# The role of seed coatings in enhancing rhizobium colonisation and yield increases in pulse crops in the northern Mallee of South Australia

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**Abstract**
The colonisation of pulse crops by rhizobia in the northern mallee of South Australia is at times highly variable and in many cases inadequate for optimum plant growth. The aim of this work was to collate recent research publications to develop a seed coating that would enhance colonisation of seed coated rhizobium onto roots in low rainfall cropping regions such as the northern Mallee of South Australia. The coating of chickpeas, peas and lentils in this trial based out of Loxton with a product based on kelp, zinc, manganese, molybdenum and bacterial suspensions (Foundation TN) at 5L per ton of seed had significant benefits in plant growth and development. There was also a visual reduction in the incidence of root disease in treated plants. Statistically significant yield results were seen with Lentils (614kg/ha control to 677kg/ha coated), Field peas (729kg/ha control to 911kg/ha coated). Increases in Chickpeas were not significant (602 to 640kg/ha) but this may have been as a result of the lower seeding rate and severe frosts at flowering. Plants that had coated seeds in conjunction with rhizobia had greater numbers of efficient colonies and reduced root pathogens suggesting that good colonisation by rhizobium suppresses pathogenic infection points. Trial results over recent years have suggested that appropriate seed coats that enhance root colonisation by rhizobium are highly cost effective and in maximising the symbiotic relationship between rhizobium and the host species.

**Key Words**
Inoculation, zinc, manganese, nitrogen fixation

## Introduction

The northern Mallee of South Australia is associated with alkaline soils and generally low rainfall in most seasons (<250mm/pa). The region has traditionally been associated with cereal production but in recent years increased plantings of canola have become widespread. The low rainfall environment and high cost of growing canola has seen plantings drop dramatically in recent years. The move to plant pulse crops was initially seen as a means to increase soil nitrogen, but profitability and success of many pulse crops in this region has seen them become a more important part of crop rotations in this region. As the planting to pulse crops has increased the importance of rhizobium inoculants and the importance of good colonization in an environment where field observations show variable degrees of colonization has taken on increased significance.. This work has focused on enhancing colonization of rhizobium and the implications for crop morphology when rhizobium colonization and effectiveness, is enhanced occurs.

The importance of legumes in crop rotations was noted by the early Romans in maintaining soil fertility and more recently in the 19$^{th}$ century. Stainer (1978) writes that the infection of the root hair resembles the infection of the plant by pathogenic bacteria in that there is an initial destruction of the root hair, followed by penetration and proliferation of the bacteria then abnormal growth of the surrounding with the rhiozbia fixing nitrogen for plant growth and development (Burges 1998).

Burges (1998) notes that while peat is the most commonly used carrier for rhizobia other alternatives exist that can be considered. Jung et al(1982) noted on their work on inoculant carriers that polyacrylamide, alginate and gums were also suitable. The importance of carriers to enhance rhizobia colonisation of root hairs highlights the need to consider a range of carriers that may suit certain environmental conditions than others. Alginates can be seen to be useful as carriers and polymerises with alginic acid a useful sticker spreader. Burges (1998) notes, that many microbes retain greater viability and the potential to use fungicides and fertilisers with alginate formulations without affecting spore production in this case *Aspergillus flavus* with Dichloran after 2 years storage at 8 C.

Velnai et al (2014) researched relationships between yeasts and strains of rhizobia in producing a novel fertiliser that resulted in increased vegetative growth over control plants. In this research they used activated carbon as the carrier. It was this work in conjunction with the role of alginates that suggested that a high carbon alginate source would be worth considering in the development of any formulation for coating pulse crops.

In the development of the formulation used in this experiment it was aimed to access research across a number of fields to put together a formulation that captured a number of publications and establish a regional protocol that would maximise rhizobia colonisation onto pulse crops. Soils of South Australia are generally highly zinc and manganese responsive and as such the importance of adding zinc and manganese into the formulation was regarded as critical to early plant growth. The use of Seasol (tn liquid kelp extract) as a source of alginate for enhancing rhizobia during the seeding phase, in conjunction with molybdenum in the form of a lignate chelated product. The aim of using Foundation (tn) which contains a mixture of bacillus spores from *Bacillus pmilus, megaterium* and *licheniformis* was supported by some of the published literature. Hoult and Tuxford (1991) noted that strains of *Bacillus pumilus* had some cytopathic effects which could play a role in disease suppression. *Bacillus megaterium* has been reported to play a role in degradation of enzymes (Brock 1974) and *Bacillus licheniformis* has been seen to be effective against many types of fungi (Burges (1998).

This project has involved trial work which originally commenced in the northern South Australian Mallee in 2003. Overtime as new understandings and results have come to light the importance of creating an environment that enhances rhizobia root colonisation has become apparent. This has led to field experiments that focus on enhancing survival of rhizobia in low rainfall alkaline environments and also ensuring rapid root growth and development in germination.

## Methods

*Rhizobia*
Seed for all varieties was all treated with peat based inoculums as recommended for the pulse variety supplied by BASF. Peas were coated with E, chickpeas F and lentils with F inoculums at recommended label rates. The aim of the experiment was to compare the impact of the coating product in conjunction with recommended rhizobia colonization.

*Seed Coating*
Trials plots of chickpeas, lentils and peas were treated with Rhizobia as recommended coated with a product formulated with 3% Foundation (tn), 2.4% zinc as zinc sulphate, 2.2% manganese as manganese sulphate, 0.08% Molybdenum as SJB Lig Moly (tn) and 30% liquid kelp (Seasol tn). Product was applied at 5L per ton of seed to all varieties in conjunction with the recommended rhizobia for each variety.

The trial site was located at the Loxton property of Bulla Burra and ran the plots in full paddock strips with alternating plots all sown with rhizobia but with and without seed coating additives.

Details of sowing depth, varieties as noted in Table 1. Different sowing equipment was used due to seed size variance.

**Table 1. Sowing Date and Crop Details**

| Sowing Date | Varieties | Sowing Equipment | Trial Strips |
|---|---|---|---|
| 9-11 May 2015 | Blitz Lentils | 42' John Deere | Eight (fp and cp) to 12 replicates |
| | Twilight Peas Striker Chickpeas | Conserva Park Seeder | |

## Results

Seasonal rainfall conditions were 40mm less than the long term average for April-October and 67mm less for September October. Severe frosts during the late August early September period were also seen to influence crop yields across many areas within the district.

Lentils and peas were harvested on the 28/10/15 and Chickpeas on the 21/11/15.

**Table 2: Compiled yield data across varieties Bulla Burra Field Site Loxton South Australia, yield in kg/ha**

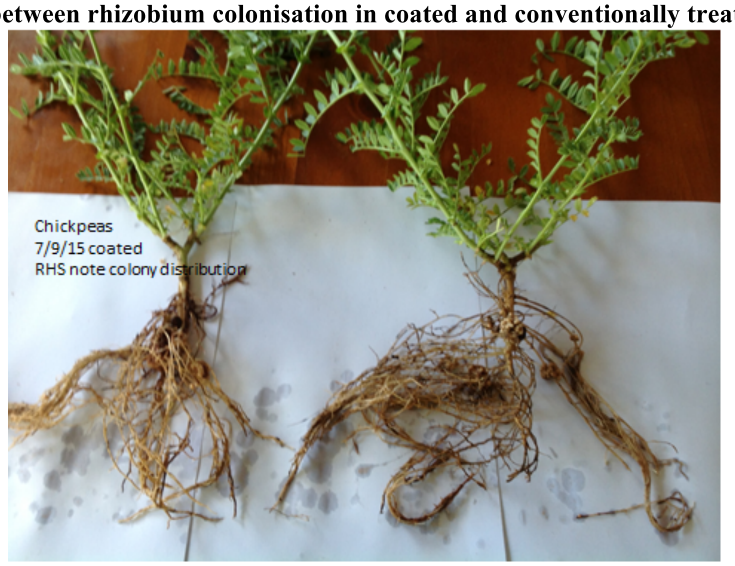
| Crop | Control | Treatment | *P <0.05* | *LSD (5%)* | *CV %* |
|---|---|---|---|---|---|
| Lentils | 614 | 777 | 0.0012 | 115 | 10.5 |
| Peas | 729 | 911 | 0.0053 | 159 | 12.2 |
| Chickpeas | 602 | 640 | 0.2733 | 75 | 14.3 |

Inspection of crops over the growing season showed significantly greater visual plant growth vegetative responses between the seed coated and conventionally sown crops. In the case of the lentils plant colour was also increased in the coated seeds. There was also a noted reduction in rhizoctonia infections on the coated plants suggesting a possibly biological suppression as a result of increased rhizobia colonisation.  Picture 1 below best highlights the difference in plant performance between the coated seeds in terms of plant growth and development which resulted in significant yield increases at harvest. Inspection of root nodules in all crops also showed increased nodulation and greater colour intensity within the nodules suggesting increased nitrogen fixation within the pulse crops. The only difference between treatments was the addition of the seed coating formulation.

**Picture 1: Plant growth responses between inoculated and seed coated and uncoated lentil plants August 2015.**

Both plots have been coated with Rhizobia F, the picture on the RHS was indicative of the nutrient coat applied in conjunction with Rhizobia inoculant. Growth responses were also visually significant in peas and chickpeas

**Picture 2: Comparison between rhizobium colonisation in coated and conventionally treated Chickpeas**




Picture 2 shows what was seen as a uniform response to root growth in all the treatments. In plantings of peas, chickpeas and lentils coating the seed with the seed coating formulation resulted in larger nodules and more of them in comparison to what is regarded as conventional rhizobia management onto pulse crops. This suggests that colony formation could be enhanced by creating an environment around the seed that is favourable for the survival and colonisation of emerging roots under harsh mallee farming conditions.

## Conclusion

The trial focused on the impact that a nutrient coat in conjunction with recommended rhizobia inoculants would have on pulse crops in the northern Mallee. Increases in plant vegetative growth and yield were seen in pulse crops coated with the seed coating mix described in this paper. The cost of coating is estimated at between $2-3/ha on top of rhizobia coating costs.  Similar responses have been seen in sites through the Upper South East of South Australia and Southern South Australian Mallee suggesting that in low rainfall low soil fertility environments seed coating to enhance the colonisation of rhizobia species on pulse crops is worth considering. Further work to investigate specific variances between species and coatings to enhance rhizobia colonisation of pulse crops in acidic soils needs also to be considered. The potential of biological nutritional coatings as part of integrated management in disease suppression also is worth further investigation.

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