Enhancing *Pinus radiata* health and vigour using beneficial microbes and natural products

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CORE project 1**: Bio-Protectant formulation for *Pinus radiata* wound protection against *Nectria fuckeliana*/flute canker.**

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Milestone : To develop a wound bio-protectant formulation which is effective against the disease Nectria Flute Canker

($25K excluding GST)

Microorganisms were isolated from (mainly pruning) wounds on Pinus radiata trees in South Island forests and screened for activity in laboratory assays. The most active microbes from these assays, together with the best antifungal microbes from the BioDiscovery collection, were fermented and formulated into three wound bio-protectant treatments for evaluation in a Tokoiti Forest pruned stub trial (established 28/29 June 2006). This trial, which includes the commercial fungicidal paste "Garrison", a Primaxa formulation and controls, will be monitored for 'flute canker' symptoms and the establishment of Nectria over the next 1 – 2 years.

Objectives: The key elements involved in this project were:-

- Collection of microbes from wound surfaces (mostly pruning wounds) of *P. radiata* trees growing in forests within the known range of Nectria Flute Canker
- Isolation of pure cultures
- Testing a representative selection of these microbes for potential activity to protect wounds in laboratory assays
- Selection of the best performing microbes
- Selection of the best microbes from the BioDiscovery collection of microorganisms
- Fermentation of the selected microbes
- Formulation into wound-protectant treatments
- Evaluation of the selected bio-protectant formulations in a pruned stub trial in a selected South Island forest site

Results and Discussion:

Over one thousand microbes were isolated from wound surfaces. These were screened in the laboratory for their potential antifungal activity (to protect wounds). The best of these microbes, together with the most active microbes in the BioDiscovery collection, were identified (Milestone 1).

A rapid and inexpensive fermentation system was developed to grow these selected microbes (Milestone 2).

Suitable wound-protectant formulations were prepared using the selected microbes (Milestone 3).

In collaboration with Ensis and City Forests Ltd, a suitable trial site was identified and a pruned stub trial was designed and installed in the Tokoiti Forest in June 2006 (Milestone 4).

All objectives and milestones for this project have been fully achieved.

The Tokoiti Forest trial will be monitored from 3 months onwards for symptoms of Nectria Flute Canker and the establishment and spread of *Nectria fuckeliana*. This ongoing trial will be funded partly through FRST (Bio-Protection Forestry Objective) and partly through FIDA funding (administered through the FBRC).

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The Forest Biosecurity Research Council (FBRC){funding support}
ENSIS {research collaboration}
City Forests Ltd {invaluable 'in kind' support; trial site selection, establishment}

CORE Project 2: Improved management of bark beetles in *Pinus radiata* plantations using a novel biocontrol fungal pathogen and attractants

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Milestone : Determine the best method to combine, and the effectiveness, of the fungus *Beauveria* and attractants as a potential control measure for *Hylastes*. 100% completed.
($20K excluding GST)

The fungi, *Beauveria* spp., are common pathogens of insects. Several isolates found in New Zealand are active against bark beetles (*Hylastes* and *Hylurgus* spp.), which are pests of pine. These fungi can be readily mass-produced on artificial substrates, and biopesticides based on isolates with activity against other pests have been developed overseas. We are assessing native strains of *B. caledonica* and *B. bassiana* for their use against bark beetles in re-stocking plantations.

In FRST funded work, field evaluation of selected *Beauveria* strains will determine the most appropriate method of delivery (e.g., seedling potting mix and/or soil inoculum around stumps). In the FBRC funded work, attractants are being tested with formulated spores in the laboratory to develop an effective combination for use in a 'lure and infect' delivery system for the biopesticide. Formulations must maintain spore viability over an extended period (shelf life) in storage and under field conditions, and be infective to beetles in the field.

Initial studies focused on developing a biopesticide "pellet" containing *B. caledonica* spores and attractants for *H. ater* and *H. ligniperda*. After lab investigations showed that the spores do not survive in direct contact with refined α- and β-pinenes or ethanol, ground pine cambium (natural source of α- and β-pinenes), a waste product of the wood processing industry, was used as an attractant. Considerable efforts were made to develop a range of biopolymer-cambium-*Beauveria* formulations using patentable technology. Spore viability over time was monitored and insect bioassays done using field-collected *H. ater* adults. Assays were also carried out using attractive pine billets coated with a biopolymer-*Beauveria* gel formulation. In all of the tests, insect mortality levels were low, suggesting that the formulations were ineffective. However, the spore loadings used in the formulations may also have been too low to effect high levels of insect infection. A new series of test formulations have subsequently been prepared, incorporating spores into a clay-based matrix containing cambium/ground bark (attractive pellet), granules (for soil application), and a biopolymer gel (for application to pine roots/billets), and an inert clay based matrix for use with external attractants. Spore survival was excellent in all of the formulations in the first replicate. These trials are currently being repeated and efficacy against beetles will be evaluated.

As well as developing a biopesticide pellet, a couple of low cost, simple to use, lure and infect traps have been designed that incorporate bark beetle attractants as well as the biopesticide pellet. The effectiveness of these traps to trap and kill beetles will be tested in the continuing programme.

CORE Project 3: Mycorrhizal colonisation of nursery *Pinus radiata* seedlings.

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Milestone: Identify the ectomycorrhiza species colonizing *Pinus radiata* seedlings in a commercial nursery

($5K excluding GST)

Ectomycorrhizal colonization of *Pinus radiata* seedlings is important for seedling establishment and health both in the nursery and in out-plantations. The ectomycorrhizal species colonizing *Pinus radiata* seedlings in a commercial nursery (FORENZA) were surveyed and the main species identify using molecular techniques. This will provide information for work within the Bio-Protection looking at the effect of *Trichoderma* bio-inoculants on ectomycorrhizal colonization of nursery seedlings.

Pinus radiata seedlings from the commercial nursery were collected and ectomycorrhizal roots grouped into morphological types by examining the roots microscopically for mantle colour and surface structure, and branching patterns. Three main morphological groups were identified. PCR grade DNA was extracted from representative samples of these main morphotypes and this used for ITS sequencing for species identification.
Wilcoxinia mikolae, *Inocybe* spp., *Thelephora* spp. and *Rhizopogon roseolus/rubescens* were identified as colonizing *Pinus radiata* roots in the nursery. These ECM species have been reported to colonise pine seedlings in forest nurseries in overseas studies.
This information will be used to investigate the effect of *Trichoderma* bio-inoculants on the colonization and diversity of ectomycorrhizal in *Pinus radiata* nursery system in the following year.

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