Bioprotection of *Acacia mangium* using *Trichoderma* in Malaysia

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Abstract

In Sarawak, *Acacia mangium* seedlings were inoculated with 50 different *Trichoderma* isolates obtained from a wide variety of healthy plants in the Planted Forest Zone. The best *Trichoderma* isolates were selected after 10 trials at the Samarakan Nursery between August 2008 and August 2009. *Trichoderma* inoculation increased seed germination and seedling establishment (by up to 36%), seedling height and stem diameter (by an average of 25%) and the proportion of healthy seedlings meeting specifications for planting out into the forest (by an average of 41%), compared with untreated controls. Seedlings that received multiple fungicide sprays (standard nursery practice) generally performed slightly less well than the untreated controls. A new *Trichoderma* inoculum production facility has been built at Samarakan Nursery and *Trichoderma* inoculation of seedlings will replace the use of fungicide sprays. The economic benefit from increased productivity is estimated to be NZ $2.5 million per year.

Introduction

In New Zealand, forestry bioprotection research over the past 20 years using selected *Trichoderma* isolates (e.g. ArborGuard™) has resulted in improved health and vigour of radiata pine nursery stock by 10–20% and reduced losses from *Armillaria* disease by 25–50% in forestry plantations (Hill 2005). This success led to an invitation to Robert Hill to investigate the potential for *Trichoderma* bioprotection of *Acacia mangium* in the Planted Forest Zone (PFZ) of Sarawak, Malaysia near Bintulu, in partnership with Grand Perfect Sdn Bhd and Sarawak Planted Forests. The project involved isolating novel *Trichoderma* isolates from the locality and testing these in nursery trials with *Acacia mangium* seedlings to select the best performing isolates. After large-scale validation in the nursery, seedlings inoculated with the best *Trichoderma* treatments were tested in pilot-scale forestry trials versus *Ganoderma* disease. These trials were established in August 2009 and are still in progress.

Materials and methods

Root samples were taken from a wide variety of healthy plants, e.g. *Hymenocallis littoralis* (Figure 1), in the locality of the Samarakan Nursery and the surrounding districts (Bintulu and Tatau). The root samples were placed in clean, labelled self-sealing bags and kept moist. In the laboratory, roots were thoroughly washed in tap water, then surface sterilised with either a 1% sodium hypochlorite solution for 10 minutes or 1% Vircon® solution for 10 minutes, followed by further rinsing in sterile distilled water. After treatment the root fragments were placed on clean paper towels in Petri dishes to absorb excess moisture for approximately 1 hour and then plated onto Tap Water Agar (TWA), 1% Malt Extract Agar (MEA) or *Trichoderma* Selective Medium (1% MEA and Rose Bengal)(TSM). Plated root fragments were incubated at ambient temperature on the laboratory bench in the light for 4–7 days (Figure 2). *Trichoderma* isolates that grew from the root fragments were isolated into pure culture (Figure 3). These *Trichoderma* isolates were assigned a TS (*Trichoderma*/Samarakan) culture number. The subcultures are being kept at the Samarakan Nursery, at the Sarawak Forestry Corporation, Forest Research Centre, Kuching, and at the Bio-Protection Research Centre, Lincoln University, New Zealand.

Figure 1. White lily (*Hymenocallis littoralis*).

Figure 2. *Trichoderma* growing from surface-sterilised white lily roots.

Figure 3. Five individual *Trichoderma* isolates, each taken from one of the following plant species: *Turnera subulata*, *Mimosa* sp., *Turnera ulmifolia*, *Bambusa* sp. and *Piper nigrum*. These isolates were subsequently combined into a mixture called TS1.

Inoculum from about 50 different *Trichoderma* isolates was grown on MEA plates for the Samarakan Nursery trials with *Acacia mangium* seedlings. Each individual *Trichoderma* isolate was tested by itself, and some isolates were tested together as a mixture of several different isolates. Treatments were applied as an aqueous suspension of conidia, sprayed onto the growing mix in the 96-cell standard trays used by the nursery (Figure 4). Ten nursery trials were conducted between August 2008 and August 2009. Each of the first 10 trials had 12 treatments replicated at random in five blocks, with two trays per treatment per block. Treatments were applied soon after the seed was sown in standard nursery conditions (growing mixture, watering, etc.). Seed germination and seedling establishment were assessed during the first month. At 108 days after sowing the number of seedlings meeting the specifications for planting out into the forest was determined, using the standard nursery criteria, which included seedling height, stem diameter and a visual assessment of seedling health. *Trichoderma* treatments and untreated controls were not sprayed with any fungicides. Fungicide controls were sprayed according to the standard nursery regime.

Figure 4. Application of *Trichoderma* treatments in Samarakan Nursery trials.

Results and discussion

The best performing *Trichoderma* isolates tested in 10 replicated trials between August 2008 and 2009 had been isolated from the roots of the following plants: *Piper nigrum* (black pepper), *Turnera subulata* (white morning glory), *Turnera ulmifolia* (yellow morning glory), *Mimosa pudica* (mimosa), *Bambusa* spp. (bamboos) (Figure 3), *Helianthus* sp. (sunflower), *Hymenocallis littoralis* (white lily) (Figure 1), *Musa* sp. (banana), *Crytostachys renda* (red palm), *Koompassia excelsa* (Tualang, bee tree) and several others. Table 1 shows a summary of the results from the first 10 trials.

Figure 5. *Acacia mangium* seedlings that had been inoculated with *Trichoderma* TS mixture 1 (right) and the untreated control (left).

The increase in healthy tree production for *Trichoderma* versus untreated controls was 41.4% (Figure 5) and for *Trichoderma* versus fungicide controls was 57.7%.

Three sites (site 1, T2 D007; site 2, T1 C006; site 3, T1 A063) were selected for pilot-scale forest plantation trials in areas affected with *Ganoderma* disease (Lee 2000). The best performing 6 *Trichoderma* treatments from all trials were planted, which included two *Trichoderma* mixes (5 isolates in each) and 4 single

Trichoderma isolate treatments. Fungicide treated as well as untreated seedlings were included as controls. Seedlings will be monitored for tree health, growth and mortality from *Ganoderma* root disease.

Table 1. Overall mean number of seedlings meeting specifications for planting out into the forest and seedling height at 108 days after seed sowing from the first 10 trials. Each tray had 96 seeds sown. Trays were thinned twice, first to 72, then to 50 seedlings per tray for final harvest.

Treatment	Number of seedlings/tray	Height (cm)
Untreated control	29	30.6
Fungicide control	26	27.8
Trichoderma-inoculated	41	38.3

Conclusions and future priorities

Trichoderma isolates, both individual, single isolates and selected mixtures, have given a substantial increase in healthy tree production at the Samarakan Nursery, compared with both untreated and fungicide (standard nursery practice) controls. *Trichoderma* inoculation is only required once at the time of seed-sowing and it is expected that no further treatment is needed during the life of the crop. The best *Trichoderma* treatments appear to be rhizosphere competent and penetrate the roots of the *Acacia mangium* seedlings, growing with the developing tree.

A *Trichoderma* inoculum production facility has been built to supply the Samarakan Nursery needs (approximately 200,000 Acacia seedlings per day). Stringent quality control procedures will ensure inoculum production that is free from contamination with other micro-organisms. Further work is also needed to optimise the application system for the nursery and for *Trichoderma* production.

References

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