From Forest Nursery Notes Winter 2013

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Forest Pathology WILEY-BLACKWELL

For. Path. 42 (2012) 75–78 doi: 10.1111/j.1439-0329.2010.00710.x

SHORT COMMUNICATION

Trichoderma atroviride promotes growth and enhances systemic resistance to *Diplodia pinea* in radiata pine (*Pinus radiata*) seedlings

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Summary

Root drench application of *Trichoderma atroviride* isolates R32, R33, R40 and R84 promoted the growth of potted radiata pine seedlings. After 6 weeks, seedlings treated with R33 and R84 had thicker stems and greater stem and root biomass ($p < 0.05$) than untreated controls. Treatment with R32 increased seedling root biomass whilst R40 increased stem diameter. None of the isolates affected seedling height. One isolate, R33, induced systemic resistance to stem inoculation with *Diplodia pinea* and reduced dieback incidence by 20% compared with untreated controls. To our knowledge, this is the first report of systemic induced resistance by *Trichoderma* in a pine species. Furthermore, seedlings that were treated with R33 (root drench) plus foliar application of methyl jasmonate (MeJA) expressed elevated peroxidase activity in their stems 2 weeks later, compared with seedlings treated only with MeJA. Because R33 itself did not affect peroxidase activity, this may be indicative of treatment synergy or defence potentiation by R33. Curiously, R33 + MeJA induced terpenoids but suppressed phenylalanine ammonia-lyase activity suggesting possible trade-offs between phenolic and terpenoid defence pathways in the treated seedlings.

1 Introduction

Trichoderma spp. are ubiquitous soil fungi and have been widely studied for their ability to promote plant growth and to suppress plant disease, with some species being marketed as biopesticides, biofertilizers and soil amendments (Harman 2006). The suppression of plant disease by *Trichoderma* spp. results from direct antagonism whereby pathogenic fungi are displaced from the rhizosphere via competition, antibiosis and parasitism and indirectly by the enhancement of host resistance to pathogenic attack (Shoresh et al. 2005; Harman 2006). Whilst pathogen suppression can increase plant productivity, it has also been shown that *Trichoderma* spp. can actively enhance root growth and stimulate the uptake of nutrients thereby directly promoting plant growth (Harman 2006; Contreras-Cornejo et al. 2009).

A few studies have investigated the use of *Trichoderma* spp. in forestry crops. Amendment of growth media with *Trichoderma harzianum* has been shown to suppress damping-off caused by various soilborne fungi including, *Phytophthora cinnamomi* on shortleaf pine (*Pinus echinata* Mill) (Kelley 1976), *Fusarium oxysporum* in Douglas fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco) (Mousseaux et al. 1998) and *Sclerotium rolfsii* Sacc. in tropical pine (*Pinus merkusii* Jung & De Vriese) (Widyastuti et al. 2003). In field studies, seed coat application of *T. harzianum* and *T. atroviride* (Arbor-Guard™; P.F Olsen and Company Ltd., Rotorua, New Zealand) enhanced the establishment and survival of containerized *Pinus radiata* D. Don (radiata pine) (Hill et al. 2007). Spray application of *T. harzianum* and *T. polysporum* (BinabT®; Binab Bio-innovation AB, Helsingborg, Sweden) reduced grey mould incidence (*Botrytis cinerea*) on needles of containerized Scots pine (*Pinus sylvestris* L.) (Capieau et al. 2004).

In this study, four isolates of *T. atroviride* were evaluated for their effects on the growth of radiata pine seedlings and for their potential to induce systemic resistance against *Diplodia pinea* (Desm.) Kickx. This pathogen typically infects via wounds and causes shoot blight, crown wilt, canker and sap stain on a wide range of conifer species throughout the world and can cause mortality in young seedlings.

2. Materials and methods

2.1 Growth assays

Radiata pine seed (GF16; Olsen Seeds, PF Olsen and Company Ltd., Rotorua, New Zealand) was germinated in trays with vermiculite and then individually transplanted after 4 weeks into 0.5 l pots containing unsterilized bark-based potting mixture. Seed germination and growth was performed in controlled environment rooms maintained at 21°C with a 16 h light/8 h dark cycle. *Trichoderma atroviride* isolates R32, R33, R40 and R84 were obtained from the Bio-Protection Research Centre, Lincoln University, New Zealand. The isolates were stored in glycerol at −80°C, plated on potato dextrose agar (PDA; Difco, Becton, Dickinson and Company, Sparks, MD, USA) and incubated for 1 week at 20°C in the dark before use. Spore suspensions of each *T. atroviride* isolate were prepared by flooding fresh cultures with 0.01% sterile Tween 80 and scraping the spores with a sterile scraper. The suspensions were filtered through a 100-μm nylon cell strainer (BD Biosciences, Sparks, MD, USA) and then diluted to a concentration of 5×10^6 spores ml^{-1} with the aid of a haemocytometer. The suspensions were then applied as a root drench around the base of the stems of 4-month-old *P. radiata* seedlings (5×10^7 spores per pot). There

Received: 15.7.2010; accepted: 17.12.2010; editor: J. Roux