Extended Abstract

Bio-inoculant formulations for enhanced seedling emergence and pasture growth

D.R.W. KANDULA[1], A. STEWART[1], J. MCDERMID[2], D. GALE[2] and J. SWAMINATHAN[3]
[1]*Bio-Protection Research Centre, PO Box 84, Lincoln University, Lincoln 7647, New Zealand*
[2]*Agrimm Technologies Limited, PO Box 35, Lincoln, New Zealand*
[3]*AgResearch Limited, PO Box 60, Lincoln 7647, Canterbury, New Zealand*
Wadia.kandula@lincoln.ac.nz

Abstract
Soil-borne damping-off diseases are a major constraint limiting pasture seedling emergence and yield. Large scale laboratory and glasshouse screening of beneficial micro-organisms against several host/pathogen systems was conducted to identify efficient bio-control and growth-promoting microbes. This was followed by field-testing of the best microbes and their formulations for efficient application techniques. The research identified a number of fungal (*Trichoderma* spp.) and bacterial (*Paenibacillus* spp.) isolates which suppressed damping-off diseases and promoted plant growth of various pasture species. Preliminary field-trials with granule and seed-coat formulations confirmed the glasshouse results. Intensive research trials with a mix of four *T. atroviride* isolates, providing growth promotion and disease control activity, led to the development of a prototype pasture seed additive product.
Keywords: damping-off diseases, *Paenibacillus* spp., seed-coating, *Trichoderma* spp.

Introduction

Pastures in New Zealand are under severe pressure as production intensifies and, with this, the impacts of pests and diseases are growing. Demand for increased and sustainable pasture production necessitates the need to identify environmentally acceptable crop-protection methods to overcome these constraints. As part of a larger programme focused on developing new systems for biologically-based pest and disease suppression in pastoral ecosystems, we have investigated the potential for beneficial micro-organisms and their formulations to improve plant persistence and/or reduce soil-borne diseases. This paper summarises the results of laboratory, glasshouse and field trials conducted over a 4-year-period.

Methods and Results

In vitro and glasshouse trials

Preliminary screening of isolates of *Trichoderma* spp. and spore forming bacteria in an *in vitro* dual inoculation agar plate assay and mycelial inhibition assay respectively, identified ten *Trichoderma* isolates (five of *T. atroviride*; two of *T. virens*; and one each of *T. koningii*, *T. hamatum*, and *T. viride*) and four *Paenibacillus* isolates (three of *P. polymyxa*; and one of *P. macerans*) with suppressive activity against four soil-borne pathogens (*Rhizoctonia solani*, *Sclerotinia trifoliorum, Fusarium culmorum* and *Pythium ultimum*). Selected isolates and their formulations were then tested in glasshouse pot trials for growth promotion of perennial ryegrass, red and white clover and for disease control activity in three host-pathogen systems (perennial ryegrass/*R. solani*, red clover/*S. trifoliorum*, white clover/*P. ultimum*). In preliminary trials, pathogen inoculum levels and methods of inoculation were established which gave approximately 50% disease levels at trial completion. Seedling emergence

was recorded at 3 weeks and shoot and root dry weights and disease severity were recorded after 7 weeks. Initial pot trials were conducted using potting mix and then selected treatments were re-tested using pasture soil. Two isolates of *T. atroviride* gave significant growth promotion (>100% increases in shoot and root dry weight) of perennial ryegrass and also gave significant growth promotion of red clover (>40% increase in shoot dry weight). One isolate of *T. atroviride* gave the strongest biocontrol activity against *Rhizoctonia solani* on perennial ryegrass with a 14% increase in seedling emergence and >60% increase in both shoot and root dry weight compared to the control. Another isolate of *T. atroviride* was the best performing strain against *Sclerotinia trifoliorum* on red clover, while one isolate of *T. hamatum* gave greatest control of *Pythium ultimum* on white clover. The *T. atroviride* isolates that provided the growth promotion effects were not the same as the isolates which provided bio-control activity against the pathogens. In the bacterial treatments, two isolates of *P. polymyxa* and one isolate of *P. macerans* significantly increased seedling emergence by 20-30% compared to the pathogen control for both *R. solani* and *S. trifoliorum*. All bacterial treatments had consistently lower disease incidence with all the three pathogens with minor variations in seed-coat and granular formulations.

Field trials

Small scale field trials (1-8 m^2 plot sizes) were conducted at Lincoln University to test seed coating (10^7 cfu/g seed) and granule (10^8 cfu/g) formulations of each *Trichoderma* and *Paenibacillus* isolate. Two isolates of *T. atroviride* performed consistently well in all trials with 10-15% increases in seedling emergence and 20-30% increases in shoot dry weight for both perennial ryegrass and mixed ryegrass/clover pastures. On the basis of the results of the glasshouse and field trials, four isolates of *T. atroviride* were selected to form a mixed isolate prototype product with both growth promotion and disease control activity for expanded field testing with mixed pastures over the 2006-08 seasons. The product, formulated as a prill by Agrimm Technologies Ltd, was mixed with pasture seeds and applied using standard seed-drilling equipment at a rate of 25-30 kg/ha. Results showed that pasture seed additive application increased seedling emergence significantly by 37% compared to the control and dry matter (kg/ha) increases of 8.8-18.5% were recorded for the first 6 pre-grazing yield assessments (covering a period of 7 months from sowing) in the first spring sown trial. In the second spring sown trial, no increase in emergence was observed but similar increases in dry matter (up to 18.7%) were recorded over the five assessment times. In an autumn sown trial with annual ryegrass, increased seedling emergence of 23% was recorded and pre-grazing yield assessments for the first three months have revealed increases of 8-30% in pasture dry matter compared to the control. Increased dry matter (4-11%) in PSA treated plots compared to the control was observed in some locations even for the second year.

Discussion

Trial results to date have shown significant establishment and yield benefits in pastures after application of *Trichoderma* prill to the soil. It is postulated that the benefits arise from a combination of plant growth promotion and disease biocontrol but mechanism of action studies are required to validate this. Trials are also underway to test the efficacy of the bio-inoculants applied as a seed-coat. Large scale testing under different pastoral zones and improved understanding of this biological control product will result in enhanced seedling emergence and increased pasture growth, providing farmers with an additional management tool for sustainable pasture production.