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RHIZOBIAL INOCULANTS FACT SHEET

GRDC Grains Research & Development Corporation

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NORTHERN, SOUTHERN AND WESTERN REGIONS

HARVESTING THE BENEFITS OF INOCULATING LEGUMES

Inoculating legumes with rhizobia can achieve substantial increases in legume nodulation, grain and biomass yield, nitrogen fixation and post-crop soil nitrate levels.

KEY POINTS

- The benefits of inoculating legumes with rhizobia (soil bacteria that fix nitrogen) have been recognised in Australian agriculture for more than 100 years.
- Inoculation can result in increases in legume nodulation, grain and biomass yield, nitrogen fixation and post-crop soil nitrate levels.
- These gains are highest when the legume is grown in nil-rhizobia or low-rhizobia soil.
- Benefits can be marginal in soils already containing high numbers of compatible rhizobia.
- Legumes have specific requirements for rhizobia: there are 39 different inoculant groups produced commercially in Australia.
- Formulations for inoculants include peat, clay and peat granules, freeze-dried cultures and liquid cultures.
- Inoculant quality is underpinned by commercial in-house testing and the National Code of Practice and Quality Trademark (Green Tick Logo).

Legumes have been a source of food since mankind first tilled the soil many thousands of years ago.

From very early times, legumes have been recognised as 'soil improvers'. The farmers

PHOTO: BRAD COLLIS

Nitrogen-fixing nodules have become a vital resource for improving the productivity of Australian soils.

of ancient Mesopotamia grew peas and beans in their agricultural systems because they realised that cereals were healthier and higher yielding when grown after a legume break crop.

Those legumes would have been nodulated with compatible, effective rhizobia: the group of soil bacteria that infect the roots of legumes to form root nodules.

Rhizobia live in a modified form in nodules, and fix nitrogen gas (N_2) from the atmosphere.

Essentially all of the nitrogen (N) that is fixed by the rhizobia is exported out of the nodules and used in legume growth.

In return, the plant provides the rhizobia with habitat, nutrients and energy.

This mutually beneficial arrangement is called symbiosis.

Eventually, when the legume dies, the nodule breaks down and its rhizobial content is released back into the soil.

Compatible rhizobia

N fixation by the legume-rhizobia symbiosis does not happen in all soils. Compatible, effective rhizobia must exist before nodulation and N fixation can occur.

When a legume is grown for the first time in a particular soil, it is unlikely that the correct rhizobia will be present. Therefore the rhizobia must be supplied in highly concentrated form as inoculants.

Early days

Australian farmers have embraced legumes and legume inoculation from the outset.

Australian agricultural soils are naturally low in plant-available N but the use of fertiliser N has not always been an affordable option.

Cultivated legumes, mainly pasture and forage species, have had to be able to effectively fix N in the soil.

In 1896, the famous agricultural chemist F.B. (Frederick) Guthrie wrote about legume N fixation in the *Agricultural Gazette of New South Wales* saying that "it will prove to be one of the most valuable contributions ever made by science to practical agriculture."

More than 100 years later, Australian farmers sow inoculated legume seed on about 2.5 million hectares annually.

All of the estimated 2.7 million tonnes of N fixed annually by legumes, growing on some 25 million hectares of land (includes newly sown crop and pasture legumes and perennial and regenerating pasture legumes), can be attributed to either current or past inoculation.

From the turn of the 20th century until around 1950, work on rhizobia was the domain of the state departments of agriculture.

After 1950, however, the area sown to legumes – particularly subterranean clover and annual medics – increased dramatically and the demand for inoculants increased.

Manufacture was passed over to the private sector, and nodulation failures followed, which led to the creation of an independent quality control service, UDALS, at the University of Sydney.

Manufacture of inoculants remains with the private sector, now serviced by an independent quality control laboratory, Australian Inoculants Research Group (AIRG).

Inoculant groups

The relationships between rhizobia and particular legumes are very specific so individual inoculants are produced for the different legumes used in Australian agriculture.

An inoculant or inoculation group is a cluster of legumes nodulated by the same species of rhizobia (Table 1).

TABLE 1 Examples of legume inoculant groups used in Australian agriculture and their rhizobia. Currently, 39 different legume inoculants are manufactured in Australia, covering about 100 legume species

Rhizobia	Commercial inoculant group	Legumes nodulated
Sinorhizobium spp.	AL	Lucerne, strand and disc medic
	AM	All other annual medics
Rhizobium leguminosarum bv. trifolii	B	Perennial clovers
	C	Most annual clovers
Bradyrhizobium spp.	G[1]	Lupin, serradella
	S[1]	Serradella, lupin
Mesorhizobium ciceri	N	Chickpea
Rhizobium leguminosarum bv. viciae	E[2]	Field pea and vetch
	F[2]	Faba bean and lentil
Bradyrhizobium japonicum	H	Soybean
Bradyrhizobium spp.	I	Cowpea, mungbean

1 Both inoculant groups G and S can be used for lupin and serradella
2 Although group E is recommended for pea/vetch and group F for faba bean/lentil, if required group E can also be used for faba bean/lentil and group F used for pea/vetch

The rhizobia in each of the inoculation groups can be quite different. For example, lupins are nodulated by the slower-growing, acid-tolerant *Bradyrhizobium* spp., while medics are inoculated by the fast-growing, acid-sensitive *Sinorhizobium* spp.

The groupings provide a framework for:

- considering if inoculation is needed, based on the type of legume previously grown in a paddock; and
- choosing the correct inoculant for the particular legume to be sown.

Inoculants are produced and marketed commercially according to these inoculant groups. Choosing the correct inoculant group for a particular legume host (indicated by letters) is critical for effective nodulation and N fixation to occur.

PHOTO: DAVID HERRIDGE

High-yielding inoculated soybean ready for harvest, grown in rotation with sugar cane in north-coastal New South Wales.

Benefits of inoculation

Benefits of inoculation can be dramatic.

After more than 100 years of legume cultivation, many Australian soils have developed substantial populations of rhizobia that are able to nodulate commonly grown agricultural legumes.

However, suitable rhizobia may still be absent from the soil if the legume has not been grown previously or where the soil is not conducive to long-term rhizobial survival.

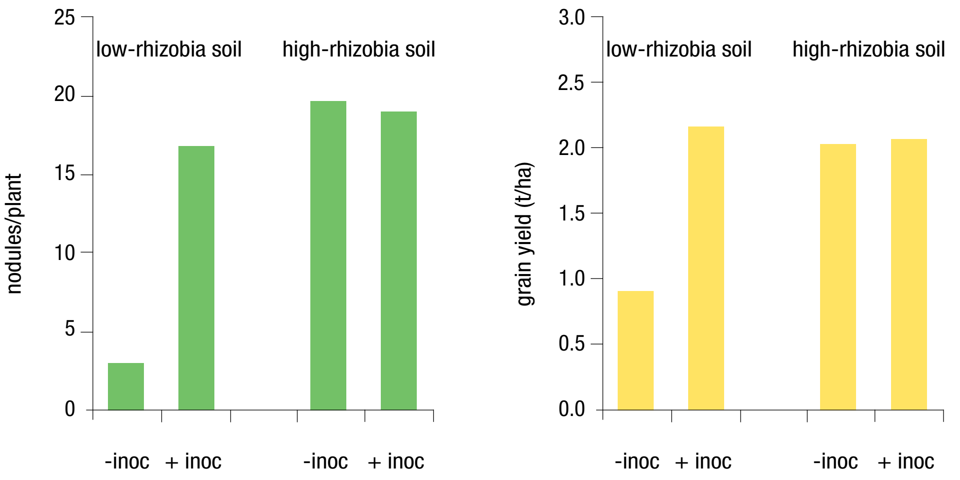
Soil acidity, for example, can reduce the chances of rhizobia persisting. Medic, lucerne and pea (including faba bean, lentil and vetch) rhizobia are particularly sensitive to acid soils.

In soils containing adequate populations of rhizobia, the communities are diverse and become less effective at fixing N over time when compared to commercial inoculant strains.

N fixation by symbioses between established soil rhizobia and the host legume is often less than 50 per cent of the symbioses that is achieved between the rhizobia in commercial inoculants and the host legume.

However, the presence of less effective, established rhizobia populations is not an

FIGURE 1 **Effects of inoculation on (A) nodulation and (B) grain yield of faba bean in low-rhizobia and high-rhizobia soils. Data aggregated from 18 experiments in the National Rhizobium Program (NRP) across WA, Victoria and NSW during 1997–2003**



impediment to the use of more effective inoculant strains.

When applied in sufficiently high numbers inoculant strains can successfully compete with established soil rhizobia and replace them.

An example of the effects of adding inoculated rhizobia can be seen in Figure 1, which shows the results of trials of faba bean sown into soils that contained either nil-to-low populations of rhizobia or high populations.

In the nil-to-low rhizobia soils, inoculation increased nodulation by more than 400 per cent and grain yield by 140 per cent.

There were no nodulation or grain yield benefits of inoculation in the high-rhizobia soils.

N fixation

There are other, less obvious benefits of inoculation than nodulation and grain (or biomass) yield.

Inoculation can also increase the amount of N fixed by the legume and the post-harvest (post-fallow) levels of soil nitrate.

In an experiment in southern NSW, inoculation increased grain yield of faba bean by 54 per cent and the amount of crop N fixed by a massive 700 per cent. See Table 2.

The benefit extended to post-fallow soil nitrate with levels increasing by 24 per cent, or 50kg N/ha.

With lupin, the grain yield benefit of inoculation was marginal (six per cent) and the benefit for post-fallow soil nitrate was also marginal (10 per cent), but the benefit for N fixation was large (360 per cent).

When do I inoculate?

The challenge facing farmers is knowing when to inoculate.

In the southern and western grain regions, only half of pea, vetch and lupin crops are inoculated, or about 500,000 out of one million hectares.

On the other hand, almost all of the 500,000 hectares of chickpea, mungbean and soybean in the northern grains belt are inoculated. Farmers are clearly making decisions about inoculation based on their experiences.

Table 2 and Figure 1 show that inoculation does not always translate into increased grain yield, although there may be other benefits such as increased N fixation and post-crop soil nitrate levels.

The book *Inoculating Legumes – a Practical Guide* includes guidelines for whether or not to inoculate, see Useful Resources. The book contains fact sheets with guidelines for the 15 major legume inoculant groups. For guidelines for inoculant groups E (pea, vetch) and F (faba bean, lentil), see Table 3.

The aim is to increase, not reduce, the percentage of crops that are inoculated. The practice represents a minor production cost with a potentially substantial payoff in terms of extra grain or biomass yield and extra N in the soil.

Inoculant formulations (products)

There are several different commercial inoculant formulations available to farmers to allow flexibility of application.

- Peat inoculants – the oldest and most common form of inoculant

TABLE 2 **Increased grain yields, crop N fixed and post-crop soil nitrate levels from inoculation of faba bean and lupin in southern NSW**

Legume	Grain yield (t/ha)		Crop N fixed (kg N/ha)*		Post crop soil nitrate (kg N/ha to 1.7m depth)	
	Nil	Plus	Nil	Plus	Nil	Plus
Faba bean	1.75	2.70	32	280	203	252
Lupin	3.50	3.70	52	240	190	209

* includes an estimate of below-ground N

SOURCE: DR MARK PEOPLES, CSIRO, FROM THE GRDC CROP SEQUENCING INITIATIVE, AND DR MATTHEW DENTON AND DR LORI PHILLIPS, FROM THE GRDC NATIONAL RHIZOBIUM PROGRAM

TABLE 3 **Likelihood of response to inoculation group E and F legumes**

High	– Soils with pH (Ca) below 6.0 and high summer temperatures (>35ºC for 40 days); or – Legume host (pea, faba bean, lentil, vetch) not previously grown.
Moderate	– No legume host (pea, faba bean, lentil, vetch) in last four years; or – Last host crop not inoculated or lacked good nodulation.
Low	– Loam or clay soils with neutral or alkaline pH and a recent history of host crop with good nodulation.

Guidelines for inoculation of the group E (pea, vetch) and F (faba bean, lentil) legumes, taken from the field pea, vetch, faba bean and lentil inoculation fact sheet in Inoculating Legumes – a Practical Guide, *see Useful Resources.*

PHOTO: ERIC ELLIOT

Lupin plant with nitrogen-fixing root nodules clearly visible.

used in Australia. These are prepared by introducing rhizobial broths into gamma-irradiated (sterilised) finely milled peat.

- Granular pellets or chips containing rhizobia made from either peat or clay.
- Freeze-dried powder, where a rhizobial broth culture is concentrated as a powder in a glass vial after all the water has been removed. The powder is reconstituted later on-farm.
- Liquid inoculants – suspensions of rhizobia in a protective liquid formulation.

Peat, freeze-dried and liquid inoculants can be applied either to seed or directly to soil in the seeding furrow. Granular inoculants are applied in-furrow.

If peat, freeze-dried or liquid inoculants are applied directly to soil, they are first suspended in clean potable water so they can be evenly distributed over the cropping area.

Inoculant quality

Inoculants manufactured and sold in Australia contain strains of rhizobia that have undergone thorough testing under laboratory, glasshouse and field conditions to ensure their local suitability.

Selection criteria include:

- strains that are highly effective in nodulation and N fixation across related legume species and cultivars of a particular species;
- strains that survive well on seed and in the soil after being introduced;
- strains that are genetically stable; and
- strains that are suitable for the manufacturing process.

In July 2010, the *National Code of Practice and Quality Trademark for Legume Microbial Inoculant Products used in Australian Crops and Pastures*, see Useful Resources, was introduced as part of a program to continually improve the quality and efficacy of biological inoculants marketed to Australian farmers.

The Green Tick Logo indicates the packet or container of inoculant meets quality standards set and monitored by the Australian Inoculants Research Group (AIRG).

The quality trademark is the Green Tick Logo, which indicates that the packet/ container of inoculant meets certain quality standards set and monitored by the AIRG.

At the time of publication, companies that are signatories to the National Code of Practice for legume inoculants and that are producing and selling inoculants carrying the Green Tick Logo are:

- Becker Underwood Pty Ltd;
- New Edge Microbials Pty Ltd; and
- Novozymes Biologicals Australia Pty Ltd.

USEFUL RESOURCES

Inoculating Legumes – a Practical Guide, December 2012
Ground Cover Direct
1800 110 044
www.grdc.com.au/bookshop

National Code of Practice
www.dpi.nsw.gov.au/__data/assets/pdf_file/0008/361295/Web-Version-of-the-NATIONAL-CODE-OF-PRACTICE-and-TRADE-MARK-LOGO-2Nov2010_final.pdf

MORE INFORMATION

Matthew Denton, University of Adelaide
08 8313 1098
matthew.denton@adelaide.edu.au

Ron Yates, Department of Agriculture and Food Western Australia
08 9368 3665
ronald.yates@agric.wa.gov.au

Nikki Seymour, Agri-Science Queensland
07 4639 8837
nikki.seymour@daff.qld.gov.au

David Herridge, University of New England Primary Industries Innovation Centre
02 6766 4504
david.herridge@industry.nsw.gov.au

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