Ecological Studies of *Trichoderma* Bio-Inoculants in the Soil Ecosystem of *P. radiata*

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Summary

Three isolates of different *Trichoderma* species were selected for this study. *T. hamatum* (LU592) and *T. harzianum* (LU686), were known to stimulate growth and improve establishment of *P. radiata* seedlings, and T*. atroviride* (LU132) had no stimulatory activity. The ability of these isolates to establish and survive in bulk soil, the rhizosphere, rhizoplane or endorhizosphere of *P. radiata* seedlings was determined. In addition, the effect of each isolate on *P. radiata* seedling development and growth was assessed and is currently being confirmed in a large scale commercial experiment. From the nursery experiment, the *Trichoderma* isolate which was the most effective at colonising all *P. radiata* root subsystems (LU592) was selected for more detailed ecological studies using genetically marked strains. Current experiments are focussed on the spatial dynamics of *Trichoderma* within the rhizosphere of *P. radiata*. The implication of the results in relation to the use of *Trichoderma* bio-inoculants in the nursery will be discussed.

Introduction

The soil-borne fungus *Trichoderma* is well known as a biological control agent (BCA) active against a wide range of diseases of crops, including pine trees (Harman *et al.*, 2004; Mousseaux, 1998). Several isolates of *Trichoderma* have been shown to improve establishment and reduce pathogen infection of *Pinus radiata* in the nursery and in forestry plantations (Hood *et al.*, 2006). Based on this work, a commercial product, ArborGuard^{TM}, consisting of a mixture of six different *Trichoderma* isolates has been developed. However, to provide more predictable and effective bio-inoculants for use in soil ecosystems, it is essential to determine the establishment and population dynamics of introduced *Trichoderma* spp. in natural habitats (Lo *et al.*, 1998).

Methods

Nursery experiment:

Each *Trichoderma* isolate was applied either as a seed coat formulation (4 x 10^5 spores/ml) or a spore-suspension (5 x 10^6 spores/ml), sprayed directly after sowing the *P. radiata* seeds. *P. radiata* seeds were grown in root-pruning containers, maintained for 7 months and conditions reflected those used in the commercial PF Olsen nursery. Health and growth assessments were made including mortality rate, shoot height and shoot and root dry weight measurements. During the 7 month trial period, *Trichoderma* populations were enumerated in the bulk potting mix, rhizosphere, rhizoplane and endorhizosphere subsystems using the dilution plating method (McLean *et al.*, 2005). Recovered *Trichoderma* colonies were morphologically and molecularly characterised after 20 weeks to differentiate between introduced and indigenous species.

Transformation of *T. hamatum* LU592:

A fluorogenic marker gene, green fluorescent protein (GFP), and a Hygromycin B resistance gene were inserted into the genome of *Trichoderma* using *Agrobacterium*-mediated transformation. After mitotic stabilisation of the transformants by single-spore isolation, the transformants were compared to the wild type by measurement of the following physiological characteristics: mycelium growth rates on different media, sporulation behaviour and spore germination. Additionally, the soil-sandwich technique, as described by Wakelin *et al.* (1999), was used to compare mycelium growth rate in non-sterile soil conditions.

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Results and Discussion

T. hamatum LU592 performed the best out of the three introduced isolates. Mortality rate was reduced from 5.2% for the control to 0.2% for LU592 and 0.4% for *T. harzianum* LU686 when applied as a seed coat. LU592 and LU686 as a seed coat also increased shoot height by 17% and 11%, respectively. Results also indicated that *T. atroviride* LU132 increased the root/shoot ratio.

Trichoderma populations of all spray application treatments (SA) were significantly higher in the rhizosphere (by 2.1 to 3.3 times) compared to the control. *T. hamatum* LU592 was the only isolate clearly dominating all four subsystems top bulk, rhizosphere, rhizoplane and endorhizosphere.

This experiment verified the beneficial impacts of *Trichoderma* on *P. radiata* in a previous large-scale experiment and another large-scale experiment at the commercial PF Olsen nursery is currently being carried out to confirm the results for *T. hamatum* LU592 and obtain some additional information of the biological impact on the seedlings.

The spray application method clearly promoted the establishment of the introduced isolates and the *Trichoderma* population assessments demonstrated the general tendency of *Trichoderma* to be rhizosphere competent. The ability of *Trichoderma*, LU592 in particular, to penetrate the roots is a valuable attribute for a BCA. Even though the highest concentrations of LU592 were found in the rhizosphere when applied as a spray application, the best biological impacts were obtained as a seed coat with an isolate's proportion of 17% in the rhizosphere. These results suggest that the use of *T. hamatum* LU592 as a BCA will achieve best results when established in an optimal balance, rather than being predominant in the rhizosphere. Subsequent experiments including several pot and microcosm trials are focussing on the use of a fluorescent marked isolate of LU592 to examine the following aspects: (i) verification of rhizosphere competence, (ii) spatio-temporal distribution within the rhizosphere (iii) endophytic activity including interactions with ectomycorrhizae.

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