection or suspicion of the presence of the specified organism
Article 3	Surveys for the presence of the specified organism in the territory of Member States
Article 4	Establishment of demarcated zones
Article 5	Eradication measures in demarcated zones
Article 6	Movement of specified plants within the Union
Article 7	Movement of specified wood and isolated bark out of demarcated

LEGISLATION

COMMISSION IMPLEMENTING DECISION (EU) 2019/2032 (specific for *Fusarium circinatum*)	
Article 8	Movement of wood packaging material out of the demarcated areas
Article 9	Introduction of specified plants into the Union
Article 10	Introduction into the Union of specified wood and isolated bark
Article 11	Official controls on the introduction into the Union of specified plants, and of wood and isolated bark originating in non-European third countries
Article 12	Compliance
Article 13	Repeal
Article 14	Addressees

LEGISLATION

13938 *REAL DECRETO 1190/1998, de 12 de junio, por el que se regulan los programas nacionales de erradicación o control de organismos nocivos de los vegetales aún no establecidos en el territorio nacional.*

Establishment of national programmes for the **eradication or control of harmful organisms to plants** not yet established in the national territory.

9317 *Resolución de 27 de mayo de 2009, de la Subsecretaría, por la que se dispone la publicación del Acuerdo de encomienda de gestión entre la Secretaría General de Medio Rural y el Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, para la realización del proyecto Etiología, epidemiología y control de «Fusarium circinatum».*

Research project carried out by numerous research groups from all over Spain that aims to address different aspects related to the **aetiology, epidemiology and control of pine pitch canker**.

PLURIFOR Project

Objective

Contribute to the development of regional and transnational risk management plans for forest areas susceptible to biotic and abiotic risks

Contingency plan for PPC (*Fusarium circinatum*)

PREVENTION

Preventive measures

1) Risk estimation of pathogen entry routes

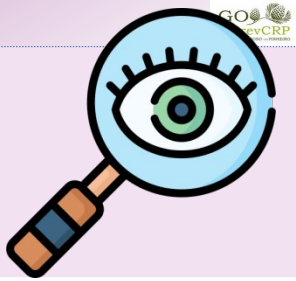
- Control seed imports
- Prevent transmission of spores that can occur through:

  Movement of wood, insects, pollen, compost, footwear and clothing, packages, etc.

2) Disinfection of workplaces and machinery

3) Information and awareness campaigns aimed at technical staff and the general public on the behaviour of the pathogen and prevention and control measures

MONITORING



Surveillance measures

1) Periodic surveys in forest stands of the genus *Pinus*

- Phytosanitary condition of trees
- Presence of symptoms or signs of disease

→ Samples (bark, phloem and xylem)

2) Periodic surveys of nurseries producing plants (sampling symptomatic as well as asymptomatic plants)

3) Surveys of forest reproductive material

4) Control of insect vectors (scoliids, pine c and trunk borers and pine cone suckers)

MANAGEMENT

Management measures for infection-free areas

1) **Heat treatment of seeds** with hot water in nurseries (52°C for 30 minutes)

2) **Sterilisation of containers and trays** by autoclaving (121ºC for 30 minutes)

3) **Regular disinfection** of tools, equipment and gloves with 70% ethanol or water vapor

4) **Use of native seeds** in infection-free areas (to avoid introductions)

MANAGEMENT

Management measures for infected areas

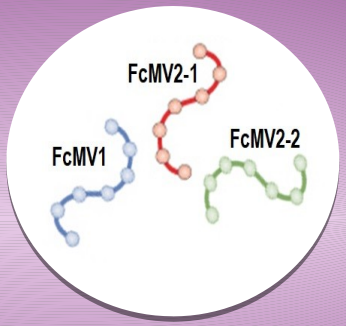
1) **Avoid planting the most susceptible pine species**

2) **Pruning** of infected branches and dying trees in the cold and dry seasons, and controlled burning of plant rests

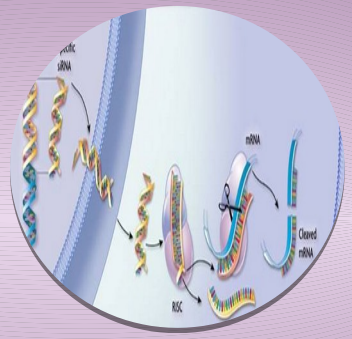
3) **Effective control of insect vectors** or wound-causing insects

Environmentally-Friendly Methods for *F. circinatum* Management

Endophytic fungi



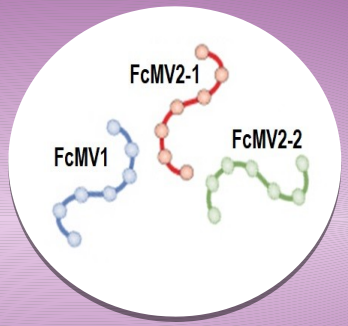
Mycoviruses



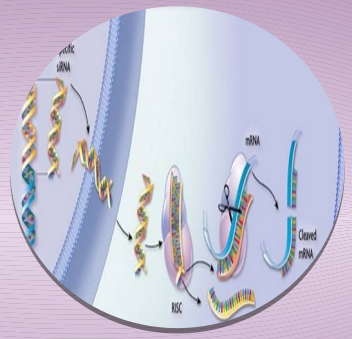
RNAi

BIOLOGICAL CONTROL of *Fusarium circinatum*

Endophytic fungi

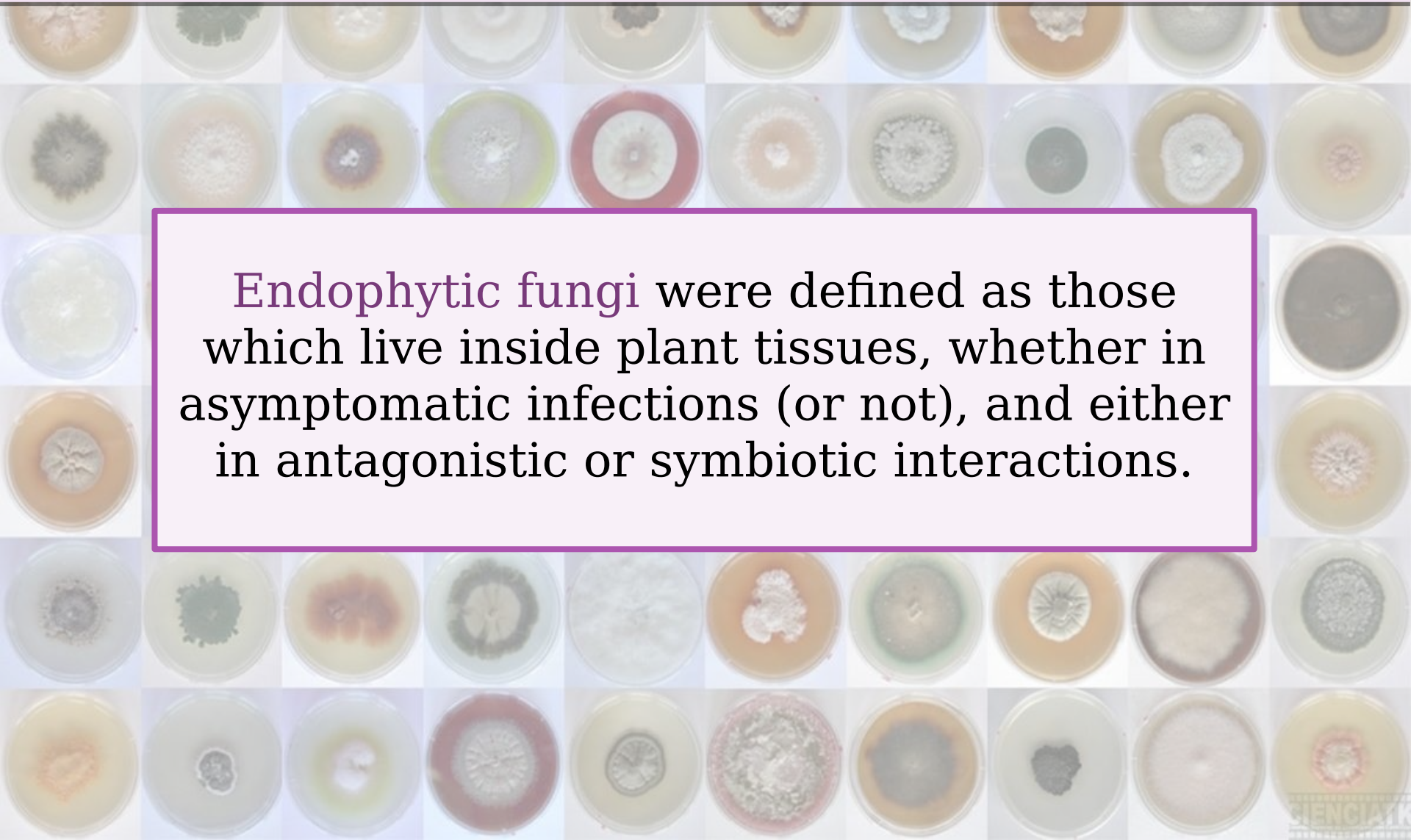


Mycoviruses



RNAi

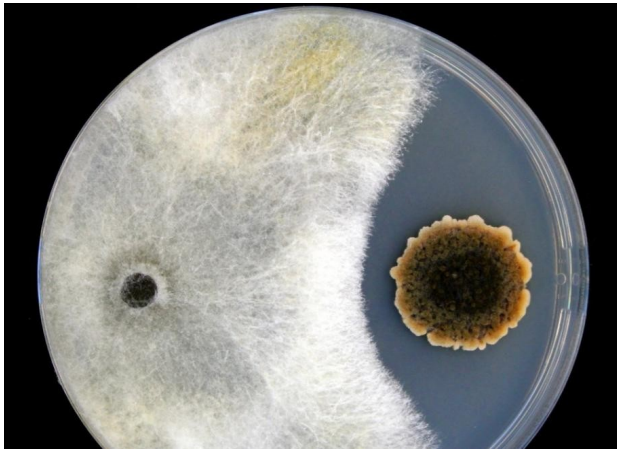
BIOLOGICAL CONTROL: ENDOPHYTES



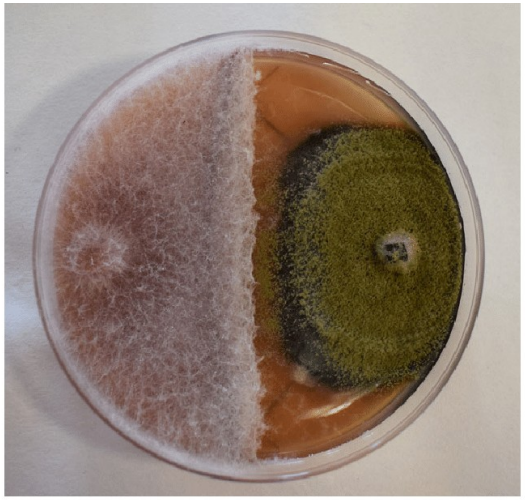
Endophytic fungi were defined as those which live inside plant tissues, whether in asymptomatic infections (or not), and either in antagonistic or symbiotic interactions.

BIOLOGICAL CONTROL: ENDOPHYTES

Endophytic fungi inhabit a similar ecological niche to that occupied by phytopathogens, thus being able to protect their environment and control them through competition, production of antagonistic substances, direct parasitism or even inducing resistance or tolerance.



ENDOPHYTES AGAINST *Fusarium circinatum*



Environmentally–friendly methods for controlling pine pitch canker

Article *in* Plant Pathology - February 2019
DOI: 10.1111/ppa.13009

Antagonistic effect of *Chaetomium aureum* (right) against *Fusarium circinatum* (left) in vitro

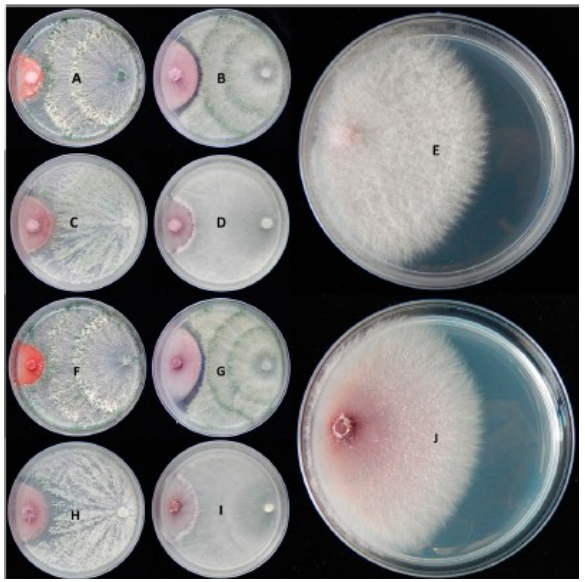
forests

MDPI

Article

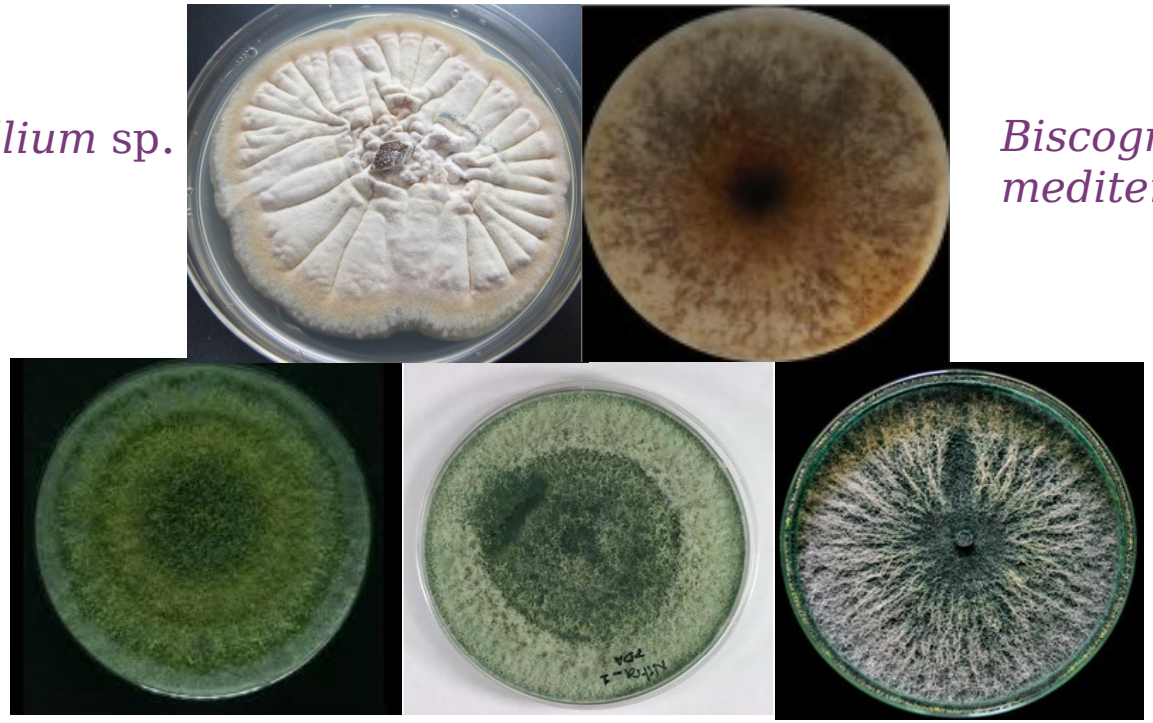
Application of *Trichoderma* spp. Complex and Biofumigation to Control Damping-Off of *Pinus radiata* D. Don Caused by *Fusarium circinatum* Nirenberg and O'Donnell

Carmen Morales-Rodríguez, Giorgia Bastianelli, MariaPia Aleandri, Gabriele Chilosi and Andrea Vannini *



ENDOPHYTES AGAINST *Fusarium circinatum*

Penicillium sp.



Biscogniauxia mediterranea

Trichoderma viridae, T. asperellum and *T. harzianum*

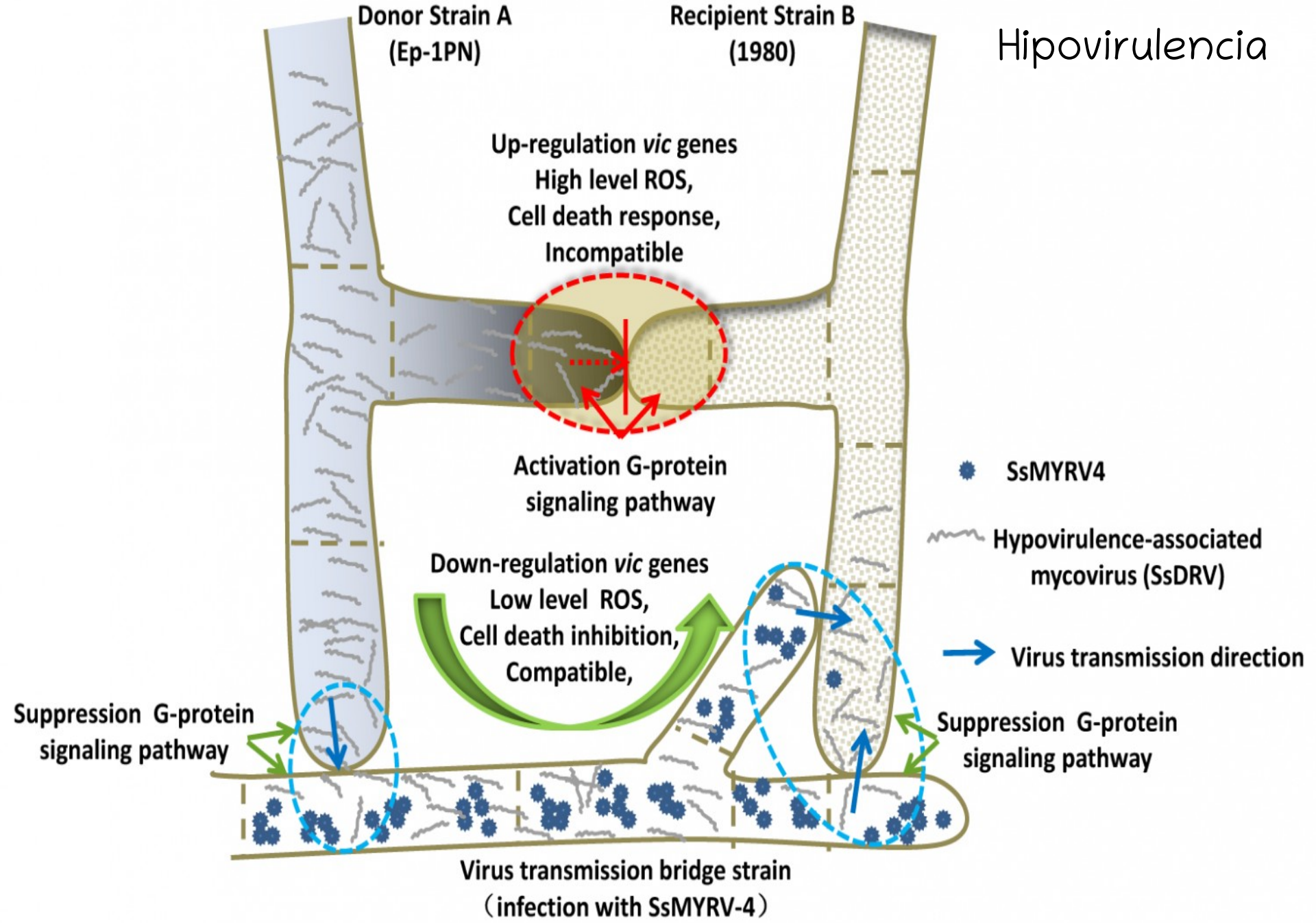
BIOLOGICAL CONTROL: MYCOVIRUSES

Mycoviruses are viruses that infect fungi. These viruses have been described infecting most taxonomic groups of fungi, including plant pathogenic fungi.

Most of these viruses of these viruses have RNA genomes, either double-stranded (dsRNA) or single-stranded (ssRNA), although they are (ssRNA) genomes, although a few have been described with circular DNA (ssDNA) genomes.

Fusarium circinatum
mycoviruses
(+)ssRNA

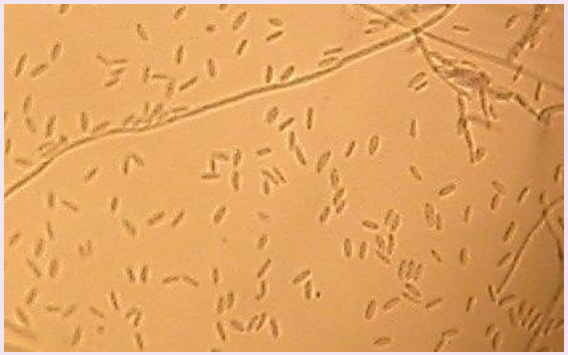
Hipovirulencia



Generally, mycoviruses lack an extracellular route of infection, and are only between isolates by **hyphal anastomosis** (horizontal transfer), or during **spore production** (vertical transfer).

Infection ways

Hyphal anastomosis (horizontal transfer)

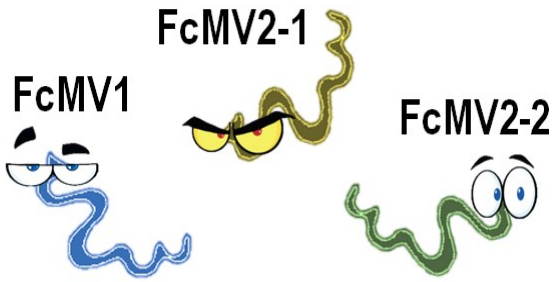


During spore production (vertical transfer)

Most **mycovirus infections are cryptic,** i.e. they do not have an effect on their fungal host, but in some cases the viral infection has been associated with the alteration of certain phenotypic traits,
being the most relevant the **virulence reduction**

HYPOVIRULENCE

Fusarium circinatum MYCOVIRUSES



***Fusarium circinatum* mycoviruses:**

Belong to genus *Mitovirus* and family *Narnaviridae*
(+)ssRNA
lack capsid
ORF – RNA-dependent RNA polymerase

GOBIERNO DE ESPAÑA MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

Project: NGSFORFUSARIUM

Perfil de expresión génica de *Fusarium circinatum* en respuesta a la infección del micovirus FcMV1

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SHORT COMMUNICATION

OPEN ACCESS

Effect of mycoviruses on growth, spore germination and pathogenicity of the fungus *Fusarium circinatum*

Juan Asdrúbal Flores-Pacheco[1,2,3], Emigdio Jordan Muñoz-Adalia[1,2], Pablo Martínez-Álvarez[1,2], Valentín Pando[1,4], Julio J. Diez-Casero[1,2] and Jorge Martín-García[1,5]

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ELSEVIER

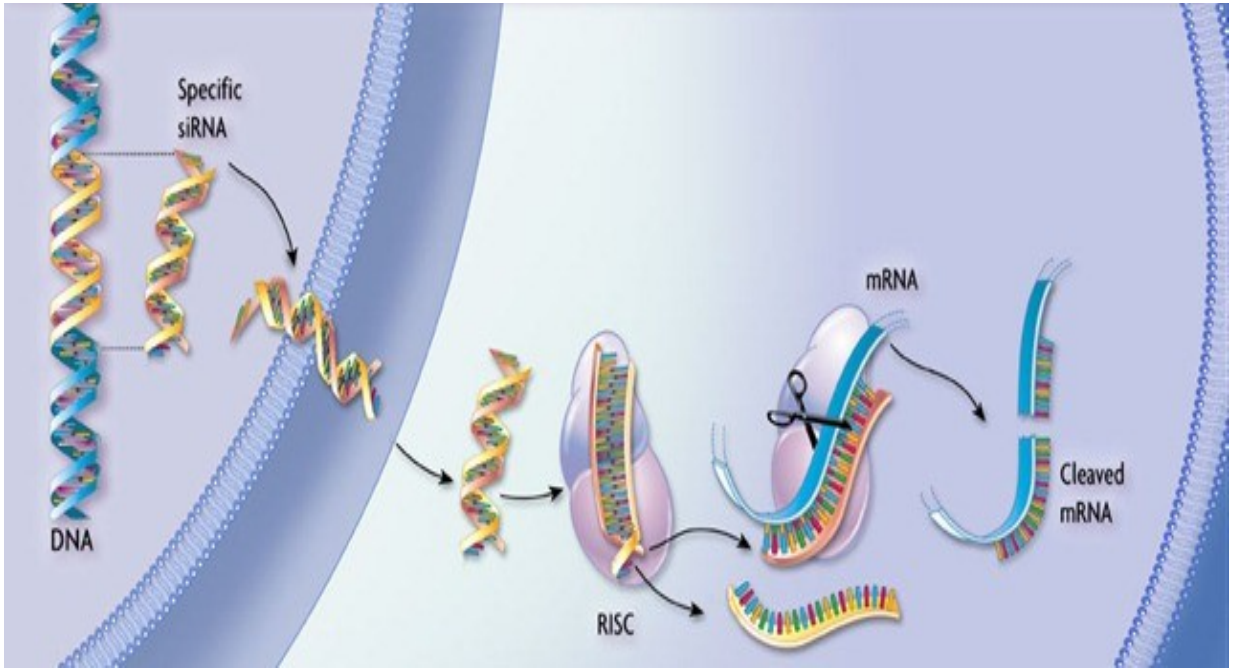
Effect of mycoviruses on the virulence of *Fusarium circinatum* and laccase activity

E.J. Muñoz-Adalia [a, b, *], J.A. Flores-Pacheco [a, b, c], P. Martínez-Álvarez [a, b], J. Martín-García [a, b], M. Fernández [a, d], J.J. Diez [a, b]

BIOLOGICAL CONTROL: RNAi

RNAi

- Is **mechanism of post-transcriptional silencing of specific genes**, naturally occurring in cells.
- It is **triggered by RNA molecules** that, being complementary to a messenger RNA, usually lead to its degradation.



Potential control of *F. circinatum* via gene silencing by RNA interference (RNAi)

dsRNA molecules

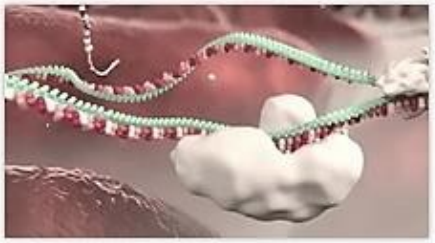
- Double stranded RNAs (dsRNAs) trigger RNAi machinery

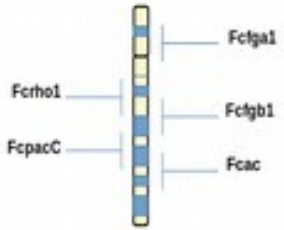
Target mRNA

- Due to its complementarity to a messenger RNA (mRNA) lead its degradation

Silencing

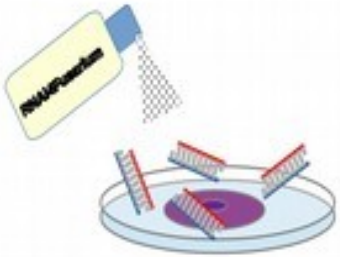
- When mRNA is degraded the expression of that specific gene is supressed





Gene Mapping and Selection

Design and Synthesis of RNAi



Spray-Induced Gene Silencing (SIGS)

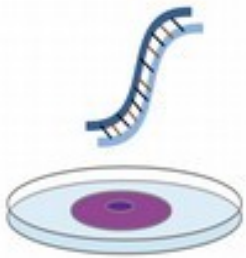
a. Effect on Gene Silencing

RNAi (dsRNA)

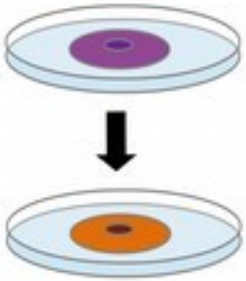
b. Effect on *Fusarium circinatum*

c. Effect on PPC

Transcriptome of *Fusarium circinatum*

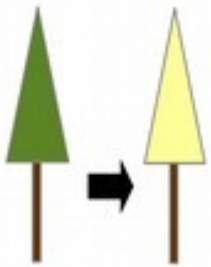


Colony Effects



Transcriptome in *Pinus* sp.

Pathogenicity

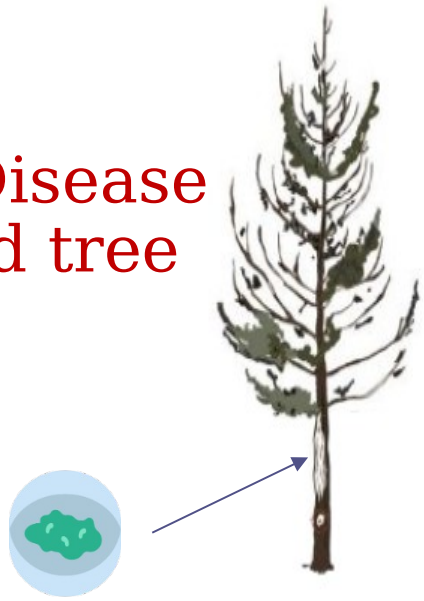


RNAi application to Forestry: SIGS technology

Spray-induced gene silencing (SIGS):
spraying artificially synthesized dsRNA molecules that **target susceptible pathogens genes on plant surfaces** resulted in a reduction of the disease

Spray dsRNAs targeting *F. circinatum* genes can decrease the fungal growth and virulence in pines

Disease d tree



SIGS pathogen prevention

Future of *F. circinatum* and hosts

-*F. circinatum* will be with us forever: **eradication not posible**

-**Difficult to manage** in the nurseries

-In the forest is a problem of some species: ***P. radiata*** **and** ***P. pinaster***

-**Synergistic effect** with *Lecanostica acícola and Dothistroma pini and D. septosporum*

-***P. radiata*** **is not an alternative anymore**

-What are the **alternative species**? Sequoia, Cunningamia...

-**Breeding of native** species as the best alternative

FP1406 - Pine pitch canker - strategies for management of Gibberella Circinata in greenhouses and forests (PINESTRENGTH)

Downloads Team

Description

Gibberella circinata is a highly virulent pathogen damaging pines, causing damping-off in nurseries and pitch canker in forests. It was first detected in North America, since when the pathogen has spread into Central and South America, South Africa, Asia and, more recently, Europe. *G.circinata* is now considered the most important pathogen affecting *Pinus* seedlings and mature trees in many countries globally; asymptomatic seedlings may be planted out, resulting in very serious losses in forests. Nevertheless, there has been little research on *G. circinata* in Europe to date and little information is available overall on host range in Europe, pathogen spread and disease control. The main aim of this Action is to establish a European-focused network to increase knowledge of the biology, ecology and pathways of spread of *G. circinata*, to examine the potential for the development of effective and environmentally-friendly prevention and mitigation strategies and to deliver these outcomes to stakeholders and policy makers. To that end, a multidisciplinary approach will be taken, including researchers, forest managers and policy makers from (initially) 27 countries focused on the common problem of pitch canker making PINESTRENGTH highly innovative.

Action Details

MoU - 100/14

CSO Approval date - 13/11/2014

Start date - 08/05/2015

End date - 07/05/2019

http://www.pinestrength.eu/

Twenty Seven Countries

- **Initial contributions from 22 COST Countries** (Bulgaria, Switzerland, Czech Republic, Estonia, Greece, Spain, Finland, France, Italy, Lithuania, Macedonia, Malta, Netherlands, Norway, Poland, Portugal, Romania, Sweden, Slovenia, Slovakia, Turkey, United Kingdom).

- And **5 non-COST Countries** Chile, South Korea, New Zealand, United States, South Africa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Thank you very much for your attention

PINESTRENGTH

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