



National Institute for
Agrarian and Veterinary
Research



**PLURIFOR PROJECT
PITCH CANKER OF PINE WORKSHOP:**

TOOLS FOR FAST DISEASE DIAGNOSTIC

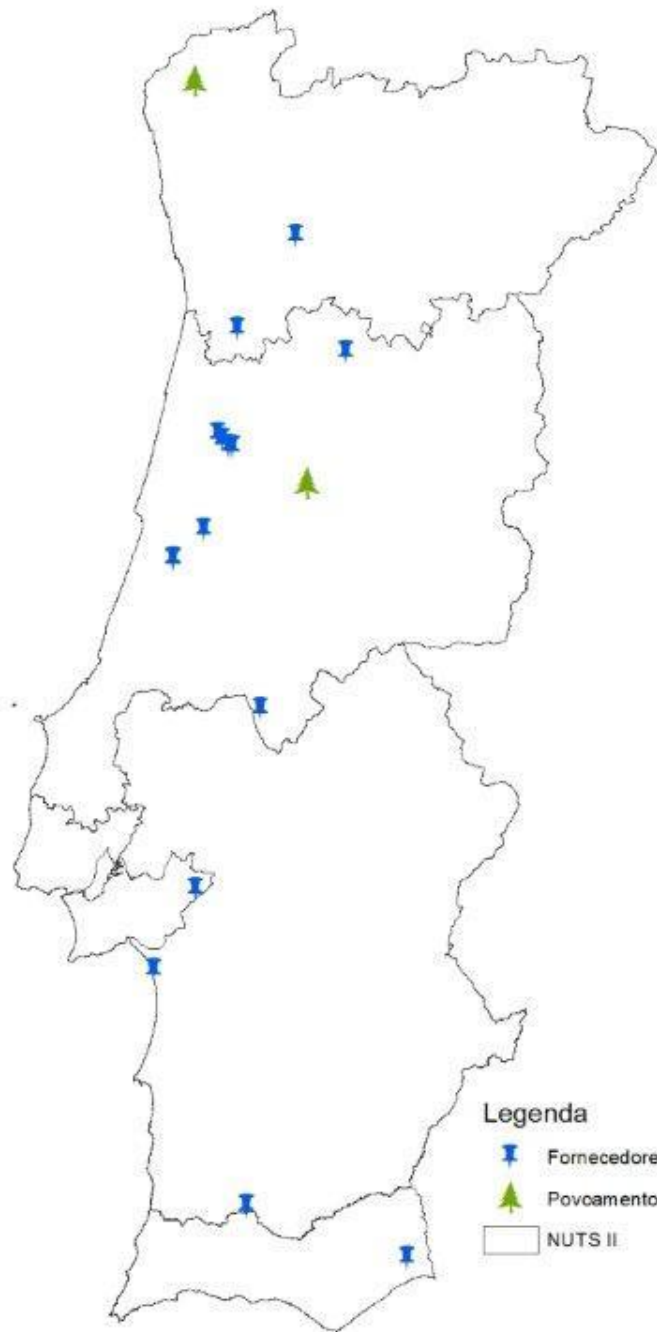
Spore Traps Combined With Real Time PCR



**PORTUGUESE
REPUBLIC**

AGRICULTURE, FORESTRY
AND RURAL DEVELOPMENT

**Helena Bragança
Aveiro, 3rd october 2017**



12



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



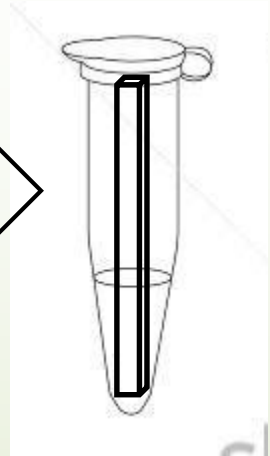
Rotor rod spore traps



Sticks with double side adhesive tape



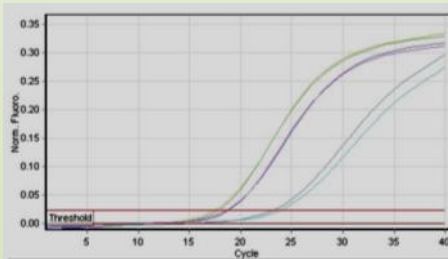
DNA extraction from traps



Real time PCR



Results



Spore Traps



Spore Traps

collect spores in 2 supports



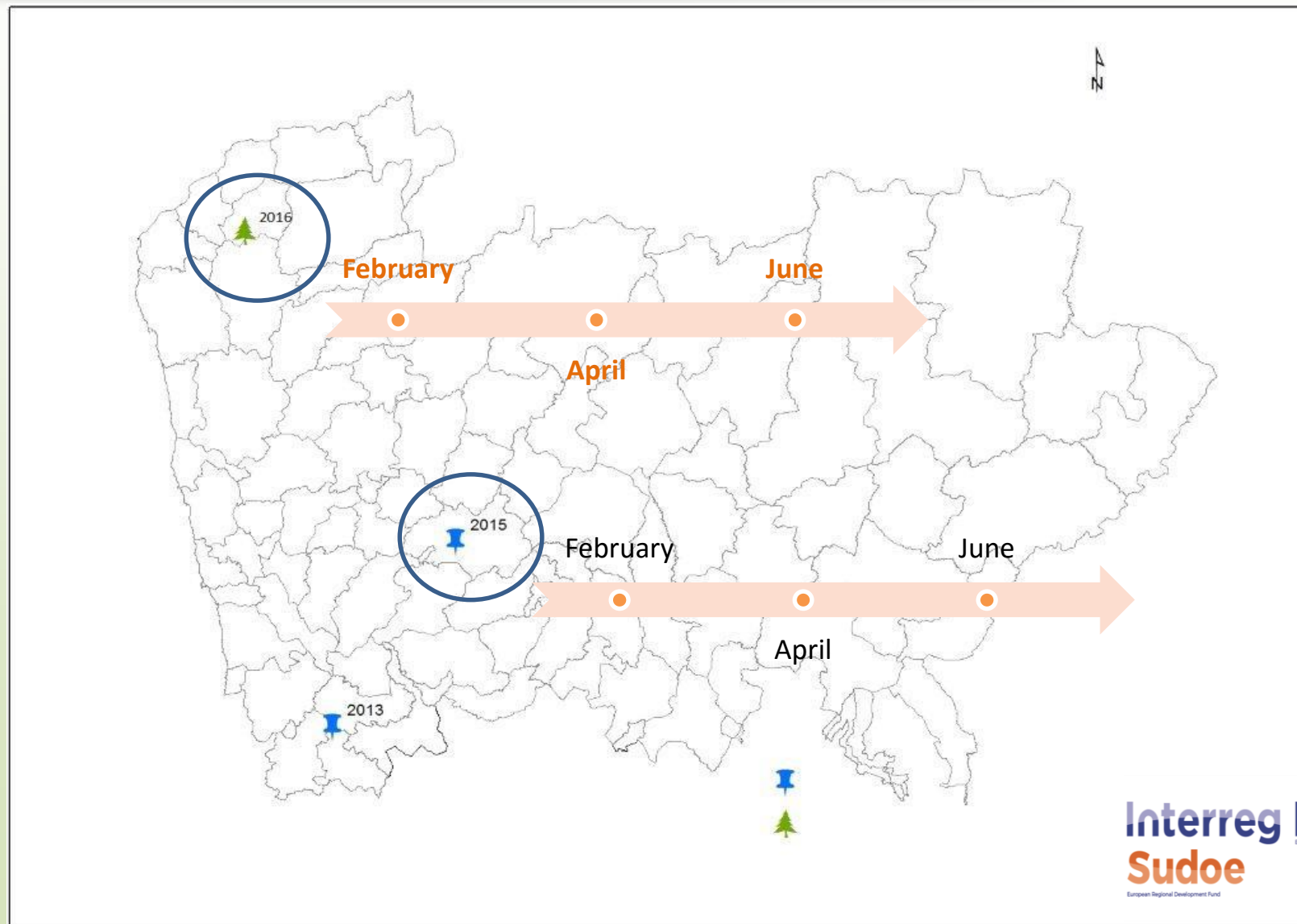
Spore Traps

continuous rotation movement



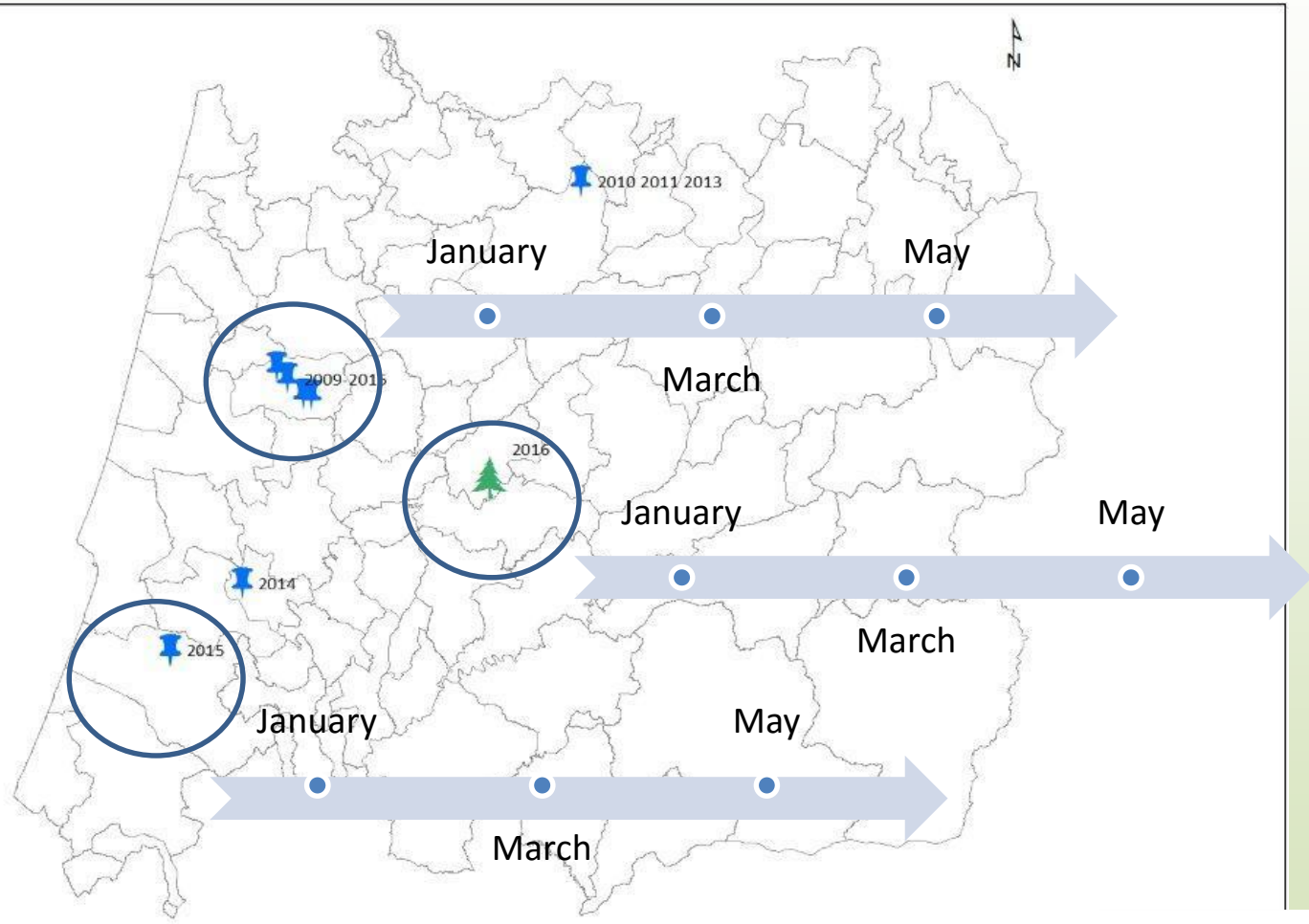
Field work planning ICNF/INIAV

3 spore traps during one week in each site



Field work planning ICNF/INIAV

2 spore traps during one week in each site



Implementation and optimization of molecular methods for the detection/identification of *F. circinatum*




Eugénia de Andrade
3rd october 2017

Available methods


-  Conventional PCR

-  Real-time PCR and Interpretation of results

 -  using hydrolysis probes

 -  loos *et al.*, 2009

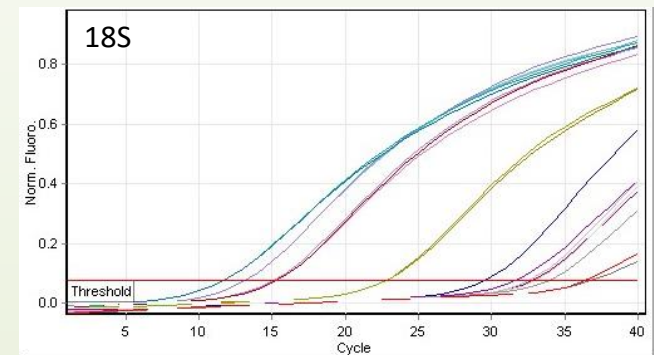
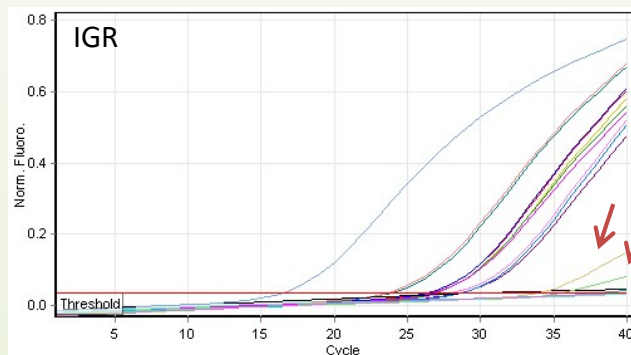
 -  Lamarche *et al.*, 2015

 -  SybrGreen

 -  Schweigkofler *et al.*, 2007

 -  Dreaden *et al.*, 2012

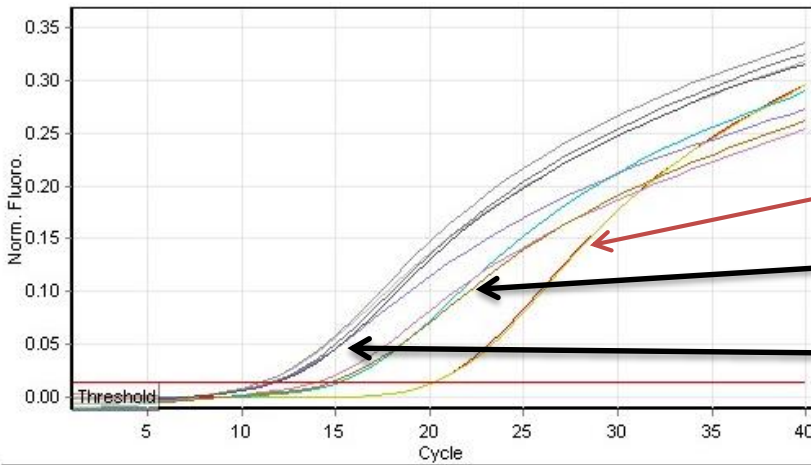
- Target: Intergenic Spacer Region (149 bp)
- Primers: FCIR-F and FCIR-R/Probe: FCIR-P
- Nothing is mentioned concerning
 - Sensitivity/LOD
 - Specificity
- In use since 2010
- In-house pre-validation
- Cut-off value – Ct=34.69





Specificity – cross-reaction with *F. subglutinans*

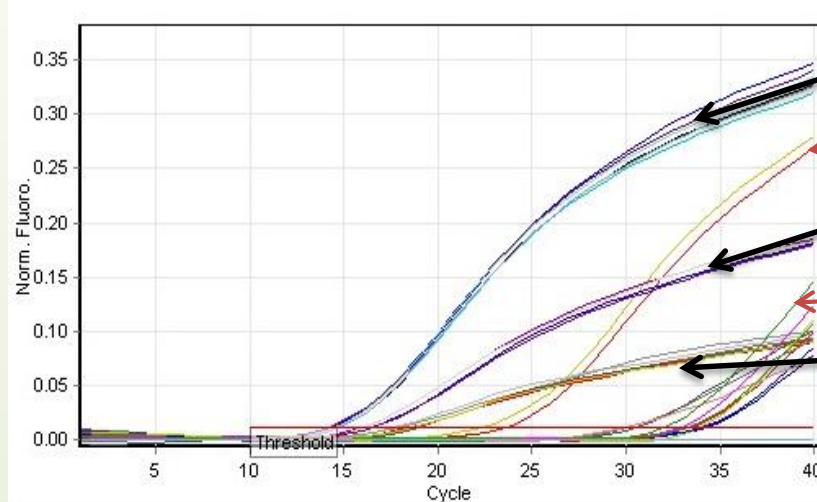
- no cross-reaction with *F. oxysporum* and *F. verticilioides*



Controllo positivo

F. subglutinans

F. circinatum



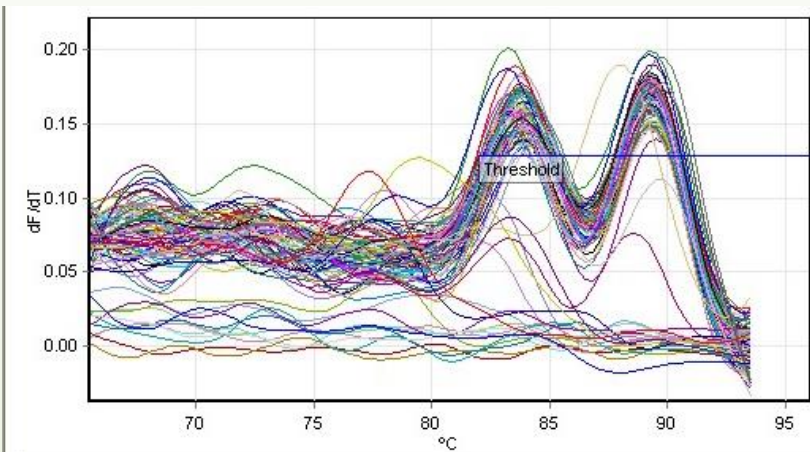
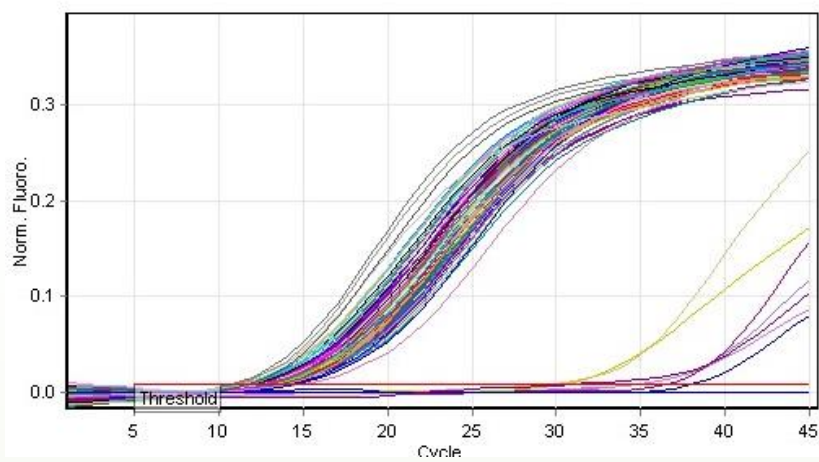
F. circinatum?

Controllo positivo

F. circinatum?

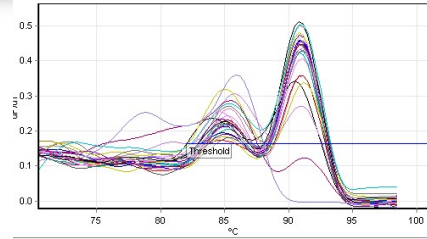
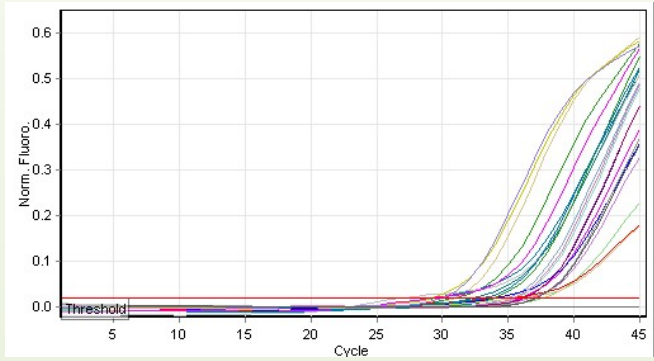
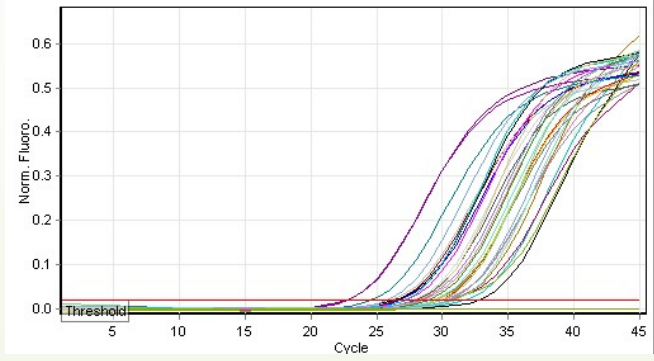
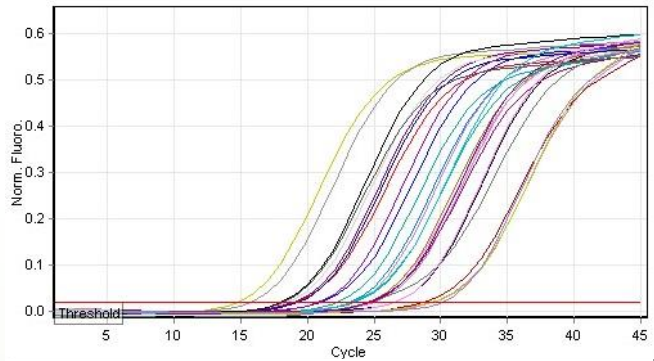
Negativo

F. circinatum?

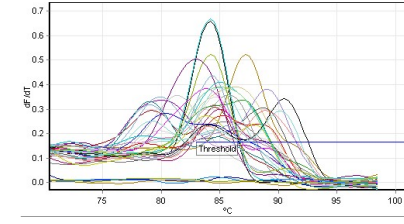


Name	Genotype	Peak 1	Peak 2	F41		83.5	89.3
F1		83.8	89.3	F41		83.5	89.3
F2		84.0	89.5	F42		83.8	89.3
F2		84.2	89.3	F42		83.8	89.3
F3		84.0	89.3	F51		88.0	
F3		84.2	89.3	F51			
F4		83.7	89.2	F52			
F4		83.8	89.2	F52			
F5		83.2	89.3	F53			
F5		83.2	89.2	F53			
F6		83.3	89.3	F54			
F6		83.3	89.3	F54			
F7		83.7	89.3	F61			
				F61			

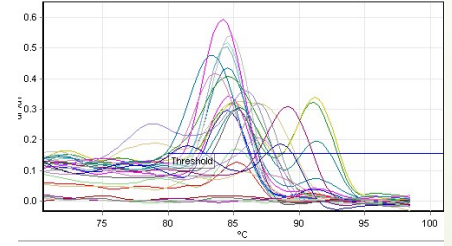
C+ F circ		84.0	89.2
C+ F circ		83.8	89.2
C-amb			
C-amb			



No.	Colour	Name	Genotype	Peak 1	Peak 2
λ1	Red	F1		84.7	91.0
λ2	Yellow	F1		85.0	91.0
λ3	Blue	F2		85.0	91.0
λ4	Purple	F2		85.0	91.0
λ5	Pink	F3		85.2	91.0
λ6	Cyan	F3		85.2	91.0
36	Purple	F11		85.0	91.0
311	Green	F14		85.3	91.0
37	Black	F26		84.0	90.5
λ2	Purple	F29		85.7	91.0
λ3	Pink	F30		85.3	91.0



No.	Colour	Name	Genotype	Peak 1	Peak 2
A11	Black	F6		84.2	
A12	Cyan	F6		84.2	
B7	Pink	F12		84.5	
B8	Red	F12		84.5	
B9	Yellow	F13		87.2	
C1	Blue	F15			
C2	Purple	F15			
C3	Pink	F16	85.0		
C4	Grey	F16	84.5		
C5	Grey	F17			
C6	Grey	F17	85.8		
C7	Grey	F18	85.7		
C8	Grey	F18	88.7		
C9	Red	F19	85.2	88.5	
C10	Yellow	F19	83.8	88.0	
C11	Blue	F20	83.7		



No.	Colour	Name	Genotype	Peak 1	Peak 2
λ9	Green	F5		84.8	91.0
λ10	Pink	F5		84.7	
31	Yellow	F7		85.5	
32	Green	F7		85.2	
33	Cyan	F8		84.5	
34	Blue	F8		84.5	
λ8	Blue	F32		84.5	
λ1	Grey	F35		84.7	
λ5	Red	F37			
λ6	Yellow	F37		85.0	91.2
λ7	Blue	F38		84.5	
λ11	Green	F40		85.3	91.3

Spore Traps – collect spores in 2 supports



F. circinatum team



Mycology Lab

Helena Bragança

Eugénio Diogo

Ana Lança

Joana Henriques (no photo 😊)

Isabel Lourenço

Florinda Medeiros (no photo 😊)

Molecular Biology Lab

Eugénia Andrade

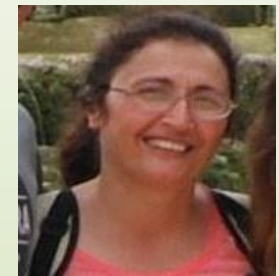
Clara Fernandes (no photo 😊)



Partners:

Dina Ribeiro

José M. Rodrigues (no photo 😊)





Thanks for your attention

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