

# A new Real-time PCR-method for the quantification of the Pine Pitch Canker fungus *Fusarium circinatum*.



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typical brown flagging of Pine Pitch Canker

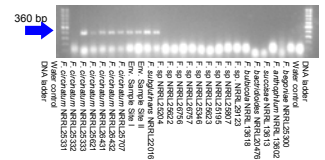
## INTRODUCTION

- Monterey Pine (*Pinus radiata*) - native to Coastal California and Northern Mexico- is the most widely planted tree for timber production worldwide and plays an important role in the forestry of AUS, NZ, ZA and CL, among other countries. The major threat for commercial Monterey Pine production is Pine Pitch Canker, caused by *Fusarium circinatum* (syn. *F. subglutinans* f.sp. *pini*). Symptoms include brown flagging of twigs, stem cankers associated with a strong ooze, dieback and finally (but not necessarily) tree death. Spreading of the disease is probably enhanced by airborne spores, insect vectors and the movement of infected plant material. Detection and quantification of the pathogen before the outbreak of symptoms could help to limit the impact of the disease.

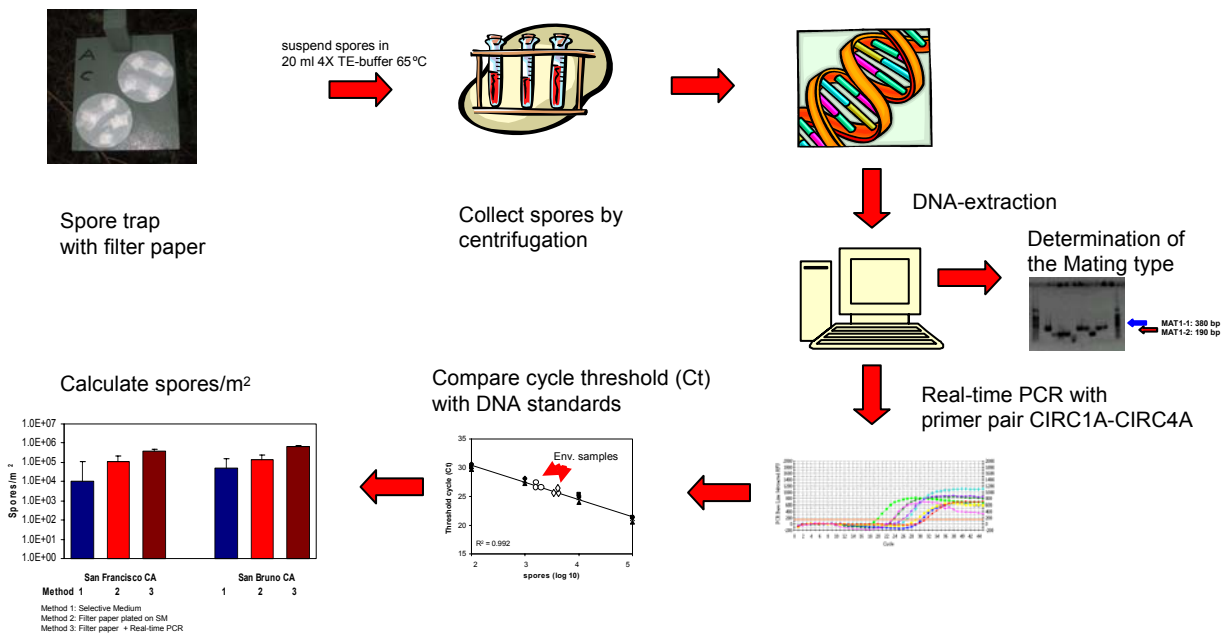
## OBJECTIVE

- The aims of our study were (i) to establish a fast and reliable test for the presence of *F. circinatum* in the absence of disease symptoms (ii) to test the suitability of the method for quantification of spore numbers from environmental samples and (iii) to determine the mating type of the fungal isolates to get an idea on the potential for sexual recombination.

Specific detection of *F. circinatum* with primer pair CIRC1A-CIRC4A (IGS-region)



## WORKING SCHEME



## RESULTS

We developed a specific primer pair (CIRC1A-CIRC4A) for the detection of *F. circinatum*, which amplifies a 360 bp fragment in the IGS-region. *F. circinatum* can be distinguished from closely related species in the *Gibberella fujikuroi*-complex. 10<sup>1</sup> pg of fungal DNA can be detected with a single PCR reaction.

We used a new trapping approach with filter papers to collect aerial spores in areas affected by Pine Pitch Canker. Spores were suspended in hot TE-buffer, DNA was extracted and Real-time PCR used to calculate the starting copy numbers of target sequences (= fungal spores) present in each reaction by comparing the threshold cycle (Ct) of unknown spore samples to the Ct-values of DNA-standards. First results indicate, that the method is both more selective and sensitive than using traditional spore traps with Selective Medium. In addition, we determined the mating type of the collected spores. So far, in California only MAT1-1 was found.

**This method is currently used for long-term studies in both native and planted Monterey Pine stands to determine the impact of climatic and bio-geographical factors on the spread of *F. circinatum*.**

### ACKNOWLEDGMENTS

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