Use of quality legume inoculants to get the most from nitrogen fixation

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Key points

- Rhizobia symbiotically fix close to three million tonnes of nitrogen a year worth $4 billion.
- The Australian Inoculants Research Group (AIRG) and its predecessors have controlled and maintained the quality of legume inoculants through collaboration with industry, universities and R&D bodies for close to 60 years.
- A symbol of inoculant quality assessment by an independent laboratory is the display of the green tick logo.
- There are different inoculant groups specific for different legumes.
- Correct storage is important to maintain high numbers of rhizobia in the inoculants.
- The AIRG continues to monitor the quality of legume inoculants at different points in the supply chain and works closely with the manufacturers to ensure that Australia's growers have ongoing access to efficacious products.

Introduction

Each year in Australia, legumes grown in association with their mutually beneficial rhizobia are estimated to symbiotically fix close to three million tonnes of nitrogen, worth $4 billion. The biological process of nitrogen fixation, where rhizobia located in legume root nodules convert atmospheric di-nitrogen (N_2) into ammonia (NH_3) for plant growth, is one of the great agriculture success stories. About 120 years ago in the *Agricultural Gazette of New South Wales*, Guthrie (1896) stated 'It will prove to be one of the most valuable contributions ever made by science to practical agriculture. It is of special interest to us in Australia'.

For close to 60 years, AIRG and its predecessors have undertaken a state and national responsibility for independent quality control and quality assurance of legume inoculants and, more recently, other beneficial microbes. AIRG controls and maintains the quality of legume inoculants through collaboration with industry, universities and R&D bodies.

The outcome of this collaboration between AIRG and industry has improved the quality of legume inoculants over the years. The AIRG laboratory pass rate for quality testing inoculants has increased to be consistently greater than 90% at the point of manufacture (Figure 1).

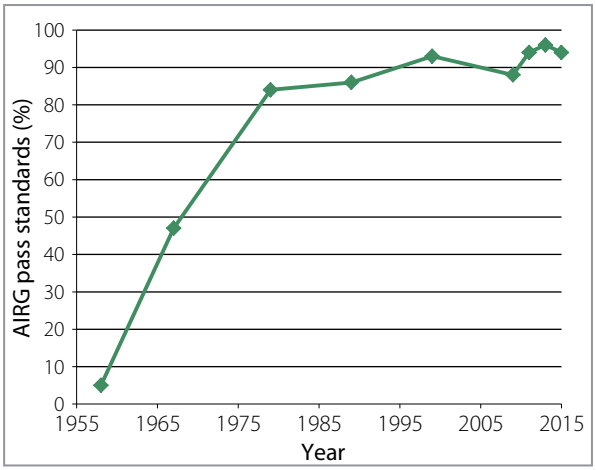

Figure 1. The AIRG laboratory pass standards from 1955 to 2015.

To achieve this:

- AIRG, part of NSW DPI's Soils Unit, has an Australia-wide mandate to ensure that the country's growers and producers have access to high quality legume inoculants and inoculant products containing prescribed numbers of either effective root nodule bacteria (rhizobia) or other beneficial microbes.
- There is a continual Australia-wide commitment to legume inoculant R&D by federal and state governments, universities and various R&D funding bodies.

» AIRG bridges the gap between inoculant R&D and commercial legume inoculant production.
» AIRG conducts quality assessments of rhizobial inoculants at point-of-manufacture and at point-of-sale.
» AIRG has a link with NSW DPI, University of Sydney and other institutions' researchers for ongoing collaborative R&D.
» AIRG operates under ISO9001: 2008 certification.

Each year, AIRG supplies a fresh set of cultures of recommended inoculant strains for temperate and tropical legumes to the inoculant manufacturers for commercial use (Figure 2). These cultures must meet stringent criteria before being released by AIRG. AIRG also holds and distributes a limited number of non-rhizobial cultures used in commercial manufacture.

Figure 2. Rhizobia 'mother' culture preparation.

Code of practice

AIRG has developed a code of practice that incorporates a set of independent protocols and standards for assessing legume inoculant products for quality. Manufacturers produce commercial batches of legume inoculants from the cultures supplied by AIRG.

A sample of each inoculant batch produced is submitted to AIRG and assessed to ensure that inoculants released for sale have:

» the approved and correct strain of rhizobia for the legume group
» high numbers of live rhizobia (the standard for all inoculants is 1,000 million/g fresh peat except Lotononis, which is 500 million/g peat)
» minimal numbers of other contaminant microorganisms i.e. less than 1 million/g peat
» optimum peat moisture for growth and survival of the rhizobia after manufacture and during storage
» optimum capacity for nitrogen fixation with its legume test plant.

Only when these standards have been met does AIRG approve the batch for sale and supplies an expiry date. Inoculant companies, who are signatory to the code and produce inoculants that comply to the standards, can display a NSW DPI registered trade mark green tick quality logo on their product (Figure 3).

Figure 3. NSW DPI green tick registered trademark.

During the period 2005–15, AIRG:

» processed and quality tested >15,000 inoculants at time-of-manufacture and point-of-sale
» processed and quality tested >700 samples of pre-inoculated and custom-inoculated seed
» supplied 1–4 manufacturers with subcultures of 35–40 strains of rhizobia annually (total of about 3,200 subcultures)
» ratified strain changes for 20 inoculant groups
» developed and ratified standards for the new inoculant formulations (granular, liquid and freeze-dried)
» maintained the core Rhizobium strain collection of nearly 2,000 strains.

How to maximise the nitrogen fixation potential of inoculated legumes

To ensure users, i.e. growers, maximise the nitrogen fixation potential of inoculated legumes, AIRG advises the following:

» use quality inoculants
» don't use inoculants that have passed the expiry date
» sow freshly inoculated seed as soon as possible and definitely within 24 hours of inoculation
» use clean potable water when preparing liquids or slurries and make sure holding tanks are free from chemical and fertiliser residues
» never mix rhizobial inoculant with pesticides, or mineral or organic fertilisers, as many are toxic to rhizobia
» rhizobia can be compatible with seed pickles or dressings for a limited time before sowing (see Inoculating legumes: a practical guide from GRDC (2012) for manufacturer's guidelines and

Table 5.4 on page 40). Always apply the seed dressing first and allow it to fully dry before applying the rhizobia as a second process

» match the correct inoculant group to the specific legume.

Inoculants for some of the common legume species, legume inoculant groups and the amount of seed that can be treated by a 250 g bag of peat inoculant are shown in Table 1.

Storing inoculant

One of the main factors affecting the quality of legume inoculants is storage temperature. Legume inoculants contain live rhizobia. It is important to make sure they are kept in moderate temperatures (less than 30 °C and not frozen) away from sunlight and chemicals. For many years, AIRG staff members have monitored inoculant quality at the point of sale. Inoculants were purchased from retail outlets covering the grain cropping areas across Australia and the number of rhizobia in each packet or container counted. Temperatures and conditions of storage were recorded. Generally, rhizobial numbers in the inoculants remain high because the product at the point of manufacture had been prepared in a suitable carrier that prolongs rhizobial survival. However, numbers for the common legume host groups decline in rhizobial number when stored at ambient to high temperature compared to those stored at less than 10 °C (refrigerated) (Table 2).

Table 1. Common legumes species, legume inoculant groups and the amount of seed that can be treated by a 250 g bag of peat inoculant.

Common legume	Legume inoculant group	Seed size	Maximum weight of seed treated with 250 g peat inoculant
Grains			
Pea, field pea, vetch, narbon bean, lathyrus	E	Large	100 kg
Faba bean, broad bean, lentil	F	Medium–large	50–100 kg
Lupin	G	Large	100 kg
Chickpea	N	Large	100 kg
Cowpea, mungbean (green and black gram)	I	Large	100 kg
Peanut or groundnut	P	Large	100 kg
Soybean	H	Large	100 kg
Pastures			
Lucerne, strand and disc medics, melilotus albus	AL	Small	25 kg
Barrel, burr, snail, sphere, gama, murex medics	AM	Medium	50 kg
White, red, strawberry, alsike, Berseem, cluster or ball, suckling, talish clovers	B	Small	25 kg
Subterranean, crimson, cupped, helmet, purple, rose, arrowleaf, balansa, bladder, gland, persian clovers	C	Small–medium	25–50 kg
Greater lotus	D	Small	10 kg
Serradellas	S	Medium	50 kg
Biserrula	Biserrula	Small	10 kg
Birdsfoot trefoil	Lotus	Small	10 kg
Sulla	Sulla	Medium	10 kg

Table 2. Effect of retail storage temperature on rhizobial survival in peat inoculants.

Group	Legume	Million rhizobia/g peat		
		Refrigerated <10 °C	Ambient >20 °C	Difference (%)
E	Pea/field pea	831	614	-26
F	Faba bean	934	612	-34
G	Lupin	1,661	1,226	-26
N	Chickpea	2,282	1,764	-23
AL	Lucerne	2,503	2,888	15
AM	Annual medic	1,212	1,366	13
C	Subclover	676	526	-22

For the groups E, F, G, N and C there is a reduction of 22% or more in rhizobial numbers when the products are not refrigerated. Lucerne and annual medic were not affected by storage temperatures higher than 20 °C.

Carefully handling inoculants before, and at, sowing ensures that the maximum number of viable rhizobia is applied to the seed or seed furrow. The potential response to inoculation is thus maximised for crop or pasture yield. The AIRG continues to monitor the quality of legume inoculants at different points in the supply chain and work closely with the manufacturers to ensure that Australia's growers have ongoing access to efficacious products.

Reference

Guthrie, FB 1896, 'Inoculation of soil for leguminous crops', *Agricultural Gazette of New South Wales*, vol. 7, pp. 690–694.

Acknowledgments

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