Microbial inoculation of seed - issues and opportunities

M. O'Callaghan, D. Wright, J. Swaminathan, S. Young and P. Wessman
AgResearch Limited, Private Bag 4749, Christchurch 8140, New Zealand

Abstract

Beneficial rhizosphere microorganisms are viable alternatives to the use of chemical pesticides and fertilisers in agriculture. Microbial inoculation of seeds is an ideal mechanism for delivery of high densities of beneficial microorganisms to soil, where they can colonise emerging plant roots. However, the use of seed microbial inoculants has been limited by technical challenges in maintaining high numbers of functional microorganisms on seed during seed treatment and storage. Increased understanding of the interactions between microorganisms, formulation components and the seed is essential in development of new seed inoculant products. There are significant opportunities for seed inoculants in New Zealand agriculture, in particular where conventional agricultural practice cannot be used, such as organic seed production.

Additional keywords: formulation, shelf-life, beneficial microorganisms, biopesticide, rhizosphere

Introduction

Beneficial rhizosphere microorganisms that act as biological control agents and/or increase growth and nutrient uptake by plants are viable alternatives to the use of chemical pesticides and fertilisers in agriculture and horticulture. Interest in the use of microbes for control of plant insect pests and diseases has increased in response to concerns about the environmental impacts and likely withdrawal of some pesticides from the market in the future (Glare *et al*., 2012). There is also renewed interest in plant growth-promoting microorganisms with the commercial market for these inoculants increasing by 10% each year (Berg, 2009).

Microbial inoculation of seed is an ideal mechanism for delivery of high densities of microorganisms to the soil, where they are well positioned to colonise emerging plant roots. Application of beneficial microorganisms to seed is not a new idea; the inoculation of legume seed with nitrogen-fixing bacteria has a long history and underpinned the development of pastoral agriculture in New Zealand. The aim of legume seed inoculation is to maximise legume yield potential by providing high numbers of viable effective rhizobia to the rhizosphere to allow rapid colonization, nodulation and nitrogen fixation by a selected inoculant strain (Deaker *et al*., 2012). Other microorganisms also promote plant nutrient acquisition and some seed inoculant products for improved plant uptake of phosphorus are commercially available (e.g. Rizophos® for maize (Rizobacter, 2009)).

However, despite clear laboratory demonstration of the potential of beneficial microorganisms to improve plant performance there are still relatively few examples of commercially available microbial seed inoculants, largely because of the difficulty of maintaining viability of the microorganisms during seed treatment and storage. To avoid issues with instability of inoculants on stored seed, many companies provide the inoculant formulated for application to seed immediately prior to sowing. For example, the biofertiliser fungus *Penicillium bilaii* (JumpStart®, Novozymes, 2010a) is applied to seeds within 24 hours of sowing.

Key challenges in application of inoculants to seed

The seed as a substrate

While seed is an efficient delivery vehicle for the targeted application of microbes to soil, it is a very complex substrate. Water relationships between the seed and coatings are poorly understood. Seeds are generally stored under dry conditions and even small increases in moisture content can cause increased respiration, and consequently heat, resulting in reduced longevity. Many microbial inoculants require some hydration to remain viable and this may be detrimental to the seed during storage. Other physical constraints include the nature of the seed coat and size of seed with small seeds able to carry only a limited cell loading. This is further complicated by natural seed microflora that may compete for space on the seed surface. Some legume seeds produce inhibitory exudates that can limit survival of inoculants on seed (Deaker *et al*., 2004).

Compatibility with current seed treatment processes

Modern seed coating plants utilise relatively small batch sizes combined with rapid processing. A rapid drying process (often at high temperature) is generally needed to maintain economically acceptable throughput rates. While many beneficial microbes can tolerate elevated temperatures (<40°C) for short periods, the rapid desiccation that occurs in commercial practice is a significant challenge to maintaining viability of inoculants on seed. The compatibility of micro-organisms with other seed coat components, particularly fungicides and insecticides, must be also assessed.

Shelf life

There have been few published studies on the survival of microorganisms on seeds during storage, despite adequate shelf life being a critical factor in the commercial success of inoculant products. The mechanisms behind cell death during storage are poorly understood. Unsurprisingly, survival of microorganisms on seed is improved at low temperatures (e.g. O'Callaghan *et al*., 2006), as is seed longevity, but this is rarely commercially feasible. Improving the survival of microorganisms on seeds can involve a trade-off between conditions favouring high initial loadings and those favouring long term stability. Drying of lucerne seeds after coating significantly reduced viable rhizobia, but higher numbers were maintained on dried seed during storage (Deaker *et al*., 2012).

Overcoming the challenges

Strain selection

In the first instance, selection of microorganisms with natural tolerance to environmental stresses is a reasonable strategy, thus spore-forming microorganisms are considered ideal candidates as seed inoculants. A formulated concentrate of the spore-forming bacterium *Bacillus subtilis* has been used widely in the US as a seed inoculant to protect cotton from soil-borne plant pathogens (Brannen and Kenney, 1997). Marketed as Kodiak®, the bacterium was readily amenable to seed application by techniques that differed little from standard seed coating processes. However, there are numerous beneficial non spore-forming bacteria (e.g. *Pseudomonas* spp.) that are more sensitive to environmental stresses and hence less amenable to conventional seed treatment processes. There is potential to exploit natural variation between the strains of the same species, for example, the most commonly used rhizobial inoculants for white clover *Rhizobium leguminosarum* biovar *trifolii* strains TA1 and CC275e differ in their ability to tolerate environmental stresses (Lowther and Kerr, 2011). Non spore-forming microorganisms can also be protected through appropriate formulation.

Formulation

A formulation includes: the active ingredient, here beneficial microorganisms; the seed; and any additional ingredients (e.g. protective compounds, desiccants, binders, coatings). The basic functions of formulation are to:

(1) stabilise the microorganism,
(2) aid in its delivery,
(3) protect the microbe at the target zone, and
(4) enhance the functionality of the microbe against the target.

While the importance of formulation is well recognized, formulations have often been developed without fundamental understanding of the complex interactions between formulation components and cell physiology. Numerous methods have been used for seed inoculation with various degrees of success (McQuilken *et al*., 1998). However, many of the techniques are unsuitable for scale-up to commercial application for various reasons including cost of materials (particularly for high volume, low margin seed) and technical difficulties in scale-up, such as viscosity of formulation components. These factors need to be considered early when developing a new seed inoculant product.

Details of formulations are generally commercially sensitive and not disclosed but product claims by some new inoculant products suggest that companies have been successful in stabilising inoculants to levels not previously possible. For example, rhizobial inoculant Nitragin gold® (Novozymes, 2010b) is reported to survive on clover seed for 6-12 months. Cedomon® and Cerall® (Lantmännen BioAgri AB, 2007) are registered for control of several seed-borne diseases on cereals. The active ingredient in the products is the naturally occurring soil-dwelling bacterium *Pseudomonas chlororaphis* which is formulated in oil (Cedomon® for barley and oats) or water (Cerall® for wheat). The treated seeds can be stored for up to one year under ambient conditions, and large amounts of Swedish cereal seed are treated with the inoculant each year.

Opportunities for microbial inoculation of seeds in New Zealand

Current chemical seed treatments are typically aimed at aiding seedling establishment, but the colonisation of the rhizosphere by beneficial microbes applied as seed treatments may potentially provide benefits to the plant well beyond the seedling emergence stage. With further development, microbial seed inoculation has potential to add value to many seed treatments in New Zealand.

Inoculation of legumes

There is a long history of legume seed inoculation, and factors affecting rhizobial survival on seed have been reviewed extensively (Deaker *et al*., 2004; 2012). However, the seed coating industry still faces problems with poor survival of rhizobial inoculants, particularly on clover seed; surveys in Australia indicate that rhizobial loadings on seed rarely satisfy numerical standards (Gemell *et al*., 2005; Deaker *et al*., 2012). Novel solutions are needed to address this well recognised problem.

Improved plant establishment and growth

Many rhizophere microorganisms have demonstrated potential as seed inoculants for New Zealand pasture species. Selected bacterial strains were shown to counteract the effects of root parasitic nematodes during establishment of white clover in pot experiments, resulting in seedling dry weight increases of up to 30% (Sarathchandra *et al*., 2010), with some isolates causing reductions in root cyst nematode populations. Biopriming has been used to inoculate tall fescue seeds with a range of beneficial bacteria (Monk *et al*., 2009), resulting in significant increases in dry matter production. Seed treatments based on *Trichoderma* spp. are available elsewhere for corn, wheat and soybeans (e.g. SabrEx™, Advanced Biological Marketing, 2010) and could be developed for selected crops in New Zealand.

Protection from insect feeding

Soil dwelling insect pests and diseases are difficult to control by any means. Seed treatment is useful way of introducing microbial control agents into the root zone and increasing the likelihood of contact with root pathogens or root-feeding pests. Treatment of seeds with the non spore-forming bacterial entomopathogen *Serratia entomophila* controlled the New Zealand grass grub (*Costelytra zealandica*) as effectively as chemical controls for wheat and ryegrass in pot trials (Young *et al*., 2009; 2010). Insect pathogens such *S. entomophila* establish within the pest population and often lead to longer term suppression of the pest population than is typically achieved using chemical pesticides.

Plant establishment in challenging environments

Tussock grasslands in New Zealand are typically devoid of rhizobia effective on pastoral legumes and effective seed inoculation with rhizobia is essential for nodulation of legumes sown in these environments. In addition, legume establishment in hill country and tussock grassland can be improved by treatment of seed with insecticides (Barratt *et al*., 1995). New Zealand pioneered the aerial delivery of coated clover containing lime and rhizobia to rugged locations in the 1950s and there is a clear need to develop this technology further for a range of pasture species as increasingly more challenging land is brought under cultivation by sheep and beef farmers and issues with poor pasture establishment are common.

Organic production

There is considerable interest among vegetable seed companies, both worldwide and in New Zealand, in producing certified organic seed. Canterbury is a globally significant area for seed production but growers have suffered significant crop losses through soil-borne fungal diseases of seedlings and feeding damage caused by *C. zealandica.* Experimental seed coatings with *S. entomophila* significantly reduced damage from *C. zealandica* to emerging carrot seedlings in pot trials (Wright *et al*., 2005).

Future directions

The combination of biological and chemical controls may increase reliability of crop protection under conditions that are not optimal for performance of the biological control agent alone and where chemical seed treatments do not give prolonged protection from pests. The nematicidal properties of *Bacillus firmus* has been combined with the insecticide clothianidin in the seed treatment product Poncho®/VOTiVO® for corn and soyabean (Bayer CropScience, 2012). The combination of microbial inoculants with soft pesticides, such as insect growth regulators, is an attractive option, as is the use of co-inoculants with different modes of action or target pests.

Many microbial inoculants currently available commercially are applied to seed just prior to sowing and adoption of this practice may facilitate rapid uptake of seed inoculants in New Zealand in the short term and provide options for growers in niche markets such as organic seed producers. For full commercial success and uptake, growers would need to be able to purchase inoculant-treated seed with similar shelf life properties and costs as conventionally treated seed. Long term survival of inoculants on seed will rely on identification of formulation and storage conditions that may be specific to particular microorganisms and seeds. To take full advantage of beneficial microorganisms as seed inoculants, greater understanding of the interactions between seeds, microorganisms and the formulation components are needed.

References

Advanced Biological Marketing. 2010. Treat Your Corn. Retrieved on 15 June 2012 from http://www.treatyourcorn.com

Barratt, B.I.P., Lowther, W.L. and Ferguson, C.M. 1995. Seed coating with insecticide to improve oversown white clover (*Trifolium repens* L.) establishment in tussock grassland. *New Zealand Journal of Agricultural Research* 38: 511-518.

Bayer CropScience. 2012. Retrieved on 15 June 2012 from http://www.bayercropscience.us/products/seed-treatments/poncho-votivo/

Berg, G. 2009. Plant-microbe interactions promoting plant growth and health: Perspectives for controlled use of microorganisms in agriculture. *Applied Microbiology and Biotechnology* 84: 11-18.

Brannen, P.M. and Kenney, D.S. 1997. Kodiak[R] - a successful biological-control product for suppression of soil-borne plant pathogens of cotton. *Journal of Industrial Microbiology and Biotechnology* 19: 169-171.

Deaker, R., Roughly, R.J. and Kennedy I.R. 2004. Legume seed inoculation technology - a review. *Soil Biology and Biochemistry* 36: 1275-1288.

Deaker, R., Hartley, E. and Gemell, G. 2012. Conditions affecting shelf-life of inoculated seed. *Agriculture* 2: 38-51.

Gemell, L.G., Hartley, E.J. and Herridge, D.F. 2005. Point-of-sale evaluation of preinoculated and custom-inoculated pasture legume seed. *Australian Journal of Experimental Agriculture* 45: 161-169.

Glare, T., Caradus, J., Gelernter, W., Jackson, T., Keyhani, N., Kohl, J., Marrone, P., Morin, L. and Stewart, A. 2012. Have biopesticides come of age? *Trends in Biotechnology* 30: 250-258.

Lantmännen Bioagri AB. 2007. Retrieved on 5 June from http://www.bioagri.se/index_eng.html

Lowther, W.L. and Kerr, G.A. 2011. White clover seed inoculation and coating in New Zealand. *Proceedings of the New Zealand Grassland Association* 73: 93-102.

McQuilken, M.P., Halmer, P. and Rhodes, D.J. 1998. Application of microorganisms to seeds. pp. 255-285. *In*: Formulation of microbial biopesticides: beneficial microorganisms, nematodes, and seed treatments. Ed. Burges, H.D. Kluwer Academic Publishers, Dordrecht.

Monk, J., Gerard, E., Young, S., Widdup, K. and O'Callaghan, M. 2009. Isolation and identification of plant growth-promoting bacteria associated with tall fescue. *Proceedings of the New Zealand Grassland Association* 71: 211-216.

Novozymes. 2010a. Retrieved on 5 June 2012 from http://www.bioag.novozymes.com/en/products/canada/wcanada/JumpStart/Pages/default.aspx

Novozymes. 2010b. Retrieved on 5 June 2012 from http://www.bioag.novozymes.com/en/products/canada/wcanada/nitragin-gold/Pages/default.aspx

O'Callaghan, M., Swaminathan, J., Lottmann J. and Wright D. 2006. Seed coating with biocontrol strain *Pseudomonas fluorescens* F113. *New Zealand Plant Protection* 59: 80-85.

Rizobacter, Argentina, S.A. 2009. Retrieved on 26 May 2012 from http://www.rizobacter.com.ar/biofertilizers.html

Sarathchandra, S.U., Bell, N.L., Burch, G., Aalders, L.T. and Eden T.M. 2010. Beneficial bacteria for improving white clover establishment. *New Zealand Plant Protection* 63: 285.

Wright, D.A., Swaminathan, J., Blaser, M. and Jackson, T.A. 2005. Carrot seed coating with bacteria for seedling protection from grass grub damage. *New Zealand Plant Protection* 58: 229-233.

Young, S.D., Townsend, R.J. and O'Callaghan, M. 2009. Bacterial entomopathogens improve cereal establishment in the presence of grass grub larvae. *New Zealand Plant Protection* 62: 1-6.

Young, S.D., Townsend, R.J., Swaminathan, J. and O'Callaghan M. 2010. *Serratia entomophila*-coated seed to improve ryegrass establishment in the presence of grass grubs. *New Zealand Plant Protection* 63: 229-234.