Progress in CoRE Biocontrol Research

Bio-protection for plantation forestry

Robert Hill

Bio-Protection

Bioprotection science for New Zealand

Pest & Disease Biocontrol

Selected beneficial micro-organisms Especially *Trichoderma* and *Beauveria* isolates for enhanced establishment and control of key pests and diseases in forest nurseries and plantations

Bio-Protection

Utilizing *Trichoderma* spp. to deliver plant health benefits in forest nurseries and plantations in New Zealand and Sarawak

Bio-Protection

PF Olsen & Co Ltd Nursery Containerised system

Bio-Protection

PF Olsen & Co Ltd Nursery Containerised system

Bio-Protection

Cuttings Trial 2009/2010 – established May 2009, assessed December 2009

	% healthy cuttings (n/1296)
• ArborGuard (formulation 1)	73
• Arborguard (formulation 2)	72
• Humate extract	71
• Humate + seaweed extract	71
• New Trichoderma 1	70
• New Trichoderma 2	67
• Seaweed extract	65
• New trichoderma 3	60
• Untreated control	57

Timberlands Te Ngae Nursery & Rangiora Nursery – soil bed system

Bio-Protection

Timberlands Ltd

Te Ngae Nursery

Pinus radiata seedling establishment and growth increased by 10 percent with ArborGuard™

1g treats 10,000 trees applied as seed coating

Bio-Protection

Cuttings trial 2009/2010 (Rangiora)

Established June 2009, assessed Feb 2010

	% healthy cuttings
• Humate extract	52.2
• New Trichoderma mix (1,2,3)	51.4
• PSA Trichoderma mix	50.3
• Seaweed extract	43.4
• AG Trichoderma	37.8
• Untreated control	36.4

Pinus radiata cuttings biochar + beneficial microbes

A- Biochar + growing mix + selected beneficial fungi

B- Biochar + growing mix

Forest plantation trials
Trichoderma vs *Armillaria*

Economic benefit:

$6.2m per annum from ArborGuard™

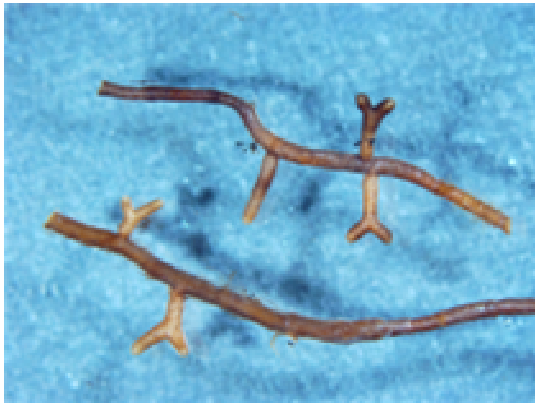
(Based on conservative estimate of $37m total loss from *Armillaria*, 50% ArborGuard™ uptake and 30% disease reduction)

Bio-Protection

Effect of *Trichoderma* bio-inoculants on ectomycorrhizal colonisation of *Pinus radiata* seedlings

Rhys Minchin
MSc project

Investigate effect of ArborGuard™ inoculation on ectomycorrhiza



- ArborGuard™ did not reduce ectomycorrhizal colonisation of *Pinus radiata* seedlings

Lincoln University

Bio-Protection

Ecological studies of *Trichoderma* bio-inoculants in the soil ecosystem of *Pinus radiata*

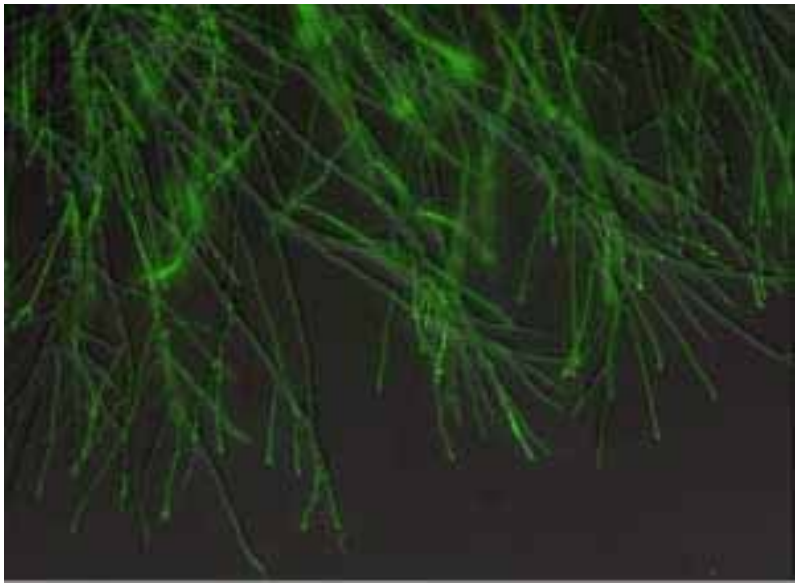
Establishment and distribution of T*richoderma* in soil and root zone

Use of genetically marked strains

Ecology, population dynamics of *Trichoderma* bio-inoculants

Effect on *P. radiata* growth and health

NZ Biotron facility used



Lincoln University

Bio-Protection

FOREST NURSERY
TRAY DISINFESTATION

- New versus Old trays
- Chemical dips

  - expensive and disposal a problem
- Heat treatment

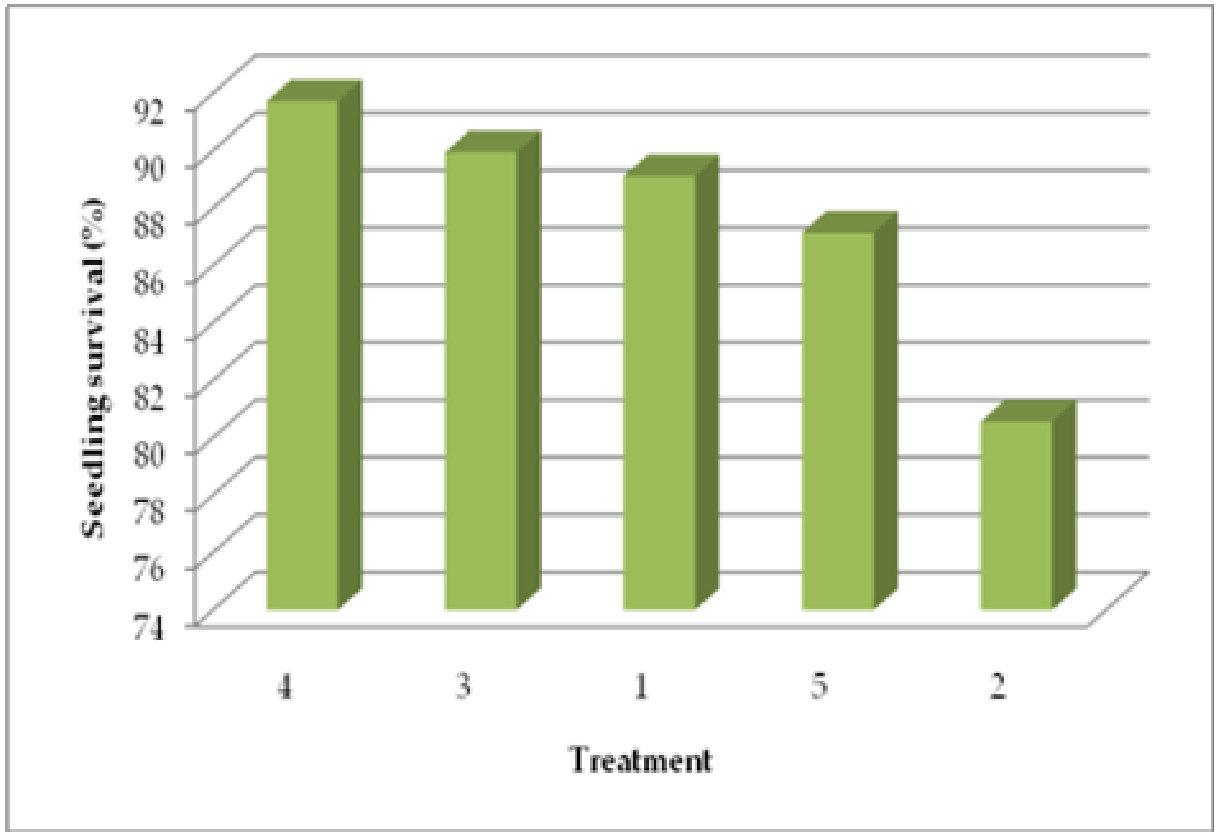
  - labour, time, cost
- Rapid, inexpensive, environmentally acceptable alternatives?
- Trichoderma suspension/ np


SAFE
IRST


GTP


TP11/T4
TP11/T4
TR11/T4
TR11/T4

Samarakan Nursery Sarawak



T1	New trays sprayed with *Trichoderma* [TS26] after sowing the seeds
T2	Old trays without *Trichoderma*
T3	Old trays sprayed thoroughly with Trichoderma [TSmix1] before media potting
T4	1st application:- Old trays sprayed with Trichoderma before media potting 2nd application;- Trichoderma sprayed after sowing
T5	New trays

PF Olsen Nursery Waiuku

	Survival of cuttings (%)
• ArborGuard (2)	81
• Humate extract (1:300)	82
• New Trichoderma mix (1,2,3)	83
• Untreated control	74

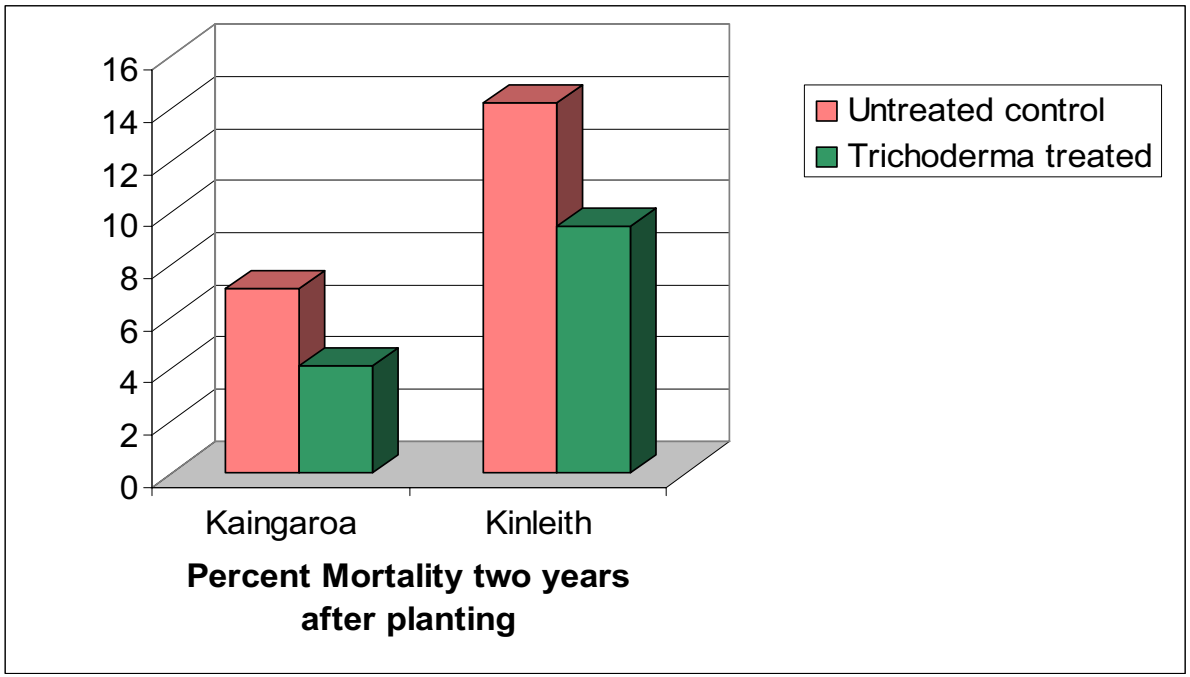
Forest plantation trials
Armillaria and flute canker

Forestry trials

- **Kaimai-Mamaku**
- **Kaingaroa**
- **Kinleith**
- **Rotoiti**
- **Tokoiti**

Bio-Protection

Trichoderma vs *Armillaria* forestry trials



Bio-Protection

The *Trichoderma* vs *Armillaria* forest plantation trials at Rotoiti (established in 2007) and Kaingaroa (established in 2001), were assessed for tree health, mortality and for growth in June 2009.

In May 2008 over 7,000 trees in this trial were assessed for health and mortality. The ArborGuardTM treatment had the highest health score and lowest mortality from *Armillaria* disease.

Bio-Protection

Rotoiti Trial

Total number of trees included in this assessment = 7,129

Total number of dead trees = 38 (0.5%)

Average health score = 3.6 overall, on a scale of 0 (=dead) – 4 (= very healthy)

Treatment	**Mean health score (%)**	**Mean mortality (%)**
T 1 bacterial mixture	3.5	0.6
T 2 new *Trichoderma*	3.7	0.8
T 3 ArborGuard TM	3.7	0.4
T 4 Untreated control	3.5	0.7
Overall mean	3.6	0.5

In June 2009 this trial was reassessed ArborGuardTM was best for health and had the lowest mortality.

Bio-Protection

Nectria flute canker pruned stub trial
Toikiti Forest (City Forests Ltd)

- Treatments reduced fluting and *Nectria* fruiting bodies
- Cost of pruned stub treatment probably uneconomic
- Future bio-protection research focus on *Nectria* control with selected root-zone and/or endophytic beneficial microbes

Bio-Protection

Biocontrol: a new approach to manage *Dothistroma* needle blight of *Pinus radiata*

Rosie Bradshaw
Rebecca McDougal
Beccy Ganley

Bio-Protection

Potential BCAs for *Dothistroma*

Pine seedlings sprayed with *Trichoderma* spores

a) One lot of trees placed in the field for natural inoculation under *Dothistroma*-infected trees
b) Another lot kept at Scion, sprayed with *Dothistroma* spores, kept under semi-controlled condition (watered two hourly, shade cloth cover in hot weather)

Bio-Protection

Initial Field Trials

Pine seedlings sprayed with *Trichoderma* spores

a) One lot of trees placed in the field for natural inoclutaion under *Dothistroma*-infected trees

b) Another lot kept at Scion, sprayed with *Dothistroma* spores, kept under semi-controlled conditions (watered 2 hourly shade cloth cover in hot weather)

Bio-Protection

a) Natural inoculation

b) Spray inoculation

Bio-Protection

Cyclaneusma minus

Screened with strains that performed best against *Dothistroma*

Also antagonistic toward *C. minus* – loss of GFP observed

Mode of interaction yet to be determined

Bio-Protection

INDUCED RESISTANCE

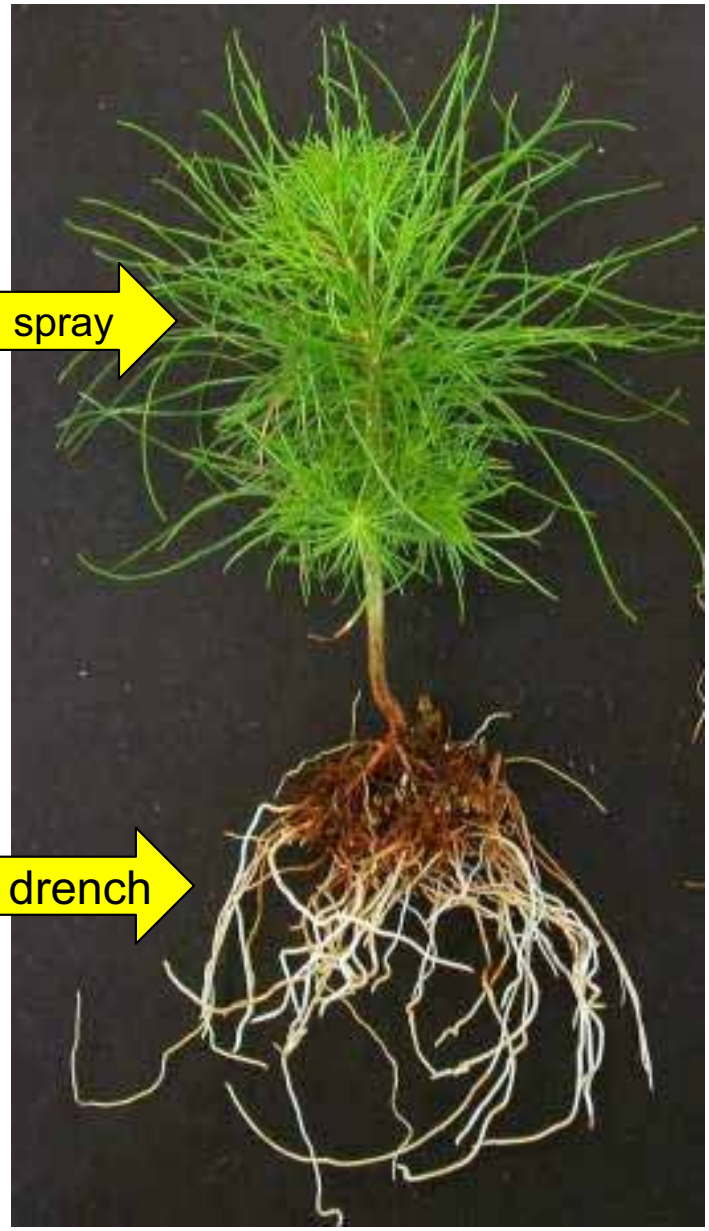
Tony Reglinski and team

- FRST Milestones (2008-2010)
- Integrate Trichoderma treatment with foliar elicitors to suppress phytophthora root rot and diplodia die-back in radiata pine seedlings.
- Determine impact of treatments on defence induction.
- Evaluate integrated strategies in a commercial forest nursery.
- Communicate results to industry.

Conditioning Treatment ⇢ Response

Elicitor

Trichoderma



- activation of defences
- accumulation of phenolics, terpenoids
- cell wall lignification
- release of interplant signals

- Colonisation of root zone
- Antagonism – competition, antibiosis, mycoparasitism.
- Seedling growth promotion
- Induced systemic resistance

Phytophthora Studies

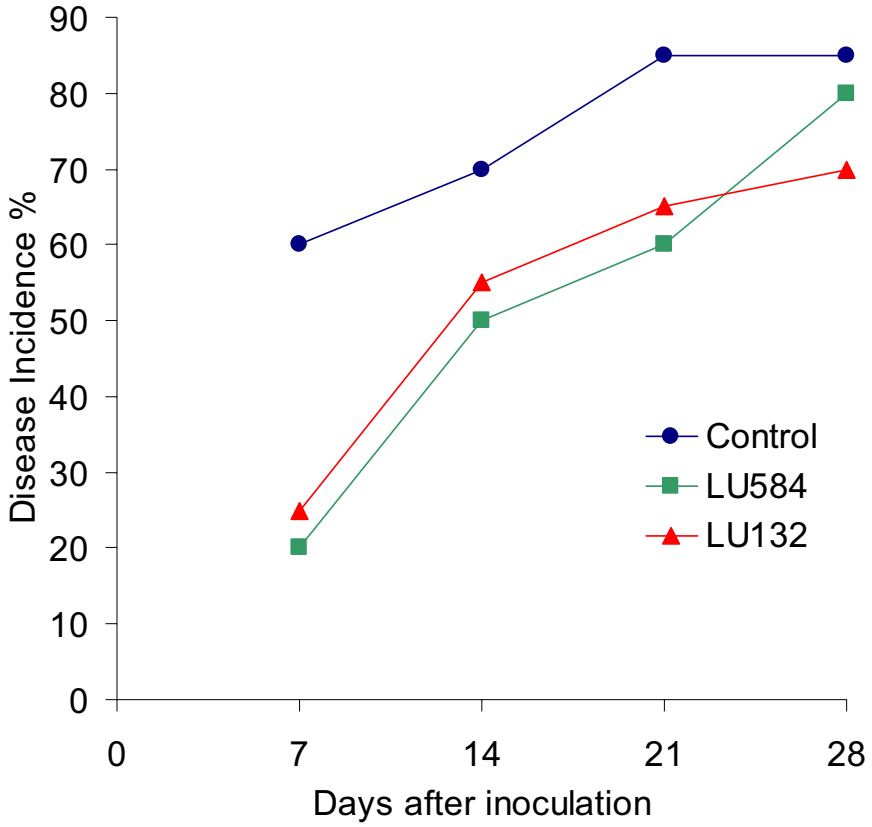
- Trichoderma was applied as a seed coating treatment or as a potting mix amendment.
- In laboratory trials Trichoderma did not suppress *Phytophthora* root rot in radiata pine seedlings under high disease pressure conditions.
- Root rot was inhibited when Trichoderma treatments were combined with foliar applications of phosphorous acid (this compound directly inhibits the pathogen and promotes plant defences)
- In commercial nursery trials the integrated treatment suppressed Phytophthora root rot by over 98% (two consecutive trials). Disease control was mainly attributed to the action of phosphorous acid.
- Results have been published:
  - scientific publication (Plant Pathology 58:723-730)
  - industry journal (NZJF – in press)
  - wall chart (to be available on NZFHRC website).

Wilting symptoms indicative of root rot

Nursery trials were part funded by NZFHRC and conducted In collaboration with Scion

Diplodia studies

- Foliar application of methyl jasmonate (MeJA) can induce resistance to Diplodia pinea in pine seedlings.
- MeJA-induced resistance is associated with enhanced peroxidase activity and the accumulation of terpenoids at the infection site.
- Root application of some Trichoderma isolates has shown potential to significantly delay the onset of diplodia development ($p<0.05$).
- Studies are in progress to identify the biochemical basis for the systemic induced resistance response.
- Published studies
  - Can J For 38:677-684 (2008)
  - Physiol Mol Plant Pathol – in press



Effect of Trichoderma root treatment on diplodia development

Inoculation of Pinus radiata tissue culture with selected beneficial microbes

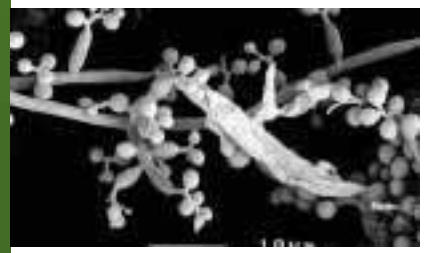
The Tree Lab

Jenny Aitken

Bill Dyck

Entomopathogen research in Forest Bioprotection

Mike Brownbridge, Steve Reay, Tracey Nelson, Travis Glare



- Research on biological control of forestry pests using insect pathogenic fungi.
- Developing *Beauveria* spp. a practical control agents
- Looking at application, formulation and efficacy in soil, and compatibility with *Trichoderma.*

Bio-Protection

Bioprotection strategies for introduced bark beetles:

- *Hylastes ater*
- *Hylurgus ligniperda*
- Maturation feeding can kill pine seedlings
- Significant impact on regeneration
- Vector sapstain and other disease-causing fungi
- Feeding wounds serve as points of entry for soil borne diseases
- Risk of infestation delays planting
- Currently >$20m p.a. in damage and delayed planting

Bio-Protection


UGA1428133
UGA1428131

H. ligniperda infected with *M. anisopliae* 275 (top) and *B. bassiana* 361 (bottom)

Relative virulence of new isolates:

- *Metarhizium anisopliae* 275
- *Beauveria bassiana* 361

Faster kill, higher levels of mortality than comparative *B. caledonica* strains, but low natural incidence

Importance of project to the future of NZ forestry

- Renewed interest in plantation forestry in NZ; contributes to NZ's future, C sequestration, etc.
- Current Pests: Protect forest establishment from *H. ater* damage
- Biosecurity: development of bio-protection technology for use against potential future bark beetle introduction, or the establishment of significant bark beetle vectored fungi (e.g. pine pitch canker)
- Use of control technologies applicable to overseas pests e.g., *Hylobius abietis* in northern Europe, *H. ater/H. ligniperda* in South America (Chile) and South Africa

Dungley et al. 2003. NZ Journal of Forestry Science on pine pitch canker:

"There is a real possibility that this fungus could become established in New Zealand, and it would most likely spread rapidly due to the presence of potential vectors and suitable climate (Dick 1998)…a possible vector for this pathogen (*Hylastes*) is already present in New Zealand…"

Bio-Protection

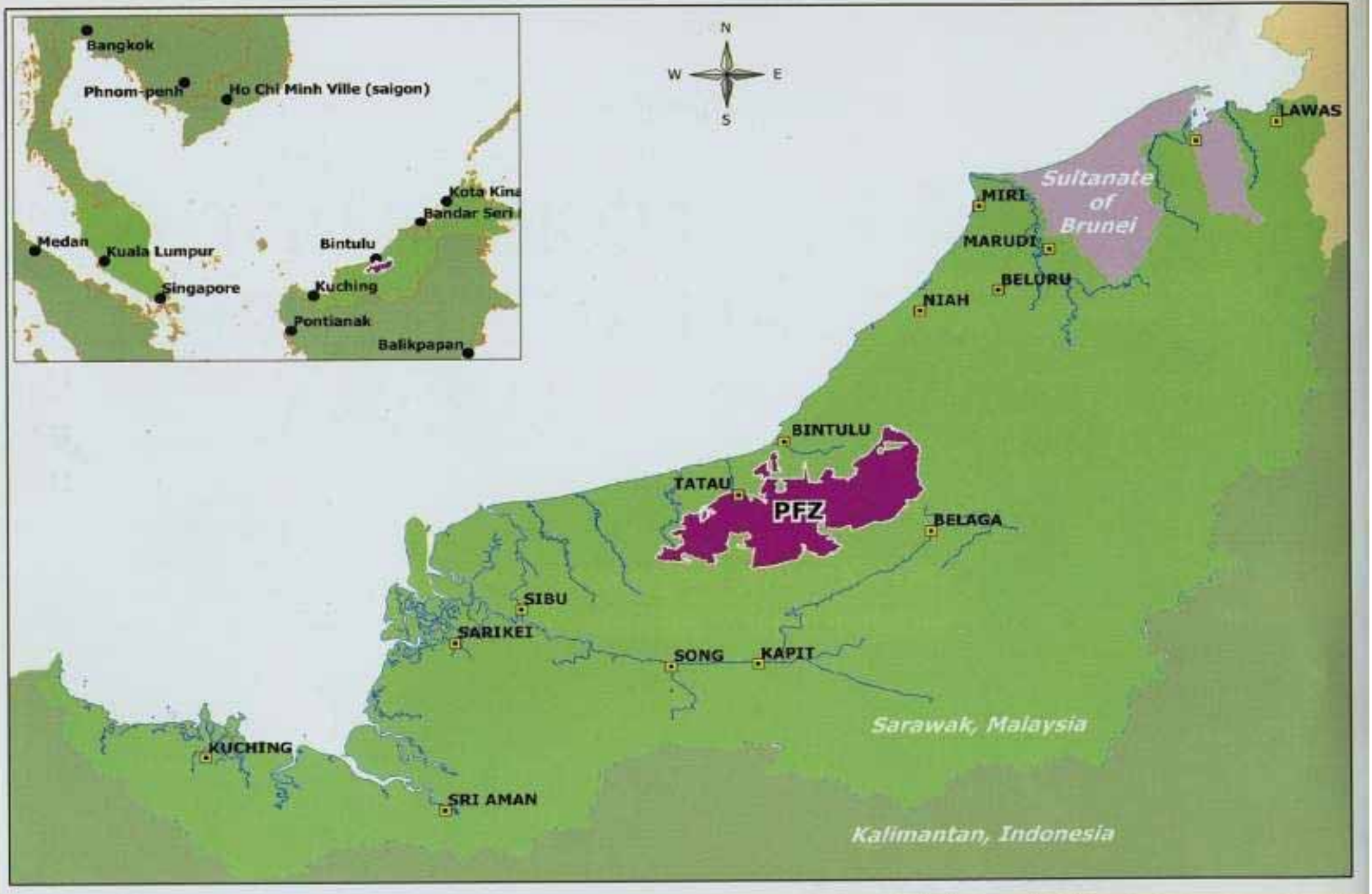
SARAWAK FORESTRY PROJECT OUTLINE

- *Trichoderma* isolations
- Inoculum production
- Nursery trials
- Select best *Trichoderma* treatments
- Validate results on large scale
- Pilot scale plantation trials
- Large scale plantation trials
- Large scale *Trichoderma* inoculum production

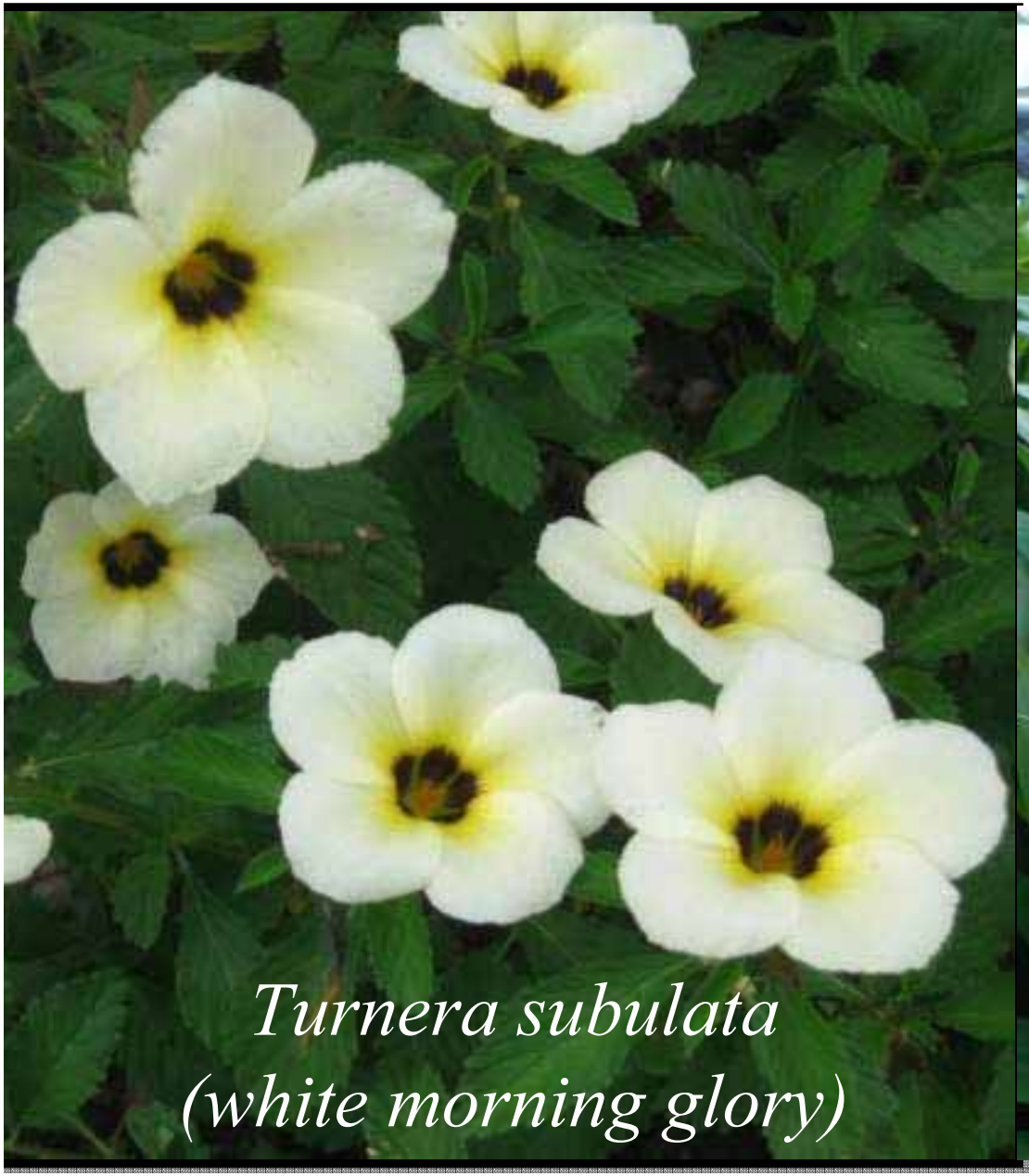
Bio-Protection

Sarawak Forestry Project

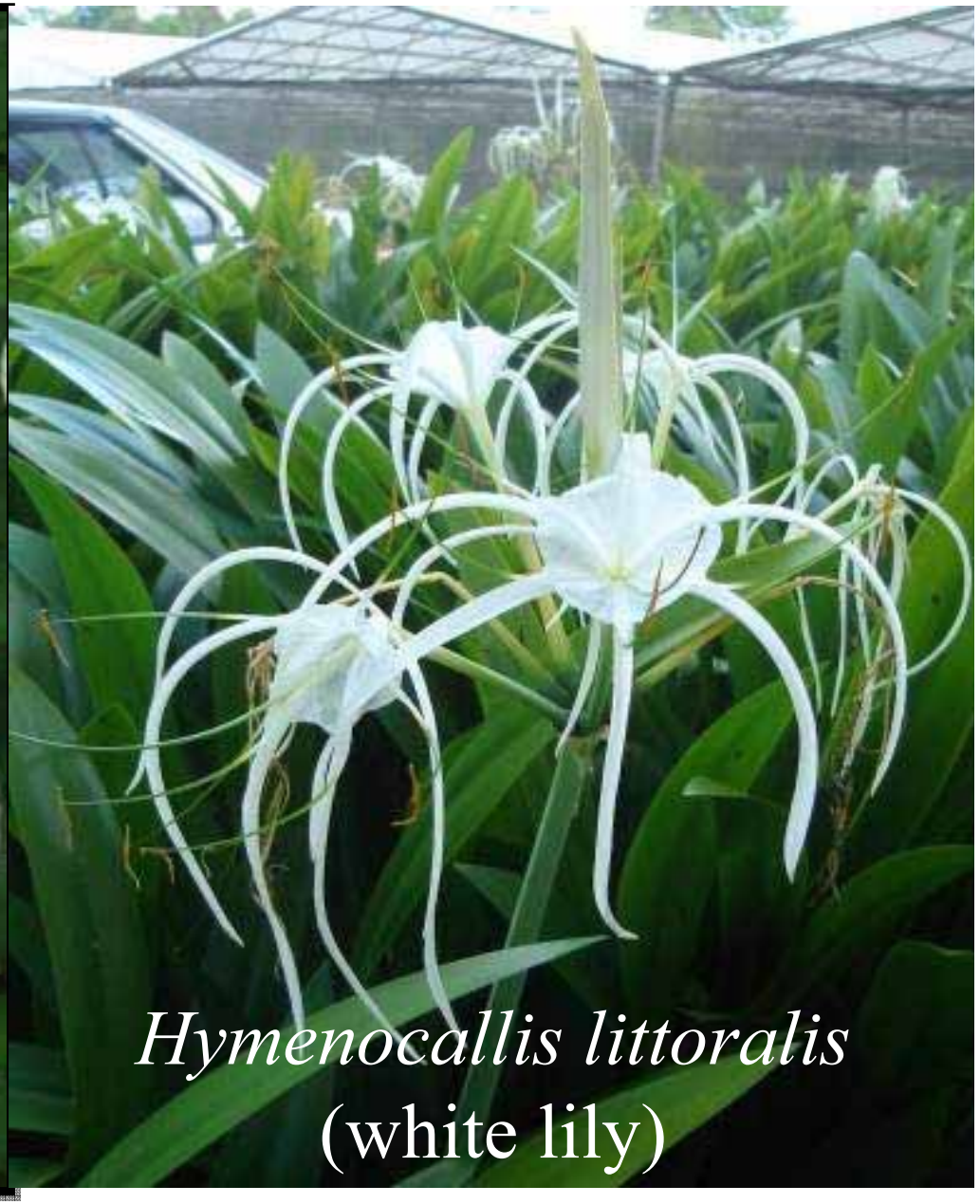
Map 1 : Location of Planted Forest Zone (PFZ) in Sarawak




STATE GOVERNMENT OF SARAWAK
PLANTED FORESTS (PULP and PAPER) PROJECT
SAMARAKAN
NURSERY



Turnera subulata
(white morning glory)



Hymenocallis littoralis
(white lily)

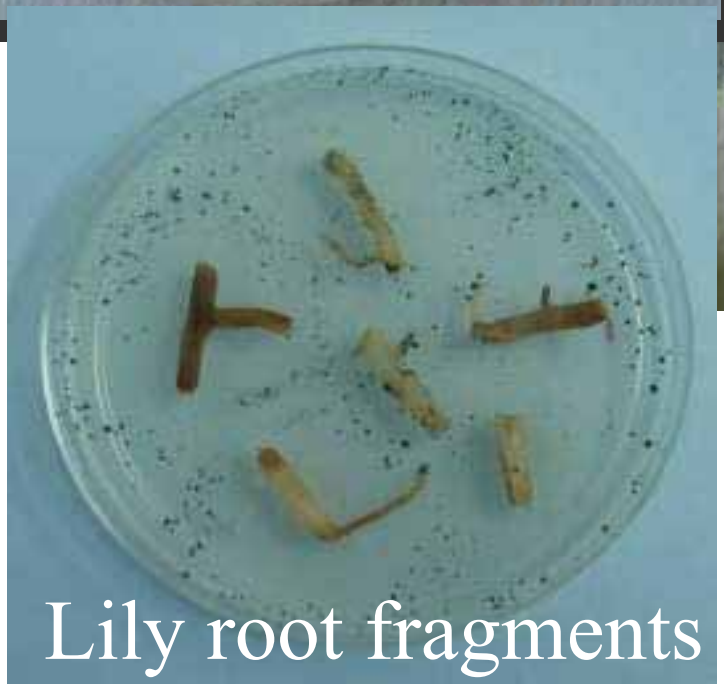
Koompassia excelsa (Tualang)

Bambusa sp.
(bamboo)

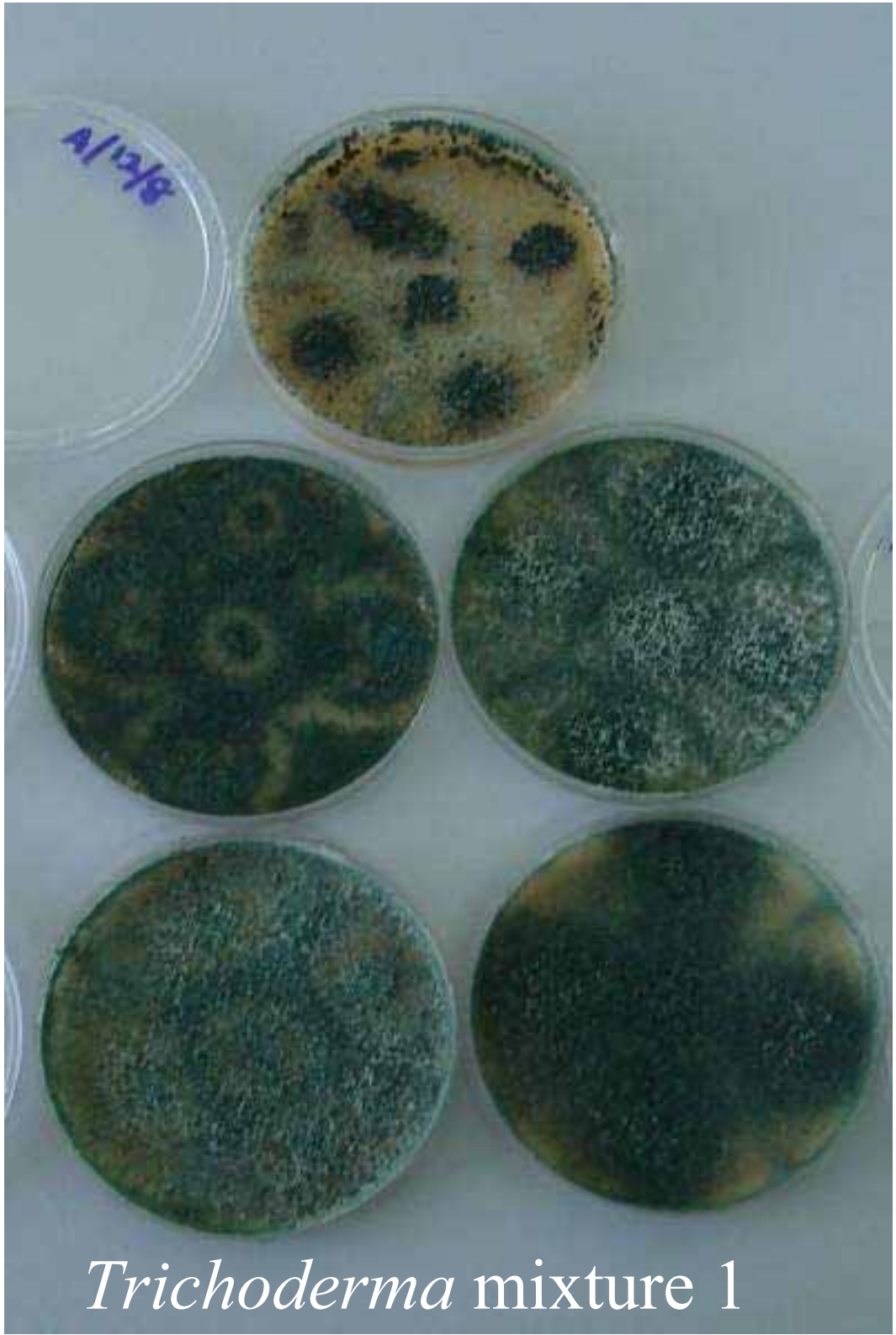
Similajau National Park - Bintulu

Surface Sterilisation of root fragments

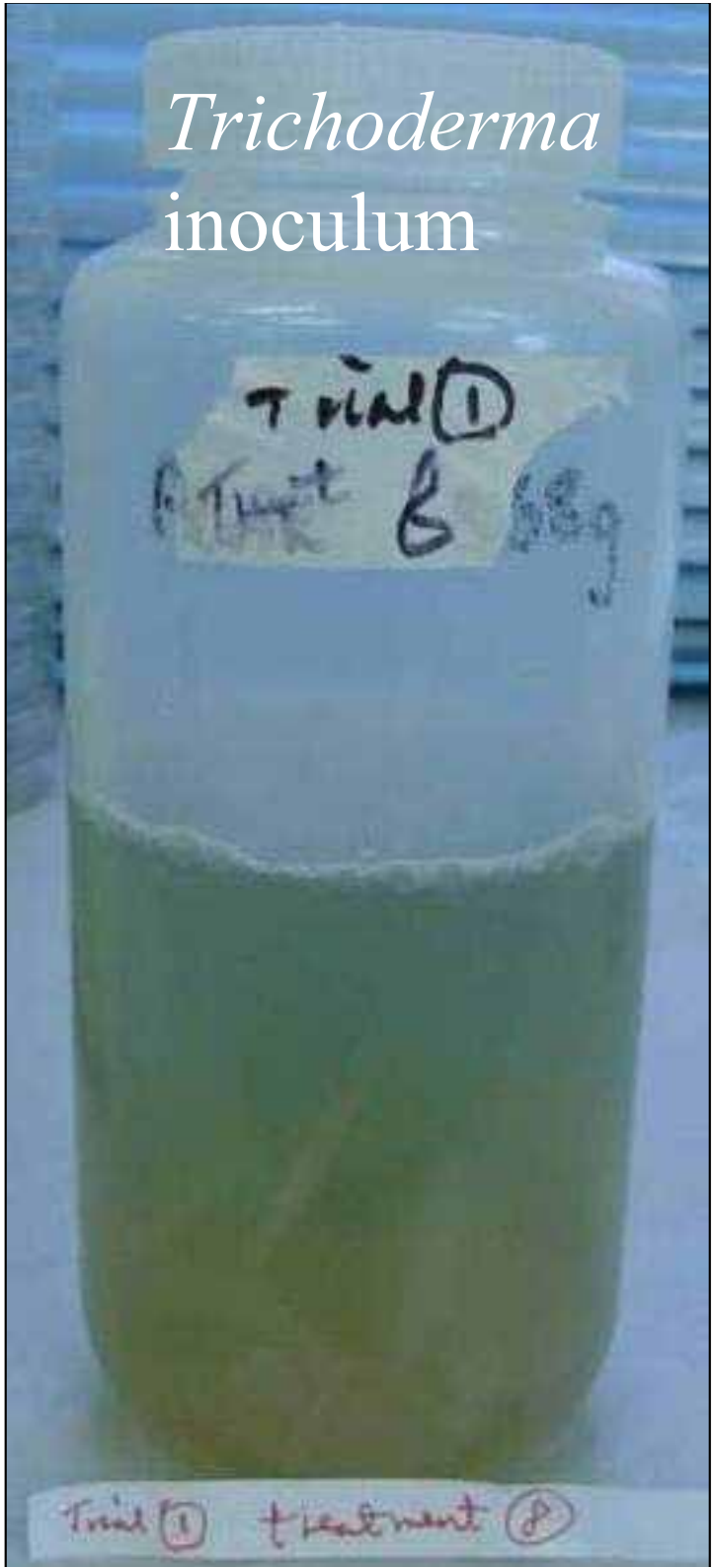
Growing *Trichoderma* inoculum for nursery trials at Samarakan

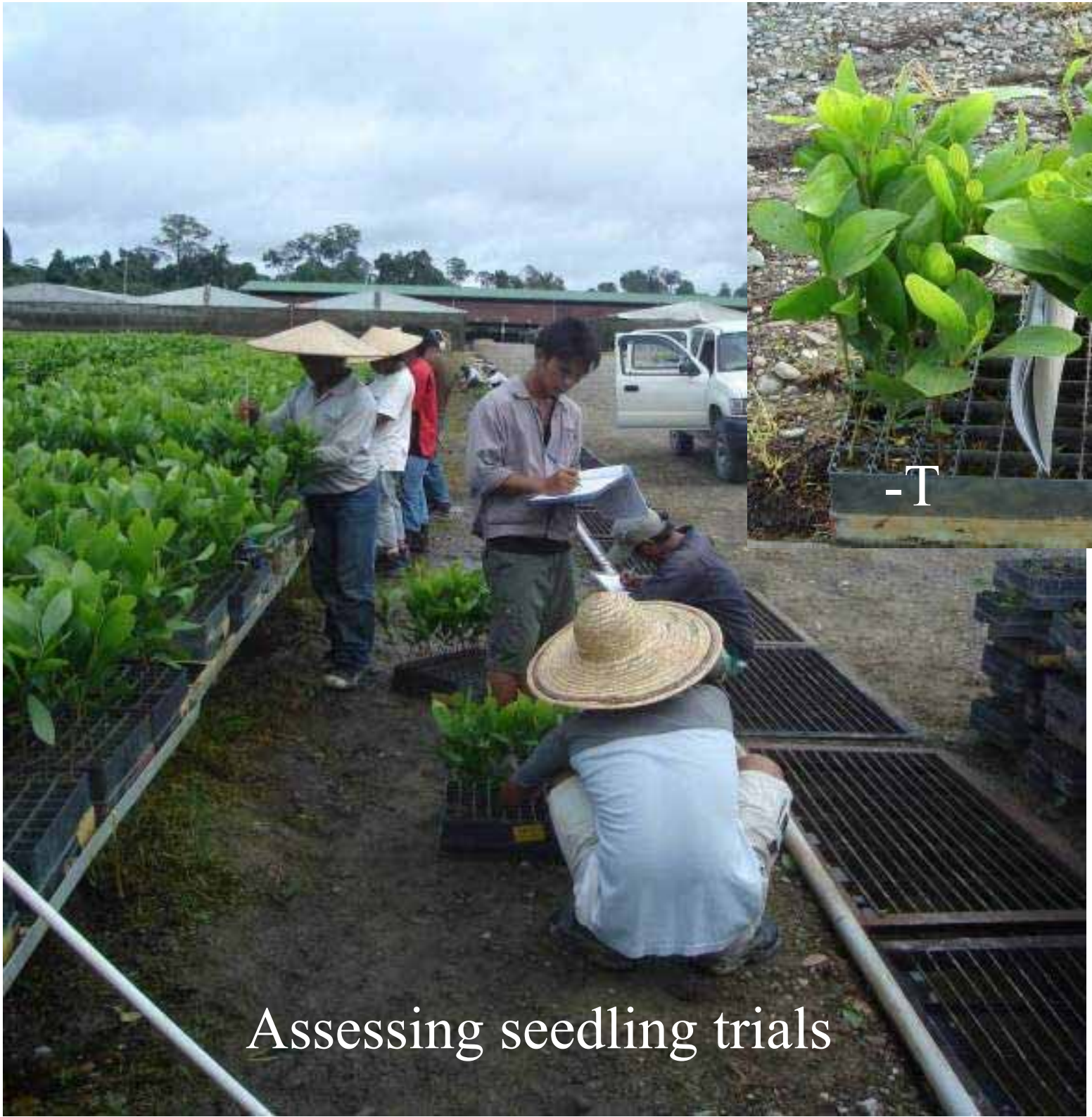


Sub-culturing *Trichoderma*



Trichoderma mixture 1





Assessing seedling trials


NO FUNGICIDE SPRAY
23/01/2010


23/01/2010

Best *Trichoderma* isolates	Plant source
TS mix 1	*Piper nigrum, Turnera subulata, T.ulmifolia, Mimosa pudica, Bambusa* sp.
TS mix 2	*Bambusa* sp.
TS 26	*Helianthus* sp.
TS 37	*Hymenocallis littoralis*
TS 42	*Musa* sp.
TS 47	Crytostachys *renda*

Bio-Protection

Seedlings meeting specifications for planting into forest and average seedling height (August 08 – 09 trials)

TREATMENT	AVERAGE SEEDLING HEIGHT (cm)	NUMBER OF SEEDLINGS
Trichoderma Treatment	38.3	350
Untreated Control (Without Treatment)	30.6	225
Fungicide Control (Standard Nursery Practices)	27.8	211

Bio-Protection

Economic benefit, based on 30 million trees/annum and 25 sens per tree,
Trichoderma vs Fungicide = RM 5,000,000 per annum

*for increased productivity- does not include,

[cost of fungicides + cost of fungicide application + labour for fungicide application]]

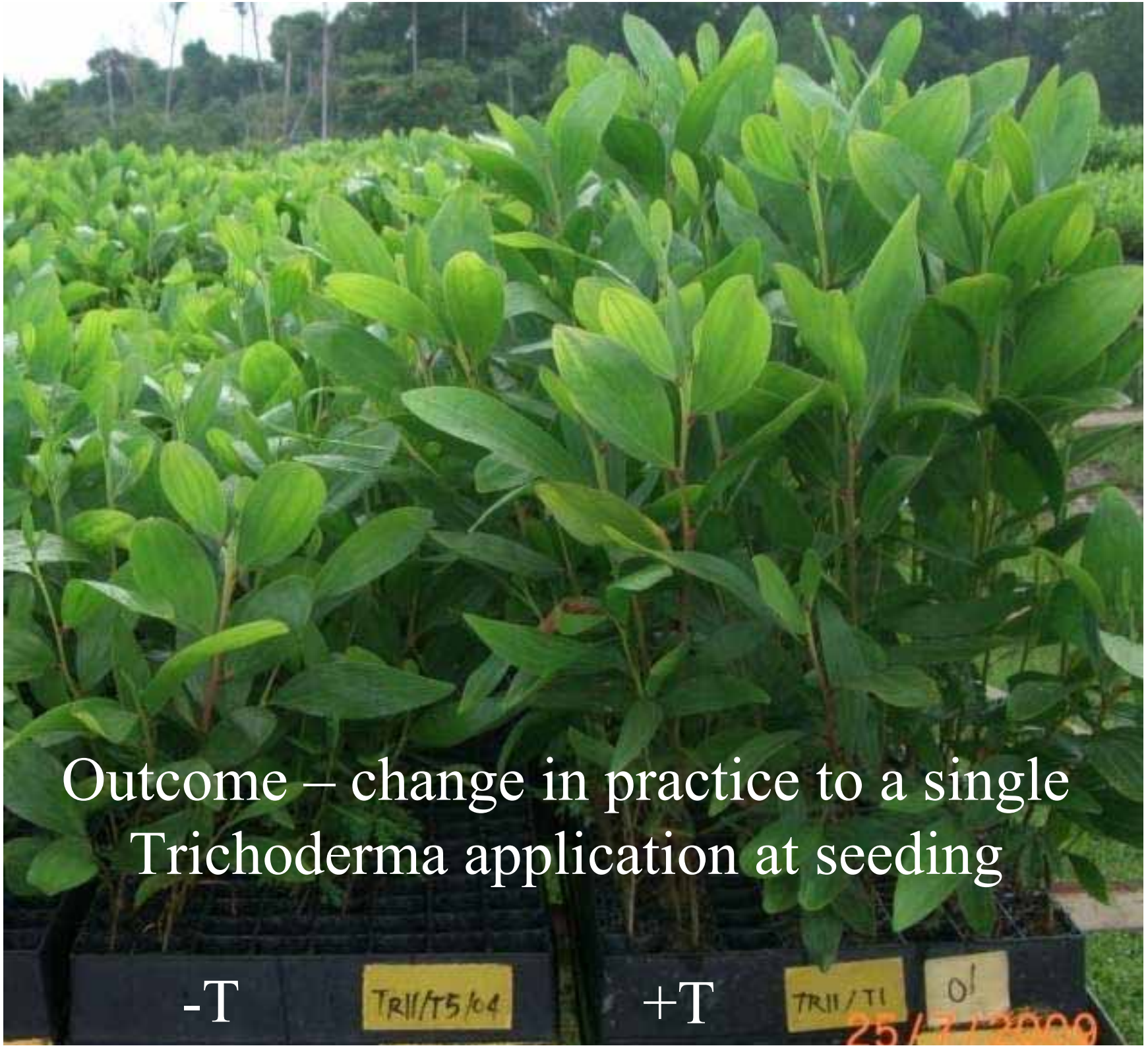
VS

[Cost of Trichoderma 1 application at sowing]

Bio-Protection

Seedlings being transported to forestry plantation sites

Forestry
trial
Site 1
(T2D 007)


Outcome – change in practice to a single Trichoderma application at seeding
-T
TR11/T5/04
+T
TR11/T1