

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)

fungus isolation not always feasible



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

ORIGINAL ARTICLE

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

F. Guglielmo¹, S.E. Bergemann², P. Gonthier¹, G. Nicolotti¹ and M. Garbelotto²

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.



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 Guglielmo¹, Paolo Gonthier¹, Matteo Garbelotto² & Giovanni Nicolotti¹

¹Department of Exploitation and Protection of the Agricultural and Forestry Resources, University of Torino, Grugliasco, TO, Italy; and ²Department 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)

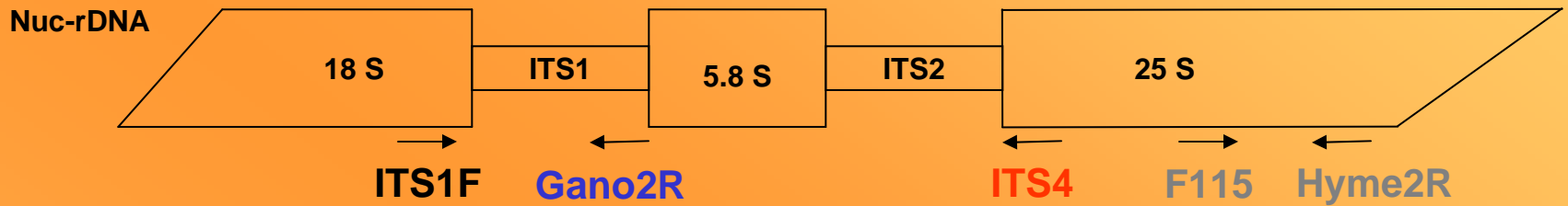
Samples



**DNA
extraction**

M1

M1



Fungi

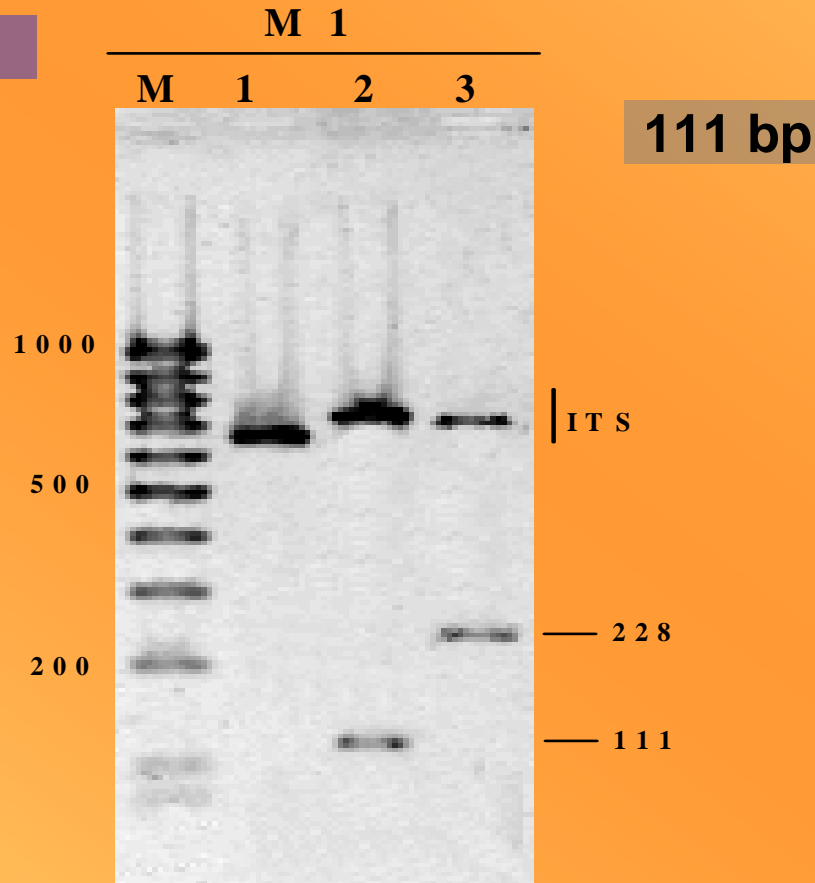
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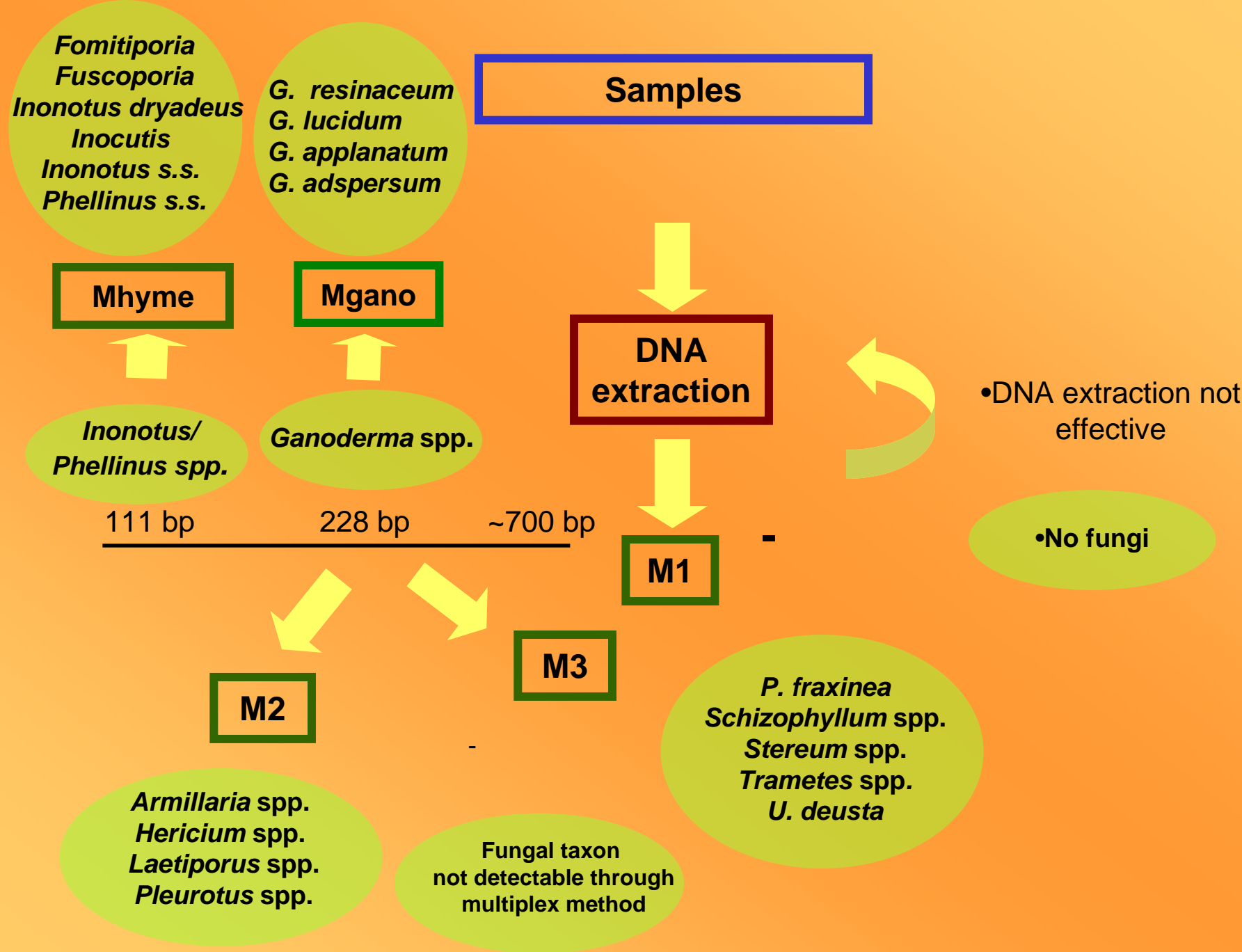
Ganoderma spp.

228bp

Inonotus/
Phellinus spp.

1. *Trametes versicolor*
 2. *Phellinus punctatus*
 3. *Ganoderma resinaceum*
- M. DNA ladder 100 bp





Aims:

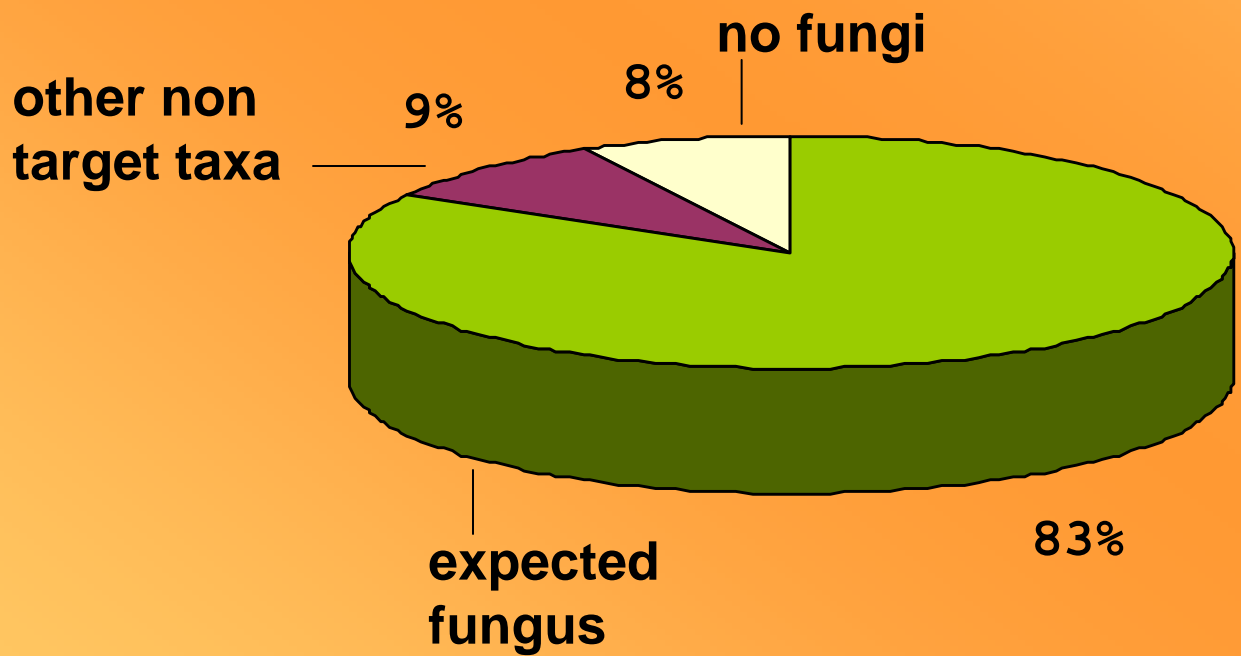
- 1. to validate the method on wood samples**
- 2. to develop an efficient drilling-based sampling method**
- 3. to infer ecological features of decay agents based on the application of the method in northern Italy**

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**

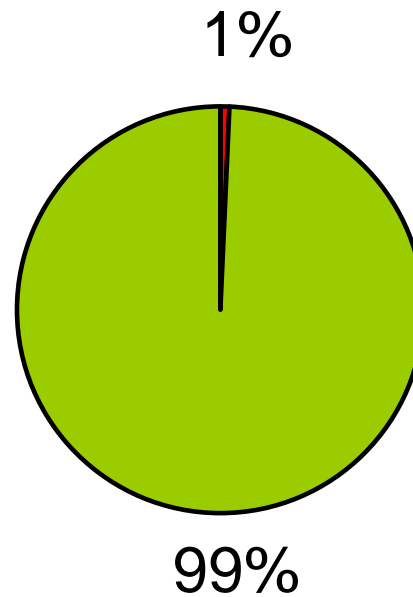
Validation II

efficiency



Validation III

specificity



aspecific amplification



no aspecific amplification

Sampling method I testing and optimization

Many trees infected by more than one target fungus



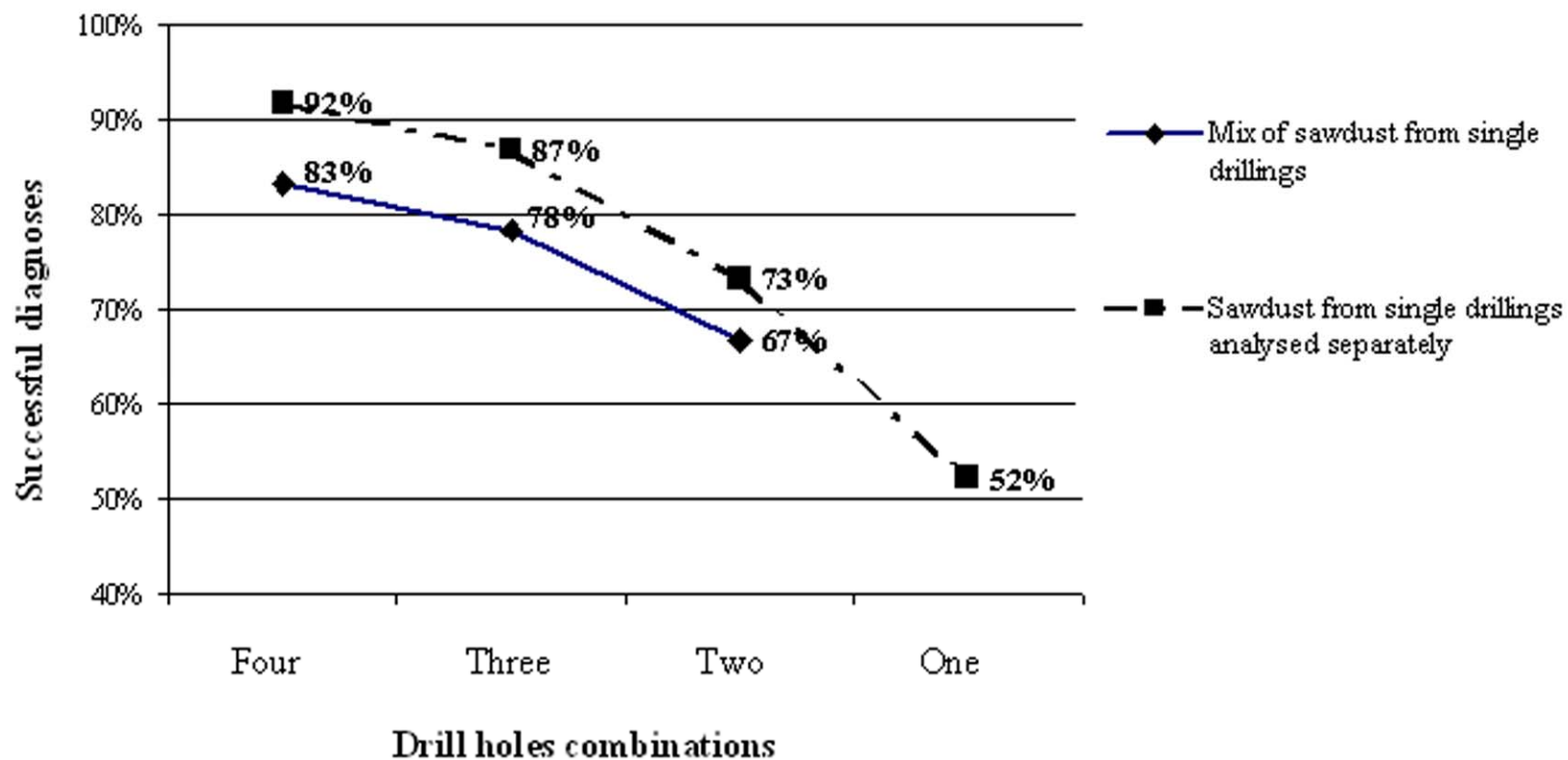
Drilling close to the collar



Wood chips



Sampling method II testing and optimization



Conclusions:

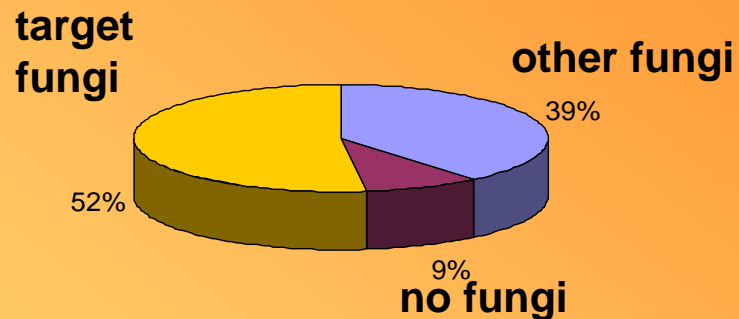
The molecular method is highly specific and efficient

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



optimal n.: 4 or 3

Perspectives:



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



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 | <input type="checkbox"/> | <input type="checkbox"/> |
| 2. <i>Armillaria</i> spp. | <input type="checkbox"/> | <input type="checkbox"/> |
| 3. <i>Fomitiporia</i> (<i>P. punctatus</i> , <i>P. robustus</i>) | <input type="checkbox"/> | <input type="checkbox"/> |
| 4. <i>Fuscoporia</i> (<i>P. contiguus</i> , <i>P. gilvus</i> , <i>P. torulosus</i>) | <input type="checkbox"/> | <input type="checkbox"/> |
| 5. <i>Ganoderma</i> spp. | <input type="checkbox"/> | <input type="checkbox"/> |
| 6. <i>Ganoderma adspersum</i> | <input type="checkbox"/> | <input type="checkbox"/> |
| 7. <i>Ganoderma applanatum</i> | <input type="checkbox"/> | <input type="checkbox"/> |
| 8. <i>Ganoderma lucidum</i> (Eu) | <input type="checkbox"/> | <input type="checkbox"/> |
| 9. <i>Ganoderma resinaceum</i> | <input type="checkbox"/> | <input type="checkbox"/> |
| 10. <i>Hericium</i> spp. | <input type="checkbox"/> | <input type="checkbox"/> |
| 11. <i>Inocutis</i> (<i>I. dryophilus</i>) | <input type="checkbox"/> | <input type="checkbox"/> |
| 12. <i>Kretzschmaria deusta</i> | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> |
| 13. <i>Inonotus dryadeus</i> | <input type="checkbox"/> | <input type="checkbox"/> |
| 14. <i>Inonotus</i> s.s. (<i>I. andersonii</i> , <i>I. hispidus</i> , <i>I. obliquus</i>) | <input type="checkbox"/> | <input type="checkbox"/> |
| 15. <i>Inonotus/Phellinus</i> spp. | <input type="checkbox"/> | <input type="checkbox"/> |
| 16. <i>Laetiporus</i> spp. | <input type="checkbox"/> | <input type="checkbox"/> |
| 17. <i>Perenniporia fraxinea</i> | <input type="checkbox"/> | <input type="checkbox"/> |
| 18. <i>Phellinus</i> s.s. (<i>P. ignarius</i> , <i>P. lundellii</i> , <i>P. tremulae</i> , <i>P. tuberculosus</i>) | <input type="checkbox"/> | <input type="checkbox"/> |
| 19. <i>Pleurotus</i> spp. | <input type="checkbox"/> | <input type="checkbox"/> |
| 20. <i>Schizophyllum</i> spp. | <input type="checkbox"/> | <input type="checkbox"/> |
| 21. <i>Stereum</i> spp. | <input type="checkbox"/> | <input type="checkbox"/> |
| 22. <i>Trametes</i> spp. | <input type="checkbox"/> | <input type="checkbox"/> |

Diagnosis:

Diagnosis:

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