



Bio-Protection

BioDiscovery
New Zealand Limited

D.E. Paderes, R. A. Hill and Wei-Young Wang

◆ Growth Enhancement of
Containerised *Pinus radiata*
Seedlings Following Application of
Trichoderma Commercial Product
(ArborGuard™) Formulations



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Professional Forestry Services **ltd**



Forenza
Superior planting stock **ltd**



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◆ Retrieval and DNA Fingerprinting of
the *Trichoderma* Commercial
Product (ArborGuard™)



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Objectives



- ◆ Determine the effect of ArborGuard™ on germination and plant growth
- ◆ Quality assurance that the seedlings contain ArborGuard™ before selling
- ◆ Identify the best application method
- ◆ Establish the best rate and frequency of application for ArborGuard™
- ◆ Differentiate ArborGuard™ *Trichodermas* from native *Trichodermas*



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Trichoderma

- ◆ Fungus, worldwide distribution
- ◆ Well known as biological control agent, for growth promotion activities and for industrial uses (enzymes and antibiotics)
- ◆ Comprises 1/3 of commercial fungal biocontrol products
- ◆ Limited work using commercial products in forestry

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Country	Product	Manufacturer
New Zealand	Trichopel, Trichojet Trichodowels, Trichoseal ArborGuard DRH	Agrimm, Christchurch GrowChem, Porirua
USA, Sweden	Binab	BioInnovation, Sweden
USA, Europe	RootShield, BioTrek 22G, Suppresivit, T-22G	Wilbur-Ellis, Borregaard BioWorks Inc. USA
Israel	Trichodex, RootPro, Root Protato	Makhteshim, Israel
USA	Promot	J.H. Biotech, CA, USA
India	Trieco	Ecosense, Mumbai, India

Product	Disease/Pathogen	Crop
Binab	Wilt, take-all, root rot, Wood wound decay	Ornamentals, turf, vegetables
Bio-Fungus, Suppresivit	<i>Sclerotinia</i> , <i>Phytophthora</i> <i>Pythium</i> , <i>Fusarium</i> <i>Verticillium</i> , <i>Rhizoctonia</i>	Strawberries, trees, vegetables
RootPro, Root Protato	<i>Rhizoctonia</i> , <i>Pythium</i> , <i>Fusarium</i> , <i>Sclerotium</i>	Vegetables, potatoes
RootShield, PlantShield	<i>Pythium</i> , <i>Rhizoctonia</i> , <i>Fusarium</i>	Ornamentals, vegetables

Product	Disease/Pathogen	Crop
Thichodex	<i>Botrytis, Colletotrichum, Plasmopara, Monilia, Fulvia, Pseudoperonospora</i>	Vegetables, sunflower, fruit trees
Trichopel	<i>Armillaria, Pythium, Rhizoctonia, Nectria, Botryosphaeria, Fusarium, Chondrosternum, Phytophthora</i>	Turf, ornamentals, field crops
Trieco	<i>Rhizoctonia, Pythium, Fusarium</i> , root rot, red rot, seedling rot, collar rot, damping-off, <i>Fusarium</i>	Oilseeds, mustard, spices, vegetables, sunflower, fruit trees
DRH	<i>Armillaria, Colletotrichum, Diaporthe, Endothia, Fusarium, Fusicladium, Helminthosporium, Phytophthora, Pythium, Rhizoctonia, Rhizopus, Sclerotinia, Sclerotium,</i>	Turf grass, fruit trees, vegetables



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Materials and Methods

- ◆ ArborGuard™ - GrowChem NZ Ltd, Porirua
- ◆ Seed coating and drench
- ◆ Pellet and Granules- soil incorporation
- ◆ Nursery management (Forenza, Waiuku)
- ◆ Block design – GenStat



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DNA Fingerprinting

- ◆ Isolation and purification of native *trichoderma* isolates plus the AG commercial isolates- semi-selective medium
- ◆ DNA extraction- Puregene extraction kit
- ◆ Run UPPCR using five primers
- ◆ 1% gel electrophoresis
- ◆ Band pattern and phylogenetic tree

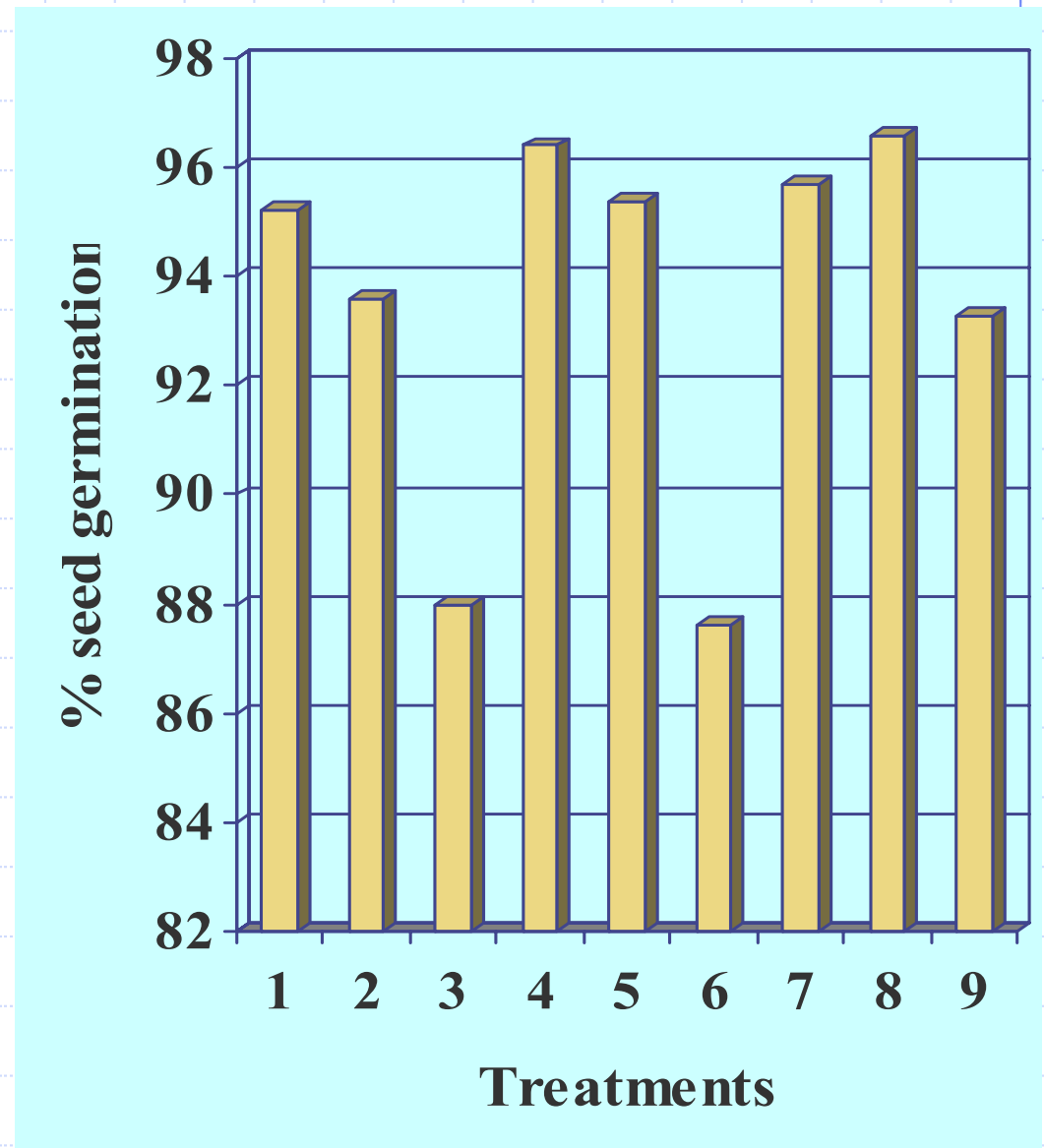


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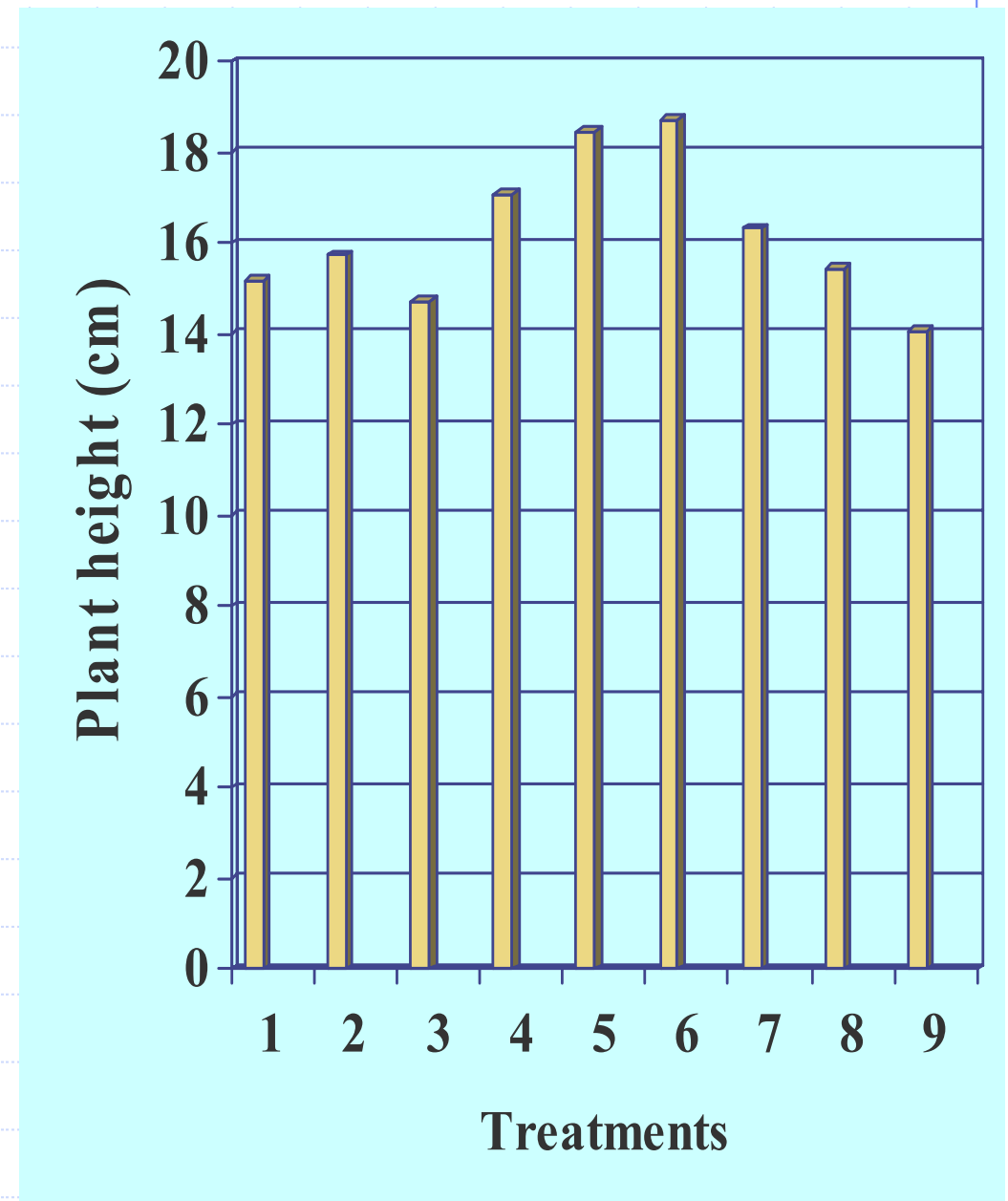


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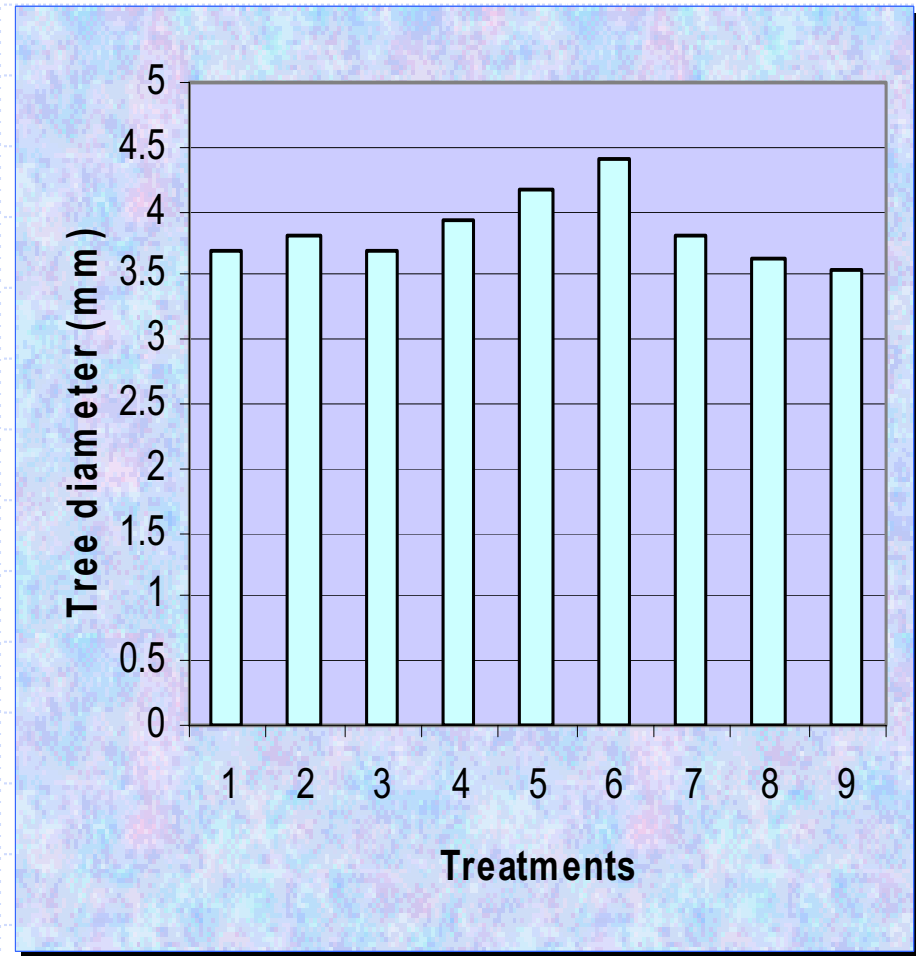
- ◆ 1-AG seed coated + AG sprayed 0 month
- ◆ 2-AG seed coated + AG sprayed 3 months after
- ◆ 3-AG seed coated + AG sprayed 3,6 and 9 months after
- ◆ 4- AG seed coated
- ◆ 5-Thiram coated + bacteria mix
- ◆ 6-Thiram coated + single bacteria
- ◆ 7-Thiram coated + LU isolate
- ◆ 8- Thiram coated (control)
- ◆ 9- Untreated control



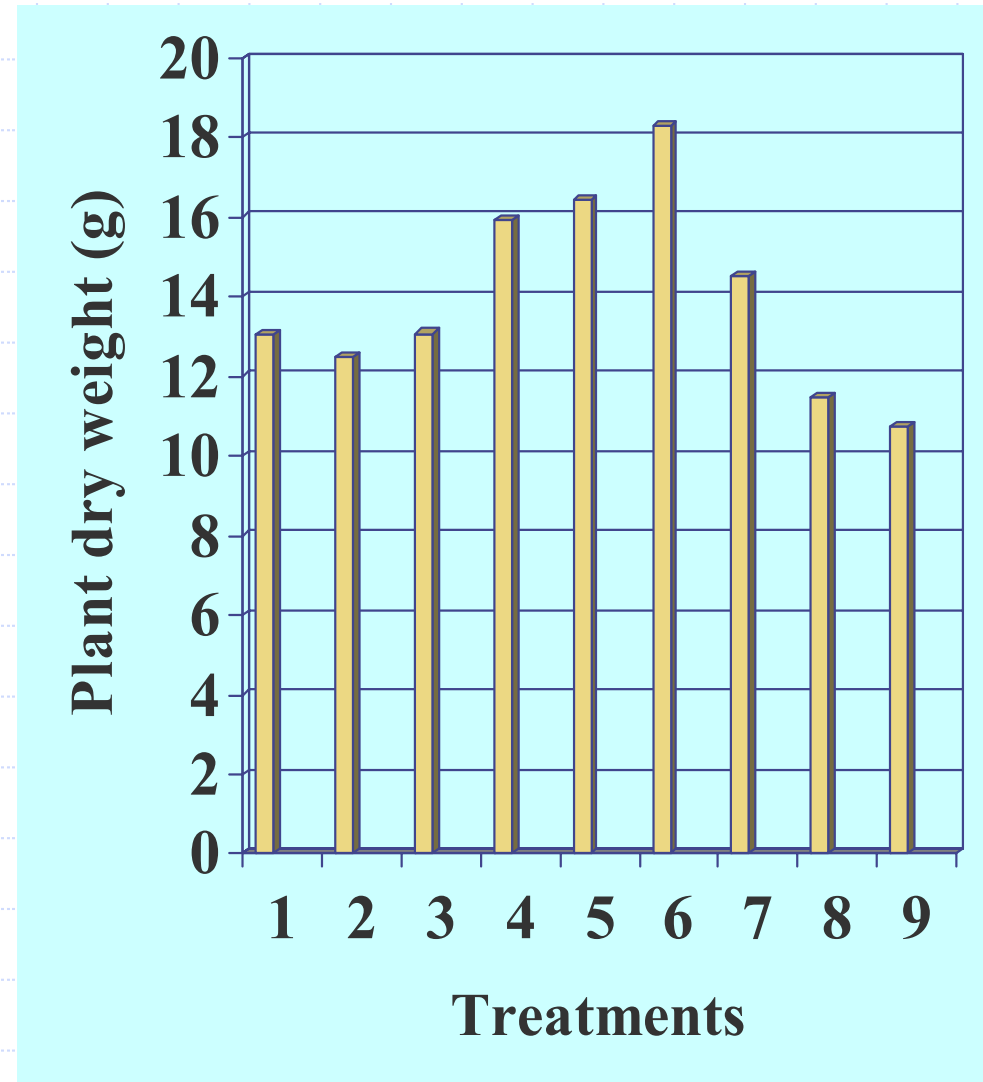
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Genetic Groups

- ◆ **Group I AG 1**
- ◆ **Group II AG 2, AG 3, AG 4, AG 5,
AG 6, NT 14 and NT 19**
- ◆ **Group III NT's**
- ◆ **Group IV NT's**



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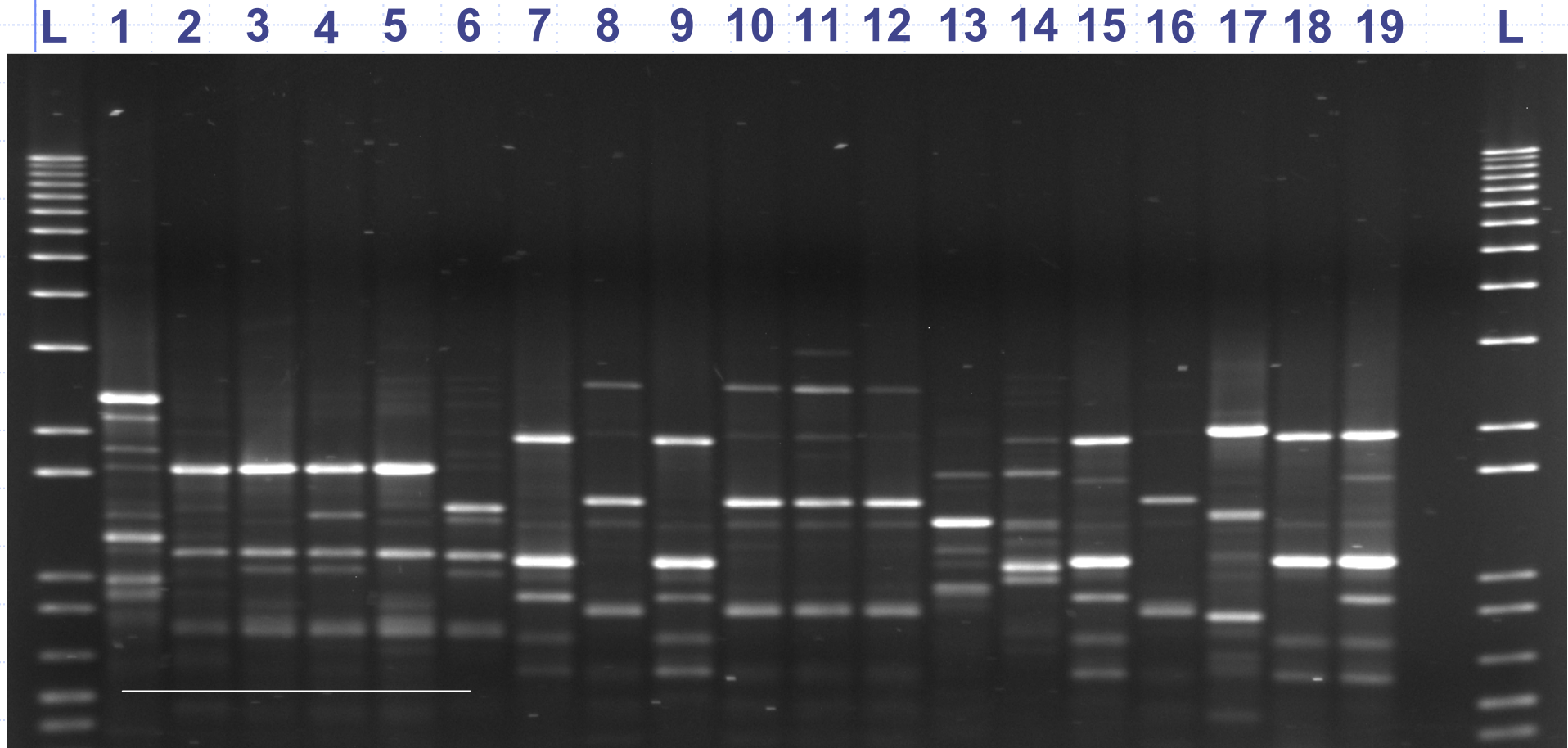


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DNA Band Pattern of *Trichoderma* using UP-PCR



ArborGuard



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Summary

- ◆ The best formulation and delivery of the commercial product was identified
- ◆ A reliable, cheap and easy to use semi-selective medium was developed
- ◆ Differentiation of the commercial isolates from native *Trichoderma* using DNA technology was accomplished
- ◆ Other beneficial organisms were recognised



Future Work

- ◆ Combine application of ArborGuard™ with other groups of microorganisms or other substances/materials
- ◆ Follow up field trial of the treated seedlings in naturally infested *Armillaria* plantation forest
- ◆ Test its effect on pine cuttings (in progress)



Acknowledgement

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- ◆ Technology New Zealand
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- ◆ BioDiscovery New Zealand Ltd. Auckland
- ◆ Forest Owners Association (FOA)
- ◆ Forest BioSecurity Research Council (FBRC)
- ◆ Ministry of Agriculture and Forestry (MAF)

Summary

- ◆ Biological control of plant diseases (1987-2002)
- ◆ Discovery of effective bio-protection isolates research, mode of action
- ◆ Pilot-scale field/forestry trials (1989-)
- ◆ Large-scale field/forestry trials (1990's, 2001-, 2006)
- ◆ Nursery trials

Summary

- ◆ Commercial product development (2000-)
- ◆ Discovery of novel, superior bio-protectants
- ◆ New applications