

PLURIFOR PROJECT - PITCH CANKER OF PINE

TOOLS FOR FAST DISEASE DIAGNOSTIC

Spore Traps Combined With Real Time PCR (qPCR)

Bragança H., Andrade E. Diogo E. and Rodrigues I.
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TOOLS FOR FAST DISEASE DIAGNOSTIC

objectives:

early detection in field

(High priority to sites with recent positive detections)

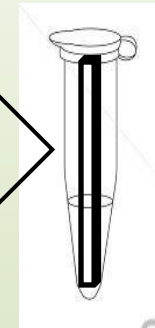
Rotor rod spor traps



Sticks with double side adhesive tape



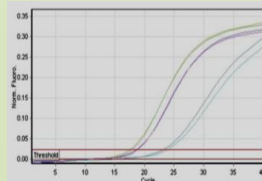
DNA extraction from
traps



Real time PCR

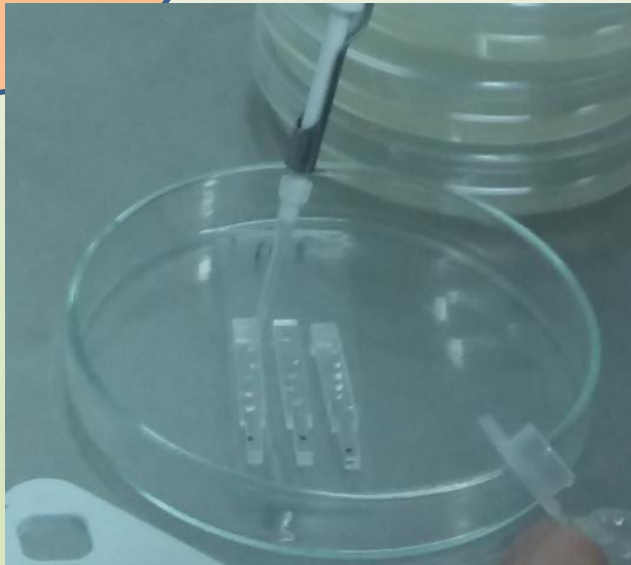


Results



Methods

Method pre
in-house
validation



Extractability and sensitivity

- ✓ 5 independent serial dilutions of spores of *F. circinatum* were prepared
- ✓ Drops are placed on the Sticks with double side adhesive tape
- ✓ Dynamic range: 10^7 to 10^3 spores/stick
- ✓ DNA extraction and qPCR

Methods

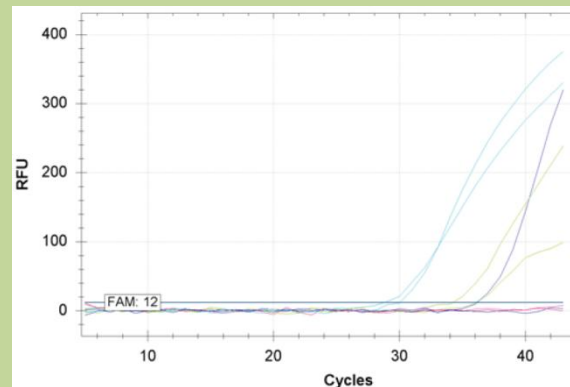
DNA extraction

Method
optimization

Extractability and sensitivity

✓ The concentration 10^7 was used to compare the extraction methods

Method	Ct value
Chandelier <i>et al.</i> (2014)	28.25
DNeasy Blood & Tissue	35.38
NucleoSpin Plant – Support protocol for fungi”	31.51



Methods

Lab work - Sticks preparation



Rotor rod spore traps



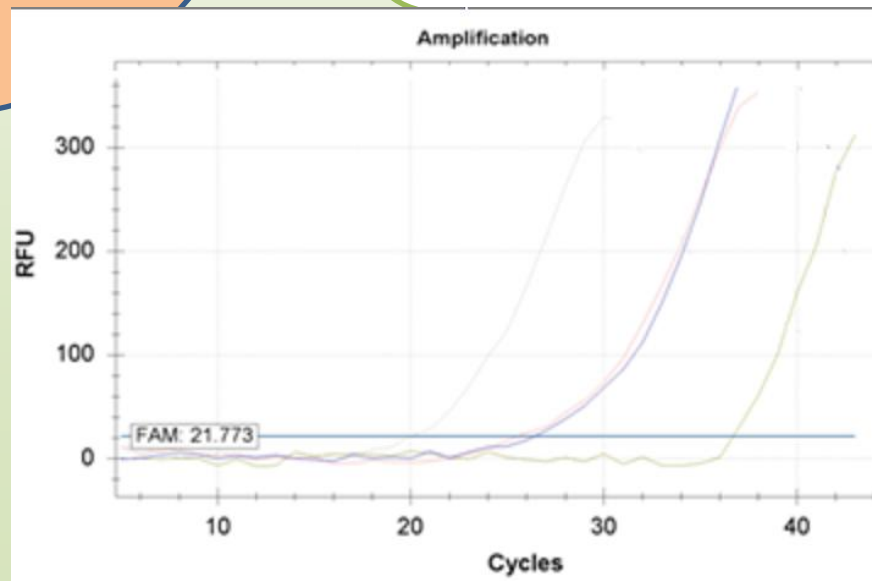
Field work - spore traps in field

Results

Analysis of the sticks positioned in field : sites with recent positive detections

Preliminary results

- ✓ Positive detections on the expected sites
- ✓ Spores concentration varying with the season
 - March - Ct = 21.25/21.94
 - June - Ct = 26.41/36.70



F. circinatum Plurifor team



Mycology Lab

Helena Bragança

Eugénio Diogo

Ana Lança

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

Instituto Nacional de Investigação Agrária e Veterinária, I.P.

Av. da República, Quinta do Marquês, 2780-157 Oeiras, Portugal

Tel : (+ 351) 21 440 3500 | Fax : (+ 351) 21 440 3666

www.inia.v.pt