1 **Title**

2 In vitro evaluation of antagonism by *Trichoderma* spp. towards *Phellinus noxius* associated with rain

3 tree (*Samanea saman*) and Senegal mahogany (*Khaya senegalensis*) in Singapore

4

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24 **Abstract**

25 A series of laboratory tests was conducted to screen and identify *Trichoderma* spp. for the biological

26 control of *P. noxius*. In total, 7 *Trichoderma* isolates were evaluated for their biological fitness and

27 antagonism towards 2 different *P. noxius* isolates associated with Senegal mahogany and rain tree in

28 Singapore. The competitive ability of various *Trichoderma* isolates was assessed by their germination

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1   rates, growth rates, and chlamydospore production; and the antagonism of *P. noxius* by *Trichoderma*

2   was assessed by the interaction of these fungi in dual culture and on wood blocks. In this study, the

3   *Trichoderma* isolates showed greater competitiveness and antagonism towards *P. noxius* compared to

4   similar reports in the literature. A majority of the *Trichoderma* isolates germinated and grew at

5   consistently high rates, but there was considerable variability in the production of chlamydospores

6   among isolates. All *Trichoderma* isolates routinely antagonized *P. noxius* in the different bioassays,

7   but there was significant variability in the antagonistic capacity of various isolates and in the

8   susceptibility of the *P. noxius* isolates to confrontation with *Trichoderma*. Relative to the controls, *P.*

9   *noxius* cultures grew significantly slower in the presence of volatile organic compounds emitted by

10   most *Trichoderma* isolates, and a large majority of *Trichoderma* isolates caused a significant

11   reduction to the dry weight loss of wood blocks inoculated with *P. noxius*. Although no single

12   *Trichoderma* isolate consistently antagonized *P. noxius* better than all others in every test, *T.*

13   *harzianum* 9132 and *T. virens* W23 notably did so more regularly than others.

14

15   **<u>Introduction</u>**

16   Trees are pruned for many reasons, including aesthetics, clearance, and safety. However, the exposed

17   wood on pruning wounds is susceptible to wood decay infection (Barry et al., 2000; Deflorio et al.,

18   2007), and these infections are a concern to people interested in preserving the environmental benefits

19   and commercial value of trees. Historically, arborists often applied various treatments to pruning

20   wounds to create a physical barrier or inhibit wood decay fungi (Lonsdale, 1984). Although some of

21   these treatments improved wound occlusion (Mercer, 1983) and temporarily prevented infection

22   (Mercer et al., 1983), the physical barriers frequently deteriorated over time due to weathering, insect

23   damage, and growth stress that, in some cases, exacerbated decay compared to untreated wounds

24   (Mercer et al., 1983). Similarly, some fungicides were reported as effective at limiting infection but

25   their phytotoxicity often caused harmful cambial dieback (Mercer et al., 1983; Mercer, 1979) that

26   inhibited wound occlusion and increased wood decay by expanding the exposed wound surface. In

27   addition to their limited efficacy, the use of synthetic chemicals, especially on trees planted in densely

28   populated urban areas, creates environmental and human health risks that many consider

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1   unacceptable. As a result, most arboriculture industry standards discourage wound treatment

2   (Anonymous 2008).

3

4   Biological control agents, however, offer a promising alternative to traditional wound treatments. In

5   particular, many fungal antagonists have been selected from the genus *Trichoderma* for biological

6   control of plant diseases, including economically important fungal pathogens affecting fruit trees

7   (Ricard and Highley, 1988) and conifers (Kallio and Hallaksela, 1979). Notably, Pottle and Shigo

8   (1975) reported that basidiomycetes were absent from 1-year-old red maple [*Acer rubrum* L.

9   (Sapindaceae)] wounds preventatively treated with *T. viride*, but decay fungi were present in 80% of

10   untreated control wounds after the same period. Mercer and Kirk (1984b) similarly reported a

11   significant reduction in wood decay fungi infection of European beech [*Fagus sylvatica* L.

12   (Fagaceae)] wounds treated with a different isolate of *T. viride*, compared to control wounds. Notably,

13   *T. viride* was more effective and persisted longer than other biological controls (Mercer and Kirk,

14   1984b). More recently, Schubert et al. (2008a) demonstrated the ability of a *T. atroviride* isolate to

15   significantly reduce wood decay infection rates among more than 1,400 wounds treated from 6

16   different broadleaf tree species.

17

18   *Trichoderma* spp. (Samuels, 1996) are ubiquitous, globally distributed saprophytes that occur

19   primarily in highly organic soils (Papavizas, 1985), but they are capable of colonizing a wide range of

20   natural substrates (Klein and Eveleigh, 1998). Many *Trichoderma* spp. are avirulent plant symbionts

21   (Harman et al., 2004) that antagonize phytopathogenic fungi through mycoparasitism (Lorito et al.,

22   1996a), antibiosis (Ghisalberti and Sivasithamparam, 1991), enzyme production (Markovich and

23   Kononova, 2003), and competition for resources (Sivan and Chet, 1989). In most cases, a single

24   *Trichoderma* spp. isolate simultaneously employs more than one of these antagonistic mechanisms to

25   suppress disease-causing fungi; there are several reports of synergistic enhancement between multiple

26   antagonistic processes and substances (Di Pietro et al., 1993; Lorito et al., 1994a; Lorito et al., 1994b;

27   Lorito et al., 1996b). In addition to suppressing harmful microbes, soil application of *Trichoderma*

28   spp. enhances root growth and nutrient uptake efficiency, resulting in increased overall plant growth

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1   and reproductive yield (Harman et al., 2004). Several authors have also demonstrated that some

2   *Trichoderma* spp. cause systemic induced resistance (Pieterse and van Loon, 1999) in plants by

3   observing physical separation between the introduced *Trichoderma* spp. and area of disease reduction

4   (Bigirimana et al., 1997; Djonovic et al., 2006; Yedidia et al., 1999).

5

6   Similar to mycorrhizae, many benefits accrue to plants after *Trichoderma* spp. colonize the outermost

7   epidermal layers of their roots (Yedidia et al., 2000). Although most *Trichoderma* spp. are avirulent, a

8   few isolates are pathogenic or phytotoxic (Koike et al., 2001), and it is important to screen for

9   potential biological control agents with representative laboratory tests that mimic the temperature,

10   moisture, and nutrient availability conditions expected for the desired field application (Howell,

11   2003). Moreover, many authors caution that individual *Trichoderma* spp. isolates usually do not have

12   the intrinsic ability to exert all of the reported biocontrol mechanisms (Harman, 2000; Howell, 2003),

13   and hybridization, genetic modification, or mixing may be needed to combine the desirable

14   characteristics of multiple isolates (Brunner et al., 2005; Djonovic et al., 2007).

15

16   However, most studies addressing biological control with *Trichoderma* have been conducted in

17   temperate climates with associated species. *Phellinus noxius* (Corner) G. Cunn (Hymenochaetaceae)

18   is an aggressive, often lethal wood decay fungus that infects the root systems of many tree species

19   throughout the global tropics and subtropics (Rossman and Farr, 2014). Macroscopically, the wood of

20   infected trees appears brown and the adjacent root flare is often covered by a dark brown mycelial mat

21   (Chang, 1995). Although the disease is commonly called brown root rot, *P. noxius* actually causes a

22   simultaneous rot by degrading cellulose and lignin at the same time (Nicole et al., 1995). A few recent

23   studies have evaluated the ability of *Trichoderma* to antagonize *P. noxius* obtained from root system

24   infections in Australia (Schwarze et al., 2012) and Hong Kong (Ribera et al., 2016). Recent work in

25   Singapore, however, demonstrated the ability of *P. noxius* to colonize pruning wounds, invade host

26   defensive responses, and cause severe wood decay in the aboveground parts of Senegal mahogany

27   [*Khaya senegalensis* (Desr.) A. Juss. (Meliaceae)]; this work revealed that *P. noxius* occupies a

28   broader ecological niche than previously understood with physiological capacity for multiple trophic

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1   strategies (Burcham et al., 2015). At the same time, diagnostic efforts have shown that *P. noxius*

2   similarly infects other trees in Singapore, including rain tree [*Samanea saman* (Jacq.) Merr.

3   (Fabaceae)].

4

5   Collectively, Senegal mahogany and rain tree comprise more than 10% of Singapore's urban forest

6   and generate a significant part of the total benefits rendered to the surrounding community. In general,

7   the ubiquity of pruning wounds creates considerable opportunity for *P. noxius* infections, and limiting

8   these infections is an important strategy to protect susceptible trees and, by extension, the health of

9   the urban forest. As a result, the objectives of this study were (1) to screen *Trichoderma* spp. isolates

10   for antagonistic potential against *P. noxius* associated with pruning wounds on Senegal mahogany and

11   rain tree and (2) to identify the most competitive isolate(s) for field testing. To this end, a series of

12   bioassays were designed to evaluate antagonism by *Trichoderma* spp. isolates towards *P. noxius* in

13   laboratory conditions that resembled situations expected for landscape application in Singapore.

14

15   **Material and methods**

16   *Origin of fungal isolates*

17   *P. noxius* isolates were obtained from decayed wood columns that were excised near existing branch

18   pruning wounds on Senegal mahogany and rain tree in Singapore. Among several obtained from these

19   tree species, the most aggressive *P. noxius* isolate was selected for screening biological control agents

20   based on its superior ability to cause dry weight loss to species-specific sapwood blocks in laboratory

21   tests (data not shown). In total, two *P. noxius* isolates were selected–one caused maximum dry weight

22   loss to Senegal mahogany and the other to rain tree wood blocks. Separately, 7 *Trichoderma* spp.

23   isolates were obtained from the fruiting bodies of basidiomycetes colonizing various plant species and

24   organic substrates in Singapore. The identity and origin of fungal isolates used in this study is given in

25   Table 1.

26

27   *Cultural media and growth conditions*

5

1   Three cultural media were used, including a malt extract agar (MEA, Thermo Fisher Scientific,

2   Waltham, MA, USA); a low nutrient agar (LNA) prepared according to Huttermann and Volger

3   (1973), with each liter containing 0.013 g H_2O: L-asparagine, 1 g KH_2PO_4, 0.3 g $MgSO_4$, 0.5 g KCL,

4   0.01 g $FeSO_4$, 0.008 g $MnSO_4$ $4H_2O$, 0.002 g $ZnSO_4$ $6H_2O$, 0.05 g $CaNO_3$ $4H2O$, 0.002 g $CuSO_4$,

5   0.008 g NH_4NO_3, 5 g D-glucose, and 10 g agar; and a basidiomycete selective medium (BSM)

6   modified from Sieber (1995), with each liter of media containing 50 g MEA, 105.75 mg

7   thiabendazole dissolved in 2 ml of concentrated lactic acid, 200 mg chloramphenicol, and 300 mg

8   streptomycin sulfate. Unless indicated otherwise, cultures were incubated consistently in the dark at

9   28 °C and 50–70% RH on 90 or 50 mm Petri dishes containing approximately 20 or 11 ml,

10   respectively, of media. All plates were inoculated with 8 mm mycelial discs extracted from the

11   actively growing margins of pure cultures on MEA and sealed with plastic paraffin film (Parafilm®,

12   Pechiney Plastic Packaging, Chicago, IL, USA).

13

14   *Germination and growth rates*

15   Growth rates of the *Trichoderma* isolates were evaluated on MEA and LNA. The LNA was chosen

16   because its higher C:N ratio better replicated the nutritional status of wood (Srinivasan et al., 1992)

17   than MEA. To assess growth, 8 mm mycelial discs of *Trichoderma* were inoculated centrally onto

18   MEA or LNA in a 90 mm Petri dish. Growth rates were determined by recording the average of 2

19   orthogonal fungal culture diameter measurements at regular intervals over a 36-hour period.

20

21   To determine germination rates, a conidial suspension of each *Trichoderma* isolate was prepared by

22   flooding mature cultures with sterile water, dislodging conidia by physical agitation, and skimming

23   buoyant conidia from the surface. The concentration of suspensions was assessed using a

24   haemocytometer and adjusted to obtain approximately 10^5 colony forming units (CFU) $\cdot ml^{-1}$.

25   Subsequently, 10 μl of each suspension was placed in 3 separate locations on a single Petri dish

26   containing LNA. Test plates were covered, sealed with paraffin tape, and incubated at constant

27   conditions for 18 hours. After incubation, conidia were observed at 10× magnification with a light

28   microscope (Nikon Eclipse E200, Tokyo, Japan) in 5 randomly selected locations in each Petri dish.

1   The germination rate was calculated for each plate as the average percent of germinated conidia

2   contained in these fields of observation.

3

4   *Chlamydospore production*

5   To assess the production of thick-walled resting spores during adverse environmental conditions,

6   chlamydospores were counted on 14-day old mature cultures of *Trichoderma* growing on LNA in a 50

7   mm Petri dish. The number of chlamydospores was determined by counting those observed at 10×

8   magnification in 5 randomly selected locations on each Petri dish using a digital camera system

9   (Nikon Digital Sight DS-Fi1, Tokyo, Japan) attached to a light microscope. The number of

10   chlamydospores on each plate was computed as the average number observed across the different

11   locations.

12

13   *VOC inhibition*

14   Inhibition of wood decay fungi by volatile organic compounds (VOCs) produced by *Trichoderma*

15   isolates was evaluated using tests described by Dennis and Webster (1971). Initially, 8 mm mycelial

16   discs of *Trichoderma* isolates were placed in the center of a Petri dish containing MEA. At the same

17   time, each of the *P. noxius* isolates was inoculated identically onto a separate Petri dish. Each

18   *Trichoderma* isolate was then paired with a *P. noxius* isolate by inverting the bottom of one Petri dish

19   containing *Trichoderma* and replacing the top of another containing *P. noxius*. The rims of the paired

20   Petri dishes were aligned and sealed. The diameter of *P. noxius* cultures was recorded as the average

21   of 2 orthogonal measurements at regular 24-hour intervals over a 5-day period. Similar Petri dishes

22   without *Trichoderma* isolates served as a control.

23

24   *Interaction tests*

25   Mycoparasitism was assessed in dual culture and on wood blocks broadly according to Highley et al.

26   (1997) and Schubert et al. (2008a), respectively. For dual culture tests, 8 mm mycelial discs were

27   removed from 14-day old, actively growing pure cultures of each *P. noxius* isolate and placed

28   individually at a regular distance (~ 10 mm) from the edge of a 90 mm Petri dish containing MEA.

1   After incubation for 72 hours, equivalent mycelial discs were removed from 7-day old actively

2   growing pure cultures of each *Trichoderma* isolate, inserted on a Petri dish opposite the growing

3   wood decay fungus, covered, and sealed. The Petri dishes were incubated under constant conditions

4   for 4 weeks. The ability of *Trichoderma* isolates to parasitize *P. noxius* isolates was assessed by

5   removing 6 mycelial discs from the final extent of the wood day fungus culture and placing discs on

6   BSM. The media's inhibition of *Trichoderma* allowed a selective determination of the lethal effect of

7   *Trichoderma* isolates, calculated as one minus the percent of these 6 discs yielding *P. noxius*:

8
$$L = \left(1 - \frac{n}{6}\right) \times 100,$$

9   where n is the number of mycelial discs

10

11   For wood block interaction tests, wood blocks ($10 \times 8 \times 30$ mm) were removed from healthy sapwood

12   in Senegal mahogany and rain tree. Individual wood blocks were oven-dried at 100 °C for 48 hours,

13   cooled in a vacuum desiccator, and weighed using a precision balance. The blocks were subsequently

14   autoclaved twice at 121 °C for 30 min. Each wood block was inoculated by immersion for 10 minutes

15   in a conidial suspension containing 10^5 CFU $\cdot$ml^{-1} of a *Trichoderma* isolate, 0.2% D-glucose, and

16   0.1% urea. The inoculated wood blocks were incubated at constant conditions in a 50 ml sterile

17   centrifuge tube for 4 weeks. Afterwards, the Senegal mahogany and rain tree wood blocks pre-treated

18   with *Trichoderma* were placed on a 14-day old pure culture of one *P. noxius* isolate growing on MEA

19   in a 50 mm Petri dish. Test plates were covered, sealed, and incubated under constant conditions for

20   12 weeks. Untreated, sterilized wood blocks were similarly placed on 14-day old actively growing

21   pure cultures of *P. noxius* and served as controls. After incubation, the wood blocks were cleaned by

22   removing surface mycelia, oven-dried, cooled, and reweighed. The dry-weight loss was then

23   determined for each wood block on a percentage basis.

24

25   *Statistical analysis*

26   For all experiments, 5 replicates were maintained of each treatment combination. Since germination

27   rates and chlamydospore counts were not measured under experimentally manipulated conditions, the

1   results were not statistically analyzed but instead presented as descriptive data. The effect of

2   experimental treatments on the measured response was determined using analysis of variance in SAS

3   9.4 (SAS Institute, Inc., Cary, NC, USA). For growth rate bioassays, model fixed effects included

4   media type, *Trichoderma* isolate, and their interaction. Since the rate of change in culture diameter

5   with respect to time was constant, growth rates were analyzed as the slope of a linear function (Gams

6   and Bissett, 1998) fit to paired observations of culture diameter and time using least squares

7   regression. For the remaining bioassays, model fixed effects included *Trichoderma* isolates, *P. noxius*

8   isolates, and their interaction. Given our primary interest in antagonism by *Trichoderma* isolates,

9   significant interactions were separated to determine differences among *Trichoderma* isolates within

10   specific levels of the other fixed effect. Mean separation was performed using Dunnett's one-way test

11   and Tukey's honestly significant difference for experiments with and without controls, respectively.

12

13   **<u>Results</u>**

14   Except for *T. reesei* 1056TD1, all *Trichoderma* isolates germinated, on average, at nearly maximum

15   rates between 99 and 100%. Still, light microscopy revealed that a considerable majority (87%) of *T.*

16   *reesei* 1056TD1 conidia germinated on LNA. In contrast, there was sizeable variability in the

17   production of chlamydospores among *Trichoderma* isolates. The average number of chlamydospores

18   observed among 5 randomly-selected visual fields at 10× magnification on each Petri dish ranged

19   between 6 (SD 2) for *T. saturnisporum* SPH20109132 and 189 (SD 30) for *T. virens* W23 (Table 2).

20

21   Overall, there were significant differences in growth rates among *Trichoderma* isolates ($F = 103.46$;

22   $df = 6, 24$; $p < 0.001$), and these growth rates differed significantly between the 2 media types ($F =$

23   107.83; $df = 1, 24$; $p < 0.001$). Specifically, *Trichoderma* isolates grew significantly faster on MEA

24   than on LNA (Table 3). However, *Trichoderma* isolates and media types interacted significantly to

25   affect growth rates ($F = 34.55$; $df = 6, 24$; $p < 0.001$). There were significant differences in growth

26   rates among *Trichoderma* isolates growing on both LNA and MEA, but the relative ranking of

27   *Trichoderma* isolates within each media type was not consistent (Table 3). Notably, the growth rate of

28   *T. virens* W23 was significantly slower than all other *Trichoderma* isolates, except *T. virens* W26, on

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1   MEA, but its growth rate actually increased by 8.0% on LNA to a value not significantly different

2   from the 4 other fastest growing isolates. The growth rate of *T. harzianum* 9132 decreased marginally

3   by only 2.9% on LNA compared to MEA; the other *Trichoderma* isolates grew between 11.6 and

4   40.2% slower on LNA (Table 3).

5

6   Compared to the controls, all *P. noxius* cultures grew slower, in absolute terms, in the presence of

7   VOCs emitted by *Trichoderma*. Overall, growth rates of *P. noxius* varied significantly in the presence

8   of VOCs produced by *Trichoderma* isolates ($F = 5.40$; df = 7, 28; $p < 0.001$), and the growth of *P.*

9   *noxius* SSN3P cultures was inhibited significantly more by *Trichoderma* VOC emission than *P.*

10   *noxius* KSK6 ($F = 24.14$; df = 1, 28; $P = 0.008$). However, *Trichoderma* and *P. noxius* isolates

11   interacted significantly to affect growth rates because VOCs emitted by *Trichoderma* isolates did not

12   inhibit the 2 *P. noxius* isolates in a proportionally consistent way; *Trichoderma* isolates significantly

13   inhibiting one *P. noxius* isolate did not necessarily inhibit the other equally well. Compared to the

14   controls, 6 *Trichoderma* isolates caused a significant reduction to the growth rate of *P. noxius* SSN3P,

15   but only 4 *Trichoderma* isolates caused similar reductions to *P. noxius* KSK6 (Table 4). Separately, *T.*

16   *harzianum* 9132 and *T. virens* W23 caused the greatest inhibition to *P. noxius* KSK6 and *P. noxius*

17   SSN3P, respectively (Table 4).

18

19   In dual culture tests on MEA, most of the *Trichoderma* isolates exhibited comparably high

20   antagonistic potential with percent mortality to *P. noxius* ranging between 80 and 100%. All

21   *Trichoderma* isolates overspread and colonized the *P. noxius* cultures; none were deadlocked in

22   distant confrontation or evaded by the wood decay fungus. Overall, there were no significant

23   differences in the percent mortality of *P. noxius* challenged by various *Trichoderma* isolates in dual

24   culture tests ($F = 0.98$; df = 6, 12; $p = 0.479$), and there were no significant differences in the

25   susceptibility of the 2 *P. noxius* isolates to antagonism by *Trichoderma* ($F = 0.61$; df = 1, 12; $p =$

26   0.517). However, *Trichoderma* and *P. noxius* isolates interacted significantly to affect percent

27   mortality ($F = 3.25$; df = 6, 12; $p = 0.039$). Although the lethal effect of various *Trichoderma* isolates

28   towards *P. noxius* SSN3P in dual culture did not vary significantly, the same was not true for *P.*

10

1   *noxius* KSK6. Specifically, the percent mortality of *P. noxius* KSK6 confronted by *T. harzianum* 9132

2   was significantly less than that caused by the other *Trichoderma* isolates (Table 5).

3

4   Overall, dry weight loss of wood blocks varied significantly according to their pre-treatment with

5   various *Trichoderma* isolates (F = 7.65; df = 7, 28; p < 0.001), and pre-treatment with *Trichoderma*

6   isolates gave significantly better protection against decomposition by *P. noxius* SSN3P than *P. noxius*

7   KSK6 (F = 20.52; df = 1, 28; p = 0.011). The interaction between *Trichoderma* and *P. noxius* isolates,

8   however, was not significant (F = 1.11; df = 7, 28; p = 0.386) because *Trichoderma* isolates inhibited

9   decomposition by the 2 *P. noxius* isolates similarly. Compared to the controls, pre-treatment with 6

10   different *Trichoderma* isolates caused a significant reduction to the dry weight loss of wood blocks

11   inoculated with *P. noxius*. Among all isolates, *T. harzianum* 9132 offered the greatest protection to

12   wood blocks against decomposition by *P. noxius*, and *T. virens* W26 was the only isolate that did not

13   significantly inhibit wood block decomposition compared to the controls (Table 6).

14

15   **<u>Discussion</u>**

16   Despite apparent variability, all of the *Trichoderma* isolates tested in this study antagonized both *P.*

17   *noxius* isolates in the different bioassays. In contrast with similar studies (Mercer and Kirk, 1984a;

18   Ribera et al., 2016; Schubert et al., 2008a; Schwarze et al., 2012), none of the *Trichoderma* isolates

19   enhanced the activity of *P. noxius* isolates in culture. Still, no single *Trichoderma* isolate was

20   consistently superior among all others in its ability to antagonize *P. noxius* across every bioassay. In

21   addition, the presence of *Trichoderma* caused a greater reduction to the growth (Table 4) and wood

22   decay rates (Table 6) of *P. noxius* SSN3P than *P. noxius* KSK6, indicating the greater susceptibility of

23   the former to biological control by *Trichoderma*. Although the wood block interaction tests were

24   performed on different wood substrates corresponding to the tree species from which each *P. noxius*

25   isolate was originally obtained (Table 1), the equally greater antagonistic effect of *Trichoderma* VOC

26   emission on *P. noxius* SSN3P suggests that the disparity is not entirely caused by differences in test

27   conditions associated with wood anatomy. In agreement with existing reports (Schwarze et al., 2012;

1   Wells and Bell, 1979), this intraspecific variability and target specificity affirms the importance of

2   screening potential biological control agents against the desired pathogen in laboratory tests.

3

4   Germination rates were nearly complete for the conidia of *Trichoderma* isolates cultured on LNA

5   (Table 2). Although exogenous nutrients are required for germination, existing reports indicate that

6   leached nutrients and secondary metabolites can provide adequate resources to support germination

7   (Danielson and Davey, 1973; Naar and Kecskes, 1998). Since conidia were unwashed, germination

8   rates reported in this study may not reflect those for *Trichoderma* in sterile environments. Practically,

9   these results suggest that conidial preparations for biological control can be amended with modest

10   supplemental nutrients to support high germination rates. Analogously, Schubert et al. (2008a)

11   reported that an unmodified conidial suspension and another amended with glucose and urea persisted

12   equally well on tree wounds over a 30-month period. In addition, high germination rates for

13   introduced conidia valuably limits the availability of dead propagules as a nutrient source to other

14   deleterious microbes that might interfere with biological control by competing with *Trichoderma* or

15   infecting the plant host (Harman et al., 1991).

16

17   In contrast, however, there was considerable variability in the production of chlamydospores among

18   *Trichoderma* isolates (Table 2). Relatively few reports exist on *Trichoderma* chlamydospore biology;

19   Lewis and Papavizas (1984) reported that their production rates similarly varied among *Trichoderma*

20   isolates. Although it is generally accepted that these resting spores enhance survival during

21   unfavorable conditions in the soil, it is not clear to what extent they might serve a similar purpose on

22   other substrates, such as wood. Practically, chlamydospores are valuable for commercial *Trichoderma*

23   formulations since these propagules better tolerate desiccation and storage than conidia or mycelial

24   fragments (Li et al., 2016).

25

26   Growth rates varied significantly among *Trichoderma* isolates, but all isolates grew at faster rates than

27   reported for others selected from temperate or sub-tropical climates for similar biological control

28   applications (Ribera et al., 2016; Schubert et al., 2008a; Schwarze et al., 2012). In agreement with

1   existing work (Schubert et al., 2008b), growth rates decreased for isolates cultured under nutrient

2   limiting conditions on LNA. However, the increased growth of *T. virens* W23 under these conditions

3   is a notable exception (Table 3). Although difficult to demonstrate experimentally (Harman, 2000),

4   competition for space and nutrients is likely an important mechanism for biological control by

5   *Trichoderma*.

6

7   It is useful to note that germination and growth rates were determined under constant environmental

8   conditions chosen to replicate typical values for Singapore. At this location, the climate is

9   representative of the equatorial tropics with stable temperatures ranging daily between 24° and 32° C,

10   abundant rainfall approaching 250 cm annually, and elevated humidity between 60 and 90%

11   (Micheline and Ng, 2012). In general, the optimum temperature for the growth of many *Trichoderma*

12   spp. is between 25° and 30° C (Klein and Eveleigh, 1998). There is considerable experimental

13   evidence that *Trichoderma* germination and growth rates are proportional to water activity and

14   temperature (Kredics et al., 2003), up to some limiting value that depends on the climatic origin of a

15   given *Trichoderma* isolate (Ribera et al., 2016; Schubert et al., 2008b; Schwarze et al., 2012); but it

16   was assumed that tolerance to a wide range of unfavorable temperature and moisture conditions is not

17   critical for biological control by *Trichoderma* in Singapore.

18

19   Compared to similar work on wood decay fungi (Mercer and Kirk, 1984a; Schubert et al., 2008b),

20   including *P. noxius* (Ribera et al., 2016; Schwarze et al., 2012), VOC emission by *Trichoderma*

21   isolates had a comparatively large effect on the growth of *P. noxius* cultures in this study. Ribera et al.

22   (2016) reported that less than half of all tested *Trichoderma* isolates inhibited the radial growth rates

23   of *P. noxius* isolates associated with landscape trees in Hong Kong. Similarly, Schwarze et al. (2012)

24   reported that only one out of 16 possible combinations of *P. noxius* and *Trichoderma* isolates resulted

25   in a significant decrease to the radial growth rate of the wood decay fungus compared to their

26   respective controls. Based on similar observations, Schubert et al. (2008b) postulated that antibiosis

27   might not be an important mechanism by which *Trichoderma* antagonizes wood decay fungi, but

28   these results provide contrasting evidence that VOCs can significantly inhibit the growth of *P. noxius*

13

1   cultures. Notably, *T. harzianum* 9132 and *T. virens* W23 caused a 68.6% and 81.3% reduction to the

2   growth rates, respectively, of *P. noxius* KSK6 and *P. noxius* SSN3P.

3

4   The lethal effect of *Trichoderma* on *P. noxius* in dual culture tests was consistently high, except for

5   the interaction between *P. noxius* KSK6 and *T. harzianum* 9132 (Table 5). The uniformly high lethal

6   effect might be partially explained by the favorable habitability of MEA; other authors have reported

7   that inhibition of wood decay fungi by *Trichoderma* in dual culture tests was significantly greater on

8   MEA than LNA (Schubert et al., 2008b; Srinivasan et al., 1992). Specifically, Srinivasan et al. (1992)

9   reported that soluble metabolites and volatile antibiotics were produced in significantly greater

10   quantities by *Trichoderma* growing on MEA. As a result, the results from this test preclude

11   discrimination among candidate *Trichoderma* isolates for biological control, but there was much

12   greater variability in the protection afforded by various *Trichoderma* isolates to wood blocks against

13   decay by *P. noxius*.

14

15   In this study, almost all *Trichoderma* isolates significantly inhibited wood decay by *P. noxius*

16   compared to the controls (Table 6). Although the *Trichoderma* isolates failed to completely prevent

17   wood decay, the sizeable reduction is in broad agreement with existing studies (Ribera et al., 2016;

18   Schubert et al., 2008a; Schubert et al., 2008b; Schwarze et al., 2012). There are 2 possible

19   explanations for the incomplete reduction of dry weight loss to wood blocks by *Trichoderma*. First,

20   the wood blocks were inoculated by introducing an artificially large amount of *P. noxius* inoculum,

21   and the disproportionate size of *P. noxius* and *Trichoderma* cultures may have favored the former.

22   Second, a fraction of the total dry weight loss to wood blocks was probably caused by the

23   decomposition of non-structural carbohydrates by *Trichoderma* (Klein and Eveleigh, 1998). Still, the

24   significant reductions to the dry weight loss of wood blocks shows the strong antagonistic potential of

25   several *Trichoderma* isolates, especially *T. harzianum* 9132 (Table 6).

26

27   After incubation for 12 weeks, the percent dry weight loss to control wood blocks inoculated with *P.*

28   *noxius* KSK6 and SSN3P was much greater than reported for balsa [*Ochroma pyramidale* (Cav. ex

14

1   Lam.) Urb. (Malvaceae)] wood blocks inoculated with different *P. noxius* isolates obtained from

2   landscape trees in Hong Kong (Ribera et al., 2016), but the same values in this study are more

3   comparable to dry weight losses reported for wood blocks removed from several landscape tree

4   species associated with *P. noxius* in Brisbane, Australia (Schwarze et al., 2012). Collectively, these

5   results demonstrate the importance of using species-specific wood substrates to represent the host-

6   fungus interaction in which *Trichoderma* must intercede as a biological control agent, and this

7   distinction gives confidence to the observations made on Senegal mahogany and rain tree wood

8   blocks in this study.

9

10 In this study, the systematic investigation of 7 *Trichoderma* isolates demonstrated their promising

11 antagonism towards *P. noxius*. Although the 2 *P. noxius* isolates differed in their susceptibility,

12 several *Trichoderma* isolates showed a significant ability to inhibit growth and reduce decomposition

13 by this aggressive wood decay fungus. None of the *Trichoderma* isolates increased the activity of *P.*

14 *noxius*, and the deliberate collection of *Trichoderma* isolates from an ecological niche that resembled

15 the desired target specificity, i.e., from basidiomycete fruiting bodies, may have been an effective

16 selection strategy for biological control agents. In agreement with existing reports, there was no clear

17 evidence of a primary mechanism by which *Trichoderma* antagonized *P. noxius*, and the results from

18 different bioassays should be given equal weight in the selection of *Trichoderma* isolates. Although

19 no single Trichoderma isolate outperformed all others in every test, there were a few that did so more

20 than others. In particular, *T. virens* W23 produced the most chlamydospores, best inhibited the growth

21 of *P. noxius* SSN3P, and uniquely grew faster on LNA. On the other hand, *T. harzianum* 9132 best

22 inhibited the growth of *P. noxius* KSK6 and prevented dry weight loss to wood blocks. Still, these

23 results are only indicative of the ability of *Trichoderma* to antagonize *P. noxius* on tree wounds, and

24 these competitive *Trichoderma* isolates should be further examined under field conditions to confirm

25 their efficacy. Laboratory conditions were more favorable and simplified than those governing host-

26 fungus interaction in dynamic natural systems.

27

28 **<u>Acknowledgments</u>**

15

4

5 **Bibliography**

6 Anonymous. American National Standard for Tree Care Operations - Tree, Shrub, and Other Woody
7 Plant Management - Standard Practices (Pruning),2008 Tree Care Industry Association, Inc,
8 Londonderry, NH, USA.

9 Barry, K.M., Pearce, R.B., Mohammed, C.L., 2000. Properties of reaction zones associated with
10 decay from pruning wounds in plantation-grown *Eucalyptus nitens*. Forest Pathol. 30, 233-245.

11 Benson, D.A., Boguski, M.S., Lipman, D.J., Ostell, J., 1997. GenBank. Nucleic Acids Res 25, 1-6.

12 Bigirimana, J., de Meyer, G., Poppe, J., Elad, Y., Hoefte, M., 1997. Induction of systemic resistance
13 on bean (*Phaseolus vulgaris*) by *Trichoderma harzianum*. Int S Crop. 62, 1001-1008.

14 Brunner, K., Zeilinger, S., Ciliento, R., Woo, S.L., Lorito, M., Kubicek, C.P., et al., 2005.
15 Improvement of the fungal biocontrol agent *Trichoderma atroviride* to enhance both antagonism and
16 induction of plant systemic disease resistance. Appl Environ Mirob. 71, 3959-3965.

17 Burcham, D.C., Wong, J.Y., Ali, M.I.M., Abarrientos, N.V., Fong, Y.K., Schwarze, F.W.M.R., 2015.
18 Characterization of host-fungus interactions among wood decay fungi associated with *Khaya
19 senegalensis* (Desr.) A. Juss (Meliaceae) in Singapore. Forest Pathol. 45, 492-504.

20 Chang, T.-., 1995. Decline of nine tree species associated with brown root rot caused by *Phellinus
21 noxius*. Plant Dis. 79, 962-965.

22 Danielson, R.M., Davey, C.B., 1973. Effects of nutrients and acidity on phialospore germination of
23 *Trichoderma* in vitro. Soil Biol Biochem. 5, 517-524.

24 Deflorio, G., Barry, K.M., Johnson, C., Mohammed, C.L., 2007. The influence of wound location on
25 decay extent in plantation-grown *Eucalyptus globulus* and *Eucalyptus nitens*. Forest Ecol Manag. 242,
26 353-362.

27 Dennis, C., Webster, J., 1971. Antagonistic properties of species-groups of *Trichoderma* II.
28 Production of volatile antibiotics. T Brit Mycol Soc. 57, 41-48.

29 Di Pietro, A., Lorito, M., Hayes, C.K., Broadway, R.M., Harman, G.E., 1993. Endochitinase from
30 *Gliocladium virens*: Isolation, characterization, and synergistic antifungal activity in combination with
31 gliotoxin. Mol Plant Pathol. 83, 308-313.

32 Djonovic, S., Pozo, M.J., Dangott, L.J., Howell, C.R., Kenerley, C.M., 2006. Sm1, a proteinaceous
33 elicitor secreted by the biocontrol fungus *Trichoderma virens* induces plant defense responses and
34 systemic resistance. Mol Plant Microbe In. 19, 838-853.

1   Djonovic, S., Vittone, G., Mendoza-Herrera, A., Kenerley, C.M., 2007. Enhanced biocontrol activity
2   of *Trichoderma virens* transformants constitutively coexpressing β-1,3- and β-1,6-glucanase genes.
3   Mol Plant Pathol. 8, 469-480.

4   Gams, W., Bissett, J., 1998. Morphology and identification of *Trichoderma*, in: Kubicek, C.P.,
5   Harman, G.E. (Eds.), *Trichoderma* and *Gliocladium* Volume 1. Taylor & Francis, London, England,
6   UK, pp. 3-31.

7   Ghisalberti, E.L., Sivasithamparam, K., 1991. Antifungal antibiotics produced by *Trichoderma* spp.
8   Soil Biol Biochem. 23, 1011-1020.

9   Harman, G.E., 2000. Myths and dogmas of biocontrol: Changes in perceptions derived from research
10 on *Trichoderma harzianum* T-22. Plant Dis. 84, 377-393.

11 Harman, G.E., Howell, C.R., Viterbo, A., Chet, I., Lorito, M., 2004. *Trichoderma* species-
12 opportunistic, avirulent plant symbionts. Nat Rev Microbiol. 2, 43-56.

13 Harman, G.E., Jin, X., Stasz, T.E., Peruzzotti, G., Leopold, A.C., Taylor, A.G., 1991. Production of
14 conidial biomass of *Trichoderma harzianum* for biological control. Biol Control. 1, 23-28.

15 Highley, T.L., Padmanabha, H.S.A., Howell, C.R., 1997. Control of wood decay by *Trichoderma*
16 (*Gliocladium*) *virens* II. Antibiosis. Mater Organismen. 31, 157-166.

17 Howell, C.R., 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant
18 diseases: The history and evolution of current concepts. Plant Dis. 87, 4-10.

19 Huttermann, A., Volger, C., 1973. Induction of aryl-β-glucosidase in *Fomes annosus* by cellobiose.
20 Archiv Mikrobiol. 93, 195-204.

21 Kallio, T., Hallaksela, A.M., 1979. Biological control of *Heterobasidion annosum* (Fr.) Bref. (*Fomes*
22 *annosus*) in Finland. Eur J Forest Pathol. 9, 298-308.

23 Klein, D., Eveleigh, D.E., 1998. Ecology of *Trichoderma*, in: Kubicek, C.P., Harman, G.E. (Eds.),
24 *Trichoderma* and *Gliocladium* Volume 1. Taylor & Francis, London, England, UK, pp. 57-74.

25 Koike, N., Kyakumachi, M., Kageyama, K., Tsuyumu, S., Doke, N., 2001. Induction of systemic
26 resistance in cucumber against several diseases by plant growth-promoting fungi: Lignification and
27 superoxide generation. Eur J Plant Pathol. 107, 523-533.

28 Kredics, L., Antal, Z., Manczinger, L., Szekeres, A., Kevei, F., Nagy, E., 2003. Influence of
29 environmental parameters on *Trichoderma* strains with biocontrol potential. Food Technol
30 Biotechnol. 41, 37-42.

31 Lewis, J.A., Papavizas, G.C., 1984. Chlamydospore formation by *Trichoderma* spp. in natural
32 substrates. Can J Microbiol. 30, 1-7.

33 Li, Y.Q., Song, K., Li, Y.C., Chen, J., 2016. Statistical culture-based strategies to enhance
34 chlamydospore production by *Trichoderma harzianum* SH2303 in liquid fermentation. J Zhejiang
35 Univ-Sc B. 17, 619-627.

36 Lonsdale, D., 1984. Available treatments for tree wounds: An assessment of their value. Arboric J. 8,
37 99-107.

1   Lorito, M., Farkas, V., Rebuffat, S., Bodo, B., Kubicek, C.P., 1996a. Cell wall synthesis is a major
2   target of mycoparasitic antagonism by *Trichoderma harzianum*. J Bacteriol. 178, 6382-6385.

3   Lorito, M., Hayes, C.K., Di Pietro, A., Woo, S.L., Harman, G.E., 1994a. Purification,
4   characterization, and synergistic activity of a glucan 1,3-β-glucosidase and an *N*-acetyl-β-
5   glucosaminidase from *Trichoderma harzianum*. Phytopathology. 84, 398-405.

6   Lorito, M., Peterbauer, C., Hayes, C.K., Harman, G.E., 1994b. Synergistic interaction between fungal
7   cell wall degrading enzymes and different antifungal compounds enhances inhibition of spore
8   germination. Microbiology. 140, 623-629.

9   Lorito, M., Woo, S.L., D'Ambrosio, M., Harman, G.E., Hayes, C.K., Kubicek, C.P., et al., 1996b.
10   Synergistic interaction between cell wall degrading enzymes and membrane affecting compounds.
11   Mol Plant Microbe In. 9, 206-213.

12   Markovich, N.A., Kononova, G.L., 2003. Lytic enzymes of *Trichoderma* and their role in plant
13   defense from fungal diseases: A review. Appl Biochem Microbiol. 39, 341-351.

14   Mercer, P.C., Kirk, S.A., Gendle, P., Clifford, D.R., 1983. Chemical treatments for control of decay in
15   pruning wounds. Ann Appl Biol. 102, 435-453.

16   Mercer, P.C., 1983. Callus growth and the effect of wound dressings. Ann Appl Biol. 103, 527-540.

17   Mercer, P.C., 1979. Phytotoxicity and fungitoxicity tests for tree wound paints. Ann Appl Biol. 91,
18   199-202.

19   Mercer, P.C., Kirk, S.A., 1984a. Biological treatments for the control of decay in tree wounds I.
20   Laboratory tests. Ann Appl Biol. 104, 211-219.

21   Mercer, P.C., Kirk, S.A., 1984b. Biological treatments for the control of decay in tree wounds II.
22   Field tests. Ann Appl Biol. 104, 221-229.

23   Micheline, F., Ng, L.K., 2012. The Weather and Climate of Singapore, Meteorological Service
24   Singapore, National Environment Agency, Singapore.

25   Naar, Z., Kecskes, M., 1998. Factors influencing the competitive saprophytic ability of *Trichoderma*
26   species. Microbiol Res. 153, 119-129.

27   Nicole, M., Chamberland, H., Rioux, D., Xixuan, X., Blanchette, R.A., Geiger, J.P., et al., 1995.
28   Wood degradation by *Phellinus noxius*: Ultrastructure and cytochemistry. Can J Microbiol. 41, 253-
29   265.

30   Papavizas, G.C., 1985. *Trichoderma* and *Gliocladium*: Biology, ecology, and potential for biocontrol.
31   Annu Rev Phytopathol. 23, 23-54.

32   Pieterse, C.M.J., van Loon, L.C., 1999. Salicylic acid-indpendent plant defence pathways. Trends
33   Plant Sci. 4, 52-58.

34   Pottle, H.W., Shigo, A.L., 1975. Treatment of wounds on *Acer rubrum* with *Trichoderma viride*. Eur J
35   Forest Pathol. 5, 274-279.

1   Ribera, J., Tang, A.M.C., Schubert, M., Lam, R.Y.C., Chu, L.M., Leung, M.W.K., et al., 2016. In-
2   vitro evaluation of antagonistic *Trichoderma* strains for eradicating *Phellinus noxius* in colonised
3   wood. J Trop For Sci. 28, 457-468.

4   Ricard, J.L., Highley, T.L., 1988. Biocontrol of pathogenic fungi in wood and trees, with particular
5   emphasis on the use of *Trichoderma*. Biocontrol News and Information, 133-142.

6   Rossman, A.Y., Farr, D.F., 2014. Fungal Databases. 2014.

7   Samuels, G.J., 1996. *Trichoderma*: A review of biology and systematics of the genus. Mycol Res.
8   100, 923-935.

9   Schubert, M., Fink, S., Schwarze, F.W.M.R., 2008a. Evaluation of *Trichoderma* spp. as a biocontrol
10   agent against wood decay fungi in urban trees. Biol Control. 45, 111-123.

11   Schubert, M., Fink, S., Schwarze, F.W.M.R., 2008b. In vitro screening of an antagonistic
12   *Trichoderma* strain against wood decay fungi. Arboric J. 31, 227-248.

13   Schwarze, F.W.M.R., Jauss, F., Spencer, C., Hallam, C., Schubert, M., 2012. Evaluation of an
14   antagonistic *Trichoderma* strain for reducing the rate of wood decomposition by the white rot fungus
15   *Phellinus noxius*. Biol Control. 61, 160-168.

16   Sieber, T.N., 1995. *Pyrenochaeta ligni-putridi* sp. nov., a new coelomycete associated with butt rot of
17   *Picea abies* in Switzerland. Mycol Res. 99, 274-276.

18   Sivan, A., Chet, I., 1989. The possible role of competition between *Trichoderma harzianum* and
19   *Fusarium oxysporum* on rhizosphere colonization. Phytopathology. 79, 198-203.

20   Srinivasan, U., Staines, H.J., Bruce, A., 1992. Influence of media type on antagonistic modes of
21   *Trichoderma* spp. against wood decay basidiomycetes. Mater Organismen. 21, 302-321.

22   Wells, H.D., Bell, D.K., 1979. Variable antagonistic reaction in vitro of *Trichoderma harzianum*
23   against several pathogens. Phytopathology. 69, 1048-1049.

24   Yedidia, I., Benahmou, N., Kapulnik, Y., Chet, I., 2000. Induction and accumulation of PR proteins
25   activity during early stages of root colonization by the mycoparasite *Trichoderma harzianum* strain T-
26   203. Plant Physiol Bioch. 38, 863-873.

27   Yedidia, I., Benhamou, N., Chet, I., 1999. Induction of defense responses in cucumber plants
28   (*Cucumis sativus* L.) by the biocontrol agent *Trichoderma harzianum*. Appl Environ Microb. 65,
29   1061-1070.

30

Table 1: Identity, origin, and accession numbers of the fungal isolates collected for use in this study

Species	Isolate	Accession No.	Substrate	Origin	Reference	Identity (%)
a. Wood decay fungi						
P. noxius	KSK6	KF233592	ex. *Khaya senegalensis*	Bugis, Singapore	FR821770 (Schwarze et al., 2012)	99
P. noxius	SSN3P	KP202302	ex. *Samanea saman*	Clementi, Singapore	FR821769 (Schwarze et al., 2012)	98
b. *Trichoderma* spp.						
T. reesei	1056TD1	KY025557	*Ganoderma boninense* on *Cocos nucifera*	Singapore	JQ979435.1 (Kuhls et al., 1996)	99
T. asperellum	3	KY025555	*Ganoderma* sp. on coarse woody debris	Bukit Batok, Singapore	AJ230669.1 (Lieckfeldt et al., 1999)	100
T. harzianum	9132	KY025556	*Ganoderma boninense* on *Cyrtostachys renda*	Singapore	KR856210.1 (Lieckfeldt et al., 2001)	99
T. saturnisporum	SPH20109132	KY025558	*Ganoderma boninense* on *Cyrtostachys renda*	Singapore	NR_103704.1 (Kuhls et al., 1997)	99
T. harzianum	W21	KY025559	*Ganoderma boninense* on *Ptychosperma macarthurii*	Woodlands, Singapore	KJ000324.1 (Lieckfeldt et al., 2001)	99
T. virens	W23	KY025560	*Ganoderma boninense* on *Ptychosperma macarthurii*	Woodlands, Singapore	KP009289.1 (Ottenheim et al., 2015)	100
T. virens	W26	KY025561	*Ganoderma boninense* on *Ptychosperma macarthurii*	Woodlands, Singapore	KR296867.1 (Ottenheim et al., 2015)	100

Note: Fungal ITS sequences were deposited in GenBank (Benson et al., 1997).

Table 2: Germination rates (%) and chlamydospore counts (*n*) for various *Trichoderma* spp. isolates cultured on a low nutrient agar (LNA)

Trichoderma spp.	Germination rate (%)	Chlamydospore count (*n*)
1056TD1	87 (21)	9 (4)
3	100 (0)	20 (15)
9132	100 (0)	20 (13)
SPH20109132	100 (0)	6 (2)
W21	100 (0)	39 (8)
W23	99 (1)	189 (30)
W26	99 (1)	165 (44)

Note: Values listed are mean (SD). See Table 1 for the taxonomic identity of fungal isolates.

Table 3: Growth rates (cm·day^{-1}) of *Trichoderma* spp. isolates cultured on 2 different media, including a malt extract agar (MEA) and low nutrient agar (LNA)

Trichoderma spp.	Media type	
	MEA	LNA
1056TD1	7.96 (0.11) a	4.76 (0.04) a
3	4.61 (0.01) c	3.12 (0.27) c
9132	4.74 (0.02) c	4.60 (0.02) ab
SPH20109132	8.04 (0.10) a	5.28 (0.48) a
W21	5.07 (0.11) b	4.48 (0.76) ab
W23	4.38 (0.10) d	4.73 (0.07) a
W26	4.41 (0.08) d	3.62 (0.97) bc
Mean	5.61 (1.56)	4.37 (0.84)

Note: Values listed are mean (SD). Means followed by the same letter within each column are not significantly different at the $\alpha = 0.05$ level. See Table 1 for the taxonomic identity of fungal isolates.

Table 4: *Phellinus noxius* KSK6 and *P. noxius* SSN3P growth rates (cm·day^{-1}) in the presence of VOCs emitted by various *Trichoderma* spp. isolates

Trichoderma spp.	*P. noxius*	
	KSK6	SSN3P
Control	1.75 (0.01)	1.34 (0.08)
1056TD1	1.48 (0.19)	0.42 (0.47)*
3	1.46 (0.09)	0.68 (0.49)*
9132	0.55 (0.45)*	0.69 (0.29)*
SPH20109132	0.90 (0.32)*	0.86 (0.21)*
W21	1.18 (0.36)*	1.04 (0.25)
W23	1.52 (0.23)	0.25 (0.33)*
W26	0.94 (0.59)*	0.39 (0.55)*
Mean	1.22 (0.48)	0.71 (0.48)

Note: Values listed are mean (SD). Within each column, asterisks (*) indicate a significant reduction in growth rates compared to the control at the $\alpha = 0.05$ level. See Table 1 for the taxonomic identity of fungal isolates.

Table 5: Percent mortality (%) to *Phellinus noxius* KSK6 and *P. noxius* SSN3P due to mycoparasitism by various *Trichoderma* spp. isolates in dual culture

Trichoderma spp.	*P. noxius*	
	KSK6	SSN3P
1056TD1	89 (19) a	89 (10)
3	83 (17) a	94 (10)
9132	33 (58) b	100 (0)
SPH20109132	94 (10) a	89 (10)
W21	78 (10) a	94 (10)
W23	89 (10) a	67 (58)
W26	100 (0) a	100 (0)
Mean	81 (29)	90 (22)

Note: Values listed are mean (SD). Within left-hand column, means followed by the same letter are not significantly different at the $\alpha = 0.05$ level. See Table 1 for the taxonomic identity of fungal isolates.

Table 6: Dry weight loss (%) caused to Senegal mahogany (*Khaya senegalensis*) and rain tree (*Samanea saman*) wood blocks by *Phellinus noxius* KSK6 and *P. noxius* SSN3P, respectively, after pre-treatment with various *Trichoderma* spp. isolates

Trichoderma spp.	*P. noxius*		Mean
	KSK6	SSN3P	
Control	12.7 (4.4)	10.8 (5.0)	11.7
1056TD1	8.0 (2.6)	3.6 (5.0)	5.8*
3	4.6 (0.5)	2.8 (0.8)	3.7*
9132	5.0 (0.8)	1.8 (1.3)	3.4*
SPH20109132	7.7 (2.4)	4.0 (4.2)	5.7*
W21	8.7 (0.8)	3.6 (2.9)	6.2*
W23	7.2 (1.2)	5.8 (4.2)	6.5*
W26	8.2 (2.1)	8.6 (1.7)	8.4
Mean	7.8 (3.1) a	5.1 (4.3) b	

Note: Values listed are mean (SD). Within right-hand column, asterisks (*) indicate a significant reduction in dry weight loss compared to the control at the $\alpha = 0.05$ level. Means followed by the same letter within the bottom row are not significantly different at the $\alpha = 0.05$ level. See Table 1 for the taxonomic identity of fungal isolates.
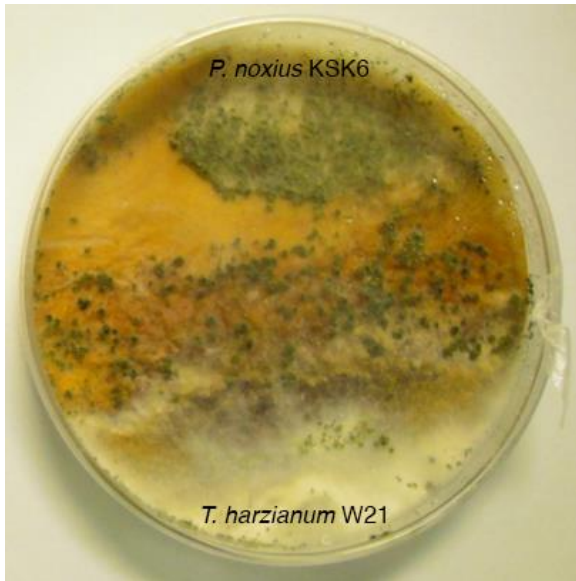
Figure 1: In one dual culture test, *T. harzianum* W21 (bottom) overgrew *P. noxius* KSK6 (top) during confrontation. The distribution of *T. harzianum* W21 over the wood decay fungus is evident by its green conidia, likely formed in response to its antagonistic interaction with *P. noxius* KSK6.