Molecular methods reveal aerial spread of *Fusarium circinatum*, causal agent of Pine Pitch Canker, in Coastal California

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DOMINICAN UNIVERSITY of CALIFORNIA

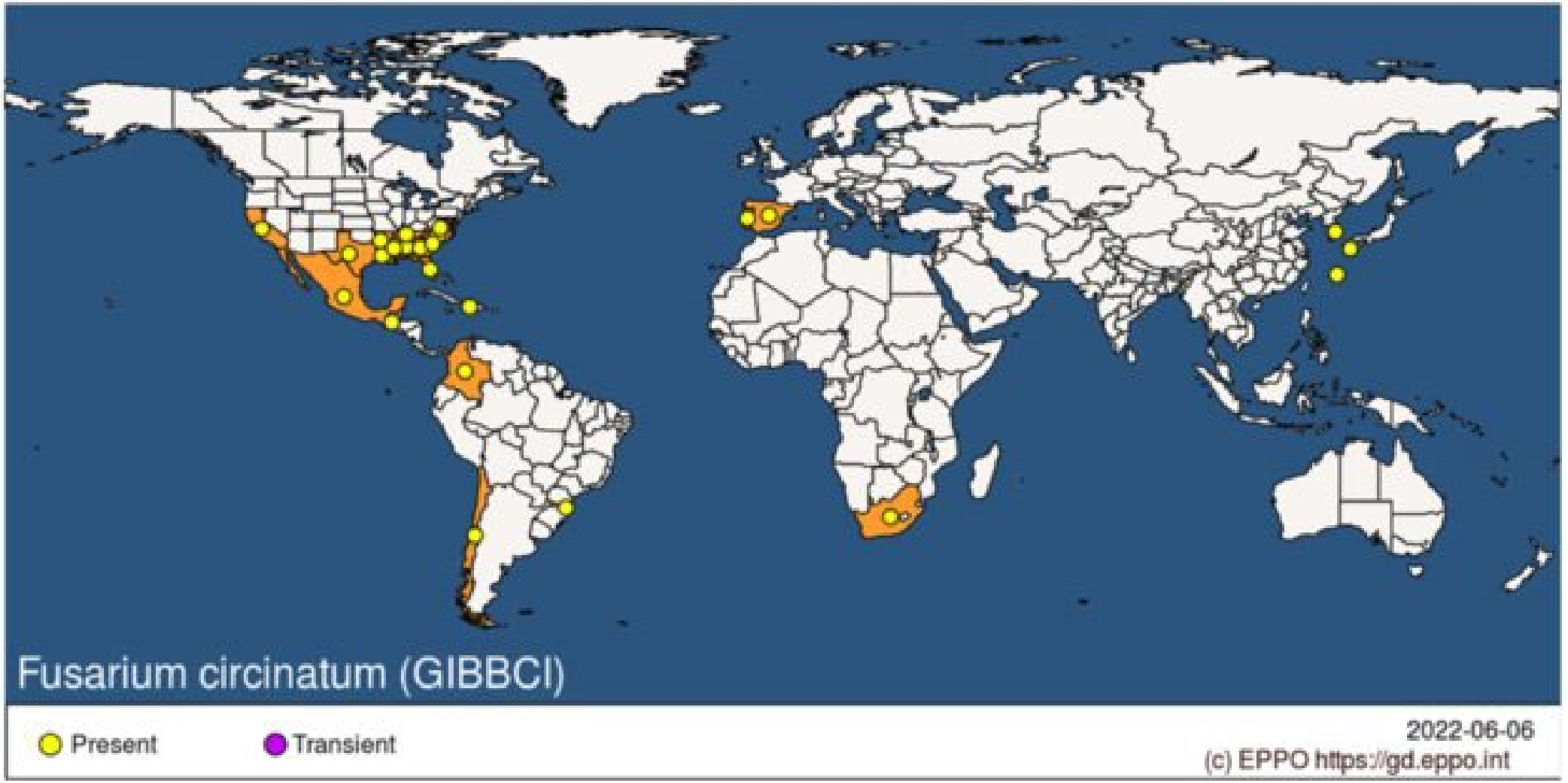
Monterey Pine – a worldwide success story?

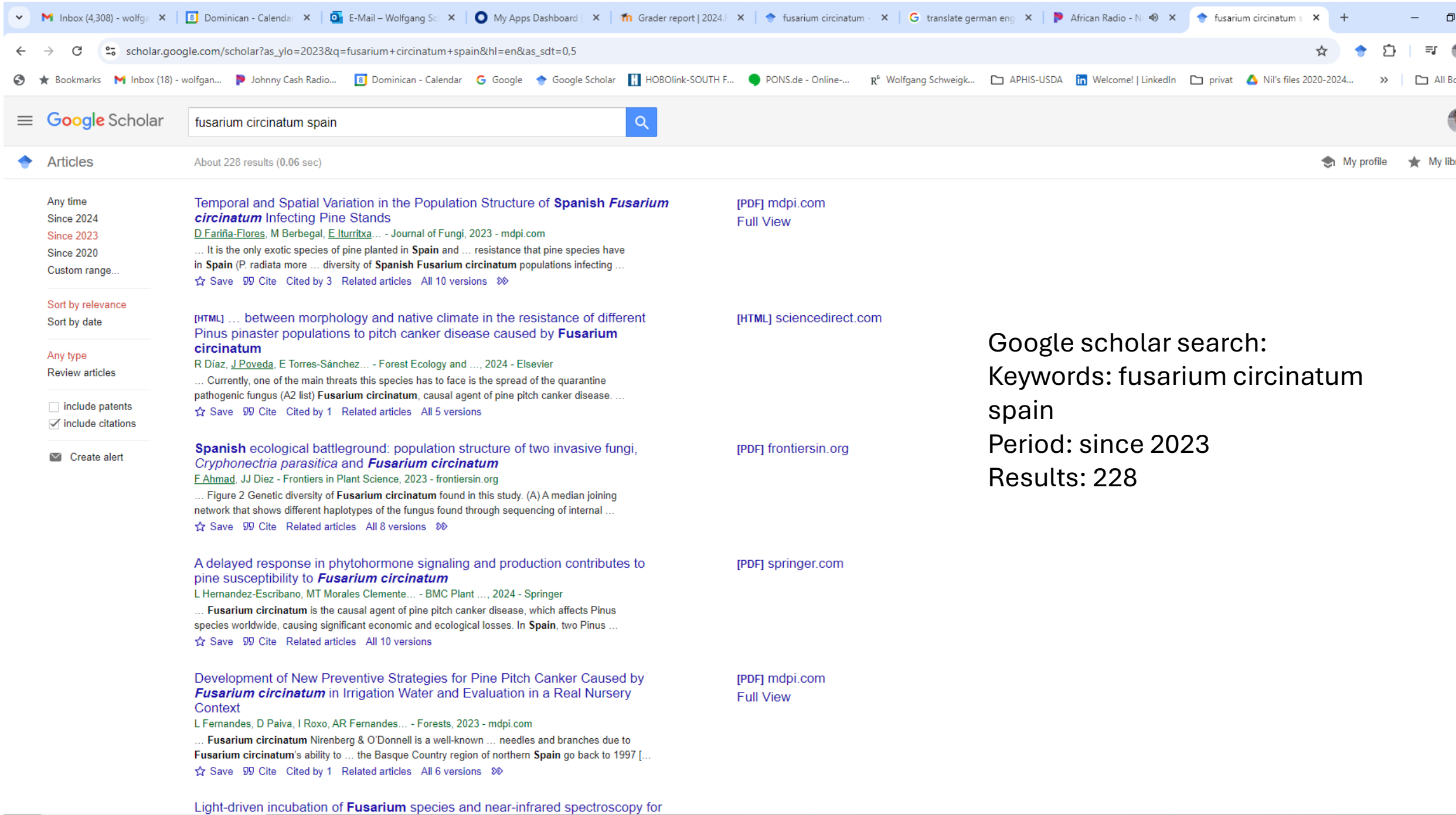
- It occupies some 4 million ha as fast-growing plantations, an area around 500 times its natural extent. Of this area over 90% is located in the Southern Hemisphere.
- The main growing countries are Chile and New Zealand, followed by Australia, then Spain and then South Africa. (CABI)
- In Europe, *P. radiata* is mainly planted in Spain

‘Super’ trees against global warming

https://neiker.eus/en/news/super-trees-against-global-warming/ 2022

Current distribution of *Fusarium circinatum* showing as of May 2022 (https://gd.eppo.int/taxon/GIBBCI)





Google scholar search:
Keywords: fusarium circinatum spain
Period: since 2023
Results: 228

Global spread of *F. circinatum*

This fungus is believed to have originated in Mexico.

It spread to the eastern United States in 1946 and by 1986 had reached the western United States (California).

It was first recorded in Japan in the 1980s, in South Africa in 1990, in Chile and Spain in the mid-1990s and in Italy in 2007.

The fungus was often isolated in nurseries: is international plant trade responsible for long distance spread?

How does it spread short- to mid-range? Insect vectors, airborne inoculum…

F. circinatum: air-borne spores

Two different kinds of asexual spores:

macroconidia are 3-septate, with slightly curved walls
microconidia are single-celled, ovoid.

Ascospores (=sexual spores) are maybe produced in rare cases, but little is known about their transmission.

Esporas de *Fusarium circinatum*.

Microconidia

Ascospores

What was the purpose of our project?

Matteo Garbelotto,
UC Berkeley

- Develop a selective and sensitive molecular tool for the detection of airborne inoculum of n *F.circinatum*
- Compare it with traditional spore trapping approaches
- Use the test for environmental sampling in Coastal California

Specific Primers for *F. circinatum*

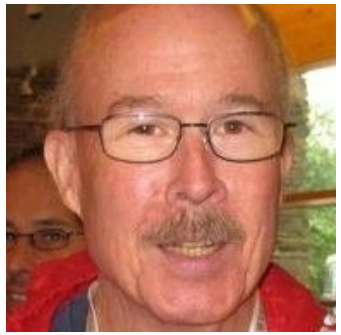
IGS: intergenic spacer region

Ribosomal gene cluster

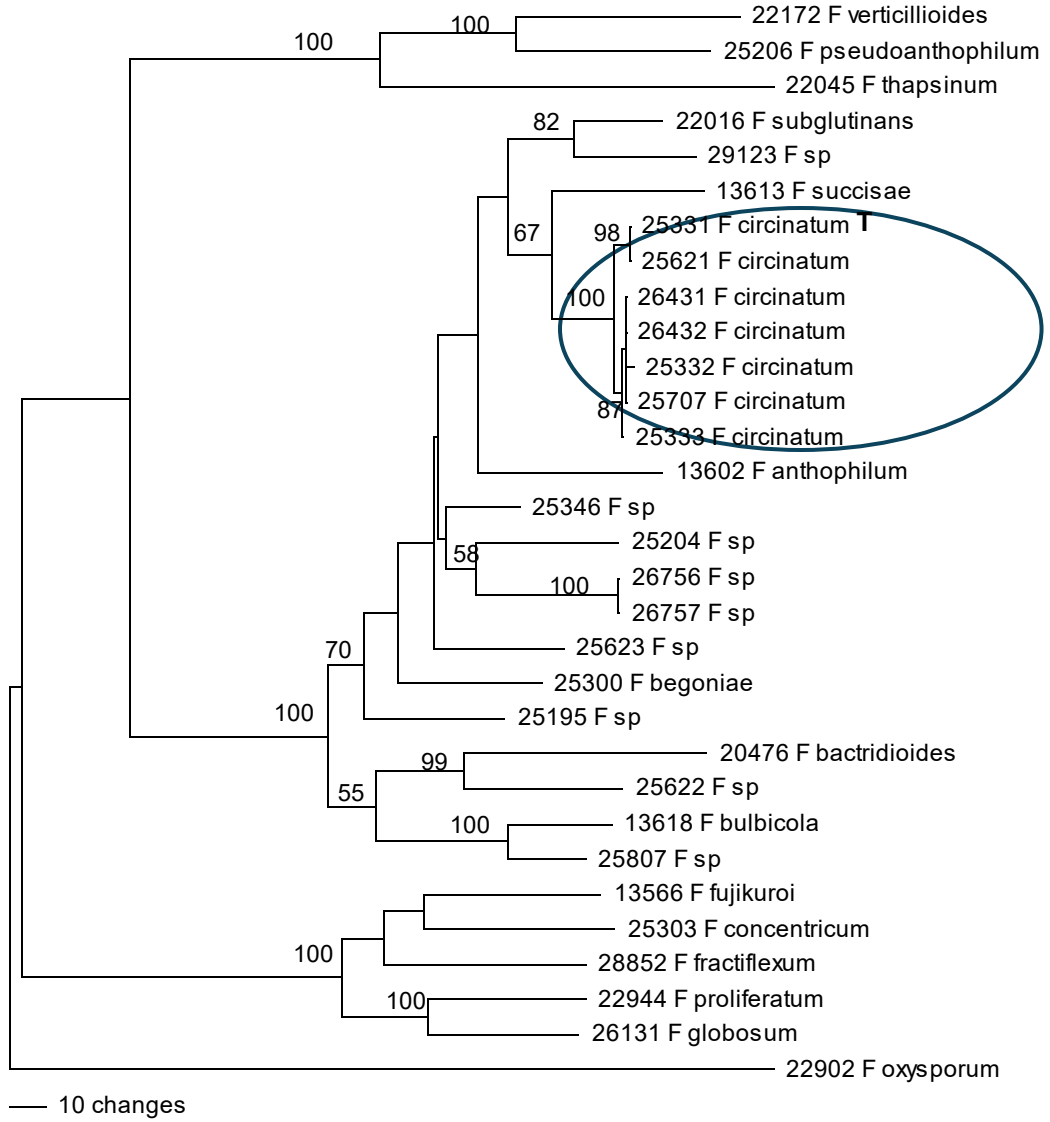
Non-coding

Multi-copy sequence: ca. 100 copies/genome

Parsimony analyses of the *Gibberella* (*Fusarium*) *fujikuroi*-complex based on 2593 bp in the Intergenic Spacer (IGS) region



Kerry O'Donnell
USDA, Peoria, IL



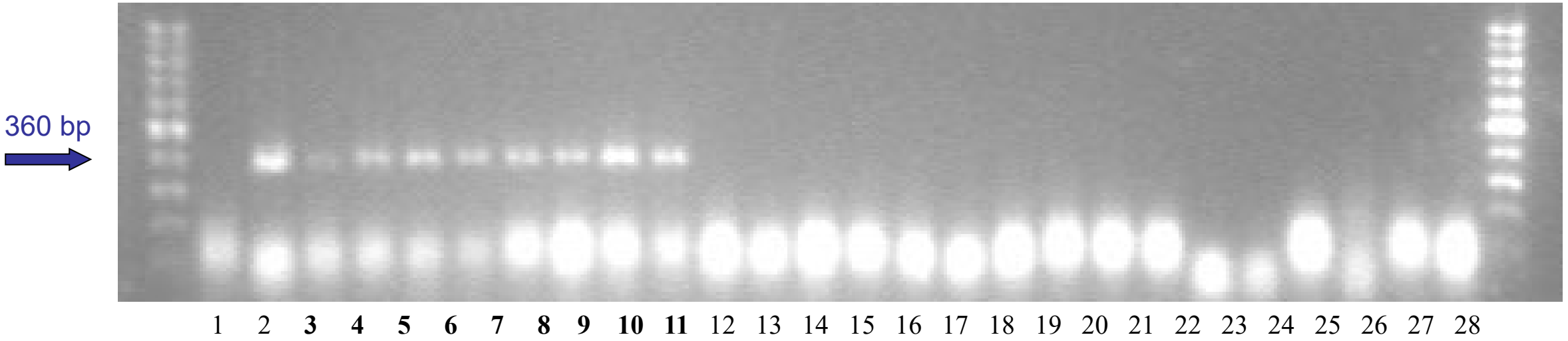
Specific primer pair CIRC1A-CIRC4A (IGS region)

CIRC1A **CTTGGCTCGAGAAGGG**
CIRC4A **ACCTACCCTACACCTCTCACT**

TGCCAGAACGCGGTGATACCACCCGCACGTATAGACGGACAAGAATAGGCTTCGGCTTAGTGTCTTAGCAGGCGATTCTTCCA
CGGCGCTCGAAGCGCGTCGTGGTATTTCGCGTATTGTAATTTCAACACGAGCGGGGTCAAATCCTTTGCAGACGACTTAGCTGT
GCGAAACGGTCCTGTAAGCAGTAGAGTAGCCTTGTTGTTACGATCTGCTGAGGGTAAGCCGTCCTTCGCCTCGATTTCCCCAAT
GGGTTCTCCGGATTTCTGGAGACTTGTAGGGGTTGTGGGCTTTTTTCGATGTGTCGTCTCTGGACGGGCGGTGCAGGGTAGTC
GAGTTGGACTTGGTGGAATTTCATTCCGTCGAGTCTGGCGGGACTTTGTGCGGCTGTGTGCTGGACGGTGCAGGGTAGGCTGC
TTGGTCTTGGTCGATTTGAGGATCGATTCGAGGGCTGGGCGCTGGTAGCTTGAGGTGTATGCGGTCTAGGGTAGGCTGGTTTG
TCTTGGTCGAATTTGATGTTGGCTTCCGTGCGGACCAGATCGAGCGTGGTACAGGTTAGGTACAGGGTAGGCGGCTTAGACTT
GGTCGATCTGGATGTCGGTTCTCTGGCTGACGGATCTCAGAGCTTTGGAACGAGATGGGAGTGGTGCAGGGTAGGCAACTTCT
GTCTTTGCCAGGATGCGATTCGCGTTGAGATGTGCGGTCTAGGTTAGGTACAGGGTAGGTAGAATCTCAGTTTCGTCGCTACAG
TTTGCCATAGGTGTAGGGTAGGTACAGGGTAGGCAGATTTCTCCTCCGGCCAGTACTTGCTTATGCGTGCGAAATGGTTCTGTG
GTCTGGTGGGCGTGAGTCGATTTTTTTGTTTTGCCATACAAACGAATTTTGCGGGAAATCAAAAGTGGCCCACGAGTCGGGTCT
GGCGTGCGTCCGACTGGAACATCGTCGGTGGACATATGAGAGGGAGCAATCCGGCCGGGCCGGGGCCCATCGCGACCTGCA
GGGTAGGTAAAAGTAAAAAAGTTGTTAAGAGGCGCGGTGTCGGTGTGCTTGTATTTGAGGAAGAGAATTTTCTGGAGAGGTAGA
AGGGCAGGAAGACTTGGACGGATCTGGTCCGGGAACGGGTCTGGGCTCGGGTTTTGGGGTGGTGCAGGGTAGGCAGGTTTA
GATAGACGGTCCAGGGTAGGTACAGGGTAGGCACAGGGTAGGTAGAAGTCCGGTAAATAGTATGGGCGGGTGTAGGGTAGGC
TCGGATACCGTTTTGTTTCACGCCCTTAC**CTTGGCTCGAGAAGGG**ACAATCTTGGCCGGGATCGA
AGTCTAGGGTAGGCTGAATGTATGATTATGTAAGCTGTATAGCTCTAGGGTAGGTAAA
AATCCCATACAAATCTGATTGGAATTGGTGAAGATGTGATTTGGCTGGAGAGATGGA
CGAAAGTGTAATGCAGAACTGTATGTGATTTTGCAAAAGTGAGTGTAAAAATGGAAA
GTCGATGTTCCCGAGCAGGTGAGAGCACGTTTGAGACGTCATCGGATGCGCCTCG
TCGAGACGAGCACAACGGTGTAGCCCATGTGTGTGCACGACTCCTGTGCGTCCGT
CCAGGGCGGGATAAGTAGAAAA**GTGAGAGGTGTAGGGTAGGT**CGTCACACCCACGACCCGA
TCACCTCGTCCGTCGATTCGTCTTTATCTGTGGGACAGGTCTAGGGTAGGCCAAAGTCAGGTCTAGGGTAGGCAACTTCCAACC
TCAAAGCTGCCTACCCGGGAGCCAATTTCAATCGCCTCTCACGCCTCCCACAGACCTCGCACGAGGATGCGACCACCACCATT
GAACTCGCCTTGGTCGAAATAGTCGGTATATGCACTTTTGAAAAAAAGCGTGCAAAATGGTTTTGTGGTTTGGTGGCCGTGAGT
CGATTTTTTTGTTTTCCCATACAAATGAATTTTGCGGAAAATAAAAAGTGGCCCACGAGGCGGTTCTGGCGTGCGGCCCACTAAA
ACGGTCTCGGAGGGTATATGAGAAGGGAGCAAATCCGGCCGAGCCTGAAAGGGCGAGGGAAAACCGGACAAGCAACCTCTCG
TACCTGATCTTAAAGACTTCCATTGCGTGTCCCTCTGTACAGCTTTGCAGGCTCCGGCCTCGGCAGCGGGGGGTTCATAGTGGT
CGTCGACCTCCACGAAACTGCTCGCCCCGGCGTGACAGCGTACTGGGGATGCCTGTGTTTACGCAGTCCGGGCTTGCCTGGA
CCGCCAGCAGATGGGCTCTGTGGATGACTGGCCGCTGGCTAGACCTGAAACCTGAGCAACGGGAGGTAACCTCTCGCCGTGG
ACACCGGAATGGTAGAAGCGGCGTGCTGCGTCCTCCTCTTGGGGCCCCTAAGCCACACCTCCCACAGCGGGTTCGGTGCGGC
GGACGGACGCCCTGGGGAATTTAGAGGGGGAAAGCGGATTGCCCTAGCGGTGCTGTTGGCCCTGCCGACCTCACTGCGAAAG
GCGCGACTTCACCGTCGCCACCCAGTAACTTGTCTCTCCGGCGCCTCACGGCGCTGGTGGCCAACCCCGGCACACGATAGTTA
C

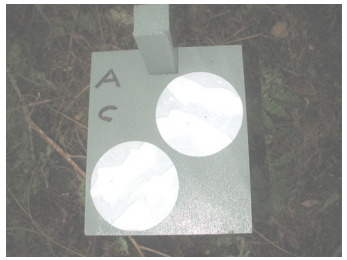
360 bp

Detection of *F. circinatum* using the primers CIRC1A-CIRC4A



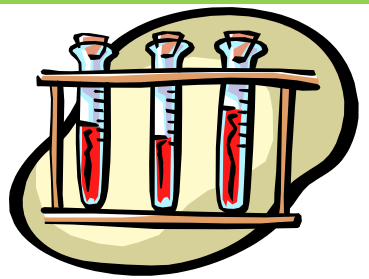
Lane 1: DNA ladder, 2: water control, **3: *F. circinatum* 25331, 4: *F. circinatum* 25332, 5: *F. circinatum* 25333, 6: *F. circinatum* 25621, 7: *F. circinatum* 26431, 8: *F. circinatum* 26432, 9: *F. circinatum* 25707, 10: environmental sample from San Francisco (Site I), 11: environmental sample from San Bruno, CA (Site II),** 12: *F. subglutinans*, 13: F. sp. 25204, 14: F. sp. 25622, 15: F. sp. 26756, 16: F. sp. 26757 17: F. sp. 25346, 18: F. sp. 25623, 19: F. sp. 25195, 20: F. sp. 25807, 21: F. sp. 29123, 22: *F. bulbicola*, 23: *F. bactridioides*, 24: *F. succisae*, 25: *F. anthophilum*, 26: *F. begoniae*, 27: water control, 28: DNA ladder.

“Molecular method” Working Scheme



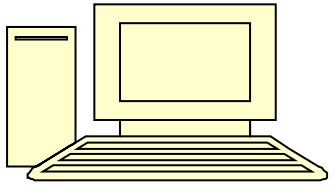
Spore trap
with filter paper

suspend in
20 ml 4X TE 65ºC



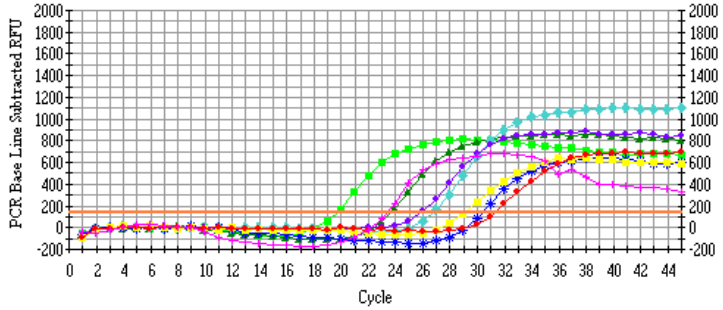
Centrifugation
Spore concentration

DNA-extraction

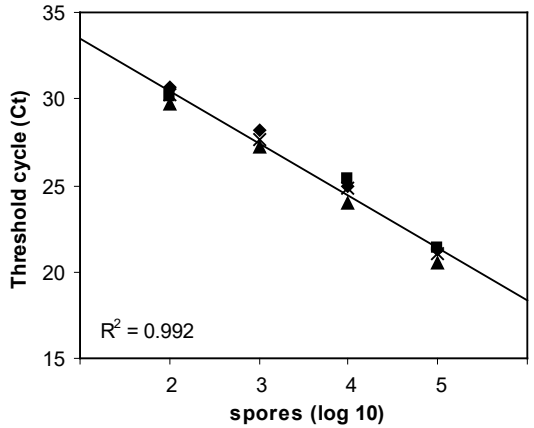


Mating type
determination

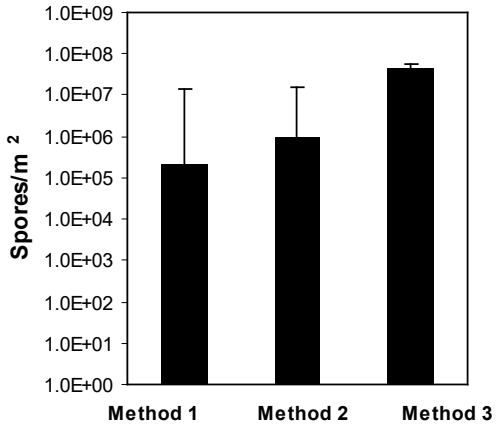
RT-PCR using
CIRC1A-4A and SYBR Green



Compare with Spore (DNA) standard

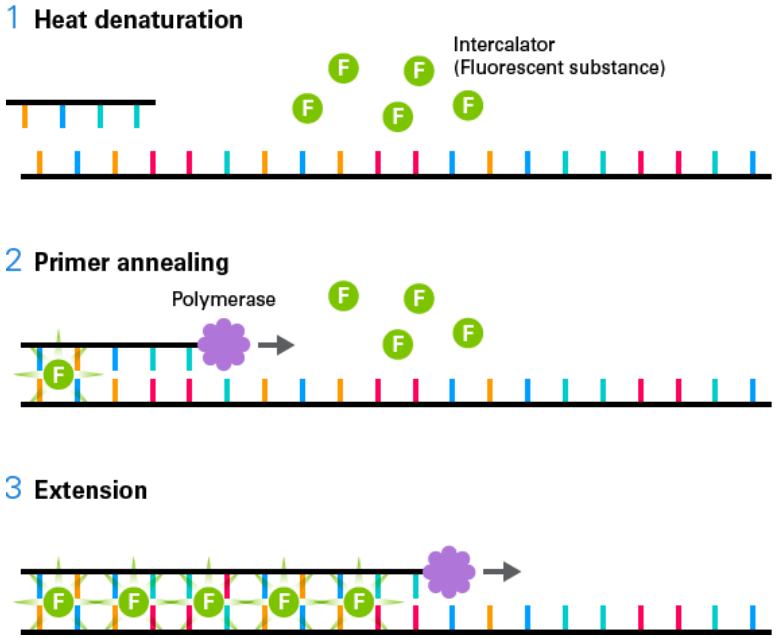


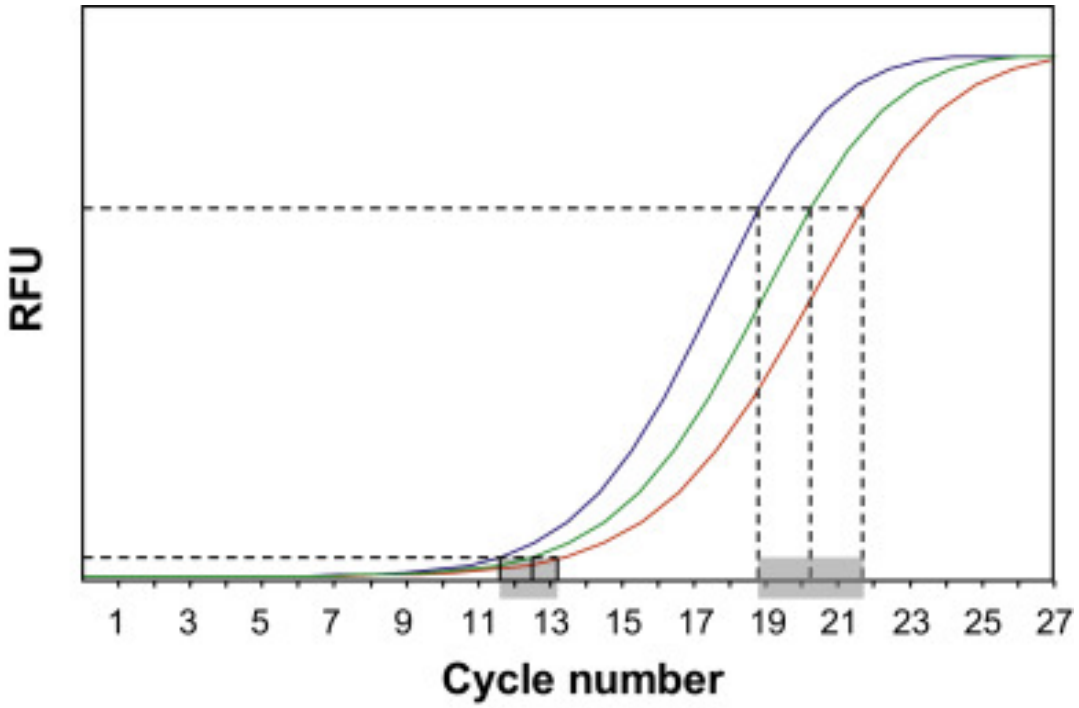
Calculate spores/m^2



How does the quantification work?

- qPCR: quantitative PCR
- The DNA target gets amplified during each cycle, exponentially
- SYBR Green is a fluorescent dye which binds to double-stranded DNA
- The fluorescent light is detected by a laser
- The more DNA is present at the start of the PCR, the earlier it crosses a threshold (ct number)
- By comparing with a standard curve the DNA content of the sample can be calculated.

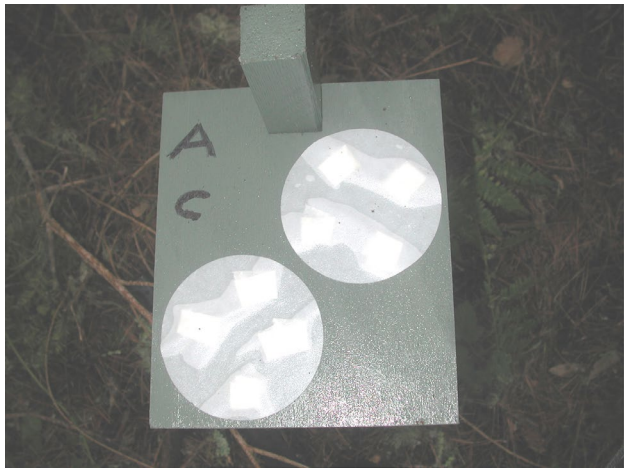




“Traditional Method”

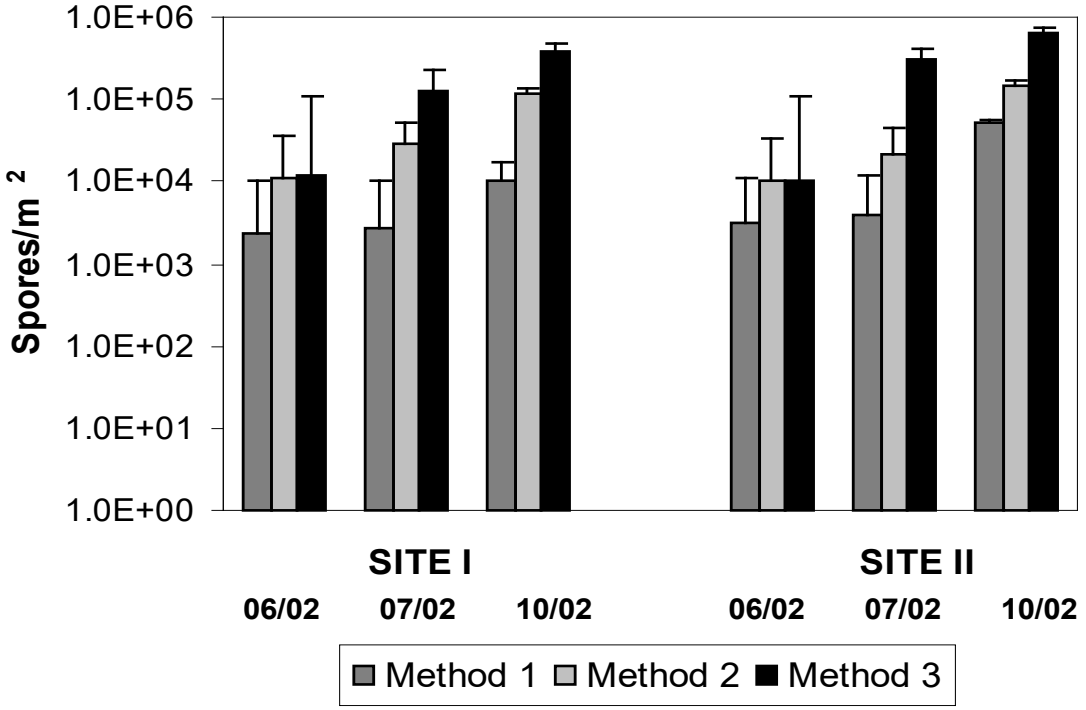
Spore traps using Petri-dishes with Selective Medium
Spore traps with Filter Paper

Count growing cultures

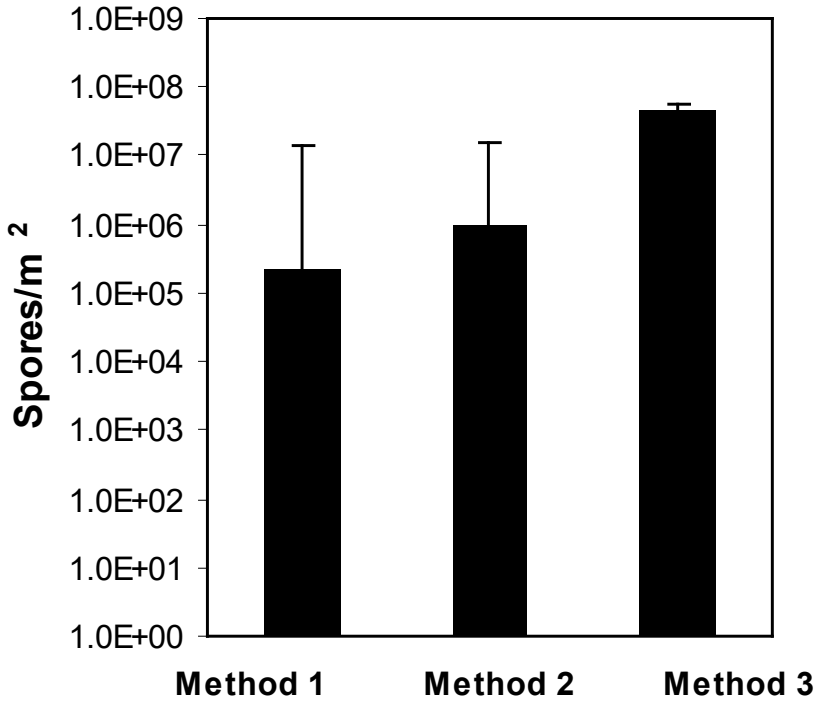


Count growing cultures

Comparison of methods 1 (Field study in San Francisco and San Bruno)



Quantification of *F. circinatum* from spore traps collected at San Francisco (Site I) and San Bruno, CA (Site II) after 2 weeks in the field

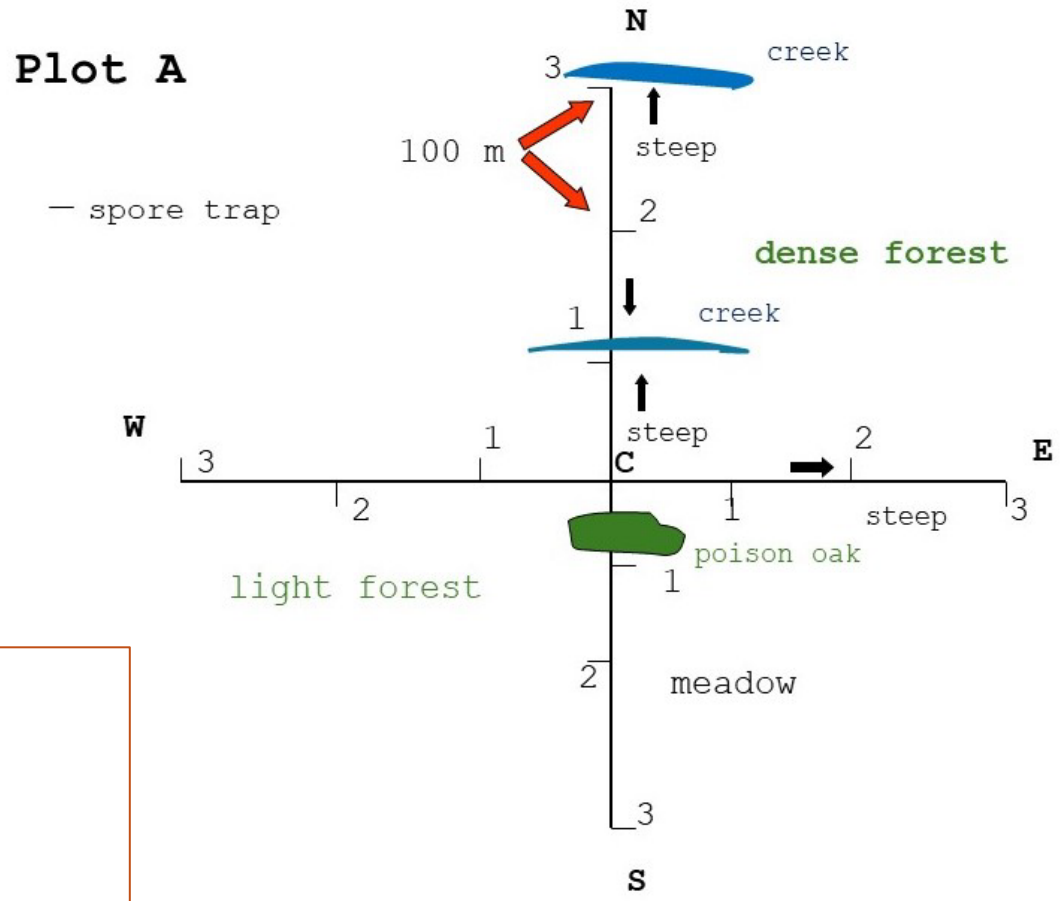


Combined samples from all sites and sampling periods

Comparison of methods 2

- All methods used ‘passive spore sampling’, which is maybe less efficient as an active, volumetric trap (e.g. Burkard 7-Day volumetric trap)
- Plates with media try out rather quickly and cannot be used during the rainy season
- Filter paper was surprisingly sturdy, but eventually also suffers in bad weather (we used 2 per trap); plating of filter paper on selective media is extremely time-intensive; risk of contamination.
- Molecular method is time-intensive; good calibration is crucial.

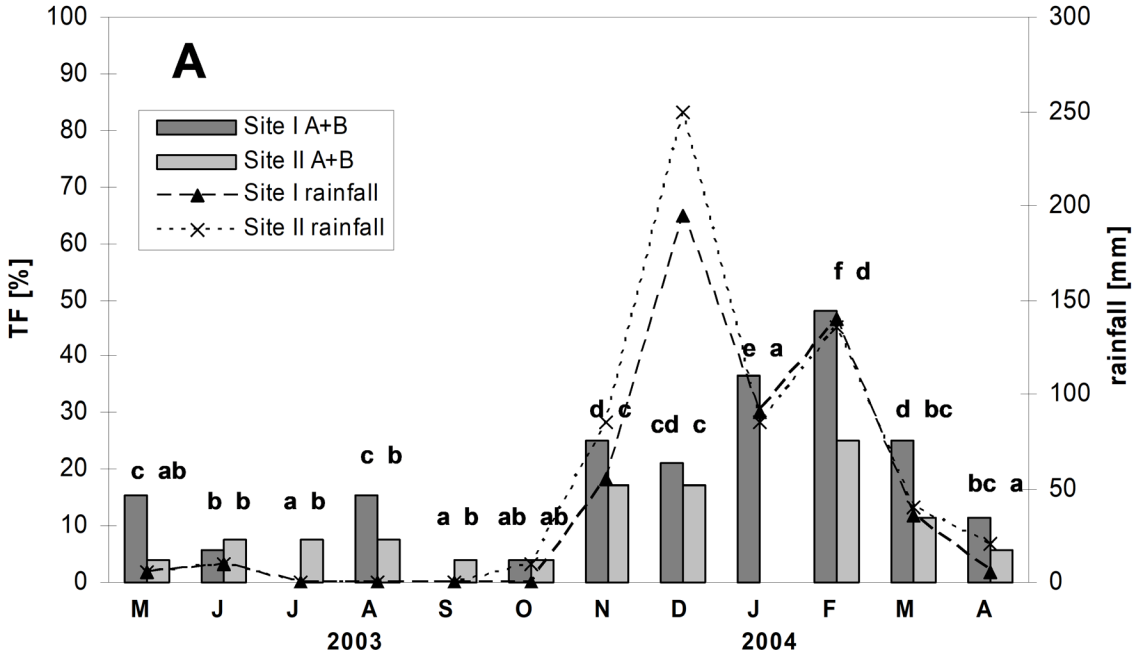
Long term study-1 year
San Francisco (Presidio) and
Santa Cruz Mountains (Swanton Pacific Ranch)

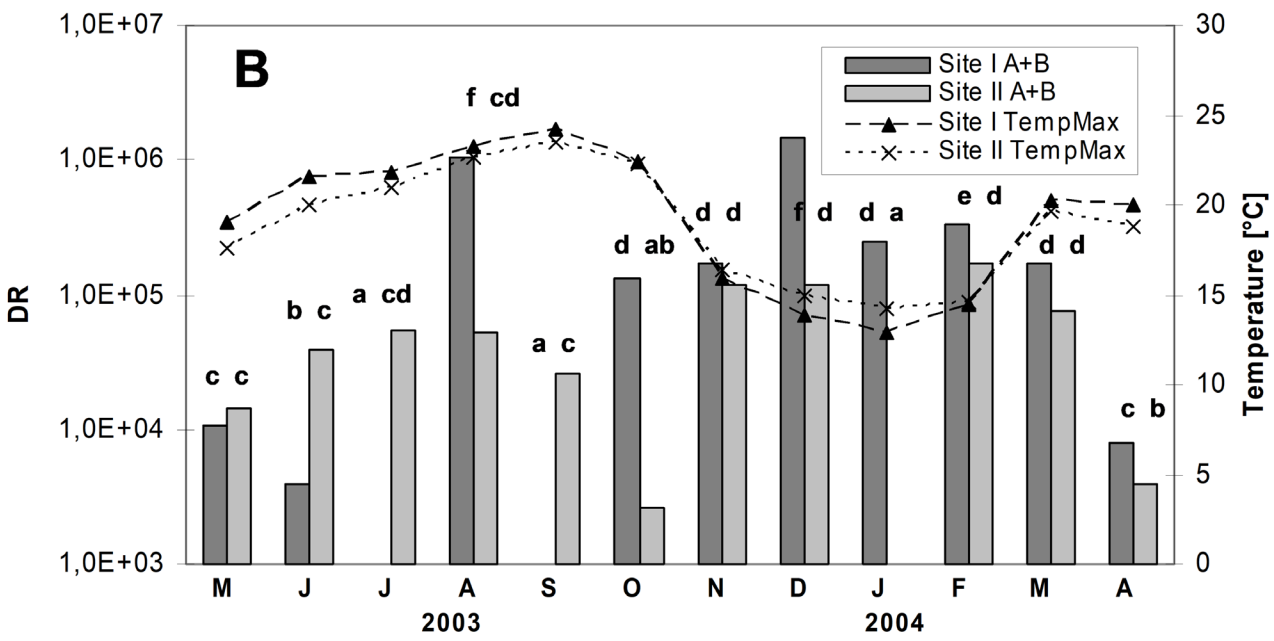


2 sites (SF and SC)
2 plots per site
13 spore traps per plot
52 total traps
Distance between traps: 100 m
Length of plot (N>S; W>E: 600 m

Long term study- results

- Trapping frequency (TF) was higher during the rainy season (November to April), than during the dry season (May to October)
- Spore deposition rate (DR) were very variable: 0-1.3x105 spores/m2
- DR were higher near trees with moderate symptoms than trees with no or very strong (or dead symptoms)
- The detection of spores on traps at distances larger than 200 m from any Monterey pine suggest at least midrange aerial dispersal.





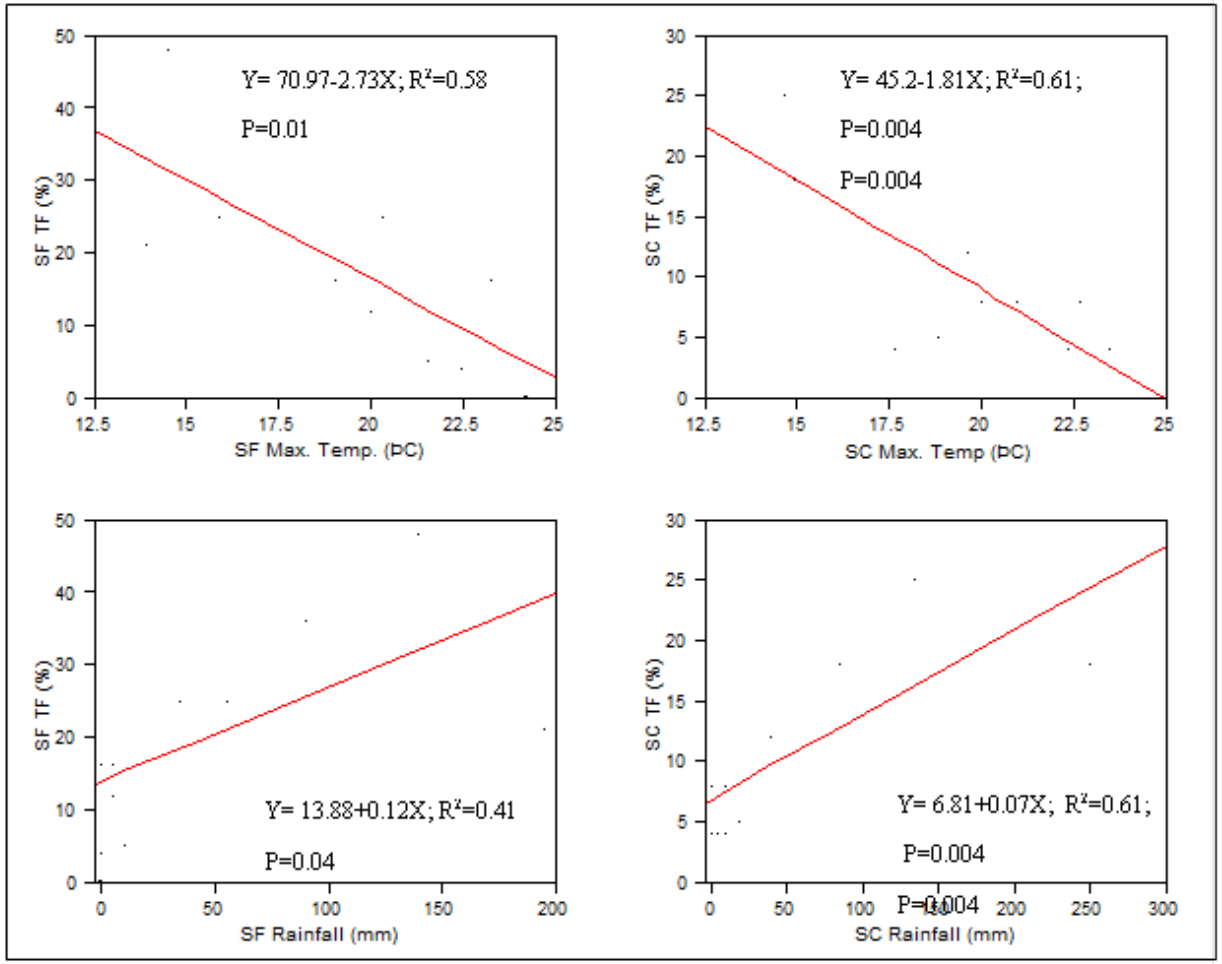
Trapping frequency of *F. circinatum* is correlated with rainfall and maximum temperature in both San Francisco (SF) and Santa Cruz (SC)

Temperature
(negative correlation)

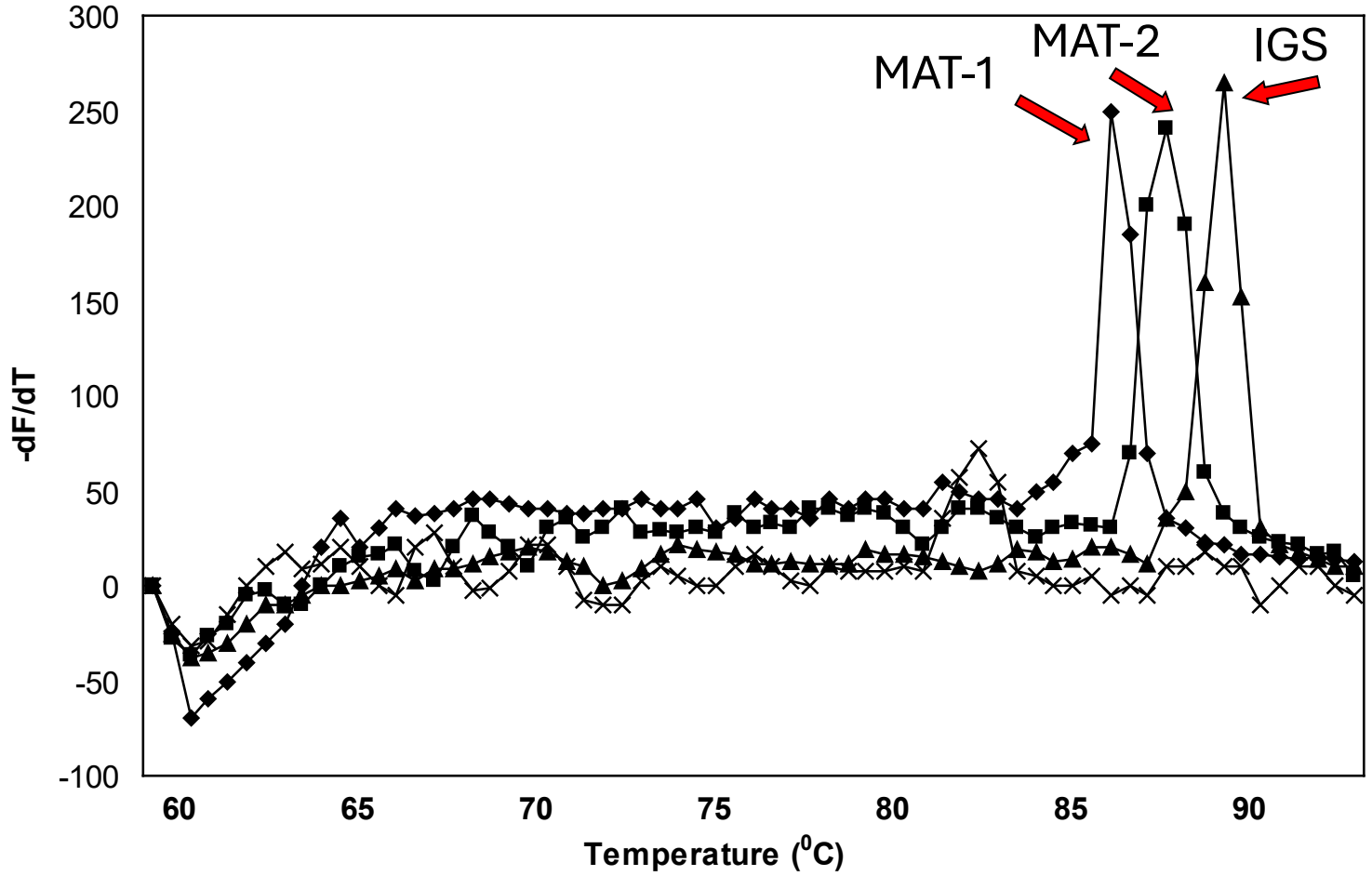
Rainfall
(positive correlation)

SF

SC



Only one mating type (*Mat 1*) was detected in California, indicating the pathogen is non-native and no sexual recombination occurs



Results from other countries

- Spain: *F. circinatum* found in *P. radiata* plantations throughout the year; no clear patter; but lower temperatures seem to be correlated to higher inoculum numbers (experiment used active spore sampling). Dvorak et al. 2017

- South Africa: *F. circinatum* occurred at relatively low levels in the nursery throughout the year and that its distribution was spatially sporadic. The data suggest that standard nursery sanitation practices in the test nursery maintained the airborne inoculum of *F. circinatum* at low levels. The uneven distribution of infection also suggests that airborne inoculum does not represent the primary source of inoculum for the *F. circinatum*-associated seedling disease. Fourie et al 2014

Spore trap developed by Quesada et al. 2018, Florida

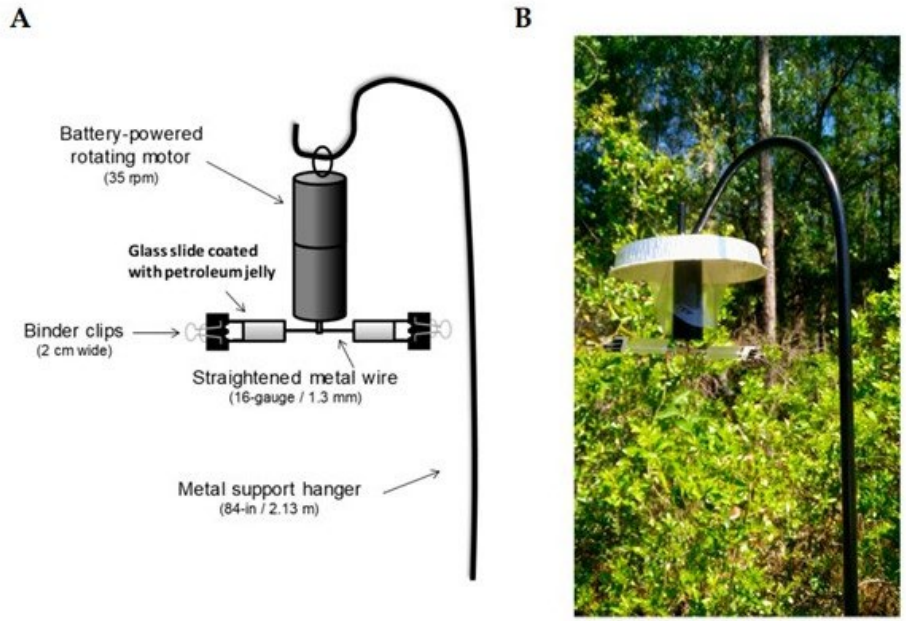
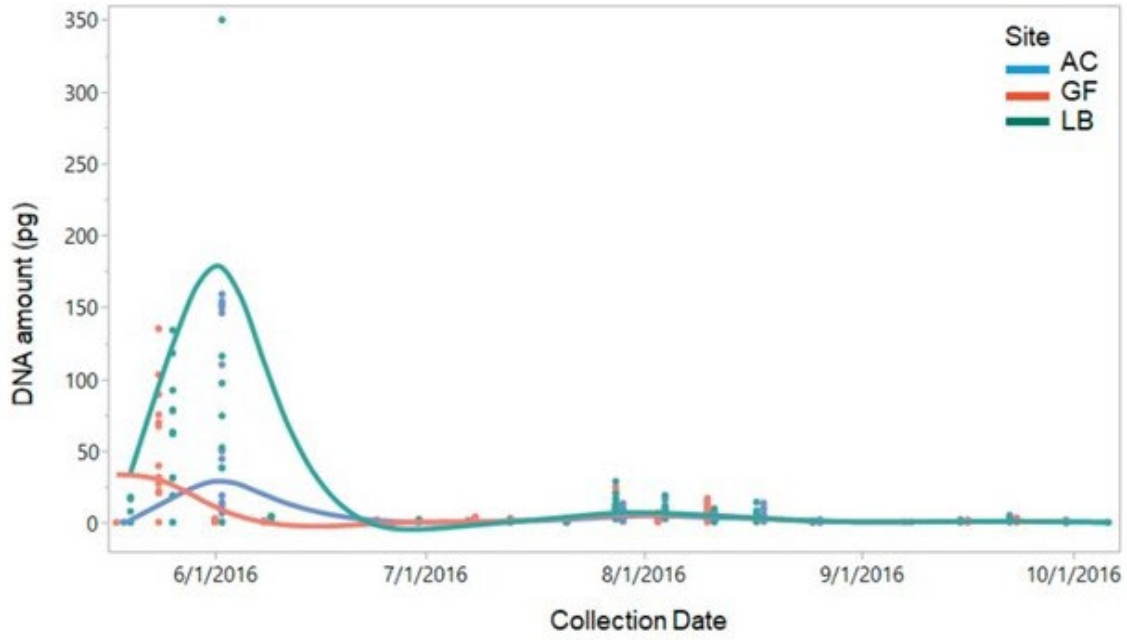


Figure 1. (**A**) Diagram of the in-house spore trap with specifications for its construction. (**B**) Photograph of spore trap in the field.



Loblolly pine (*P. taeda*)
Slash pine (*P. elliottii*)
Longleaf pine (*P. palustris*)

Conclusion 1

- *F. circinatum* is a invasive pathogen witch expresses some typical features (aggressive, limited genetic variability)
- Aerial inoculum most probably plays an important role for short to mid-distance spread
- Conidia can be produced and spread throughout the year, depending on climatic conditions
- The methods available are relatively laborious and not easily used for long-time studies with an significant number of samples

Conclusion 2

Sporulation

i) is enhanced during cool-wet conditions

ii) does not occur (or reduced) when temperatures are below 0°C

iii) may occur in warm conditions in the absence of rainfall if high humidity is caused by coastal fog

iv) spore densities are very variable among sampling points, suggesting a strong local effect

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UC Berkeley

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Thank you!