IN-VITRO EVALUATION OF ANTAGONISTIC *TRICHODERMA* STRAINS FOR ERADICATING *PHELLINUS NOXIUS* IN COLONISED WOOD

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RIBERA J, TANG AMC, SCHUBERT M, LAM RYC, CHU LM, LEUNG MWK, KWAN HS, BAS MC & SCHWARZE FWMR. 2016. In-vitro evaluation of antagonistic *Trichoderma* strains for eradicating *Phellinus noxius* in colonised wood. The aim of the present in-vitro studies was to identify *Trichoderma* strains from Hong Kong with highly antagonistic potential against the basidiomycete *Phellinus noxius*. Dual culture and interaction tests using samples of balsa wood (*Ochroma lagopus*) as well as studies on fungal growth at different temperatures and water activities were conducted. The impact of *Trichoderma* strains on wood colonisation and decomposition by three *P. noxius* isolates were quantitatively analysed by measuring dry weight loss and rate of eradication of the pathogen from the test wood. Most *Trichoderma* strains revealed antagonistic potential against *P. noxius*. In the wood blocks incubated with *P. noxius* and then treated with *Trichoderma* T-TMS1 for 24 weeks, 100% eradication of three *P. noxius* isolates was recorded. The results indicated that application of *Trichoderma* strains might be a promising and environmentally benign method of eradicating *P. noxius* from wood debris in soils.

Keywords: Brown root rot disease, survival, wood debris, inoculum, biological control

INTRODUCTION

Phellinus noxius, the cause of brown root rot disease, is an aggressive and destructive pathogen that thrives in soil and attacks roots of susceptible plants (Ann et al. 2002, Mohd et al. 2009, Sahashi et al. 2012). It mainly infects roots and lower trunk of trees, causing the roots to rot and resulting in chronic or acute crown dieback and plant death after impairment of its water-conducting system (Hodges & Tenorio 1984, Ann et al. 2002). Brown root rot disease has a wide range of host and has been reported on more than 200 plant species in subtropical and tropical regions (Ann et al. 2002). In Hong Kong, a number of trees species (e.g. *Acacia confusa*, *Aleurites moluccana*, *Bombax ceiba*, *Celtis sinensis*, *Delonix regia*, *Ficus elastica*, *F. microcarpa*, *F. benjamina*, *Gleditsia fera*, *Lophostemon confertus*, *Macaranga tanarius*, *Mallotus paniculatus*, *Mangifera indica*) have recently been confirmed as hosts of the disease. *Ficus microcarpa,* especially, has attracted much concern among different tree management units and the general public in Hong Kong. *Phellinus noxius* can survive in the soil and roots of dead host plants for more than 10 years (Chang 1996). Infected root debris is the primary source of inoculum (Chang 1996). Removal of this debris or killing the fungus within the debris has been considered the most effective method of control, but even for a small tree, complete removal of the infected root debris is very difficult.

Traditionally, chemical fungicides have been widely used to protect plants from fungal pathogens. Three systemic fungicides (Calixin,

Bayleton and Nustar) inhibit mycelial growth of *P. noxius* on agar medium (Chang & Chang 1999), but none was effective for killing the fungus in infested wood. Volatile ammonia, generated from urea added to soil, is able to kill *P. noxius* in infested wood (Chang & Chang 1999). Urea and ammonia solution produce similar effects and destroy *P. noxius* in the wood without direct contact. None of the *P. noxius*-infested wood fragments buried in the soil or placed on top of the soil survived 1 month after treatment (Chang & Chang 1999). Volatile ammonia generated from urea is fungicidal to *P. noxius* in infested wood. However, ammonia gas is severely irritating to the respiratory tract, eyes, moist skin and mucous membranes of humans (Leduc et al. 1992). It would be more desirable to devise a strategy to control plant diseases without excessive application of such fungicides. Alternatives to biocidal control of plant pathogens have now become more important owing to legal restrictions of conventional biocides on urban sites. Thus, biological control of *P. noxius* may be a promising strategy. Biocontrol methods of removing roots infected with *P. noxius* and eradicating the fungus in small debris could greatly enhance the effectiveness of other control measures.

Two antagonistic strains of *Trichoderma* against *P. noxius* in Australia have been identified (Schwarze et al. 2012). These *Trichoderma* strains were applied to the mulch around five *Phellinus*-infected *F. benjamina* trees and one *F. benghalensis*. After treatment, all trees started to develop new roots within 6–8 weeks and the mycelium of *P. noxius* was killed after 8–11 weeks exposure to *Trichoderma* (K Foster, personal communication).

The aim of the present work was to assess the potential of highly antagonistic *Trichoderma* strains that can be applied against the wood-decay basidiomycete *P. noxius* from different sites in Hong Kong. For comparison we used the European *T. atroviride* strain 15603.1, which is known to have high antagonistic potential against a range of wood-decay basidiomycetes (Schubert et al. 2008a, b, c) together with the Hong Kong native *Trichoderma* strains. Special emphasis was given to whether highly antagonistic *Trichoderma* species can be used for eradication of *P. noxius* in incubated wood.

MATERIALS AND METHODS

Micromorphological and molecular identification

All cultures were microscopically identified and the internal transcribed spacer (ITS1-5.8S-ITS2 region) of the rDNA was amplified and sequenced for each strain. The origins of the *Trichoderma* strains and wood-decay basidiomycetes are provided in Tables 1 and 2. All cultures were maintained on 2% malt extract agar (MEA) at 4 ± 1 °C. For further studies, Petri dishes with MEA were inoculated with 5-mm diameter agar plug cut from the growing edge of colonies of the strains and stored in the dark at 25 ± 1 °C and 70% ambient relative humidity. The sequences were deposited in the European Molecular Biology Laboratory Nucleotide Sequence Database.

Bioassays for growth under different conditions

The effects of temperature (20, 25, 30, 35 °C) and water activity (0.928, 0.955, 0.978, 0.998) on hyphal growth were monitored on 2% MEA. All agar plates (90 mm) were inoculated centrally with a 5-mm disc of the respective *Trichoderma* species and wood-decay fungi taken from the margin of growing cultures and incubated at 25 ± 1 °C and 70% relative humidity. For each experimental treatment (water activity and temperature), five replicates of each fungal strain were performed. The growth rate (mm day^{-1}) was determined by colony radial measurements carried out along two perpendicular axes after 24 hours (Schubert et al. 2009). Water activity of the substrate was controlled by addition of appropriate weights of glycerol prior to autoclaving (Dallyn 1978).

Inhibitory effects of volatile compounds produced by *Trichoderma* species on *P. noxius* strains

The effects of *Trichoderma* strains on wood-decay fungi of volatile organic compounds were evaluated using the following techniques as described by Dennis and Webster (1971). *Trichoderma* strains were centrally inoculated onto

Table 1 Origin of *Trichoderma* strains used in the present study

Trichoderma	Isolate code	EMBL no.	Origin
T. atroviride	T-15603.1	FR178524	EMPL culture collection, Switzerland
T. gamsii	T-PSL	LN558861	Soil at Pat Sin Leng, Hong Kong
T. harzianum	T-HV1	LN558862	Soil at Happy Valley, Hong Kong
T. harzianum	HKP	LN558863	Soil at Hong Kong Park, Hong Kong
T. harzianum	T-TMS1	LN558864	Soil at Tai Mo Shan, Hong Kong
T. harzianum	T-YWE	LN558865	Roots of *Ficus microcarpa* at Yu Wan Estate, Hong Kong
T. koningiopsis	T-PC	LN558866	Soil at Plover Cove, Hong Kong
T. koningiopsis	T-TL	LN558867	Soil at Tai Lam, Hong Kong
T. koningiopsis	T-TMS2	LN558868	Soil at Tai Mo Shan, Hong Kong
T. spirale	T-SMCP	LN558869	Soil at Shing Mun Country Park, Hong Kong
T. spirale	T-TPK	LN558870	Soil at Tai Po Kau, Hong Kong

EMBL = European Molecular Biology Laboratory

Table 2 Origin of *Phellinus noxius* isolates used in the present study

Isolate code	EMBL no.	Infected root
JCHS	LN558871	*Celtis sinensis*, Jockey Club Hing Shing Road Playground, Kwai Fong, Hong Kong
SBR	LN558872	*Ficus microcarpa*, Society for the Relief of Disabled Children, Sandy Bay Road, Hong Kong
SNRP	LN558873	*Mallotus paniculatus*, Sau Nga Road Playground, Kwun Tong, Hong Kong
YMT97	LN558874	*F. microcarpa*, LCSD YMT/97, Kowloon Park, Hong Kong
E35	LN558875	*Gleditsia fera*, LCSD E/35, Victoria Park, Hong Kong
SSP14	LN558876	*F. elastica*, LCSD SSP/14, Osmanthus Road Rest Garden, Hong Kong
YTM65	LN558877	*F. microcarpa*, LCSD YTM/65, Kowloon Park, Hong Kong

EMBL = European Molecular Biology Laboratory

2% MEA by placing 5-mm discs taken from the margin of 7-day-old cultures and incubating the plates at 25 ± 1 °C and 70% relative humidity for 4 weeks. MEA plates were inoculated centrally with 5-mm discs with the wood-decay fungi and the top of each plate was replaced with the bottom of a *Trichoderma*-inoculated plate. Replicates without *Trichoderma* species were used as control. Five replicates were maintained for each treatment. The pairs of Petri dishes were fixed and sealed together with Parafilm and incubated at 25 ± 1 °C and 70% relative humidity. The radii of all listed wood-decay fungi colonies were measured after an incubation period of 7 days.

Dual culture and interaction tests

Mycoparasitism of all *Trichoderma* species against the selected wood-decay fungi was assessed in dual cultures (Schubert et al. 2008a). Mycelial discs (5 mm) were removed from 1-week-old MEA cultures of each of the seven wood-decay fungi and placed equidistantly at the margin of Petri dishes (90 mm) containing 2% MEA. These were incubated at 25 ± 1 °C and 70% relative humidity for 3–4 days. The discs were removed from the margins of actively growing 1-week-old cultures of *Trichoderma* species and placed at opposite sides of the dish and incubated in the dark at 25 ± 1 °C and 70% relative humidity for 4 weeks. Petri

dishes without *Trichoderma* were used as control. Five replicates were used for each experiment. Petri dishes were examined at regular intervals. Sporulation tufts and pustules of *Trichoderma* fungi were used as indication of their activity (Naár & Kecskes 1998). In order to check whether the antagonist was able to overgrow and parasitise the wood decay fungus, five agar discs (5 mm) were removed from non-sporulating regions of the mycelium of the wood-decay fungus and placed on a *Trichoderma*-selective medium (Askew & Laing 1993). After 7 days of incubation at room temperature, discs were observed for *Trichoderma* colonies. Competition (mycoparasitism rate) was assessed as follows: 0 = no overgrowth, 1 = slow overgrowth, 2 = fast overgrowth and 3 = very fast overgrowth and deadlock of *P. noxius* strains. The ability of *Trichoderma* species to eliminate *P. noxius* strains (lethal effect) during the 4 weeks incubation period was evaluated by aseptically transferring 5-mm discs from the test plates to MEA with 5 mL of 2% thiabendazole dissolved in lactic acid (T-MEA). T-MEA suppresses the growth of *Trichoderma* species but allows growth of wood-decay fungi (Sieber 1995). The lethal effect of *Trichoderma* species was expressed as the percentage of *P. noxius* strains eliminated.

Evaluation of antagonistic activity in wood blocks

Interaction tests in wood blocks of *O. lagopus* (15 mm × 25 mm × 50 mm) were performed as described by Schubert et al. (2008a) with the following modifications. For evaluation of the preventative and curative effects of *Trichoderma* species against *P. noxius* isolates, sterilised wood blocks with ethylenoxid were placed in autoclavable plastic containers (40 cm × 60 cm × 15 cm) with 2% MEA and incubated for 6 weeks at 25 ± 1 °C and 70% relative humidity (Figure 1). Separate containers were used for measuring weight loss of controls. After 6 weeks, wood blocks inoculated with 5-day-old fresh cultures from 2% MEA plates of *Trichoderma* T-TMS1 (pretreatment) and those infected with 7-day-old fresh cultures from 2% MEA of one of three pretreatments with *P. noxius* isolates (SSP14, YTM65, YMT97) were placed together in the plastic containers containing vermiculite at 25 ± 1 °C and 70% relative humidity (Figure 1). Before testing, moisture content and water-holding capacity of vermiculite were determined according to EN 807 (CEN 2001). The volume of water needed to bring the substrate to 75% of its water-holding capacity was calculated and added to the vermiculite. In total, 15 wood blocks for each incubation period were used, i.e. five each pretreated with *Trichoderma* T-TMS1, *Phellinus* and *P. noxius* (control). From the 10 strains isolated in Hong Kong, T-TMS1 showed high potential in laboratory tests, i.e. growth rates in different conditions, volatile organic compounds and mycoparasitism test. Wood blocks were incubated for a further 12, 16 and 24 weeks in a climate chamber at 25 ± 1 °C and 70% relative humidity. All containers were monitored weekly and moisture content was adjusted by adding sterile water. After incubation, specimens were removed from the containers, cleaned and dried for determination of dry weight loss (Schwarze & Fink 1998).

Eradication of three *P. noxius* isolates (SSP14, YTM65 and YMT97) by *Trichoderma* T-TMS1 was examined under the same conditions described above. After 12, 16 and 24 weeks of incubation, five wood blocks were removed, cleaned and five small wood fragments were extracted from the centre parts and placed on Petri dishes containing selective medium with thiabendazole (Sieber 1995) (Figure 1). The Petri dishes were incubated at 25 ± 1 °C and 70% relative humidity and any growth of *P. noxius* isolates was monitored over a period of 4 weeks. Lethal effect of the *Trichoderma* strains was expressed as percentage of eliminated *P. noxius* isolates.

Statistical analysis

Analysis of variance (ANOVA) was used to assess significance of the effect of the water activity and temperature on the growth behaviour of *P. noxius* isolates and *Trichoderma* strains. The effects of both factors were performed in a multifactorial ANOVA test and p-value < 0.05 was considered to be significant. Bonferroni intervals were computed for multiple comparisons in order to identify significant differences in the average growth of fungi by levels of temperature and water activity. A box and whisker representation was illustrated for the set of data with optimal

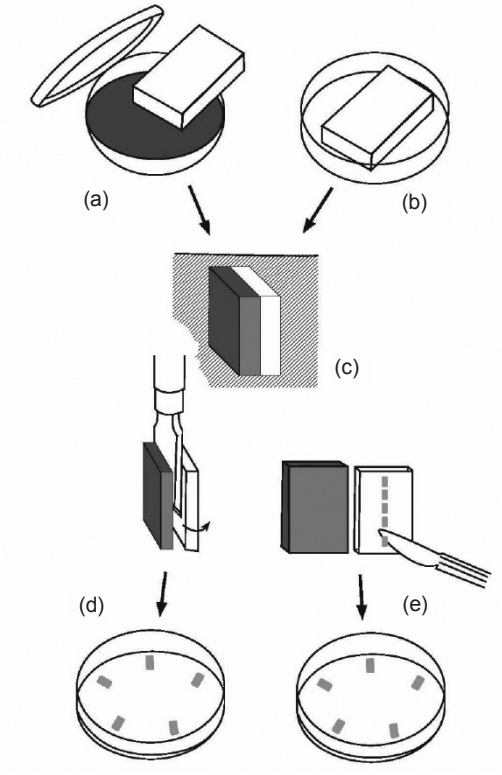

Figure 1 Process of incubating wood blocks with *Trichoderma* T-TMS1 and three *Phellinus noxius* isolates; (a) wood blocks incubated with *Trichoderma* T-TMS1 and (b) *P. noxius* for 6 weeks, (c) wood blocks placed together in containers with vermiculite for 12, 16 and 24 weeks, (d) wood blocks split to extract fragments for examination of the preventative effects of *Trichoderma* T-TMS1 and (e) wood blocks split to extract fragments for examination of the eradicative effects of *Trichoderma* T-TMS1 on *P. noxius*

conditions of temperature and water activity for growth of the fungi in order to study the variability in growth of different fungi. Statistical analysis was performed with Statgraphics Centurion XVI.II software.

RESULTS

Growth rate of *Trichoderma* strains and wood-decay fungi under different conditions

ANOVA results showed that temperature and water activity influenced growth of *Trichoderma* strains significantly ($p < 0.05$ for both factors and for the interaction). Effects of temperature and water activity on mean growth rates of wood-decay basidiomycetes and *Trichoderma* strains are shown in Figure 2. Low water activity resulted in reduction in growth rates for all fungi at all temperatures. However, high water activity affected growth rates differently depending on the temperature. For *P. noxius* isolates, the most conducive temperature for growth was 30 °C, whereas for the *Trichoderma* strains, the highest growth rate was recorded at 25 °C. All *P. noxius* isolates could grow at water activity values of 0.998 and 0.978, but at 0.955 and 0.928 growth was inhibited (Figure 2b). In contrast, slight growth in *Trichoderma* strains was observed at water activity 0.955 (Figure 2a). These results were also obtained by pair-wise comparisons of the levels of each factor by calculating Bonferroni intervals (Figure 3). *Phellinus noxius* E35 showed the highest growth rate and *P. noxius* SBR, the lowest (Figure 4b). *Trichoderma harzianum* (T-HV1) showed the highest growth rates under all the

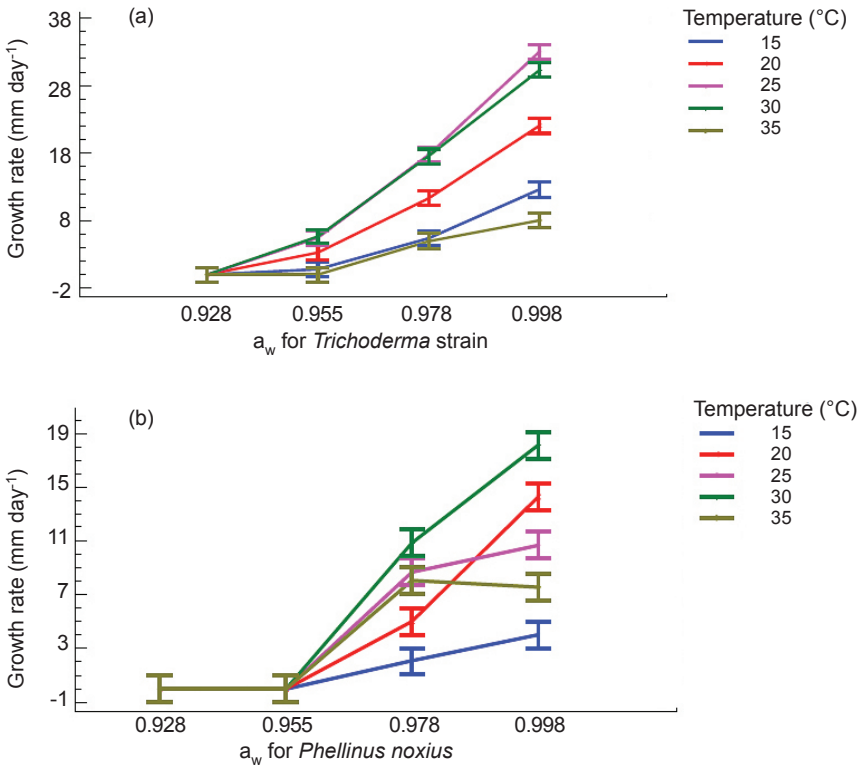

Figure 2 Mean growth rates at 95% confidence intervals (radial measurements in mm day^{-1}); (a) eleven *Trichoderma* strains and (b) seven *Phellinus noxius* under different temperature and water activity (a_w) conditions

test conditions while *T. koningiopsis* (T-TMS2), the lowest (Figure 4a).

Inhibitory effects of volatile organic compounds produced by *Trichoderma* strains on wood-decay fungi

The effect of volatile organic compounds produced by *Trichoderma* strains on the growth of *P. noxius* is shown in Figure 5. After 5 days of incubation, volatile organic compounds produced by T-PSL, T-15603.1 and T-TMS1 caused the highest inhibition of the growth of *P. noxius* isolates. In particular, isolates with higher reduction in growth under the influence of T-TMS1 were YMT97, YTM65 and SSP14 compared with control. The weakest influence against the *P. noxius* isolates was demonstrated by T-PC and T-TL.

Antagonistic activity in dual cultures

Growth of all *P. noxius* isolates was suppressed by one or more of the *Trichoderma* strains, with the exception of *P. noxius* SSP14, which showed high tolerance to *Trichoderma*. Interactions between *P. noxius* isolates and *Trichoderma* strains were observed in all cases, but the capacity to overgrow and parasitise the mycelia of *P. noxius* isolates depended on the antagonistic ability of each *Trichoderma* strain and the resistance mechanisms of the confronted *P. noxius* isolates (Table 3). Most *Trichoderma* strains were able to overgrow the *P. noxius* isolates. However, no differences between the *Trichoderma* strains could be determined regarding the ability to mycoparasitise *P. noxius* isolates. Strains T-15603.1 and T-TMS1 showed high antagonistic potential against four *P. noxius* isolates (Table 3). Strains

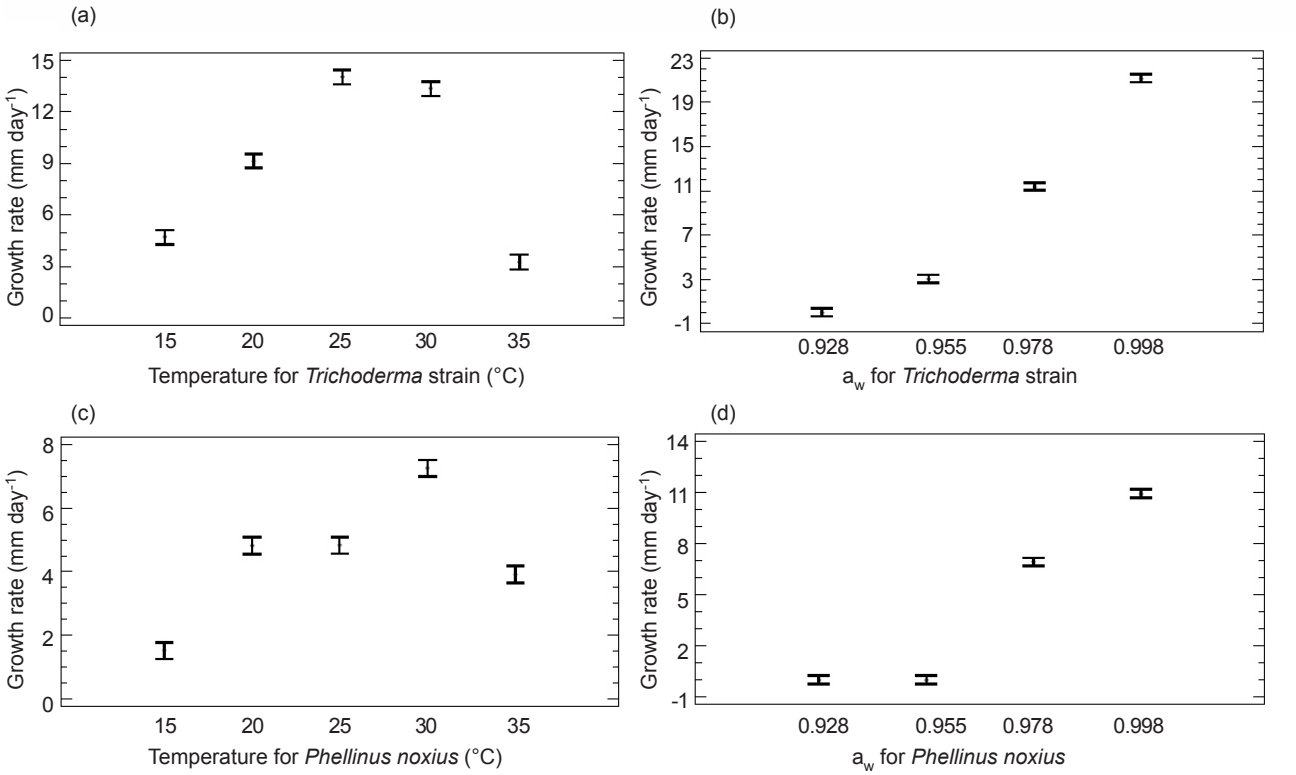

Figure 3 Mean growth rate values (radial measurements in mm day^{-1}) and Bonferroni intervals at 95% confidence intervals: (a) eleven *Trichoderma* strains under different temperature, (b) eleven *Trichoderma* strains under different water activity (a_w), (c) seven *Phellinus noxius* isolates under different temperature and (d) seven *P. noxius* isolates under different water activity

T-SMCP and T-TMS2 revealed weaker effects against *P. noxius* isolates. Highest vulnerability of *P. noxius* to antagonism of *Trichoderma* strains was observed for isolate YMT97.

Antagonistic activity in wood blocks

All isolates of *P. noxius* completely colonised control wood samples but exhibited low potential to decompose the wood. Increases in weight loss after 12, 16 and 24 weeks were minimal (Table 4). In the test evaluating eradication of *P. noxius* by *Trichoderma* T-TMS1, a clear trend was observed. With increasing length of incubation period, the survival of *P. noxius* was significantly reduced (Table 5). After 12 weeks, T-TMS1 had high effect on survival of the *P. noxius* isolates and could eradicate the isolates SSP14, YTM65 and YMT97 to 16, 32 and 50% respectively. After 16 weeks of incubation, *P. noxius* isolate YMT97 was completely eradicated and isolates SSP14 and YTM65 showed increased eradication of 44 and 60% respectively. After 24 weeks, *P. noxius* isolates were completely eradicated (Table 5).

DISCUSSION

Trichoderma species can be successfully used to suppress growth of a range of wood-decay fungi. In the wood block test, *T. harzianum* T-TMS1 was used against three moderately to highly resistant *P. noxius* isolates (YMT97, YTM65 and SSP14). Weight loss of *P. noxius* in control blocks of balsa wood was low in comparison with previous studies but increased over time. Mass losses of 1–35% was recorded for *Araucaria bidwillii*, *Delonix regia*, *Ficus benjamina* and *Jacaranda mimosifolia* by Australian *P. noxius* strains after 12 weeks incubation (Schwarze et al. 2012). Balsa wood, which consists of 92% parenchymal cells, is highly resistant to a range of brown rot fungi (*Poria placenta*, *Gloeophyllum trabeum*, *Coniophora puteana* and *Fomitopsis pinicola*) but is susceptible to the white rot fungus *Trametes versicolor* (Schwarze 2007). Variations

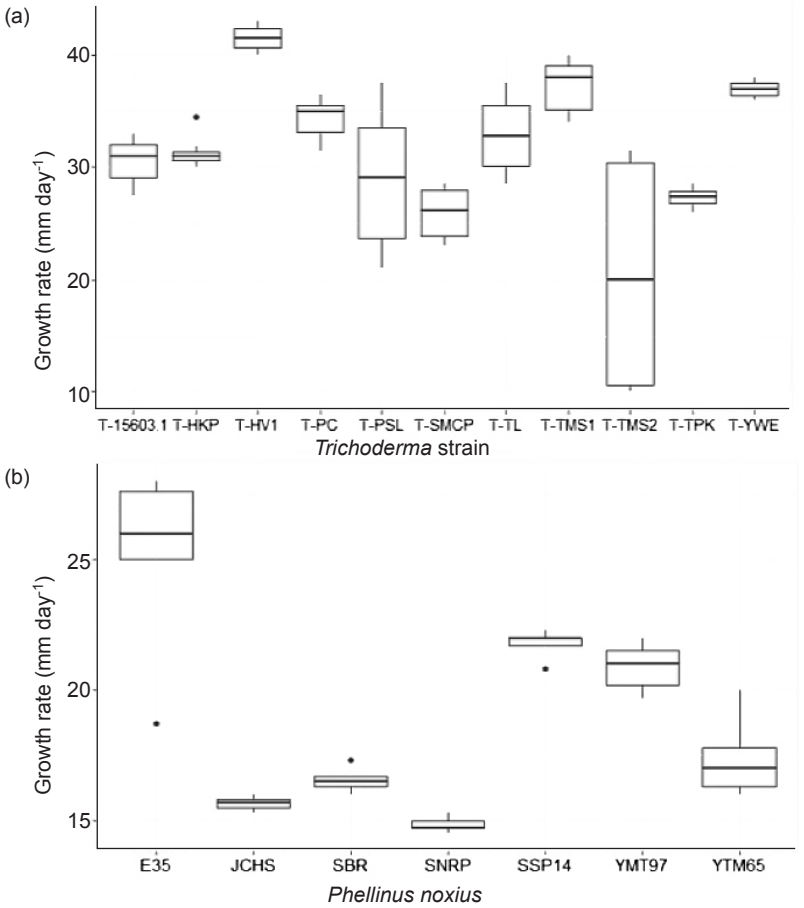

Figure 4 Box and whisker plots of mean growth rate values (radial measurements in mm day^{-1}) of (a) *Trichoderma* strains at 25 and 30 °C and 0.998 water activity conditions and (b) *Phellinus noxius* isolates at 30 °C and 0.998 water activity

in weight loss may be explained by anatomical structure and specific lignin composition of the different wood species or the presence or absence of polyphenolic deposits (Whetten & Sederoff 1995, Schwarze et al. 2012). Preferential degradation of polyphenolic deposits in wood of London plane (*Platanus* × *hispanica*) by the highly invasive white rot fungus *Ganoderma adspersum* resulted in significantly higher weight losses than in sound wood without deposits (Schwarze & Ferner 2003). In our comparison with untreated controls, a reduction in weight loss was recorded after 24 weeks incubation of *P. noxius* isolate SSP14 in wood blocks pretreated with *Phellinus* and *Trichoderma* T-TMS1. After 24 weeks all three *P. noxius* strains were completely eradicated from the wood blocks by *Trichoderma* T-TMS1.

Replanting of susceptible trees in soil infested with *P. noxius* is not recommended because they may become infected after contact with mycelium in the rhizosphere or in buried roots (Ann & Ko 1992). Many soil treatments have been tested and numerous experiments have been performed to find effective ways of eliminating such inoculum. Currently, the most efficient method of destroying residual inoculum is by flooding the area. *Phellinus noxius* was not recovered from soils containing infested root debris after 1 month of flooding (Chang 1996). This may explain the apparent preference of the organism for sandy soils, which are generally well drained (Chang 1996). Various fungicides have activity against *Phellinus* strains. In-vitro studies demonstrated that 7 of 45 fungicides strongly inhibited growth of *P. noxius* (Mappes & Hiepko

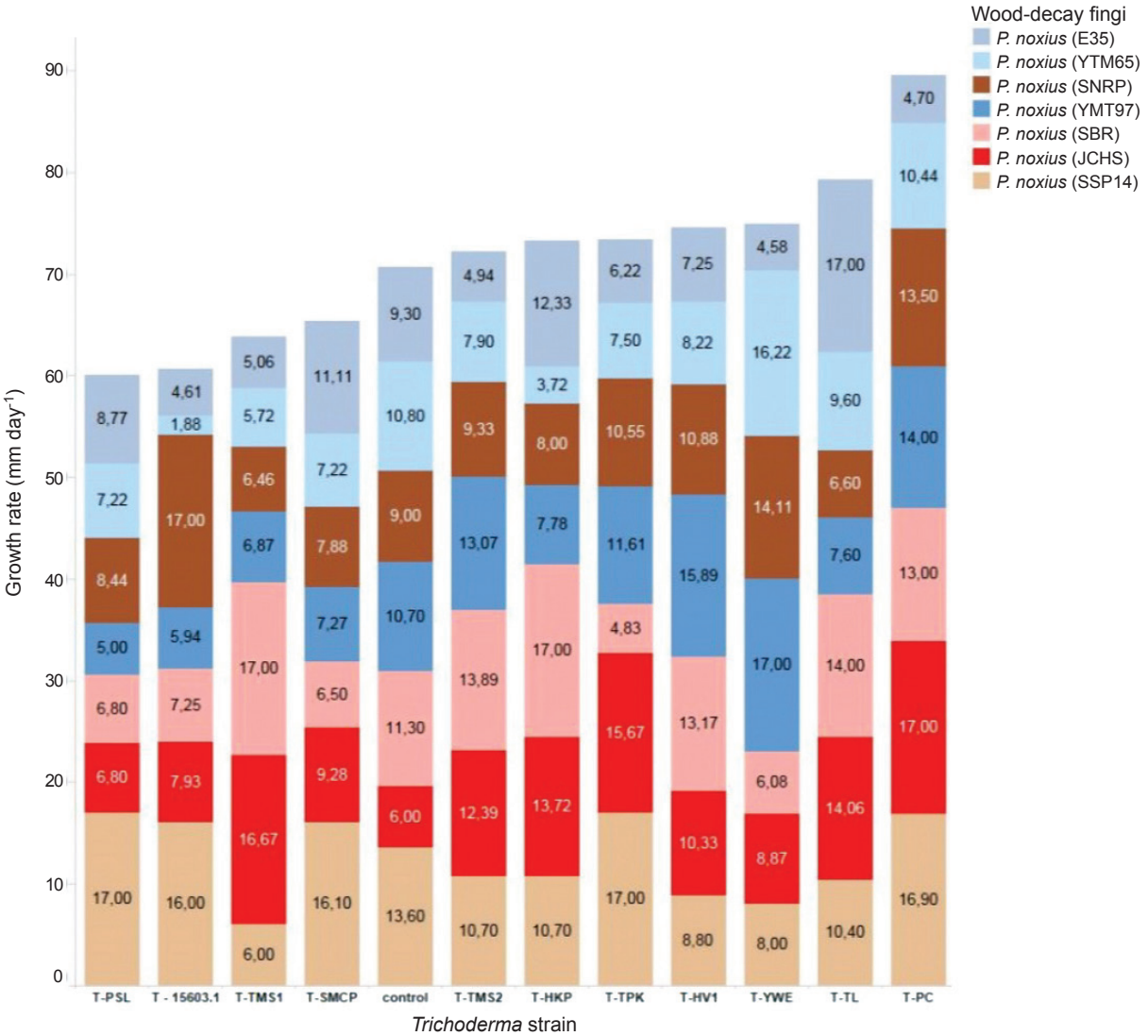

Figure 5 Influence of the radial growth (mm day^{-1}) of wood-decay fungi by volatile organic compounds produced by *Trichoderma* strains; height of each column shows the mean growth rate values of all *Phellinus noxius* isolates under the influence of each evaluated *Trichoderma* strain, colour of each column shows the mean growth rate values of each *P. noxius* isolated under the influence of each evaluated *Trichoderma* strain; control shows the mean growth rate values of *P. noxius* without *Trichoderma* influence

1984, Lim & Teh 1990). These fungicides were further evaluated for their ability to control the disease in greenhouse experiments and the systemic fungicides, Triadimefon, Prochloraz and Mepronil, reduced disease incidence without phytotoxicity (Ann et al. 2002). Efficacy of fungicide treatment, however, reduces with increasing levels of infection (Ismail & Shamsuri 1998). Fungicides should therefore be applied only to newly infected trees or trees with mild levels of infection. Other chemicals that are effective eradicants of infections are soil fumigants (e.g. ammonia-based chemicals (Chang & Chang 1999) or methyl isothiocyanate (Fu et al. 2012) but are not used on a large scale because of their prohibitive cost and potential danger to users.

Sahashi et al. (2012) identified 53 host trees of *P. noxius* in residences, parks, roadsides and sightseeing places in different areas of Japan, indicating that infected trees were associated with sites having high human activities. This

Table 3 Degree of competition of different *Trichoderma* strains against different *Phellinus noxius* isolates on malt extract agar after 4 weeks

Trichoderma strain	*P. noxius* JCHS	*P. noxius* SBR	*P. noxius* SNRP	*P. noxius* YMT97	*P. noxius* E35	*P. noxius* SSP14	*P. noxius* YTM65
T-15603.1	3.0 (100)	1.3 (80)	3.0 (100)	3.0 (100)	2.3 (60)	1.3 (40)	2.3 (80)
T-PSL	2.0 (80)	1.3 (80)	1.6 (80)	3.0 (100)	2.0 (60)	1.3 (40)	1.6 (60)
T-HV1	1.6 (60)	0.6 (20)	2.6 (80)	3.0 (100)	2.3 (80)	1.6 (80)	1.3 (60)
T-HKP	2.6 (100)	1.3 (60)	2.6 (80)	3.0 (100)	2.3 (80)	1.3 (60)	1.6 (60)
T-TMS1	2.6 (100)	1.6 (80)	2.6 (100)	2.6 (100)	2.6 (80)	2.3 (20)	2.3 (100)
T-YWE	2.6 (100)	2.3 (80)	3.0 (80)	3.0 (80)	3.0 (80)	2.6 (20)	1.3 (60)
T-PC	1.6 (80)	1.6 (100)	2.3 (80)	2.6 (100)	2.6 (100)	1.3 (40)	1.3 (60)
T-TL	2.3 (100)	2.6 (100)	1.3 (60)	2.6 (100)	2.6 (100)	1.6 (60)	1.3 (60)
T-TMS2	2.0 (80)	1.3 (20)	1.3 (40)	1.3 (40)	1.6 (40)	1.6 (60)	1.3 (20)
T-SMCP	1.3 (60)	0.3 (0)	2.3 (60)	3.0 (100)	1.6 (80)	1.3 (40)	2.3 (60)
T-TPK	1.3 (60)	0.6 (0)	2.6 (100)	3.0 (100)	1.3 (60)	1.0 (20)	2.0 (60)

System used to classify the rate of competition in dual cultures: 0 = no overgrowth, 1 = slow overgrowth, 2 = fast overgrowth, 3 = very fast overgrowth and deadlock of the wood decay fungi; figures within parentheses indicate the ability of *Trichoderma* strains to eliminate the wood-decay fungi (lethal effect in %)

Table 4 Dry weight loss (% ± standard deviation) caused by three *Phellinus noxius* isolates in treated controls and in wood blocks pretreated with *Trichoderma harzianum* T-TMS1 and *P. noxius*

Treatment	Incubation period (weeks)		
	12	16	24
SSP14			
Control	1.39 ± 0.82	1.87 ± 0.45	2.31 ± 0.58
Pretreated with T-MS1	1.52 ± 1.00	1.59 ± 0.50	1.24 ± 0.90
Pretreated with *Phellinus*	2.23 ± 0.79	1.64 ± 0.46	1.33 ± 0.05
YTM65			
Control	1.50 ± 0.62	2.63 ± 0.80	2.69 ± 0.84
Pretreated with T-TMS1	1.62 ± 0.31	1.98 ± 0.27	1.47 ± 0.27
Pretreated with *Phellinus*	1.48 ± 0.30	1.57 ± 0.51	2.05 ± 0.58
YMT97			
Control	0.95 ± 0.30	1.32 ± 0.14	1.73 ± 0.38
Pretreated with T-TMS1	2.44 ± 0.42	1.48 ± 0.20	1.81 ± 0.96
Pretreated with *Phellinus*	1.10 ± 0.19	1.47 ± 0.42	3.17 ± 0.99

Table 5 Eradication (% ± standard deviation) of *Phellinus noxius* isolates in balsa wood blocks by *Trichoderma harzianum* T-TMS1 after 12, 16 and 24 weeks incubation

Balsa wood	Incubation period (weeks)		
	12	16	24
SSP14	16.0 ± 8.0	44.0 ± 15.0	100.0 ± 0.0
YTM65	32.0 ± 9.8	60.0 ± 17.9	100.0 ± 0.0
YMT97	50.0 ± 9.8	100.0 ± 0.0	100.0 ± 0.0

study is the first in-vitro study to demonstrate that *Trichoderma* species could be used to eradicate *P. noxius* in colonised wood. As the viability of *P. noxius* declines quickly in soils without host plant debris, with no recovery of fungus after 5 months, successful application of antagonist such as *T. harzianum* T-TMS1 has significant benefit on the control of brown root rot disease in urban sites in Hong Kong.

A dual approach of replanting sites with resistant tree species (Ann et al. 1999, Chang 2002, Wu et al. 2011) and applying a biocontrol agent such as *T. harzianum* T-TMS1 could be a promising alternative to chemical methods that either fail to eradicate *P. noxius* or have adverse effects on the soil, root growth and the environment. Positive results obtained from in-vitro studies are only indicative because such experiments do not reflect all environmental conditions encountered. Field studies are, therefore, essential to test the potential of *Trichoderma* species for eradicating *P. noxius* in woody debris of naturally infected soils.

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