
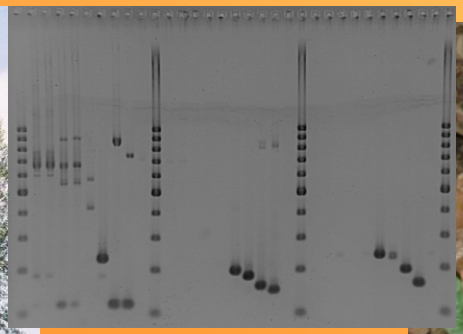


# Molecular diagnosis of wood rotting fungi in ornamental trees

**Matteo Garbelotto**
**U.C. Berkeley- UCCE**

# wood decay fungi

## diagnosis and identification

↓

## prognosis

Prediction of **severity** and **evolution** of **decay** process

↓

- appropriate FRC (Failure Risk Classification)
- appropriate management practices

**rapidly progressing root** and **butt rot agents**

# Identification of wood decay fungi in standing trees

**traditionally based on macro- and micro-morphology of fruiting bodies**

**Overlapping** morphological characters

*Ganoderma resinaceum*

*Perenniporia fraxinea*

Fruiting bodies **rarely visible**
**advanced stage**

# Identification of wood decay fungi in standing trees

**pure culture analysis (i.e. Stalpers 1978)**

**fungal isolation** not always feasible

identification **time-consuming** and often **complicated**

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ORIGINAL ARTICLE

# A multiplex PCR-based method for the detection and early identification of wood rotting fungi in standing trees

F. Guglielmo[1], S.E. Bergemann[2], P. Gonthier[1], G. Nicolotti[1] and M. Garbelotto[2]

1 Department of Exploitation and Protection of Agricultural and Forestry Resources, Plant Pathology, University of Torino, Grugliasco , Italy
2 Ecosystem Sciences Division, Department of Environmental Science, Policy and Management, University of California Berkeley, California, USA

**Keywords**
internal transcribed spacers, mitochondrial small subunit, nuclear large subunit, molecular diagnostic, wood decay.

**Abstract**

**Aims:** The goal of this research was the development of a PCR-based assay to identify important decay fungi from wood of hardwood tree species in northern temperate regions.

www.wooddecay.org

2008

RESEARCH LETTER

# A PCR-based method for the identification of important wood rotting fungal taxa within *Ganoderma*, *Inonotus* s.l. and *Phellinus* s.l.

Fabio Guglielmo[1], Paolo Gonthier[1], Matteo Garbelotto[2] & Giovanni Nicolotti[1]

[1]Department of Exploitation and Protection of the Agricultural and Forestry Resources, University of Torino, Grugliasco, TO, Italy; and [2]Department of Environmental Science, Policy and Management, Ecosystem Sciences Division, University of California, Berkeley, USA

**Correspondence:** Giovanni Nicolotti, Department of Exploitation and Protection of the Agricultural and Forestry Resources, University of Torino, via L. da Vinci 44, I-10095 Grugliasco (TO), Italy. Tel.: +39 011 6708544;

**Abstract**

Two multiplex PCRs, based on 10 taxon-specific primers designed on rRNA gene regions, were developed for the identification of taxa within the lignivorous genera *Ganoderma*, *Inonotus* s.l. and *Phellinus* s.l., each comprising both secondary and

# Decay fungi included in the method

- *Armillaria* spp. (Agaricales, Marasmiaceae)
- *Ganoderma* spp. (Polyporales, Ganodermataceae)
- *Hericium* spp. (Russulales, Hericiaceae)
- *Inonotus/Phellinus* spp. (Hymenochaetales, Hymenochaetaceae)
- *Laetiporus* spp.(Polyporales, Polyporaceae)
- *Perenniporia fraxinea* (Polyporales, Polyporaceae)
- *Pleurotus* spp. (Agaricales, Pleurotaceae)
- *Schizophyllum* spp. (Agaricales, Schizophyllaceae)
- *Stereum* spp. (Russulales, Stereaceae)
- *Trametes* spp. (Polyporales, Polyporaceae)
- *Ustulina deusta* (Xylariales, Xylariaceae)

4 groups

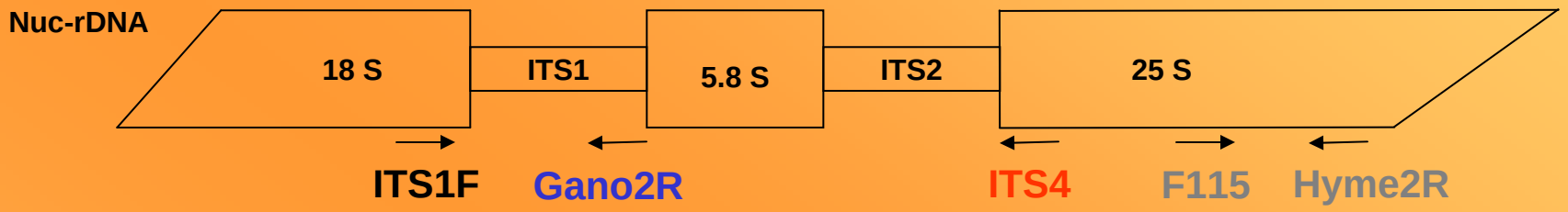
6 groups
(Wagner and Fischer 2002)

# Samples

**DNA extraction**

**M1**

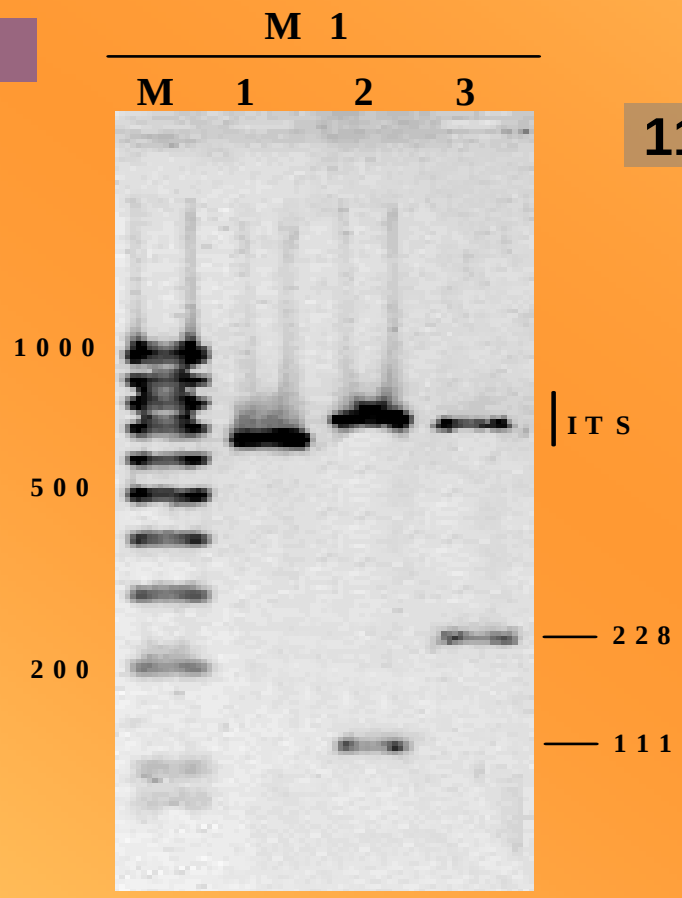
# M1




**Fungi** — **600-800bp**

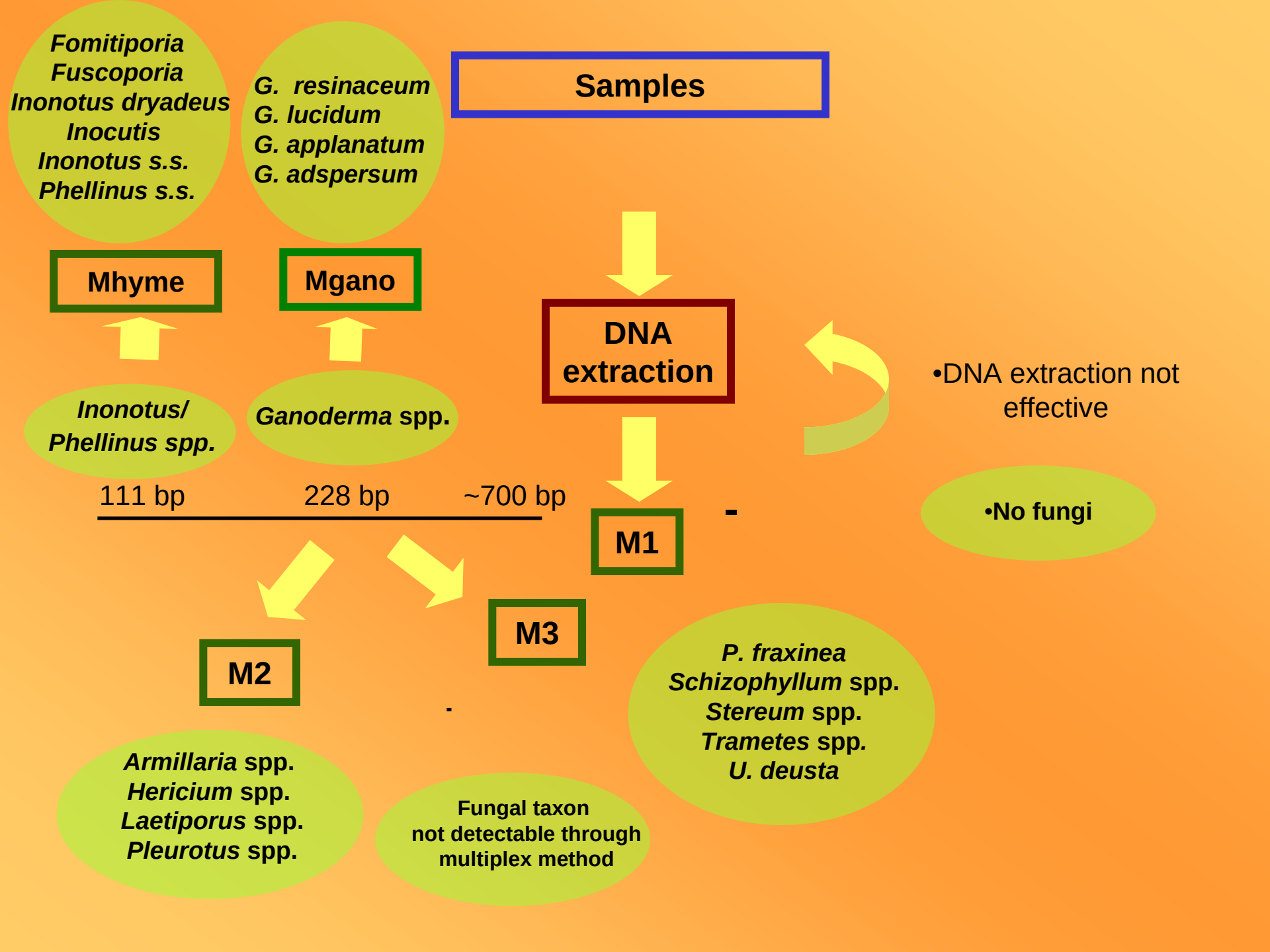
***Ganoderma* spp.** — **228bp**

***Inonotus/ Phellinus* spp.** — **111 bp**




1. ***Trametes versicolor***
2. ***Phellinus punctatus***
3. ***Ganoderma resinaceum***

M. DNA ladder 100 bp


Fomitiporia
Fuscoporia
Inonotus dryadeus
Inocutis
Inonotus s.s.
Phellinus s.s.
G. resinaceum
G. lucidum
G. applanatum
G. adspersum
Samples
Mhyme
Mgano
DNA extraction
•DNA extraction not effective
Inonotus/ Phellinus spp.
Ganoderma spp.
111 bp
228 bp
~700 bp
M1
-
•No fungi
M2
M3
P. fraxinea
Schizophyllum spp.
Stereum spp.
Trametes spp.
U. deusta
Armillaria spp.
Hericium spp.
Laetiporus spp.
Pleurotus spp.
Fungal taxon not detectable through multiplex method

## Aims:

1. to validate the method on wood samples

2. to develop an efficient drilling-based sampling method

3. to infer ecological features of decay agents based on the application of the method in northern Italy

# Validation I

•**114** wood samples collected (through a swedish increment borer) from decay-affected trees in central California and northern Italy

• Wood DNA extraction through QIAmp DNA Stool mini kit (Qiagen)

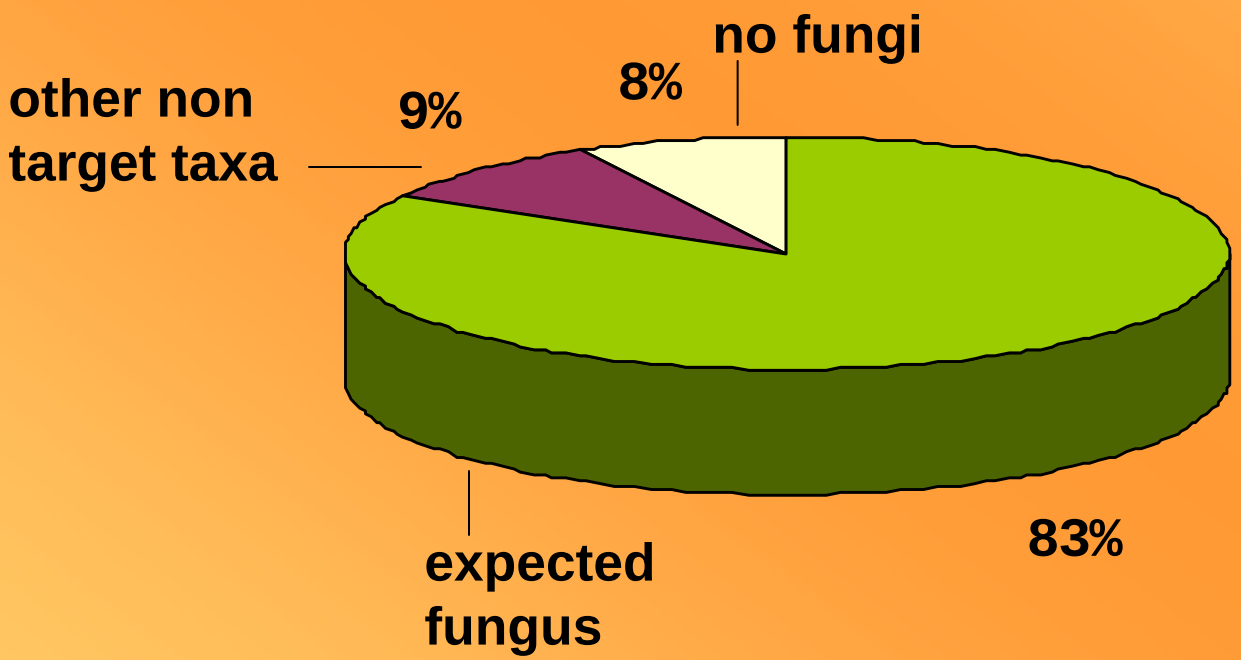
•**Obtained results**
- ✓From multiplex PCR protocol developed

•**Expected results**
- ✓From analysis of visible fruiting bodies or sequencing

•**Comparison** between **expected** and **obtained results**

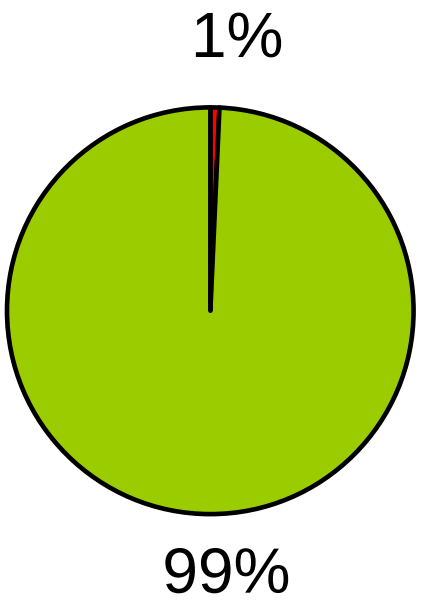
# Validation II

efficiency



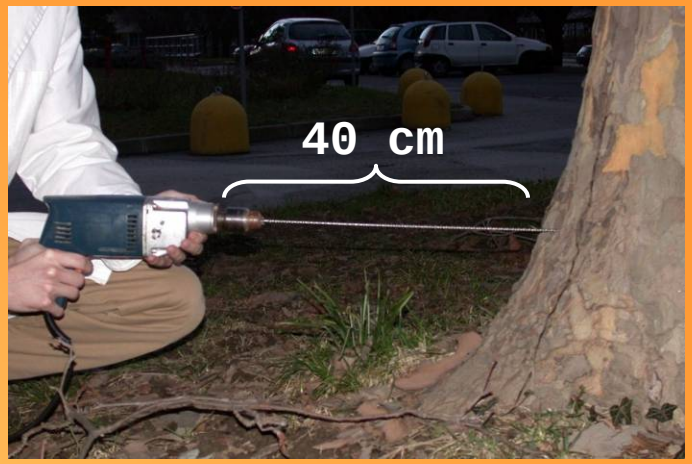
# Validation III

specificity



# Sampling method I
# testing and optimization

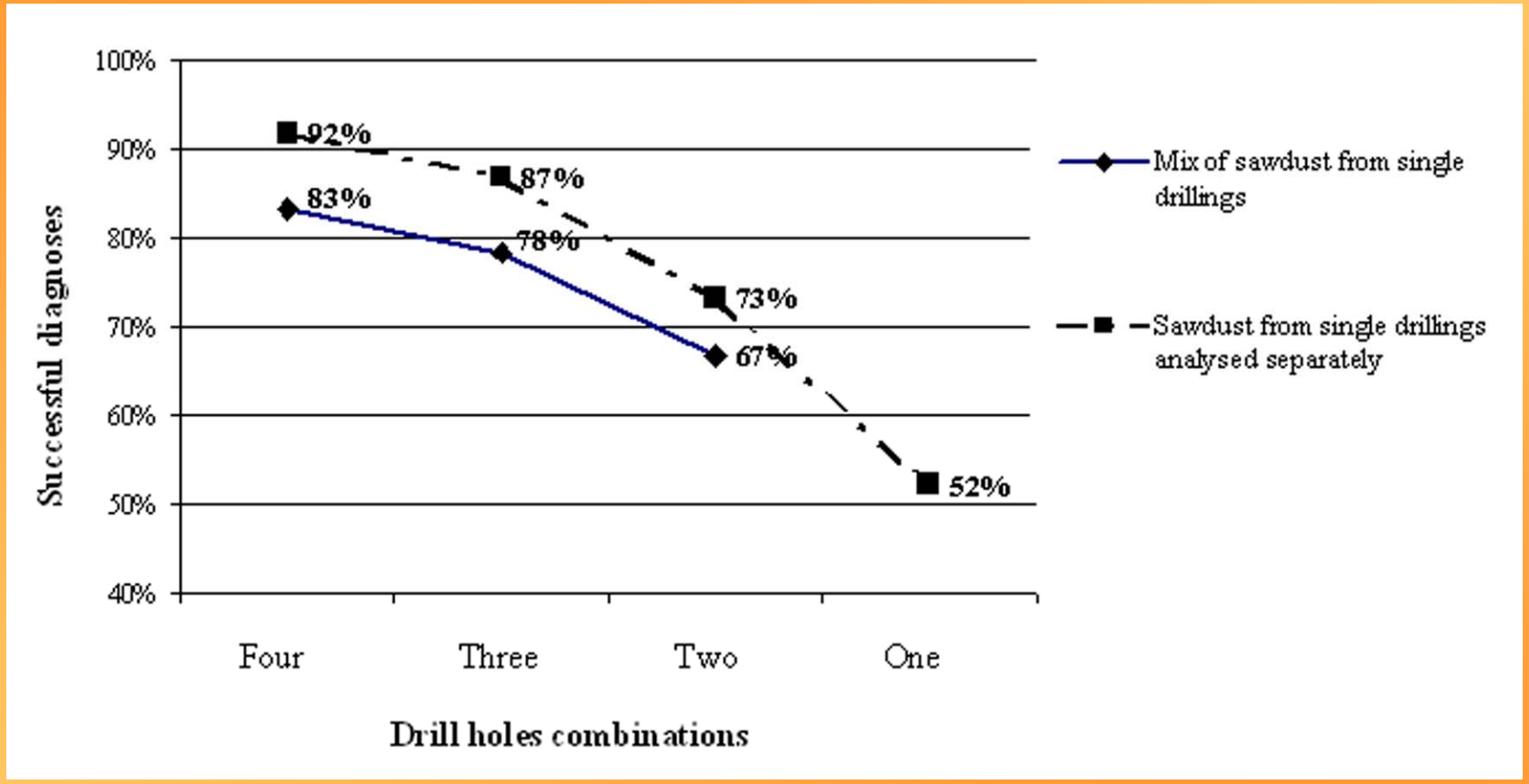
Many trees infected by more than one target fungus




**Drilling close to the collar**

**Wood chips**

# Sampling method II testing and optimization



## Conclusions:

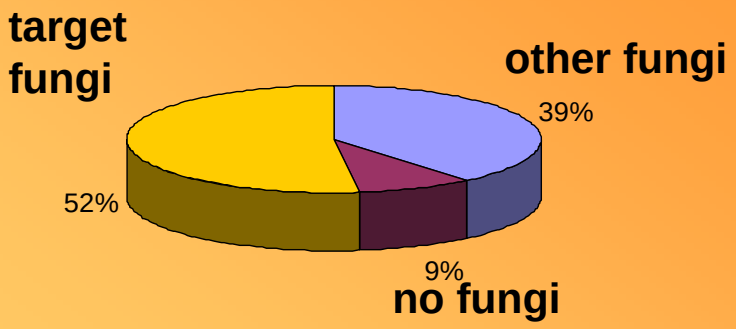
**The molecular method is highly specific and efficient**

**Fungal DNA may be successfully extracted from mixtures of sawdust obtained from drillings**

↓

**optimal n.: 4 or 3**

## Perspectives:

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Sequencing

*Bjerkandera* sp., (*Aesculus* sp.)
*Oxyporus populinus*, (*Platanus* sp.)
*Gymnopus* (=*Collybia*) *fusipes*, (*Quercus robur*)
*Spongipellis spumeus*, (*Aesculus* sp.)
*Phellinus cavicola*, (*Platanus* sp., *Aesculus* sp.)
*Hyphodontia* sp., (*Platanus* sp.)

# Wood Decay Diagnostic - Sample Submission Form

Date: _____ Sample Name / ID _____

Name/Affiliation: _____

Contact Email: _____

Location of Tree (township, county and State, GPS, more information is better than less)

_____

_____

_____

_____

_____

Tree Species: _____

Reason for Sending Sample: _____

_____

Was there a failure?_____ When? _____

Additional Notes:_____

_____

_____

# SENDING SAMPLES

- Sample should be sent with “next day “ service within 24 hours of collection
- Do not send samples in on Fridays
- Need to let us know you are sending samples with either a phone call or email
- Place samples in paper envelope and fill in one form for each sample

Forest Pathology & Mycology Laboratory, UC Berkeley

# Wood Decay Diagnostic Results

ID Code: Collection Date: _____

_____

Submitted by: Received: _____

_____

Tree Species: Done by Technician:

_____ _____

Location: _____

Reason For Submission: Wind Throw ❑ Hazard Tree ❑ Survey ❑

| Targets | Results Sample | Control |
|---|---|---|
| 1. Fungal DNA | ❑ | ❑ |
| 2. *Armillaria* spp. | ❑ | ❑ |
| 3. *Fomitiporia* (*P. punctatus, P. robustus*) | ❑ | ❑ |
| 4. *Fuscoporia* (*P. contiguous, P. gilvus, P. torulosus*) | ❑ | ❑ |
| 5. *Ganoderma* spp. | ❑ | ❑ |
| 6. *Ganoderma adspersum* | ❑ | ❑ |
| 7. *Ganoderma applanatum* | ❑ | ❑ |
| 8. *Ganoderma lucidum* (Eu) | ❑ | ❑ |
| 9. *Ganoderma resinaceum* | ❑ | ❑ |
| 10. *Hericium* spp. | ❑ | ❑ |
| 11. *Inocutis* (*I. dryophilus*) | ❑ | ❑ |
| 12. *Kretzschmaria deusta* | ❑ | ❑ |
| 13. *Inonotus dryadeus* | ❑ | ❑ |
| 14. *Inonotus* s.s. (*I. andersonii, I. hispidus, I. obliquus*) | ❑ | ❑ |
| 15. *Inonotus/Phellinus* spp. | ❑ | ❑ |
| 16. *Laetiporus* spp. | ❑ | ❑ |
| 17. *Perenniporia fraxinea* | ❑ | ❑ |
| 18. *Phellinus* s.s. (*P. igniarius, P. lundelii, P. tremulae, P. tuberculosus*) | ❑ | ❑ |
| 19. *Pleurotus* spp. | ❑ | ❑ |
| 20. *Schizophyllum* spp. | ❑ | ❑ |
| 21. *Stereum* spp. | ❑ | ❑ |
| 22. *Trametes* spp. | ❑ | ❑ |

)iagnosis:

)iagnosis:

❑ Positive For:

_____

_____

❑ Negative for all targets but positive for fungal DNA control (decay caused by non-target fungi.)

❑ Assay inconclusive due to excessive decay or inhibition of DNA analysis.

# RESULTS

- In about 5-6 weeks unless differently agreed
- Assay only targets specific fungi not all decay fungi
- For legal purposes, we stand behind our positives, but it is the collector's responsibility to make sure samples are collected correctly and in the best of ways; so our assay will not stand in trial by itself but it needs to be matched by the professional's diagnosis and correct collecting approach
- IT ALL DEPENDS ON HOW GOOD YOU ARE AT SAMPLING

# All info on:

WWW.WOODDECAY.ORG

or

WWW.MATTEOLAB.ORG