Evaluation of *Trichoderma* spp. as biocontrol agent against wood decay fungi in urban trees

Prof. Dr. Francis W.M.R. Schwarze
Biological control has attracted attention from researchers for over 30 years, primarily because of the interest in developing more environmentally “friendly” means of disease management in the absence of pesticides.

Despite considerable effort in the area of biological control, few practical applications have become established for the control of plant diseases. There are considerably more success stories involving control of insect pests.
Although biological control consists of diverse methods and approaches to suppress plant disease, in most cases antagonists to pathogens are added to the ecosystem. Most approaches of biocontrol are directed at suppressing initial disease induced by a soil-borne pathogen or the application of an avirulent isolate of the pathogen that “competes” with the virulent pathogen on or in the host.

One example of biological control is the application of *Trichoderma* spp., which are known to parasitize hyphae of pathogens.
What is *Trichoderma*?

A genus of fungi, including many species that can be used to control phytopathogenic fungi. *Trichoderma* spp. are, generally, soil dwelling saprophytes. They have a rapid growth rate, sporulate abundantly, compete well with other soil microorganisms, show resistance to chemical pesticides and produce various antibiotics (e.g., gliotoxin and viridin). *Trichoderma* spp. have been investigated for the control of wood-rotting, wound-infecting and soil-borne fungal pathogens of seedlings and mature plants.
**Taxonomy of *Trichoderma***

*Trichoderma* spp. | Class | Order
--- | --- | ---
*T. harzianum* |  |  |
*T. viride* | *Deuteromycetes* | *Moniliales*
*T. koningii* |  |  |
*T. hermatum* |  |  |

Hypocreales usually *Hypocrea* spp. (Ascomycetes) are the sexual teleomorphs of *Trichoderma*.
Microscopic features of *Trichoderma*

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.
Mechanisms of biological control with \textit{Trichoderma} spp.

- Mycoparasitism
- Antibiosis
- Competition for nutrients or space
- Tolerance to stress through enhanced root and plant development
- Induced resistance
- Inactivation of the pathogen’s enzymes
Growth rates of *Trichoderma* and wood decay fungi

Schubert et al. (2006)
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*
Treatment of pruning wounds

- **Dujesiefken, 1992; 1995** – treatment with wound sealants not effective.
- **Legislation, 1998** – wound treatment with chemicals not always legally permitted.
- **Dubos & Ricard, 1974** – curative treatment with *Trichoderma* spp. against *Chondrostereum purpureum*.
- **Pottle & Shigo, 1975** – treatment of *Acer rubrum* wounds with *Trichoderma viride* resulted in a reduction in isolates of Hyphomycetes and Basidiomycetes.
- **Pottle et. al., 1977** - treatment of *Acer rubrum* wounds with *Trichoderma harzianum* protected colonisation against Basidiomycetes for two years.
- **Smith, 1981** - *Trichoderma* spp. counteracted modification of polyphenols by suppressing the growth of Hyphomycetes.
- **Mercer & Kirk, 1982** – Wound treatment with *Trichoderma* spp. partly protected host from infection by Basidiomycetes.
- **Lonsdale, 1992** – Successful wound treatment with *Trichoderma* sp. Against *Chondrostereum purpureum*. Even five years after treatment Trichoderma was present in the wood.
Objectives of the investigations

- To evaluate the potential of different *Trichoderma* spp. as biocontrol agents
- To identify a competitive species that can be used for the treatment of pruning wounds on urban trees against colonisation by wood decay fungi
- To establish method that promotes colonisation and survival of a selected *Trichoderma* isolate in pruning wounds.
Target organism *Inonotus hispidus*

Main hosts:
- Plane
- Apple tree
- Ash
- Walnut

Strong sporulation

[Image showing a tree with a fungus, labeled "Main hosts"]
**Trichoderma** isolates and wood-decay fungi

<table>
<thead>
<tr>
<th><strong>Trichoderma spp.</strong></th>
<th><strong>Source and origin</strong></th>
<th><strong>Isolat-No.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichoderma atroviride</em></td>
<td>Armillaria mellea - Germany</td>
<td>15603.1¹</td>
</tr>
<tr>
<td><em>Trichoderma atroviride</em></td>
<td>Peel of <em>Citrus aurantium</em> - Israel</td>
<td>CBS 351.93²</td>
</tr>
<tr>
<td><em>Trichoderma atroviride</em></td>
<td>Forest soil - USA</td>
<td>CBS 396.92²</td>
</tr>
<tr>
<td><em>Trichoderma fasciculatum</em></td>
<td>Bark of <em>Betula</em> spp. - Netherlands</td>
<td>CBS 338.93²</td>
</tr>
<tr>
<td><em>Trichoderma virens</em></td>
<td>Decayed wood - Germany</td>
<td>CBS 126.65²</td>
</tr>
<tr>
<td><em>Trichoderma harzianum</em></td>
<td>BINAB TF WP</td>
<td>IMI 206039³</td>
</tr>
<tr>
<td><em>Trichoderma. polysporum</em></td>
<td>BINAB TF WP</td>
<td>IMI 206040³</td>
</tr>
</tbody>
</table>

¹Isolates from the Forest Botany, University of Freiburg.
²Isolates from Centraalbureau voor Schimmelcultures, the Netherlands.
³BINAB Bio-Innovation AB, Sweden.

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<table>
<thead>
<tr>
<th><strong>Wood decay fungi</strong></th>
<th><strong>Isoliert aus</strong></th>
<th><strong>Isolat-No.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Polyporus squamosus</em></td>
<td><em>Tilia cordata</em> Mill.</td>
<td>291101.2¹</td>
</tr>
<tr>
<td><em>Ganoderma adspersum</em></td>
<td><em>Fagus sylvatica</em> L.</td>
<td>086699.2¹</td>
</tr>
<tr>
<td><em>Ganoderma lipsiense</em></td>
<td><em>Fagus sylvatica</em> L</td>
<td>250593.1¹</td>
</tr>
<tr>
<td><em>Kretzschmaria deusta</em></td>
<td><em>Acer pseudoplatanus</em> L.</td>
<td>271098.1⁴</td>
</tr>
<tr>
<td><em>Inonotus hispidus</em></td>
<td><em>Fraxinus excelsior</em> L.</td>
<td>200792.1¹</td>
</tr>
<tr>
<td><em>Inonotus hispidus</em></td>
<td><em>Platanus x hispanica</em></td>
<td>221105.1</td>
</tr>
</tbody>
</table>

¹¹Isolates from the Forest Botany, University of Freiburg
*In vitro* evaluation of the antagonistic activity

- Bioassays to evaluate growth and germination rates
- Inhibitory effects of volatile compounds produced by *Trichoderma* in the presence of wood decay fungi
- Dual culture tests on MEA and LNA
- Evaluation of antagonistic activity in wood.
Mean growth rate of the *Trichoderma* isolates under different conditions (mm d\(^{-1}\)). ±SE.

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>MEA (a_w) 0.892</th>
<th>MEA (a_w) 0.995</th>
<th>MEA (a_w) 0.998</th>
<th>LNA (a_w) 0.892</th>
<th>LNA (a_w) 0.995</th>
<th>LNA (a_w) 0.998</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
<td>2.9 ±0.18</td>
<td>0</td>
<td>0</td>
<td>2.7 ±0.22</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>0</td>
<td>8.9 ±0.21</td>
<td>0</td>
<td>0</td>
<td>7.0 ±0.16</td>
</tr>
<tr>
<td>25</td>
<td>0</td>
<td>6.4 ±0.15</td>
<td>18.9 ±0.28</td>
<td>0</td>
<td>7.5 ±0.16</td>
<td>12.7 ±0.25</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>5.6 ±0.23</td>
<td>12.9 ±0.17</td>
<td>0</td>
<td>6.9 ±0.21</td>
<td>13.1 ±0.22</td>
</tr>
</tbody>
</table>

Mean germination rate of *Trichoderma* spp. under different conditions (% d\(^{-1}\)). ±SE.

<table>
<thead>
<tr>
<th>Water activity (a_w)</th>
<th>Temperature °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>(a_w) 0.892</td>
<td>0</td>
</tr>
<tr>
<td>(a_w) 0.995</td>
<td>0</td>
</tr>
<tr>
<td>(a_w) 0.998</td>
<td>0</td>
</tr>
</tbody>
</table>
Inhibition of radial growth (%) of wood-decay fungi by volatile organic compounds (VOCs) produced by *Trichoderma* spp.
Dual culture tests

Trichoderma / Ganoderma adspersum

Trichoderma / Polyporus squamosus

Dual culture tests

Mycoparasitism

Classification of the degree of mycoparasitism of different *Trichoderma* spp. on MEA. ± SE

<table>
<thead>
<tr>
<th></th>
<th>MEA</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T-15603.1</td>
<td>T-351.93</td>
<td>T-396.92</td>
<td>T-Binab</td>
<td>T-126.65</td>
<td>T-338.93</td>
</tr>
<tr>
<td><em>I. hispidus</em></td>
<td>2.2 ± 0.14 [100]b</td>
<td>2.9 ± 0.98 [100]</td>
<td>2.4 ± 0.65 [83]</td>
<td>2.3 ± 0.77 [100]</td>
<td>3.0 ± 0.89 [100]</td>
<td>2.1 ± 0.89 [67]</td>
</tr>
<tr>
<td></td>
<td>3.0 ± 0.10 [100]</td>
<td>2.5 ± 0.18 [100]</td>
<td>2.9 ± 0.12 [100]</td>
<td>2.4 ± 0.67 [100]</td>
<td>2.9 ± 0.14 [100]</td>
<td>0.7 ± 0.09 [17]</td>
</tr>
<tr>
<td><em>G. adspersum</em></td>
<td>2.3 ± 0.11 [83]</td>
<td>2.4 ± 1.23 [100]</td>
<td>1.9 ± 0.21 [67]</td>
<td>1.9 ± 0.23 [83]</td>
<td>2.3 ± 0.54 [100]</td>
<td>0 ± 0.0 [0]</td>
</tr>
<tr>
<td><em>G. lipsiense</em></td>
<td>3.0 ± 0.36 [100]</td>
<td>2.8 ± 1.31 [100]</td>
<td>2.6 ± 0.33 [100]</td>
<td>2.8 ± 0.42 [100]</td>
<td>2.9 ± 0.56 [100]</td>
<td>1.8 ± 0.36 [67]</td>
</tr>
<tr>
<td><em>K. deusta</em></td>
<td>2.2 ± 0.56 [83]</td>
<td>1.8 ± 1.05 [67]</td>
<td>2.4 ± 0.66 [83]</td>
<td>1.7 ± 0.11 [83]</td>
<td>2.9 ± 1.45 [100]</td>
<td>0 ± 0.0 [0]</td>
</tr>
<tr>
<td><em>P. squamosus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a* = Following system was used to classify the rate of mycoparasitism: 0 = no overgrowth; 1 = slow overgrowth; 2 = fast overgrowth; 3 = very fast overgrowth and deadlock of the wood decay fungi within 4 weeks.

*b* = Lethal effect as percent was measured by the ability of *Trichoderma* spp. to eliminate the wood decay fungi during the incubation time of 4 weeks.

### Classification of the degree of mycoparasitism of different *Trichoderma* spp. on LNA. ± SE

<table>
<thead>
<tr>
<th></th>
<th>T-15603.1</th>
<th>T-351.93</th>
<th>T-396.92</th>
<th>T-Binab</th>
<th>T-126.65</th>
<th>T-338.93</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>I. hispidus</em></td>
<td>1.9 ± 0.43 [83](^a)</td>
<td>2.4 ± 0.89 [100]</td>
<td>2.2 ± 0.73 [83]</td>
<td>1.9 ± 0.09 [100]</td>
<td>1.9 ± 1.32 [100]</td>
<td>1.9 ± 0.10 [67]</td>
</tr>
<tr>
<td><em>G. adspersum</em></td>
<td>2.3 ± 0.07 [100]</td>
<td>2.2 ± 0.82 [100]</td>
<td>2.4 ± 0.44 [100]</td>
<td>2.1 ± 0.17 [100]</td>
<td>1.8 ± 0.89 [83]</td>
<td>0.8 ± 0.14 [17]</td>
</tr>
<tr>
<td><em>G. lipsiense</em></td>
<td>1.1 ± 0.33 [17]</td>
<td>1.3 ± 0.07 [33]</td>
<td>0.9 ± 0.69 [17]</td>
<td>0 ± 0 [0]</td>
<td>1.8 ± 0.14 [83]</td>
<td>0 ± 0 [0]</td>
</tr>
<tr>
<td><em>K. deusta</em></td>
<td>2.9 ± 0.33 [100]</td>
<td>2.3 ± 0.11 [100]</td>
<td>2.3 ± 0.74 [100]</td>
<td>2.7 ± 0.19 [100]</td>
<td>3.0 ± 1.20 [100]</td>
<td>1.3 ± 0.12 [33]</td>
</tr>
<tr>
<td><em>P. squamosus</em></td>
<td>0 ± 0.0 [0]</td>
<td>0 ± 0.0 [0]</td>
<td>0.6 ± 0.75 [17]</td>
<td>0 ± 0 [0]</td>
<td>2.3 ± 1.01 [83]</td>
<td>0 ± 0.0 [0]</td>
</tr>
</tbody>
</table>

\(^a\) = Following system was used to classify the rate of mycoparasitism: 0 = no overgrowth; 1 = slow overgrowth; 2 = fast overgrowth; 3 = very fast overgrowth and deadlock of the wood decay fungi within 4 weeks.

\(^b\) = Lethal effect as percent was measured by the ability of *Trichoderma* spp. to eliminate the wood decay fungi during the incubation time of 4 weeks.

Evaluation of antagonistic activity in wood

Symbols with different letters indicate significant (p≤0.05) differences in weight losses according to Ryan-Einot-Gabriel-Welsch-Test (REWGQ).
Interaction studies in wood blocks

Ganoderma adspersum / Trichoderma

Interaction studies in wood blocks

*Kretzschmaria deusta / Trichoderma*

Interaction studies in wood blocks

Polyporus squamosus / Trichoderma

## Classification of the degree of Mycoparasitism of different *Trichoderma* spp.

<table>
<thead>
<tr>
<th>In vitro studies</th>
<th>Index of dominance (I_D)</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dual culture tests</td>
<td>I_D I</td>
<td>1 – 5</td>
</tr>
<tr>
<td>Affect of VOCs (volatile organic compounds)</td>
<td>I_D II</td>
<td>1 – 5</td>
</tr>
<tr>
<td>Interaction studies in wood</td>
<td>I_D III</td>
<td>1 – 5</td>
</tr>
<tr>
<td>Growth rates</td>
<td>I_D IV</td>
<td>1 – 5</td>
</tr>
<tr>
<td>Germination of conidia</td>
<td>I_D V</td>
<td>1 – 5</td>
</tr>
</tbody>
</table>

I_D-Index = \[ \frac{\sum I_{Dx}}{\sum T_x} \]  

*ID-Index = Sum of indices IDx / Sum of tests Tx.*
Classification of the degree of Mycoparasitism of different *Trichoderma* spp.

Index of Dominance:

<table>
<thead>
<tr>
<th><em>Trichoderma</em> spp.</th>
<th>Isolat-Nr.</th>
<th>ID-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichoderma atroviride</em></td>
<td>15603.1¹</td>
<td>3,6 (a)</td>
</tr>
<tr>
<td><em>Trichoderma atroviride</em></td>
<td>CBS 351.93²</td>
<td>3,6 (a)</td>
</tr>
<tr>
<td><em>Trichoderma atroviride</em></td>
<td>CBS 396.92²</td>
<td>3,0 (b)</td>
</tr>
<tr>
<td><em>Trichoderma fasciculatum</em></td>
<td>CBS 338.93²</td>
<td>2,2 (c)</td>
</tr>
<tr>
<td><em>Trichoderma virens</em></td>
<td>CBS 126.65²</td>
<td>3,6 (a)</td>
</tr>
<tr>
<td><em>Trichoderma harzianum</em></td>
<td>IMI 206039³</td>
<td>3,2 (b)</td>
</tr>
<tr>
<td><em>Trichoderma. polysporum</em></td>
<td>IMI 206040³</td>
<td></td>
</tr>
</tbody>
</table>

Values with the same symbols denote significant differences according to the Ryan-Einot-Gabrial-Weltsch-test (REGWQ) (p≤0,05).

ID -value 0-1 = no antagonistic potential  
ID -value 1-2 = weak antagonistic potential  
ID –value 2-3 = moderate antagonistic potential  
ID -value 3-4 = high antagonistic potential  
ID -value 4-5 = very high antagonistic potential
Environment
1 UV radiation
2 Temperature
3 Precipitation
4 Emissions

Biotic interactions
1 *Fusarium* sp.
2 Bacteria
3 Other organisms / endophytes

Tree
1 Nutrients
2 Stress metabolites
3 Reaction-/barrier zones
4 Wood moisture
5 Water conducting system
6 Sap-/heartwood
Field studies to evaluate the efficiency of *Trichoderma* (T-15603.1) for biological control

- Treatment of pruning wounds of a range of hosts on different sites with conidial suspensions of *Trichoderma atroviride* 15603.1
- Reisolation and analysis *Trichoderma atroviride* 15603.1
- Identification of *Trichoderma atroviride* 15603.1
- Impact of *Trichoderma atroviride* 15603.1 on woundwood formation
Field studies

I. Wound treatment:

Spring / summer 2003

9 pruning wounds on 37 trees in Strasbourg and on 81 trees in Ludwigshafen, BASF AG were treated with conidial suspensions of *Trichoderma atroviride* 15603.1

II. Infection trials with *Trichoderma atroviride* 15603.1 and wood decay fungi:

9 pruning wounds on 78 trees were treated with *T. atroviride* 15603.1 at a site in Freiburg. Three weeks after treatment wounds were additional inoculated with *Inonotus hispidus*, *Ganoderma adspersum* and *Polyporus squamosus*. 
Range and number of tree species inoculated with *Trichoderma atroviride* 15603.1

*Platanus x hispanica* Münchh. 91
*Acer pseudoplatanus* L. 40
*Tilia cordata* L. 24
*Populus nigra* L. 16
*Quercus* spp. L. 16
*Robinia pseudoacacia* L. 9

Total trees: 196
Total wounds: 1764

Schubert (2006)
Treatment of pruning wounds with *Trichoderma atroviride* 15603.1

Method **A**: Suspension [CFU: 10^5 conidia/ml]
Method **B**: Suspension [CFU: 10^5 conidia/ml + 0.2% glucose + 0.1% urea]
Method **C**: Suspension [CFU: 10^5 conidia/ml + 0.2% glucose + 0.1% urea + 0.4% Luquasorb® (Natriumpolyacrylat)] *
Method **K**: Control – no treatment
*For method **C** a Hydrogel (Luquasorb® 10 30) developed by BASF AG/ Polymer Department was used as a carrier substance.

At the site in Strasbourg the Hydrogel Luquasorb® was not applied. For this reason an alternative method was used:

Method **CS**: Suspension [CFU: 10^5 conidia/ml + 0.5% Glucose + 0.2% Urea]

Schubert (2006)
Field studies

Experiment A: Treatment of pruning wounds:

2 months 8 months 12 months 18 months 24 months 30 months

Schubert (2006)
Reisolation of *Trichoderma atroviride* 15603.1 from pruning wounds

\[ W_{\text{grad}} = \frac{(P_x[\%] - B[\%]) \times 100}{P_x[\%]} \]

\[ K_{\text{coeff[\%]}} = 100 - \frac{\left(\frac{\pi}{3} \times h \times b \right)}{\pi \times r^2} \times 100 \]

Schubert (2006)
Monitoring of *Trichoderma atroviride* 15603.1 in the field studies

Conventional methods

![Conventional method image]

Molecular biological methods

![Molecular biological method image]

Identification of *Trichoderma atroviride 15603.1* with RAPD-PCR

Lane 1 = applied strain T-15603.1 (reference); lanes 2–6 = *Trichoderma* isolated from the treated pruning wounds after 30 months; lane 7 = *Trichoderma virens*; lane 8 = *Trichoderma fasciculatum*.

Versuch A

Reisolation results of *T. atroviride* at the site in Ludwigshafen

Symbols with different letters indicate significant (p≤0.05) differences in weight losses according to Ryan-Einot-Gabriel-Welsch-Test (REWGQ).
Versuch A

Reisolations of *T. atroviride* at the site in Ludwigshafen and Straßburg

Symbols with different letters indicate significant (p≤0.05) differences in weight losses according to Ryan-Einot-Gabriel-Welsch-Test (REWQ).

Schubert et al. (2006)
Reisolation rates in relation to climate conditions

Schubert (2006)
Reisolation rate of *Trichoderma atroviride* 15603.1 from sap- and heartwood
Reisolation rate of *Trichoderma atroviride 15603.1* in relation to wound size
Range and number (%) of microorganisms isolated from wounds

Schubert (2006)
Measurement of the woundwood coefficient

\[ C\% = 100 - \left( \frac{\pi \cdot h \cdot b}{\pi \cdot r^2} \right) \times 100 \]

- \( a, b, c \) denote significant differences \((P < 0.05)\) of wound occlusion for tree species. \(<30\%31-60\%>60\%\)

<table>
<thead>
<tr>
<th>Tree Species</th>
<th>&lt;30%</th>
<th>31-60%</th>
<th>&gt;60%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Populus nigra</td>
<td>79.6%</td>
<td>72.7%</td>
<td>69.2%</td>
</tr>
<tr>
<td>Acer pseudoplatanus</td>
<td>63.9%</td>
<td>69.2%</td>
<td>72.7%</td>
</tr>
<tr>
<td>Quercus rubra</td>
<td>69.2%</td>
<td>72.7%</td>
<td>63.9%</td>
</tr>
<tr>
<td>Tilia platyphyllos</td>
<td>79.6%</td>
<td>69.2%</td>
<td>72.7%</td>
</tr>
</tbody>
</table>

T-15603.1
WOOD DECAY FUNGI
CONTROL

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ISA Inaugural Asia Pacific Conference, Brisbane, Australia
Diversity index $H'$ calculated from wounds treated with different conidial suspensions of T-15603.1

Reduction of infection rate by wood degrading basidiomycetes after wound treatment with *Trichoderma atroviride* 15603.1

Infection rate (%) without and with Trichoderma treatment for different fungal species:

- *I. hispidus*
- *G. adspersum*
- *P. squamosus*
- Total

Efficency [%]. Asterices denote significant reduction in the infection rate: * = significant (p ≤ 0.05); ** = highly significant (p ≤ 0.001).

Summary

- *Trichoderma atroviride* was consistently and highly competitive against most wood decay fungi with the exception of *Polyporus squamosus*.

- In comparison to untreated control wounds, *Trichoderma atroviride* 15603.1 significantly suppressed growth of wounds colonised by three basidiomycetes (*Inonotus hispidus*, *Ganoderma adspersum* and *Polyporus squamosus*).

- Monitoring results with RAPD-PCR showed that conidial suspensions of T-15603.1 applied in a humidity storing gel as a carrier substance significantly increased germination rate and colonisation by the antagonist.
Ganoderma basal stem rot

- Seedling disease
- Basal stem rot
- Upper stem rot
Ganoderma australe
Ganoderma boniense
Gandoerma colossus
Ganoderma lucidum
Ganoderma pseudoferreum
Ganoderma tropicum
Ganoderma zonatus
Identification with RAPD PCR

9438_eHS3_17.10 *Ganoderma boninense*

AAGCTGTAGACATAGGGTTGTTAGCTGGCCTTTCCAGGAGCATCTGGCACGCCCCCCGTCCATC
CACTCTACACCTGTGCACTTTACTGGGTATAGAYCGTGTTGAGCGAGCTCGTTTTCTGCT
GACGAGTTTGCAGACGCGTGCTGTGCGCCTGCTGCTTTTTTTWYMMMAAMNNNNWWAANNANTAAA
ANGGGGNTTGGGGANGGNANGNNYNNTNTNCMANTTTCNNNNACNGANNNCTTGGGNTNNC
CNNNTCNNNLAAANANNNNCNAAGGNANNARNNNANGGGNANTGNNAANTNNNNNNGNAN
CNNCCNANCNTTNANNNNNNCTGNNNTCCNTGGNATTNCNAAGANNTGNCNNGGTTNAN
CGGNNNNANCCNCTCNANCNNANCCNTNCGGNNNTTTTGNNNGNTTGGGANTTNGANGC
GGGCCNCCCCNTNNNTGNNNCNGNTTCCCTCAAANGNNTAANNNNTNGNNTCCCTTGGNNATCC
GNNNGNCGNNGNNNNNTNAANGNNTNNNNCNACGGGNNNNNCNGNNNGTGANCTANNC
ACTCGANTNNNGNGNCTCTCTTNNTATNCNNNCCTCCATNNNCANNNCNGNNNAACNNTATCTN
NATNAANNNNAANATGAGNNNN
## Competition studies

### Dual culture tests:

![Diagram of G. boniense and Pilzart X](image)

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Trichoderma atroviride</td>
<td>15603.1¹</td>
<td>Ganoderma boniense</td>
<td>70¹³</td>
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<tr>
<td>Trichoderma atroviride</td>
<td>351.93²</td>
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<tr>
<td>Trichoderma virens</td>
<td>511³</td>
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</tr>
</tbody>
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¹ = Isolate obtained from Institut für Forstbotanik, Universität Freiburg  
² = Isolate obtained from Centraalbureau voor Schimmelcultures – Niederlande  
³ = Isolate obtained from NP Singapur, isolated at EMPA, St. Gallen

### (1) Standard media (MEA, pH- 5 ):
- 2% Malt extract (OXOID LP39)  
- 2% Agar Technical (OXOID LP13)

### (2) Low Nutrient Agar (LNA, pH- 5 ):
- L-asparagine 0.013g  
- KH2PO4 1g  
- MgSO4 0.3g  
- KCL 0.5g  
- FeSO4 0.01g  
- MnSO4 4H2O 0.008g  
- ZnSO4 6H2O 0.002g  
- CaNO3 4H2O 0.05g  
- CuSO4 0.002g  
- NH4NO3 0.008g  
- D-glucose, 5g; Agar 10g

*Schubert et al. (2008a). Arboric Journal in press  
Dual culture tests between *Ganoderma boninense* and *Trichoderma atroviride* 15603.1

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