



National Institute for  
Agrarian and Veterinary  
Research



# ***Fusarium circinatum* in Portugal - monitoring, epidemiology, control**

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**PLURIFOR PROJECT - PITCH CANCKER OF PINE WORKSHOP**



**PORTUGUESE  
REPUBLIC**

AGRICULTURE, FORESTRY  
AND RURAL DEVELOPMENT

**Interreg  
Sudoe**  
European Regional Development Fund



# Portuguese forest

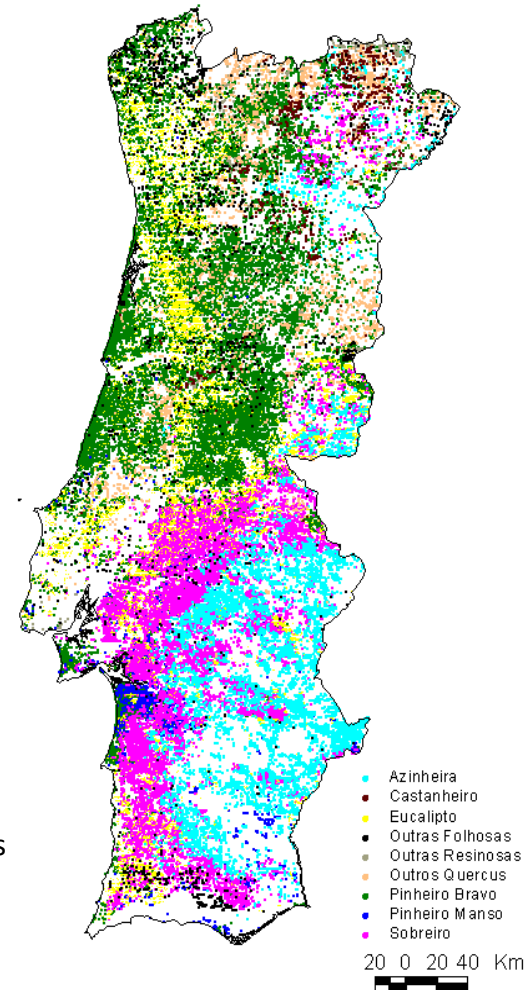
**Forest surface**  
**3.154.800 ha**  
**(36% of country area - 2010)**

**811.943 ha**  
**(26% of forest area)**

**736.775 ha**  
**(23% of forest area)**

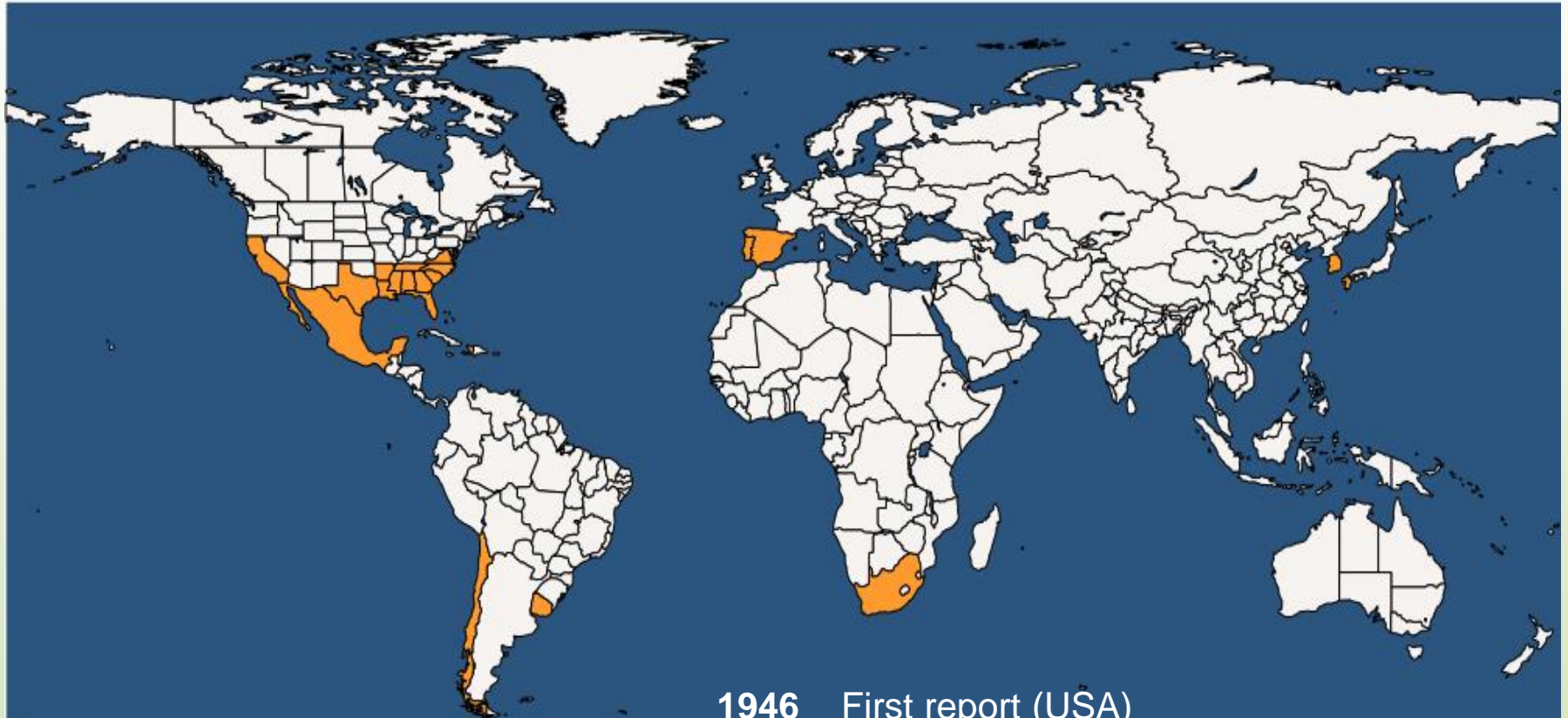
**714.445 ha**  
**(23% of forest area)**

- Holm oak
- Chestnut
- Eucalypt
- Cork oak
- Maritime Pine
- Stone pine
- other oaks
- other broadleaves
- other Conifers



# *Fusarium circinatum*

## Geographic distribution



**1946** First report (USA)

**2001** First report in Europe

*Gibberella circinata* (GIBBCI)

# *Fusarium circinatum*

## 2009 First report in Portugal



BRAGANÇA H., E. DIOGO, F. MONIZ, P. AMARO 2009. First Report of Pitch Canker on Pines Caused by *Fusarium circinatum* in Portugal. *Plant Disease* 93 (10): 1097.



***P. pinea***

***P. radiata***

***P. pinaster***

Experiments confirm pathogenicity for most important pine species in Portugal

# Portugal – *F. circinatum*

Action plan since 2010



(Forest authority): coordination & crisis management

In collaboration with the Phytosanitary authority (DGAV) and INIAV

The image shows the cover of the action plan document on the left and a vertical flowchart on the right. The document cover is orange and features the ICNF and POSF logos at the top. The title is 'PLANO DE AÇÃO PARA PROSPECÇÃO E ERRADICAÇÃO DO FUNGO GIBBERELLA CIRCINATA/FUSARUM CIRCINATUM CAUSADOR DO PRINHEIRO'. Below the title, it states 'Trabalhos de ação em prevenção, controlo e erradicação do agente causador da praga no território nacional em articulação com a Direção da Praga em Portugal.' At the bottom left, it says 'Janeiro, 2014'. At the bottom right, it says 'Associação Forestal Pinheiros e Pseudotsuga'. The flowchart on the right is a vertical line of five white circles connected by a green line, with each circle next to a green horizontal bar containing a white text label: 'Survey', 'Control and eradication', 'Prevention', 'Inspection', and 'Entities'.

National law applies: Portaria n.º 294/2013, de 27 de setembro. D.R. n.º 187, Série I. It establishes extraordinary phytosanitary protection measures to prevent the introduction and dissemination of the fungus

# Portugal – *F. circinatum* action plan

## Eradication, control and contingency



- When the pitch canker fungus is detected, a demarcated area is established. It is composed of an infested zone and a buffer zone. The buffer zones measures at least 1 km wide around the infested zone.
- The main control measure is the destruction of seeds, symptomatic seedlings, plants, trees, within the infested places.
- For the rest of the host plant species without symptoms within the demarcated area, a two-year quarantine is applied. During this period their circulations is forbidden and they are intensively monitored and sampled.

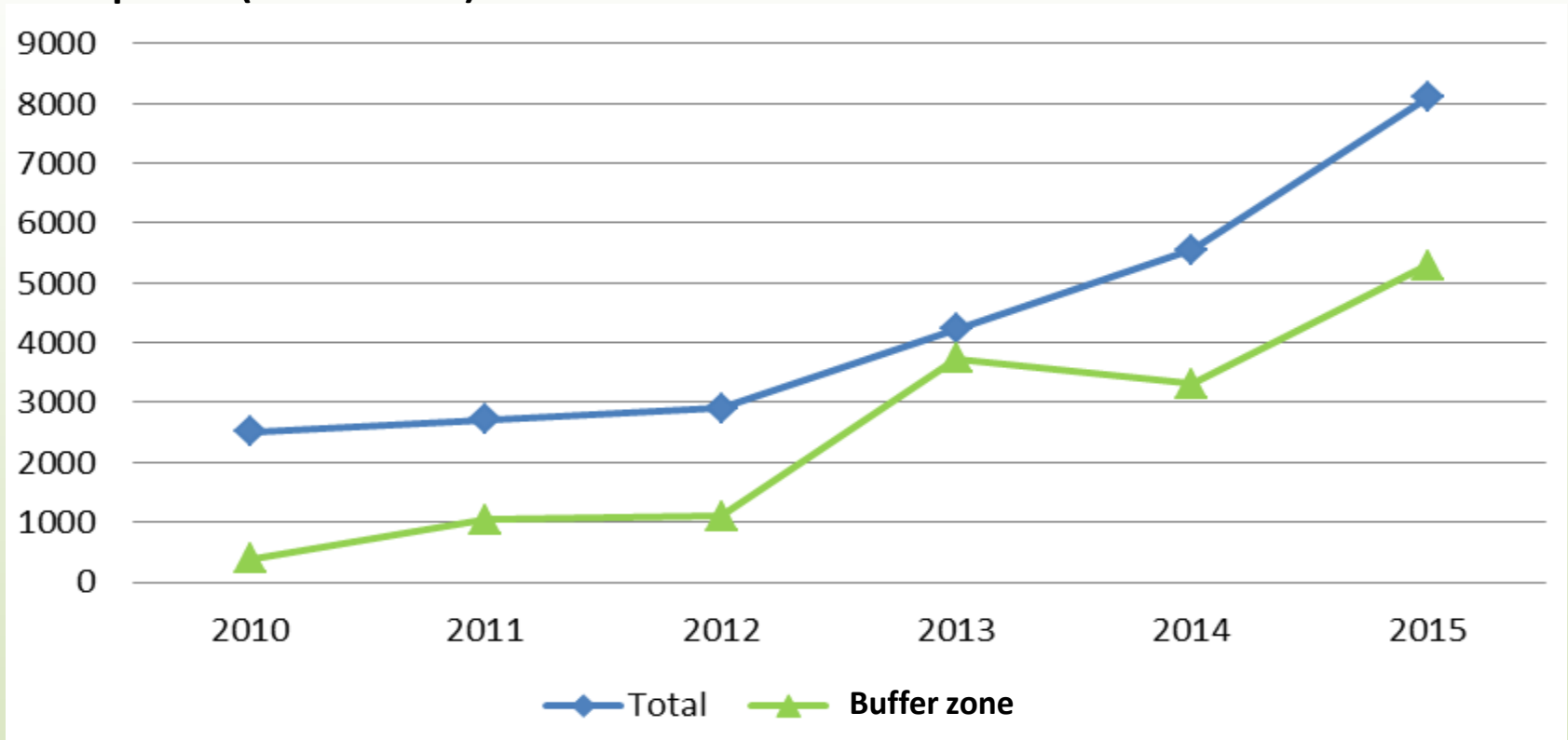


# *F. circinatum*

## Pine forest survey



Grid points (2 km x2 km) between 2010 and 2015



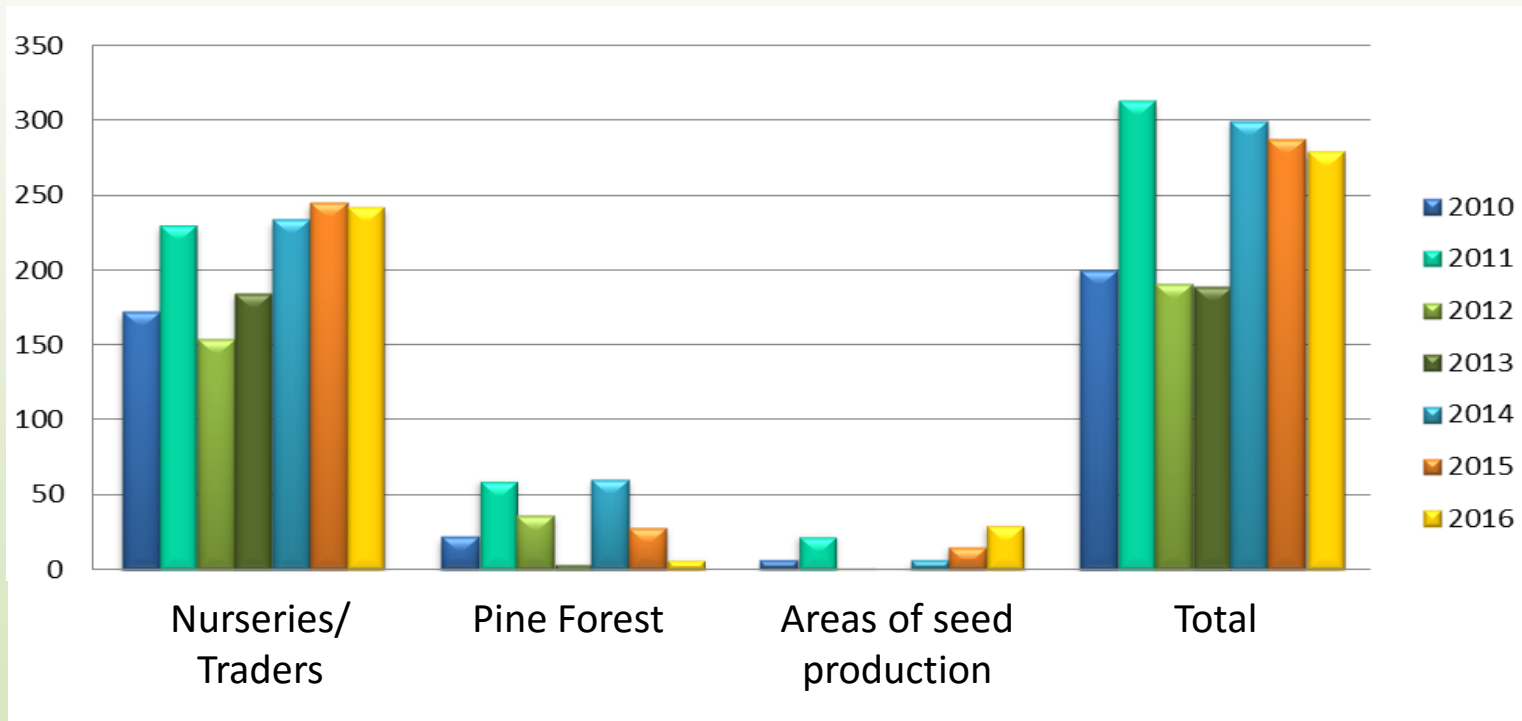
*F. circinatum* surveyed in defined high risk areas - (ICNF data)

# *F. circinatum*

## Global survey



### Collected samples 2010-2016

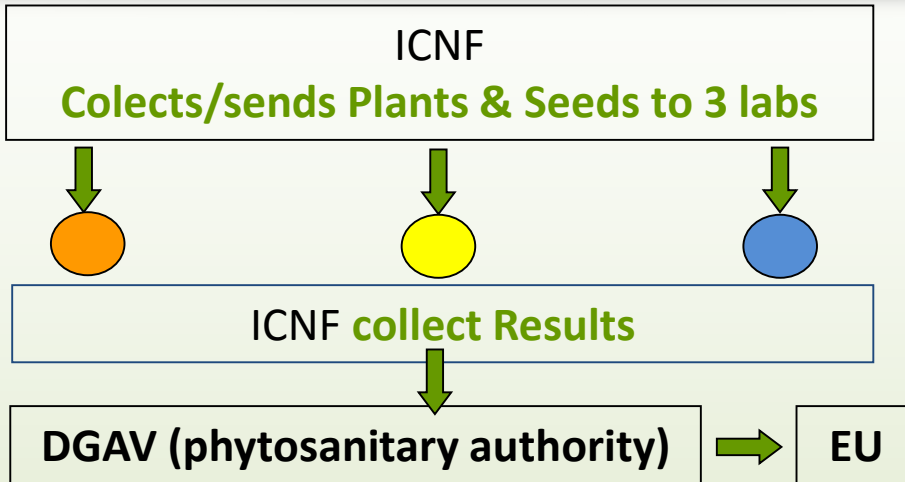


(ICNF data)



# Portugal – *F. circinatum* action plan

## Survey and detection



INIAV (Oeiras)



# Laboratory procedures

## Forest plants



Branches/Trunks - Bark removed to locate necrotic wood



# Laboratory procedures

## Seedlings



Each sample: 60 seedlings



- 2 pieces per seedling
- surface-sterilized in a 1.5% solution of sodium hypochlorite, and rinsed in sterile distilled water



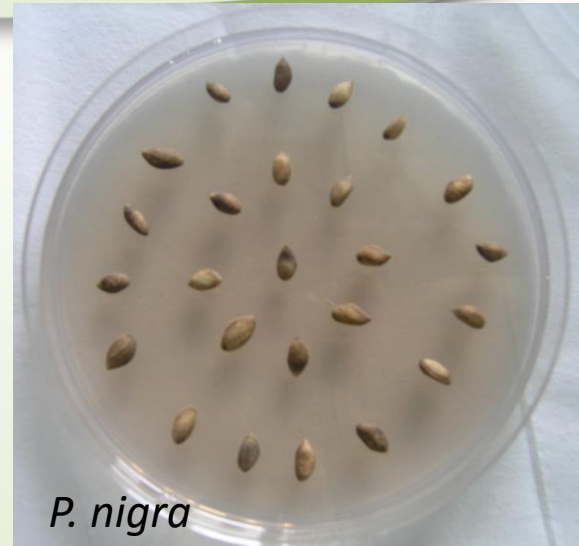
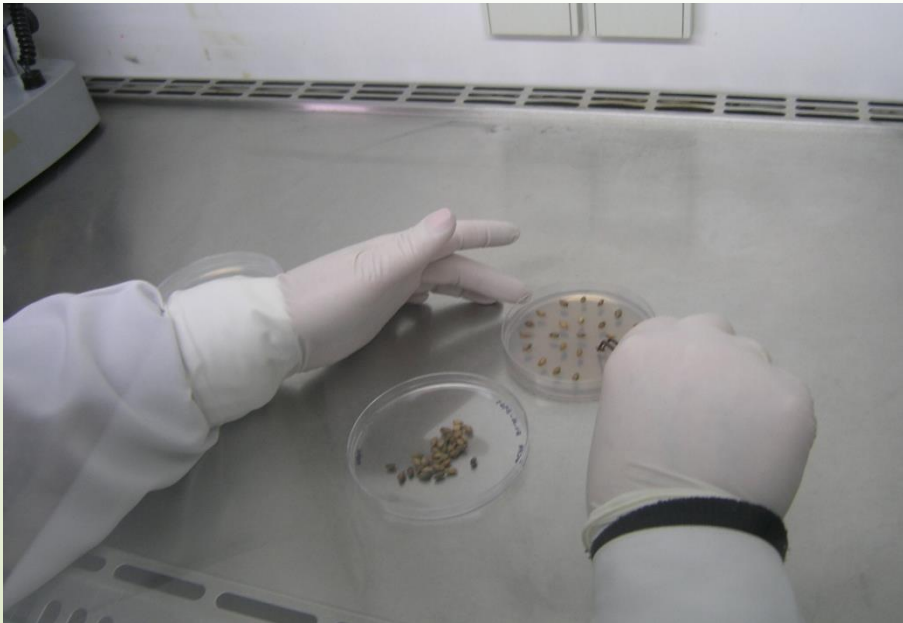
(4 seedlings/90 mm PDAs plate)

# Laboratory procedures

## Seeds by (DCPA) Dichloran Chloramphenicol Peptone Agar medium



Each sample 400 seeds

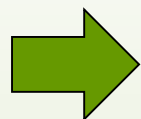


- Plated without surface disinfection
- Incubation at 25 °C or room temperature
- First observation between 4 to 7 days and up to three weeks

# Morphological Identification by Spezieller-Nährstoffarmer Agar (SNA) medium



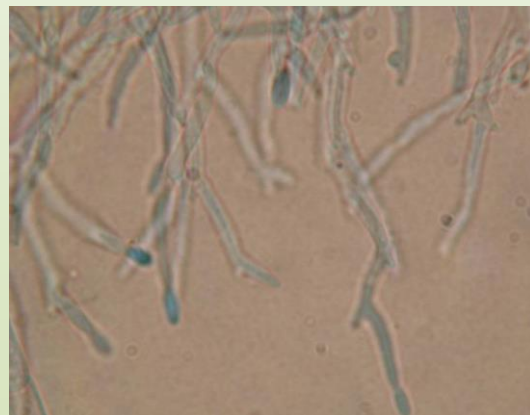
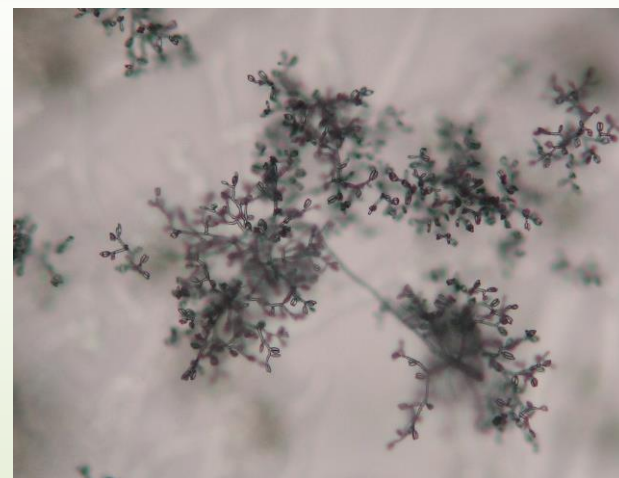
*Fusarium* colonies subcultured to PDA then SNA



SNA



SNA Cultures-  
Examination  
after 10 days:



- macro and microconidia
- coiled sterile hyphae
- polyphialidic conidiophores
- absence of clamidospores

Positives: confirmation by IGS rDNA region conventional or RT PCR

# Laboratory procedures

## Seeds by Biological enrichment & Real-time PCR

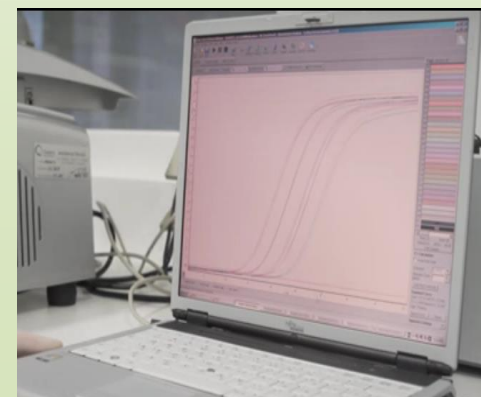


- **Biological enrichment:** seeds incubated at  $22 \pm 3C$  for 72 h (PDB Difco)
- Crushing by a **Micotron MB 550**
- DNA extraction (Kit)
- **Real-time PCR** (IGS rDNA)



**Ioos *et al.* (2009)**

**EPPO - Protocol Bulletin**  
**39(3):298–309** (Appendix 6)



# *F. circinatum* - Current situation



## *F. circinatum* – detections



Year	Nurseries/traders with positive results	Stands with positive results	Positive samples	Destroid plants
2009	7	-	13	800.000
2010	4	-	10	214.000
2011	3	-	4	111.000
2012	0	-	0	0
2013	2	-	2	52.000
2014	4	-	6	170.000
2015	2	-	3*	79.520
2016	2	2	5	132.508 (1300 trees in forest stands)
Total	24	2	43	1.559.028

- **2015 first report in seeds** - \*two positive seed samples of *P. radiata* were detected;
- **2016 first report in field** - ICNF tracked the origin of the positive seeds and the *P. radiata* stand of origin situated in the north of country were more intensively surveyed;
- **2016 second report in field** – positive results in young plantation of *F. radiata* were found in the centre of the country.



# 2016 – young *P. radiata* plantation

In the centre of the country



# *Fusarium circinatum* in Portugal

## Needs (owners/forest services)



### From scientists/from government :

- To understand the pathways of dispersion to implement good management procedures;
- Methods for **early detection**;
- **Risk maps**;
- **Rehabilitation plan** for affected forest areas;
- **Compensatory measures** should be implemented for laboratory analyses and vegetal material destruction. (Owners support all at their own expense).



F. Quirino-INIAV

# *Fusarium circinatum*

On going/Further research



- Characterization of Portuguese isolates/epidemiology (**INIAV/Valencia University colaboration**);
- Improved methods for detection (**PLURIFOR & COST Action FP1406 – PINESTRENGTH**);
- Improved good methods for disinfection of seeds, containers and subtracts (new project approved: **+PrevCRP**)
- Screening for susceptibility of the two most important pines in Portugal, *P. pinaster* and *Pinus pinea*, based on the knowledge already gathered in a previous breeding program;
- Evaluation of the potential of insects as passive or active vectors of the fungus in Portuguese forest and nurseries;
- Development of control measures by testing chemicals and/or natural products/organisms (**INIAV Master thesis & +PrevCRP**).





# Thanks for your attention

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