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Understanding *Trichoderma* in the root system of *Pinus radiata*: associations between rhizosphere colonisation and growth promotion for commercially grown seedlings

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ABSTRACT

Two *Trichoderma* isolates (*T. hamatum* LU592 and *T. atroviride* LU132) were tested for their ability to promote the growth and health of commercially grown *Pinus radiata* seedlings. The colonisation behaviour of the two isolates was investigated to relate rhizosphere competence and root penetration to subsequent effects on plant performance. *Trichoderma hamatum* LU592 was shown to enhance several plant health and growth parameters. The isolate significantly reduced seedling mortality by up to 29 %, and promoted the growth of shoots (e.g. height by up to 16 %) and roots (e.g. dry weight by up to 31 %). The introduction of LU592 as either seed coat or spray application equally improved seedling health and growth demonstrating the suitability of both application methods for pine nursery situations. However, clear differences in rhizosphere colonisation and root penetration between the two application methods highlighted the need for more research on the impact of inoculum densities. When spray-applied, LU592 was found to be the predominant *Trichoderma* strain in the plant root system, including bulk potting mix, rhizosphere and endorhizosphere. In contrast, *T. atroviride* LU132 was shown to colonise the root system poorly, and no biological impact on *P. radiata* seedlings was detected. This is the first report to demonstrate rhizosphere competence as a useful indicator for determining *Trichoderma* bio-inoculants for *P. radiata*. High indigenous *Trichoderma* populations with similar population dynamics to the introduced strains revealed the limitations of the dilution plating technique, but this constraint was alleviated to some extent by the use of techniques for morphological and molecular identification of the introduced isolates.

Introduction

Trichoderma spp. are known to form mutualistic relationships with plants (Harman *et al.* 2004). The ability of *Trichoderma* strains to proliferate and function in association with plant roots has been identified as one of the most important factors in determining their potential to control root pathogens (Lewis & Papavizas 1984; Ahmad & Baker 1987, 1988; Harman *et al.* 2004). However, the relationship between root colonisation by *Trichoderma* isolates and their enhancement of plant performance has had limited investigation. An effective bio-inoculant should preferably penetrate the roots to not

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only directly antagonise root pathogens, but also benefit plant growth and vigour through various mechanisms such as nutrient mobilisation and induction of host defences (Vinale *et al.* 2008). Several species of *Trichoderma* (including *T. hamatum*) have been shown to elicit such promoting effects on different perennial woody plants (including *Pinus radiata* and *Pinus sylvestris*) (Paderes *et al.* 2005; Adams *et al.* 2007; Grodnitskaya & Sorokin 2007). However, the distribution and the fate of these *Trichoderma* isolates in the root system were not examined and, to the best of our knowledge, there have been no reports on the association between *Trichoderma* root colonisation and plant growth performance for *P. radiata*.

In New Zealand, approximately 40 million *P. radiata* seedlings/cuttings were sold in 2009 with an estimated value of approximately US$12M (W.Y. Wang, pers. comm.). Seedlings/cuttings are either grown in a bare-root system where the plant is placed directly into soil in raised nursery beds or increasingly in a container-grown system which allows quicker production of planting stock and an extension of the planting season (Menzies *et al.* 2001). Since, tree stock sales are expected to increase 2- to 5-fold within the next 10 y. Even small improvements in plant growth and health can result in significant economic benefits.

In a previous screening assay conducted by members of the research team, a range of *Trichoderma* strains were evaluated for their ability to enhance growth of *P. radiata* seedlings. *Trichoderma hamatum* LU592 was shown to significantly enhance seedling growth, whereas *Trichoderma atroviride* LU132 (previously designated as C52), a highly successful bio-inoculant against *Sclerotium cepivorum* in onion and *Botrytis cinerea* in grapevine (McLean & Stewart 2000; McLean *et al.* 2008), showed little biological impact on *P. radiata* (GroChem NZ Ltd, commercial trial). These two *Trichoderma* isolates, with different effects on plant performance, were chosen for this study to identify distinctive colonisation patterns which may be related to biological activity. The two most common methods of applying bio-inoculants to tree nurseries are by seed coating or spray application. Seed coating is usually more cost effective due to the lower application rates required and has been shown to be an effective strategy to improve *Trichoderma* establishment in the spermosphere and suppress soil-borne pathogens such as *Pythium ultimum* and *Fusarium oxysporum* (Mousseaux *et al.* 1998; Bell *et al.* 2000). Spray application is also widely used due to its ability to introduce high concentrations of viable spores into the soil system.

The objective of this study was (i) to compare different soil and root colonisation patterns shown by two *Trichoderma* isolates and relate these to effects on plant performance, and (ii) to evaluate the effect of two different application methods on rhizosphere colonisation. This study focussed on the container-grown system, simulating the conditions of commercially grown *P. radiata* seedlings in root-pruning pots.

Materials and methods

Fungi and plants

Two *Trichoderma* isolates were used in this study. *Trichoderma atroviride* LU132 was originally isolated from soil, Pukekohe, Auckland, NZ in 1991 (previously designated as C52; McLean *et al.* 2005) and *Trichoderma hamatum* LU592 from soil, Christchurch, NZ in 1997 (Rabeendran *et al.* 1998). Cultures of LU132 and LU592 were stored at −80 °C in 25 % glycerol and routinely cultured on potato dextrose agar (PDA; Difco™ Laboratories, USA) at 20 °C in darkness.

Seeds of the *Pinus radiata* seedlines 283:539 × 875:242 and AO 880:692 × 268:539, obtained from PF Olsen Nursery Ltd (New Zealand), were used in this study. Seedline 283:539 × 875:242 was used for experiment 1 and AO 880:692 × 268:539 for experiments 2 and 3. The seeds were stratified prior to use to enhance germination. The seeds were soaked in sterile distilled water (SDW) for 1 d after which the excess water was drained off and the seeds were then stored in a closed container for 1 month at 0–2 °C before sowing.

Growth conditions

Plants were grown in potting mixture consisting of 50 % composted pine bark, 25 % peat and 25 % pumice with the addition of 12.5 kg m^{-3} slow-release fertiliser (1.5 kg m^{-3} calcium magnesium carbonate, 2 kg m^{-3} calcium sulphate dihydrate coarse, 2 kg m^{-3} calcium sulphate dihydrate fine, 1 kg m^{-3} hydraflo II G wetting agent, 6 kg m^{-3} osmocote [N:P:K:Mg 15:4:9:1.5]). For experiments 1 and 2, seedlings were grown outside during the emergence period and irrigated at a rate of 200 mL m^{-2} every 2 h between 6 am and 6 pm. The growing containers were covered with a mesh to protect seeds from birds. After 6 weeks, seedlings were irrigated once per day at a rate of 3 L m^{-2}. A biological fertiliser (Peters Plant Starter; Scotts, The Netherlands) was added to the water at an adjusted electrical conductivity of 1.1–1.2 siemens m^{-1}. The herbicide Valzine500 (425 g L^{-1} terbuthylazine and 75 g L^{-1} hexazinone; AgPro NZ Ltd, New Zealand) was added once per month to the water at a concentration of 1 L ha^{-1} to reduce weed competition. After 6 m, shoot tips were cut to maintain a favourable root/shoot ratio following commercial practice. For experiment 3, the seedlings were first grown in the glasshouse (average $T_{max} = 25$ °C (day), average $T_{min} = 16$ °C (night); 60 % shading effect) for 6 weeks, before being transferred to an open area. Half a teaspoon of the solid fertiliser Osmoform® (Scotts, The Netherlands) was added to each cell (see experimental procedure below) after 10 and 20 weeks. An automatic irrigation system, consisting of six spray nozzles, was set up during the glasshouse period at a rate of 50 mL m^{-2} every hour between 8 am and 5 pm and 17 mL m^{-2} every hour between 5 pm and 8 am. After transfer from the glasshouse, seedlings were irrigated once per day at a rate of 3 L m^{-2}.

Inoculum preparation and application

Both *Trichoderma* isolates were applied either as a seed coat preparation or spore suspension sprayed directly after sowing of the *Pinus radiata* seeds into the potting mixture. Concentrations for both application methods were based on optima determined in previous *P. radiata* pot experiments (seed coat at 4×10^5 spores $seed^{-1}$ and spray application at 5×10^6 spores $cell^{-1}$; GroChem NZ Ltd, commercial trial). Spore suspensions were prepared as described in Rabeendran *et al.* (2006). Spore suspensions of both isolates were prepared by