EMPA

Materials Science & Technology

Fungi: foes or friends of veteran trees?

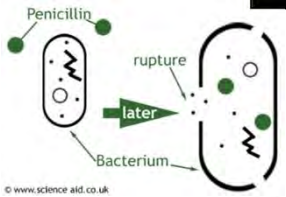
I Monumentali – Convegno Internazionale sulla cultura, filosofia e tecnica degli alberi veterani

Prof. Dr. Francis W.M.R. Schwarze
Applied Wood Science
Bio-engineered Wood

Varese, 23. November 2012

Fungi as beneficial microorganisms

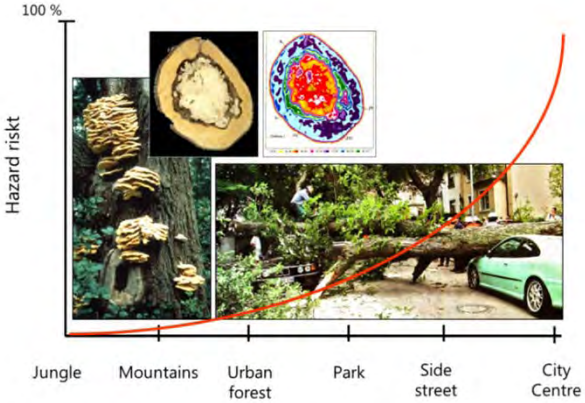
Pharmaceuticals/Food



Symbionts

Mycotecture

Philip Ross/University of San Francisco



Antagonists

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Wood decay fungi as catalyst for biodiversity

The habitat created by wood decay fungi helps to make old trees so important for wildlife diversity.

Decay is not a static condition and as the habitat changes, especially as trunks and branches hollow and the bark ages, new habitats are created and exploited by increasingly specialized wildlife.

Once cavities have been created, they are occupied by creatures as diverse as bats and great spotted woodpeckers.

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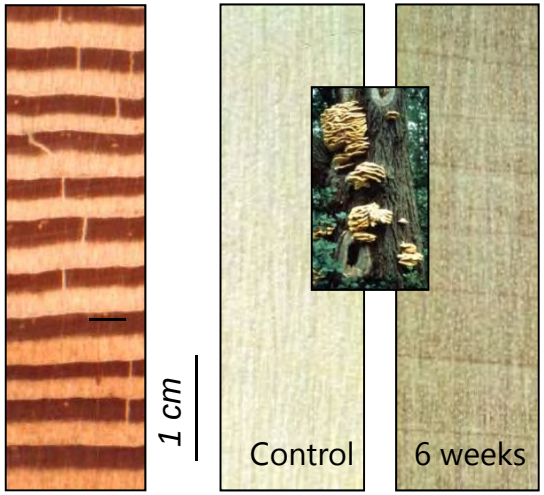
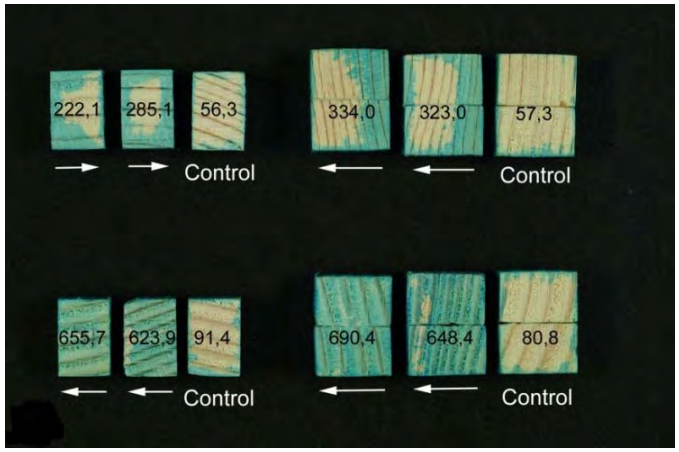
Fungal biotechnology at Empa

Tree ring analysis



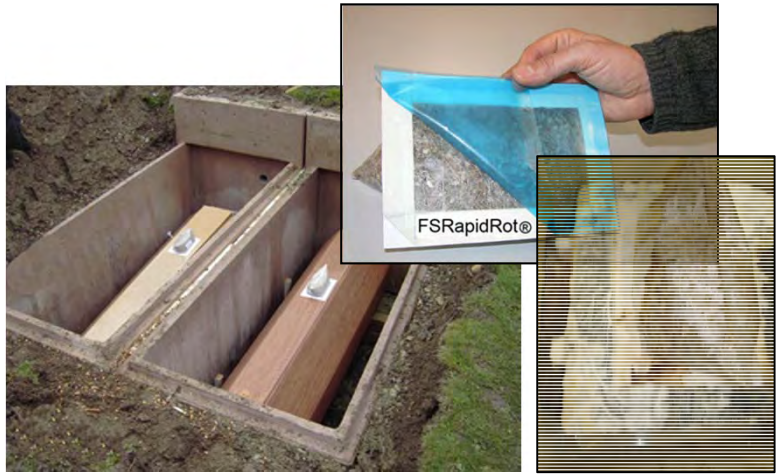
DeFlorio et al. (2005) Dendrochronologia 22, 123-130.

Increase in wood permeability



Schwarze et al. (2006) Holzforschung.

Decomposition of coffins



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Improvement of acoustics

Schwarze et al. (2008). New Phytologist 179, 1095-104.

"Mycowood" project

- Influence of climate on the quality of resonance wood
- Old Cremona violins passed their zenith
- Increasing size of concert halls.

Concert hall Lucerne

How can we improve the acoustic properties of contempory resonance wood to compensate supply shortages?

Schwarze et al. (2008). New Phytologist 179, 1095-104.

Violins of Antonio Stradivari (1644-1737)

Stradivari's earliest extant label is dated 1666 and his last 1737. His finest instruments were made after 1700. He produced some 1,100 instruments, of which about 540 violins, 12 violas and 50 cellos are known. His workmanship brought the violin to perfection.

Year	Stradivarius	Manufactured	Price
1998	„Kreutzer“	1727	€ 1,5 Mio
2005	„Lady Tennant“	1699	€ 1,5 Mio
2006	„Hammer“	1707	€ 2,8 Mio
2007	„Solomon“	1729	€ 2,0 Mio
2007	„Joseph Joachim“	1715	€ 6,8 Mio
2008	„Barrow“ Eigentlich „de Barrau“	1715	€ 7,2 Mio
2011	„Lady Blunt“	1721	€ 11 Mio

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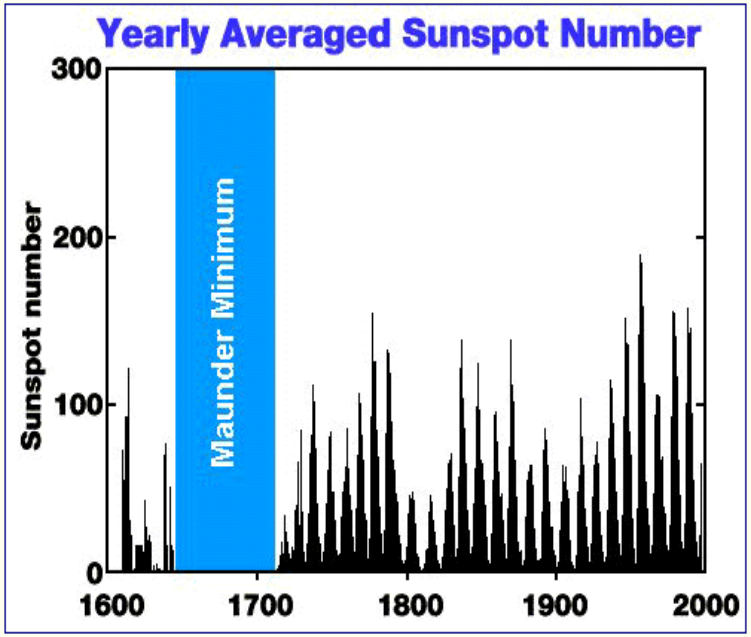
Maunder Minimum



During the coldest part of the Little Ice Age, from 1645 to 1715, there is believed to have been a decrease in the total energy output from the Sun, as indicated by little or no sunspot activity.

Known as the Maunder Minimum, astronomers of the time observed only about 50 sunspots for a 30-year period as opposed to a more typical 40-50,000 spots.

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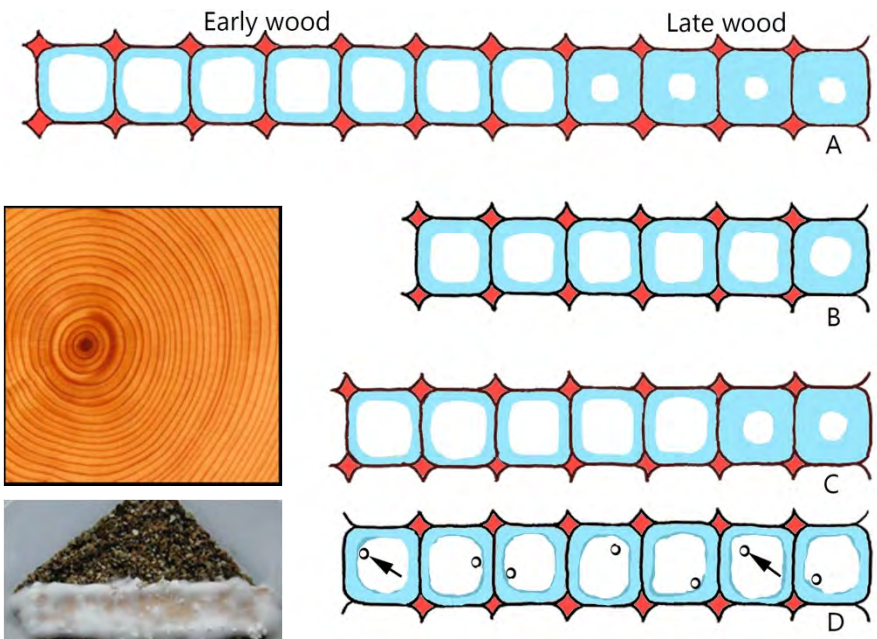
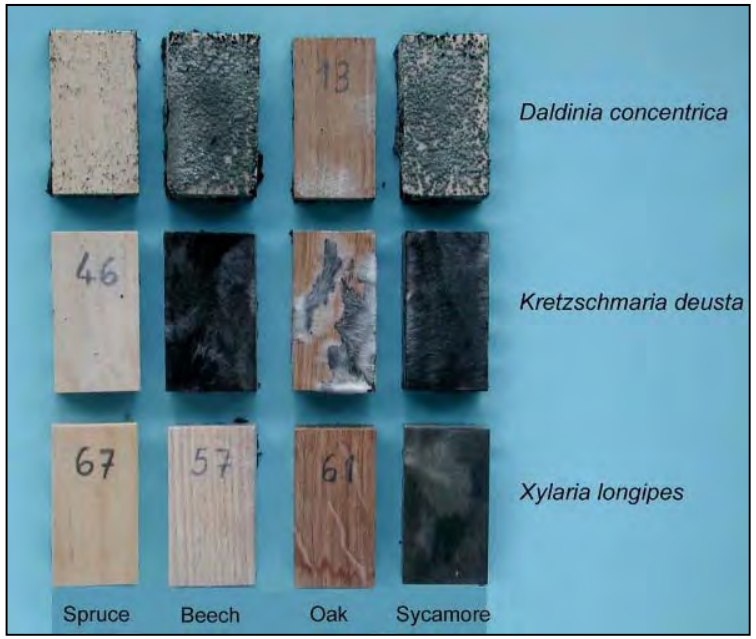
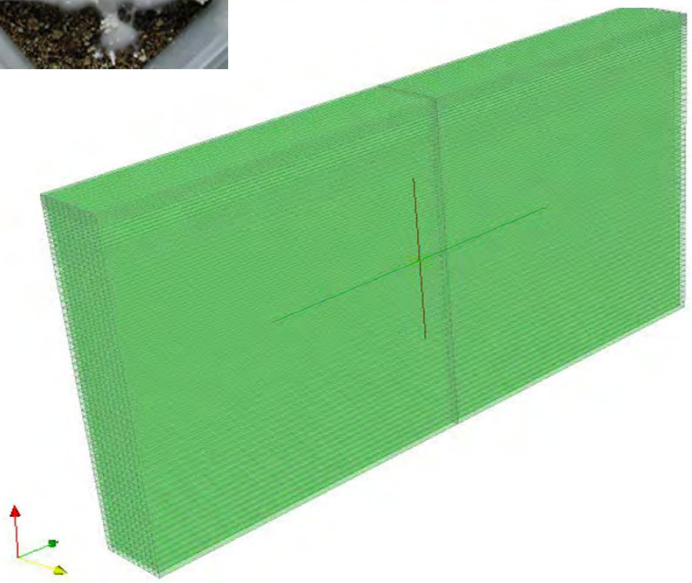
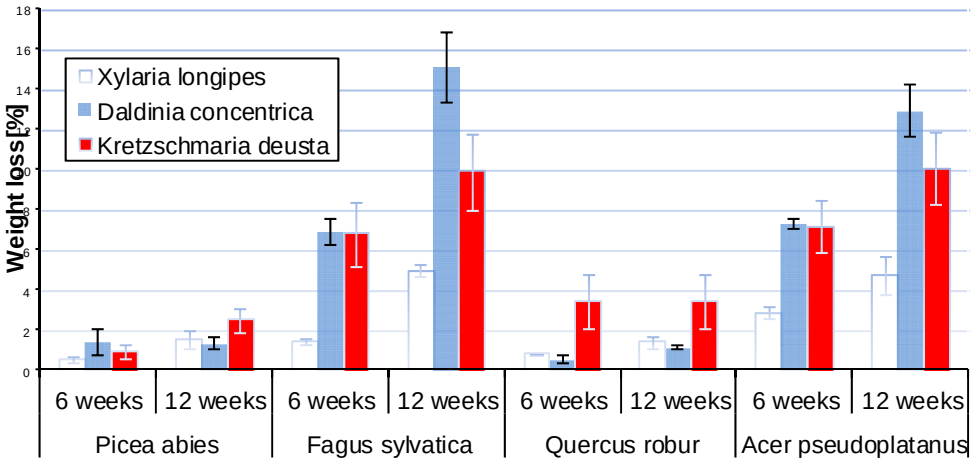
Schwarze et al. (2008). New Phytologist 179, 1095-104.

Wood decay fungi as a substitute cold climate









Schwarze (2008). Fungal Biology Reviews 21, 33-170.

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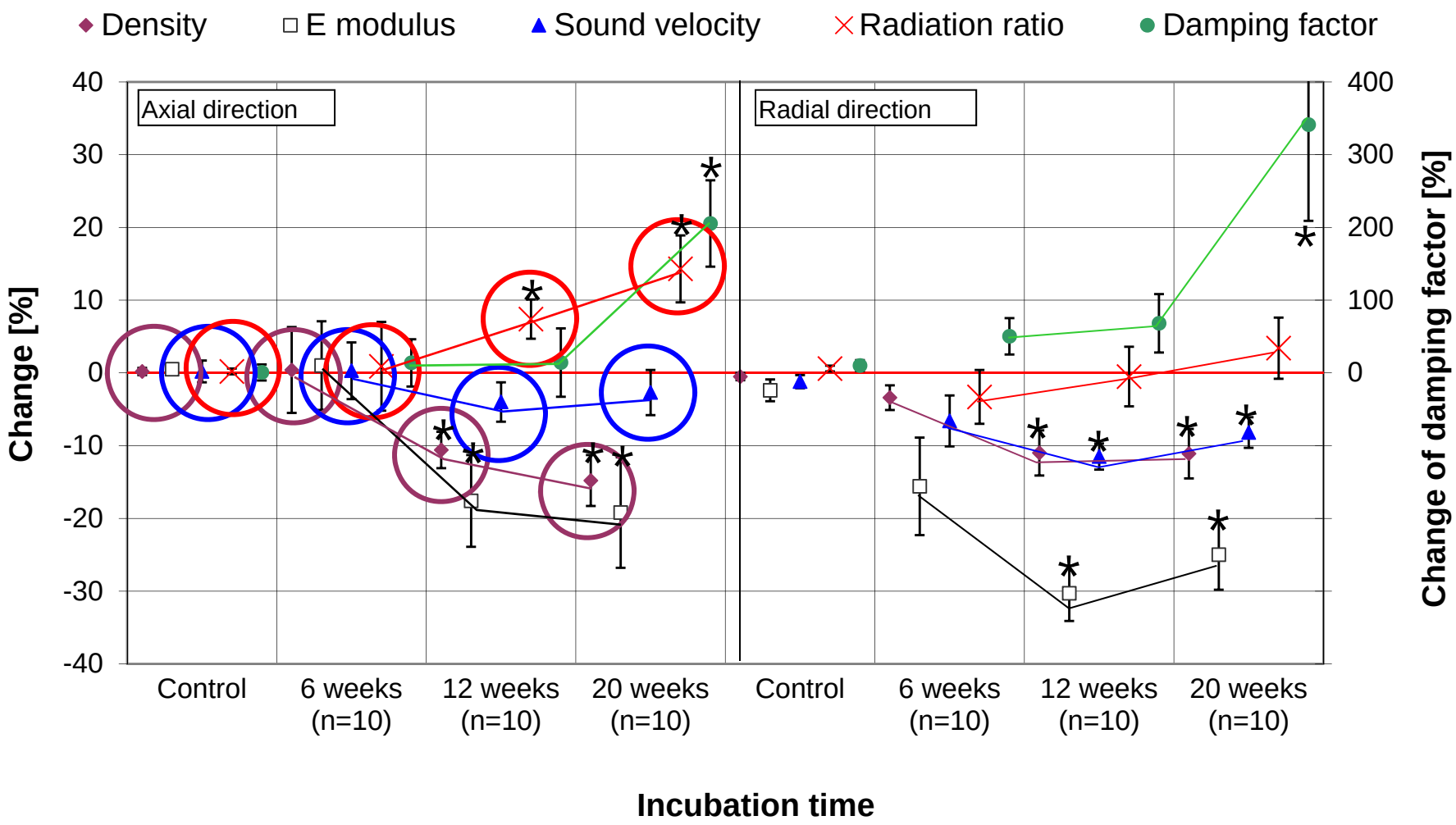
Norway spruce wood incubated with *Physisporinus vitreus*



Schwarze et al. (2008). New Phytologist 179, 1095-104.

Manufacture of fungally treated wood for violins

Opus 58 / CT Scans
Berend Stoel, Leiden University Medical Center, NL.

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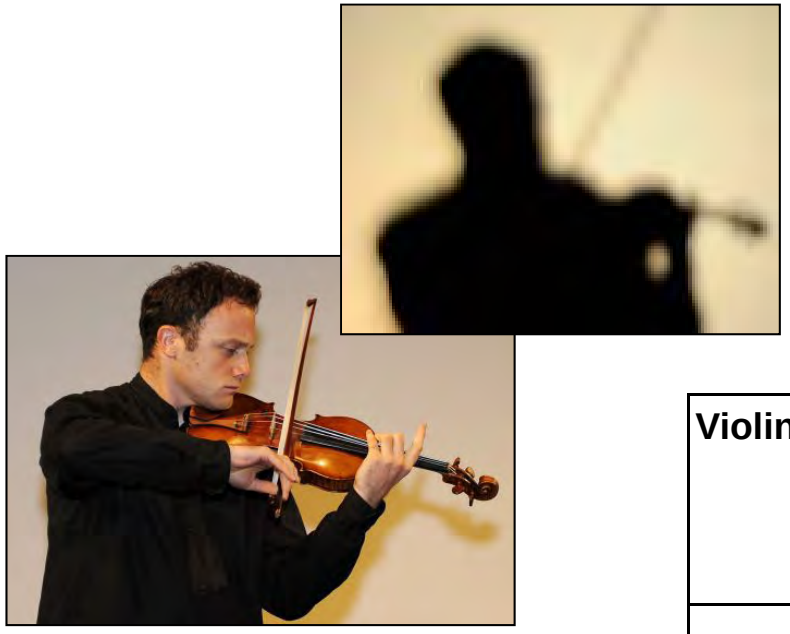
Blind test at the 27. Osnabrücker Baumpflegetagen (2009)



Mathew Trusler

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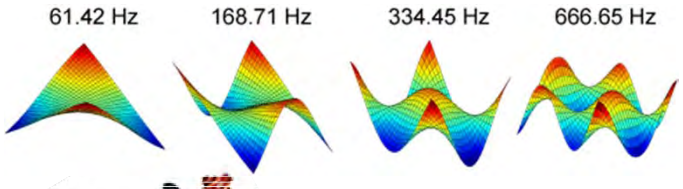
Violin	Opus 55 untreated	Opus 56 6 months fungal-treated	Opus 58 9 months fungal-treated	Stradivarius	Opus 54 untreated
Which violin did you like most?					
No. votes	9	24	90	39	19
Which violin was the Stradivari?					
No. votes	3	15	113	25	17

TIME

Walter Fischli-Foundation



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Rot offers fresh sound for violin makers

ABC News

Sounds like a Strad? Must be the mushrooms

ScienceDaily
Your source for the latest research news

Fungus-treated violin outdoes Stradivarius

TIMESONLINE

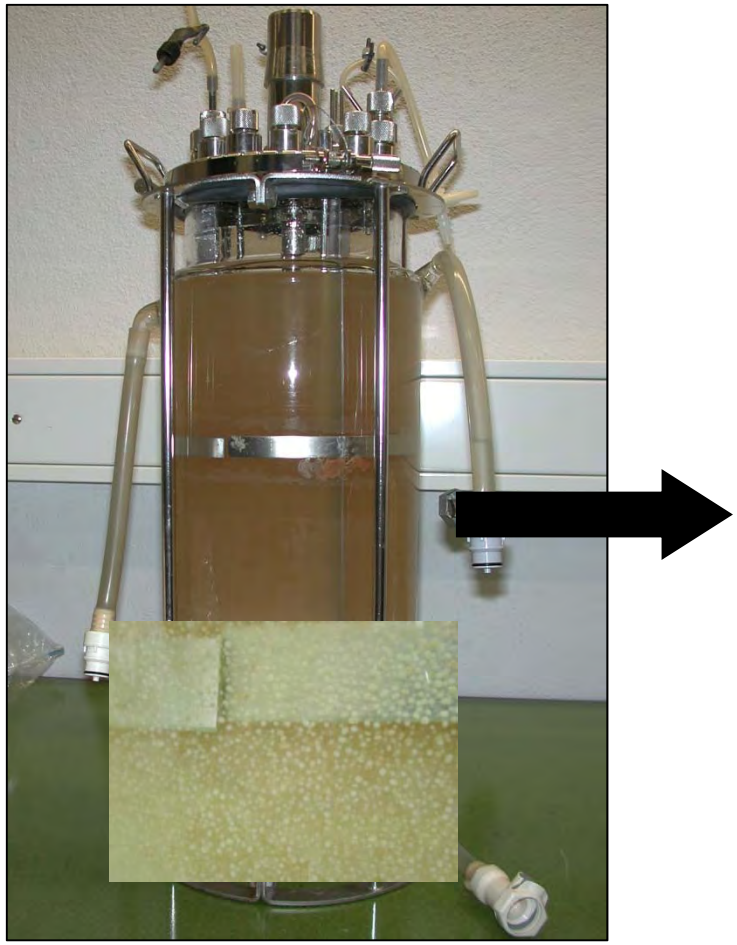
Biotech impostor rivals Stradivarius violin

nature biotechnology

The biotech Stradivarius

- Development of a quality assurance programme for the production of superior resonance wood.

Incubation method



Cultivation of the fungus in a bioreactor.

Contaminations of the wood by *Trichoderma* spp.

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What is *Trichoderma*?

Trichoderma spp. are free-living fungi that are common in soil and root ecosystems.

Recent discoveries show *Trichoderma* spp. are opportunistic, avirulent plant symbionts, as well as being parasites of other fungi.

Harmann (2004). Nature Reviews Microbiology 2, 43-56

Taxonomy of *Trichoderma*

Mycota

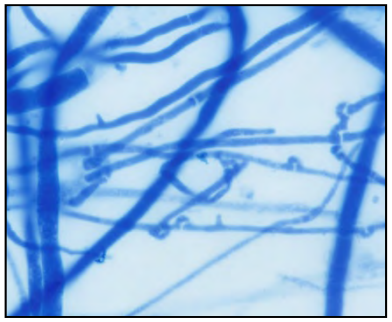
Divison	Class	Order	Family
Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae

Important species used for biocontrol:

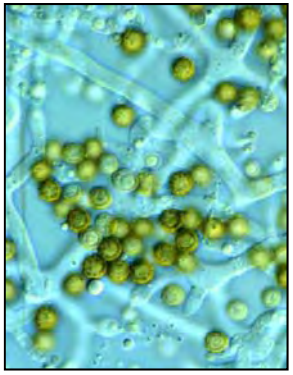
Trichoderma atroviride
Trichoderma harzianum T22
Trichoderma hermatum
Trichoderma virens

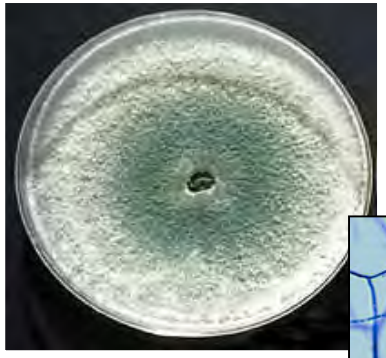
Microscopic features of *Trichoderma*

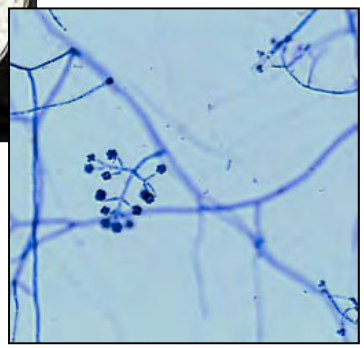
Chlamydospores



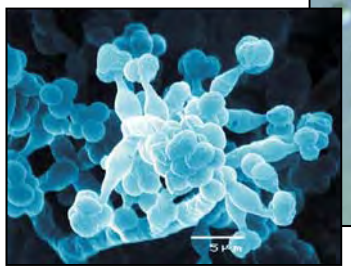
Hyphae

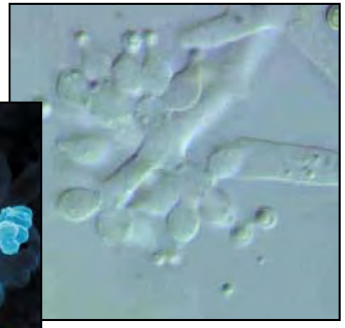






Conidiophores





Phialides

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Septate hyaline hyphae. Conidiophores are hyaline, branched, phialides are hyaline, flask-shaped, and inflated at the base.

The colour of the conidia is mostly green. *Trichoderma* spp. may also produce chlamydospores.

Bumble bees as bee-delivery technique for the dispersal of *Trichoderma*

- Insertion of *Trichoderma* conidia into the bee hive
- Bees deposit conidia on flowers they visit as they search for pollen and nectar
- Bee-delivery is twice as effective as spraying
- Strawberry yields improve by 20 -30 %.

Trichoderma is highly effective when applied to blossoms or fruits for control of *Botrytis cinerea*

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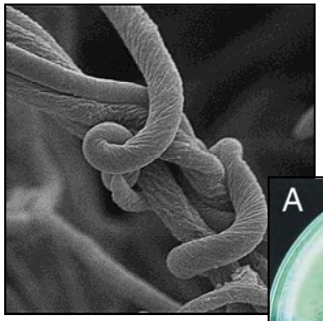
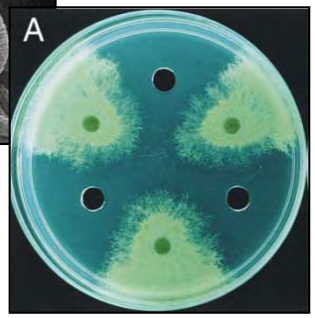
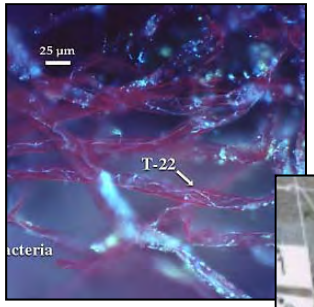
Mechanisms of biological control with *Trichoderma* spp.

- Mycoparasitism
- Antibiosis
- Competition for nutrients or space
- Tolerance to stress through enhanced root and plant development
- Solubilization and sequestration of inorganic nutrients
- Systemic aquired resistance (SAR)
- Inactivation of the pathogen´s enzymes

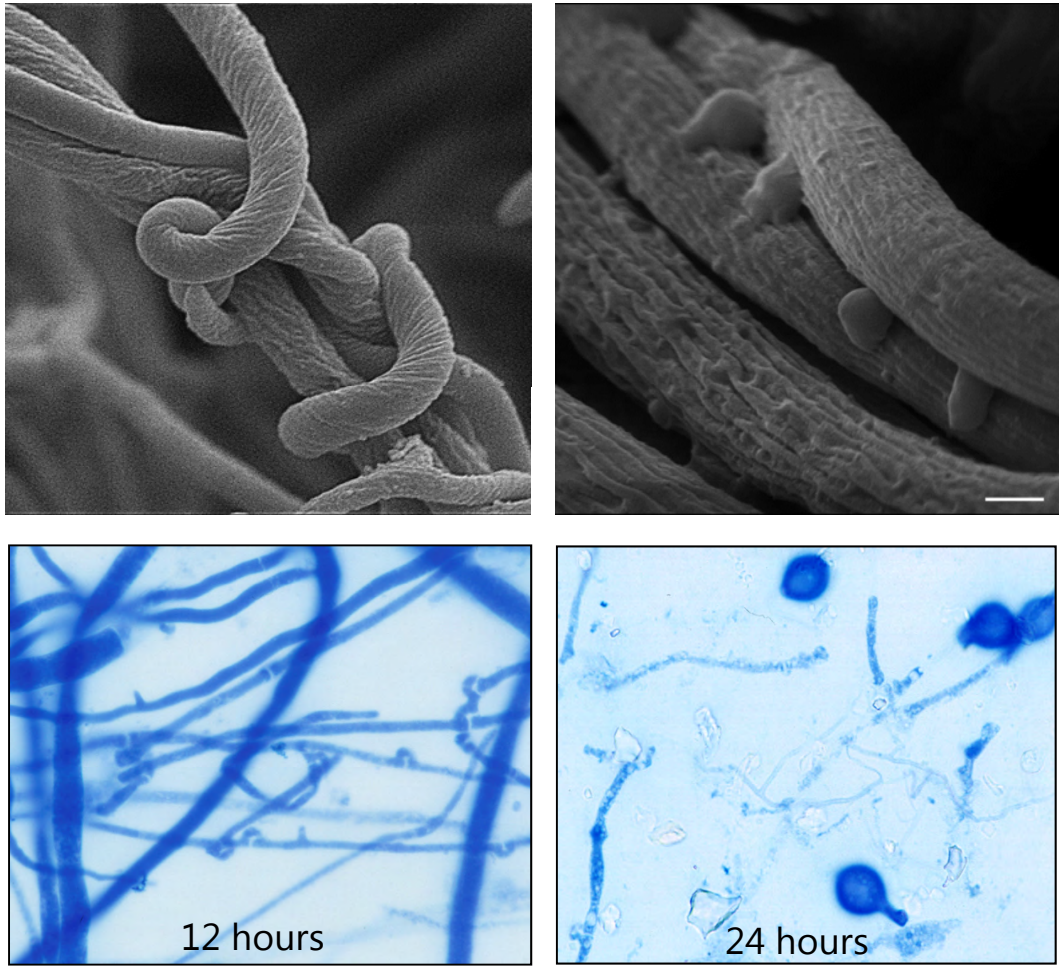






Mycoparasitism

Trichoderma spp. grow tropically toward hyphae of other fungi, coil about them in a lectin-mediated reaction, and degrade cell walls of the target fungi.

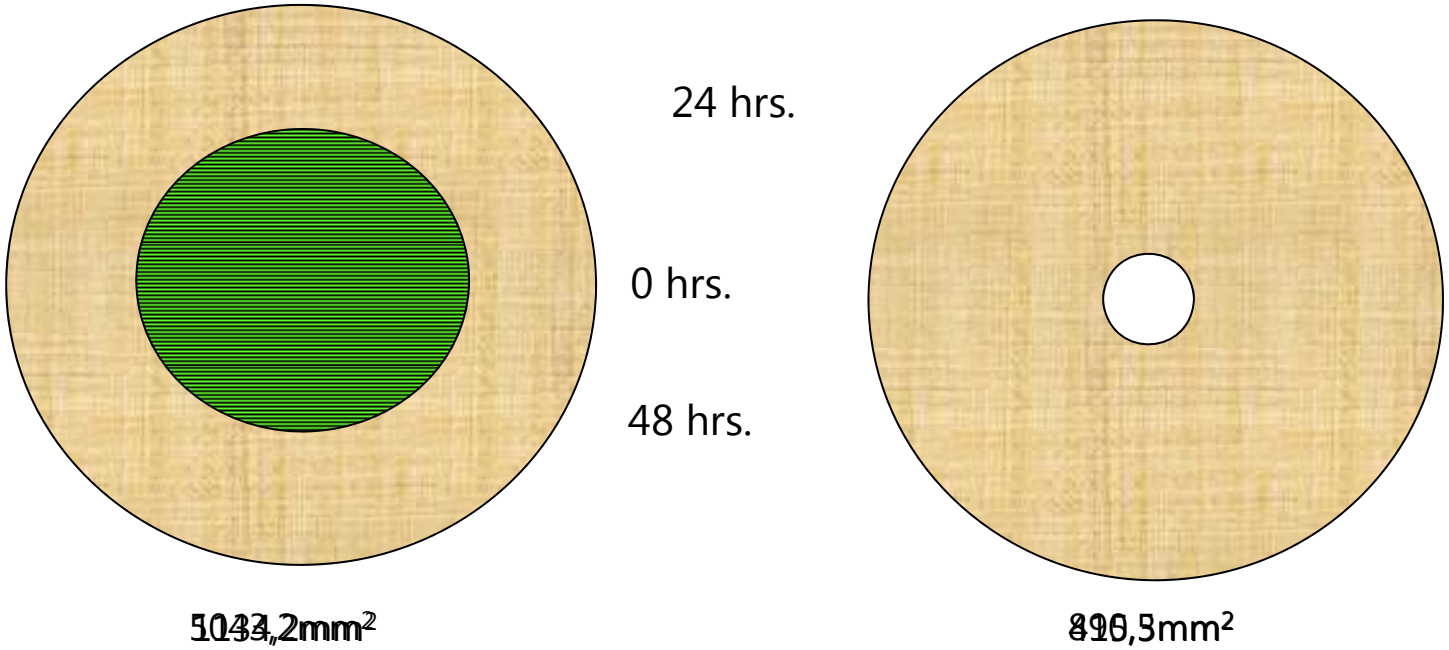


Schubert et al. (2008). Biological Control 45, 111-123.

Growth rates of *Trichoderma* and wood decay fungi

Trichoderma spp.

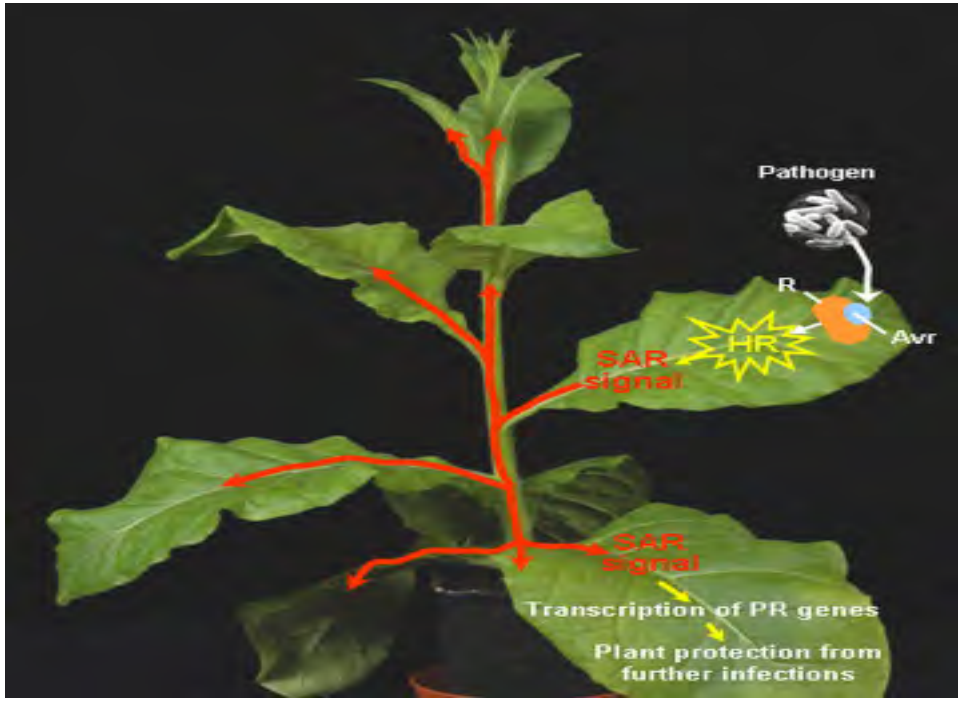
Wood decay fungi



Ø 5fold stronger growth rate!

Schubert et al. (2008). Biological Control 45, 111-123.

Systemic aquired resistance (SAR)



Development of systemic acquired resistance (SAR) that is dependent on the phytohormone salicylic acid. A pathogen secretes effectors (Avr) that are recognized by resistance (R) proteins and trigger the development of hypersensitive response (HR). HR can activate a SAR signal that leads to the production of pathogenesis related (PR) proteins and can potentiate other plant tissues against a broad spectrum of same or other pathogens (Hofius et al., 2007).

Harmann (2004). Nature Reviews Microbiology 2, 43-56

Some *Trichoderma* strains clearly are potent inducers of SAR-like responses. When inoculated onto roots or leaves they can provide control of disease caused by *Botrytis cinerea* on leaves spatially separated from the site of application of the biocontrol agent.

Biological control of wood decay fungi with *Trichoderma* species

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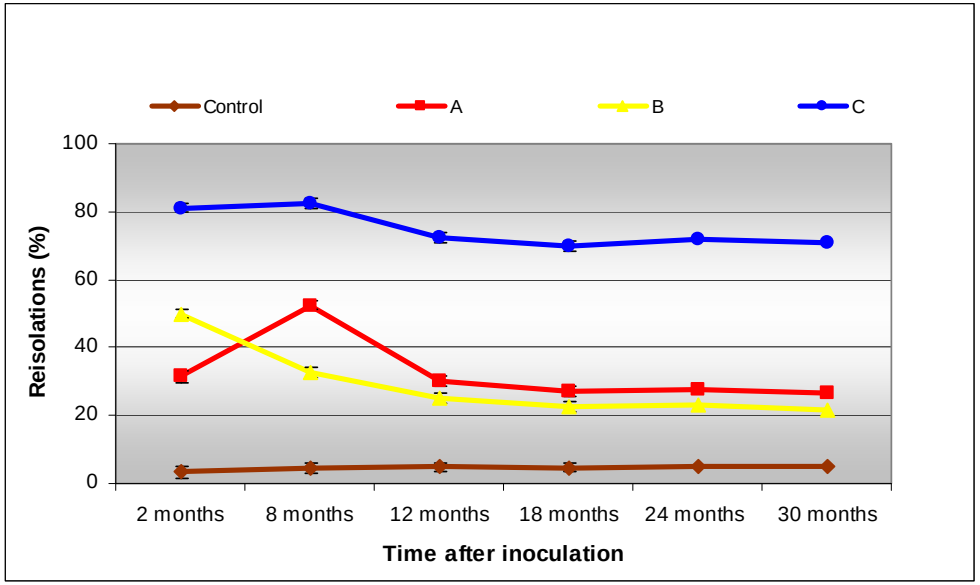
BIO-INNOVATION AB



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Schubert et al. (2008). Biological Control 45, 111-123.

Trichoderma Research Singapore

Pruning wounds as infection courts for wood decay fungi

- Disturbs resource allocation among growth, defense, reproduction and storage
- Degrades material strength and load-bearing capacity of branches
- Results in sunscald, tissue necrosis, and cambial dieback

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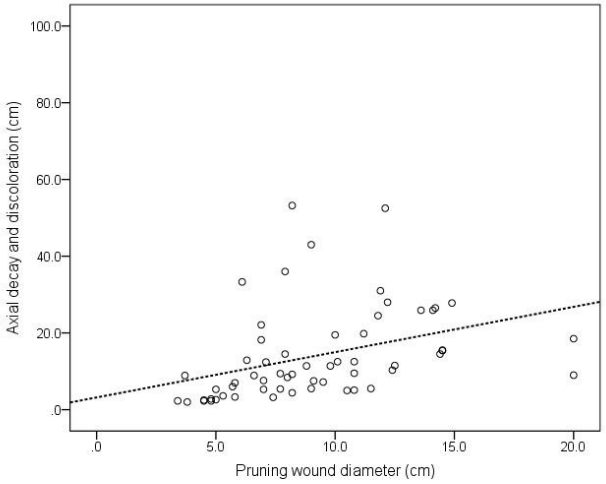
Identify causal fungi

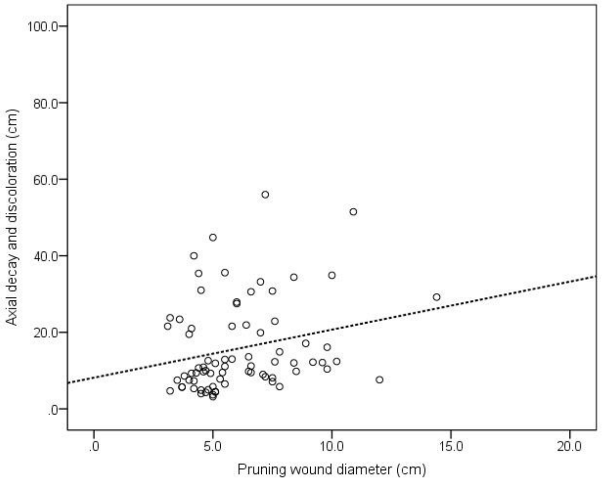
Host/ fungus interactions

Biological control

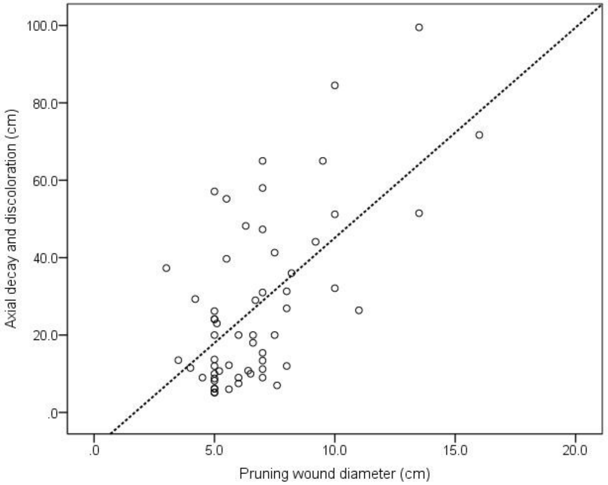
Khaya senegalensis



Peltophorum pterocarpum



Samanea saman



Identify causal fungi → Host/ fungus interactions → **Biological control**

1. Treat fresh pruning wounds with *Trichoderma* conidial suspensions
2. Monitor colonization and persistence of *Trichoderma* on pruning wounds
3. Evaluate the effect of *Trichoderma* on wound occlusion and infection rates

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Wood decay triangle

Total of conditions favouring susceptibility

HOST

Each side of the triangle represents one of three components. If the three components of the decay triangle could be quantified, the area of the triangle would represent the amount of decay.

The more unfavourable the conditions that help the pathogen the shorter the pathogen side would be and the smaller the potential amount of decay.

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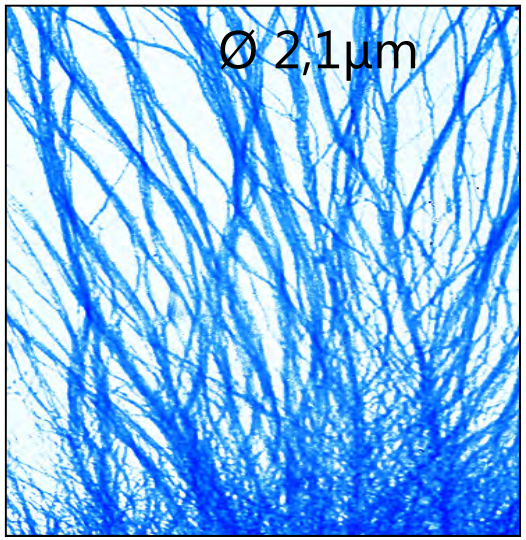
Ganoderma species

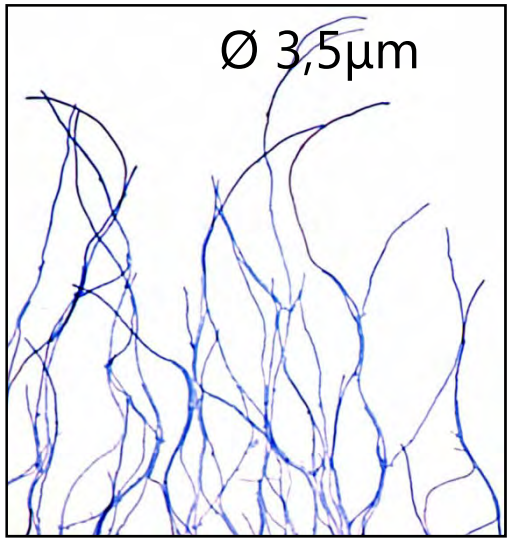
G. adspersum

G. lipsiense

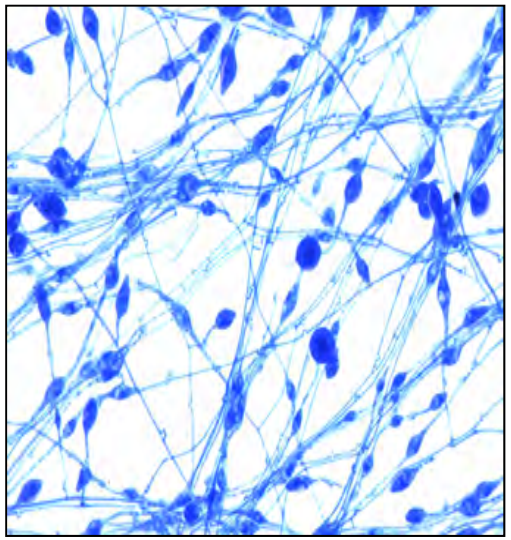
G. resinaceum



Hyphal strands at the colony margin



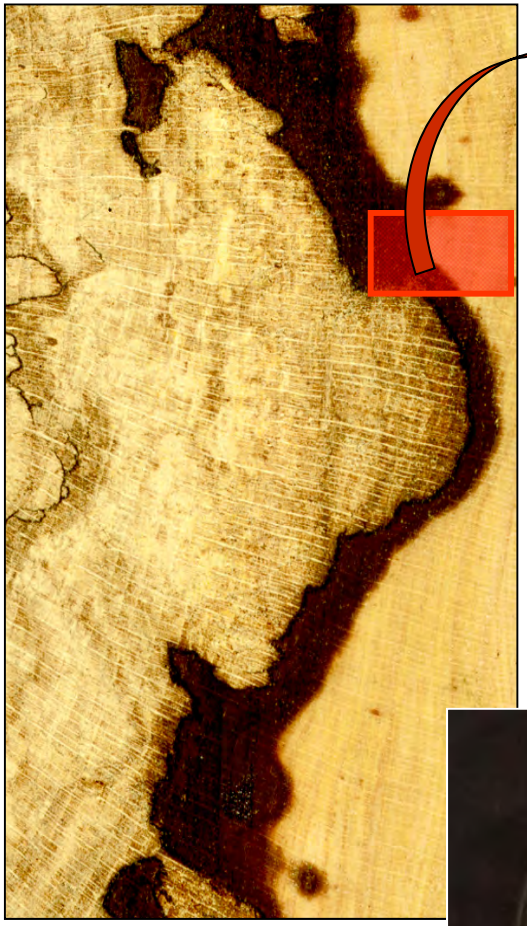
Hyphal strands absent

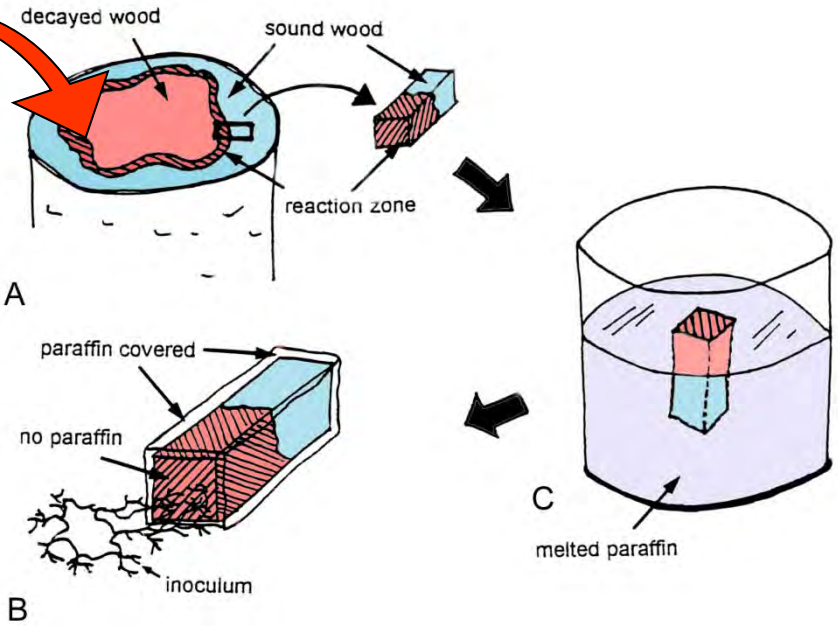


Chlamydospores

Schwarze & Ferner (2003). Arboric. Journal 27, 59-77.

Reaction zones challenged by *Ganoderma* species





Schwarze & Baum (2001)
New Phytol. 146, 129-140.

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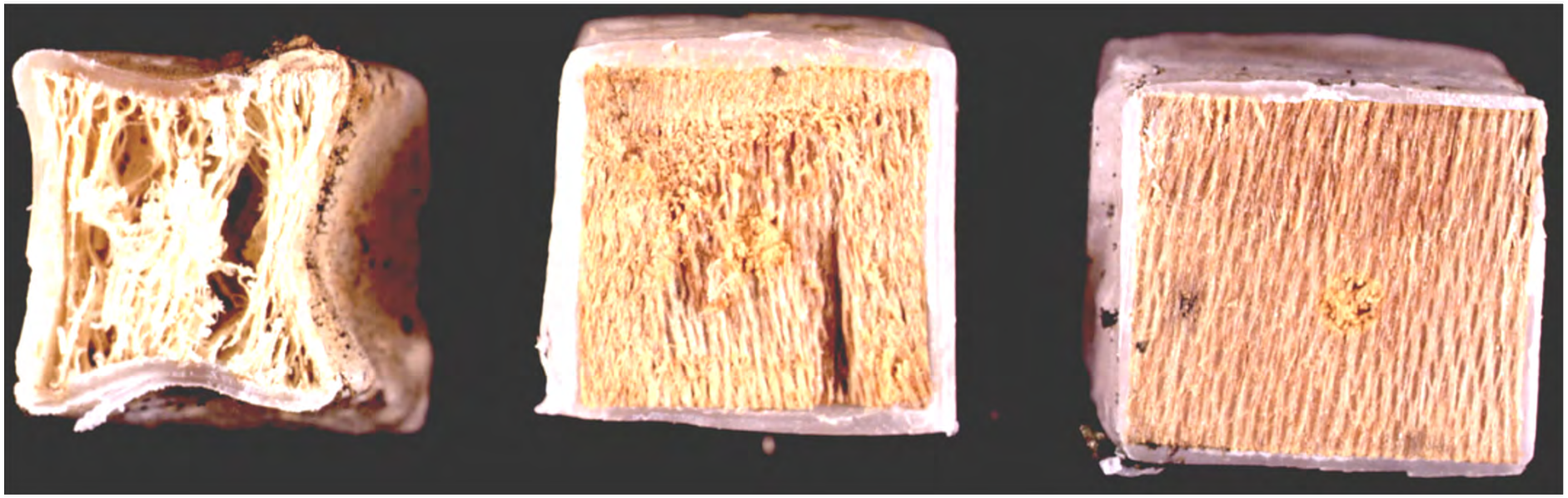
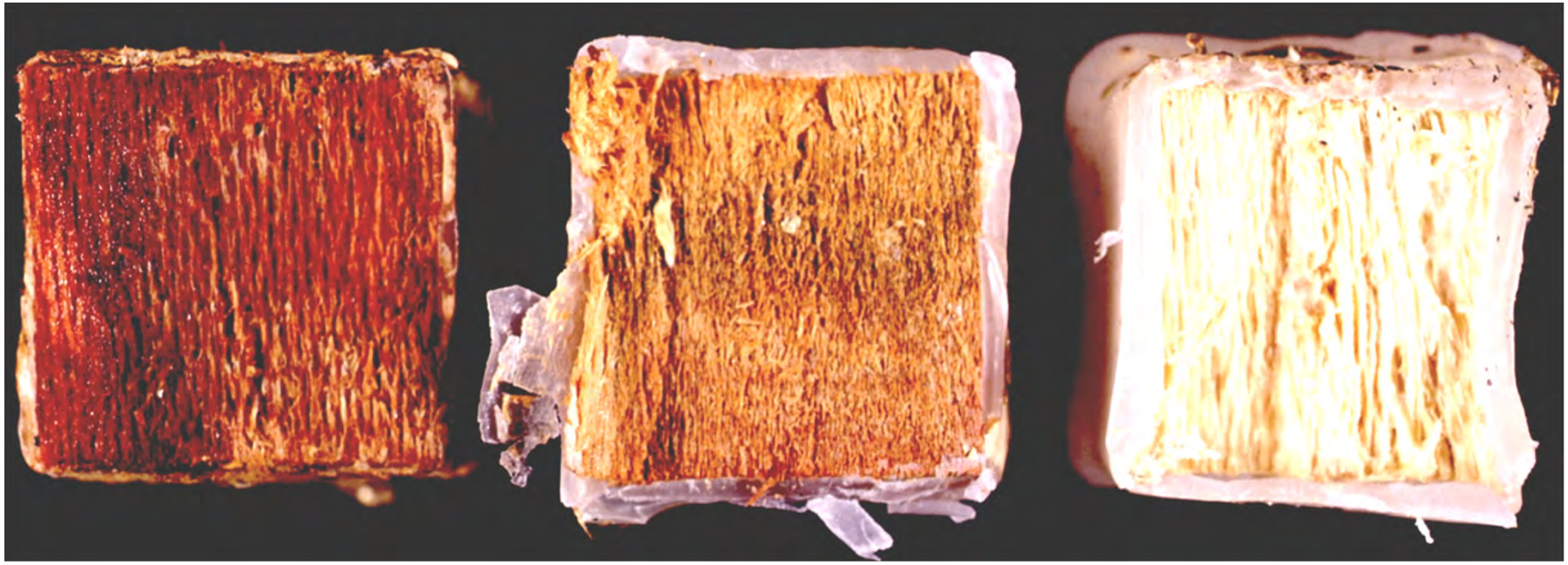
London plane wood blocks without reaction zones challenged by *Ganoderma* species



G. lipsiense *G. resinaceum* *G. adspersum*

Schwarze & Ferner (2003). Arboric. Journal 27, 59-77.

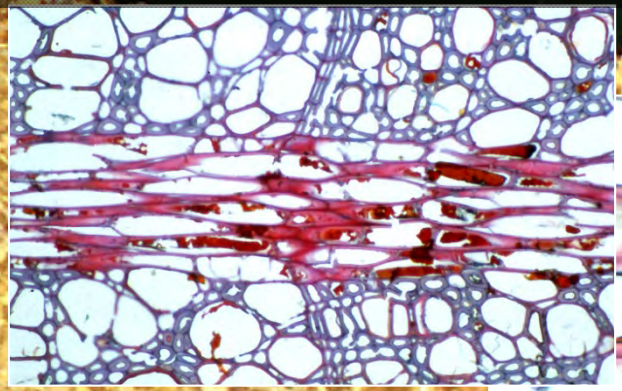
London plane wood blocks containing reaction zones challenged by *Ganoderma* species



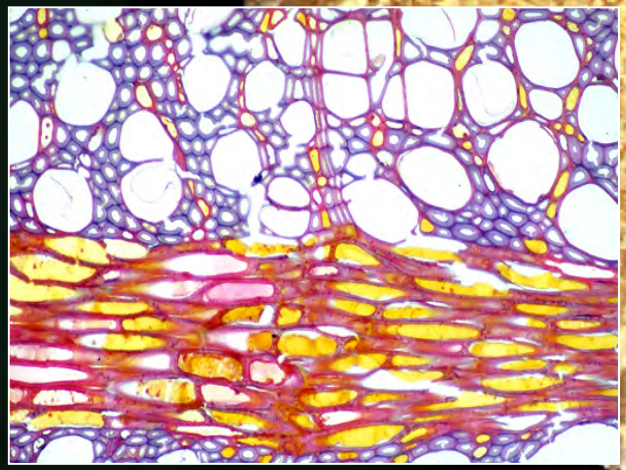
G. lipsiense *G. resinaceum* *G. adspersum*

Schwarze & Ferner (2003). Arboric. Journal 27, 59-77.

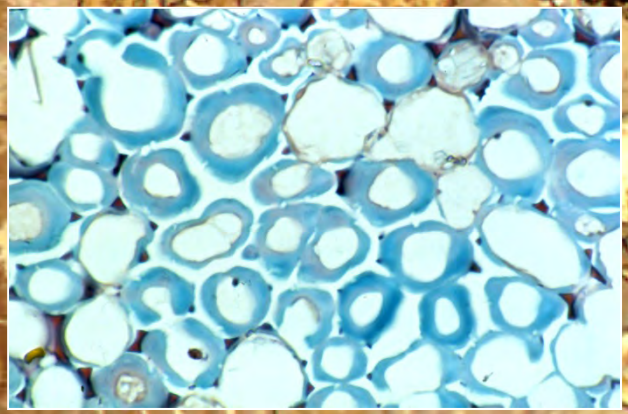
Features of London plane reaction zones challenged by *Ganoderma adspersum*



Preferential degradation of polyphenols



Abundant polyphenols

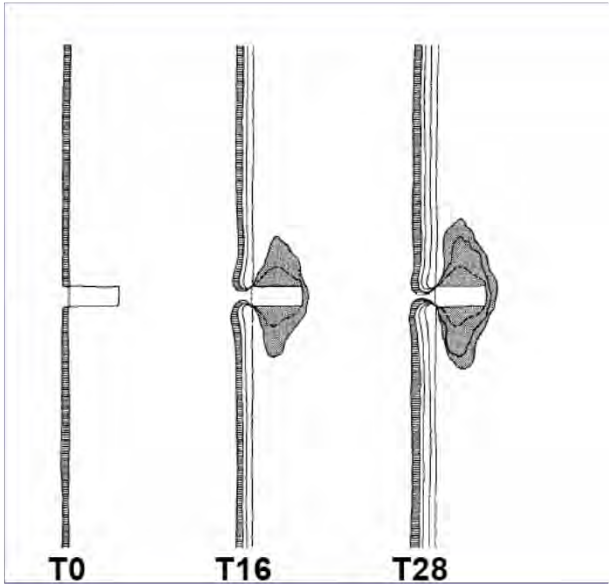


Selective delignification

Schwarze & Baum (2001)
New Phytol. 146, 129-140.

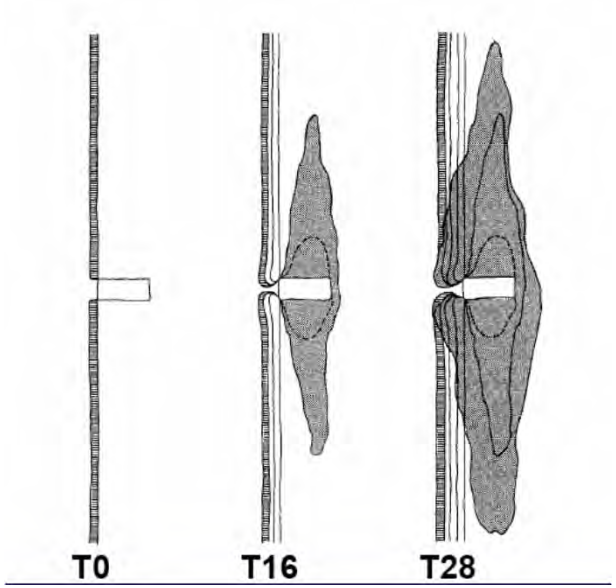
Inoculation trials with wood decay fungi

Saprophyt



Ganoderma lipsiense

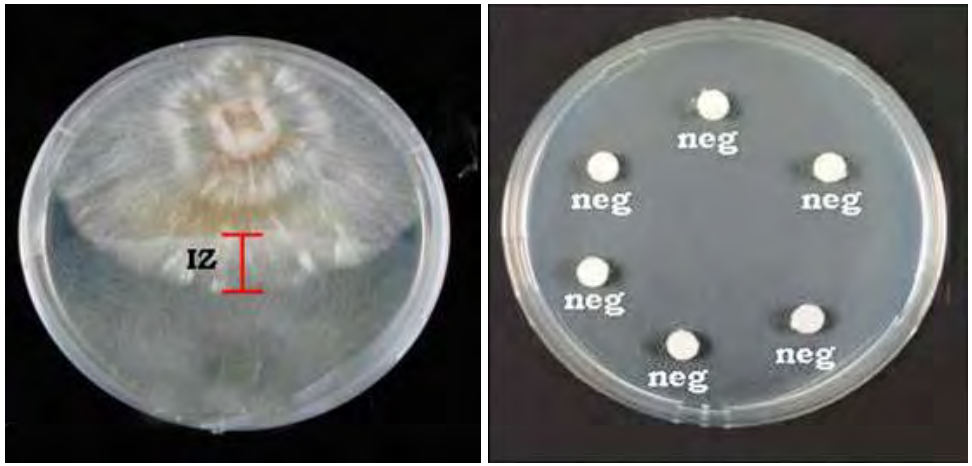
Facultative parasite



Ganoderma adspersum

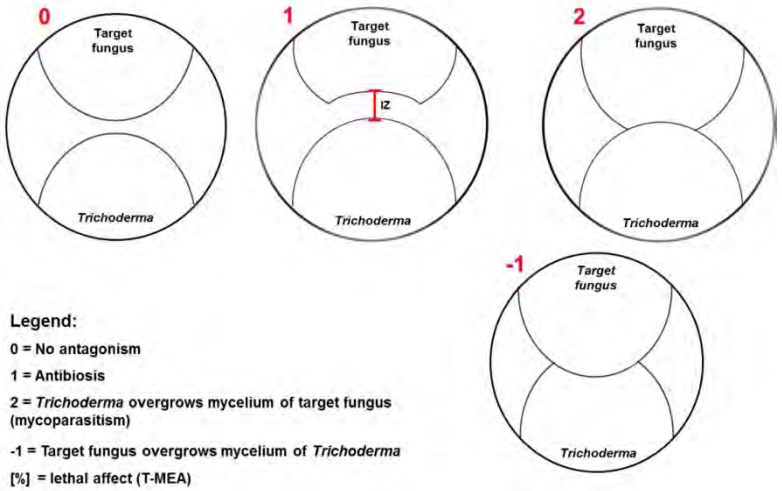
Deflorio et al. (2005) Botany 87, 26-35.

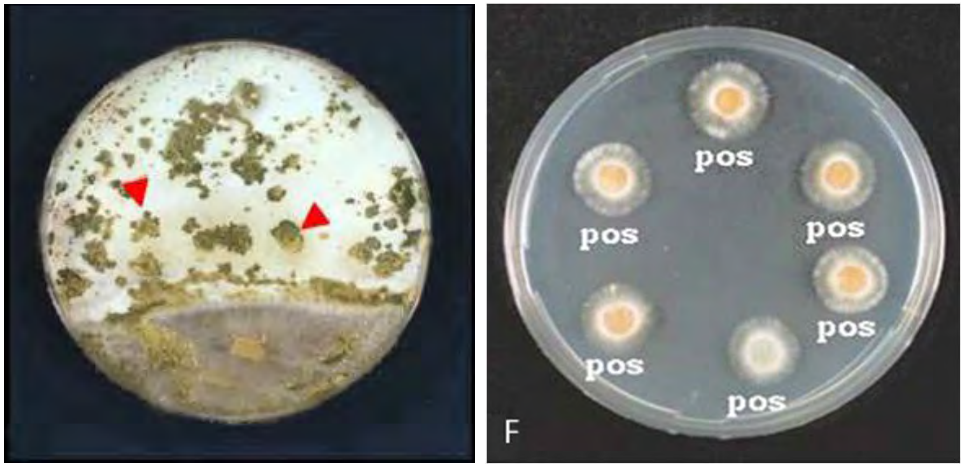
Dual culture test



Ganoderma adspersum

[100%] = lethal affect (T-MEA)

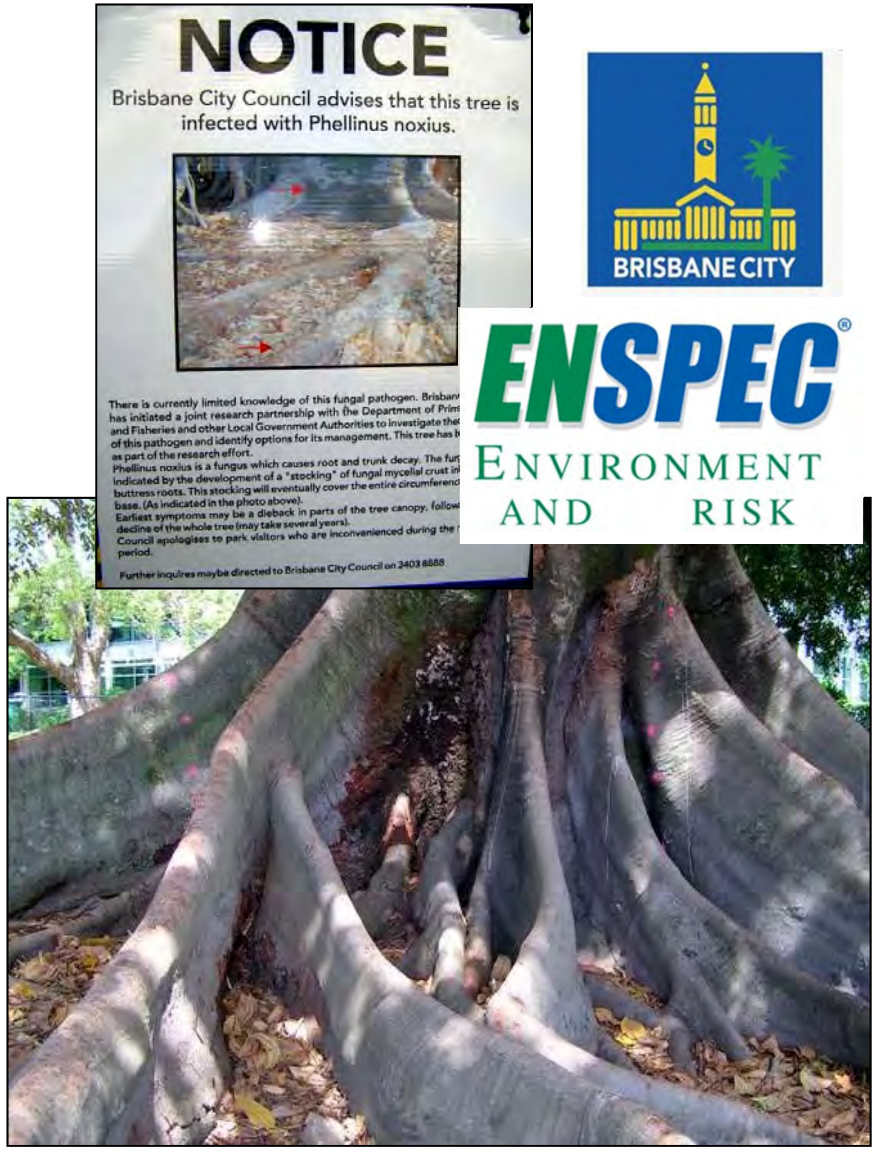




Ganoderma lipsiense

[0%] = lethal affect (T-MEA)

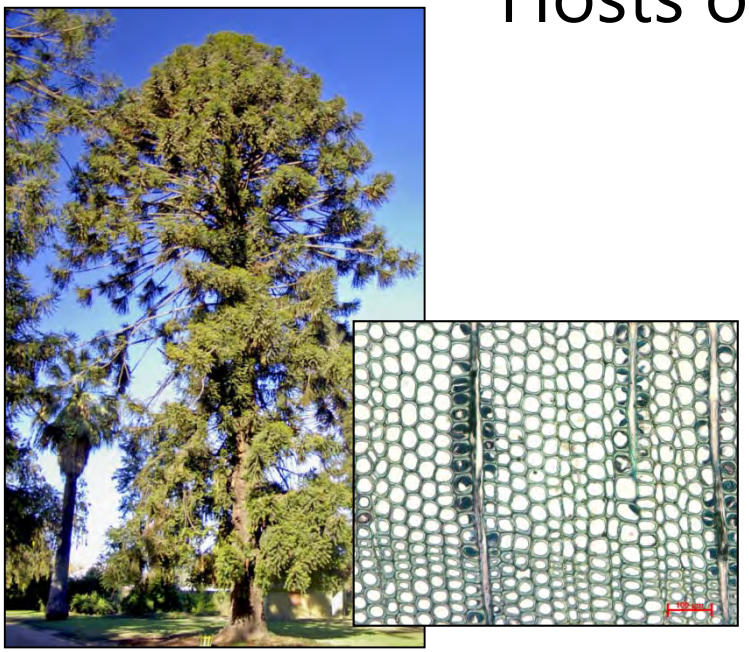
Phellinus noxius (Brown root rot)



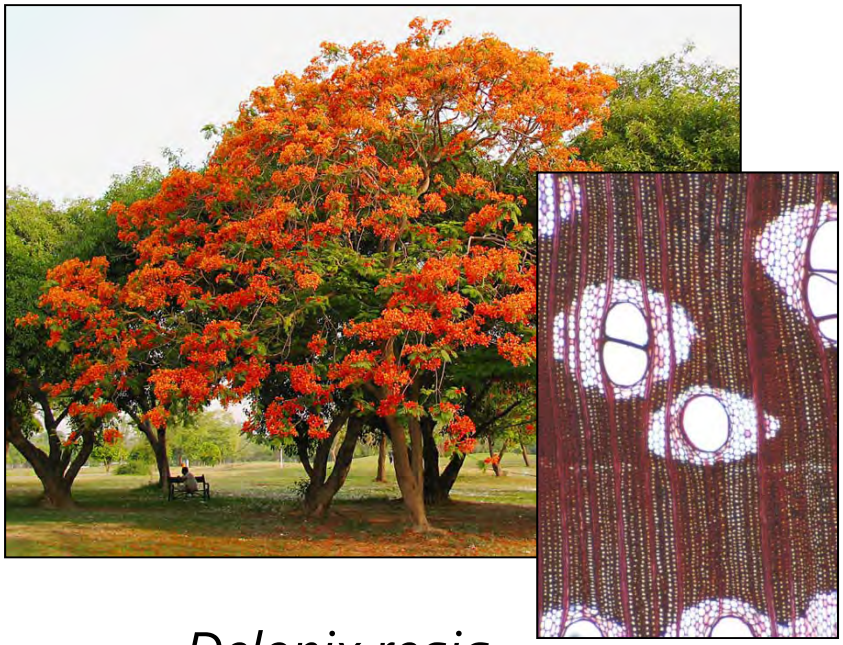
Oil palm plantation

Schwarze et al. (2012) Biological Control 61, 113-184.

Hosts of *Phellinus noxius*



Araucaria bidwillii



Delonix regia

Ficus benjamina

Jacaranda mimosifolia

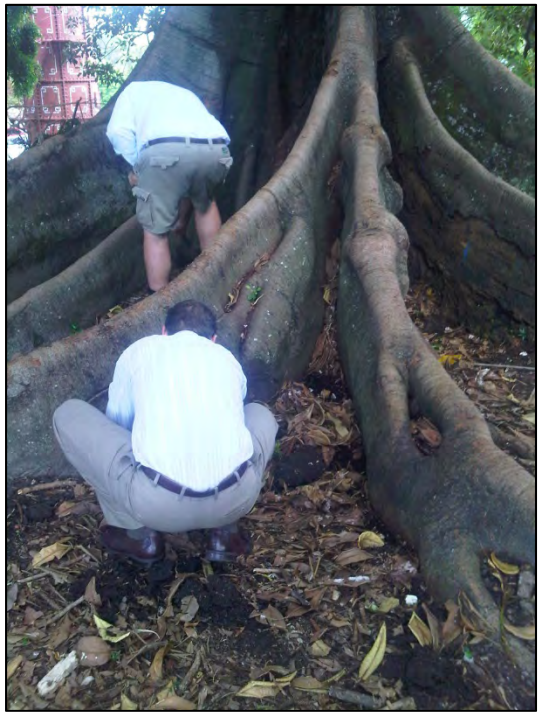
I Monumentali, Varese

In vitro screening of an antagonistic *Trichoderma* strain against *Phellinus noxius*

- Bioassays to evaluate growth rates
- Inhibitory effects of volatile organic compounds (VOCs) produced by *Trichoderma* in the presence of wood decay fungi
- Dual culture tests on MEA
- Evaluation of antagonistic activity in wood.

Schwarze et al. (2012) Biological Control 61, 113-184.

Biocontrol of *Phellinus noxius* (Brown root rot)



Keith Foster, Senior Coordinator Arboriculture, Brisbane City Council.

Biocontrol of *Phellinus noxius* (Brown root rot)

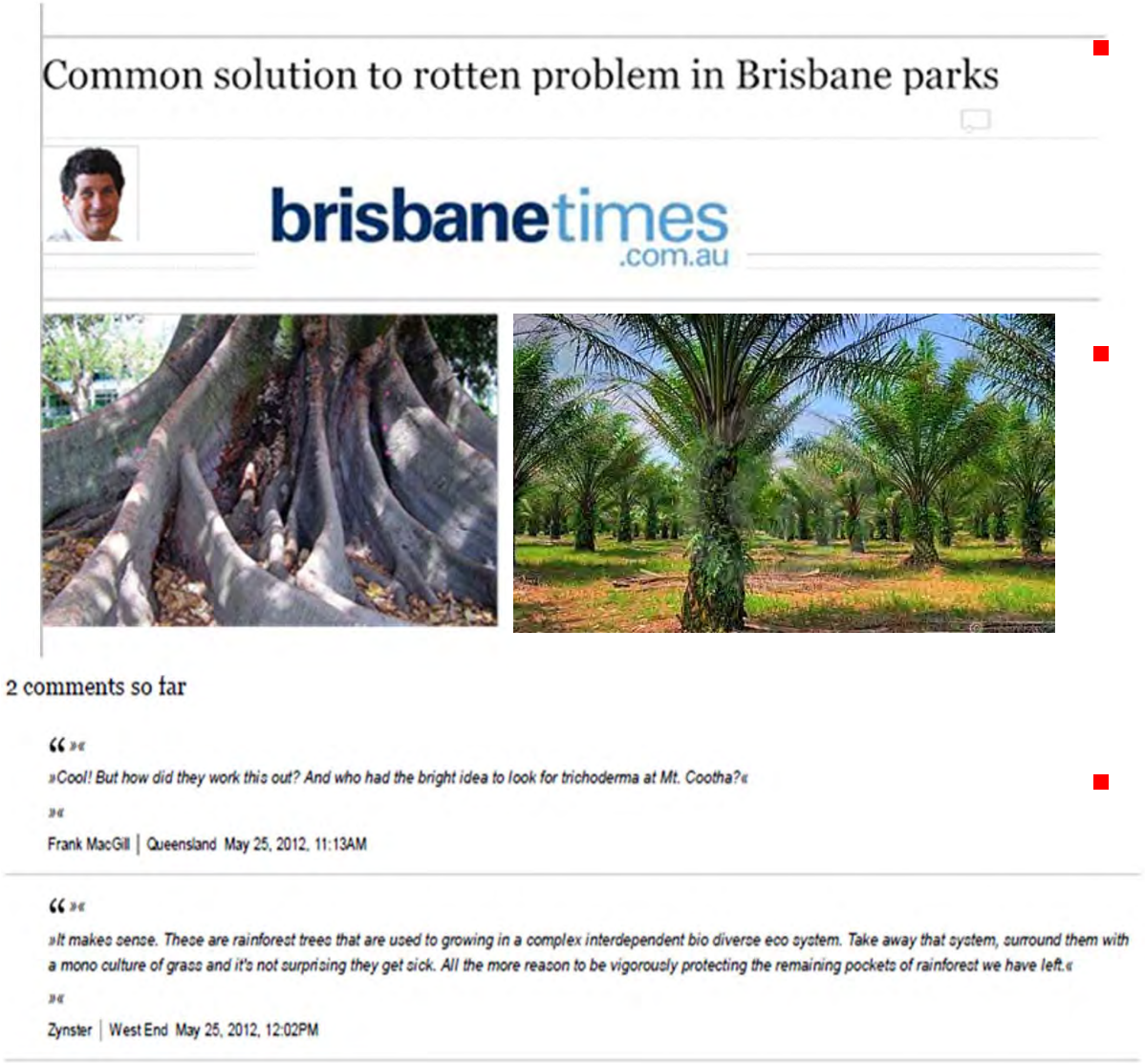
Keith Foster, Brisbane City Council:

"A great outcome, I would like to thank you so much for your help, the trees that we have used the *Trichoderma* on are all recovering. The Banyan fig at Shorncliffe prior to introducing the *Trichoderma* had little leaf in the canopy and what leaf was left were yellow, now it has a new canopy of leaf with a dark green color. Amazing.

Once again thank you, without you help, we would not have been in a situation where we can manage the diseased trees, they would have been lost.

I Monumentali, Varese

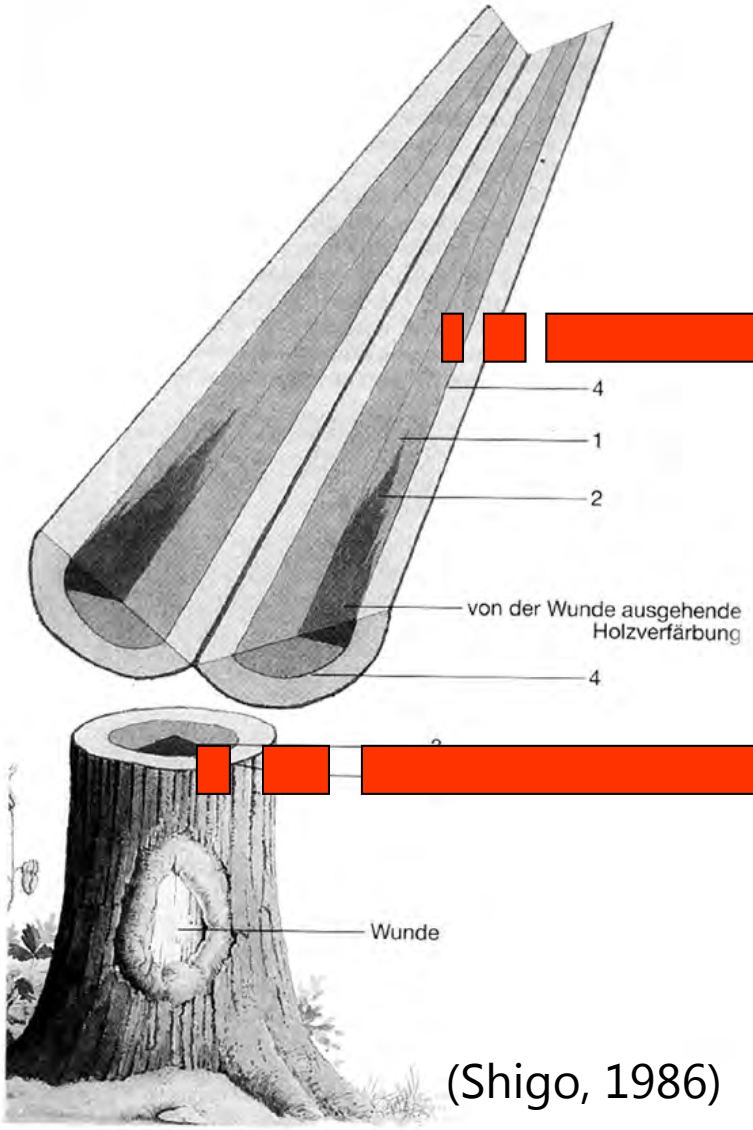
Summary



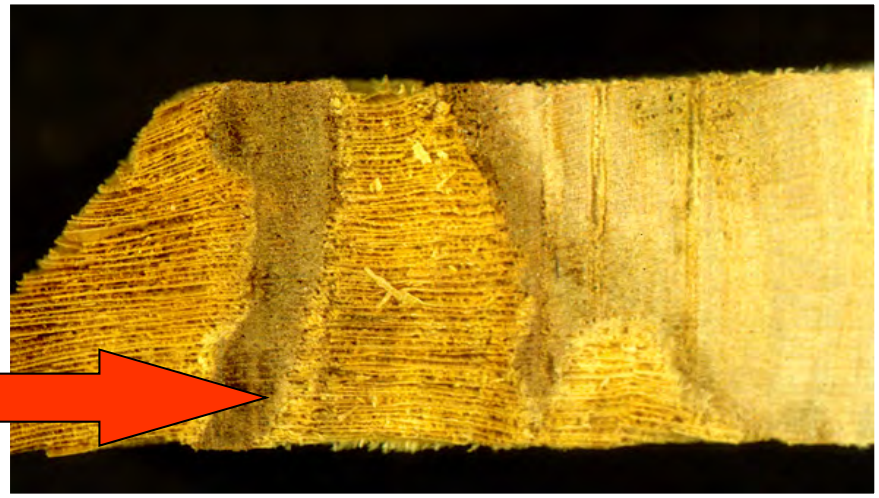
- Biocontrol methods may be beneficial for urban trees growing on sites with low soil biodiversity
- Circumstantial evidence suggests that carefully selected antagonists can be applied against wood decay fungi that are weak competitors
- *Trichoderma* spp., can reduce decomposition rates of opportunistic wood decay fungi in trees and induce SAR.

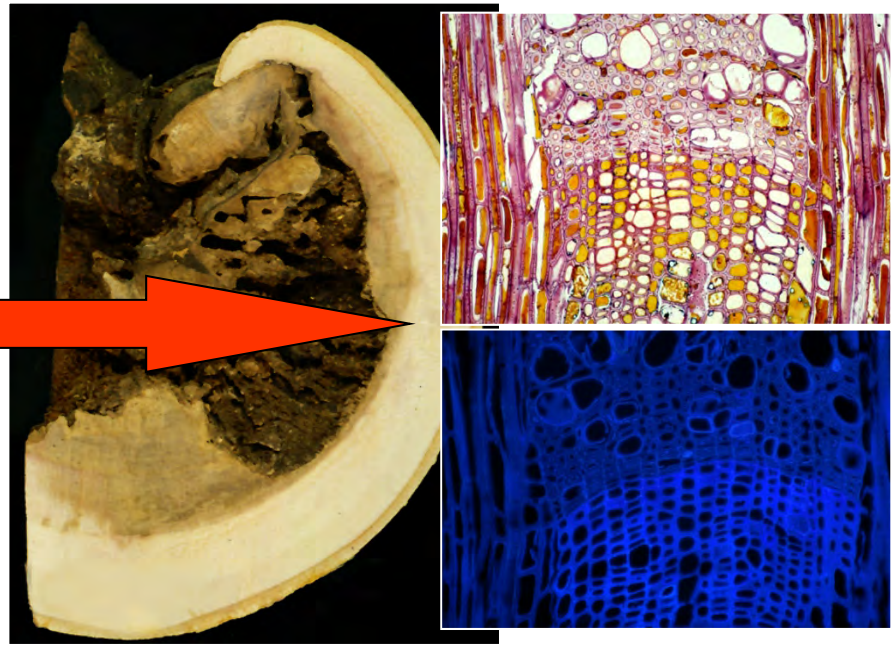
Epilog

Reaction zones and barrier zones



(Shigo, 1986)





(Schwarze, 2008)

Decay associated symptoms

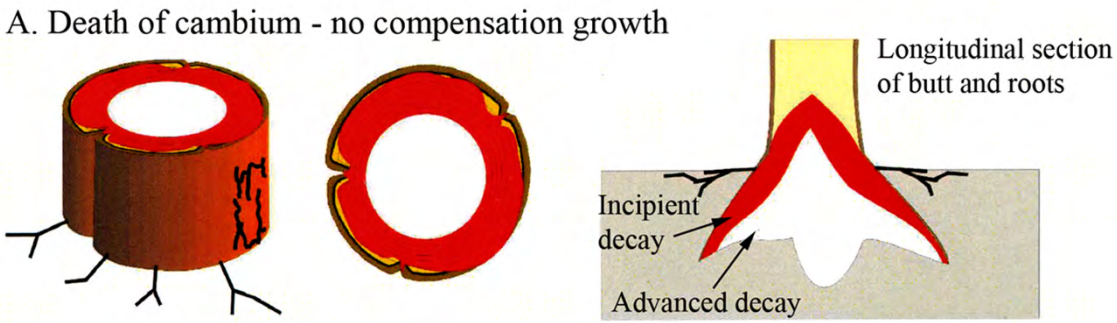
(Schwarze, 2008)

I Monumentali, Varese

Compensation growth – no!

If a tree shows damage due to decay, without compensation growth (e.g. advanced butt rot without the development of a broader root plate), strength and stability of the tree is most likely impaired.

The absence of compensation growth is probably an indication for a reduction in the strength and stability of a tree.

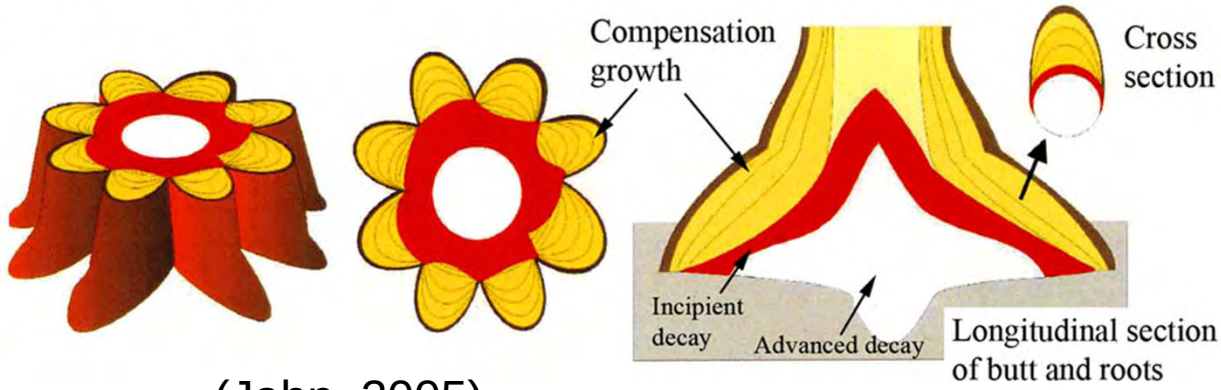


(Jahn, 2005)

Compensation growth – yes!

If a tree responds to damage due to decay with compensation growth (i.e. development of a broader root plate or secondary root growth), they can compensate damage for a long time.

B. Compensation growth and strengthening of root plate stability



(Jahn, 2005)

Development of decay in butt and roots (Jahn, 2005)

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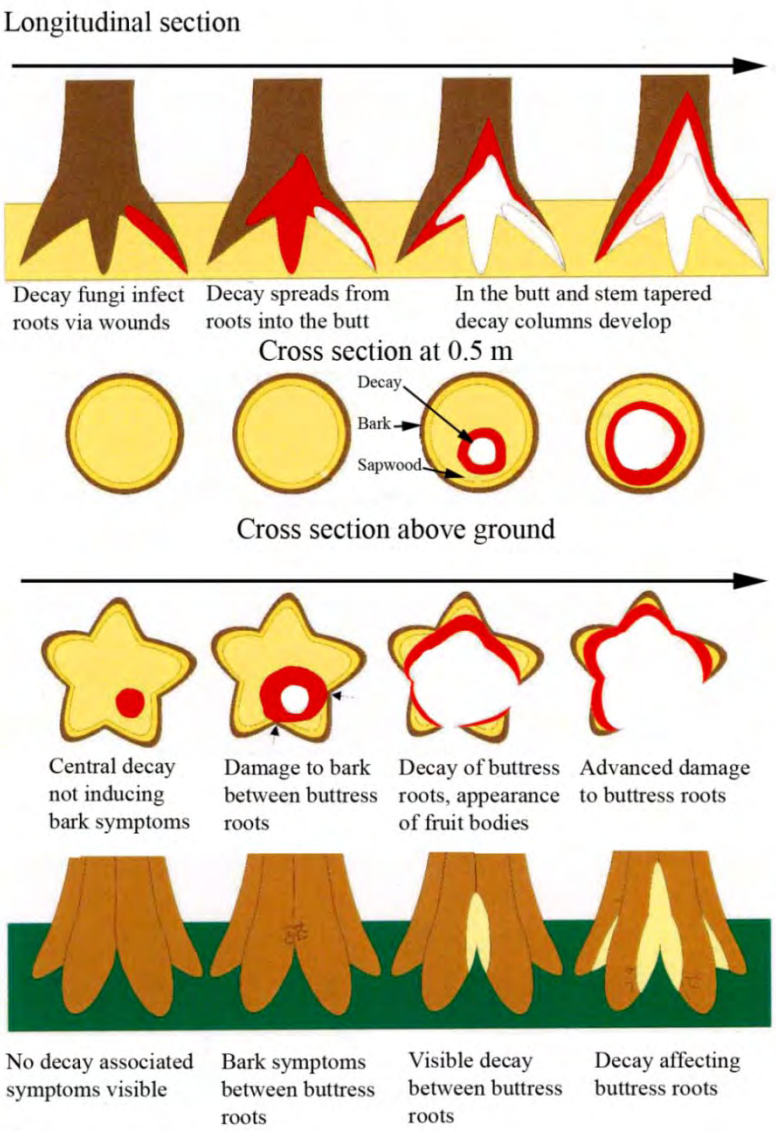
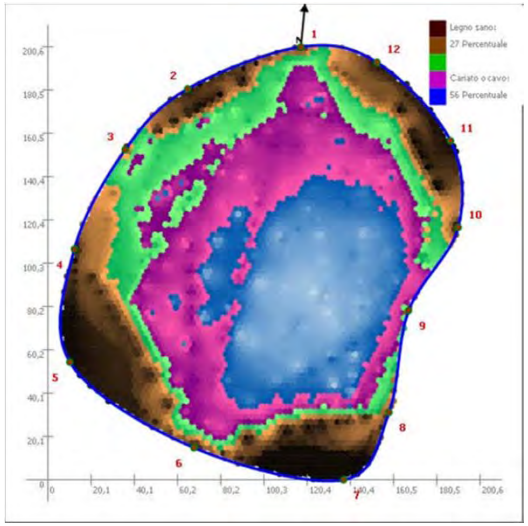
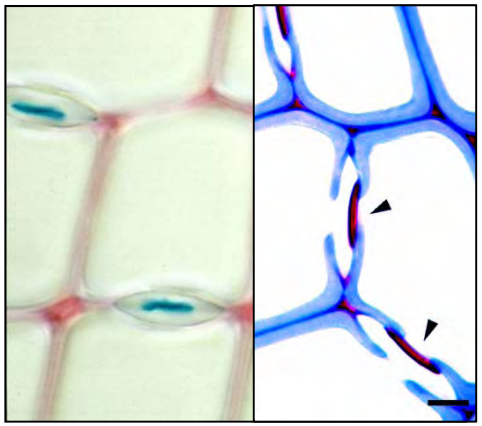
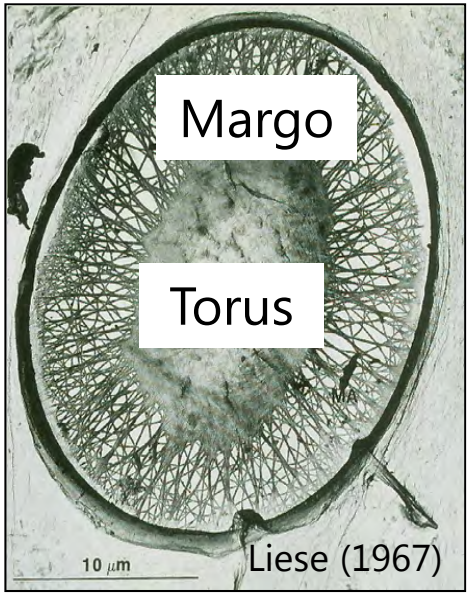




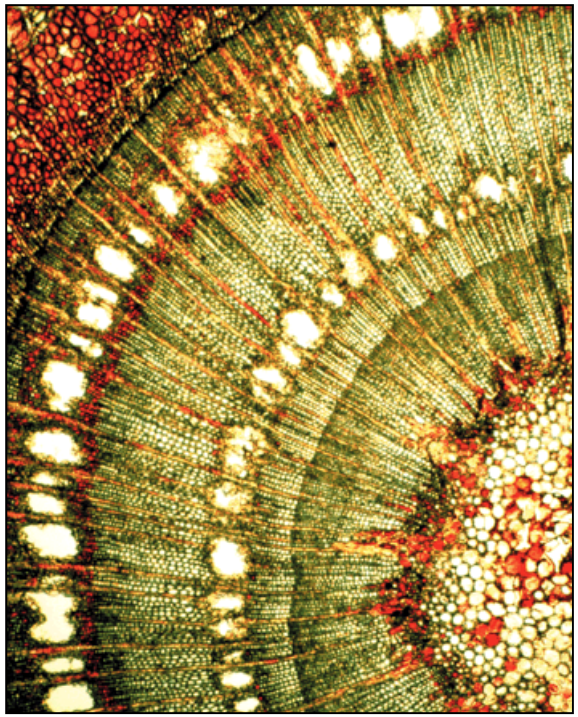
Host response in Cedrus





Ingress is counteracted by aspiration of bordered pit membranes.

Resin ducts are absent in the xylem of *Cedrus atlantica*.



Two concentric rings of traumatic resin ducts formed in response to cambial damage.

INTERACTIONS IN THE XYLEM OF TREES

COMPARTMENTALIZATION

Stem wounds

SAPWOOD

DYSFUNCTION!

Response against ingress of air and infection by deuteromycetes and wound parasites

Occlusion of cells

Composition of polyphenols

Anatomy

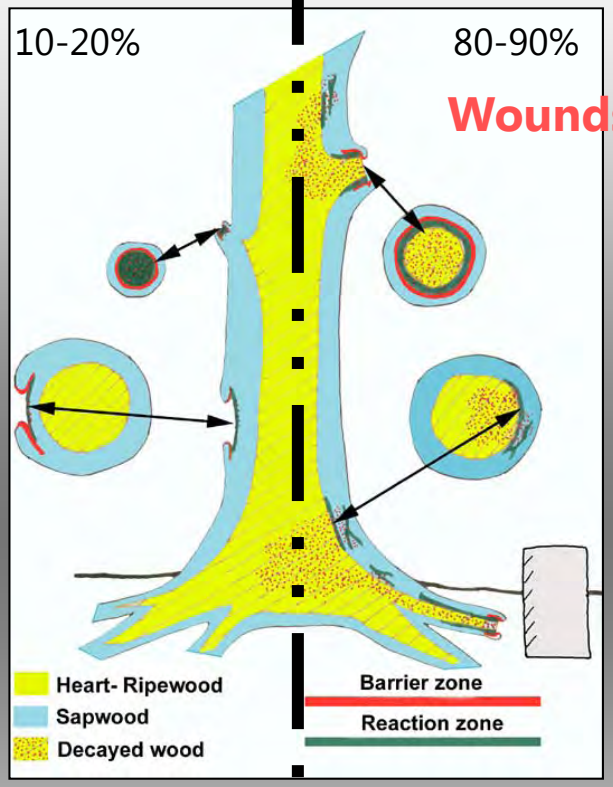
Host response

Wood moisture

Suberin layers

Vitality and season

Effective compartmentalization



(Schwarze, 2008)

COMPARTMENTALIZATION

Wounds to large branches and roots

HEART- & RIPEWOOD

DECAY!

Response against infection by wood decay fungi

Enzymatic Potential

Soft rot mode

Fungal invasiveness

Dual degradation modes

Inoculum potential

Adaptation to substratum

Weak compartimentalization

I Monumentali, Varese

Effectiveness of compensation growth

I Monumentali, Varese

PCR Identification of the casual decay fungus on cedar

Phellinus torulosa
(synonym Fuscoporia torulosa)

I Monumentali, Varese

NNNNNNNNNNNNNNNGTGGGTCTCTTGAGCTGGCATTGCAGGTGCTCGGAGGCTCCCATTCTCATCCACTC
AACCCCCTGTGCACTTCTGAACGCAAGTGAGTCGTCAGTCCCCATGCTGGGATTGACTTGTATTTACTTCGTTT
ATTACAAACTCTTTTTATGTCTTGTAGAATGCATTGCCTCTTTACAGGCGAAATGTAATACAACTTTCAACAAC
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NNNCCNNGGNNNNNNAAAANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNC
NNNNNNNNNNNNNNNNNNNNNNGNNNNNNNNNNNNNNNNNNNNNTNNNNNNNNNNNNNNNNNN
NGNGNNNNNNNNTNNNN

>emb|AM269803.1| **Phellinus torulosus** (Pers.) Bourdot & Galzin 18S rRNA gene (partial), ITS1, 5.8S rRNA

gene, ITS2 and 28S rRNA gene (partial), isolate 759

Length=656

Score = 749 bits (405), Expect = 0.0

Identities = 405/405 (100%), Gaps = 0/405 (0%)

Strand=Plus/Plus