Title

Field evaluation of *Trichoderma* spp. as a biological control agent to prevent wood decay on Benin mahogany (*Khaya grandifoliola*) and rain tree (*Samanea saman*) in Singapore

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Abstract

In paired field experiments, two *Trichoderma* sp. isolates were evaluated for their ability to separately prevent wood decay on the pruning wounds of Benin mahogany (*Khaya grandifoliola*) and rain tree (*Samanea saman*) in Singapore. For each species, 150 pruning wounds were created among 10 trees and received either a conidial suspension of the biological control agent or no treatment. At regular

intervals, the ability of the isolates to colonize wounds was evaluated using selective fungal isolations, and wound wood occlusion was simultaneously monitored by successive wound diameter measurements. After 18 months, the wounds were harvested and dissected to measure the size of wood discoloration columns. Overall, relatively superior outcomes for the biological control of wood decay were observed on rain tree compared to Benin mahogany. *Trichoderma* spp. were approximately twice as abundant on the treated wounds of rain tree than Benin mahogany at all times during the experiment. Although the *Trichoderma* spp. isolates were effectively inoculated onto the pruning wounds of both species, they were isolated at rates that declined by approximately half over the 18-month experiment. Compared to non-treated controls, rain tree pruning wounds treated with *T. harzianum* 9132 had significantly less wood discoloration and greater wound wood occlusion, but the same treatment effects were not observed on Benin mahogany using *T. virens* W23. The results demonstrate that *T. harzianum* 9132 is an effective biological control agent for wood decay on rain tree, and the treatment effects offer a valuable way to limit the biological and mechanical costs of tree pruning.

Keywords

Antagonism; Wood decay; Pruning wounds; Wood discoloration; Wound occlusion

- The prevention of decay on pruning wounds by *Trichoderma* sp. isolates was tested.
- *Trichoderma* sp. isolates germinated and persisted on pruning wounds for 18 months.
- Tested isolates differed in their ability to prevent decay on two tree species.
- Wounds treated with *T. harzianum* 9132 had less decay and were more occluded.

Introduction

The mechanical wounds created during tree pruning are often infected by wood decay fungi (Wiseman et al., 2006). In response, trees confine infections with a variety of inherent and induced antimicrobial modifications to their wood anatomy (Morris et al., 2016; Pearce, 1996), but each host-fungus interaction uniquely determines the severity of the resulting decay (Baum and Schwarze, 2002; Schwarze and Baum, 2000). During this process, the consumption of non-structural carbohydrates for defense alters the tree's cellular growth processes (Herms and Mattson, 1992), limiting total resources available for tolerating additional environmental disturbance. For example, Arbellay et al. (2012) reported that mechanical injury altered the structure of European ash [*Fraxinus excelsior* L. (Oleaceae)] wood for several years; new xylem had a greater proportion of small vessels and radial parenchyma, reflecting a shift in its anatomy towards hydraulic safety and mechanical strength at the expense of water conduction. In many cases, the adaptive growth must reinforce tree parts whose mechanical strength has been weakened by wood decay (Niklas, 1992). Regardless of a species' ecological strategy, the costs associated with mechanical injury may limit longevity (Loehle, 1988). As a result, most professional tree care standards recommend limiting the severity of pruning to less than 25% of leaf area (TCIA, 2008).

Still, trees are frequently pruned in urban areas to maintain spatial clearance, improve aesthetics, or reduce risk (Gilman and Lilly, 2008), and many have considered the use of various wound treatments to minimize costs associated with the resulting decay (Lonsdale, 1984). In the past, arborists often treated wounds using various physical sealants or chemical fungicides (Lonsdale, 1984). Although some of these initially prevented wood decay (Mercer et al., 1983) and improved wound occlusion (Mercer, 1983), the benefits often eroded over time as sealants physically deteriorated from weathering and growth stress (Mercer et al., 1983). In addition, the short-term preventative benefits of fungicides are offset by concerns about their environmental and human health risks. As a result, pruning wound treatment is currently discouraged by most arboriculture industry standards (TCIA, 2008).

However, some natural antagonists of wood decay, especially fungi belonging to the genus *Trichoderma* (Samuels, 1996), provide effective biological control (Ricard and Highley, 1988). *Trichoderma* spp. occur widely as saprophytes in highly organic soils (Klein and Eveleigh, 1998), and several have been identified as biological control agents of various diseases affecting economically important crops (Harman, 2006). Consistent with work on other plant diseases (Howell, 2003), *Trichoderma* spp. antagonize wood decay fungi by direct parasitism, antibiosis, enzyme production, and competition for resources (Bruce et al., 1984; Highley, 1997; Schubert et al., 2008a). Most reports indicate that none of these antagonistic mechanisms is independently responsible for control (Highley et al., 1997; Highley, 1997), but the most effective inhibition occurs by the synergistic enhancement of several mechanisms acting simultaneously (Lorito et al., 1996). In most cases, *Trichoderma* propagules are applied prophylactically to plants to facilitate confrontation between fungi (Harman et al., 1991).

However, *Trichoderma* spp. vary considerably in their ability to antagonize phytopathogenic fungi, and it is important to screen for antagonism in representative laboratory tests that mimic conditions expected for the intended application (Mercer and Kirk, 1984a; Schubert et al., 2008a). Some authors have investigated the ability of *Trichoderma* to antagonize wood decay fungi in laboratory tests and identified isolates that are highly antagonistic towards one (Ribera et al., 2016; Schwarze et al., 2012) or more (Mercer and Kirk, 1984a; Schubert et al., 2008a) wood decay fungi. However, there have been relatively few long-term studies investigating the ability of selected *Trichoderma* spp. isolates to prevent wood decay under natural field conditions (Mercer and Kirk, 1984b; Schubert et al., 2008a). To be effective, the applied *Trichoderma* sp. isolate must germinate and persist on the wound surface, especially during adverse environmental conditions; but this outcome can be affected by the chosen conidial formulation, its method of application, or the site-specific environmental conditions (Schubert et al., 2008a). More studies are needed to investigate these factors and optimize processes associated with field application.

In a similar effort, several *Trichoderma* spp. isolates were identified for their unique antagonism towards *Phellinus noxius* (Corner) G. Cunn. (Hymenochaetaceae) associated with mechanical wounds on Senegal mahogany [*Khaya senegalensis* (Desr.) A. Juss. (Meliaceae)] and rain tree [*Samanea saman* (Jacq.) Merr. (Fabaceae)], respectively, in Singapore (Burcham et al., 2017). In many places, *P. noxius* is known to cause a lethal root system infection on a wide range of tree species (Ann et al., 2002; Bolland, 1984), but recent work demonstrated that this fungus occupies a broader ecological niche by also infecting mechanical wounds on aboveground tree parts (Burcham et al., 2015). Although others have identified *Trichoderma* spp. isolates that antagonized *P. noxius* in laboratory tests (Ribera et al., 2016; Schwarze et al., 2012), the studies involved isolates obtained from the rhizosphere that were intended for preventative application to root systems, and they were selected from geographic regions with dissimilar climate conditions. Moreover, none of these *Trichoderma* spp. isolates selected for antagonism towards *P. noxius* were tested on living trees to confirm their efficacy.

Practically, it is useful to prevent the infection of mechanical wounds by highly invasive wood decay fungi, such as *P. noxius*, to limit the associated costs to individual trees and sources of inocula in the urban landscape. As a result, an experiment was designed to evaluate the ability of selected *Trichoderma* spp. isolates to separately prevent wood decay on the wound surfaces of Benin mahogany [*Khaya grandifoliola* C. DC. (Meliaceae)] and rain tree, respectively, in Singapore. Based on laboratory tests (Burcham et al., 2017), *T. virens* W23 and *T. harzianum* 9132 (Table 1) were selected for field testing separately on Benin mahogany and rain tree, respectively. Although *T. virens* W23 was selected originally for its antagonism towards *P. noxius* on wood harvested from a different, congeneric tree species (Senegal mahogany), it was tested in this study on Benin mahogany to evaluate its potential application to a different tree species. Specifically, the objectives of the study were to determine the effect of *Trichoderma* application to pruning wounds on the development of wound wood occlusion and the severity of associated wood discoloration. In addition, the study was designed to examine the effect of different conidial suspension formulations and application methods on the persistence of *Trichoderma* on wound surfaces.

Materials and methods

Experimental site and species

Ten rain trees and 10 Benin mahoganies were selected from two adjacent urban landscapes near Kallang, Singapore (latitude 1° 17' N, longitude 103° 52', elevation 10 m). The trees were large, mature specimens growing in small even-aged homogenous stands that were not maintained after planting on an unknown date. Trees with similar size and shape were selected for use in the study, and their crowns were cleaned at the start by removing dead, diseased, damaged, or broken branches as recommended (TCIA, 2008).

To monitor environmental conditions at each site, a weather station was installed in one representative tree per species. On each weather station, three sensors continuously recorded temperature, T (°C), and relative humidity, RH (%), (S-THB-M002, Onset Computer Corporation); global irradiance, G ($W \cdot m^{-2}$), (S-LIB-M003, Onset Computer Corporation); and wind speed, U ($m \cdot s^{-1}$), (S-WSB-M003, Onset Computer Corporation). A trailing period average was recorded for each parameter at five-minute intervals. To record conditions near pruning wounds, the weather stations were rigidly attached to a large branch and oriented towards the center of each tree crown during the experiment.

Preparation

A conidial suspension of each *Trichoderma* sp. isolate was prepared by flooding mature cultures with sterile water, dislodging conidia by physical agitation, and skimming buoyant conidia from the surface. The concentration of suspensions was checked with a haemocytometer and adjusted to obtain approximately 10^5 colony forming units (CFU) $\cdot ml^{-1}$. Two water-based formulations of each conidial suspension were tested:

Suspension 1: 10^5 CFU$\cdot ml^{-1}$ *Trichoderma* conidia, 0.2% D-glucose, 0.1% urea, 0.1% surfactant (Tween® 20, Sigma-Aldrich, St. Louis, Missouri, United States)

Suspension 2: 10^5 CFU·ml^{-1} *Trichoderma* conidia, 0.2% D-glucose, 0.1% urea, 0.1% surfactant (same as above), 0.4% hydrogel (Sodium polyacrylate $C_3H_3NaO_2$, Sigma-Aldrich, St. Louis, Missouri, United States)

Since the antagonistic *Trichoderma* spp. isolates were selected in host-specific tests, two sets of conidial suspensions were prepared for the experiment. One set was prepared using conidia harvested from *T. virens* W23 for application to Benin mahogany pruning wounds, and a second set was prepared with conidia harvested from *T. harzianum* 9132 for application to rain tree pruning wounds. The surfactant Tween 20® was added to increase dispersion and adhesion of the conidial suspension to vertical wound surfaces; laboratory bioassays indicated that the adjuvant did not inhibit germination rates of either *Trichoderma* sp. isolate.

Treatment application

Fifteen pruning wounds were made on each tree by removing branches with either reduction or removal cuts according to the tree care standards. Removal cuts were placed immediately outside the swollen branch collar to prevent damage to the trunk, and reduction cuts were oriented perpendicular to the longitudinal axis of the shortened branch immediately distal to the retained branch bark ridge to minimize the size of wounds. To prevent uneven cut surfaces and torn bark, branches were removed with a sharp chainsaw. In addition to the type of pruning cut, several attributes of each wound surface were recorded, including the diameter, D (cm), inside the bark along 2 orthogonal axes oriented approximately along the major and minor wound dimensions; orientation, γ (°), towards geographic directions; and inclination, θ (°), relative to the horizontal plane. In addition, a branch aspect ratio, $\mathfrak{R}_B$ (dimensionless), was computed to describe the size of the removed branch relative to its subtending member by dividing the diameter of the wound into the subordinate; $\mathfrak{R}_B$ was computed using diameters measured outside of the bark for consistency. Length measurements were recorded using a steel tape measure (Fisco Satellite, Essex, England) and angles were recorded using a handheld compass and inclinometer (Suunto MC-2, Vantaa, Finland).

In each tree, pruning wounds received either Suspension 1, Suspension 2, or no treatment, i.e., control. Suspension 1 was applied using a spray bottle, and Suspension 2 was applied using a paint brush. The suspensions were applied until the surface was uniformly saturated and runoff occurred. The suspensions were prepared seven days before application, and they were only applied once immediately after pruning at the beginning of the study. During application, the non-treated control wounds were made last to prevent their inadvertent contamination with the applied *Trichoderma* spp. isolates. Wound treatments were replicated five times in each tree, and each pruning wound was labeled with water-resistant paint for subsequent identification.

Treatment monitoring

The presence of both applied and naturally occurring *Trichoderma* spp. isolates on pruning wounds was monitored at 4, 8, 12, and 18 months after treatment application. Small wood shavings were removed from the center and periphery of each wound surface with a sterile chisel, and the wood samples were divided into subsamples that were placed on a sterile *Trichoderma* selective medium (TSM), with each liter of media containing 0.2 g $MgSO_4$, 0.9 g KH_2PO_4, 0.15 g KCl, 1 g NH_4NO_3, 3 g glucose, 0.3 g p-dimethylaminobenzenediazo sodium sulfonate, 0.25 g chloramphenicol, 0.2 g pentachloronitrobenzene, 0.15 g rose bengal, and 20 g agar (Elad et al., 1981). If a wound was completely occluded, wood samples were not collected. Unless indicated otherwise, fungal cultures were incubated consistently in the dark at 28 °C and 50–70% RH in this study. In addition, the occlusion of pruning wounds was monitored at identical intervals. The occluded area was estimated, using repeated diameter measurements, as the absolute difference between the initial elliptical wound surface area and that measured at a given time.

Experimental harvest

After 18 months, the treated pruning wounds were removed from trees by making two cuts perpendicular to the longitudinal axis of the branch subtending each wound: the first immediately distal to and the second approximately 1 m basal to the wound surface. Axial branches originating within this region were similarly removed. The branch samples were labeled and immediately

transported to a laboratory for processing within seven days. At the laboratory, each pruning wound was dissected with a chainsaw along a radial longitudinal plane bisecting the center of each pruning wound. The dissected surfaces were photographed adjacent to one another, and the visible area of the wood discoloration column was computed as the average of that measured photogrammetrically in the two halves using Adobe Photoshop CS6 Extended (Adobe Systems, Inc., San Jose, California, United States).

At the same time, small wood shavings were extracted with a sterile chisel from the exposed wood discoloration column at three positions: near the wound surface, in the center, and at the advancing margin of the infection. The wood samples were sub-sectioned and placed on 3 different sterile media types, including a basidiomycete selective medium (BSM) modified from Sieber (1995), with each liter of media containing 50 g malt extract agar (MEA), 105.75 mg thiabendazole dissolved in 2 ml of concentrated lactic acid, 200 mg chloramphenicol and 300 mg streptomycin sulphate; TSM; and 2% MEA. All fungal cultures obtained from the harvested wounds were transferred into pure cultures and grown in 90 mm Petri dishes containing 2% MEA. The cultures were identified based on macro- and micro-morphological features using taxonomic keys (Gams and Bissett, 1998; Stalpers, 1978). Shannon's diversity index (Shannon and Weaver, 1949), *H*, was used to calculate the diversity of fungal species colonizing pruning wounds as:

$$H = -\sum_{i=1}^{S} p_i \ln (p_i)$$

where *S* is the total number of fungal species and p_i is the proportion of individuals belonging to the *i*th species.

In addition, RAPD-PCR was used to evaluate the similarity between the *Trichoderma* sp. isolate initially applied to pruning wound surfaces and that subsequently isolated during the experimental harvest. After selective isolation, *Trichoderma* spp. isolates from the harvested pruning wounds were grouped using micro- and macro-morphological features. Subsequently, a representative sample of isolates was selected from each of these morphologically similar groups for characterization with

RAPD-PCR. The *Trichoderma* spp. isolates were grown at 30 °C in potato dextrose broth liquid media for 72 hours on an orbital shaker set to 150 revolutions·min^{-1}. Mycelia were harvested by vacuum filtration through a Buchner funnel on filter paper, and harvested mycelia were washed with distilled water. Approximately 15 mg of mycelia were macerated in a 2 ml micro centrifuge tube using a micro pestle and liquid nitrogen. DNA was extracted using a commercial kit (DNeasy Plant Mini Kit, Qiagen, Hilden, Germany) according to the manufacturer's instructions. A spectrophotometer (Gene Quant Pro, Biochrom, Cambridge, Massachusetts, United States) was used to determine DNA concentration by calculating the ratio of absorbance at 260 and 280 nm. Preserved laboratory cultures of the two biological control agents, *T. virens* W23 and *T. harzianum* 9132, were similarly used for comparison.

RAPD characters (Williams et al., 1990) were developed with primer 1 (5′-CACGGCGAGT-3′) and 2 (5′-CTGTCCAGCA-3′) (Sigma-Aldrich, St. Louis, Missouri, United States). The 50 µl PCR sample volume contained 1 µl *Trichoderma* DNA, 30.2 µl distilled water, 2.5 µl 50mM $MgCl_2$, 5 µl 10× buffer, 5 µl primer, 1 µl 10mM deoxynucleoside triphosphate mix, and 0.3 µl 1.5U of *Taq* polymerase. In addition, a negative control was prepared using a reaction mixture without DNA. The samples were amplified in a thermal cycler (GeneAmp PCR System 2700, Applied Biosystems, USA) at the following conditions: initial denaturation, annealing and extension at 92, 48, and 74 °C, respectively, for 2 minutes each; 39 cycles of denaturation at 92 °C for 1 minute, annealing at 48 °C for 1 minute, elongation at 74 °C for 2 minutes; and a final extension at 74 °C for 10 minutes. PCR products were separated by electrophoresis on a 2% agarose gel in 1× Tris-borate EDTA buffer at 70 V for 2 hours. The fragments were visualized by staining with ethidium bromide (3µl /60 ml buffer) and viewed under UV illumination with a gel documentation system (G:BOX EF, Syngene, Cambridge, United Kingdom). The RAPD characters were validated against other *Trichoderma* spp. isolates in the laboratory before the field experiment.

Gel images were analyzed using GelCompar II (Applied Maths, Sint-Martens-Latem, Belgium) to determine the location, i.e., fragment length (base pairs), and magnitude of peaks in stained DNA

fragments for all analyzed PCR products. In each image, the size of fragments was determined by normalizing values against a reference DNA ladder (GeneRuler 100bp Plus, ThermoFisher Scientific, Waltham, Massachusetts, United States). In each lane, only clearly amplified polymorphic fragments were analyzed with peak intensity values greater than 25% of the absolute maximum; binary vectors were constructed to indicate the absence (0) or presence (1) of a band at specific locations relative to other lanes. Subsequently, binary vectors were concatenated and used to construct a similarity matrix for all analyzed samples by computing the Jaccard similarity coefficient (Jaccard, 1908). Based on these values, isolates were clustered into groups using the unweighted pair-group method with arithmetic averages. Isolates assigned to a cluster containing a reference DNA sample extracted from the applied *Trichoderma* sp. isolate were considered to be the same; others were considered to be different, naturally occurring *Trichoderma*.

Experimental design and data analysis

The experiment was designed as a randomized complete block trial with five replicates of all wound treatments blocked in 10 trees per species. The data for Benin mahogany and rain tree were analyzed separately because a different *Trichoderma* sp. isolate was tested on each species. Linear mixed effects models for repeated measures ANOVA were fit to the occluded wound area and *Trichoderma* isolation rates using proc mixed in SAS 9.4 (SAS Institute, Inc., Cary, NC, USA). The fixed effects were wound treatment and time (months); random effects included replicate wounds and replicate wounds nested in trees. Model variance-covariance matrix structures were evaluated using visualization techniques (Dawson et al., 1997) and information criteria (Wang and Goonewardene, 2004). The covariance structure with the algebraically lowest corrected Schwarz's Bayesian Information Criteria (BIC) was selected to preserve test power (Wang and Goonewardene, 2004). The Kenward-Roger (Kenward and Roger, 1997) correction was used to limit Type I error (Guerin and Stroup, 2000) by obtaining error degrees of freedom adjusted for the selected covariance structure. Significant interactions were separated to determine the effect of wound treatments at a given time. In addition, linear mixed effects models were fit to the measured area of wood discoloration columns and *H*. For these models, the fixed and random effects were identical to those used for the area of

wound wood occlusion and *Trichoderma* spp. isolation rates, with one exception: the fixed effect of time was removed because only one observation was made during experimental harvest at 18 months. For the models fit to the area of wound wood occlusion and discoloration columns, measurements were not normalized as ratios or percentages, but a continuous covariate equal to the initial wound area was tested to account for differences in initial size. In addition, a covariate equal to $\mathfrak{R}_B$ was included in the models fit to the area of wood discoloration columns to account for anatomical differences among branch attachments. For means associated with specific levels of a continuous independent variable, total sums of squares were partitioned into single-degree-of-freedom orthogonal polynomial comparisons to assess the significance of individual polynomial terms. Based on these results, least squares regression was used to determine the associated polynomial coefficients. Separately, the proportion of wounds that were infected with basidiomycetes among the three treatment groups was compared with Fisher's exact test of independence using proc freq in SAS 9.4.

Results

Experimental site and species

Environmental conditions were typical for Singapore and comparable between the two sites. Elevated *T* and *RH* values were inversely proportional to one another with diurnal ranges of 26–29 °C and 80–95%, respectively. For most of the day, *G* and *U* were consistently higher at the Benin mahogany site than the rain tree site with peak values occurring at mid-day for both sites (Figure 1).

The size of trees used in this study were typical for mature Benin mahogany and rain tree, with the former being much taller, on average, than the latter. There was similar variability in *D* for pruning wounds created on both species, but one large (44.5 cm) pruning wound on rain tree caused its mean (13.0 cm) to slightly exceed the same for Benin mahogany (12.4 cm). Wound γ and θ were widely distributed among all possible positions for both species. A greater number of reduction cuts made on Benin mahogany caused its $\mathfrak{R}_B$ to exceed one, but the same was not true for rain tree (Table 2). For each removal cut, there were approximately 2.1 and 0.4 reduction cuts made on Benin mahogany and rain tree, respectively. However, the average $\mathfrak{R}_B$ of each treatment group occupied a relatively narrow

range for Benin mahogany (1.29–1.37) and rain tree (0.88–0.92), indicating that a reasonably consistent proportion of the 2 cut types existed among treatment groups within each species.

Fungal communities colonizing wounds

Although not statistically compared, the applied and naturally occurring *Trichoderma* spp. were isolated, overall, more frequently from the pruning wounds on rain tree than Benin mahogany. On average, isolation rates for pruning wounds treated with Suspension 1 and 2 on rain tree were approximately twice that for Benin mahogany. For both tree species, *Trichoderma* spp. were isolated from wounds treated with Suspension 1 and 2 at rates that declined by approximately half over the 18-month experiment.

For Benin mahogany, isolation rates for the applied and naturally occurring *Trichoderma* spp. varied significantly among wound treatments. Compared to the controls, *Trichoderma* spp. were isolated at significantly higher rates from wounds treated with Suspension 1 and 2. Although isolation rates decreased significantly over time, wound treatments interacted significantly with time to affect the presence of *Trichoderma* spp. on pruning wounds. Specifically, the rate at which *Trichoderma* spp. were isolated from wounds treated with Suspension 1 and 2 decreased over time, but these isolation rates increased over time on the non-treated control wounds. As a result, isolation rates for wounds treated with Suspension 1 and 2 were significantly greater than the control at 4, 8, and 12 months; but these differences were no longer significant at 18 months (Table 3).

For rain tree, isolation rates for the applied and naturally occurring *Trichoderma* spp. similarly varied among wound treatments; the rates for wounds treated with Suspension 1 and 2 were significantly greater than the control. Overall, *Trichoderma* spp. were isolated at rates that decreased significantly over time, but the interaction between wound treatments and time was significant. Although isolation rates for wounds treated with Suspension 1 and 2 decreased over time, these rates remained relatively constant, with respect to time, on the non-treated control wounds. As a result, *Trichoderma* spp. were

isolated from wounds treated with Suspension 1 and 2 at rates that were significantly greater than the non-treated controls at all times considered in this study (Table 3).

During experimental harvest, 36 and 43 *Trichoderma* spp. isolates were recovered from the dissected Benin mahogany pruning wounds treated with Suspension 1 or Suspension 2, respectively. On the other hand, 29 *Trichoderma* isolates were similarly obtained from the dissected non-treated control wounds. Since the isolates displayed considerable morphological variation in culture, all of the isolates (n = 108) acquired from wounds examined in the study were used for characterization with RAPD-PCR. Based on gel electrophoresis of RAPD-PCR products (Figure 2), 91% and 78% of the isolates acquired from wounds treated with Suspension 1 or Suspension 2, respectively, showed band patterns that were similar to *T. virens* W23, indicating an equivalence between the biological control agent and a majority of isolates acquired from treated wounds. However, none of the isolates acquired from non-treated control wounds were similar to *T. virens* W23 (Figure 2). Overall, 82% of the *Trichoderma* spp. isolates were acquired from the wound surface; a smaller proportion of all isolates were found in the middle (12%) or bottom (6%) locations of the wood discoloration column.

At the same time, 109 and 87 *Trichoderma* spp. isolates were recovered from the dissected rain tree wounds treated with Suspension 1 or Suspension 2, respectively; and 47 *Trichoderma* spp. isolates were similarly obtained from non-treated control wounds. Since most of these isolates appeared morphologically homogenous in culture, isolates were sampled from wounds treated with Suspension 1 (n = 47), Suspension 2 (n = 36), or non-treated control wounds (n = 22) for characterization with RAPD-PCR. Based on gel electrophoresis of RAPD-PCR products (Figure 3), 91% and 98% of the isolates obtained from wounds treated with Suspension 1 or Suspension 2, respectively, showed band patterns that were similar to *T. harzianum* 9132, indicating an equivalence between the biological control agent and a majority of isolates acquired from treated wounds. However, 19% of the isolates acquired from non-treated control wounds also showed band patterns that were similar to *T. harzianum* 9132, suggesting there was some contamination of non-treated control wounds on rain tree by the biological control agent. Compared to Benin mahogany, the *Trichoderma* spp. isolates

recovered from rain tree were less concentrated at the wound surface. Overall, 60% of these isolates were acquired from the wound surface; 25% and 15%, respectively, were recovered from the middle and bottom locations of the wood discoloration column.

During experimental harvest, many of the same species of fungi were discovered on the pruning wounds of both species (Table 4). Although not statistically compared, fungal diversity was slightly higher on Benin mahogany pruning wounds compared to others on rain tree. On average, *H* was 1.4 (SD 0.4) and 1.2 (SD 0.4), respectively, among all wounds examined on Benin mahogany and rain tree. Although fungal diversity was slightly greater, in absolute terms, on wounds treated with Suspension 1 and 2 for both species (data not shown), these differences were not statistically significant for Benin mahogany ($F = 1.07$; df = 2, 18; $p = 0.362$) or rain tree ($F = 1.54$; df = 2, 18; $p = 0.241$).

Among the pruning wounds examined in this study, the biological control agents reduced the rate of infection by wood decay fungi in all cases, except for Benin mahogany wounds treated with Suspension 1. For Benin mahogany, the rate of infection for pruning wounds treated with Suspension 1 or 2, respectively, was 54% and 46%, but a similar proportion (54%) of the non-treated controls were similarly infected. For rain tree, the rate of infection for wounds treated with Suspension 1 or 2, respectively, was 30% and 34%, and a slightly higher proportion (42%) of the controls were similarly infected. However, Fisher's exact test indicated that the infection rates did not differ significantly among the treatment groups for Benin mahogany ($p = 0.676$) or rain tree ($p = 0.489$).

Wound wood occlusion

Although interspecific statistical comparisons were not made, the pruning wounds on Benin mahogany had greater wound wood occlusion, on a relative basis, than others on rain tree. After 18 months, the pruning wounds were, on average, 55% and 27% occluded on Benin mahogany and rain tree, respectively. At the same time, wound wood occlusion covered the entire surface of 31 (21% of total) Benin mahogany pruning wounds, but none of the rain tree pruning wounds were completely

occluded by the end of the experiment. For both species, the initial area of pruning wounds accounted for a highly significant amount of variance in the area of wound wood occlusion (Table 4). Fit indices showed that homogeneous covariance structures best described the wound wood occlusion datasets for Benin mahogany and rain tree; for both species, the algebraically lowest AICC and BIC was obtained by fitting models to data with the homogeneous Toeplitz covariance structure (data not shown).

After accounting for differences in initial size, the area of wound wood occlusion on Benin mahogany pruning wounds did not vary among experimental treatments, but the occluded area increased significantly over time. The interaction between wound treatments and time, however, was not significant because the pruning wounds in each treatment group occluded at a similar rate (Table 5). Orthogonal polynomial comparisons revealed that the relationship between the area of wound wood occlusion and time (months) was cubic, reflecting an initial increase in the rate of occlusion followed by its eventual tapering over the 18-month period evaluated in this study (Table 5). The mixed model regression equation is:

$$z = 0.87x + 0.43x^2 - 0.02x^3 + 0.32(y),$$

where x is time (months) and y is the initial wound area (cm^2). Based on this equation, the average Benin mahogany pruning wound, with an initial wound area of 133 cm^2, would be fully occluded in 28.6 months.

After accounting for differences in size, the area of wound wood occlusion on rain tree did not vary among experimental treatments, but the occluded area increased significantly over time. However, wound treatments interacted significantly with time to affect the area of wound wood occlusion. Although the area of wound wood occlusion did not vary among experimental treatments after 4 and 8 months, the occluded area of wounds treated with Suspension 1 was significantly greater ($p = 0.032$) than the non-treated control wounds after 12 and 18 months. On the other hand, the difference between the occluded area of wounds treated with Suspension 2 and the non-treated controls was not significant ($p = 0.061$) after 12 and 18 months (Table 5).

Area of wood discoloration columns

After 18 months, the initial area and $\mathfrak{R}_B$ of pruning wounds accounted for a highly significant amount of variance in the area of discoloration associated with pruning wounds on Benin mahogany. However, after accounting for variability in the initial size and $\mathfrak{R}_B$, there were not significant differences in the area of discoloration associated with wound treatments (Table 6). For rain tree, $\mathfrak{R}_B$ accounted for a significant amount of variance in the area of wood discoloration columns. However, the same was not true for the initial size of wounds, and this covariate was removed from the final model. After accounting for differences in $\mathfrak{R}_B$, there were significant differences in the area of discoloration among wound treatments. Specifically, the discolored area associated with wounds treated with Suspension 1 was significantly less ($p = 0.010$) than the non-treated controls. On the other hand, the difference in discolored area between wounds treated with Suspension 2 and non-treated controls was not significant ($p = 0.100$) (Table 6).

Discussion

These experiments revealed important differences in the efficacy of biological control and confirmed the importance of testing laboratory-based approaches in the field, and they contribute valuable observations to a limited body of evidence on the biological control of wood decay. Collectively, the results demonstrated relatively superior outcomes for the biological control of wood decay fungi on rain tree. During the 18-month experiment, applied and naturally occurring *Trichoderma* spp. were more abundant on the treated pruning wounds of rain tree than Benin mahogany (Table 3). In addition, rain tree wounds treated with *T. harzianum* 9132 had significantly greater wound wood occlusion and smaller wood discoloration columns than non-treated controls, but the same was not true for Benin mahogany (Tables 4, 6). Similarly, there was a larger reduction in the percent of wounds infected by wood decay fungi on rain tree than Benin mahogany at the end of the experiment.

In general, the tested application methods and conidial formulations effectively facilitated the establishment of *Trichoderma* spp. on pruning wounds. Selective fungal isolations from pruning

wounds revealed that the two *Trichoderma* spp. isolates successfully colonized the wound surfaces of both species. For most of the experiment, the rate at which *Trichoderma* spp. were isolated from Benin mahogany and rain tree wounds treated with *T. virens* W23 and *T. harzianum* 9132, respectively, was significantly greater than non-treated control wounds (Table 3). These differences corresponded with the experimental inoculation of wounds and indicated the presence of the applied biological control agent. At the end of the experiment, analysis of RAPD-PCR products confirmed that the applied *Trichoderma* spp. isolates were still present on a majority of the treated wounds for both tree species (Figures 2–3). Still, the abundance of *Trichoderma* spp. consistently declined over time on the treated wounds of both species, and it may be necessary to treat large wounds more than once to enhance the persistence of the biological control agent. Since parasitized fungi provide nutrients for *Trichoderma* spp., these repeated applications may be especially important for uninfected wounds until the surface is fully occluded.

However, there was little difference between the rate at which *Trichoderma* spp. were isolated from Benin mahogany and rain tree wounds treated with Suspension 1 or 2 (Table 3), and this suggests that the additional moisture stored by the hydrogel did not improve wound colonization or persistence by the introduced *Trichoderma* isolates. In contrast, Schubert et al. (2008a) reported that a similar conidial suspension amended with hydrogel significantly increased the presence of *T. atroviride* T-15603.1 on pruning wounds relative to two different conidial suspensions over a 30-month period. This distinction is probably caused by the different climate zones in which the studies were conducted; the desiccation of propagules before germination is likely a more serious problem in dry temperate than humid equatorial climates (Schubert et al., 2008a; Schubert et al., 2008b). In Singapore, stable, elevated *T* and *RH* (Figure 1) are favorable for biological activity throughout the entire year, and these results suggest that hydrogel can be omitted from conidial suspensions intended for use in similar tropical conditions. Still, the long term viability of spores and the shelf life of the suspensions are unknown, and additional experiments are needed to optimize the production, storage, and delivery of these *Trichoderma* spp. isolates.

Although RAPD-PCR indicated that some of the non-treated control wounds on rain tree were contaminated by *T. harzianum* 9132, this is primarily an experimental concern with few practical implications. At most, the inadvertent contamination of the rain tree control wounds may have caused a slight underestimation of the beneficial effect of *T. harzianum* 9132 by mitigating the severity of wound conditions on the associated controls. Since the control wounds were created last during treatment application, it is not immediately clear how this contamination occurred, but it is possible that conidia formed by *Trichoderma* colonies on treated surfaces were passively transferred by aerial dissemination onto adjacent control wounds at some point during the experiment.

Although statistically insignificant, the slight increase in fungal diversity on treated wounds, relative to the non-treated controls, is interesting and different from existing reports that showed *Trichoderma* inhibited fungal diversity on pruning wounds (Schubert et al., 2008a). Presumably, the two conidial suspensions increased the moisture content and available nutrients on wound surfaces, but their application did not significantly affect the ecology of fungal communities colonizing these exposed surfaces. Similarly, *Trichoderma* spp. were isolated from non-treated control wounds at higher rates than reported for similar studies (Schubert et al., 2008a). In most cases, analysis of RAPD-PCR products suggested that these isolates were naturally occurring *Trichoderma* spp. that were not similar to either *T. harzianum* 9132 or *T. virens* W23 (Figures 2–3).

Notably, this is the first study to demonstrate that *Trichoderma* application to wound surfaces improved wound wood occlusion. In a related study, Schubert et al. (2008b) reported that *T. atroviride* T-15603.1 did not affect wound wood occlusion on six different temperate broadleaf species. In contrast, the significant increase in the occluded area of rain tree wounds treated with *T. harzianum* 9132, relative to non-treated controls, is practically meaningful because it facilitates the restoration of a physical barrier between the wound and environment. This external barrier contains an array of secondary metabolites that further restricts the spread of infection (Eyles et al., 2003), and it also beneficially limits gas exchange and desiccation to create internal conditions that are inimical to fungal growth (Boddy, 1992). Typically, the process is much slower on rain tree (Ow et al., 2013),

and the accelerated defensive response associated with *T. harzianum* 9132 may usefully limit the severity of wood decay infections (Metzler, 1997) on this tree species. Although the same beneficial effect was not observed on Benin mahogany, the rate of occlusion is typically greater on Benin (Table 4) and Senegal mahogany (Ow et al., 2013) compared to many other species.

For non-treated wounds, most existing research has demonstrated that the extent of wood discoloration columns is proportional to the size of pruning wounds (Danescu et al., 2015; Grabosky and Gilman, 2007; Ow et al., 2013), and the covariate representing the initial area of wounds similarly accounted for a significant amount of variance in the area of wood discoloration columns on Benin mahogany. However, the same covariate did not account for a significant amount of variance on rain tree, indicating that the treatment effect associated with *T. harzianum* 9132 obscured the expected proportionality (Table 6), and this further corroborates other evidence of an exclusive treatment effect on this tree species. Practically, the significant reduction in the size of wood discoloration columns on rain tree is especially valuable, since these columns are generally larger, for a given wound size, on rain tree than Senegal mahogany (Ow et al., 2013). For these treated wounds, the smaller wood discoloration columns should have beneficially limited the extent of defensive anatomical modifications, resulting in lower mechanical and biological costs for treated pruning wounds.

On the other hand, the covariate representing $\mathfrak{R}_B$ accounted for a significant amount of variance in the area of wood discoloration columns for both tree species. In agreement with existing studies (Eisner et al., 2002), this suggests that the anatomy of branch attachments significantly affects the severity of decay after pruning, it will be important to account for this source of variation in future studies examining wound treatment. Usefully, this means that arborists can synergistically reduce the size of wood decay columns by removing branches with a small $\mathfrak{R}_B$.

However, it is important to note that only the size of wood discoloration columns was analyzed in this study. Measurements did not permit an evaluation of the severity of decay associated with experimental treatments. Lundborg and Unestam (1980), for example, reported that the frequency of

transverse boreholes created by *Heterobasidion annosum* in Norway spruce [*Picea abies* (L.) Karst (Pinaceae)] was reduced by the presence of *T. harzianum*, even in cases where the wood decay fungus remained present in quantities similar to non-treated controls. In future investigations, material infected by wood decay fungi should be examined to study the altered fungal degradation patterns or physical characteristics of wood inoculated with *Trichoderma* spp. Still, the lower wood decay infection rates associated with most experimental treatments in this study necessarily implies an equivalent reduction to the total number of wood decay columns in a given tree, regardless of their severity.

Overall, these results consistently showed increasingly desirable outcomes for biological control on rain tree, and there are several possible explanations for the relatively inferior outcomes on Benin mahogany. First, the results may simply reflect the superior antagonistic capacity of *T. harzianum* 9132 towards naturally occurring wood decay fungi on rain tree. The evidence from existing laboratory tests supports this possibility. Compared to *T. virens* W23, *T. harzianum* 9132 caused a greater absolute decrease to the growth, viability, and decay rates of *P. noxius* in controlled laboratory tests (Burcham et al., 2017). Second, the substitutionary use of Benin and Senegal mahogany may have neglected the inherent host-specificity of the biological control. For example, there may have been important differences in the physical and chemical wood properties of these two species that account for the comparative scarcity of *T. virens* W23 on Benin mahogany pruning wounds. Third, it is possible that observed differences in G and U between sites may have inhibited the growth and persistence of *T. virens* W23 on Benin mahogany. Although the increased U was likely an artefact of the nonlinear increase in wind flow above ground arising from viscous effects near the ground surface (Stull, 1988) (the weather station was installed higher above ground on the taller Benin mahogany), the increased G was probably caused by the greater use of reduction cuts that increased light penetration in the crown of this species. At present, it is not possible to objectively evaluate these possibilities, and they should be closely examined in future studies.

In this study, neither *Trichoderma* isolate completely prevented colonization by wood decay fungi, but this is similar to many related biological control applications (Schubert et al., 2008b). Schubert et al. (2008a), for example, reported that treatment efficacy varied considerably among the unique confrontations between *T. atroviride* T-15603.1 and various wood decay fungi. Still, the beneficial decrease in wood discoloration (Table 6) and increase in wound wood occlusion (Table 4) on rain tree demonstrate the successful application of *T. harzianum* 9132 as a biological control agent. In this case, the application fills a valuable niche for biological control where chemical treatments have largely proven ineffective (Gendle et al., 1981; Mercer et al., 1983; Mercer, 1979) and present undesirable risks to the environment and human health. Although there was no measured improvement to treated Benin mahogany wounds, the application of *T. virens* W23 did not, like many other wound treatments, exacerbate wound wood occlusion or wood discoloration.

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Table 1: Identity, origin, and accession numbers of the *Trichoderma* spp. isolates used in this study

Species	Isolate	Accession No.	Substrate	Origin	Reference	Identity (%)
T. harzianum	9132	KY025556	*Ganoderma boninense* on *Cyrtostachys renda*	Singapore	KR856210.1 (Lieckfeldt et al., 2001)	99
T. virens	W23	KY025560	*Ganoderma boninense* on *Ptychosperma macarthurii*	Woodlands, Singapore	KP009289.1 (Ottenheim et al., 2015)	100

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

Table 2: Average tree (a) and wound (b) attributes for Benin mahogany (*Khaya grandifoliola*) and rain tree (*Samanea saman*)

	Benin mahogany	rain tree
a) Tree attributes		
Diameter, *DBH* (m)	0.70 [0.08; 0.56–0.78]	0.83 [0.11; 0.66–1.03]
Height, *H* (m)	29.7 [1.7; 25.0–31.6]	21.5 [1.0; 20.1–23.2]
b) Wound attributes		
Diameter, *D* (cm)	12.4 [4.7; 5.0–26.3]	13.0 [5.2; 5.3–44.5]
Inclination, θ (°)	53 [24; 0–90]	61 [19; 0–90]
Orientation, γ (°)	171 [105; 0–356]	188 [114; 0–360]
Aspect ratio, $\mathfrak{R}_B$	1.45 [0.81; 0.26–5.25]	0.90 [0.25; 0.52–1.78]

Note: Values listed are mean [SD; min–max].

Table 3: Analysis of variance of *Trichoderma* spp. isolation rates on Benin mahogany (*Khaya grandifoliola*) and rain tree (*Samanea saman*) pruning wounds treated with *Trichoderma virens* W23 and *Trichoderma harzianum* 9132, respectively

Effect	df	*F*	*p*	Level	Mean (SE)
a) Benin mahogany					
Treatment	2, 18	12.83	< 0.001	Suspension 1	31.6 (4.4)*
				Suspension 2	26.5 (4.4)*
				Control	6.3 (4.4)
Time	3, 441	10.62	< 0.001		
Treatment × Time	6, 441	10.52	< 0.001		
Treatment:$Time_1$(4 mos.)	2, 441	29.96	< 0.001	Suspension 1	44.8 (5.2)*
				Suspension 2	44.5 (5.2)*
				Control	1.0 (5.2)
Treatment:$Time_2$(8 mos.)	2, 441	16.85	< 0.001	Suspension 1	28.0 (5.2)*
				Suspension 2	39.3 (5.2)*
				Control	2.4 (5.2)
Treatment:$Time_3$(12 mos.)	2, 441	3.66	0.026	Suspension 1	21.1 (5.2)*
				Suspension 2	21.3 (5.2)*
				Control	5.9 (5.2)
Treatment:$Time_4$(18 mos.)	2, 441	1.00	0.370	Suspension 1	12.2 (5.2)
				Suspension 2	21.3 (5.2)
				Control	16.0 (5.2)
b) rain tree					
Treatment	2, 18	62.06	< 0.001	Suspension 1	62.8 (4.3)*
				Suspension 2	62.7 (4.3)*
				Control	11.9 (4.3)
Time	3, 441	33.61	< 0.001		
Treatment × Time	6, 441	9.04	< 0.001		
Treatment:$Time_1$(4 mos.)	2, 441	75.74	< 0.001	Suspension 1	80.6 (5.2)*
				Suspension 2	82.1 (5.2)*
				Control	9.6 (5.2)
Treatment:$Time_2$(8 mos.)	2, 441	47.12	< 0.001	Suspension 1	69.3 (5.2)*
				Suspension 2	69.4 (5.2)*
				Control	12.7 (5.2)
Treatment:$Time_3$(12 mos.)	2, 441	36.18	< 0.001	Suspension 1	65.0 (5.2)*
				Suspension 2	61.5 (5.2)*
				Control	13.7 (5.2)
Treatment:$Time_4$(18 mos.)	2, 441	9.72	< 0.001	Suspension 1	36.3 (5.2)*
				Suspension 2	38.0 (5.2)*
				Control	11.5 (5.2)

Note: Asterisks (*) indicate that treatment mean is significantly greater than the control at the $\alpha = 0.05$ level.

Table 4: Fungal genera and species isolated from Benin mahogany (*Khaya grandifoliola*) and rain tree (*Samanea saman*) pruning wounds after 18 months

Ascomycetes	Basidiomycetes	Zygomycetes
Aspergillus sp.	*Rhizoctonia* sp.	*Cunninghamella* sp.
Aspergillus niger	*Phellinus noxius*	*Mucor* sp.
Curvularia sp.		
Fusarium sp.		
Fusarium oxysporum		
Fusarium solani		
Nigrospora sp.*		
Paecilomyces sp.		
Penicillium sp.		
Phomopsis sp.		
Pestalotiopsis sp.*		
Trichoderma sp.		

Note: Asterisks denote fungi found exclusively on Benin mahogany; all other fungi were discovered on both tree species.

Table 5: Analysis of variance of occluded pruning wound area for Benin mahogany (*Khaya grandifoliola*) and rain tree (*Samanea saman*), respectively, treated with *Trichoderma virens* W23 and *Trichoderma harzianum* 9132

Effect	df	*F*	*p*	Level	Mean (SE)
a) Benin mahogany					
Treatment	2, 38.2	0.25	0.776		
Time	3, 284	64.46	< 0.001		
Orthogonal polynomial comparisons					
Linear	1, 241	186.04	< 0.001		
Quadratic	1, 257	24.47	< 0.001		
Cubic	1, 263	12.56	< 0.001	4	20.9 (4.4)
				8	38.9 (4.4)
				12	57.2 (4.4)
				18	73.5 (4.4)
Treatment × Time	6, 325	0.45	0.848		
Initial Wound Area	1, 128	188.55	< 0.001		
b) rain tree					
Treatment	2, 18	1.55	0.240		
Time	3, 441	32.11	< 0.001		
Treatment × Time	6, 441	2.15	0.047		
Treatment:$Time_1$(4 mos.)	2, 441	0.42	0.659		
Treatment:$Time_2$(8 mos.)	2, 441	1.25	0.289		
Treatment:$Time_3$(12 mos.)	2, 441	3.07	0.047	Suspension 1	46.2 (4.6)*
				Suspension 2	44.5 (4.6)
				Control	32.3 (4.7)
Treatment:$Time_4$(18 mos.)	2, 441	3.07	0.047	Suspension 1	46.2 (4.6)*
				Suspension 2	44.5 (4.6)
				Control	32.3 (4.7)
Initial Wound Area	1, 441	94.83	< 0.001		

Note: LS means are computed using the mean value of the covariate, initial wound area: 133 and 151 cm^2 for Benin mahogany and rain tree, respectively. Asterisks (*) indicate that treatment mean is significantly greater than the control at the $\alpha = 0.05$ level.

Table 6: Analysis of variance of the area of wood discoloration columns for Benin mahogany (*Khaya grandifoliola*) and rain tree (*Samanea saman*) pruning wounds treated with *Trichoderma virens* W23 and *Trichoderma harzianum* 9132, respectively

Effect	df	*F*	*p*	Level	Mean (SE)
a) Benin mahogany					
Treatment	2, 18	2.34	0.125	Suspension 1	54.1 (10.5)
				Suspension 2	66.1 (10.4)
				Control	78.2 (10.4)
Initial Wound Area	1, 118	74.99	< 0.001		
Aspect Ratio, $\mathfrak{R}_B$	1, 118	8.66	0.004		
b) rain tree					
Treatment	2, 18	4.05	0.035	Suspension 1	74.8 (15.7)*
				Suspension 2	95.5 (15.7)
				Control	124.2 (15.6)
(Initial Wound Area)	1, 108	0.63	0.429		
Aspect Ratio, $\mathfrak{R}_B$	1, 109	16.06	< 0.001		

Note: LS means were computed using the mean value of the covariates initial wound area and aspect ratio, $\mathfrak{R}_B$. For Benin mahogany, the average initial area of wounds was 133 cm^2. For rain tree, the initial wound area (mean = 151 cm^2) was removed from the final model (denoted parenthetically) because it did not account for a significant amount of variation in the area of wood discoloration columns. See Table 2 for the average initial $\mathfrak{R}_B$ of both species. Asterisks (*) indicate that treatment mean is significantly less than the control at the $\alpha = 0.05$ level.

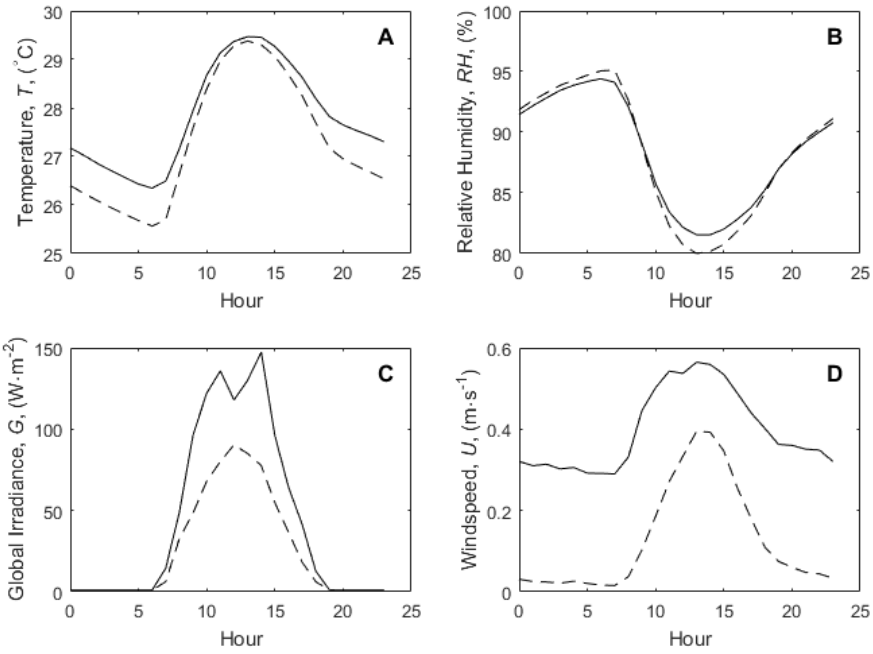

Figure 1: Average hourly weather conditions recorded during the 18-month field experiment at the two sites, including, (A) temperature, T (°C); (B) relative humidity, RH (%); (C) global irradiance, G ($W \cdot m^{-2}$); and windspeed, U ($m \cdot s^{-1}$). Solid and dashed lines, respectively, represent data observed at the Benin mahogany (*Khaya grandifoliola*) and rain tree (*Samanea saman*) sites.

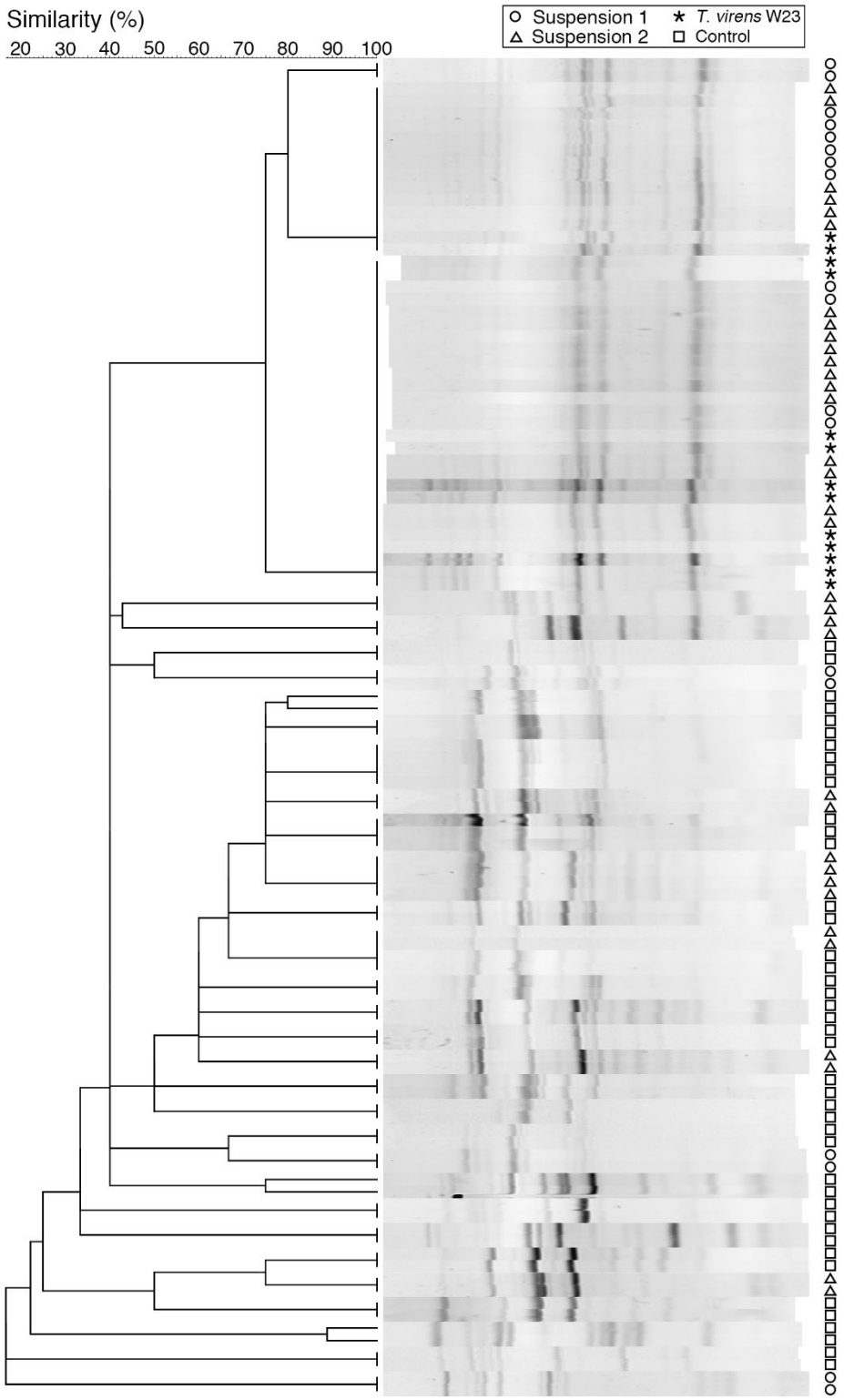

Figure 2: Dendrogram (left) shows similarity (%), based on gel electrophoresis of RAPD-PCR products (right), among groups of *Trichoderma* isolates recovered from Benin mahogany (*Khaya grandifoliola*) pruning wounds. A majority of *Trichoderma* spp. isolates recovered from wounds treated with Suspension 1 (91%, circle) or Suspension 2 (78%, triangle) were clustered in groups containing a reference isolate of *T. virens* W23 (asterisk), indicating the dominant presence of the biological control agent on treated wounds. On the other hand, none of the *Trichoderma* spp. isolates obtained from non-treated control wounds (square) were clustered together with a reference isolate of *T. virens* W23 (asterisk), indicating that they were other naturally occurring *Trichoderma* spp.

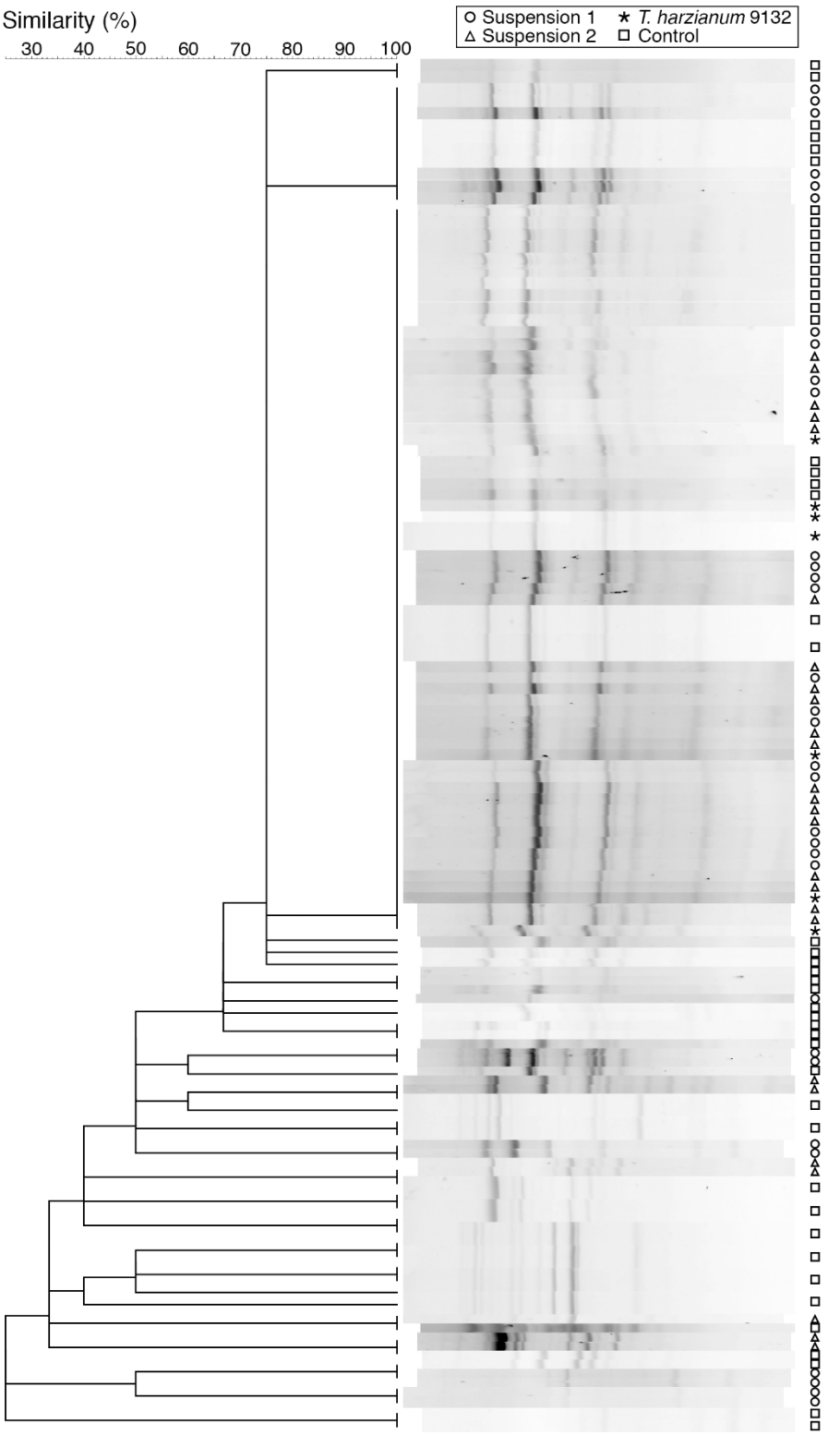

Figure 3: Dendrogram (left) shows similarity (%), based on gel electrophoresis of RAPD-PCR products (right), among groups of *Trichoderma* isolates recovered from rain tree (*Samanea saman*) pruning wounds. A majority of *Trichoderma* spp. isolates recovered from wounds treated with Suspension 1 (91%, circle) or Suspension 2 (98%, triangle) were clustered in groups containing a reference isolate of *T. harzianum* 9132 (asterisk), indicating the dominant presence of the biological control agent on treated wounds. On the other hand, 19% of *Trichoderma* spp. isolates obtained from non-treated control wounds were similar to *T. harzianum* 9132, indicating some contamination of the controls by the biological control agent.