

Update of tools used in Cantabria for dealing with *Fusarium* *circinatum*

Pablo Martínez Álvarez

Aveiro, 3rd October
2017



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* Niremburg et O'donnell.*

La enfermedad de los pinos, conocida en el mundo anglosajón como «pitch canker», provoca importantes pérdidas económicas en las producciones de numerosas especies de pinos y otras coníferas. La causa de la enfermedad es la actividad del hongo fitopatógeno *Fusarium Circinatum* Niremburg et O'donnell, cuyo teleomorfo es *Giberella circinata*, y hasta la fecha, la presencia de este organismo nocivo era desconocida en España.

Este hongo fitopatógeno tiene una elevada capacidad de diseminación a través del material forestal de reproducción, especialmente las semillas, la circulación de productos forestales, como la madera, y el concurso de insectos perforadores, como curculiónidos y escolítidos, que actúan como vectores de éste.

El organismo ha sido detectado principalmente en material forestal de reproducción de pino en el norte de la península ibérica y, sólo en algunas ocasiones, ha sido confirmada su presencia en masas forestales. Existe la sospecha de que pudiera estar presente en aquellos territorios en los que se encuentran importantes formaciones forestales de *Pinus Radiata*, especie altamente sensible al «pitch canker». En todos los casos confirmados se ha procedido a la erradicación de los focos.

Dado que se trata de un organismo nocivo cuya pre-

das en el anexo I, en lo sucesivo «especies sensibles», de acuerdo con las disposiciones del artículo 15.2 de la Ley 43/2002, de 20 de noviembre, de Sanidad Vegetal, y el 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.

2. En virtud del artículo 15.1 de la Ley 43/2002 se declaran de utilidad pública las medidas de salvaguardia incluidas en el programa y adoptadas en cumplimiento del artículo 16.2 del Real Decreto 58/2005, de 21 de enero, por el que se adoptan medidas de protección contra la introducción y difusión en el territorio nacional y de la Comunidad Europea de organismos nocivos para las vegetales o productos vegetales, así como para la exportación y tránsito hacia países terceros.

Artículo 2. *Ámbito de aplicación.*

El programa que se aprueba y las medidas de él dimanantes serán de aplicación en todo el territorio nacional.

Artículo 3. *Obligaciones de los agentes implicados.*

1. Los recolectores, productores y proveedores de semillas, viveristas, agricultores, silvicultores, operadores de industrias de primera transformación de la madera, importadores y profesionales que ejerzan actividades relacionadas con la protección vegetal deberán notificar inmediatamente al órgano competente de la comunidad autónoma o, en el caso de importadores de terceros países, a la Dirección General de Agricultura del Ministerio de Agricultura, Pesca y Alimentación la existencia de vegetales o productos vegetales de las especies relacionadas en el anexo I de este real decreto con síntomas de *Fusarium Circinatum* Niremburg et O'donnell. en lo suce-

Regional regulation (Cantabria)



CONSEJERÍA DE DESARROLLO RURAL, GANADERÍA, PESCA Y BIODIVERSIDAD

Orden DES/32/2008, de 16 de abril, por la que se establece un programa de actuación para la lucha contra la enfermedad producida por el hongo Fusarium Circinatum Niremberg et O'donnell, se habilita el procedimiento para el movimiento de vegetales y productos vegetales de las especies sensibles y se crea el registro de empresas autorizadas para tratamiento de madera procedente de zonas demarcadas.

El *Fusarium Cirnatum* (*Gibberella Circinata*) es un hongo causante de la enfermedad del chancro resinoso que produce en determinadas especies de coníferas la aparición de importantes exudaciones de resina que pueden llegar a producir la muerte del árbol. Este hongo

hasta fechas recientes desconocido en España, ha causado graves daños en países como EE.UU. Sudáfrica, Chile o México y fue detectado en Cantabria en 2005 tanto en masas forestales como en viveros sobre plantas del género *Pinus*. La importante superficie de algunas especies de pinos en Cantabria y el peligro de extensión de la enfermedad a toda la superficie repoblada aconseja establecer medidas urgentes de control de este organismo nocivo.

La Ley 43/2002, de 20 de noviembre, de Sanidad Vegetal, obliga en su título II, capítulos III y IV a adoptar medidas para conseguir la erradicación o evitar la propagación de las plagas de cuarentena, previendo además la posibilidad de establecer indemnizaciones cuando la lucha contra una plaga suponga la destrucción, deterioro o inutilización de bienes o propiedades particulares o públicas.

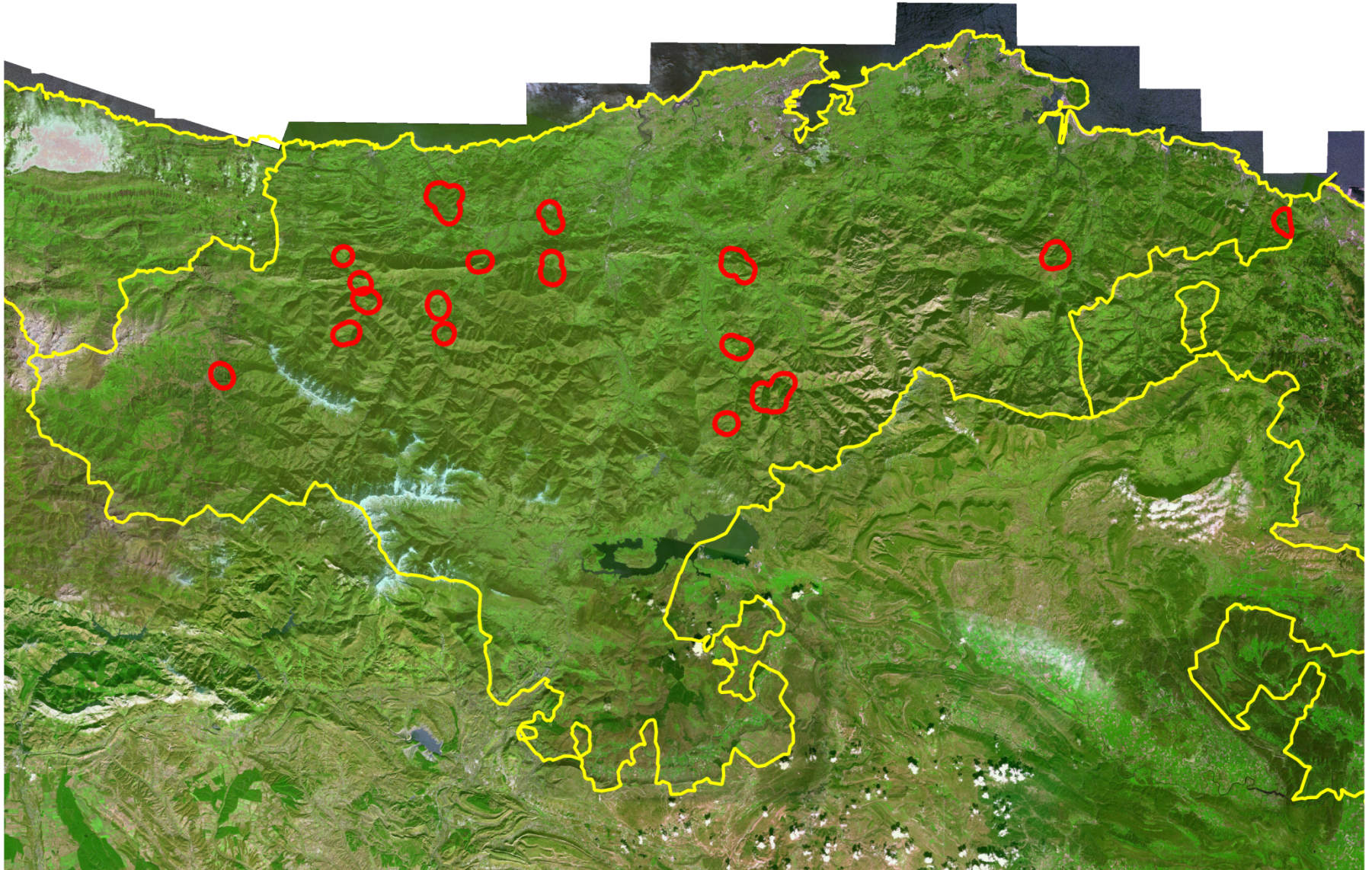


Most important measures

- **Visual inspections** in a net of 8x8 km (4x4 in pine plantations).
- Inspections in **forest nurseries** (2 inspections per year).
- Delimitation of “**zonas demarcadas**”= areas affected by the disease and **1 km buffer area**.
- **Removal** of the infected trees “*in situ*”
- To take wood out of the “zona demarcada” it must be **debarked** and **heat treated** (56 °C in the center of the wood during 30 minutes).

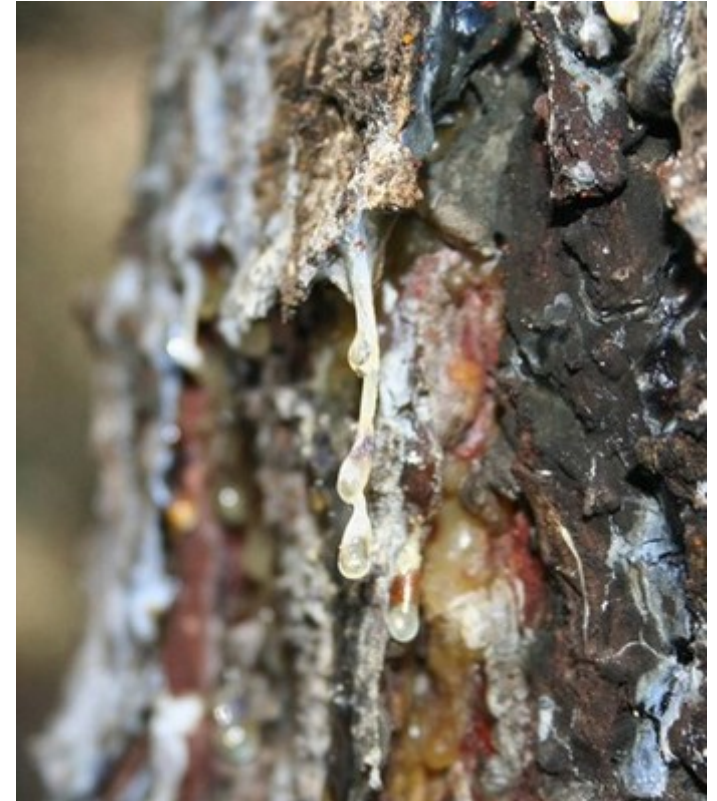


“Zonas demarcadas” in Cantabria (Spain)



Research studies on *F. circinatum* performed in Cantabria

- **Susceptibility** of different conifer species to *F. circinatum*.
- Use of **endophytes** to fight against *F. circinatum*.
- Detection of **viruses** hosted by *F. circinatum*.
- Analysis of the role of different **insects** in relation to the disease.
- Effect of pruning on the disease



Susceptibility of different conifers to *F. circinatum*



Species	Provenance
<i>Abies alba</i>	ES02 Pirineo Central
<i>Cedrus atlantica</i>	Unidentified
<i>Chamaecyparis lawsoniana</i>	Unidentified
<i>Cupressocyparis levlandii</i>	Unidentified
<i>Picea abies</i>	East Europe
<i>Pinus nigra corsicana</i>	902 Sud-ouest (France)
<i>Pinus pinaster</i>	ES08 Meseta castellana
<i>Pinus radiata</i>	Unidentified
<i>Pinus sylvestris</i>	ES10 Sierra de Guadarrama
<i>Pinus uncinata</i>	ESC Sierra de Gúdar
<i>Pseudotsuga menziessi</i>	430 Washington, Randle
<i>Sequoiadendron giganteum</i>	Unidentified
<i>Thuja plicata lobbii</i>	Unidentified



Susceptibility of different conifers to *F. circinatum*



Plant Pathology (2014) 63, 1086–1094

Doi: 10.1111/ppa.12187

Alternative species to replace Monterey pine plantations affected by pitch canker caused by *Fusarium circinatum* in northern Spain

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The pitch canker pathogen *Fusarium circinatum* was first found to cause damage in nurseries and pine plantations in northern Spain in 2004. Since then, establishment of pine plantations in the region has decreased as a result of the prohibitions placed on planting *Pinus* spp. and *Pseudotsuga menziesii* in areas affected by the disease. However, although most pine species have been found to be susceptible to the pathogen under nursery conditions, little is known about how the fungus affects the trees in the field. Furthermore, it is not known whether some of the native or exotic species commonly planted in the area are also susceptible to *F. circinatum*. The aim of this study was to evaluate the susceptibility of several conifer species commonly planted in northern Spain to the pitch canker pathogen. For this purpose, two different trials were carried out, one under controlled laboratory conditions and the other in the field. Although

Pinus uncinata

ESC Sierra de Gúdar

Pseudotsuga menziesii

430 Washington, Randle

Sequoiadendron giganteum

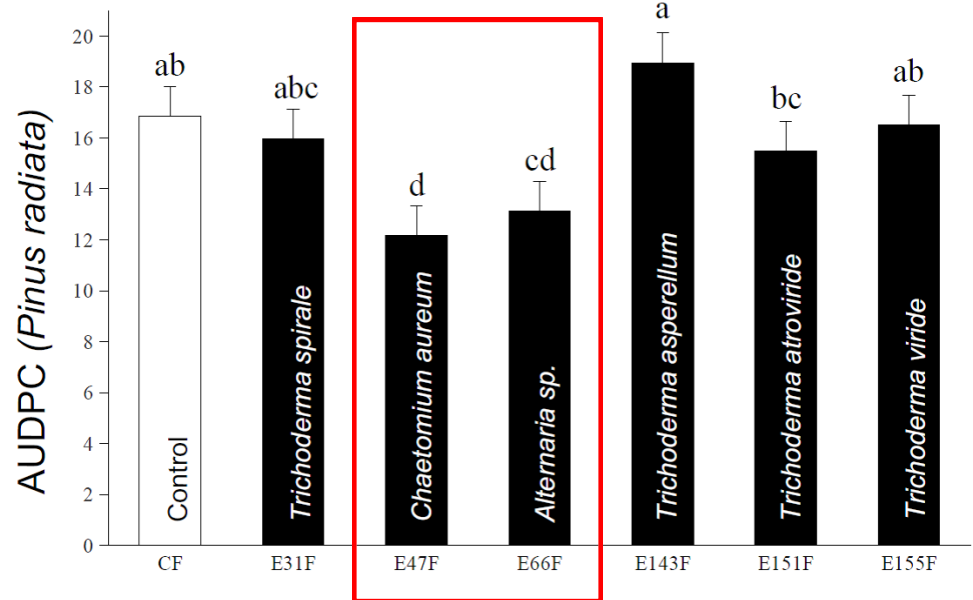
Unidentified

Thuja plicata lobbii

Unidentified



Use of endophytes to fight against *F. circinatum*



Use of endophytes to fight against *F. circinatum*



SILVA FENNICA

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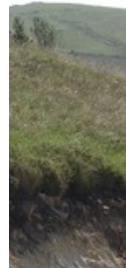
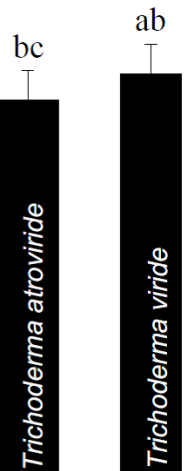
In Vitro and In Vivo Interactions between *Trichoderma viride* and *Fusarium circinatum*

Pablo Martínez-Álvarez, Fernando Manuel Alves-Santos and Julio Javier Diez

Martínez-Álvarez, P., Alves-Santos, F.M & Diez, J.J. 2012. In vitro and in vivo interactions between *Trichoderma viride* and *Fusarium circinatum*. Silva Fennica 46(3): 303–316.

Fusarium circinatum, a fungus that causes pitch canker disease, has been present in Europe since at least 2003, when it was detected in northern Spain and found to be producing severe damage in tree nurseries and pine plantations. In this study, we tested a method of biological control of the disease with *Trichoderma viride*, a fungal species successfully used against many other pathogens. In vitro and in vivo assays were carried out to test the efficacy of this antagonist in controlling *F. circinatum*. The *T. viride* isolate exerted a significant effect on the growth of *F. circinatum* in the in vitro assay, reducing the length of the pathogen colony by

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Use of endophytes to fight against *F. circinatum*

Biological Control 94 (2016) 1–10



Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/ybcon



Two fungal endophytes reduce the severity of pitch canker disease in *Pinus radiata* seedlings



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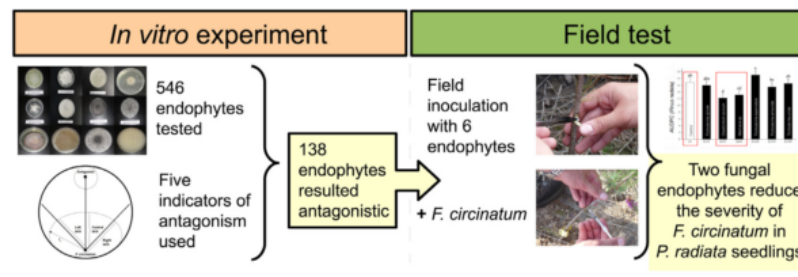
^c Calabazanos Forest Health Center, Junta de Castilla y León, Polígono Industrial de Villamuriel S/N, 34190 Villamuriel de Cerrato, Palencia, Spain

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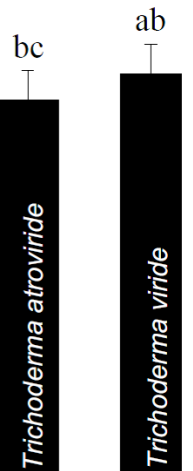
HIGHLIGHTS

- Five hundred forty-six endophytes were tested against *F. circinatum* in vitro.
- The antagonistic activity of the endophytes was quantified using five indicators.
- The six endophytes that showed the most promising results were tested in the field.
- *Chaetomium aureum* and *Alternaria* sp. reduced the damages caused by the pathogen.

GRAPHICAL ABSTRACT



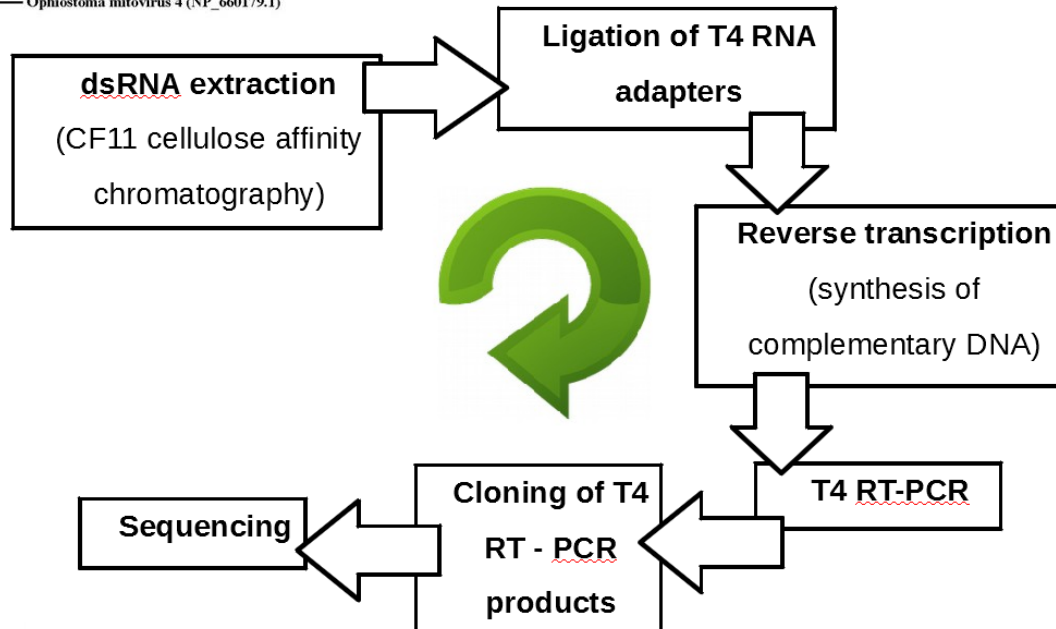
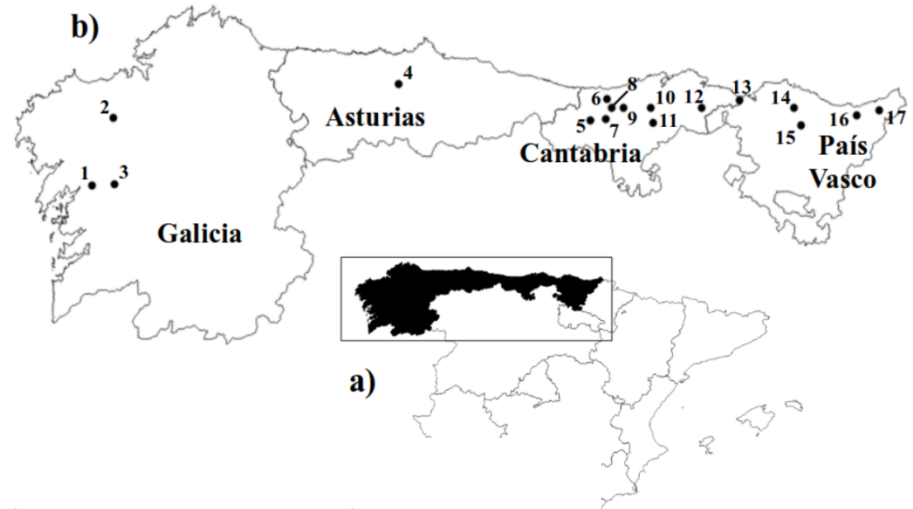
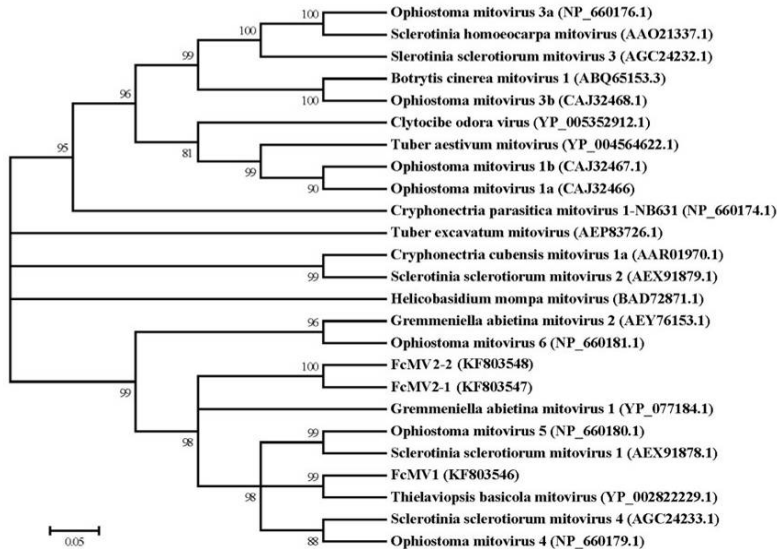
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Trichoderma atroviride

Trichoderma viride

Detection of viruses hosted by *F. circinatum*



Detection of viruses hosted by *F. circinatum*

Author's personal copy

Arch Virol (2014) 159:2153–2155
DOI 10.1007/s00705-014-2012-8

ANNOTATED SEQUENCE RECORD

Three mitovirus strains infecting a single isolate of *Fusarium circinatum* are the first putative members of the family *Narnaviridae* detected in a fungus of the genus *Fusarium*

Pablo Martínez-Álvarez · Eeva J. Vainio ·
Leticia Botella · Jarkko Hantula · Julio J. Diez

Received: 12 November 2013 / Accepted: 27 January 2014 / Published online: 12 February 2014
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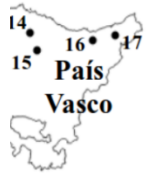
Abstract *Fusarium circinatum* Nirenberg & O'Donnell (teleomorph = *Gibberella circinata*) is the causal agent of pitch canker disease of pines. Since 2004 it has been present in Europe, particularly in northern Spain, affecting *P. radiata* and *P. pinaster* in plantations and nurseries. The disease has now also spread to other European countries, including France, Italy and Portugal. In this report, we describe three novel members of the genus *Mitovirus* from a Spanish isolate of *F. circinatum*: *Fusarium circinatum* mitovirus 1 (FcMV1), FcMV2-1 and FcMV2-2. Using a mitochondrial translation table, the complete 2419-bp genome of FcMV1 encodes an RNA-dependent RNA

polymerase similar to the other strains. However, FcMV1 shared 46 % protein-level similarity with *Thielaviopsis basicola* mitovirus. This is the first study to report viruses in *F. circinatum*, as well as the first time that mitovirus genome sequences are described from *Fusarium* spp.

Introduction

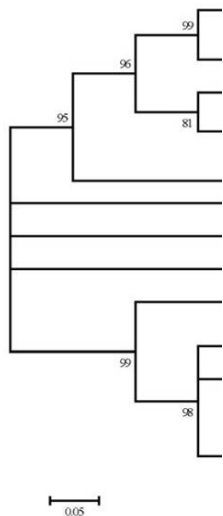
Pitch canker is a severe disease of conifers caused by the

products



0.05

Detection of viruses hosted by *F. circinatum*



Arch Virol (2015) 160:2093–2098
DOI 10.1007/s00705-015-2462-7



BRIEF REPORT

Fusarium circinatum isolates from northern Spain are commonly infected by three distinct mitoviruses

Eeva J. Vainio¹ · Pablo Martínez-Álvarez^{2,3} · Diana Bezos^{2,3} · Jarkko Hantula¹ · Julio J. Diez^{2,3}

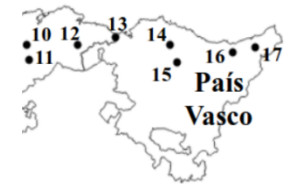
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Abstract Pitch canker is a serious disease of pines caused by the ascomycete fungus *Gibberella circinata* (anamorph = *Fusarium circinatum*). Three distinct mitovirus strains have been described in this fungus: *Fusarium circinatum* mitovirus 1 (FcMV1), FcMV2-1 and FcMV2-2. Here, we investigated the frequency and population variation of these viruses and closely related sequence variants in northern Spain using RT-PCR and sequencing. Each virus strain and similar sequence variants shared >95 % sequence identity and were collectively designated as virus types. All virus types were relatively common in Spain, with estimated prevalence of 18.5 %, 8.9 % and 16.3 % for FcMV1, FcMV2-1 and FcMV2-2, respectively.

Keywords *Gibberella circinata* · Pitch canker · ssRNA · *Narnaviridae* · Virus population

The ascomycete fungus *Gibberella circinata* Nirenberg & O'Donnell (anamorph = *Fusarium circinatum*) causes a serious disease known as pitch canker on *Pinus* spp. The pathogen initiates resinous bleeding cankers on the tree trunks or large branches, and infected trees can eventually die due to girdling or stem breakage. The fungus was first reported in North Carolina [6] and Mexico, and has since spread into Haiti, South Africa, Japan, Chile, Korea, Southern Europe, Uruguay [reviewed in reference 12], Colombia [17] and Brazil [15]. *F. circinatum* has moved between plantations areas via contaminated seed and seedlings, and the fungus spreads further through airborne spores, insect vectors and water splash. Mechanical damage that provides infection courts increases the risks of infection [1]. The fungus is considered to be native in Mexico [24], the Caribbean and the southeastern USA.

Fungi of the genus *Fusarium* are hosts to diverse viruses, including members of the families *Chrysoviridae*, *Hypoviridae*, *Partitiviridae* and *Totiviridae* [3], as well as the yet unassigned *Fusarium graminearum* virus 1, which



Detection of viruses hosted by *F. circinatum*

Physiological and Molecular Plant Pathology 94 (2016) 8–15



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journal homepage: www.elsevier.com/locate/pmpp



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



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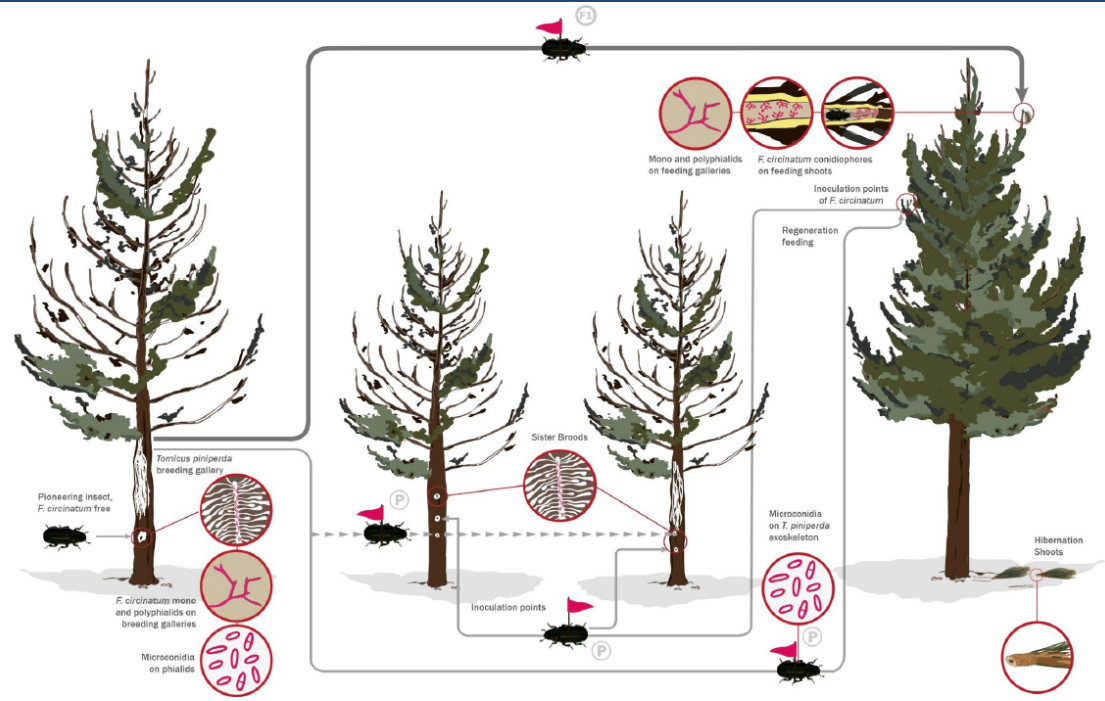
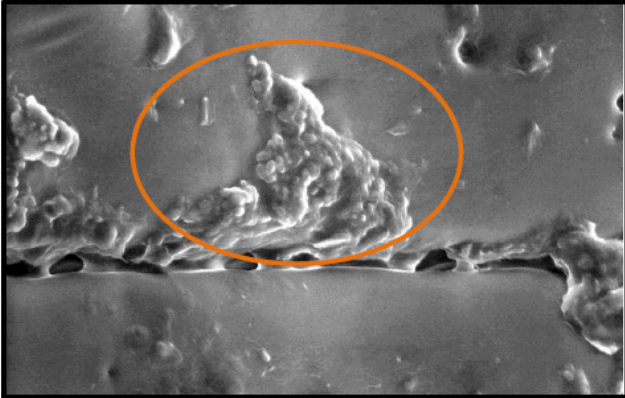
ssRNA

ABSTRACT

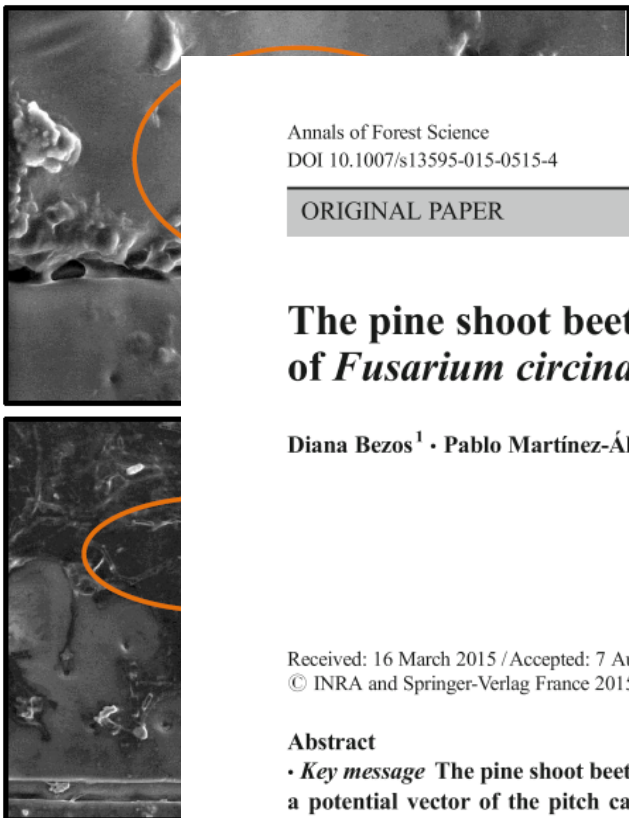
Laccase enzymes (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) play a major role in the degradation of phenolic compounds such as lignin. They are common in fungi and have been suggested to participate in host colonization by pathogenic fungi. Putative mycoviruses have recently been isolated from the causal agent of pine pitch canker disease, *Fusarium circinatum* Nirenberg & O'Donell. In this study, the effects of single and double mycoviral infections on laccase activity, growth rate and pathogenicity were investigated in fourteen *F. circinatum* strains. Extracellular laccase activity was analyzed by the Baven-damm test, image processing and a spectrophotometric method. Mycelial growth, *in vivo* pathogenicity and seedling survival probability were also determined in Monterrey pine (*Pinus radiata* D. Don) seedlings. The findings showed that (i) mycelial growth of isolates from the same fungal population was homogeneous, (ii) the presence of mycovirus appears to increase the virulence of fungal isolates, (iii) co-infection (with two mycoviruses) caused cryptic effects in fungal isolates, and (iv) laccases embody a possible auxiliary tool in fungal infection. The prospects for biocontrol, the adaptive role of *F. circinatum*

products

Role of insects in relation to the pitch canker



Role of insects in relation to the pitch canker



Annals of Forest Science
DOI 10.1007/s13595-015-0515-4



ORIGINAL PAPER

The pine shoot beetle *Tomicus piniperda* as a plausible vector of *Fusarium circinatum* in northern Spain

Diana Bezos¹ · Pablo Martínez-Álvarez¹ · Julio J. Díez¹ · Mercedes M. Fernández¹

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Abstract

• **Key message** The pine shoot beetle, *Tomicus piniperda*, is a potential vector of the pitch canker disease pathogen. The insect could transmit the pathogenic fungus during its maturation or regeneration feeding on the shoots of healthy pine crowns.

• **Context** *Fusarium circinatum*, the causal agent of pitch canker disease, currently affects *Pinus radiata* in northern Spain, causing pitch-soaked cankers and tree death. Although several species of the family Scolytinae have been reported as vectors of this pathogen, the role of the pine shoot beetle *T. piniperda* remains unclear.

• **Aims** The general objective of this study was to determine whether *T. piniperda* is a vector for the pitch canker pathogen *F. circinatum*. For this purpose, Leach's postulates (1) an

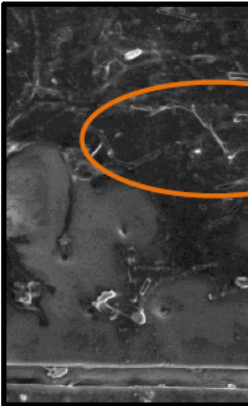
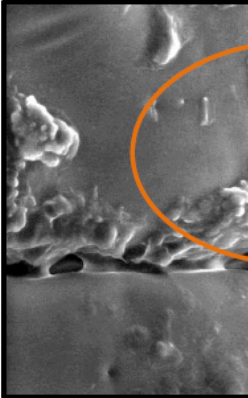
association between *T. piniperda* and trees affected by pitch canker disease; (2) regular visits by *T. piniperda* to healthy *P. radiata* trees; (3) presence of the pathogen on the insect in nature; and (4) transmission of the pathogen to disease-free host material under controlled conditions.

• **Methods** Fresh green shoots with feeding galleries were collected from the ground, breeding galleries were collected from diseased trunks and insects were collected during their dispersion flights. A laboratory experiment was conducted in which specimens of *T. piniperda* were inoculated with the pathogen prior to feeding on shoots.

• **Results** In the field, *T. piniperda* was found to be associated with both diseased and healthy *P. radiata* trees, and *F. circinatum* was found to be present, at low rates, on the exoskeleton of *T. piniperda*. In the laboratory experiment,



Role of insects in relation to the pitch canker



Entomologia Generalis, Vol. 36 (2016), Issue 1, 043–054
Published in print July 2016

Article

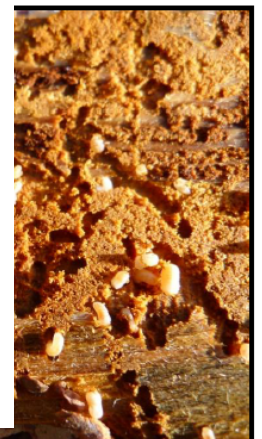
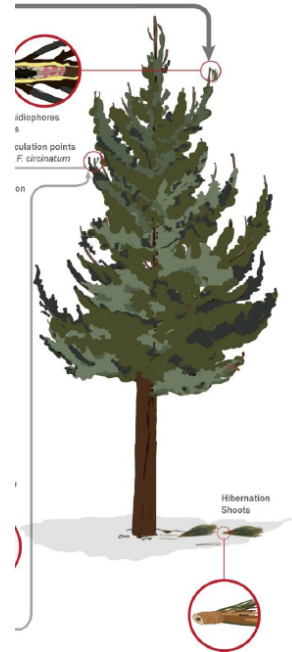
Association levels between *Pityophthorus pubescens* and *Fusarium circinatum* in pitch canker disease affected plantations in northern Spain

Diana Bezos*, Pablo Martínez-Álvarez, Julio J. Díez & Mercedes Fernández

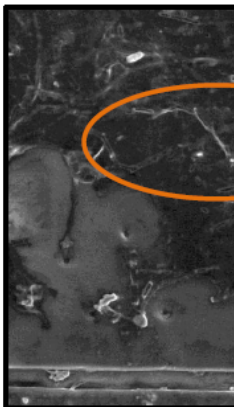
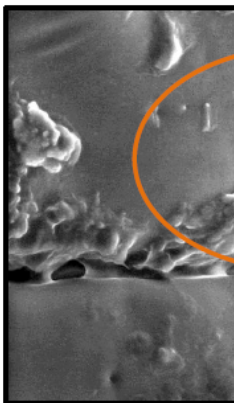
Instituto de Investigación Forestal Sostenible (Universidad de Valladolid-INIA),
Avenida Madrid 44, 34004 Palencia, España

With 2 figures and 1 table

Abstract: *Fusarium circinatum*, the causal agent of pitch canker disease (PCD), poses a threat to *Pinus radiata* plantations due to the presence of bleeding cankers on the trunk that can cause the tree to die. This pathogen has been reported to be phoretically associated with bark beetle species, specifically, with *Pityophthorus* species in California. *Pityophthorus pubescens* is a secondary pest, attacking weak trees or broken branches in healthy trees. The aim of this study was to know the association between *P. pubescens* and *F. circinatum* in PCD affected plantations in northern Spain. Specific aims were determined: i) to assess the phoretic association between *P. pubescens* and *F. circinatum*, ii) to study the presence of *F. circinatum* in *P. pubescens* infested twigs and iii) to evaluate whether PCD damages were enhanced in (E)-pityol baited *P. radiata* trees. Funnel traps baited with (E)-pityol were established and twigs from



Role of insects in relation to the pitch canker



forests

Article

Sydowia polyspora Dominates Fungal Communities Carried by Two *Tomicus* Species in Pine Plantations Threatened by *Fusarium circinatum*

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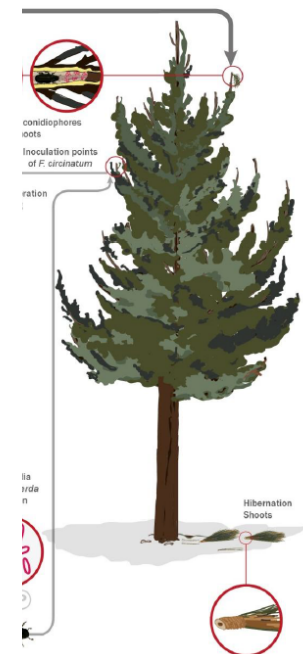
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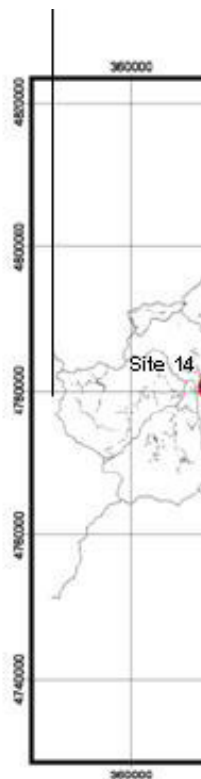
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Abstract: Bark beetles (Coleoptera, Scolytinae) carry a diverse filamentous fungal community sometimes acting as vectors or carriers of phytopathogens. In this study, mycobiota carried by two *Tomicus* species (*Tomicus piniperda* and *Tomicus destruens*) were investigated through (i) morphological and molecular identification of taxa; (ii) taxonomic richness, diversity, evenness, dominance and phoresy indices; (iii) ecological network analysis and (iv) statistical co-occurrence analysis. The studied mycobiota were formed by eleven taxa and showed a moderate fungal diversity with low evenness. The fungus *Sydowia polyspora* was significantly abundant and dominated the community. All the fungal taxa were randomly associated. Both insect species (*T. piniperda* and *T. destruens*) were collected from plantations of *Pinus radiata* infected by *Fusarium circinatum*. The ecological factors that



Effect of pruning on the disease



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Effects of pruning in Monterrey pine plantations affected by *Fusarium circinatum*

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Abstract

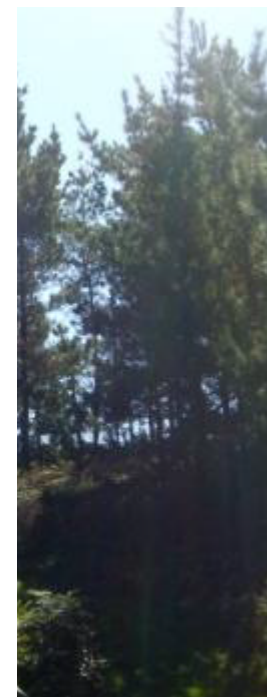
Fusarium circinatum Nirenberg and O'Donnell (1998) is the causal agent of Pitch Canker Disease (PCD) in *Pinus* species, producing damage to the main trunk and lateral branches as well as causing branch dieback. The disease has been detected recently in northern Spain in *Pinus* spp. seedlings at nurseries and in *Pinus radiata* D. Don adult trees in plantations. *Fusarium circinatum* seems to require a wound to enter the tree, not only that as caused by insects but also that resulting from damage by humans, i.e. mechanical wounds. However, the effects of pruning on the infection process have yet to be studied. The aim of the present study was to know how the presence of mechanical damage caused by pruning affects PCD occurrence and severity in *P. radiata* plantations. Fifty *P. radiata* plots (pruned and unpruned) distributed throughout 16 sites affected by *F. circinatum* in the Cantabria region (northern Spain) were studied. Symptoms of PCD presence, such as dieback, oozing cankers and trunk deformation were evaluated in 25 trees per plot and related to pruning effect. A significant relationship between pruning and the number of cankers per tree was observed, concluding that wounds caused by pruning increase the chance of pathogen infection. Other trunk symptoms, such as the presence of resin outside the cankers, were also higher in pruned plots. These results should be taken into account for future management of Monterrey Pine plantations.

Key words: pitch canker; Cantabria; Spain; wound; *Pinus radiata*.

Resumen

Efecto de la poda en plantaciones de pino radiata afectadas por *Fusarium circinatum*

Fusarium circinatum Nirenberg and O'Donnell (1998) es el agente causante de la enfermedad del chancro resinoso del pino, que afecta a especies del género *Pinus* y provoca la aparición de chancros resinosos en el tronco y en ramas gruesas, además de puntisecado en la guía terminal. Esta enfermedad fue detectada recientemente en el norte de España asociada a plántulas de coníferas en vivero y a plantaciones de *Pinus radiata* D. Don. *Fusarium circinatum* suele requerir una herida en el árbol para poder infectarlo. Estas heridas pueden estar causadas por insectos o ser de origen antrópico, como las heridas mecánicas. Con la finalidad de conocer cómo las heridas producidas durante la poda afectan a la severidad de la enfermedad del chancro resinoso del pino, se estudiaron 50 parcelas de *P. radiata* (podadas y no podadas) distribuidas a lo largo de la provincia de Cantabria. En cada una de las parcelas fueron evaluados 25 árboles, en los que se estudiaron los síntomas más característicos de la enfermedad, como son puntisecado, presencia de chancros resinosos y deformación del tronco, relacionándolos con la presencia de poda. Se observó una



A photograph of a dense forest of tall pine trees. The trees are green with brown trunks, and their branches are covered in needles. The sky is overcast and grey. The text "Obrigado!" is overlaid on a dark blue banner at the bottom of the image.

Obrigado!

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