INOCULATING LEGUMES: PRACTICE AND SCIENCE

GRDC
GRAINS RESEARCH & DEVELOPMENT CORPORATION

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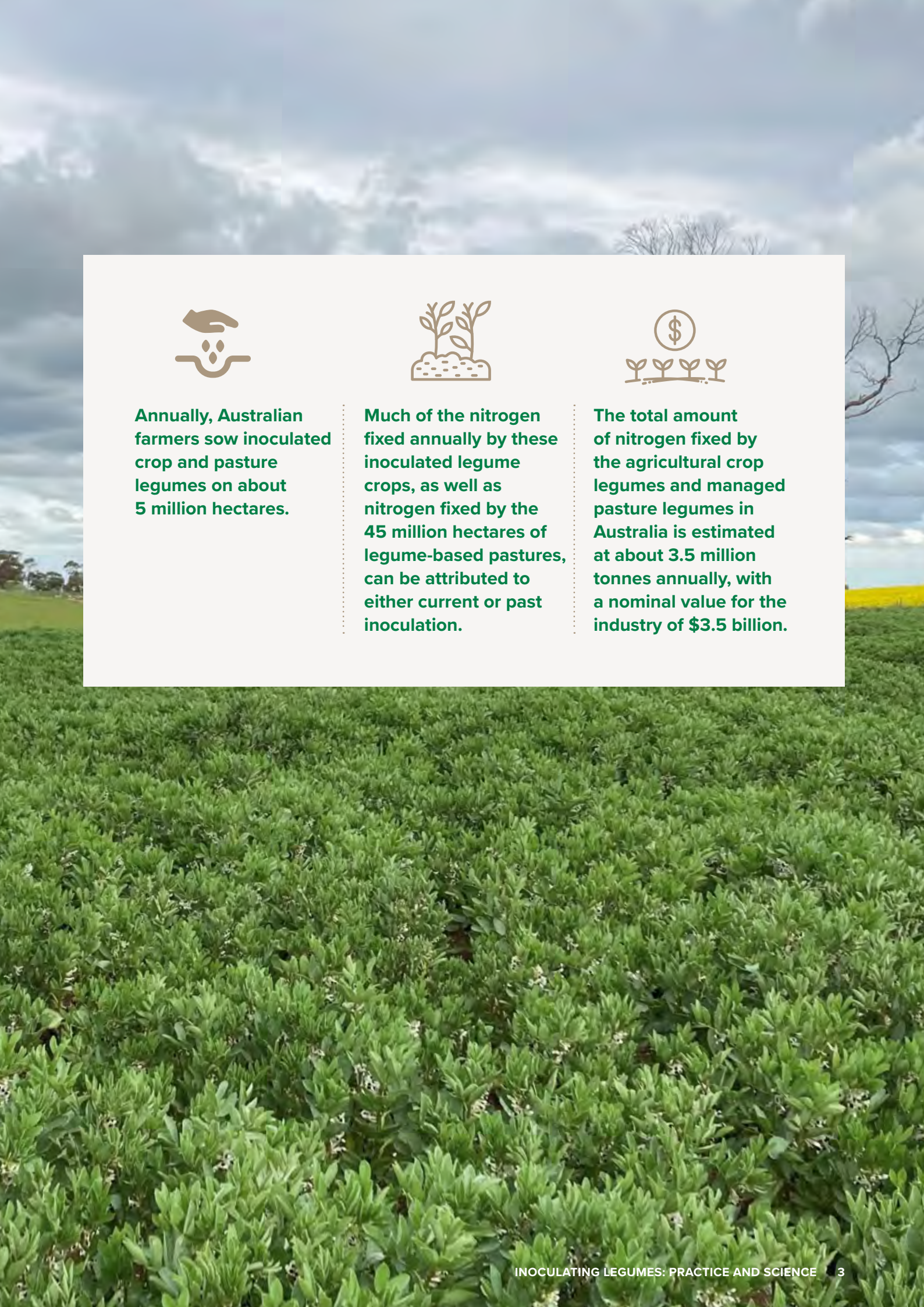
Well nodulated bean plant.
Photo: Liz Farquharson, SARDI.



Annually, Australian farmers sow inoculated crop and pasture legumes on about 5 million hectares.

Much of the nitrogen fixed annually by these inoculated legume crops, as well as nitrogen fixed by the 45 million hectares of legume-based pastures, can be attributed to either current or past inoculation.

The total amount of nitrogen fixed by the agricultural crop legumes and managed pasture legumes in Australia is estimated at about 3.5 million tonnes annually, with a nominal value for the industry of $3.5 billion.

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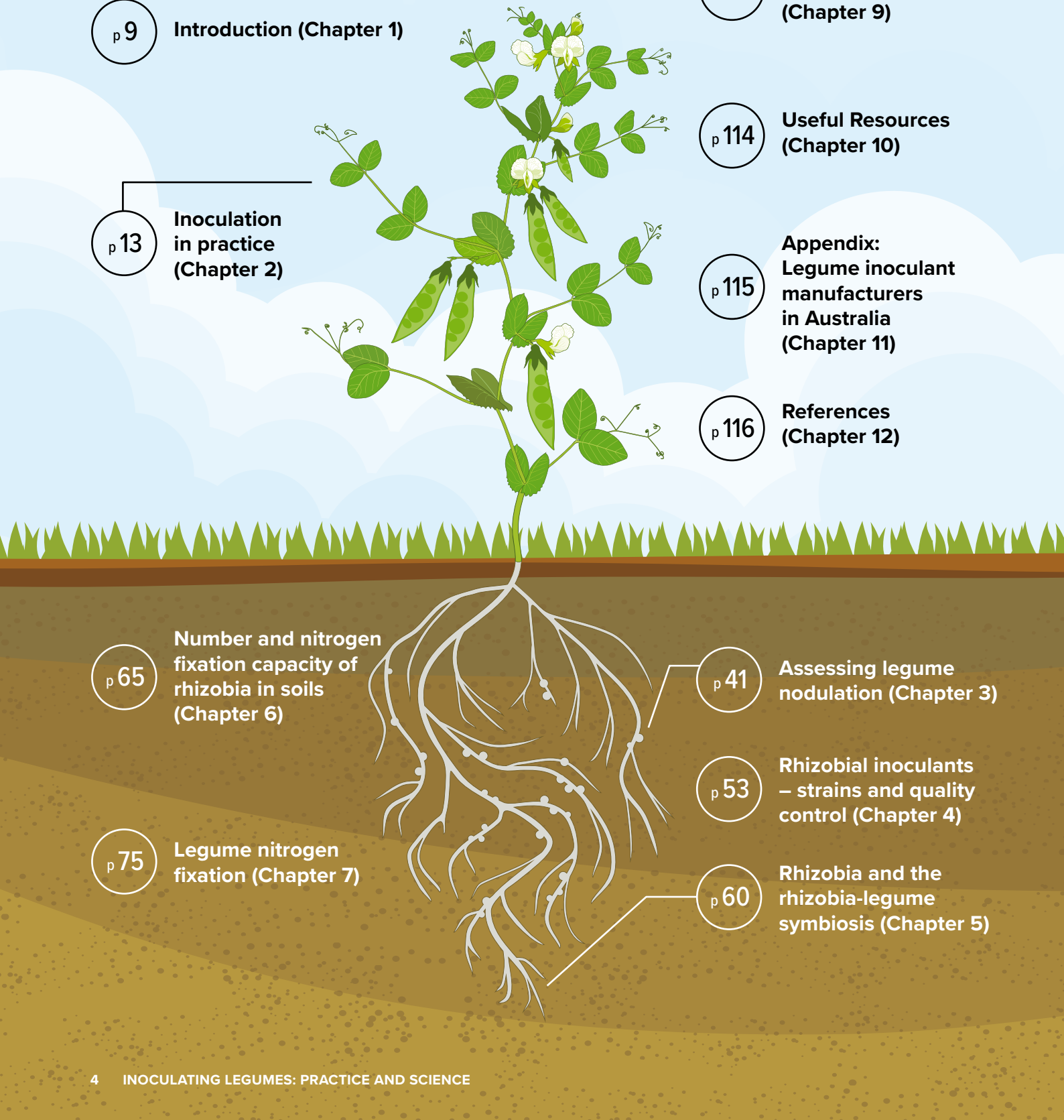

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Foreword

Atmospheric nitrogen (N) is fixed by symbiotic root-nodule bacteria (rhizobia) associated with pasture and pulse legumes and has a national value of about $3.5 billion annually.

This is based on nitrogen fixation rates of about 70 kilograms per hectare per year, crop and pasture legume areas of close to 50 million ha and fertiliser N costed to the grower at $0.76/kg, which equates to $1.00/kg plant-available N in the soil after accounting for N losses. The price of carbon-based fossil fuels, used in the production of nitrogenous fertilisers, is expected to increase in the future which will push fertiliser costs higher. Added to that are the environmental costs associated with the production, distribution and application of nitrogenous fertilisers. Therefore, the historical and ongoing interest by Australian farmers in using legumes which fix nitrogen in their farming systems makes good economic and environmental sense and needs to be sustained into the future.

Legumes must be effectively nodulated for the benefits of nitrogen fixation to be maximised. Reasons for ineffective nodulation may include:

- crops are not inoculated when they should be;
- use of inoculation practices that do not deliver sufficient rhizobia to the developing legume seedling;
- use of inoculants of sub-optimal quality;
- inoculant rhizobia are exposed to chemical toxicities during inoculation or soon after application to the soil;
- legume breeding programs release cultivars that are not matched with highly effective nitrogen-fixing rhizobial strains;
- ineffective populations of rhizobia evolve in the soil and outcompete effective inoculant rhizobia;
- populations of soil rhizobia decline because the landscapes become hostile through soil salinity, acidity or for other reasons.
- management practices such as herbicide applications damage plant root systems
- environmental constraints such as acidity, salinity or disease impact on plant health and nodulation.

To capitalise on the potential benefits of legume nodulation and nitrogen fixation, Australian farmers need to:

- understand the role of legumes in supplying nitrogen to agricultural production systems;
- manage legume nitrogen fixation and system nitrogen supply for maximum productivity and sustainability;
- inoculate legumes where and when appropriate;
- optimise inoculation outcomes through correct use of the inoculant;
- understand the limitations of inoculants, e.g. death of the rhizobia from exposure to toxic and dehydrating conditions;
- have access to the most efficacious inoculant products in the marketplace;
- understand the specific nature of the relationship between legumes and rhizobia, and use the most appropriate inoculant strain for a target legume-host;
- grow the most appropriate legume in terms of environment and soil biology; and
- manage soils to minimise plant growth-limiting factors e.g. pathogens, heavy metals, low pH, salinity, herbicides.

This handbook was originally written in 2012 by a group of Australian experts in the field of rhizobiology and nitrogen fixation from universities and state departments of agriculture and primary industries, all of whom worked at various times within the Grains Research and Development Corporation (GRDC) funded National Rhizobium Program (NRP) and national Nitrogen Fixation Program (NFP). The NRP/NFP existed as coordinated, nation-wide programs between 1998 and 2017. Since then, GRDC has funded individual projects covering the western, southern and northern regions. The value of the handbook has been recognised by GRDC, growers, advisers and researchers and so a rewrite was commissioned in 2021.

From 1998 to today, the objectives have been to address the above issues through research and extension. This latest version of the handbook includes revisions of particular sections based on the latest research findings and changes in farming systems and the inoculation industry. The major geographic focus of the handbook is the 70 million hectares of cropping and managed pasture land that produces all of Australia's grain and much of its livestock and cotton products.

The key audiences are farmers, farming systems groups, commercial and government advisers, agribusiness, research agronomists, legume breeders, seed coaters or pelleters, resellers and seed merchants. It is intended that material from this handbook can be extracted and used in training workshops.

By using this handbook and/or participating in workshops that use materials from this handbook, users should have an increased knowledge of legumes and legume nodulation in farming systems, more effectively use inoculation as a key farm practice, and achieve higher farm productivity through enhanced legume nitrogen fixation and system N supply.

Stephen Loss
GRDC Manager Soils and Nutrition - South

Photo: Liz Farquharson, SARDI.

1 | Introduction

It is envisaged that this revised edition of the preceding handbook, *Inoculating Legumes – A Practical Guide* will be easily accessible to those needing information for their own purposes or who are giving advice to growers. We hope that it will be a one-stop shop for information on rhizobia and legume inoculation.

It is also intended that this handbook will be a comprehensive resource for agronomists and other agricultural scientists in the preparation of seminars and training workshops for growers and advisers.

1 | Introduction - Legumes and Rhizobia

Legumes have been used as a source of stock feed and human food ever since humans first tilled the soil many thousands of years ago. From very early times, legumes were recognised as 'soil improvers'. The farmers of ancient Mesopotamia grew peas and beans in their agricultural systems because they realised that cereals, their mainstay crops, were healthier and higher yielding when grown after a legume break crop.

Those legumes would have been nodulated by compatible, effective rhizobia, the endemic group of soil bacteria that infect the roots of legumes to form nitrogen-fixing root nodules.

1.1 What are rhizobia?

Rhizobia are very specific beneficial soil bacteria that live in nodules and fix nitrogen gas (N_2) from the atmosphere. The first product of the nitrogen fixation process is ammonia, which is then converted to amino acids and amides within the nodules before being transported in the xylem sap to other plant parts. These products of nitrogen fixation are vital for plant growth. In return, the rhizobia are provided with habitat and supplied with nutrients and energy in the form of carbon compounds. This mutually beneficial association is called symbiosis. Eventually, when the legume begins to senesce and the flow of nutrients and energy from the plant to the nodule ceases, the nodule disintegrates and its rhizobial occupants are released into the soil. As the roots and shoots of the legumes break down nitrogen (N) is released into the soil. Other free-living, non-symbiotic bacteria live in the soil that are capable of N fixation, however, these fix relatively small amounts of N compared to rhizobia.

Although legumes had been used as rotation crops in most parts of the world through the ages, it was not until the late 19th century that the links between nodulation, nitrogen fixation and soil improvement were described scientifically. Today, it is estimated that worldwide, 50-60 million tonnes of N is fixed annually by 250 million hectares of pulse and oilseed (crop) legumes and 220 million hectares of managed pastures containing legumes. In Australia, the 2.8 million hectares of crop legumes and 47 million hectares of managed, legume-based pastures are estimated to fix about 3.5 million tonnes of N annually. The annual cost for Australian farmers to replace the N fixed by legumes with fertiliser N would be $3.5 billion.

1.2 The practice of inoculation

For nodulation and N fixation to occur in legumes, rhizobia that are compatible with that legume species and effective in N fixation must be in the soil in which the legume is growing. When a legume is grown for the first time in a particular soil or after a break of several years, it is highly likely that compatible, effective rhizobia will not be present. In such circumstances, the rhizobia must be supplied in a highly concentrated form as an inoculant.

Inoculation of legumes with rhizobia is one of the success stories of sustainable agriculture and, indeed, may be one of the most cost-effective of all agricultural practices. Millennia before the science of legume nitrogen fixation was understood, farmers used rudimentary means of inoculation such as the transfer of soil from paddocks growing well-nodulated legumes. For example, as late as 1920, Australian farmers were encouraged to inoculate lucerne seed with a mixture of glue and soil taken from paddocks containing well-nodulated lucerne (Guthrie 1896). Inoculation of legume seeds using pure cultures of rhizobia was made possible by the ground-breaking work of German and Dutch microbiologists during the last two decades of the 19th century. Within a few years, European growers had access to cultures of rhizobia for inoculating a range of legumes. Inoculation of both seed and soil were advocated. Since that time, the production and distribution of legume inoculants has become an established industry in many countries.

1.3 Inoculants and inoculation of legumes in Australia

Australian growers embraced legumes and legume inoculation from the outset of ley-farming systems. The soils that they farmed were generally low in plant-available N and the use of nitrogenous fertiliser was not an affordable option. The legumes grown, mainly pasture and forage species, had to supply N for themselves and had to be capable of effective N fixation.

In 1896, agricultural chemist, 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. It is of special interest to us in Australia," (Guthrie 1896).

Mr Guthrie had remarkable foresight because more than 100 years later, Australian farmers sow an estimated 6 million hectares of legumes annually, and about 80 per cent of the sown legumes are freshly inoculated or, in the case of pasture legumes, preinoculated. These estimates are derived from the results of two farmer surveys on inoculation, conducted in 2013 and 2017 by the GRDC-supported national Nitrogen

Fixation Program (NFP), from a 2013 report to GRDC on inoculation (SM Jones and R Deaker, personal communication) and a 2018 farm adviser survey (Hackney *et al.* 2019).

It is difficult to say how much N is fixed by the newly-sown crop and pasture legumes as a result of inoculation, but estimate that much of the 3.5 million tonnes of legume N fixed annually in Australia's crop and managed pasture lands results from either current or past inoculation.

The success of legume inoculation as a routine practice in Australian agriculture was underpinned by effective scientific research and training in the state departments of agriculture, universities and several CSIRO divisions. Centres for research on the legume-rhizobia symbiosis were established at various times in all Australian states, leading to rapid advances in knowledge and inoculant technology and placing Australia foremost in the world in inoculant development and adoption.

It was timely in 2012 that the authors of the first edition of 'Inoculating Legumes: A Practical Guide' took time out to compile a handbook that related scientific theory to the practical aspects of the legume-rhizobia symbiosis.

FIGURE 1-1. A group of the handbook's authors inspecting serradella and biserrula pastures at Neil Ballard's farm, Narrogin, WA.

Photo: David Herridge.

2 | Inoculation in practice

What is inoculation?

Inoculation is the application of root-nodule bacteria (rhizobia) to a legume seed or to the soil in which the legume is sown. Inoculation facilitates infection of seedling roots by rhizobia and subsequent nodulation of the legume by the appropriate strain of rhizobia (Chapter 5). About 100 years ago when scientists developed a basic understanding of the symbiotic relationship between the host legume and compatible rhizobia, and the beneficial effect of that symbiosis on legume nitrogen and growth, Australian farmers were encouraged to inoculate sown legumes (Figure 2-1, page 15). The first rudimentary inoculants consisted of soil collected from a paddock in which nodulated legumes of the same type were growing. The sieved soil was then mixed with glue and applied to seed. Since then, legume inoculants have become more sophisticated and efficacious, delivering greater numbers of more effective rhizobia to the roots of the emerging legume seedlings.

Australian farmers continue to inoculate legumes with recent surveys indicating approximately 80 per cent of sown legumes are either inoculated on-farm (mainly crop legumes) or preinoculated by seed coaters (pasture legumes). In some crop legumes such as chickpea in southern Australia and mungbean in the northern region, the percentage inoculated would be close to 100 per cent.

The aim of inoculation is to deliver large numbers of rhizobia from the inoculant to the young roots. As inoculants contain living organisms, care should be taken in storage and to ensure their effectiveness. The growth and survival of rhizobia can be reduced through contact with some chemicals and fertilisers, heat or freezing temperatures, long exposure to sunlight, desiccation, and acidic (low pH) or highly alkaline (high pH) environments. In circumstances that are challenging, such as sowing into dry or acidic soils, the amount of inoculant used should be increased or the formulation of inoculant considered, (Ballard *et al.* 2019, see later in this Chapter for details of the different commercial inoculant formulations), with some granular products providing improved efficacy over peat and other formulations under dry sowing conditions (Denton *et al.* 2018, Farquharson *et al.* 2018).

Photo: Alistair Lawson, AgCommunicators.

INOCULATING LEGUMES

TIPS FOR OPTIMISING NODULATION

DOS

DO take into account paddock history, including legume and inoculation history and the soil pH.

Acidic soils, especially if pH_{Ca} is less than 5.5, usually require inoculation every time for pulses, except narrow leaf lupins. Inoculate if it's been four years since the legume was grown.

DO use the correct inoculant group for the legume.

Each legume type is matched to its own inoculant group. Inoculating with the wrong inoculant group leads to nodulation failure. Using the correct group is essential to good nodulation and nitrogen fixation.

DO take care when inoculating pickled seed.

Some pickles, or seed-applied fungicides can reduce the survival of the rhizobia on the seed. For example, thiram and fungicides containing thiram (P-Pickel T®) are toxic to rhizobia and metalaxyl (Apron®) can also be inhibitory.

To avoid issues, apply rhizobia last, once the fungicide has dried on seed. Limit exposure time between inoculation and sowing to less than 6 hours where possible.

DO consider using double rate inoculant in the following situations:

- if the inoculant group for that legume hasn't been used in that paddock before (especially for chickpea)
- when sowing into dry soil
- in very acidic soils with a pH_{CaCl_2} of less than 5.5.

DO use clean equipment and containers or tanks.

Rhizobia are sensitive to chemical residues. Take care to use clean equipment when preparing, mixing and delivering inoculant.

DON'TS

DON'T use saline bore water or chlorinated tap water when preparing and applying peat slurry or freeze-dried inoculants.

Rhizobia are sensitive to chemical stresses, so it is important to use good quality rainwater or non-saline bore water. If using chlorinated water, let it sit for at least 24 hours.

DON'T mix trace elements with liquid inoculants in a tank as they can be very toxic to rhizobia.

Where freeze dried and peat slurry inoculants are applied as liquids in furrow, avoid adding trace elements or other additives to the tank mix, as they often kill the rhizobia. Alternatively, use a granular inoculant.

DON'T wait too long before sowing seed.

Sow peat inoculated seed within 24 hours, or within 6 hours if the seed has a fungicide coating.

Sow freeze-dried inoculated seed within 5 hours.

DON'T leave inoculant in direct sunlight or in temperatures higher than 25 degrees Celsius.

All types of inoculant in bags or packets, on inoculated seed, as liquid or in granule form can be damaged by warm temperatures.

DON'T mix inoculant directly with fertilisers.

Some types of fertiliser can be toxic to rhizobia and most types of inoculant granule should not be mixed with fertiliser. This is particularly the case for acidic fertilisers such as mono ammonium phosphate (MAP) and super-phosphate.

Delivery of liquid fertiliser through a separate line can be effective.

Your inoculation questions answered

2.1 When is inoculation required?

When sowing legumes, inoculation should always be considered due to the potential to increase N fixation and grain yield. The circumstances under which inoculation of specific legumes is recommended are covered in Chapters 6 and 9. In general, inoculation will result in increased nodulation, biomass and grain yield when the number of suitable rhizobia in the soil is zero or low (Figure 2-1). When the population of suitable soil rhizobia is high, it is unlikely that nodulation, biomass and grain yield will be improved through inoculation.

Important reasons to undertake inoculation include:

- If the particular legume has never been grown in the paddock;
- If it has been more than four years since that particular legume was grown in the paddock;
- To introduce newly selected rhizobial strains with increased effectiveness and survival;
- If acidic or highly alkaline soils are present in the paddock, which are likely to have reduced numbers of rhizobia already in the soil;
- If the paddock has been subjected to a particularly hot, dry summer.

2.2 Which inoculant group should I use?

Legumes can be very particular about the species and strain of rhizobia they nodulate with. For example, chickpeas and field peas each nodulate with different species of rhizobia. Commercial inoculants contain the correct species and the most effective strain of rhizobia for particular crop and pasture legumes, and these are assigned to 'inoculant groups' (Chapters 4 and 5). It is crucial that the correct inoculant is used for the crop or pasture legume. If the incorrect inoculant group is used, the likely outcome is nodulation failure. Further details of particular inoculants are provided in Table 2-1 (page 16) and Chapter 9.

2.3 Which inoculant group do I need for a mixture of pasture species?

When using mixtures of different pasture legume species, the correct inoculant groups must be supplied for each species. For example, group C that nodulates subterranean clover will not nodulate lucerne. If using peat inoculant, each pasture type should be inoculated separately with the correct inoculant group, and once dried, the seed of the different species can be mixed together. Alternatively, if using granular products, the required application rate must be used for each inoculant group. Regardless of the inoculation method, the full rate of inoculant is required for each species in a mixture. See inoculant manufacturer's details.

2.4 How do numbers of inoculant rhizobia relate to legume nodulation and yield?

Increasing the number of rhizobia inoculated onto seed can increase nodulation and grain yields (Denton *et al.* 2013). For the crop legumes, peat inoculants usually contain enough rhizobia to deliver around ten billion to one hundred billion rhizobia per hectare (see Chapter 4). The minimum recommendation for rhizobial numbers on seed at sowing when inoculated by peat slurry inoculants is 100,000 rhizobia per large seeded pulse species (chickpeas, lupins) and 10,000 for smaller seeded pulse (mungbeans, lentils). For peat and preinoculated pasture legume seeds, the minimum recommendations are 1000 rhizobia for medium-sized pasture seeds, such as subterranean, and 500 rhizobia for smaller seeded pastures, such as white clover (see Chapter 4 for testing of rhizobial numbers on preinoculated seed).

FIGURE 2-1: Effects of inoculation on nodulation and grain yield of faba bean in a low rhizobia soil (left-hand graph) and a high rhizobia soil (right-hand graph). Data are aggregated from 18 field experiments conducted in Western Australia, Victoria and New South Wales during 1997 to 2003 (DF Herridge, personal communication).

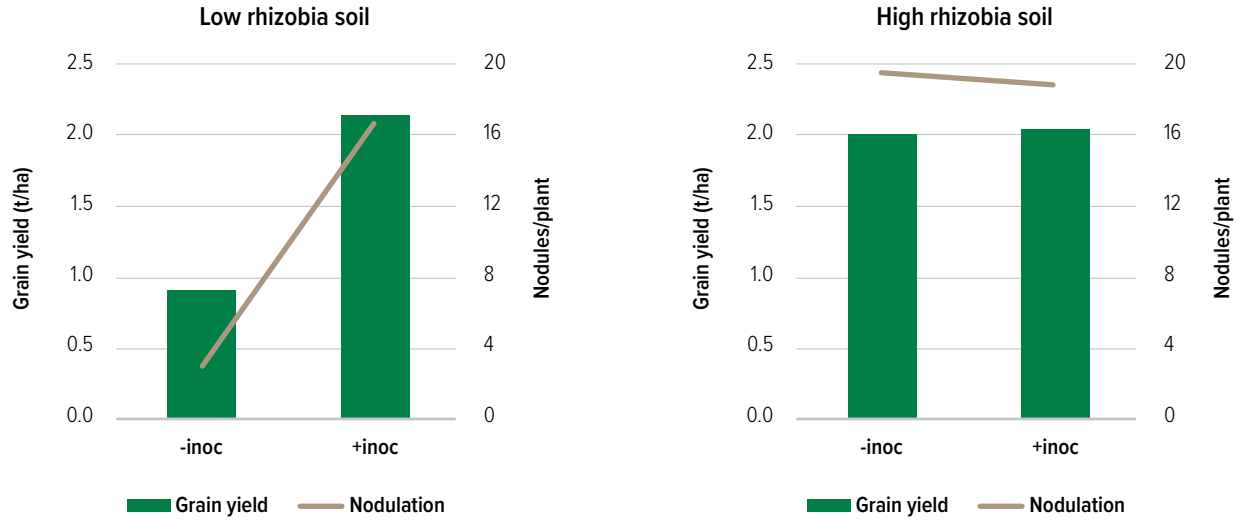


FIGURE 2-2. A faba bean trial at Telangatuk (Victoria) on a very acidic soil (pH_{CaCl_2}<4.5). Poorly nodulated beans (left) compared to well nodulated beans (right), shows the importance of good inoculation practice.

Photo: Ross Ballard, SARDI.

TABLE 2-1. Inoculant groups for some common legume species and rate of peat inoculant applied to seed. Always check manufacturers guidelines.

Inoculant group	Common name of legume	Seed size	Grams of peat inoculant required to treat 1kg seed
AL	Lucerne, strand and disc medics Sweetclover (only *Melilotus albus*)	Small	10
AM	Burr, barrel, snail, sphere, murex and gama medics	Medium	5
B	White, red, strawberry, alsike, talish, berseem/Egyptian, cluster/ball and suckling clovers	Small	10
C	Subterranean, bladder, rose, helmet, crimson clovers	Medium	5
	Arrowleaf, balansa, gland, purple, Persian clovers	Small	10
D	Greater lotus (only *Lotus pedunculatus*)	Very small	25
E	Field pea, pea (snow and snap peas etc), vetch (com-mon, woolly pod, bitter & purple), narbon bean, grass pea (*Lathyrus*)	Large	2.5
F	Lentil	Medium	5
	Faba, broad and tick bean	Large	2.5
G	Narrow-leaf, Mediterranean white, yellow and sandplain lupins	Large	2.5
H	Soybean	Large	2.5
I	Cowpea, mungbean (green and black gram)	Large	2.5
J	Pigeon pea, lablab	Large	2.5
	Horse gram	Medium	5
N	Chickpea (desi and kabuli)	Large	2.5
P	Peanut or groundnut	Large	2.5
S	French, yellow, pink, slender, hybrid and birdsfoot serradellas	Medium	5
Special Inoculants	More than 20 Special Inoculants are made for legume species not covered by the Inoculant Groups above. Legumes requiring special inoculants include Biserrula, birdsfoot Lotus, sulla, messina, tedera, Phaseolus beans (common and kidney), stylo, desmanthus, etc. The full list of Special Inoculants can be found in Section 9.1 (page 93).		

2.5 Can you use too much inoculant?

In a word, no.

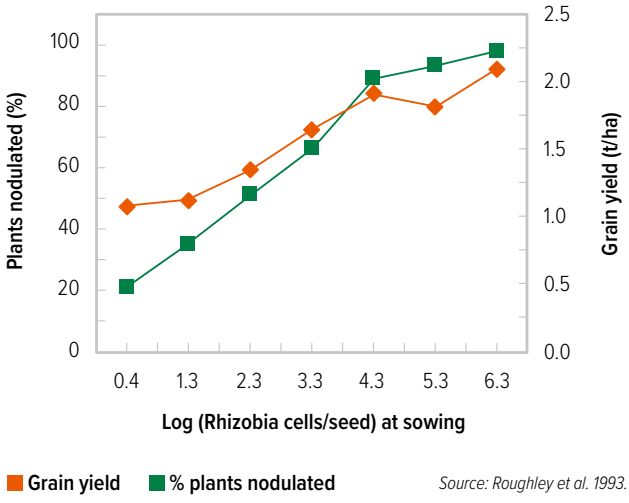
Inoculation of legumes at higher than recommended rates is not harmful to legume growth or production, and may be beneficial in some circumstances (see section 2.6 and Figure 2-3). However, over application of peat inoculants may cause the seed to stick together and create problems with flow through augers and seeders. Too much inoculant represents a comparatively small cost to production, whereas poorly nodulated and N deficient crops will cause a substantial reduction in production and profit, so it is best to err on the side of over-application rather than under-application.

2.6 Should I use higher than recommended rates of inoculant?

Nodulation can be improved for grain legume crops if the number of applied inoculant rhizobia is increased above the recommended rate, especially in adverse soil conditions e.g. dry or acidic soils for some pulses (see sections 2.11 and Chapter 6) (Ballard *et al.* 2019). We would expect similar benefits for pasture legumes, however further research is required to confirm this. Sufficient rhizobia need to be alive on or near the seed at germination to multiply around the root for good nodulation. When soil conditions are unfavourable, increasing application rates of peat inoculant on seed increases the likelihood that sufficient rhizobia will survive on the seed until plant germination occurs.

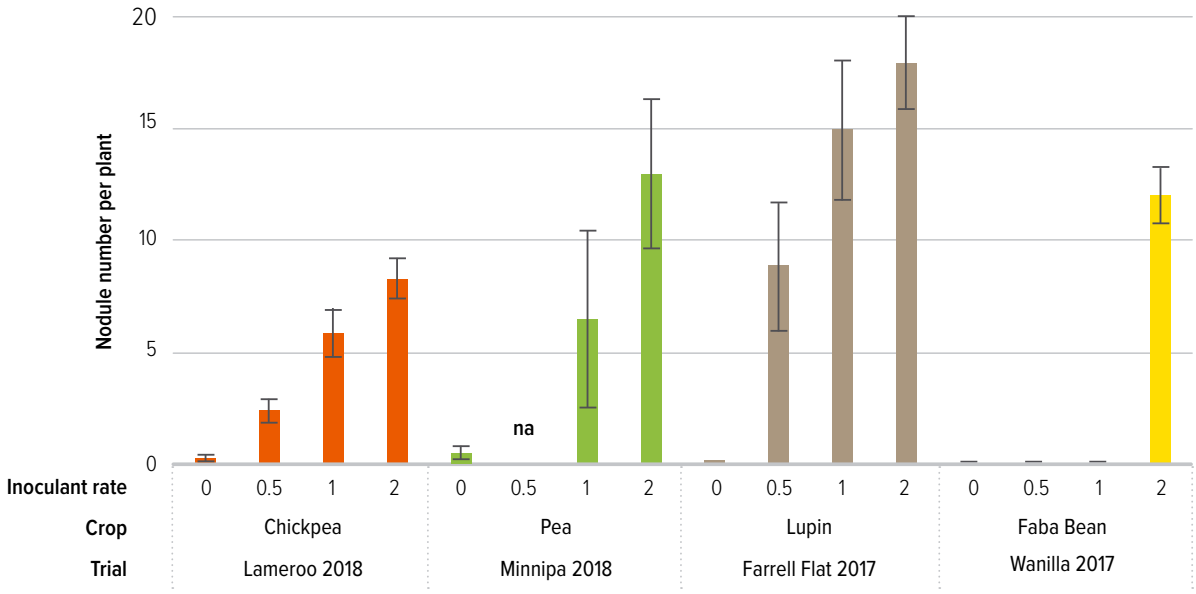
Research has shown that double the recommended inoculant rate consistently improves nodulation where the rhizobia or host plant are stressed. (Figure 2-4). When applying double rates, use twice the inoculant in the same amount of water as for the single rate. Do a small batch test first to avoid seeder blockages, especially with smaller seeded legumes. Granular inoculants may also provide benefits from increased rates under adverse sowing conditions (see section 2.11), based on limited research. See Figure 2-5 (page 18) to help determine if increased inoculation rates may be beneficial.

FIGURE 2-3. Rhizobial numbers on seed at sowing and their effect on nodulation and grain yield of narrow leaf lupin.



Source: Roughley et al. 1993.

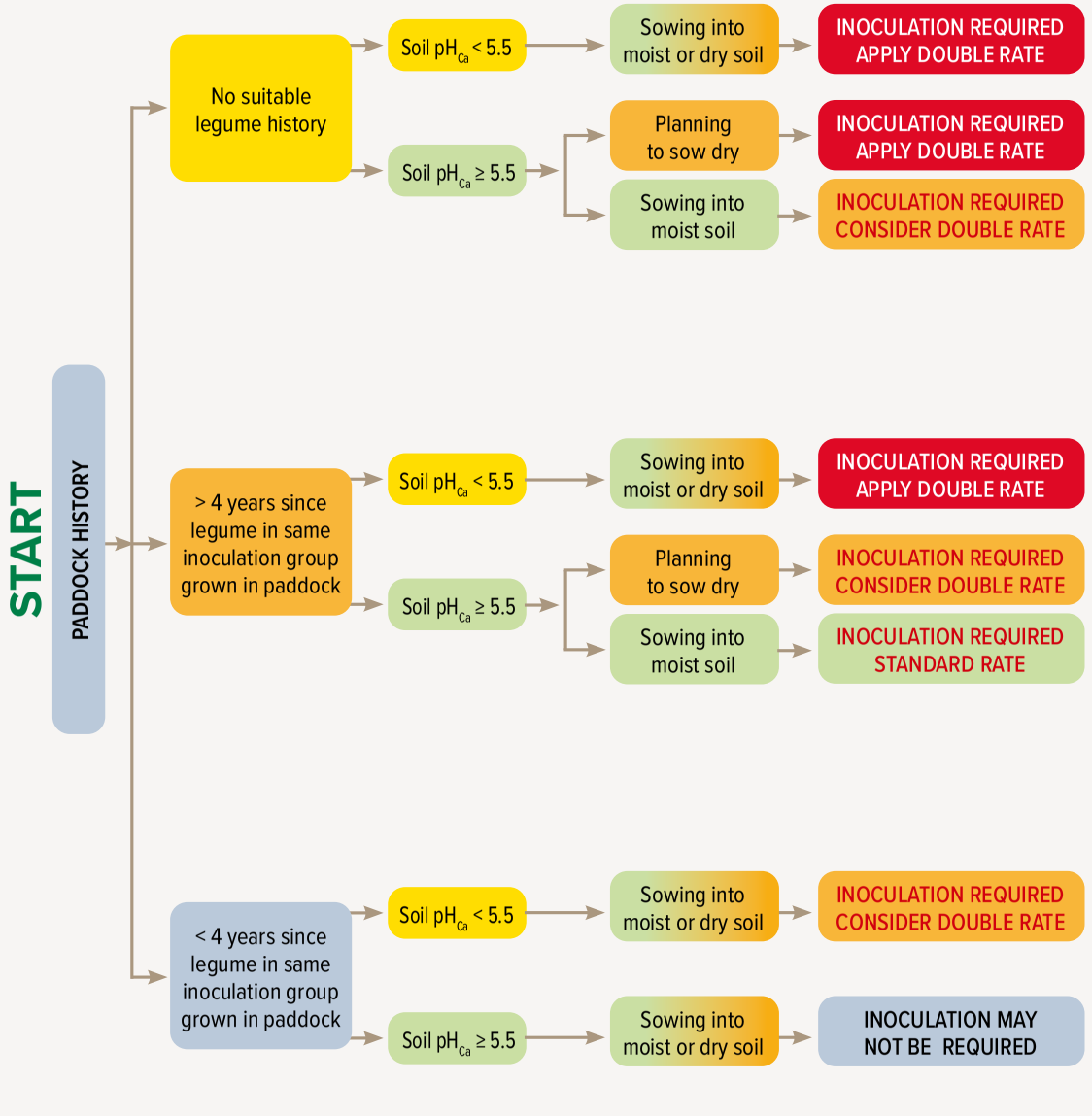
FIGURE 2-4. Nodule number responses in hostile sowing conditions to varying rates (0, 0.5, 1, 2 times standard rate) of peat inoculant applied to seed. Modified from Farquharson *et al.* 2018 & Ballard *et al.* 2019. Bars indicate standard error of mean within a trial. *Nodule weight per plant measured at Wanilla was used to estimate nodule number.



Inoculant - standard or double rates

Refer to chapter 9 to aid your decision making. The considerations for doubling inoculant rate of peat slurry on seed are similar to those used for determining whether inoculation is required in the first instance:

FIGURE 2-5. Decision flow chart to help determine if doubling the amount of peat inoculant used on seed should be considered.



***INOCULATION REQUIRED, APPLY DOUBLE RATE**

**Double rate inoculation should be used where rhizobia are absent or numbers too low for adequate nodulation, which is likely where there has been no recent legume history, AND when soil or sowing conditions are likely to reduce the survival of rhizobia on inoculated seed.

For example: No legume history AND soil pH < 5.0

Nodulation and growth of grain legumes, other than lupin, will be severely compromised when pH < 4.5. Lime soil to pH 5.5 (except for lupin) or consider acid tolerant legumes e.g. lupin and serradella.

***INOCULATION REQUIRED, CONSIDER DOUBLE RATE**

**Double rate inoculation should be considered if rhizobia are absent, even if soil conditions are non-stressful, OR when hostile soil conditions have reduced rhizobia numbers to an insufficient level and are likely to reduce the survival of rhizobia on inoculated seed.

For example: No legume history, soil moist and pH ≥ 5.5 OR recent legume history but soil pH < 5.5

***INOCULATION REQUIRED, APPLY STANDARD RATE**

When rhizobia present in soil, but not in sufficient number because a legume in same inoculation group has not been grown in the paddock in the previous four years, AND when soil conditions at sowing are conducive for survival of rhizobia on inoculated seed.

INOCULATION MAY NOT BE REQUIRED

When suitable rhizobia are likely to be present in adequate numbers because a well nodulated legume in the same inoculation group was grown in the last four years and soil conditions are conducive to rhizobia survival.

Dry sowing and seed applied fungicides are unlikely to constrain nodulation.

* Inoculation requirement for legumes in the E and F inoculation groups (faba bean, lentil, field pea and vetch) can be measured using PREDICTA rNod provided by SARDI. Tests for Group G and S, and N are under development.

** For double rate inoculation add twice the amount of peat to the same amount of water recommended for single rate. A small test batch is recommended to confirm the inoculated seed is unlikely to cause seeder blockages, especially with smaller seeded legumes.

2.7 Which formulation of legume inoculant should I use?

A range of different inoculant formulations are available to Australian legume growers. For crop legumes, the most popular inoculant formulation is peat, followed by granular, freeze-dried and liquid formulations (Figure 2-7, page 22). In the case of pasture legumes, they are commonly preinoculated by commercial seed coaters prior to purchase by the farmer. Where this is not the case, more likely in WA, pasture legumes can also be inoculated with peat and freeze-dried formulations and, with increasing frequency, granular inoculants.

Inoculant manufacturers can sign up to a voluntary code of conduct for quality control. As part of this arrangement they submit random packets or samples of inoculant from selected batches which are independently tested by the Australian Inoculants Research Group (AIRG). Only batches that pass stringent standards carry the Green Tick Logo (Figure 2-6, see Chapter 4). Each packet has a use-by-date, which should be adhered to.

In selecting an inoculant formulation, consider the following characteristics outlined in this chapter and summarised in Table 2-2 (page 22).

FIGURE 2-6. Registered trademark for inoculant quality – the Green Tick Logo.

Inoculant formulations

PEAT

- High numbers of rhizobia per gram of product mean application is very cost effective, but extra labour is needed for inoculating seed at sowing time.
- Can be applied as slurry on seed or in-furrow.
- Best results achieved when sowing into moist soils. Can be applied in some dry sowing scenarios.
- Can be applied at higher rates (e.g. double), especially under dry sowing or acidic soil conditions.
- For double rate, use twice the inoculant in the same amount of water as single rate.
- Do a small batch test first to avoid seeder blockages, especially with smaller seeded legumes.
- Some seed-applied chemicals e.g. fungicides containing thiram, are detrimental to rhizobial survival when peat is applied to seed.
- In-furrow application allows the separation of the inoculant from potentially harmful seed applications such as fungicides, insecticides and trace elements.
- Requires addition of adhesive (sticker), unless indicated by manufacturer.

FREEZE- DRIED

- High numbers of rhizobia per gram of product mean application rates are low, and can be very cost effective.
- Can be applied on seed or in-furrow.
- Suitable for sowing into moist soils. Not suitable for application when sowing into dry soils.
- Can be applied at double rate if sowing legume for the first time.
- Do not use in tank mixes with pesticides, fertilisers or trace elements.
- Recommended to be sown within five hours of seed application.
- In-furrow application allows the separation of the inoculant from potentially harmful seed applications such as fungicides, insecticides and trace elements.

GRANULES

- Several granular formulations are available using different clays or other carriers.
- Have fewer rhizobia per gram of product than peat and freeze-dried, and therefore must be applied at higher rates
- Applied in-furrow, ideally via separate seeder box.
- Granular formulations can provide good nodulation under dry sowing conditions compared to other formulations.
- Granules allow separation of inoculant from seed applied chemicals.
- Granules can be applied at variable rates using precision-farming technology where paddock conditions and rhizobia requirements are well understood.
- The ease of application compared to other formulations makes granular inoculants attractive to some farmers.

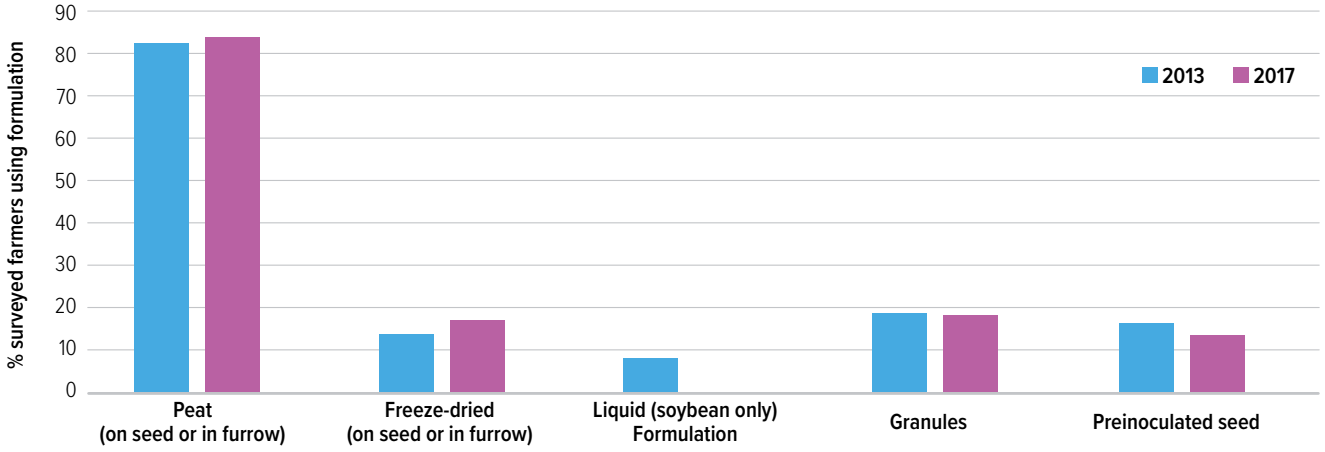
LIQUID

- Currently only available for soybean in Australia.
- High numbers of rhizobia per gram of product.
- Can be applied on seed or in-furrow.
- Suitable for sowing into moist soils. Not suitable for application when sowing into dry soils.
- Do not use in tank mixes with pesticides, fertilisers or trace elements.
- Recommended to be sown within five hours of seed application, do not expose seed to direct sunlight.
- In-furrow application allows the separation of the inoculant from potentially harmful seed applications such as fungicides, insecticides and trace elements.

PREINOCULATED PASTURE SEED

- **It is possible to purchase pasture legume seeds inoculated with rhizobia as part of a seed inoculation or coating package.**
- **May also be coated with insecticides, fungicides, plant beneficial micro-nutrients and colourants.**
- **Provides flexibility but risk of lower rhizobia survival than freshly inoculated seed.**
- **Available for lucerne, annual medics and clovers.**
- **Common in eastern states of Australia.**
- **Two week expiry for red and white clovers.**
- **Six week expiry for subterranean and annual clovers.**
- **Six months expiry for lucerne.**

FIGURE 2-7. Use of the different inoculant formulations for crop and pasture legumes by Australian farmers. The surveys were conducted in 2013 and 2017 and involved a total of 560 farmers (Ryder *et al.* 2017).



2.8 Requirements for storing and handling inoculant

TABLE 2-2. Inoculant formulations available to Australian growers.

Inoculant formulation	Composition	Storage	Application options		Sowing Conditions		Sowing Window (sow within...)	
			On Seed	In furrow	Moist soil	Dry soil	Slurry on Seed	In furrow
Peat	High organic matter soil, milled and irradiated, with rhizobia added in a nutrient suspension	4 to 10°C	✓	✓ liquid suspension	✓	maximum 7 days before rain*	24 hours	Within 6 hours of mixing
Freeze dried	Concentrated pure cells of rhizobia following extraction of water under vacuum	4 to 10°C	✓	✓ liquid suspension	✓	✗	5 hours	Immediately following dilution
Granular	Clay or peat granules impregnated with rhizobia	Cool, Dry	NA	✓	✓	✓		Not applicable
Liquid	Suspension of rhizobia in a protective nutrient solution	4°C	NA	✓ liquid suspension	✓	✗		Immediately following dilution
Preinoculated seed	Seed coated with peat inoculant, polymers and agrochemicals	Cool, Dry	NA	NA	✓	✗		2 wks-6 months #Species variable

 # 6 months for lucerne and annual medics, 6 weeks for sub clover, 2 weeks for white and red clovers and other speices. (Gemell et al. 2005)
**Double rate recommended. Efficacy in dry soils may be reduced in soils where pH_{CaCl_2} <5.5.*

Important considerations with storing inoculants:

- Always use inoculants before their use-by-date.
- Avoid exposing inoculants to temperatures above 25°C, e.g. in a vehicle. Use an insulated box to keep inoculants cool.
- Minimise exposure to direct sunlight.
- Reseal inoculant packages after opening to reduce moisture loss and avoid contamination.

Safety precautions

Rhizobia are not considered dangerous to human health, however some caution should always be taken when handling inoculant products. This is because products can be dusty and may contain organisms other than rhizobia. For this reason, a dust mask and gloves should be used when handling dry products and manufacturer guidelines with respect to safety should be followed.

USING PEAT INOCULANTS

Peat inoculants are best applied as a slurry on the seed but can be mixed with water and injected into a moist seedbed at sowing. The exception to this is Bayer Tag-Team peat which can also be applied dry using an applicator (see manufacturer for details). Simply sprinkling standard peat direct from the pack into the seed box is not recommended because the inoculant won't stick to the seed in sufficient quantity, leading to inadequate and uneven inoculation.

FIGURE 2-8. Peat inoculant is easily seen on inoculated soybeans (left) when compared with uninoculated seed (right).

Photo: Akitomo Kawasaki, AIRG, NSW DPI.

PEAT SLURRY INOCULATION OF SEED

1. Ensure you have selected the correct inoculant group for your crop or pasture (Table 2-1, page 16).
2. Use a clean container or drum and clean potable water (rainwater recommended). If possible, the pH of the water should be checked to ensure it is between 6.0 and 7.0 or rapid death of the rhizobia will likely result. It is critical to avoid chlorinated water and toxic chemicals and residues, particularly if the water is sourced from bore water or a storage tank. The water must not contain high levels of dissolved salts, spray rig washings containing pesticides or detergents, or swimming pool water that may be chlorinated.
3. Mix the packet of peat into the specified quantity of water. For pasture legumes or where specified by the manufacturer add a pre-prepared sticker if required to ensure effective contact with seed and to protect the rhizobia from desiccation. See section 'Adhesive solutions' and 'Inoculating and lime pelleting pasture legumes'.
4. Stir or use a pump to mix inoculant (Figure 2-9b, page 24 and Figure 2-10a, page 26). Allow a minimum of 15 minutes for full dispersal of peat and activation of the sticker.
5. Apply the peat solution over the correct weight of seed and mix until all seeds are evenly coated.
   a. For small operations this can be done in a concrete mixer, or by shoveling by hand on a cement floor or tarpaulin.
   b. For larger operations use a rotary coater, applicator or auger. Slurry inoculant can be applied to the seed during various pre-seeding transfers including augering of seed from a silo to truck, or truck to seeder. Care must be taken to avoid crushing or cracking the seedcoat. The slurry must be applied evenly to ensure consistent distribution across the seed lot. See field operations (Figure 2-9, page 24 and 25).
6. Sow the seed within **24 hours** of inoculation.

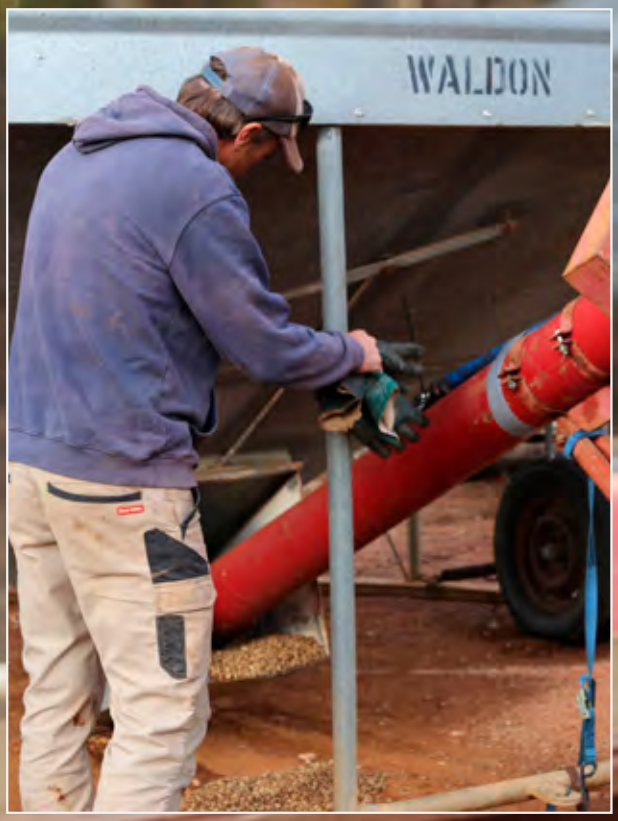
FIGURE 2-9 (a). Peat slurry inoculation of faba bean on farm – setup includes spray tank with pump, liquid slurry is transferred to auger and coats seed en route to truck/seeder bin prior to sowing.

Photos: Belinda Cay, AgCommunicators.

FIGURE 2-9 (b). Peat is added to clean water in clean spray tank where mixing occurs, pump allows for easy and adjustable flow to seed.

FIGURE 2-9 (d). Inoculated seed is deposited in truck. Freeze-dried inoculant can be applied to seed in the same way as peat slurry and according to the manufacturer's instructions.

FIGURE 2-9 (c). Hose attached to auger delivers peat to seed.



Photos: Belinda Cay, AgCommunicators.

Adhesive solutions for slurry inoculation of seed

Where adhesive is already included in commercial peat inoculants, additional sticker should not be required. Where adhesives are required, concentration will vary with the type of sticker and whether seed is lime pelleted.

Adhesives include methyl-celluloses such as Methocel™ and other proprietary products such as StickA™. To make the sticker, use potable water where possible and clean equipment. For a one litre final volume: sprinkle the appropriate quantity (see below and refer to manufacturer recommendations) of the granulated adhesive powder on to 200mL of near boiling water (~80°C), stirring vigorously, until powder is dispersed. Slowly add 800mL of cold water while still stirring vigorously, until an even gel is produced.

Where seed is to be lime pelleted (pastures), a higher concentration of sticker is needed, usually 1.5 per cent (15g in 1000mL of water) for Methocel™ and 10 per cent (100g in 1000mL water) for StickA™.

Where seed is not lime pelleted (pulses), a lower concentration of sticker is needed, usually 0.5 per cent (5g in 1000mL of water) for Methocel™ and 2 per cent (20g in 1000mL water) for StickA™.

Cool the solution to less than 30°C and thoroughly stir the solution prior to use. The sticker is best prepared the day before inoculation and should be used within three days.

Combine peat inoculant and sticker together for immediate application to seed. See instructions for 'peat slurry inoculation of seed'.

Many other adhesives have been used to apply rhizobia to seed, however, not all adhesives are compatible or protective of rhizobia (Deaker *et al.* 2004; Deaker *et al.* 2007; Hartley *et al.* 2012). It is important to use only adhesives that are recommended for legume inoculants.

Example of peat slurry inoculation for smaller operations.

FIGURE 2-10 (a). Mixing peat slurry in a bucket.

FIGURE 2-10 (b). Slowly adding peat when augering the seed. Freeze dried inoculant can be applied to seed in the same way as peat slurry and according to the manufacturer's instructions.

Photos: Alistair Lawson, AgCommunicators.

Inoculating and lime pelleting pasture legumes

Temperate pasture legume seeds are often coated with fine lime immediately after the application of the peat inoculant slurry to help dry the seed, prevent clumping and improve flow of seed through seeding equipment (Figure 2-12, page 28). Lime pelleting is also used to support the nodulation of acid sensitive legumes such as lucerne and messina in acidic soils, and more generally, improve the survival of rhizobia on seed where there are delays between inoculation and sowing. It also protects rhizobia from fertilisers, such as superphosphate. Serradella is the only temperate pasture legume that is not routinely lime pelleted because its rhizobia are tolerant of acidity (see Chapter 9 Fact sheets). If necessary, dehulled serradella seed that has been slurry inoculated can be dried off with clay or finely ground gypsum (4kg to every 50kg of seed). Tropical pasture legumes (except Leucaena and Kenya White clover) do not require lime pelleting. Grain legumes are not usually lime pelleted.

Very fine lime (plasterer's whiting, calcium carbonate) such as Omyacarb5® should be used. Slaked or hydrated lime (calcium hydroxide) and builder's lime (quicklime, calcium oxide) are too alkaline and will kill the rhizobia – these should not be used. Keep in mind that the pellet can increase the weight of the seed substantially, so sowing rates may need to be adjusted.

The amount of lime needed is mostly determined by the amount of peat slurry applied and needing to be dried. Since more inoculant slurry is added to smaller seeded legumes, more lime is needed, per kg of seed (Table 2-3, page 28). However, the amount of lime needed is also affected by the legume species (which vary in their absorption of the slurry), as well as the specifications of the adhesive and coating product. While lime rates equivalent to ten times the amount of inoculant slurry have been recommended (12.5kg of lime for each 1.25L of Methocel™ based inoculant slurry added to seed), half this rate is often sufficient, and therefore the preparation of a small trial batch is always recommended.

To lime pellet pasture seed:

1. Pour the mixture of peat and sticker over the seed and mix in a rotating drum (concrete mixer) until seeds are evenly coated.
2. Immediately add the appropriate amount of very fine lime (such as Microfine Omyacarb®5) all at once to the rotating seed, and roll for one to three minutes.
3. Allow pelleted seed to dry in a cool place out of direct sunlight.

Example of field operations for inoculating and lime pelleting pasture seed.

Photos: Neil Ballard.

FIGURE 2-11 (a). Stir inoculant (with sticker) vigorously until all dissolved. A paint stirrer attached to drill is effective.

FIGURE 2-11 (b). Add peat slurry inoculant to seed and mix in a clean cement mixer or similar.

FIGURE 2-11 (c). Add fine lime to seed.

FIGURE 2-11 (d). Mix until evenly coated.

TABLE 2-3. Lime pelleting pasture seed; Quantity of peat inoculant, adhesive solutions and recommended amount of coating agent (lime) required to treat 50kg of pasture seed.

	Materials required for 50kg of pasture seed and *indicative lime rate		
Pasture seed size (see Table 5-1, page 62)	Peat Inoculant	Methocel™ (1.5%) OR StickA™ (10%)	Coating agent (fine lime)
Very small e.g. lotus, biserrula	1,250g	5L	*31kg
Small e.g. lucerne	500g	2L	*13kg
Medium e.g. sub clover	250g	1L	*6kg
Large e.g. leucaena	125g	0.5L	*3kg

***PLEASE NOTE:** Indicative lime rate is based on half of the rates that have been previously recommended. Preparation of a small trial batch is always recommended, particularly if the process is being undertaken for the first time.*

Troubleshooting

Good quality pelleted seed is:

- evenly coated with the lime (see Figure 2-12); and
- firm enough when dry to withstand a light rolling between the fingers, without the lime flaking off.

Poor quality pelleted seed is:

- powdery, with soft pellets indicating too much lime or uneven mixing, or both.
- seed can also be pasty with the seed surface showing, which is caused by too much adhesive. This may be rectified by adding more lime.
- seed can also be clumped together, the result of too much adhesive or inadequate mixing prior to adding lime.
- hard, glossy or smooth caused by too little lime, or too much mixing after adding the lime.

FIGURE 2-12. Three different batches of lime pelleted clover seed after being inoculated with peat-slurry mix. The seeds on the left (powdery, soft pellets) display either too much lime or uneven mixing, while the seeds on the right (hard, glossy, smooth pellets) show too little lime or too much mixing after adding lime. The seeds situated in the centre indicate an even amount of mixing and lime addition.

Photo: Ronald Yates, DAFWA.

USING FREEZE-DRIED INOCULANTS

FREEZE-DRIED INOCULATION OF SEED

1. Ensure you have selected the correct inoculant group for your crop or pasture (Table 2-1, page 16).

2. Use a clean container or drum and clean potable water (rainwater recommended). If possible, the pH of the water should be checked to ensure it is between 6.0 and 7.0 or rapid death of the rhizobia will likely result. It is critical to avoid chlorinated water and toxic chemicals and residues, particularly if the water is sourced from bore water or a storage tank. The water must not contain high levels of dissolved salts, spray rig washings containing pesticides or detergents, or swimming pool water that may be chlorinated.

3. Prepare the freeze-dried vial by removing the bung and adding a small amount of cool, clean water. Follow manufacturer's instructions.

4. Dissolve the protecting agent in a container or drum using clean potable water. Follow manufacturer's instructions.

5. Add the freeze-dried vial contents to the prepared protecting solution. Stir or use pump to mix the inoculant thoroughly.

6. Apply the solution over the correct weight of seed and mix until all seeds are evenly coated.

7. For small operations this can be done in a concrete mixer, shoveling by hand on a cement floor or tarpaulin.

8. For larger operations use a rotary coater, applicator or auger. Liquid inoculant can be applied to the seed during various pre-seeding transfers including augering of seed from a silo to truck, or truck to seeder. Care must be taken to avoid crushing or cracking the seedcoat. The liquid must be applied in a calibrated flow to ensure consistent distribution across the seed lot.

9. Sow the seed within **five hours** of inoculation.

USING LIQUID AND SLURRY INOCULANTS IN FURROW

Ensure spray cart filters are 80µm mesh or coarser. Apply inoculant liquid mixture in-furrow at a rate of 50-100L/ha depending on seeding rates and water volumes able to be carried. The inoculant solution should come into contact with the seed once sown, for best results.

Tank mixes are best used as soon as possible after preparation.

LIQUID INJECTION INOCULATION USING PEAT, FREEZE-DRIED AND LIQUID INOCULANTS

1. Ensure you have selected the correct inoculant group for your crop or pasture (Table 2-1, page 16).
2. Fill a well cleaned spray tank with clean potable water (rainwater recommended). Ensure all tubing and nozzles are also well rinsed. It is important that the pH of the water is checked and is between 6.0 and 7.0 or rapid death of the rhizobia will likely result. It is critical to avoid chlorinated water, toxic chemicals and residues, particularly if the water is sourced from bore water or a storage tank.
3. Do NOT mix peat, freeze-dried and liquid inoculants with chemicals, fertilisers, fungicides or insecticides in the tank.
4. Spray tanks with agitators will aid mixing and dispersion of inoculant. Add inoculant to spray tank as follows:

   a. Freeze-dried: prepare freeze-dried vial contents and protecting agent in a clean bucket. Add mixed solution to tank. Follow manufacturer's instructions.

   b. Peat: place inoculant into a porous bag (e.g. calico, stocking) to reduce blockages and suspend bag inside the tank. Allow 15 minutes for product to disperse.

   c. Liquid: add contents to tank and allow time for dispersion.

FIGURE 2-13 (a). Spray line attached behind planting tyne/boot used to disperse inoculants into furrow (water injection).

Photos: Nikki Seymour, QDAF.

FIGURE 2-13 (b). Water tank mounted on tractor to apply inoculants by water injection.

USING GRANULAR INOCULANTS

Types of granules

Granular inoculants can be manufactured from prilled peat, clay (bentonite) with rhizobia incorporated or clay granules (attapulgite) coated with peat. Granules vary in appearance and characteristics such as particle size and uniformity (Figure 2-15, page 33). Granules should be stored in a dry, cool area, raised off the floor (e.g. on pallet) and away from direct sunlight.

Granules can be used in either dry or moist soils. It important to note products may vary in their efficacy due to a combination of rhizobia strain, formulation, and sowing conditions (soil moisture, soil temperature, soil acidity and the time between application and crop emergence) and should be used in line with manufacturer recommendations.

A common feature of granular inoculants is that they have far fewer rhizobia per gram than peat and freeze-dried inoculants (Denton *et al.* 2009, 2017). However, granular inoculants protect the rhizobia contained within them during the inoculation process, unlike the other formulations where the rhizobia are less protected and may die on the seed or in the soil. Granules are typically applied at 2.6 to 10kg/ha when sowing on 18cm row spacings, depending on the manufacturer, the strain and number of rhizobia per gram of product (Table 2-4, page 33).

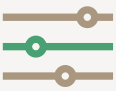
FIELD OPERATIONS FOR GRANULAR INOCULANT

1. Ensure you have selected the correct inoculant group for your crop or pasture (Table 2-1, page 16).
2. Keep granules dry during handling and application as they can absorb moisture. This will help avoid coagulation and blockages. Do not leave granules in seeder or hopper box overnight.
3. Ensure the equipment (hopper, rotor, feed mechanism, and calibration equipment, etc.) is free from chemical residues, fertiliser, and debris.
4. Granular products can be dusty and may contain organisms other than rhizobia. For this reason wear appropriate personal protective equipment (e.g. mask/respirator, safety glasses and gloves) as per manufacturer's guidelines.

5. It is generally preferable not to mix granules with fertiliser, although manufacturers' recommendations vary and should always be followed.

Excessive augering should be avoided to ensure that the particle size is maintained and to minimise dust. Granules are best distributed through a separate sowing box, rather than mixed with seed because size differences between granule and seed may result in separation, settling and uneven distribution of both granules and seed.

6. Calibrate machinery to deliver the correct amount of granule for your row spacing. This can range from 2.6kg/ha up to 10kg/ha. Check manufacturer's guidelines and rate charts. Granules can be applied at variable rates based on existing paddock knowledge of factors affecting rhizobia populations such as soil pH.



7. For best results, apply granules in-furrow, rather than broadcast. Granules can be sown into moist or dry soil.

See examples of field operations Figure 2-14, page 32

Example of field operations for use of granular inoculants

Photos: Liz Farquharson, SARDI.

FIGURE 2-14 (a). A seeder allowing separation of seed, fertiliser and rhizobia granules prior to seeding.

FIGURE 2-14 (b). Third box on the back of the seeder containing granular inoculant.

FIGURE 2-14 (c). Adding granular inoculant to the third box. This should always be done just prior to sowing and excess product should not be stored in the box for extended periods.

FIGURE 2-14 (d). For this air-seeder the seed, fertiliser and granules are mixed together in the air flow just prior to delivery to the soil furrow.

FIGURE 2-14 (e). Beans with granular inoculant being sown into a cereal stubble.

FIGURE 2-14 (f). The grower has pre-mapped this paddock for soil pH and is using GPS and variable rate technology to apply granular inoculant at higher rates on the low pH areas of the paddock and lower rates on the neutral areas.

FIGURE 2-15. Granular inoculants from left to right: clay granules (attapulgite) coated with peat, clay (bentonite) with rhizobia incorporated and prilled peat.

Photo: Ashlea Webster, AIRG, NSW DPI.

TABLE 2-4. The influence of row spacing on application rates for three different types of granular inoculant

Row spacing (cm)	17.8	20.3	23	25.4	27.9	30.5	33	35.6	38
Attapulgite clay granule rate (kg/ha)	6	5.3	4.6	4.2	3.8	3.5	3.2	3	2.6
Peat granule rate (kg/ha)	5.6	4.9	4.4	3.9	3.6	3.3	3	2.8	2.6
Bentonite clay granule rate (kg/ha)	10	10	10	10	10	10	10	10	10

2.9 Preinoculated seed

Some seed companies sell pasture legume seeds that have been inoculated with rhizobia as part of a seed inoculation or coating package. Seed coated with rhizobia may also be coated with insecticides, fungicides, plant beneficial micro-nutrients and colourants (Figure 2-16). Pre-inoculation provides farmers with more flexibility at sowing, but adds the risk of providing lower rhizobia numbers compared to freshly inoculated seed. These risks are reduced and nodulation potential increased where the time between inoculation and sowing is minimised.

Pre-inoculation is mainly used for pasture species, such as lucerne, annual medics and sub clovers and is particularly common in the eastern states of Australia. However, rhizobia survival on pre-coated seed is highly variable due to the fragile nature of some of the strains of rhizobia, particularly those for red and white clover, where 2 weeks expiry after coating is recommended. In comparison, subterranean and other annual clovers are more tolerant and a 6 week expiry after coating is recommended. If the recommended use-by date has expired, then the seed will need to be re-inoculated before sowing.

In addition, the range of additives, application and drying methods, and storage times employed by different seed coating processes can affect the number of rhizobia surviving on seed at point of sale (Hartley *et al.* 2012, Deaker *et al.* 2012). Testing of preinoculated seed samples collected from retail outlets has shown that many samples did not meet the Australian Inoculant Research Group (AIRG) standard for numbers of rhizobia on the seed required for adequate nodulation (Gemell *et al.* 2005) (see Chapter 4).

If purchasing preinoculated seed for clovers, serradella, biserrula and sulla, ensure the seed has been freshly coated, as rhizobial numbers can reduce significantly within days for these species.

FIGURE 2-16. Preinoculated lucerne seed.

Photo: Rosalind Deaker, University of Sydney.

2.10 Compatibility issues between seed-applied inoculants and fertilisers, chemicals and pesticides

As rhizobia are living microorganisms, it is very important that inoculants are kept away from toxic substances that reduce their viability, such as certain fertilisers, fungicides, insecticides and herbicides (Rathjen *et al.* 2020). In particular, inoculated seed should not come in direct contact with fertilisers, especially fertilisers such as triple super and MAP, because they can kill the rhizobia through a zone of reduced pH and high salt concentrations near the granules as they dissolve. Certain pesticides can also decrease both rhizobial survival and nodulation.

There are three major factors to be considered:

- **Are the chemicals or fertilisers acidic or alkaline in solution?** Most rhizobia are sensitive to solutions with pH_{CaCl_2} values below 5.0 or above 7.5.
- **Do the preparations contain toxic chemicals?** Metals such as manganese, copper and zinc are harmful. The effects of other active ingredients may be difficult to predict.
- **How long is the time of contact between a substance and inoculated seed?** Direct contact between the inoculated seed and other substances should be avoided where possible. If contact is unavoidable, but for only a short period, detrimental effects on the rhizobia may be reduced.

Fertiliser compatibility

Superphosphate, MAP and related products have an acidic reaction when they dissolve and create areas of high salt concentration in the soil near the fertiliser granules which are toxic to rhizobia when in direct contact with inoculated seed. Contact between seed and fertiliser should be avoided even if the seed has been lime pelleted. With the exception of bentonite inoculant granules, the mixing of fertilisers with granules is not recommended.

Most trace element seed dressings, including zinc, manganese and copper, are toxic to seed applied inoculants and should be avoided. If the legume seed has a trace element dressing, a granular or liquid inoculant separated from the seed may be the best practice. Some formulations of molybdenum are compatible with rhizobial seed inoculants.

Adding molybdenum at inoculation

Low molybdenum (Mo) in the soil can cause a reduction in nodulation and N fixation in a legume crop, particularly in soils with a low pH_{CaCl_2} (<6.0) that have reduced Mo availability. Applying Mo to seed at inoculation is cost-effective and ensures an even distribution of Mo across the paddock. Use either molybdenum trioxide (66 per cent Mo) or ammonium molybdate (54 per cent Mo) for seed application. Do not use sodium molybdate as it is toxic to rhizobia. When sowing pasture legumes in Mo-deficient soils, 50g of Mo is required per hectare. If seed treatment is not possible, then Mo-fortified superphosphate can be used at sowing. A rate of 250kg/ha of 0.02 per cent Mo superphosphate will deliver the required quantity of Mo.

In Mo deficient soils, a maintenance application of 25g of Mo per hectare (e.g. 125kg/ha of 0.02 per cent Mo superphosphate) should be applied every four to five years. In some areas, positive responses to larger quantities of Mo have occurred. Check local recommendations. However, it is important not to apply excess quantities of Mo as this can result in development of copper deficiency in grazing livestock.

Fungicide compatibility

Seed-applied fungicides are a preventive treatment to kill or inhibit fungi that may cause plant disease, such as Ascochyta of chickpea. Some seed-applied fungicides (sometimes called pickles) can reduce the survival of rhizobia on seed. Information supplied online by inoculant companies and chemical companies on fungicide-rhizobia compatibility is

TABLE 2-5. Compatibility (seed inoculation) of different rhizobia groups when mixed with seed-applied fungicides and insecticides. Information sourced from commercial product information guides (BASF and Novozymes) and research data. (Rathjen *et al.* 2020)

Inoculant group / crop	Pesticide type	Planting window of peat inoculated seed
E - field pea, vetch	Thiram (360g/L) + Thiabendazole (200g/L)	*Not compatible
	Imidacloprid (600g/L)	24 hours
F - faba bean, lentil	Thiram (360g/L) + Thiabendazole (200g/L)	*Not compatible
	Thiram (600g/L)	*Not compatible
	Imidacloprid (600g/L)	24 hours
G - lupin	Thiram	*Not compatible
	Iprodione (250g/L)	6 hours
H - soybean	not compatible with seed dressings	
N - chickpea	Thiram (360g/L) + Thiabendazole (200g/L)	*Not compatible
	Thiram (600g/L)	*Not compatible
	Metalaxyl-M (350g/L)	6 hours
peanut	not compatible with seed dressings	

** When no background rhizobia are present and pulse diseases are a significant risk, the separation of rhizobia inoculant from seed is strongly recommended.*

not always consistent. Rhizobial survival is dependent on the toxicity of the product to the rhizobia and period of time that the inoculant is in contact with the seed-applied fungicides prior to sowing. The acceptable times between coating fungicide-treated seed with inoculant and sowing are shown in Table 2-5 (page 34). Thiram is particularly toxic to rhizobia: when using fungicides containing thiram as a seed dressing, inoculant is best applied separately in furrow, either as a granular or a liquid formulation. If there is no alternative to inoculating rhizobia onto legume seed that has already been treated with a fungicide, ensure this is sown immediately into moist soil or, as per the manufacturers' recommendations.

Insecticide compatibility

- Bendiocarb and permethrin, used to protect seed from ants, are safe (although limited trials indicate that there may be some reduction in nodulation).
- Imidacloprid is safe to use with rhizobia provided treated seed is sown into moist soil as soon as possible, but no longer than 24 hours after the inoculant has been applied.
- Dimethoate can harm rhizobia.
- Follow label instructions carefully.

FIGURE 2-17. Above ground damage to lentil due to group 4 (I) herbicide residues.

Photo: Andy Bates, Bates Agricultural Consulting.

Herbicide compatibility

Herbicides can damage legume shoots and roots and reduce N fixation (Figure 2-17), even though rhizobia themselves are relatively tolerant of herbicide concentrations recommended for field use. Because of the differences in susceptibility between the different host symbioses, it is difficult to make accurate assessments of the general impact of herbicides and additives on all legumes and rhizobia, and the specific effects on plant growth, nodulation and N fixation.

Some herbicides with residual soil activity such as group 2 (B) (e.g. sulfonyl urea (SU) herbicides) and group 4 (I) (e.g. clopyralid), especially on alkaline soils, may significantly impact root health. SU herbicides have been shown to reduce root hairs (Martensson and Nilsson, 1989, Holmes *et al.* 2006), which are essential for nodulation. While the development of herbicide-tolerant legume cultivars is in part overcoming negative effects of herbicide on the legume symbiosis, adherence to all plant back requirements for herbicides is essential to avoid impacts on legume growth and, in turn, nodulation and N fixation. Herbicides can differ in terms of plant back requirements for sensitive crops depending on soil texture, pH, rainfall and application timing and rate. Research is ongoing to clarify our understanding of these interactions. See Chapter 7 – Legume Nitrogen Fixation for further information regarding impacts of some herbicides on N fixation.

2.11 Sowing inoculated legumes into hostile soils

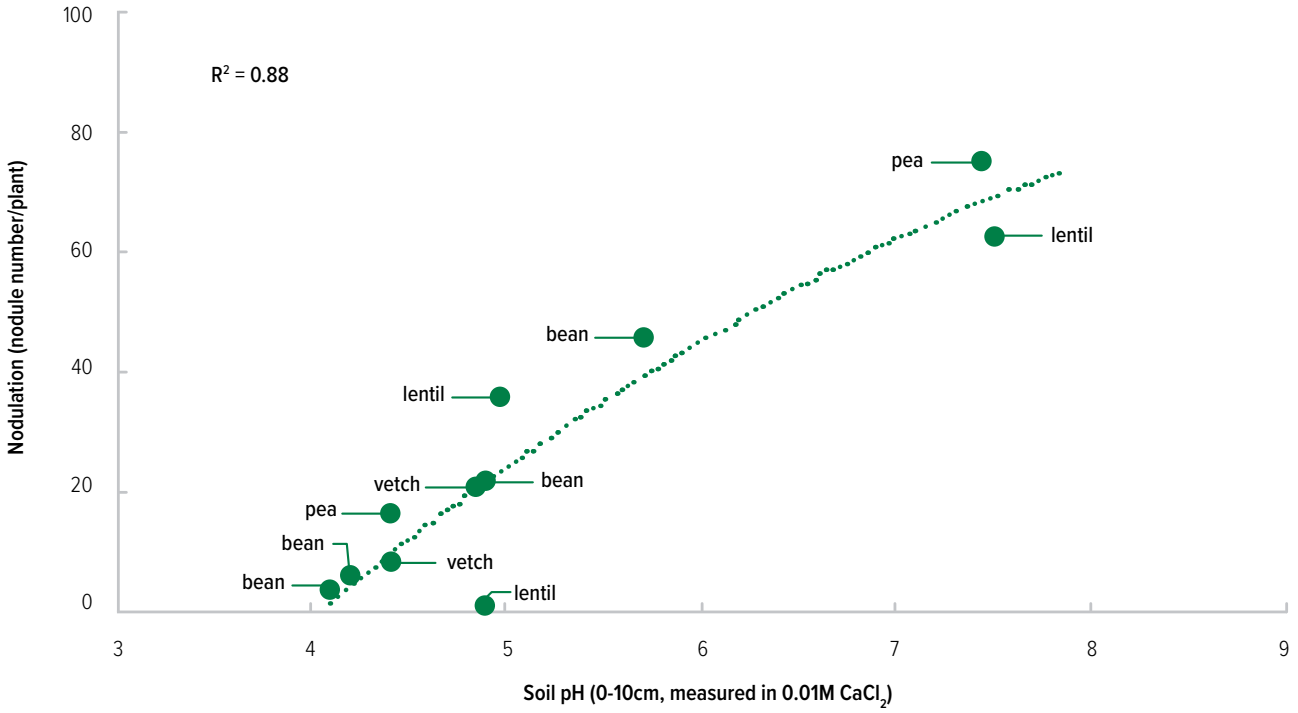
Soil acidity

Soil acidity is a widespread issue, especially in western and southern Australia. Acidity alone can have detrimental impacts on crop and pasture legumes and their associated rhizobia. However, acidity is often coupled with increasing availability of potentially toxic elements such as aluminium and manganese which can impact root growth, rhizobia survival, the nodulation process and capacity for fixation of nitrogen.

Pulse rhizobia species vary in their sensitivity to soil acidity (chapters 5 & 6). The rhizobia that nodulate legumes in the E (field pea, vetch), F (faba bean, lentil) and N (chickpea) inoculation groups are the most sensitive to soil acidity, especially below pH_{CaCl_2} 5.5. However, lupin (Group G) rhizobia are quite tolerant of acidic soils.

For pasture species, very similar rhizobia to lupin (Group G) are used to inoculate serradella (Group S), and these rhizobia have a good tolerance of acidic soils. The rhizobia used for biserrula is also relatively tolerant to soil acidity. The rhizobia used to inoculate annual (Group C) and perennial clovers (Group B) generally has intermediate tolerance while the rhizobia used to inoculate medics (Group AL or AM depending on plant species) and lucerne (Group AL) are sensitive to soil acidity. Lime pelleting can improve the survival and nodulation by rhizobia at pasture establishment for clover, medics and lucerne. However, sensitivity of the host plants to soil acidity generally follows a similar pattern to rhizobia, and consequently, overall productivity of sensitive host plant-rhizobia combinations will be limited in acidic soils.

FIGURE 2-18. Nodulation of a range of pulse crops in the E-F inoculation group showing the impact of declining soil pH on nodule number per plant (Adapted from, Ballard *et. al.* 2019).



The sensitivity of rhizobia to soil pH is discussed more fully in Chapter 6. In addition to rhizobia numbers being reduced in acidic soils, plant health and the nodulation process can also be impacted as shown in Figures 2-18 and 2-19.

Farmers are wanting to grow pulses in areas with acidic soils and this is presenting issues with nodulation, plant establishment and crop growth (Yates *et al.* 2021). These areas are likely to have low background levels of suitable rhizobia, and hence, will benefit from rhizobial inoculation, especially where grain legumes are grown in the paddock for the first time or have been infrequently grown. Sowing into acidic soils, particularly when combined with other practices which are stressful to rhizobia, such as dry sowing or the application of pesticides to seed, can profoundly reduce the survival of rhizobia, the success of nodulation, and subsequent performance of the pulse crop. There are several strategies growers can use to optimise nodulation and pulse crop performance which are outlined in Figure 2-20, page 37.

In many areas soil acidity also impacts the nodulation of pasture legumes. Recent surveys in the mixed farming and permanent pasture areas of NSW (Hackney *et al.* 2019) found 93 per cent of paddocks had inadequately nodulated pasture legumes, associated with low soil pH. Of the few paddocks where legume nodulation was adequate, all had a soil pH within the range considered adequate for optimal survival of the rhizobia associated with the host legume and for growth of the host legume. Clearly, there is a major opportunity to improve pasture legume nodulation, N fixation and production through increased lime applications and inoculation practices.

FIGURE 2-19. Faba beans grown at Wanilla in trials showing the difference between nodulation failure (left) and successful nodulation (right).

Photo: Liz Farquharson, SARDI.

Inoculating legumes in acidic soils

Growers sowing legumes into acidic soil conditions where pH_{Ca} in the 0-10cm layer is less than 5.5 have a number of options to consider to increase the chances of growing successful crops. Key considerations include:

FIGURE 2-20. FLOWCHART – Inoculating grain legumes in acidic soils.

* Number of E/F rhizobia in soil can be measured using PREDICTA rNod provided by SARDI, tests for Group G and S, and N are under development.

**Where inoculation is required, avoid direct contact between rhizobia and seed applied pesticide treatments.

Plant and rhizobia adaptation to acidic soils

One option to optimise legume production on acidic soils is choosing host legume-rhizobia combinations that are best adapted to specific soil conditions. For example, in soils where pH_{CaCl_2} is less than 4.8, highly tolerant species such as lupins and serradella and moderately tolerant species such as sub clover (except sub species brachycalycinum) will be better able to nodulate and fix N than sensitive species. Biserrula is reported to be slightly less tolerant of acidic soils than serradella. Bladder clover is recommended and has been grown in soils with pH_{CaCl_2} ranging from 4.8 to 6.0 and is generally regarded as slightly less acid tolerant than both serradella and biserrula. As soil pH increases to between 5.5 and 7.5 most crop and pasture legume species and their rhizobia are able to perform well. On strongly alkaline soils, where pH_{CaCl_2} is more than 8.0 fewer crop and pasture legumes options are suited. For example, serradella and narrow leaf lupin are intolerant of alkaline soils, particularly where free lime is present. In these more alkaline environments barrel, strand and disc medics, vetch and field pea are better suited.

While there are general associations between the pH preferences of particular crop or pasture legume species and their associated rhizobia, considerable gains have been made to select and breed genotypes of both that are adapted to soil pH levels outside the optimal range.

One example is the search for lucerne cultivars and rhizobial strains with improved acidity and aluminium tolerance. Rhizobial strain SRDI736 was selected for use with SARDI 7 Series 2 lucerne on soils of pH_{CaCl_2} 4.5-5.0 (Figure 2-21). Similar work is underway to identify potential Group E/F and Group N rhizobia strain alternatives with improved soil acidity tolerance.

Any farming system that removes product, be it in the form of grain or livestock, will acidify. Hence, without amelioration, soil acidification will continue to occur and pH will decline, especially on sandy soils with low pH buffering capacity. It must be remembered that while pursuing improved adaptation of both host legume and rhizobia to acidic soil conditions may be a good short to medium term solution to acidic soils, in the long term, amelioration of soil acidity through the application of lime must be considered to avoid further declines in soil pH and impacts on root growth and general crop health in sensitive species (Table 2-6, page 40). Liming costs can be significant in areas where local supplies are limited. However, amortised over a period of 10-15 years, the cost of liming and the benefit it can bring in terms of increasing crop and pasture choice and production, needs to be considered.

Identifying and rectifying soil acidity

Soil acidity should be determined using a careful soil sampling strategy and commercial soil testing laboratories. For the purposes of rapid in-field determination soil pH test kits available from gardening centres can provide an indication of pH. However, it must be remembered that pH test kits will generally give a pH 0.5-1.0 units higher than that given by laboratory determination of pH measured in calcium chloride.

Test kits can be useful to quickly assess variation in pH within or between paddocks or down the soil profile. In-field pH testing should be followed up by laboratory testing for accuracy.

Where subsurface acidity is likely (typically in the 7-15cm layer), growers are encouraged to sample soils in increments of 5cm to a depth of 25cm to diagnose potential stratification issues (Condon *et al.* 2020). This is because the top 2-5cm of soil is usually alkaline, and mixing this with deeper layers will provide a misleading result. A soil core to 25cm depth can be readily obtained from most soils using a 'dig-stick' allowing the pH to be determined by application of the pH kit chemicals down the length of the core (Condon *et al.* 2020), Figure 2-22, page 39. These cores can also be used to carefully collect segmented soil layers for more precise laboratory analysis. Alternatively, digging a hole with a sharp shovel to 30cm depth allows for the application of the pH kit chemicals down the vertical face of soil profile to quickly show variations in pH.

FIGURE 2-21. Rhizobia strain SRDI736 improved lucerne nodulation and dry matter production (mean of 32 assessements) across four field trials (pH_{CaCl_2} 4.1 to 4.8). SRDI736 is recommended for use with SARDI 7 series 2 lucerne on soils of pH_{CaCl_2} 4.5 to 5.0.

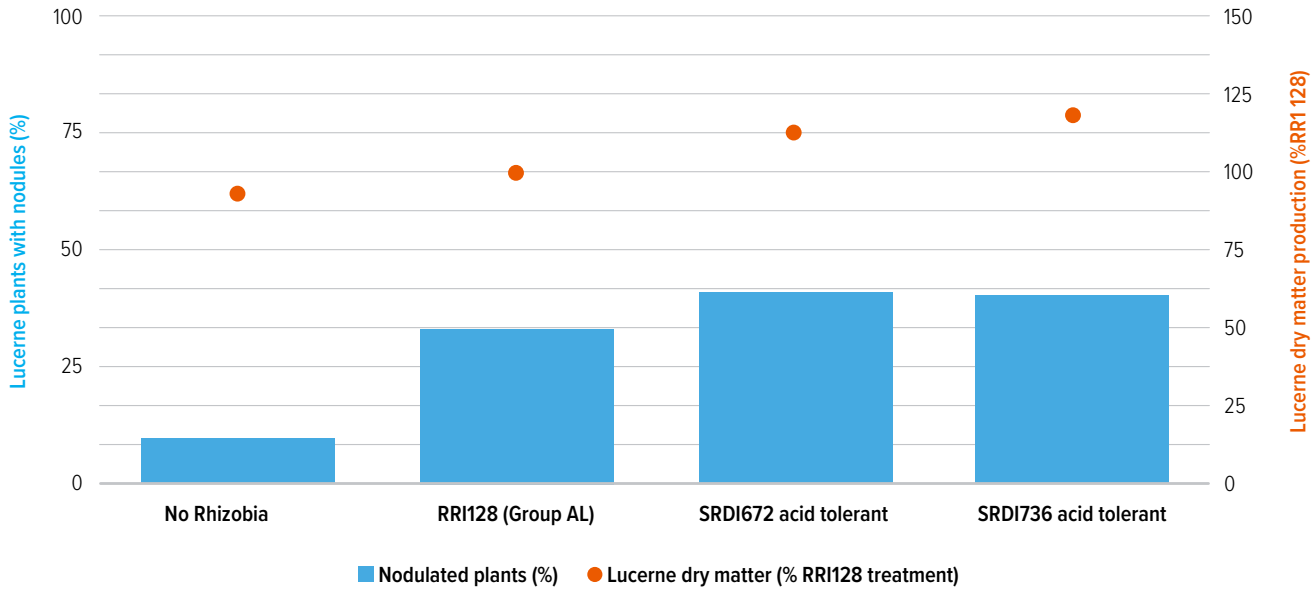


FIGURE 2-22 (left). A 'dig-stick' can be used to get a soil core to test pH and identify acidic soil conditions. FIGURE 2-23 (right). Garden centre pH kit.

Photo: Tree Logic Tools.

Photo: Belinda Cay, AgCommunicators.

Dry sowing

Dry sowing, or sowing into insufficient soil moisture for seed germination in anticipation of rain in the following 1-4 weeks, is popular throughout the western and southern Australian grain belt as it provides growers with more flexibility in their seeding schedule and allows strong crop establishment before soil temperatures fall during the colder winter months. With less reliable autumn rainfall conditions in recent decades and larger cropping programs, dry sowing is sometimes a necessity. When good nodulation is achieved, early sown crops often outperform later sown pulse crops in terms of both N fixation and yield, providing weed and disease management are in check.

For good nodulation to occur under any scenario, sufficient rhizobia need to be alive on or near the seed at germination to multiply around the root, infect the plant and form nodules. Where sufficient levels of appropriate rhizobia exist in the soil and inoculation is not required, then dry sowing is not considered a risk to legume nodulation. (See Chapter 9 for likelihood of inoculation response for given legume). However, when dry sowing legumes in paddocks with low or no suitable rhizobia in the soil, standard inoculation practices (e.g. peat slurry applied to seed at recommended rate) are unlikely to deliver satisfactory nodulation, especially when the time between sowing and rainfall sufficient for plant germination exceeds seven days, and/or there are other stresses such as soil acidity.

TABLE 2-7. Factors influencing nodulation when dry sowing.

Optimising factors	Limiting factors
More rhizobia If using peat inoculant, apply at double the recommended rate.	**Days dry after sowing (>7)** **If possible, sow within a week of forecast rain.** The longer the period between dry sowing and rain the less rhizobia present at seed germination.
Mild soil temperatures Aim for May sowing when soil temperatures are lower than April.	**Low pH** (Less than 5.5 $_{CaCl_2}$) Avoid dry sowing into acidic soils. Implement a liming strategy to increase soil pH.
Inoculant formulation Granule formulations may provide better nodulation where dry conditions exceed 7 days.	**Seed chemicals** Avoid dry sowing where both seed chemicals (e.g. P-Pickel T) and seed inoculation are required.

The key to successful nodulation with dry sown legumes is to increase the chance of sufficient rhizobia surviving the dry period between sowing and the germinating rain. Some granule formulations may provide greater protection of rhizobia from desiccation than others, and have been successfully used for establishment of pulses and pasture legumes sown under dry conditions. Where peat inoculation is used, doubling the inoculation rate and reducing other stress factors (e.g. avoiding acidic soils or seed dressings) can improve rhizobia survival and nodulation in dry sowing scenarios (Figure 2-5, page 18 and Table 2-7, page 39). Research has shown for pulses, granule formulations provided nodulation similar or better than double rates of peat on seed when the dry period exceeded seven days and the granular product had high rhizobial numbers (Denton *et al.* 2018, Farquharson *et al.* 2018).

2.12 Summer sowing of annual legume pastures using unscarified seed

Annual pasture legumes such as serradella, bladder clover, arrowleaf clover, gland clover and biserrula which allow growers to harvest seed using conventional headers has led to cheap seed produced on farm. As a result, novel methods to establish pastures such as summer sowing have been developed. The seed of these species undergoes very little scarification when harvested with a header, meaning a very high percentage retains its 'hard', impermeable seed coat. Instead of the farmer sending seed away for further processing (scarification), a method of pasture establishment was developed, whereby the unscarified seed is sown in summer, relying on natural processes to break down the hardseededness. This process is termed summer sowing (Nutt *et al.* 2021).

In summer sowing, unprocessed seed is sown in mid to late summer at a relatively high rate. Fluctuations in temperature and moisture over late summer and into early autumn break down a proportion of the hard seed enabling it to emerge and commence growth with opening season rainfall. This process means pasture can be sown before the winter cropping program commences. Often the summer sown pasture seed germinates and emerges with the first autumn rains in April or May while temperatures are higher than for pastures traditionally sown in June, and high biomass production is achieved early in the season.

High summer temperatures and relatively shallow sowing depth of the seed (<1cm) mean that where suitable rhizobia are not already present in soil, rhizobia must be delivered in a form that can survive such conditions. Clay-based bentonite granules impregnated with appropriate rhizobia applied

TABLE 2-6. Crop symptoms at different soil pH (measured in calcium chloride).	
If the soil pH is:	
More than 5.5	There will be no problems from soil acidity affecting crop growth and yield, and there could be net movement of lime beyond 10cm depth.
Less than 5.5	The effectiveness and numbers of rhizobia that fix nitrogen (N) on acid sensitive legumes (e.g. lucerne and pulses, but not narrow-leafed lupin) are reduced. Liming increases the persistence and nodulation by these rhizobia, and the amount of N fixed and grain produced.
Less than 5.0	In addition to the effects above, there is a chance of molybdenum deficiency in legumes — check for local advice. Molybdenum is important in the synthesis of amino acids and proteins and a requirement for rhizobia bacteria to fix atmospheric N. In some soils manganese can increase to toxic levels.
Less than 4.8	In some soils, aluminium (Al) starts to change from a harmless solid into a soluble form which is toxic to root growth. Aluminium tolerance among plant species varies. Reduced root growth means roots are unable to effectively explore soil for nutrients (particularly phosphorus and trace elements) and access stored subsoil water for growth or grain filling. Crop yield is reduced significantly. Reduction in root hairs occurs and so infection by rhizobia (nodulation of legumes) is reduced.
Less than 4.5	The speed of N mineralisation processes (nitrification) slows significantly, resulting in decreased N supply. In most soils Al concentrations increase further and quickly become toxic to most pasture and crop species. The nodulation of rhizobia in acid intolerant or sensitive legumes is reduced.
Less than 3.8	Soil can no longer buffer effectively against pH change and is overcome with acidity. Irreversible soil structural damage may occur.

Adapted from Fenton G (2003). Planning on liming, NSW Agriculture, Acid Soil Action leaflet No 4 (2 ed).

with the seed are commonly used to provide nodulation in summer sown pastures with considerable success (RJ Yates pers. comm. 2021). In comparison, the rhizobia in peat slurry inoculants applied to shallow sown pasture legumes in February are unlikely to survive high summer temperatures and moisture fluctuations between sowing and germination in April or May.

2.13 Formulations of inoculants containing co-inoculants

Apart from rhizobia, other microorganisms may have beneficial impacts on plant growth. Beneficial co-inoculants such as *Penicillium bilaii* are commercially available, with other co-inoculants likely to be available in the future. Some co-inoculants have the extra microorganism added during manufacture of the rhizobial peat or granular inoculant, but sometimes the extra microorganism is supplied separately. Co-inoculants are marketed as increasing root growth, nodulation, phosphorus uptake, or reducing the incidence of pathogens affecting root growth. Advice from the individual manufacturer should be sought regarding their advantages compared to rhizobial inoculation alone and the recommended application methods followed.

2.14 Concluding comments

This chapter has highlighted some considerations when inoculating legume seed to achieve effective legume root nodulation. The number of rhizobia that are applied to legume seeds or directly to the soil and their survival is of utmost importance for nodulation and the formation of an effective N fixation symbiosis. Rhizobia are living organisms; when handling inoculants, remember that many things may harm the rhizobia, including chemicals and fertilisers, high or freezing temperatures, sunlight, desiccation, and extremes of pH.

Unless the legume was grown recently and suitable rhizobia are present in the soil, legumes must be inoculated with the correct rhizobial strain (inoculant group) for maximum benefit. In Australia, inoculant rhizobia are currently available in different carriers: peat, freeze-dried powders, granules, liquids and as preinoculated seed. The shelf life of these products varies from several weeks in the case of some pre-inoculated seeds to eighteen months for some peat inoculant groups. The cost of inoculation can vary depending on the product. Peat is the most cost-effective form of inoculant to purchase, but there are additional application costs to consider in terms of time and labour. Granules are more expensive, but are easier to use and offer flexibility at sowing.

The inoculation of legumes can be an inconvenient process, especially during the busy sowing period. But by following some simple instructions and precautions, delivery of large numbers of rhizobia to the target legume roots can be readily achieved, resulting in successful nodulation, high levels of symbiotic N fixation, abundant biomass production and high grain yield.

USEFUL RESOURCES ON SOIL ACIDITY AND AMELIORATION

GRDC Publication - Legumes in Acidic Soils – Maximising production potential in south eastern Australia
grdc.com.au/legumes-in-acidic-soils

GRDC Acid soils SA
acidsoilssa.com.au/

3 | Assessing legume nodulation

KEY POINTS

- Roots of crop and pasture legumes need to have adequate numbers of active nodules to ensure optimal amounts of nitrogen will be fixed by the rhizobia.
- Nodules can form from rhizobia already present in the soil or through inoculation.
- It is helpful to investigate the extent and location of nodulation on plant roots, irrespective of whether the seed was inoculated or not.
- Conduct nodulation checks between 6 and 12 weeks after emergence.
- Aim for a paddock nodulation score of 'adequate', to be confident that your crops have a sufficient number of nodules to fix abundant N. 'Adequate' numbers of nodules per plant vary for different legumes.

LEGUME NODULATION CHECKS

A GUIDE TO NODULATION CHECKS AND TROUBLESHOOTING POOR NODULATION

DOS

DO carry out nodulation checks.

Do this 6-12 weeks after emergence and allows adequate time for nodulation to occur before conducting the counts and paddock rating.

DO collect samples from three or more separate locations within a paddock.

Store and wash samples gently and thoroughly in separate buckets. Assess each sample separately. Target different soil types within the paddock, or areas of good, average and poor growth.

DO cut open a few nodules while counting your samples.

A healthy red/pink colour inside the nodules indicates nitrogen fixation, while white or green nodules indicates ineffective fixation and the need to troubleshoot your inoculation strategies.

DO consider collecting a second group of samples if your initial nodulation scores are poor.

Sampling elsewhere in the paddock can help you identify if poor nodulation is a localised problem or if you need to review your inoculation strategy and legume management.

DO conduct a nodulation check regardless of whether you inoculated your legume seed.

Nodulation checks help inform if there are suitable existing rhizobia in the soil, or if inoculation is required in the future.

DON'TS

DON'T be rough or rushed when cleaning your plant samples.

Clean the root system of each plant carefully with water and rinse a couple of times. For heavy soils, soak in water for 30 minutes. Float the root systems in water in a tray, preferably white plastic, for easy observation.

DON'T miss the opportunity to observe legume root health in future.

Healthy roots free from diseases or nematodes are important for good nodulation and plant growth.

DON'T assume your nodulation score can't be improved.

There are a number of variables that affect nodulation and could require you to fine tune your inoculation strategy. For example, consider whether this is the first time sowing a particular legume crop in a paddock, if the soil is very acidic, or if you are dry sowing legumes. These factors can affect nodulation and could be remedied by increasing the inoculation rate.

DON'T forget that adequate nodulation numbers vary by plant type.

Ensure you consult the GRDC Tips and Tactics: Legume and Nitrogen Fixation and other resources for more information. Adequate nodulation is upwards of 50 nodules per plant for vetch, field pea, faba bean and lentil, and 10-30 for chickpea.

DON'T discount the small cost of inoculating future legume crops if you record a poor nodulation score.

Correct inoculation and successful nitrogen fixation can provide benefits to legumes and subsequent crops for several seasons.

3.1 Why check nodulation?

Legume nodulation should be monitored to determine whether inoculation was successful.

- If you didn't inoculate, to check whether there are suitable levels of appropriate rhizobia in the soil or whether you should inoculate in future.
- To help understand if poor nodulation is likely to be limiting crop performance.

3.2 How do I assess nodulation?

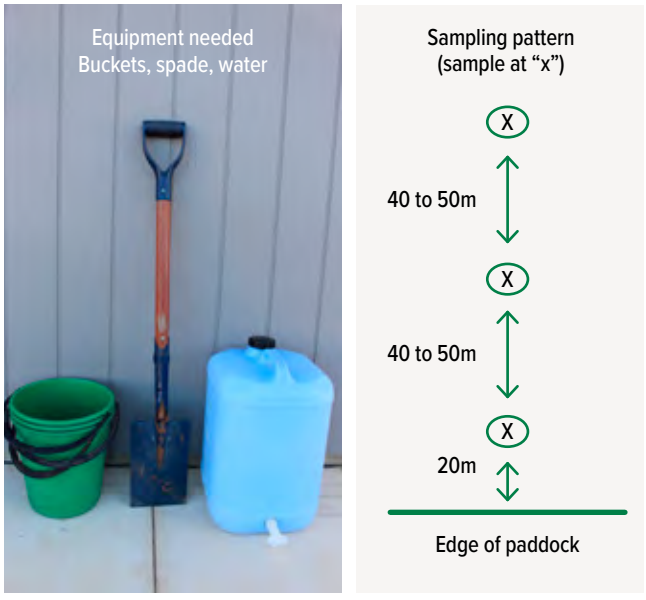
By following a few simple steps, you can check to see if your legume nodulation is adequate.

Grower paddock assessment method

See short, instructional videos at:
ua.edu.au/legume-inoculation

1. **When?** Take root samples between 6 and 12 weeks after emergence, depending on crop and location. Early to mid-flowering (6 to 8 weeks) for summer season crops; 10 to 12 weeks (or later if emergence is slow), i.e. late winter or early spring in southern and western Australia.
2. **Where?** In a single paddock, collect about 30 plants, 10 at each of three sample spots (see sample pattern diagram Figure 3-1), putting each set of 10 plants from the spots in three separate buckets.

FIGURE 3-1. Sampling equipment and suggested sampling pattern (minimum distances)



3. **How?** Dig up the plants carefully, down to at least 10cm below seeding depth if possible, to recover enough of the root system to enable inspection and scoring.
   Note: dig up the plants, don't pull them, otherwise you may lose many roots and nodules. In heavier clay soils, it helps to use a garden fork rather than a shovel, to ease the root system out from multiple directions.
4. Carefully wash off the soil in a bucket of water and rinse roots once or twice to remove remaining soil. For heavy clay soils you may need to soak the samples for ½ to 1 hour before washing.
5. Score each plant for adequate / poor nodulation.
   Refer to following photos for various legume species, showing the scoring system and examples of adequate and poor nodulation and numbers of nodules per plant. To aid assessment, float the roots in a few cm of water on a white background, e.g. in a clean white plastic drum cut in half.
6. For samples from each sampling point, sort plants into two groups: adequate scores and poorly nodulated or plants without nodules. Then work out the percentage of plants adequately nodulated at each sampling point and the average score for the three locations to get a paddock nodulation score (Table 3-1).
7. Check the colour of the interior of a few nodules by cutting or breaking the nodule open with a fingernail. This is best done as soon as possible after washing. A red or pink interior means the nodule is actively fixing N. If it is green or brown it may not be functioning as desired or senescing.

TABLE 3-1. Overall average nodulation score for a sample

Adequate	70% or more of plants rated adequate
Borderline	50 – 70% of plants rated adequate
Poor	Less than 50% of plants rated adequate
None	No nodules present (= no nitrogen fixation)

Notes: *Plants scored as adequate should have most nodules with a red/pink colour inside (actively fixing nitrogen). In mature nodules e.g. those that have begun to elongate, the red coloration may be confined to the nodule tip. (Figure 3-5, page 51)*

Nodulation will vary across a paddock, and between plants taken at a single sampling point: this is why it's advisable to take 10 plants from each of several locations.

Acknowledgements: *The grower paddock assessment method is based on the work of Janine Sounness, formerly pulse agronomist with Agriculture Victoria, Horsham.*

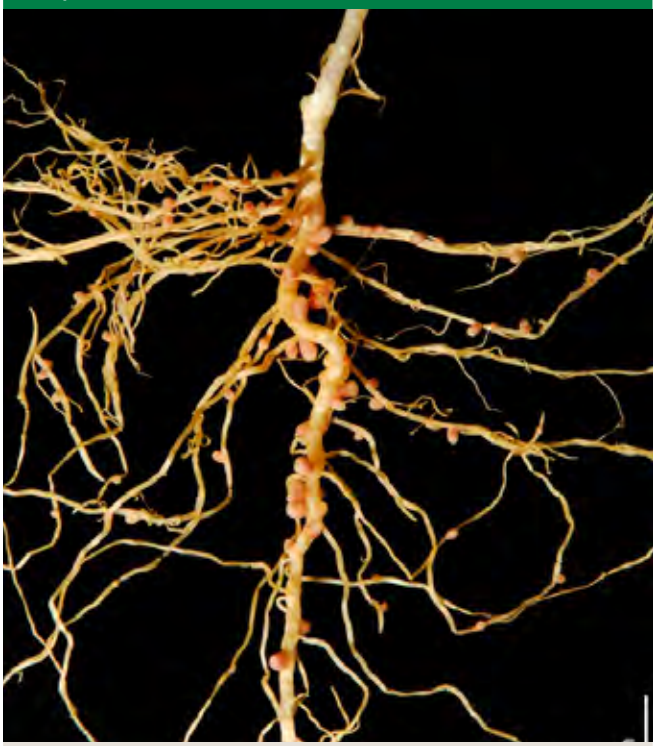
Illustrations of adequate and poor nodulation

Photos on the left illustrate adequate nodulation, and on the right, poor nodulation generally around 10 weeks after crop emergence unless specified otherwise. Desirable number of nodules per plant are included below the photos.

FIELD PEA

Adequate

Photo: Liz Farquharson, SARDI.



50 or more nodules.

Poor

Photo: Maarten Ryder, University of Adelaide.

Less than 20 nodules.

LENTIL

Adequate

Photo: Liz Farquharson, SARDI.

50 or more nodules.

Poor

Photo: Liz Farquharson, SARDI.

Less than 20 nodules.

FABA BEAN

Adequate

Photo: Maarten Ryder, University of Adelaide.

50 or more nodules per plant.

Poor

Photo: Maarten Ryder, University of Adelaide.

Less than 20 nodules.

LUPIN

Adequate

Photo: Ross Ballard, SARDI.

Nodules right around (encasing) the crown & on laterals; Plant on right: nodules have been sliced open to reveal pink interior (arrowed).

Poor

Photo: Liz Farquharson, SARDI.

Few nodules (arrowed).

CHICKPEA

Adequate

Photo: Andrew Heath.

Adequate: 10 to 30 nodules. Note multi-lobed nodules around crown.

Poor

Photo: Ross Ballard, SARDI.

Less than 10 nodules.

SOYBEAN

Adequate

Photo: Nikki Seymour, QDAF.

20 nodules or more per plant after eight to ten weeks.

Poor

Photo: Nikki Seymour, QDAF.

MUNGBEAN

Photo: Nikki Seymour, QDAF.

Adequate

20 nodules or more per plant after six to eight weeks.

Poor

Photo: Nikki Seymour, QDAF.

Less than 10 nodules per plant after six to eight weeks.

PEANUT

Adequate

Poor

Photos: Nikki Seymour, QDAF.

Peanuts form many nodules (i.e. more than 100/plant). It is not possible to state the number of nodules per plant after eight to 10 weeks of plant growth that might be considered satisfactory.

STRAND MEDIC

Adequate

Photo: Ross Ballard, SARDI.

5 to 10 nodules per plant (8 in this case).

Poor

Photo: Ross Ballard, SARDI.

3 nodules.

SUB CLOVER

Adequate

Photo: Ross Ballard, SARDI.

50 to 100 nodules.

Poor

Photo: Liz Farquharson, SARDI.

Less than 20 nodules.

LUCERNE

Adequate

Poor

Photos: Ross Ballard, SARDI.

Young plants (pictured): 10 – 15 nodules per plant at 10 weeks
Mature plants: look for nodules on the finer lateral roots

More detailed nodulation assessment methods for test strips and trials

Scoring systems have been developed for more detailed comparison of treatments applied in field or greenhouse trials, which may also be useful for comparing in-paddock test strips.

One example is the 0 to 5 scale, with some subdivisions, developed originally for chickpea and now modified for use with for other grain legumes (Table 3-2). In this scoring system, for an individual faba bean plant, a score of 3 or more is considered adequate nodulation.

TABLE 3-2. Scoring the nodulation of faba bean roots on a 0-5 scale

Nodulation score	Nodules on crown and tap root	Nodules on lateral roots
0	0	0
0.5	0	0 - 5
1	0	5 - 10
1.5	0	More than 10
2	Few (10 to 20)	0
2.5	Few (10 to 20)	Few (10 to 20)
3	Few (10 to 20)	Many (more than 30)
4	Many (more than 50)	Few
5	Many (more than 50)	Many (more than 50)

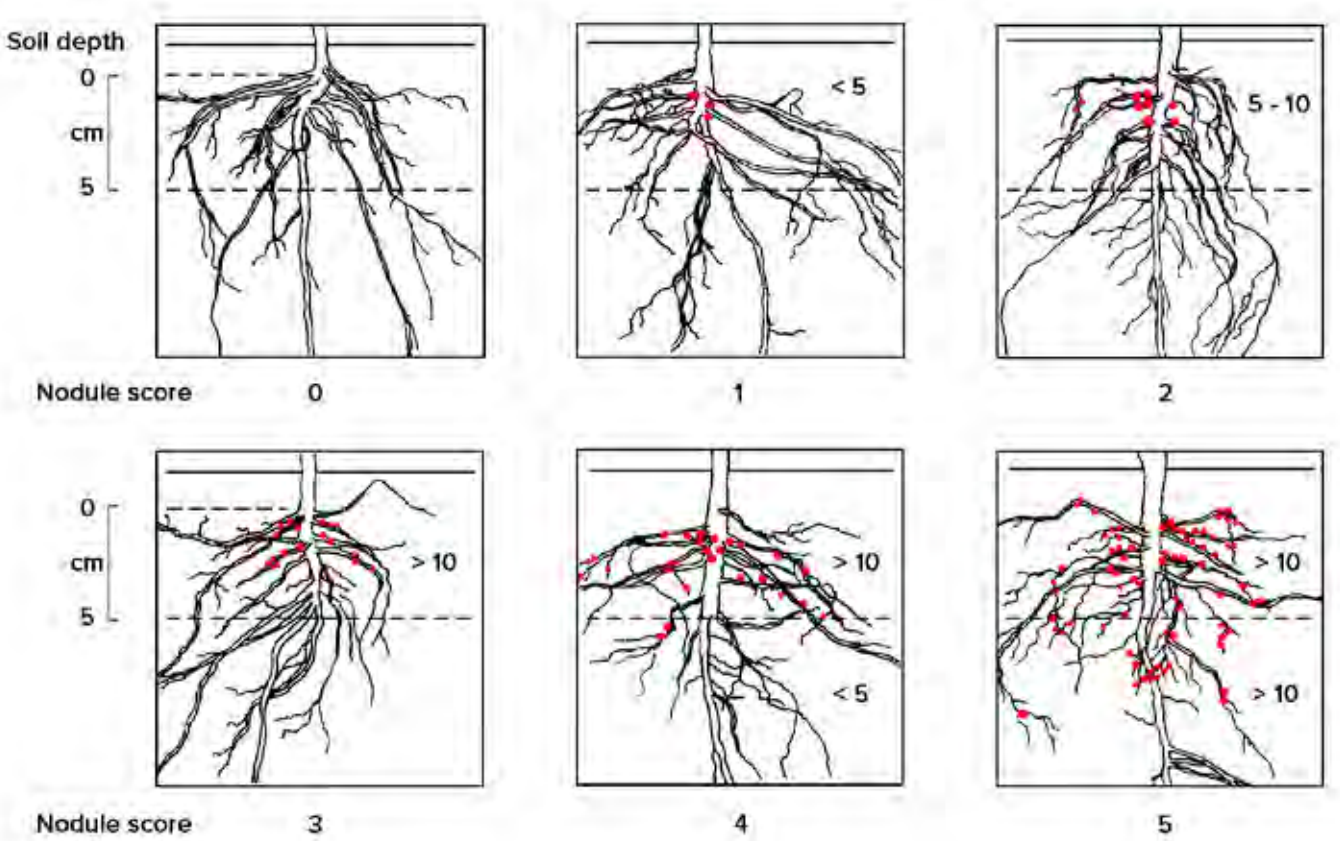
(adapted from Corbin et al., 1977)

Another useful scoring system, using a 0 to 5 scale, is shown in Figure 3-2, page 50. A score of 4 or more is considered adequate.

3.3 Nodulation patterns:

- If no background soil rhizobia are present, a seed-inoculated legume crop will tend to have nodules mainly around the crown of the plant. This indicates nodulation was prompt and able to support early N-fixation.
- A legume that has been nodulated by background rhizobia populations will have nodules more widely spread over the root system. It indicates rhizobia have colonised the soil and this may be benefical to N-fixation later in the season.
- A crop that was inoculated in-furrow below the seed may have nodules further down the root system.

FIGURE 3-2. System for scoring nodulation of legumes using a 0-5 scale. Numbers within boxes are the numbers of nodules observed, and their associated ranking (score) is given under each box. Scores between whole numbers (e.g. 2.5, 3.5) can also be incorporated. The system is suitable for most legumes species and can be modified for perennial species or species with higher nodulation targets for an adequate score. (Unkovich *et al.* 2008.)



3.4 Target numbers of nodules per plant

The relationship between nodulation of field pea and amount of N fixed has been well-researched (Figure 3-3). The fitted curve shows that N fixation is reduced below 50 nodules per plant, and therefore a target of 50 nodules per plant is considered adequate.

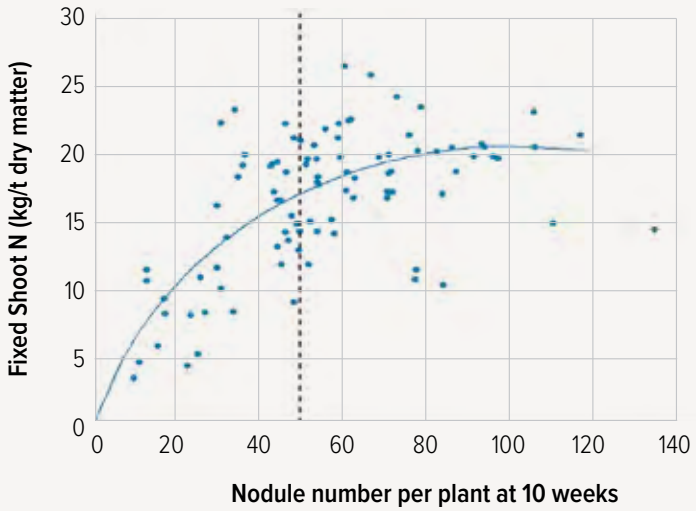


FIGURE 3-3. The relationship between amount of nitrogen fixed and nodulation of field pea in the field in SE Australia (Farquharson and Ballard, SARDI, unpublished data).

Data to support targets for levels of adequate nodulation of other grain legumes is being collected. Targets for the other pulses and pastures shown in this chapter are based partly on the developing data sets, and also on numbers of nodules per plant commonly seen on legumes that have been grown in good soil conditions with no serious root disease problems.

A common observation for chickpea is that there are fewer but larger, often multi lobed (walnut like appearance) nodules compared to those found on other pulses. The target number of nodules per plant for chickpea is lower than for other grain legumes, but nodules are often larger, so the total nodule mass is roughly equivalent.

Strand medic is known as a shy nodulator and the number of nodules per (seedling) plant regarded as adequate is quite low (<10). Barrel and burr medics typically require 10 to 20 nodules per plant.

3.5 Nodule colour

Young nodules that are coloured red or pink inside can be regarded as actively fixing nitrogen. It is desirable to see mostly pink nodules on root systems that have been sampled in the suggested time after legume emergence. Nodule colour is best observed on either a white or black background, and can often be seen without breaking the nodules open. However, breaking or cutting open nodules will reveal the interior colour more clearly.

The pink colour is due to the presence of leghaemoglobin, which is similar to haemoglobins in human and animal blood and has the function of regulating oxygen levels within the nodule, so that N fixation can occur.

Other nodule colours:

White nodules are either in an early a stage of development or have been formed by ineffective rhizobia (Figure 3-4). There is no N fixation occurring here.

FIGURE 3-4. Effective and ineffective nodules collected from inoculated burr medic plants.

Photo: Ross Ballard, SARDI.

Green or dark brown nodules are also ineffective. These may be caused by waterlogging conditions, or may be old, senescent nodules.

Large multi-lobed nodules may be green in the inner (older) regions and pink in the outer (freshly grown) regions (Figure 3-5). This is normal.

FIGURE 3-5. Mature effective nodules showing pink zone of fixation near the nodule tip and older green senescent zone near the root.

Photo: Liz Farquharson, SARDI.

Nitrogen fixing nodules go through a life cycle: initiation and early growth; then mature nodules that are actively fixing N; then a senescent, dying off stage where activity decreases then stops. Nodules are most effective at fixing N in the mid-stages of their growth cycle (see Chapter 5 for further detail).

3.6 What if nodulation is not adequate?

- Sample elsewhere in the paddock, to check if it is a localised problem or not.
- Check and compare areas of healthy green crop versus areas with yellow and poor growth.
- Complete the troubleshooting questions (see below).
- Poor nodulation can be caused by a number of factors including low rhizobia numbers, problems with incorrect use of inoculant or environmental stresses (dry or acidic soils, co-application with certain pesticides or trace elements).
- The application of N fertiliser could be considered to salvage yield in high value crops.

3.7 Reasons and solutions for poor nodulation

Poor nodulation is difficult to remediate during the growing season. In high value crops, the application of N fertiliser could be considered to salvage some yield. The solutions below are suggested for future seasons. For more detailed information on the inoculation process refer to chapter 2.

Inoculation practice

- **The crop was not inoculated, or was inoculated with the incorrect inoculant group.**
  - > Inoculate the crop with the right rhizobial group next time it is grown.
- **Was the inoculant stored correctly prior to use?**
  - > Store inoculant in cool conditions out of the sun before use; do not use inoculant past the expiry date.
- **Did you leave inoculated seed or prepared inoculant for too long prior to sowing?**
  - > Sow inoculated seed as soon as possible, preferably on the same day as inoculation to ensure highest populations of rhizobia. Preinoculated, pelleted pasture seeds can be stored for longer times, but are still best sown soon after inoculation to maximise nodulation potential.
- **Was the peat or freeze-dried inoculant mixed with low quality water (e.g. acidic, saline or chlorinated)?**
  - > Use rain water or tap water that has been allowed to stand to reduce chlorine levels; avoid water treated with algicides.
- **Was the inoculant mixed with incompatible chemicals or fertilisers either prior to application on seed or in-tank mixes?**
  - > Avoid applying inoculant on top of seed-dressing fungicides containing thiram or high levels of metalaxyl.
  - > Don't apply liquid inoculant mixed in tank with liquid trace elements.
  - > Only bentonite granules are recommended for mixing with fertilisers. Adhere to manufacturer instructions.
  - > Consider separating the inoculant physically from other treatments e.g. apply pesticide on seed, inoculant in furrow.

Sowing conditions

- **Was the legume sown into dry soil (i.e insufficient moisture for seed germination) in a paddock without a background of suitable rhizobia?**
  - > Dry sowing is not recommended for freeze-dried inoculant.
  - > For peat inoculant on seed, double the rate of inoculant. Efficacy in dry soils may be reduced in soils where pH_{CaCl_2} is less than 5.5 or the period between sowing and rain exceeds 7 days.
  - > Consider using a granular inoculant if sowing legumes into dry soil where inoculation is required and the time between sowing and rain is likely to exceed 7 days.
- **Was an acid sensitive legume sown into acidic soil (pH_{CaCl_2} less than 5.5)?**
  - > In future consider acid tolerant legumes such as lupin or serradella.
  - > Double the rate of inoculant for acid sensitive legumes sown on acidic soils.
  - > Implement a liming program to raise soil pH to 5.5 for acid sensitive legumes.
  - > For temperate pasture legumes, except serradella, lime pellet the seeds. Tropical pasture legumes (except Leucaena and Kenya White clover) do not require lime pelleting.
- **Were there factors that caused crop stress such as poor nutrition or root disease?**
  - > Ensure good crop management to correct nutritional problems and control root disease.
- **Is there evidence of herbicide damage?** For example, Group 2 (B) herbicide (e.g. sulfonylurea) residues in alkaline soils can dramatically inhibit nodulation of legumes, as these herbicides can damage plant roots and are slow to break down in these soils, especially in dry conditions.
  - > Avoid situations with high risk of damaging herbicide residues.
  - > Keep good spray records and adhere to plant back recommendations.

4 | Rhizobial inoculants – strains and quality control

Photo: Ashlea Webster, AIRG-NSW DPI.

KEY POINTS

- Strains of rhizobia used in commercial inoculants must satisfy a number of criteria, including effectiveness in fixing nitrogen (N) with the appropriate legume.
- Selection of new rhizobia strains for newly released legumes and for marginal and hostile soil environments is ongoing.
- Rhizobial inoculants are formulated and available in peat, clay or peat granules, liquids and as a freeze-dried powder.
- Most rhizobial inoculants in Australia are subjected to independent quality testing by the Australian Inoculants Research Group (AIRG) as part of a voluntary program.
- Inoculant quality is determined largely by the number of viable rhizobia contained in the inoculant, and comparatively low numbers of contaminants.
- Inoculants meeting the standards of the independent AIRG quality testing display the Green Tick Logo.
- The Green Tick Logo does not guarantee inoculant efficacy in the field, as this is influenced by many management factors.
- Testing of inoculants and preinoculated pasture legume seed at the point-of-sale generally indicates high quality of peat inoculants supplied to growers, but problems sometimes occur with very low numbers of rhizobia on preinoculated pasture seed.

4.1 What are rhizobial legume inoculants?

Legume inoculants are products containing commercially prepared cultures of live rhizobia (i.e. bacteria) protected in carriers that enable large numbers of viable rhizobia to be applied to the seed or soil at sowing for the effective nodulation of legumes. The purpose of legume inoculation is to supply elite rhizobial strains in large numbers to the roots of the legumes soon after germination, to optimise the chances of effective nodulation, and to increase symbiotic N fixation, plant and grain yield, while decreasing input costs. Some of the N fixed by legumes and rhizobia will have carryover benefits for the farming system, thereby reducing the need for N fertilisers in following crops (Chapters 7 and 8).

There are several different commercial inoculant formulations available to growers to allow flexibility of application; these are peat, granular, liquid and freeze-dried. Some seed companies sell pasture legume seeds that have been inoculated with rhizobia as part of a seed inoculation or coating package. The characteristics and use of inoculants is covered extensively in Chapter 2.

4.2 Selection of rhizobial strains for commercial inoculants

New rhizobia strains for inoculants are selected to improve the N fixing potential of legume crops and pastures, to expand the current geographic and environmental range of legumes (e.g. into more hostile soils) and to meet the needs of newly developed cultivars and species of legumes not previously grown in Australia. Inoculants in Australia contain rhizobial strains that have been selected according to the criteria outlined below, established during many years of scientific research.

(i) Nitrogen fixation capacity of rhizobia with their legume host

There are thousands of strains of rhizobia that can nodulate and potentially fix N with each legume host. However, the amount of N fixed can vary substantially, depending on the compatibility of legume host and rhizobial strain. Strains that are used in commercial inoculants are selected for their high N fixation capacity with the range of legume species/cultivars.

FIGURE 4-1. Growth of annual clover species with former inoculant strain WU95 (left) and current strain WSM1325 (right), indicating the selection of a strain that improves N fixation across multiple clover species

Photos: Ron Yates, DPIRD.

This usually translates into increased legume growth and yield, and more residual N for following crops. The benefits of using a highly effective rhizobial strain to fix N in subterranean and other annual clover plants grown in N-deficient medium is shown in Figure 4-1, page 54.

Genetic stability

A commercial inoculant strain must also maintain its symbiotic capacity (nodulation and N fixation performance) and other key traits during culture, manufacture and application. Commercial strains are assessed for genetic stability over many generations and assessed for consistent colony morphology, effectiveness, and ability to maintain these traits from season to season. In addition rhizobia that persist in the soil are prone to genetic drift and their N fixation ability may decline over a period of years – this is an area of ongoing research.

Production as commercial inoculants

Rhizobial strains must be suitable for commercial manufacture and be able to grow and survive in large numbers in commercial inoculant formulations (known as manufacturability). Inoculant companies test strains for manufacturability in their production system and for growth and survival in inoculant carriers prior to commercial release.

Survival during inoculant application

Application of peat inoculant directly to seed is the most widely used method to introduce rhizobia into the soil at sowing. Strains vary in their ability to survive on seed and in other formulations. Survival of rhizobia in peat on seed, the granule or other formulations needs to be high and this is an important part of the selection process. This is particularly important for pasture rhizobia destined for application to pre-inoculated seed, which can be stored for weeks.

Persistence in soil

Ideally, rhizobia need to be able to survive and grow in soil in the absence of the host legume plant for several years – this is known as saprophtyic competence. This trait is important for regenerating annual pastures which are entirely reliant on the soil rhizobia for nodulation, and also for pulse legumes where there is often several years between crops that are not always inoculated.

New improved rhizobial strains need to meet or exceed the above criteria if they are to be considered as potential inoculant strains. The consideration of a strain for commercial production requires the preparation of a technical brief by the proponent, for assessment by a National Steering Committee comprised of scientists with expertise in the field of N fixation and strain selection.

In addition to the five key criteria outlined above, the following is a list of additional desirable traits for rhizobial inoculant strains;

The ability to:

- form nodules and fix N in the presence of soil nitrate to help build up soil N
- colonise the soil in the absence of the legume host
- tolerate environmental stresses such as drought and acidity
- compete in nodule formation with populations of rhizobia already present in the soil
- be compatible with seed applied agrochemicals

4.3 Quality of inoculants

In Australia, during the 1940s and early 1950s, the area sown to legumes increased with the introduction of many new species, particularly pasture legumes, which prompted a shift in the manufacture of inoculants from the public to the private sector.

Adoption of the US technology using peat as a carrier and a lack of regulation of the quality of inoculants eventually led to nodulation failures. In 1954, Professor Jim Vincent, an eminent microbiologist from the University of Sydney, asserted that poor-quality inoculants cost growers in lost production and would eventually discredit the practice of inoculation. He made basic recommendations for quality control and the use of legume inoculants and established the first quality control laboratory as a joint venture between the University of Sydney and the NSW Department of Agriculture.

The quality control and assurance of legume inoculants continues today within the Australian Inoculants Research Group (AIRG) under the auspices of the NSW Department of Primary Industries (DPI), based at the Elizabeth Macarthur Agricultural Institute in Menangle. The group is funded through service agreements with inoculant manufacturers that opt in and are signatories to the Code of Practice.

We are fortunate that Professor Jim Vincent recognised the harmful implications of poor-quality inoculants at the farm level and established an independent laboratory to conduct quality assessment. Additionally, the laboratory acted as a resource to assist the industry to continually improve inoculants. Now, more than 70 years later, the system, with its clearly-stated framework, has survived essentially unchanged and has become the model that other countries follow.

The AIRG is responsible for:

- maintaining, authenticating and issuing approved rhizobial strains for commercial use to manufacturers
- assessing the quality of inoculants at point of manufacture for compliance with the National Code of Practice and Quality Trademark for Legume Microbial Inoculant Products Used in Australian Crops and Pastures
- administering and promoting the Green Tick Logo trademark.

More information on current rhizobial inoculant quality testing at AIRG, including the current Code of Practice, can be accessed at the AIRG website (dpi.nsw.gov.au/inoculants).

Inoculant manufacturers also take responsibility for ensuring their product is of high quality and conduct a number of tests in their own laboratories or via contractual arrangements with independent quality assured organisations such as the University of Sydney who work with industry on both coated seed and inoculant quality including inoculant QA. Likewise, researchers across Australia who work in suitably equipped laboratories (some who are authors of this guide) take the responsibility to test inoculant quality at their respective institution prior to conducting laboratory, glasshouse or field research.

Photo: Ashlea Webster, AIRG-NSW DPI.

How do growers know if an inoculant is high-quality?

Since July 2010, rhizobial inoculants in Australia that have been tested by the AIRG to meet strict quality standards display a registered trademark called the Green Tick Logo (Figure 4-2). The logo indicates that at the time of testing the product contained:

- the correct rhizobial strain for the target legume hosts with proven ability to nodulate a host post-manufacture;
- numbers of live rhizobia equal to or above a minimum standard;
- zero or minimal numbers of other contaminant organisms.

FIGURE 4-2. Registered trademark for inoculant quality – the Green Tick Logo. The Green Tick Logo indicates that an inoculant has been independently tested and satisfies Australian quality standards.

The logo also indicates that labelling standards have been achieved. The product label should display:

- the name of the target legume host;
- application method/s;
- storage conditions;
- expiry date/shelf life;
- guaranteed number of live rhizobia at the point of sale;
- batch number.

Inoculants will only carry the logo if a representative sample of packets from the batch has been tested. The current signatories to the voluntary code of conduct can be found on the AIRG website: dpi.nsw.gov.au/inoculants

4.4 Numerical standards for legume inoculants

In Australia, legume inoculants displaying the Green Tick Logo must contain a minimum number of rhizobia prescribed for each inoculant formulation for the shelf life of the product (Table 4-1).

These numerical standards for legume inoculants are based on scientific research that has defined the number of rhizobia required for adequate nodulation. Requirements for inoculants in an individual paddock will be affected to some extent by the environmental and soil conditions at that site. The numerical standards were developed and are applied to ensure effective nodulation is likely to be achieved with each formulation in the absence of serious stresses such as soil acidity.

Research into the quality aspects of peat inoculants has been more extensive than with other formulations which are relatively recent innovations. Standards for all inoculant formulations are under continual review and are adjusted as new data becomes available.

In addition to the requirement for high numbers of rhizobia, inoculants produced using sterile media (peat, liquid and freeze-dried) should not contain a high number of other contaminating organisms. As such, batches in which contaminants are detected at significant concentrations fail quality-assurance testing, and do not qualify to display the Green Tick.

Standards for preinoculated seed are not covered by the current code of practice, and no preinoculated seed suppliers are currently participating in the Green Tick program.

4.5 Does a high-quality inoculant guarantee efficacy in the field?

There are factors that may compromise field efficacy of an inoculant. While the quality tests ensure that inoculants contain high numbers of effective rhizobia at the time of testing, the quality of the inoculant can be affected by the way it is treated along the supply chain, in particular how it is transported, stored and applied (Chapter 2). Careful application of high-quality inoculants to legume crops increases the chances that nodulation, N fixation and yield will be optimised.

4.6 What is the quality of inoculants and preinoculated seed at the point-of-sale?

Shelf life of inoculants is determined by measuring the survival of rhizobia in inoculant formulations or on pre-inoculated seed over time in the distribution chain.

The last significant studies in this area were conducted by AIRG between 2005 and 2010, the surveys covered 266 towns across the Australian grain belt.

Inoculant quality

During this period, 1556 legume inoculants for temperate and tropical legumes were tested for quality. In all surveys, three inoculant formulations were on sale to farmers, and purchased for testing in the following proportions: peat 92 per cent, freeze-dried 3 per cent; and granular 5 per cent. Each inoculant was assessed for quality (based on AIRG standards) to determine whether it passed or failed the standards. Pass rates ranged from 87 per cent to 94 per cent.

TABLE 4-1. Australian minimum standards for legume inoculants

Product	Initial count after manufacture	Count throughout shelf life	Current Recommended Expiry time (months) after manufacture
Peat (CFU/g)	$\geq 1 \times 10^9$	$\geq 1 \times 10^8$	12*
Liquid (CFU/mL)	$\geq 5 \times 10^9$	$\geq 1 \times 10^9$	6
Granules (CFU/g)	$\geq 1 \times 10^7$	$\geq 1 \times 10^6$	6
Freeze-dried (CFU/vial)	$\geq 1 \times 10^{12}$	$\geq 5 \times 10^{11}$	6

CFU: colony forming units.

Minimum standards for inoculants applied to seed have been set to achieve particular numbers depending on seed size, outlined in Table 4-2.

Numerical standards for CB376 for Lotononis bainesii are 2 x 10^8 rhizobia/g moist peat (2 x 10^7 rhizobia/g at expiry).

Standard for liquids based on a three litre bottle used to treat one tonne of seed. Standard for freeze-dried is based on vial used to treat up to 500kg of seed. (Information on standards from Australian Legume Inoculant Research Unit Annual Report 2007)

** Based on current data, 18 months expiry applies for groups E, F, G and N stored at 4°C. Group G is applicable to strain WU425 only.*

TABLE 4-2. Australian minimum standards for rhizobia numbers on seed

Seed Size	Example Species	CFU/seed
Large	soybean, faba bean	100,000
Medium	lentil	10,000
Small	subterranean clover and lucerne	1000
Very small	white clover	500

CFU: colony forming units.

Preinoculated seed quality (rhizobia numbers)

Point-of sale surveys of preinoculated seed were conducted across 37 towns in the wheat/sheep belt, mainly in the eastern states, during 2005. The majority of samples were temperate legume pasture species. Only 5 per cent of samples met the minimum number of rhizobia. Despite many attempts by various seed coaters to improve the quality of preinoculated legume seed, numbers of rhizobia on seed collected from retail outlets had not improved since an earlier series of surveys during 1999-2003 (Gemell *et al.* 2005). Generally, survival of rhizobia on lucerne seed is better than survival on clovers (Chapter 2, Table 2-2, page 22).

Some of the samples met the rhizobial numerical standards when less than 50 days after inoculation but virtually none of the older samples (i.e. >50 days) met the standards. Growers should always use within the recommended expiry for preinoculated seed, or where possible use freshly inoculated seed.

4.7 Examples of strain selection for commercial inoculants

In the ongoing selection of new inoculant rhizobia, potential strains are sourced from genetic resource centres in Australia or overseas, or from collection trips, often to regions with particular soil types, such as acidic soils. Strains are tested initially with the target legumes in glasshouse experiments and followed by testing of the most promising strains in field trials across the range of soil types and environments appropriate for the target legume (Howieson *et al.* 2000). The protocols for assessing strains and ensuring compliance of the protocols is overseen in Australia by the National Rhizobium Steering Committee, which includes rhizobial researchers from across Australia and representatives from AIRG.

Many new strains of rhizobia have been successfully introduced in Australia during the last few decades. In the 1990s, new strains of rhizobia were selected for the field pea, lentil, vetch and faba bean group of legumes and associated species, with strain WSM1274 replacing SU303 in 1998 for this E group (Herridge *et al.* 2008). Additional experiments showed that another strain, WSM1455 produced about 10 per cent more yield of faba bean and lentil than WSM1274 over a range of field sites and fixed an average of 27 per cent more N with a wider range of species under glasshouse conditions (Howieson *et al.* 2000; O'Hara *et al.* 2002; J. Slattery, pers. comm.). In 2002, WSM1455 replaced WSM1274 as the inoculant strain for the E group (Bullard *et al*., 2005). Recent research indicated potential advantages in acidic soils of new strains developed by the Centre for Rhizobium Studies (Yates *et al.* 2021) and SARDI (Figure 4-3).

New inoculants have also been developed that have expanded the use of pasture legumes into new environments. In the first example, strain SRDI554 was developed for use with messina, *Melilotus siculus*, a herbaceous annual pasture legume adapted to saline, marshy areas. Strain SRDI554 was selected from 118 strains through a series of greenhouse, laboratory and field studies in SA and WA, and was shown to be far superior to the commercial inoculant strain WSM1115 in fixing N, producing nodules, surviving on seed and, most importantly, surviving in saline soils (Figure 4-4, Figure 4-5, both page 59). While strain SRDI554 is adapted to saline soils, it is only recommended where soil pH (Ca) is greater than 5.8 (Ballard and Peck 2021).

FIGURE 4-3. The response of inoculation of faba bean with an acid tolerant strain (left), relative to the current commercial strain WSM1455 (right).

Photo: Liz Farquharson, SARDI.

FIGURE 4-4. Growth of messina plants (~12 weeks) related to inoculation with different rhizobial strains as part of the selection process of new rhizobia for saline soils (Ballard RA, unpublished).

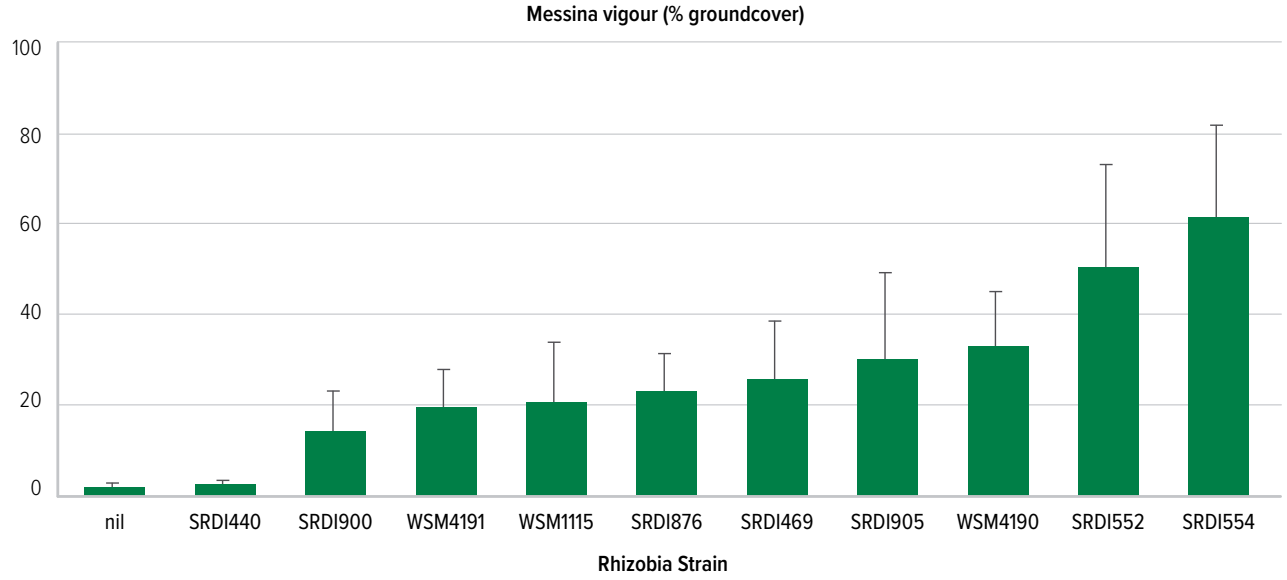


FIGURE 4-5. The growth of messina was improved by new strain SRDI554 (left) compared to the commercially available strain WSM1115 (right) which is used in medic inoculants and was initially used for messina.

Photo: Ross Ballard, SARDI.

A similar process was used to recently select inoculant strain WSM4083 for tedera, *Bituminaria bituminosa* var. *albomarginata*, a herbaceous perennial pasture legume originally from the Canary Islands that has high drought-tolerance and remains green and productive during the summer months. This makes it a valuable out-of-season feed source to fill the summer and autumn feed-gaps in Mediterranean climates (DIPIRD_WA 2020).

4.8 Concluding comments

The benefits of legume inoculants and their use is dependent on their quality, the way they are stored and applied, along with paddock conditions and crop performance. Without a strong quality assurance program, the quality of the inoculant used may be poor and the benefits from inoculation will not be realised. In countries across the globe, the successful production and use of legume inoculants is invariably associated with an effective, regulatory quality assurance program, with strong industry support and adherence. The programs mainly focus on ensuring the most highly effective rhizobial strains are contained in the inoculants in high numbers and other contaminating organisms in very low numbers. The quality assurance may be supported by appropriate legislation (e.g., Canada, Uruguay, France) or may be voluntary on the part of the inoculant manufacturers (e.g., Thailand, New Zealand, South Africa, Australia). In other countries, such as the US, regulatory control and independent testing has been considered unnecessary, with manufacturers conducting their own internal quality assurance.

Due to the vigilance of QA testing and strong linkages between researchers and the Australian inoculant industry, the field performance of rhizobial inoculants has and continues to be impressive. Efforts are continuing to select improved inoculant strains to meet the needs of changing farming systems and develop formulations that optimise the productivity of legume crops and pastures.

5 | Rhizobia and the rhizobia-legume symbiosis

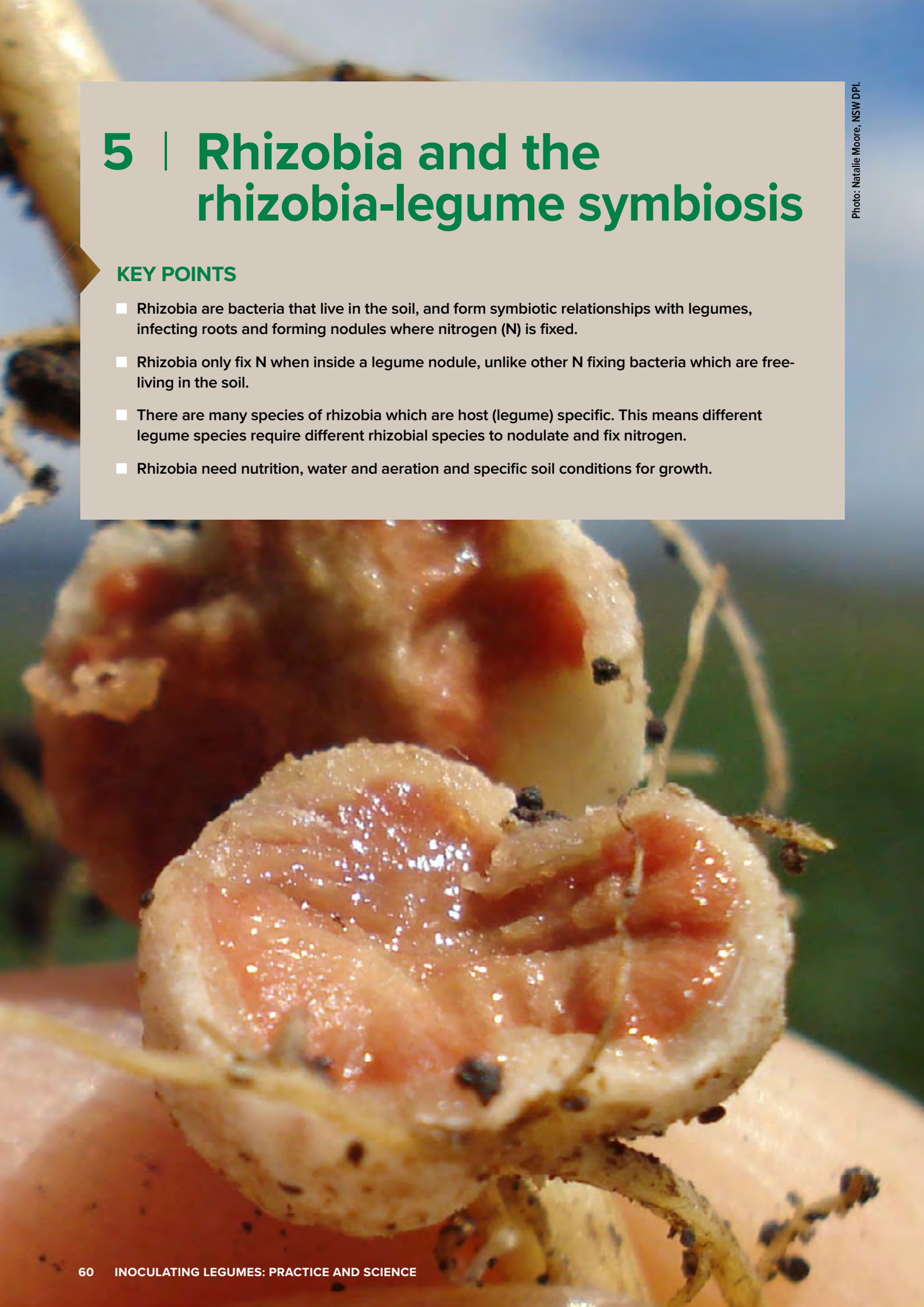


Photo: Natalie Moore, NSW DPI.

KEY POINTS

- Rhizobia are bacteria that live in the soil, and form symbiotic relationships with legumes, infecting roots and forming nodules where nitrogen (N) is fixed.
- Rhizobia only fix N when inside a legume nodule, unlike other N fixing bacteria which are free-living in the soil.
- There are many species of rhizobia which are host (legume) specific. This means different legume species require different rhizobial species to nodulate and fix nitrogen.
- Rhizobia need nutrition, water and aeration and specific soil conditions for growth.

5.1 What are rhizobia?

Rhizobia, also known as root-nodule bacteria, are specialised soil bacteria that are prominent members of microbial communities in the soil and on plant roots. Rhizobia are microscopic single-celled organisms. They are so small, being around one thousandth of a millimetre in length, that they can only be seen through a microscope. Many thousands of cells of rhizobia would fit on the head of a pin.

Due to their co-evolution with legumes, they are able to establish mutually beneficial, i.e. symbiotic, associations with the roots of legume plants to fix atmospheric N. The availability of this fixed N can make the legume independent of soil and fertiliser nitrogen. When legumes are grown in rotation with other crops, their requirements for fertiliser N are reduced due to the carryover of N for following crops, resulting in increased agricultural productivity and efficiency.

When the legume-rhizobia symbiosis is successfully established, specialised structures on the legume roots, known as root nodules, appear. Within the root nodules, the rhizobia use carbohydrates supplied from the plant and in return fix atmospheric N for use by the plant. The nitrogen (N_2) is fixed by the rhizobia into ammonia (NH_3) that is then transferred to the plant and assimilated into organic compounds for distribution via the xylem - the same part that transports water and nutrients from the soil to the shoots. Legumes are unable to fix atmospheric N in the absence of rhizobia, although they can absorb mineral N from the soil. Rhizobia only fix N when inside the root nodules.

Rhizobia can have thread-like flagella that allow them to move through water films in soil and on plant roots. However, they are spread over longer distances on seed, in dust and in soil. Although all rhizobia appear very similar under the microscope, they are genetically diverse and markedly different organisms. There are more than 200 named species of rhizobia in 18 genera, and scientists are discovering and describing about 20 new species each year. At present in Australian agriculture, we only use a small number of species of rhizobia as inoculants for the legumes we grow. As new legume genera and species with potential for agricultural use are developed, there will be a need for new species of compatible rhizobia to be used as inoculants.

Each species of rhizobia includes many thousands of genetically unique forms (strains) that vary in important characteristics that influence their interaction with the legume and adaptation to soil conditions. Commercial inoculants in Australia contain single strains of rhizobia that provide optimum N fixation with the target legume and adaptation to soils where the legume is grown.

5.2 Specificity of rhizobia

The relationships between rhizobia and particular legumes are very specific, hence unique strains are prepared as inoculants for the various legumes grown in Australian agriculture.

Only specific rhizobia will nodulate and fix N with a particular legume host – this is why we have different inoculants.

An inoculant or inoculation group is a cluster of legumes nodulated by the same species of rhizobia (Table 5-1, page 62). Different inoculation groups are nodulated by distinctly different rhizobia. For example, lupins are nodulated by the slower-growing (on agar) acid-tolerant *Bradyrhizobium* spp., whereas the medics are nodulated by the fast-growing, acid-sensitive *Sinorhizobium* spp. The groupings provide a practical framework when considering if inoculation is needed based on the type of legume previously grown in a paddock, and for choosing the correct inoculant for the particular legume to be sown. Inoculants are produced and marketed commercially according to these inoculant groups. More detail of inoculants and inoculation can be found in Chapters 2, 6 and 9.

5.3 What do rhizobia need to prosper?

Rhizobia only exist as living cells i.e. they cannot form survival structures known as spores and this makes all rhizobia very sensitive to environmental stresses. They can easily be killed by exposure to stresses such as heat, extreme pH and toxic chemicals.

TABLE 5-1. Some of the legume inoculant groups used in Australian agriculture and their rhizobia (see Chapter 9 for a complete list of the inoculant groups).

Taxonomy of 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 peas & vetch
	F[2]	Faba beans & lentil
Bradyrhizobium japonicum	H	Soybeans
Mesorhizobium ciceri bv. *biserrulae*	Biserrula special	Biserrula
Bradyrhizobium spp.	P	Peanuts
Rhizobium sullae	Sulla special	Sulla
Bradyrhizobium spp.	I	Cowpeas, mungbeans
Bradyrhizobium spp.	J	Pigeon peas

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 beans/lentils and group F used for peas/vetch*

As with all bacteria, rhizobia will grow when the conditions are suitable, i.e. when they are provided with food (carbon and other nutrients) and water at a suitable pH (Table 5-2). Rhizobia are aerobic organisms and need oxygen for respiration. Temperature also markedly affects rhizobia. The conditions listed in Table 5-2 (substrate, air, water, pH and temperature) are what inoculant manufacturers try to optimise when they produce inoculants.

On seed and in soil, rhizobia can be killed by heat (some die at 35°C), desiccation, extreme acidity or alkalinity, and the presence of toxic chemicals such as fertilisers, fungicides and heavy metals (Table 5-3). These stresses must be avoided when handling and applying inoculants to ensure the maximum number of rhizobia remain alive and are able to colonise the soil and legume roots in sufficient number to form nodules.

The acidity or alkalinity of water and other additives used during the inoculation process can determine whether rhizobia live or die. All rhizobia survive well at neutral pH (7.0), although different species vary in their sensitivity to pH (Table 5-4, page 63).

In soils below pH 5 (measured in $CaCl_2$), aluminium and manganese toxicity become additional stresses that can kill rhizobia. Moderate soil salinity is usually not a practical limitation to the growth and survival of rhizobia, but the legume is typically more sensitive to salinity stress. The legume messina is an exception as it has a high tolerance to salinity and requires an inoculant strain with comparable salinity tolerance.

TABLE 5-2. Rhizobia are living organisms with essential needs for growth and survival.

Requirement	Comment
Food and energy	Usually carbohydrates (sugars such as glucose)
Mineral nutrients	Essential macro and micronutrients
Water	Rhizobia can only grow in moist conditions
Temperature	Preferred range is 15 to 30°C
pH	Preferred range is pH 6.0 to 7.5
Air	Rhizobia are aerobes and need oxygen for respiration

TABLE 5-3. Harsh environmental conditions kill rhizobia.

High Temperatures above 35°C will kill most rhizobia	
Acidity and alkalinity	pH sensitivity of rhizobia varies (see Table 5-4 , page 63)
Toxic chemicals	Fungicides, solvents, alcohols and disinfectants kill rhizobia
Inorganic chemicals	High levels of heavy metals (Zn, Cu, Co) kill rhizobia

TABLE 5-4. Sensitivity of key rhizobia to pH_{CaCl_2}, where red is sensitive and green is optimal.

Rhizobia	Host legume	pH 4	pH 5	pH 6	pH 7	pH 8
Bradyrhizobium spp	Cowpea, lupin, serradella					
Bradyrhizobium japonicum	Soybean					
Rhizobium leguminosarum bv. *trifolii*	Clovers					
Rhizobium leguminosarum bv. *viciae*	Pea, faba bean, lentil, vetch					
Mesorhizobium ciceri	Chickpea					
Sinorhizobium spp.	Medics					

5.4 The process of nodulation

Rhizobia need adequate nutrients, moisture, temperature, pH and aeration for growth and survival.

Nodulation always begins with the colonisation of the legume roots by rhizobia. The earlier the colonisation, the sooner root nodules develop and the rhizobia begin to fix N. A specific sequence of events and optimal conditions are required for nodulation, which can commence within days of plant germination.

Nodule formation on legume roots is the result of a highly regulated process. This infection process is under the genetic control of both rhizobial and plant genes, and a high degree of genetic compatibility between partners is essential for the development of nodules containing highly effective rhizobia. This strong genetic compatibility is one of the key features of the elite inoculant strains currently available to Australian farmers.

An essential feature of nodule formation is the exchange of specific signal chemicals between the legume root and the appropriate species of rhizobia. While the rhizobia are the partner that fixes the N in this symbiosis, the legume plants generally determine the pathway of infection, and subsequently the type of root nodule that develops.

The majority of agricultural legumes grown in Australia are infected via root hairs and so anything that injures these fragile root parts can reduce nodulation. Notable exceptions are lupins which are infected between epidermal cells, and peanut and stylothanes which are infected at lateral root junctions.

For N fixation to occur, two unique compounds are produced in the nodules:

1. Nitrogenase produced by the rhizobia – this is the enzyme that facilitates the conversion of atmospheric nitrogen (N_2) to ammonia (NH_3) which can be used by the plant. The enzyme contains molybdenum (Mo), which is why this micro-element is often added as a fertiliser when legumes are sown.
2. Leghaemoglobin produced by the plant – this compound provides the characteristic pink/red colour of healthy functioning nodules.

The function of the leghaemoglobin in the nodule is similar to that of haemoglobin in blood. Both compounds act as oxygen-transport molecules with the leghaemoglobin making sure the right concentration of oxygen is available for rhizobial respiration. However, excess oxygen adversely affects the nitrogenase enzyme and stops N fixation. The colour of nodules is often used as an indicator of active N fixation as the presence of leghaemoglobin (pink colour) is a prerequisite for the process. In contrast, white nodules lack leghaemoglobin and cannot fix N. Green nodules usually indicate non-functional senesced nodules, with the green colour being a breakdown product of leghaemoglobin.

Nodule types

There are two basic types of nodules on agricultural legumes – determinate and indeterminate. The legume plant alone governs which type of root nodule occurs, irrespective of the species of rhizobia.

Soybeans, peanuts, lotus, navy beans, cowpeas and pigeon peas are legumes that form determinate nodules, see Figure 5-1a, page 64. Determinate nodules are generally spherical, less than five millimetres in diameter and lack distinct internal zones. If the internal colour of these nodules is white or green, rather than pink, then they are unlikely to be fixing N.

Field peas, faba beans, lentils, chickpeas, lucerne, medic, clover, biserrula, serradella and sulla are legumes that form indeterminate nodules. Indeterminate nodules can keep growing throughout the season and can remain functional to meet the N demand of the crop. These nodules can develop lobed finger-like projections to give a coralloid appearance Figure 5-1b, page 64.

Internally, indeterminate nodules have distinct zones, shown in Figure 5-2, page 64, and grow from the outside tip, a region called the meristem. Although some part of the nodule may go green during the growing season, if the tip is pink the nodule should still be fixing some N.

FIGURE 5-1 (a). Example of a determinate nodule of soybean.

Photo: Natalie Moore, NSW DPI.

FIGURE 5-1 (b). Example of an indeterminate nodule of chickpea showing active pink zone and green senescent zone (near the root).

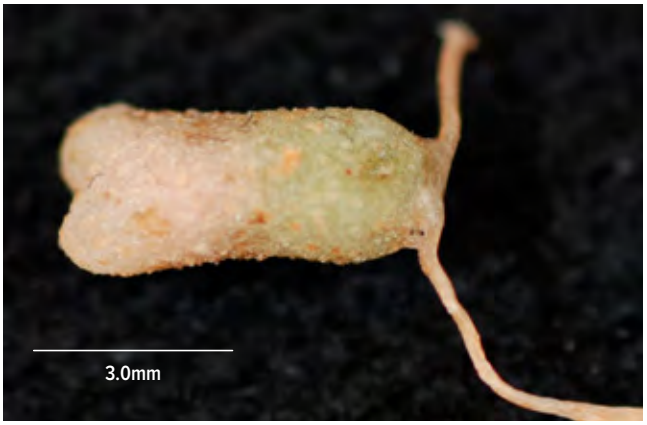


Photo: Liz Farquharson, SARDI.

5.5 Other important symbioses that fix nitrogen

i. *Acacia* (wattles) are a group of legumes that form nodules in association with rhizobia. Unlike the agricultural legumes, acacias are native to Australia and their rhizobia already reside in the soil. The acacia rhizobia are similar to the lupin and soybean rhizobia; however, there is no overlap (cross infection) between them.

ii. *Casuarina* are non-legume trees (sheoaks) that can fix N in nodules formed by very special and unusual soil bacteria, called *Frankia*. They grow as long filaments and appear more like fungi than bacteria.

5.6 Legume and rhizobia incompatibility

Although a considerable amount of time and effort is exerted to select efficacious strains of rhizobia and provide these to the inoculant manufacturers for use in commercial inoculants, these may be outcompeted by less effective rhizobial strains present in the soil.

In many situations there are already rhizobia present in the soil that can nodulate the legume in preference to the applied inoculant rhizobia (Chapter 6). Although unlikely, these resident strains may have always been present. However, it is more likely that they were introduced on seed and soil through agricultural activities or they arose from genetic changes of inoculant rhizobia after being introduced into the soil. In these situations, the quest to form a nodule becomes a competition between the improved inoculant rhizobia applied at sowing and other strains of rhizobia already in the soil. The quality of the inoculant and its survival during the process of inoculation is critical in this competition. This is covered in more detail in Chapters 4 and 6.

Scientists are just beginning to understand how resident strains of rhizobia evolve in the soil. The best understood scenario in Australia is that of biserrula. At the time biserrula was introduced to Western Australia in 1994, there were no rhizobia in soils capable of nodulating it.

All sown biserrula were inoculated with an elite rhizobial strain. Within seven years it was noticed that a small proportion of nodules formed on biserrula regenerating in the field which were small and green, and occupied by rhizobia that differed considerably from the original inoculant. Research since then has led us to understand that the original inoculant strain for biserrula has shared its nodulation genes with other bacteria that were already in the soil in Western Australia. These bacteria were able to nodulate biserrula only when they received the genes for nodulation, but they do not always have all the other genes required for high levels of N fixation.

Hence, the evolution of less effective rhizobia in soil may significantly impair N fixation of legumes if they successfully out-compete the highly effective inoculant rhizobia to form nodules. In general, this is less of a problem for the pulse legumes than for the annual pasture legumes (see chapter 6).

The only means of managing this issue is to periodically re-inoculate sown or regenerating biserrula with high numbers of the highly effective inoculant rhizobia (hoping to out-compete the soil rhizobia). More long-term research is underway to identify strains of rhizobia that do not share their nodulation genes with soil bacteria and can compete with background rhizobia populations. An alternative approach is the development of legume cultivars that inherently form more effective symbioses.

FIGURE 5-2. Cross section of a mature indeterminate nodule from sub clover stained, and imaged using a laser scanning confocal microscope. Four distinct sections of the nodule are visible; i) Active meristem where cells divide, ii) Infection zone, where rhizobia bacteria enter the plant cells through infection threads, iii) N fixation zone, each cell contains leghaemoglobin and fully differentiated bacteria called bacteroids which fix N, iv) the senescent zone, this is where older bacteroids degrade and lose function.

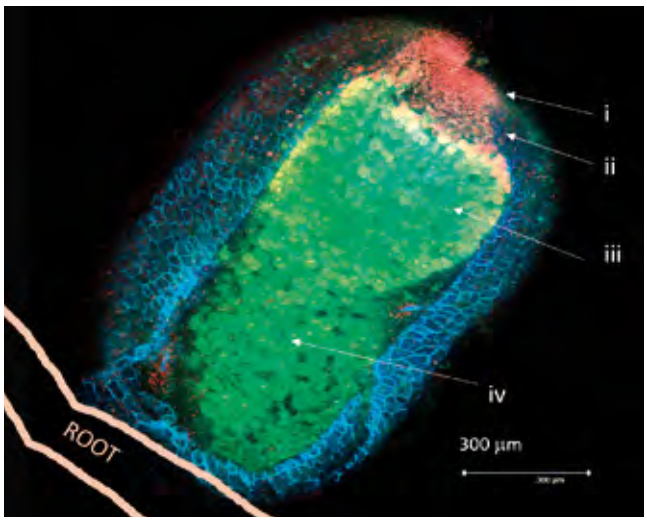


Photo: Liz Farquharson, SARDI, and Rosemary White, CSIRO.

6 | Number and nitrogen fixation capacity of rhizobia in soils

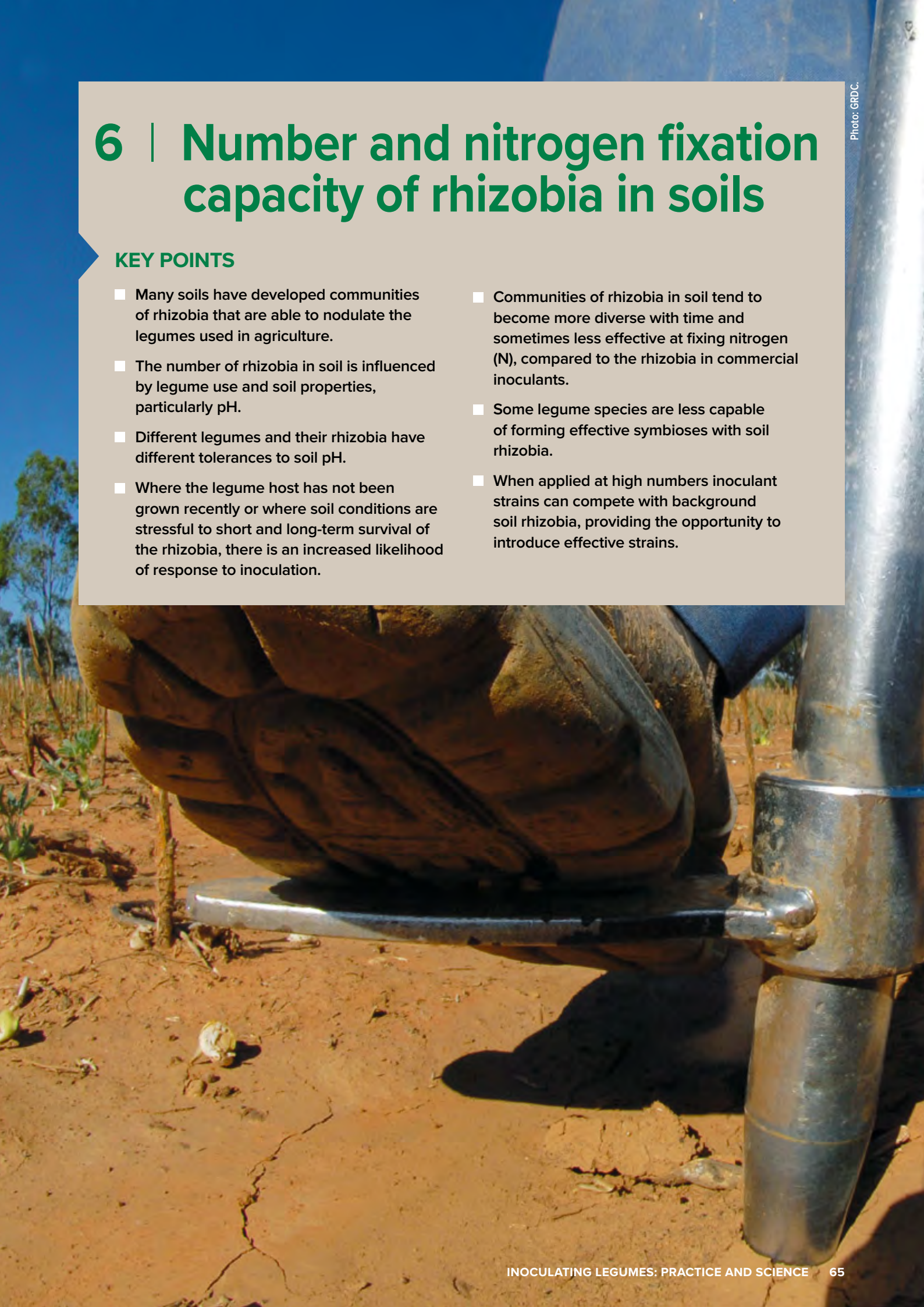


Photo: GRDC.

KEY POINTS

- Many soils have developed communities of rhizobia that are able to nodulate the legumes used in agriculture.
- The number of rhizobia in soil is influenced by legume use and soil properties, particularly pH.
- Different legumes and their rhizobia have different tolerances to soil pH.
- Where the legume host has not been grown recently or where soil conditions are stressful to short and long-term survival of the rhizobia, there is an increased likelihood of response to inoculation.
- Communities of rhizobia in soil tend to become more diverse with time and sometimes less effective at fixing nitrogen (N), compared to the rhizobia in commercial inoculants.
- Some legume species are less capable of forming effective symbioses with soil rhizobia.
- When applied at high numbers inoculant strains can compete with background soil rhizobia, providing the opportunity to introduce effective strains.

6.1 Introduction

Before European settlement, Australian soils lacked the rhizobia needed for the pulse and pasture legumes that are now commonly grown in farming systems. Now, after more than a century of legume cultivation, many soils have developed large and diverse communities of introduced rhizobia.

Rhizobia become established in soils in several ways. Many were introduced as high quality inoculants. Others arrived accidentally with the movement of dust, soil and seed around the country, and some have evolved via genetic exchange with other bacteria in the soil (see Chapters 1 and 5). However, because rhizobia are legume specific and their persistence is affected by soil characteristics and cultural practices, their diversity, number and N fixation capacity vary across the country.

This chapter examines some of the factors leading to this variability and its implications for inoculation, nodulation and N fixation by different legumes.

6.2 How do we know if a soil has the right rhizobia?

The history of legume cultivation in a paddock provides a guide as to what rhizobia might be present. If a legume species, or others with similar rhizobial requirements, has not been grown in a paddock, then there are probably insufficient numbers of soil rhizobia to effectively nodulate the legume.

Conversely, where there has been a recent history of well-nodulated and productive legumes in a paddock, there is a reasonable chance the rhizobia that nodulated the legume will remain in the soil. There can also be more than one species of rhizobia in the soil. For example, it is common to find clover, lucerne and field pea rhizobia in the same paddock, if all those legumes have been grown previously.

As a general rule of thumb, it is suggested that inoculation is not necessary if the legume host has been grown in any of the previous four years. The problem with this simplistic rule is that it fails to recognise that the level of nodulation of the previous crop can affect the current population of rhizobia in the soil and that many soils are not conducive to the survival of large numbers of rhizobia because of factors such as extremes of soil pH. Also, the communities of some rhizobial species that develop under legume cultivation can become less effective at fixing N over time. If the previous legume was well nodulated and productive, that is a good indication that the rhizobial strain was effective and the soil was conducive to rhizobial survival.

6.3 How many soil rhizobia are needed for effective nodulation?

The minimum number of soil rhizobia needed for rapid and effective nodulation lies somewhere between 100 and 1000 rhizobia per gram of soil. We say this for two reasons. Firstly, when commercial inoculants of rhizobia are applied at recommended rates, they add the equivalent of about

FIGURE 6-1. Example of rapid and abundant nodulation on a bean root collected from a paddock containing an adequate number of rhizobia.

Photo: Liz Farquharson, SARDI.

100 rhizobia per gram of soil to a 10 centimetre depth. This consistently results in prompt nodulation because these are placed close to the seed. Secondly, the evidence from many field and greenhouse experiments is that nodulation is inadequate once the number of rhizobia is less than 100 per gram of soil. High numbers of rhizobia result in rapid nodulation and plants tend to develop many nodules on the tap root, close to the top of the root system (Figure 6-1). Low numbers of soil rhizobia can result in delayed nodulation and fewer nodules mainly on the lateral roots.

6.4 Measuring the number of rhizobia in soil

Until recently, a laboratory-based plant nodulation bioassay has been used to determine the number of rhizobia in the soil. In the test, the legume of interest is inoculated with a sequence of 10-fold dilutions of the collected soil (Figure 6-2, page 67). After four weeks of plant growth, the number of plants with nodules resulting from each soil dilution is used to calculate the number of rhizobia in the original soil sample (called a most-probable number calculation). While this test is not available to growers, it has been used by researchers to quantify numbers of rhizobia in thousands of Australian paddocks. Apart from being slow and labour intensive, the plant-based test is expensive, and is generally used for experimental purposes only.

The test is generally used with soils collected from the top 10 centimetres, because this is where most rhizobia are concentrated, and therefore, where most nodulation of annual legumes occurs. Rhizobia are also found deeper in the soil profile, below 10cm. Although these rhizobia can play an important role in maintaining the N fixation of annual legumes towards the end of their growth cycle and the nodulation of deep-rooted perennial legumes such as lucerne, they are seldom measured because they are less predictive of inoculation response.

More recently, a DNA based test has been developed by SARDI to measure rhizobia number in soils. The test measures the rhizobia that nodulate legumes in the E and F inoculation group (faba bean, lentil, field pea and vetch). This test is available to growers (pir.sa.gov.au/research/services/molecular_diagnostics/predicta_b) with further tests for lupin and chickpea rhizobia in development. While the test can be used at any time of the year, it is recommended soil collected in February (2-3 months before sowing for winter crops) is used to inform inoculation requirement once the number of rhizobia in has been determined (Figure 6-3).

FIGURE 6-2. Plant assay method for counting rhizobia in soil. Plants are inoculated with different soil dilutions and the frequency of nodulation measured.

Photo: Liz Farquharson, SARDI.

FIGURE 6-3. Example report produced for the DNA test that measures E and F rhizobia number in soil.

PREDICTA rNod
Group E & F root nodule bacteria

SARDI Soil biology & Diagnostics
Gate 2A, Hartley Grove
URRBRAE SA 5064
Ph 08 8429 2236

Sample:	**RNB1171**			**Report date:**	**15/04/2021**
Paddock:	**B4**			Date sampled:	31/03/2021
Grower:	**J SMITH**			Dry weight (g):	**493**
Nearest town:	**ADELAIDE**	Region:	**Southern**	Sample condition:	Damp
				Core depth:	10cm

Pulse/legume history	Group E & F*	Chickpea	Lupin/serradella	Mungbean
Year last grown eg 2015:	2020			

*Group E & F crops include faba bean, lentil, field pea, vetch and lathyrus

Intended crop	**Soil pH(Ca):**	**Soil texture**
Beans	7.38	Loam

BENEFICAL ORGANISMS	**RESULT**		**Inoculation Requirement****
			High / Medium / Low / Nil
Rhizobium Group E & F	4.1	log(rhizobia)/g soil	

**Inoculation requirement categories may vary between regions and seasons, and may be revised over time.

Agronomist	Mobile	Accreditation Number
Pulse, Peter	0418 000 000	1000

Comments on results:

Group E and F rhizobia persist well above this pH.

Inoculation not required.

For PREDICTA®B accredited agronomists: rootdisease.aweb.net.au
Further information is available at pir.sa.gov.au/research/services/molecular_diagnostics/predicta_b
also see: Ballard *et al.* 2020.

Because most interest in rhizobia number is to understand the requirement for inoculation, rhizobial numbers are commonly measured in soils collected at the end of summer or close to the start of the growing season in Mediterranean environments. Numbers are near their lowest at this time and provide a conservative guide to the number available for legume nodulation. However, the number of rhizobia in soil changes through a growing season, particularly when a legume host is grown (Figure 6-4).

In the Mediterranean climates of the western and southern regions, rhizobial numbers start to increase at the break of the season in autumn when soils become wet and the legume host germinates. Rhizobia are stimulated to multiply in the immediate vicinity of the root or rhizosphere. Under favourable conditions they can quickly multiply to levels of 10,000 per gram of soil.

Once the rhizobia have infected the root, they multiply and change into a bacteroid form capable of fixing N. Rhizobia cannot fix N in the free-living form in the soil. Legume root cells infected with rhizobia grow and differentiate to form the nodules where the N fixation takes place. In the middle of the growing season, nodules on well established plants can contain 40 million rhizobia and rhizobia in soil close to the root can exceed one million per gram of soil.

When annual legumes set seed, their nodules begin to shut down and cease N fixation as carbohydrates that provide energy to the nodules are diverted to seed development. Eventually the nodules senesce, and rhizobia are released back into the soil.

Rhizobial numbers may then decline to less than 100 per gram of soil over the next few months if soil conditions are stressful (e.g. low pH), or they may persist at a level of many thousands under more benign soil conditions (Figure 6-4).

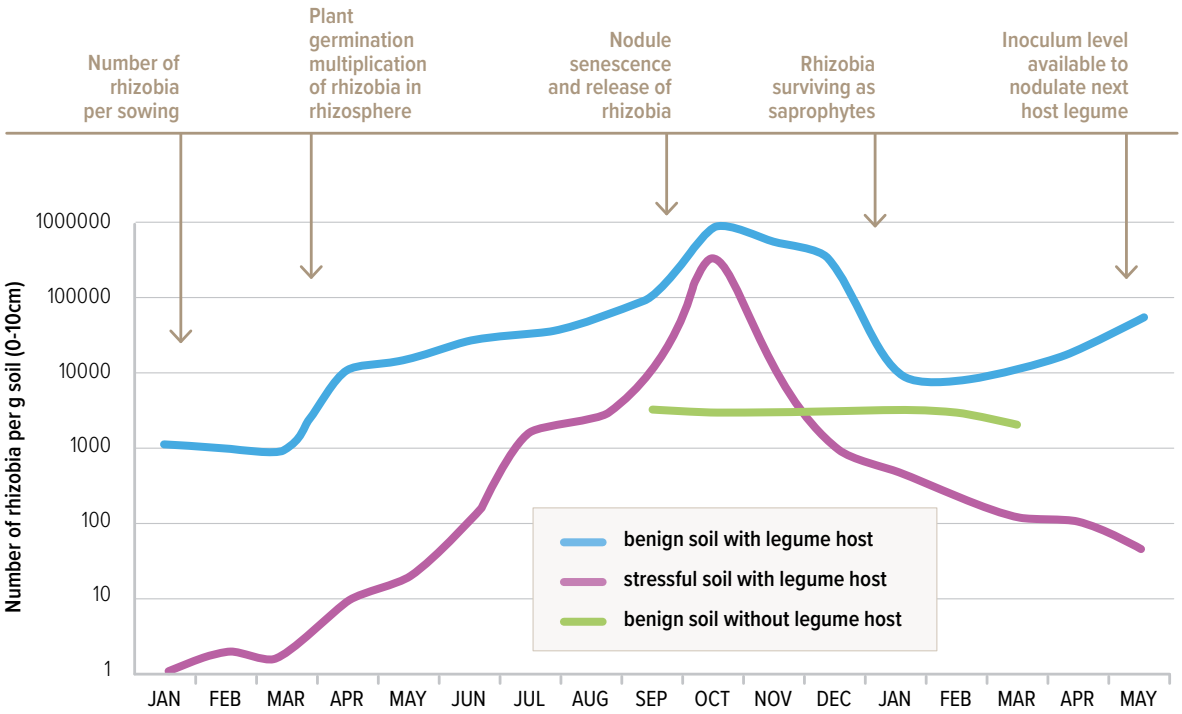
Rhizobial numbers are more stable if a legume host is not grown in the paddock. Across nine paddocks with neutral pH in southern Australia which had a good history of field pea, lentil or faba bean production, rhizobial numbers were between 2000 and 3000 when measured between September and March in the cereal crops that followed the legumes. These rhizobial numbers will gradually decline each year a non-host is grown.

6.5 What numbers of rhizobia are measured in soils?

Surveys of soils provide a snapshot of the number of rhizobia at a given time and reveal that many favourable soils support substantial populations of rhizobia. It is not unusual to measure more than 1000 rhizobia per gram in the top 10cm of soil at the end of summer. A million rhizobia per gram has been measured in some instances. To put this number in context, the proportion of rhizobia in the total population of soil bacteria is still less than 0.1 per cent. Figure 6-5 shows how the numbers of rhizobia in Australian soils vary for three pulse and two pasture legumes.

In surveys of Australian soils conducted over a number of decades, rhizobia for the pasture legumes – medic and clover – were abundant, with more than 60 per cent of soils containing 1000 or more rhizobia per gram (Figure 6-5, page 69). Large areas of sown, regenerating and naturalised pasture

FIGURE 6-4. Typical changes in the number of rhizobia through the seasonal cycle of an annual legume in southern Australia (Mediterranean climate).



legumes (currently about 40 million hectares across the country) aid the multiplication and survival of these rhizobia.

In contrast, rhizobia for pulse legumes are less abundant and seemed to struggle to persist in large numbers. For field peas, chickpeas and lupin, about 40 per cent of soils contained less than 100 rhizobia per gram. Understanding why some soils support fewer rhizobia is important to making sensible decisions about further inoculation.

6.6 Factors affecting the survival of rhizobia in soil

Regional influences can strongly affect the occurrence of rhizobia in soil. These regional effects reflect both historical differences in legume use as well as differences in the characteristics of the soils.

Influence of host legume

At a regional level, the more widely a legume has been grown, the more likely soils will contain compatible rhizobia. For example, all the chickpea soils without rhizobia shown in Figure 6-5 were from South Australia, where chickpeas are less frequently grown. The remaining soils were from an area in New South Wales where chickpeas are commonly grown and compatible rhizobia were abundant in most of these soils.

Once a well nodulated legume has been grown, the number of years since it was grown and the soil type influence how many rhizobia are likely to persist in the soil. Where soil conditions are non-stressful, rhizobia can persist in the soil for many years in the absence of a host legume, but may decline rapidly where soil conditions are stressful. The relationship between time since the last Group E/F legume crop and rhizobia number is shown in Figure 6-6. Although a general decline in rhizobia number is evident over time, at any given time rhizobia number in individual paddocks can vary greatly. In general, inoculation is recommended where a suitable host has not been grown in the previous four years, since after this time the majority of paddocks have fewer than 1000 rhizobia per gram of soil. However, even after two years absence of the host legume, some paddocks have less than 100 rhizobia per gram soil. Where more specific guidance is needed, the DNA test that measures the number of E and F rhizobia in soil (for faba bean, lentil, field pea and vetch) should be used (Section 6.4 above).

In general, pasture legume rhizobia occur more often in high numbers than pulse rhizobia, due to the widespread occurrence of sown and naturalised clover and medic species. Even so, there are some commercial species within the clovers that may not consistently nodulate with the soil rhizobia because they are incompatible with much of the soil rhizobia population.

Within the annual clovers, gland clover (cv. *Prima*) and bladder clover (cv. *Bartolo*) are more specific in their rhizobial requirement and should be inoculated even where rhizobia that nodulate other annual clovers are known to be present. However, such nodulation specificity is uncommon, and legume species within the same inoculation group almost always have similar rhizobial requirements for inoculation.

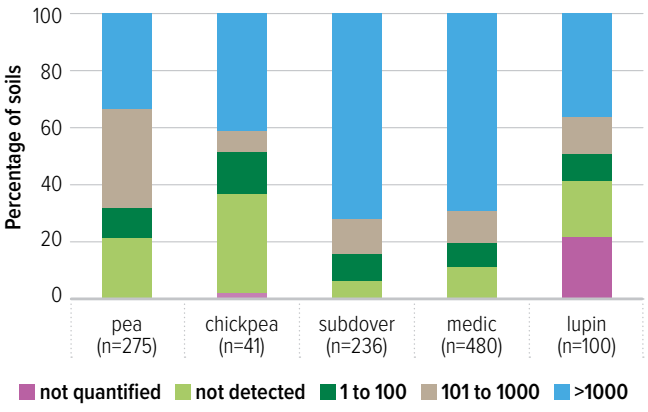
Influence of soil type

Soil chemical, physical and biological properties affect the survival of rhizobia, especially pH, texture (clay content) and organic matter.

Soil pH is the best understood of these properties. It affects both the survival of the rhizobia and the formation of nodules, and can also affect the growth of the host plant. Different symbioses have different pH preferences. Although the rhizobia tend to be a little more sensitive to pH extremes than the legumes, understanding the pH preferences of the host legume will provide a reasonable insight into the pH preferences of the legume-rhizobia symbiosis.

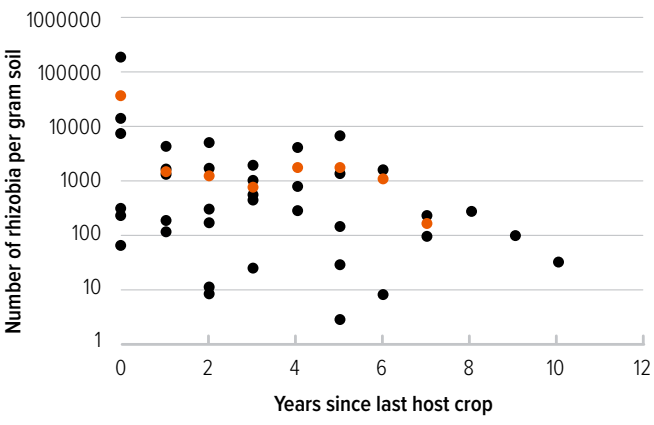
The preferred pH range (measured in calcium chloride) of some of the more common pulse and pasture legumes is

FIGURE 6-5. Percentage of soils classified according to number of field pea, chickpea, sub clover, medic, or lucerne rhizobia they contain.



SOURCE: Chatel and Parker 1973; Slattery and Coventry 1989; Fettell *et al.* 1997; McInnes 2002; Howieson and Ballard 2004; Evans 2005; Elias 2009; Drew *et al.* 2011, 2012; Ballard *et al.* Unpublished data.

FIGURE 6-6. Relationship between years of absence of the host crop (field pea, bean lentil or vetch) and number of E & F rhizobia in soil. Orange circles show the average rhizobia number at each time since last host crop.



SOURCE: Evans 2005; Ballard *et al.* 2004, Fettell *et al.* 1997; Drew *et al.* 2012; Barnett and Ballard, Unpublished data.

shown in Table 6-1. Narrow-leaf lupin and serradella rhizobia are highly tolerant of soil acidity. While these species readily form nodules at pH 4.0, they can experience nodulation problems where soil pH exceeds 7.0.

Rhizobia that nodulate field pea, faba bean, lentil and vetch are moderately sensitive to soil acidity. Data from several surveys of these rhizobia across Australia have been combined in Figure 6-7 (page 71) to demonstrate the strong effect that pH has on their number in soil. Below pH 5.5, the number of rhizobia is less than 100 per gram of soil, and without inoculation, insufficient nodulation is highly likely.

On acidic soils less than pH 5.5, more frequent inoculation is needed for field peas, faba beans, lentils and vetch, and lime application to the soil should be considered to increase soil pH if it is less than 5.5. Liming programs that increase pH benefit the number of E and F rhizobia persisting in soil (Figure 6-8, page 71) as well as legume root growth and nodulation (Figure 6-9, page 71). Liming targeting a pH of 5.5 will also benefit the performance of cereals and oilseeds in the rotation.

Lucerne and its rhizobia are also sensitive to soil acidity with rapid decreases in rhizobial survival and nodulation occurring below pH 5.0.

The strand and disc medics that are assigned to the same inoculant group as lucerne (AL) are similarly sensitive to soil acidity. Burr, sphere and murex medics are more tolerant of acidic soils, with increased tolerance attributable to the selection and use of an acid-tolerant strain of rhizobia (WSM1115, group AM inoculant) selected for use with these medics.

Subterranean clover is divided into three subspecies with ssp. *yanninicum* best adapted to acidic soils. Although more tolerant of acidity than both lucerne and medic, the effects of acidity in sub clover pastures are easily overlooked as plants compensate to a point by producing larger, but fewer nodules. It is not until nodule mass falls below the level needed to supply the plant with adequate N that the effects of the acidity become obvious. At this point the sub clover content of the pasture can decline rapidly.

There are also instances where the rhizobia in relatively undisturbed pastures may be able to avoid the acidity stress, with adequate nodulation measured in regenerating sub clover pastures, even though the pH of the bulk 0-10cm soil is less than 4.5. This is attributed to the survival of the rhizobia in small niches in the soil, often associated with soil organic matter in the top 5cm. When these soils are disturbed as a result of cropping or during pasture renovation, and the rhizobia are displaced from these protective niches, numbers decline. There is a moderate likelihood of responses to inoculation on acidic soils when pastures are renovated, even though nodulation constraints may not have been apparent previously.

The relationships between soil organic matter or clay content and rhizobia are less well understood than soil pH, but rhizobia numbers tend to be lower in soils below 1.5 per cent organic carbon and 10 per cent clay content. Consistent with this, numbers are usually lower in sandy soils, compared to loamy soils in the same paddock in South Australian/Victorian Mallee. It is worth noting that most commercial inoculants produced for growers use peat (high organic matter) or clay as a carrier, because rhizobia are known to survive well in them.

Other factors

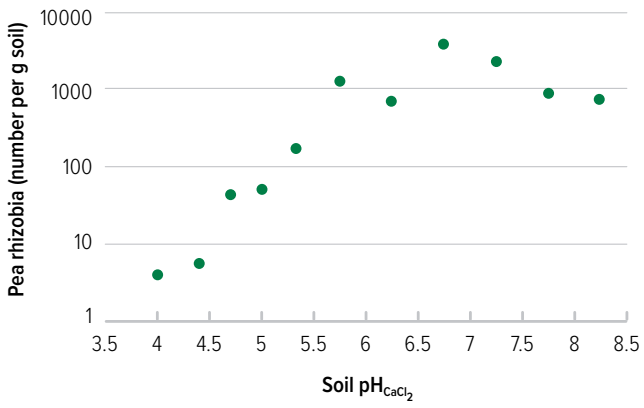
The extensive use of herbicides in farming systems may affect the legume-rhizobia symbiosis. However, their impact is mostly through damage to plant roots, rather than a direct impact on the growth, survival or effectiveness of rhizobia (sections 2.10 and 7.4). This can occur when herbicides are not used appropriately or there is carryover from one season to the next. Even where rhizobia are present in high numbers, the damage to legume root systems by some herbicides (e.g. Group 2 (B) herbicide residues in both acidic and alkaline soils in low-rainfall regions) can reduce root growth and nodulation by 50 per cent. Label recommendations on herbicide use and plant back periods should always be adhered to, especially on alkaline soils in dry conditions.

Desiccation is also detrimental to the survival of rhizobia. Rhizobia numbers in soils tend to decline by the end of a hot dry summer. Soils that experience long dry summers and are subjected to higher temperatures may have fewer rhizobia per gram of soil, particularly where clay content is low or other soil stresses are present.

Table 6-1. Recommended pH range (measured in calcium chloride) of key legumes (most acid tolerant at top).

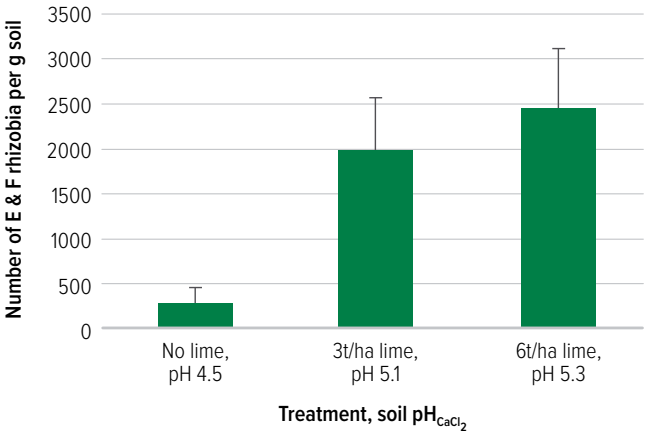
	pH 4.0	pH 4.5	pH 5.0	pH 5.5	pH 6.0	pH 6.5	pH 7.0	pH 7.5	pH 8.0	pH 8.5	pH 9.0
Narrow leaf lupin & serradella		■	■	■	■	■					
Biserrula			■	■	■	■	■	■			
Sub clover (dependant on sub species)				■	■	■	■	■	■		
Soybean				■	■	■	■				
Peanut					■	■	■				
Pea, faba bean, vetch					■	■	■	■	■	■	
Lentil						■	■	■	■		
Burr medic					■	■	■	■	■	■	
Lucerne					■	■	■	■	■	■	
Chickpea						■	■	■	■	■	
Strand and disc medic						■	■	■	■	■	

FIGURE 6-7. Relationship between soil pH and the number of field pea rhizobia in soils.



SOURCE: Drew *et al.* 2012; Ballard *et al.* Unpublished data.

FIGURE 6-8. Effect of lime application in 2018 on soil pH$_{CaCl_2}$ (0 – 10cm) and the number of E and F rhizobia persisting in the soil, when measured in February 2021. Bars indicate standard error.



SOURCE: Farquharson and Ballard, Unpublished data.

FIGURE 6-9. Impact of decreasing pH on the root growth and nodulation of faba bean (PBA Samira) inoculated with Group F rhizobia strain WSM1455.

Photo: Ross Ballard, SARDI.

pH	pH 5.0	4.5	4.3	4.0
Nodule numbers per plant	86	73	7	<1

6.7 How well do soil rhizobia fix nitrogen with legumes?

So far, we have considered the number of rhizobia in soils which impacts nodulation. However, the capacity of the rhizobia in the nodules to fix N is also important because, even where best practice inoculation is used, less effective soil rhizobia may compete with the improved inoculant strain and form a significant proportion of inefficient nodules.

The N fixation capacity of rhizobial communities in soils tends to decrease over time because, even where rhizobial inoculation is well managed, the diversity of the rhizobial community increases soon after legume introduction. The development of strain diversity can be rapid (years not decades) and is associated with the transfer of symbiosis genes between inoculant strains and other soil microbes. This is often, but not always, associated with an increase in the number of less effective rhizobia strains.

About 20 per cent of legumes sown annually and all regenerating pasture legumes are nodulated wholly or partly by a variety of rhizobia strains in the soil. DNA-based detection methods (Figure 6-10, page 72) show that more than 10 different strains of rhizobia can form nodules on a single legume plant growing in the field.

FIGURE 6-10. Different strains are shown as different 'barcodes'. Many different strains can be isolated from the nodules of a single sub clover plant.

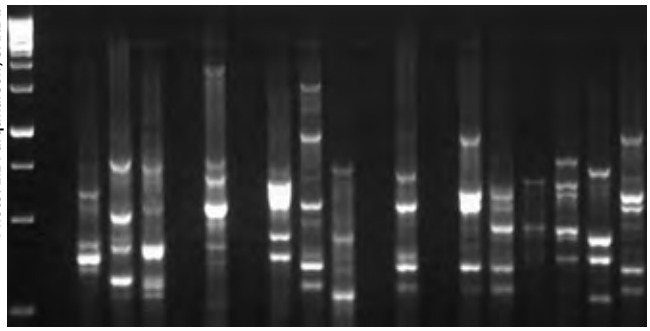


Photo: Liz Farquharson, SARDI.

Increased diversity of the rhizobial community is not always detrimental because N fixation capacity of the symbiosis is the result of the legume-rhizobia partnership, not just the rhizobia. The legume plant has substantial influence. In some situations, the plant can foster occupancy of its nodules by the more effective rhizobial strains from the soil. In other situations where less effective strains form nodules, the plant can increase nodule number to meet N demand. The extent to which different legumes are able to manage the rhizobia forming nodules means that N fixation capacity varies for different legume species. In general, pulse legumes are less prone to form ineffective symbioses than the annual pasture legumes.

The N fixation capacity of soil rhizobia is commonly measured using a whole soil inoculation method or by inoculating plants with individual strains of rhizobia isolated from nodules (Figure 6-11). The terms 'effective' and 'ineffective' are commonly used to describe differences in N fixation capacity. In Table 6-2 (page 74), the term 'effective' is used where the shoot weight of plants resulting from an inoculation treatment (rhizobia) is at least 75 per cent of plants inoculated with a highly effective strain of rhizobia. Symbiotic capacity is deemed 'moderately effective' when shoot weight is between 50 and 75 per cent and 'ineffective' when below 50 per cent.

Data about the effectiveness of soil rhizobia are more limited than for population number, especially for the tropical legumes e.g. soybeans, mungbeans and peanuts. Even so, it is apparent that while the symbioses formed by the commonly grown legumes and soil rhizobia are seldom grossly ineffective (Figure 6-12, page 73), they are often less effective than with the inoculant strain for the legume.

For example, the effectiveness of the symbioses formed between subterranean clover and the rhizobia in 43 soils ranged from 8 per cent to 99 per cent, compared to subterranean clover with the effective commercial inoculant strain (WSM1325). Most commonly, the communities of soil rhizobia were 51 to 60 per cent as effective as the inoculant strain (Figure 6-12, page 73). 32 per cent were classed as ineffective.

The average N fixation capacity of soil rhizobia with a range of different temperate legumes is shown in Table 6-2, page 74. The higher prevalence of ineffective symbioses for burr

FIGURE 6-11. Field pea from a greenhouse experiment inoculated with two strains of rhizobia of varying effectiveness. Plants formed an effective symbiosis with the commercial inoculant strain (right), but not the strain isolated from soil (left). Such ineffective symbioses are uncommon in the field because plants are nodulated by many strains of soil rhizobia and the effective strains can compensate for the ineffective ones.

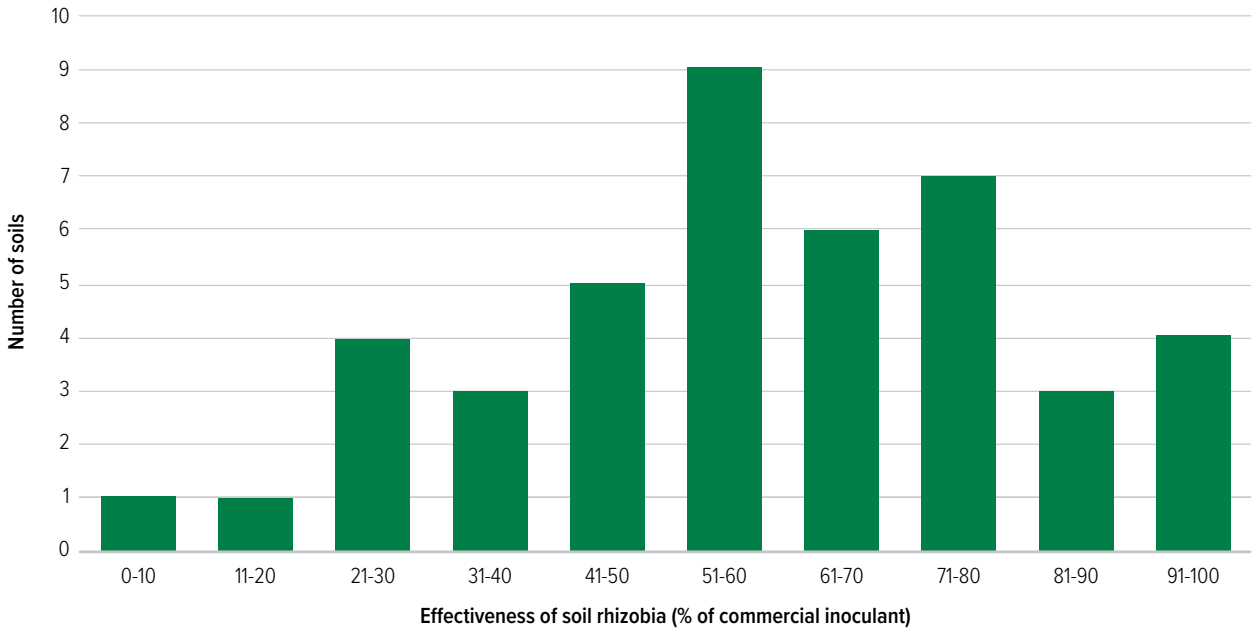
Photo: Liz Farquharson, SARDI.

medic compared to lucerne (both *Medicago* spp.) highlights the differences in how different legume species manage the rhizobia forming nodules.

Pulse legumes are less prone to form ineffective symbioses with soil rhizobia than the annual pasture legumes. For field pea and faba bean, the majority of rhizobial communities appear to be effective. Lentils and vetch are nodulated by the same rhizobia and are likely to be similar to field peas - we are not aware of data or anecdotal evidence to suggest otherwise. The same can be said for narrow-leafed lupin, which is nodulated by the same rhizobia that form effective symbioses with serradella.

While differences in rhizobial persistence can be linked to frequency of legume cultivation and soil properties such as pH, reasons for variation in symbiotic effectiveness are not well understood. Variation in symbiotic effectiveness cannot be predicted for individual paddocks, in the absence of laboratory testing.

FIGURE 6-12. Distribution of soils according to the effectiveness of their sub clover rhizobia relative to an effective inoculant strain.



SOURCE: Drew and Ballard, 2010; Drew *et al.* 2011.

6.8 Managing soil rhizobia

Where large and persistent populations of rhizobia are present in the soil, a competitive barrier for the introduction of new strains of inoculant rhizobia is created. This is not a problem where the soil community is effective with the legume host, and inoculation is not required. Where the soil rhizobia are not effective, however, high nodule occupancy by an effective inoculant strain is desirable to optimise N fixation potential. Rhizobia persist in many soils well above the threshold needed (100 rhizobia per gram) for prompt nodulation and often at numbers far greater than can be introduced through inoculation. However, rhizobia in the soil are distributed diffusely, while those applied to seed as inoculant are in close proximity to the root and are able to rapidly multiply and achieve effective nodulation.

FIGURE 6-13. Ineffective soils rhizobia (across the bottom are the log number rhizobia per gram of soil) are overcome when equivalent numbers of inoculant rhizobia are applied to the seed (shown as log number per seed)

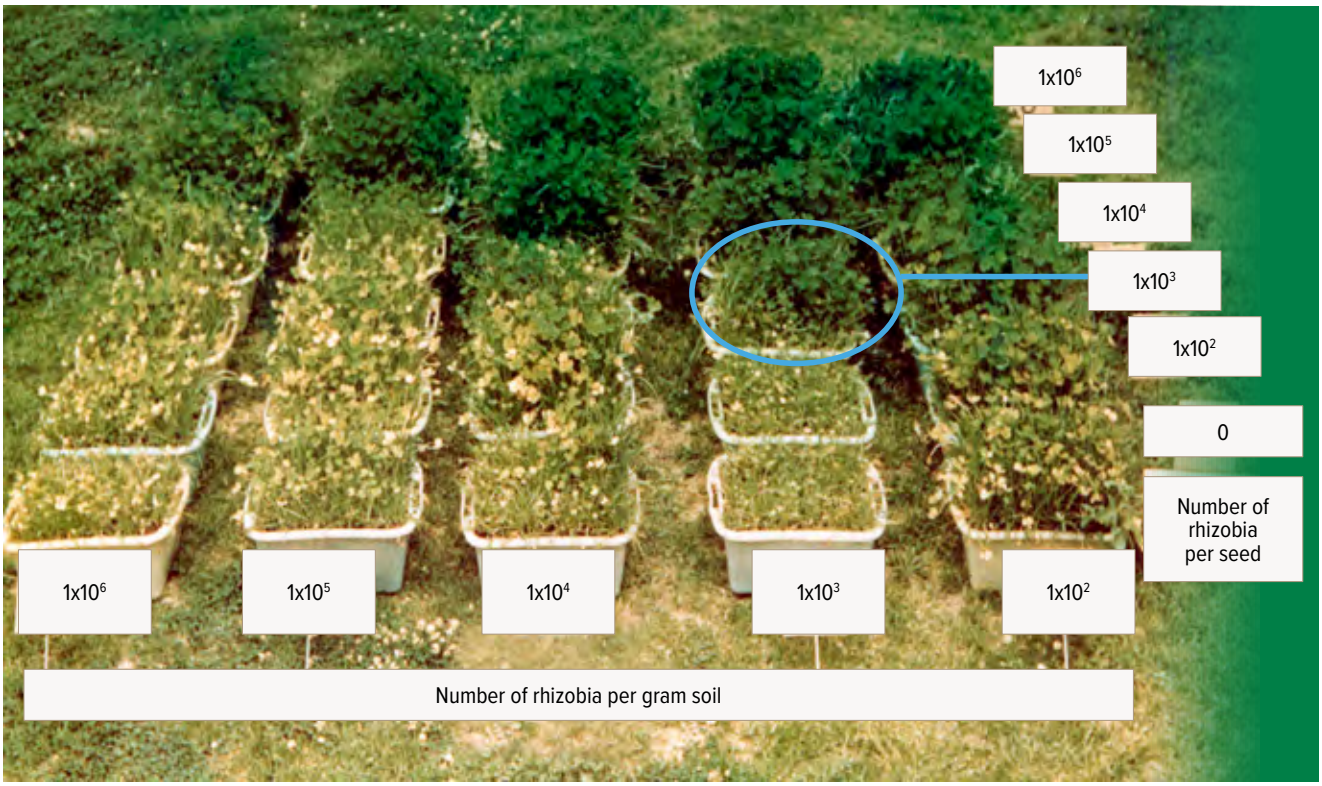


Photo: JA Ireland 1968.

TABLE 6-2. Mean symbiotic capacity of temperate legumes with soil rhizobia relative to effective inoculant strains and distribution of the communities of soil rhizobia based on their classification as effective, moderately effective or ineffective.

Legume	Mean nitrogen fixation capacity (%)	Percentage distribution of soil rhizobia communities based on their symbiotic capacity		
		Effective ≥ 75%	Moderately effective 50% to 75%	Ineffective ≤ 50%
Field pea	78	68	23	11
Faba bean	83	71	14	14
Chickpea	60	25	40	35
Yellow serradella*	>75	-	-	-
Sub clover	58	19	49	32
Strand medic	62	36	34	30
Burr medic	36	15	21	64
Lucerne	84	89	11	0
Biserrula	>75	92	-	8

** determined using individual strains isolated from soils.*

SOURCE: Bowman *et al.* 1998; Brockwell 2001; McInnes 2002; Ballard *et al.* 2003; Charman and Ballard 2004; Ballard *et al.* 2004; Elias 2009; Drew and Ballard 2010; Drew *et al.* 2011, 2012; Ballard and Barnett unpublished data.

Studies investigating the success of applied inoculants show that if the rhizobia per seed are numerically equivalent to the number of rhizobia per gram of soil, then the inoculant strain is able to form sufficient nodules to improve plant nitrogen fixation and growth, if the background rhizobia population is poorly effective. (Figure 6-13, page 73).

For example, in Figure 6-13, a growth response to inoculation was only apparent in a soil containing 1000 (i.e. 1×10^3) rhizobia per gram when the number of rhizobia applied as inoculant exceeded 1000 per seed.

This and similar studies form the basis of quality guidelines that specify minimum inoculation standards of 1000 cells per seed for subterranean clover and similarly sized pasture legumes.

Inoculant strains can compete with large background populations of rhizobia in the soil so long as they are applied in sufficient numbers.

It is common that the number of soil rhizobia can exceed 1000 per gram where a legume species has been previously grown. Responses to inoculation would only be likely where the minimum standards for inoculant on seed are exceeded.

As Australian inoculants are mostly produced in sterile peat and meet minimum standards of one billion (1×10^9) cells per gram of peat at manufacture, seed standards are easily surpassed when recommended rates of inoculant and methods of application are followed and the seed is promptly sown.

For pulse legumes, where seed size is larger, the number of rhizobia applied per seed is also larger compared to smaller pasture seeds (refer to application rates in Chapter 2). For most pulse legumes, the recommended standard is 100,000 rhizobia per seed. High numbers of rhizobia on seed combined with the annual re-sowing of pulse crops provide a good opportunity to introduce effective inoculant strains into the soil.

However, these opportunities are less frequent for regenerating pastures. Furthermore, while the benefits of effective strains introduced through inoculation will be important to pasture establishment, occupancy by the applied inoculant may be temporary and possibly insignificant if the effective strains become overwhelmed by the naturalised soil rhizobia, especially where the pasture phase extends beyond a few years.

6.9 Concluding comments

After more than 100 years of legume cultivation, many Australian soils have developed large populations of rhizobia 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, along with low clay and organic carbon levels, often decreases the persistence of the rhizobia. Medic, lucerne, field pea, faba bean, lentil, vetch and chickpea symbioses are particularly sensitive to acidic soils.

Where soils are favourable to rhizobial survival, the communities are diverse and may become less effective at fixing N over time, when compared with commercial inoculant strains. The formation of ineffective symbioses between regenerating/sown legumes and soil rhizobia is more frequent amongst the clovers and annual medics, than with pulses. Around 50 per cent of the symbioses formed by these pasture legumes with soil rhizobia are classified as ineffective. It is not possible to predict the N fixing capacity of the soil rhizobia at a paddock level.

The good news is that inoculant strains, when applied at recommended number or higher, can compete with background soil rhizobia, due in part due to their concentration around the emerging root. Inoculation therefore provides an opportunity to introduce and reintroduce effective rhizobial strains to soils growing pulse crops and renovated legume-based pastures.

7 | Legume nitrogen fixation

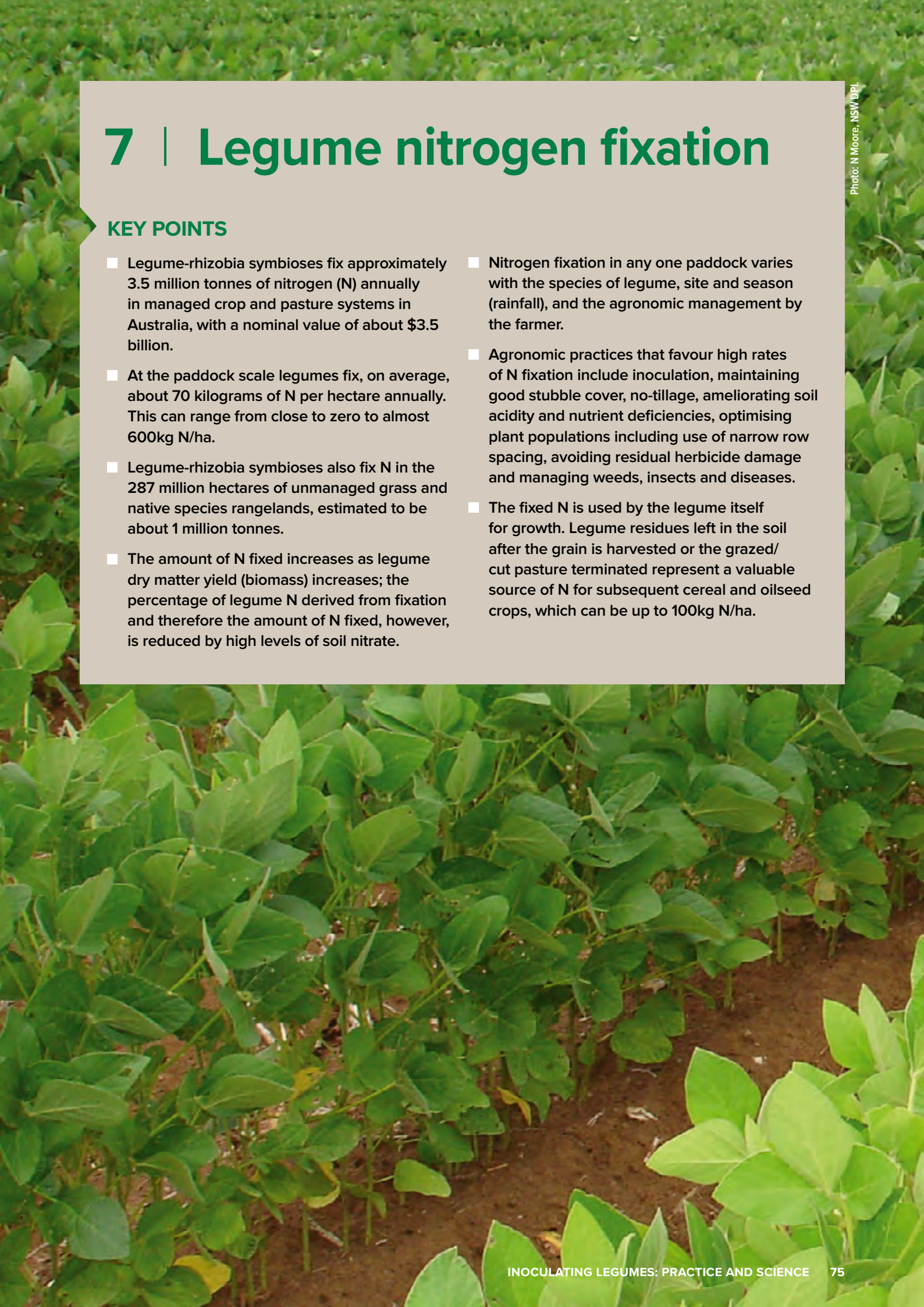
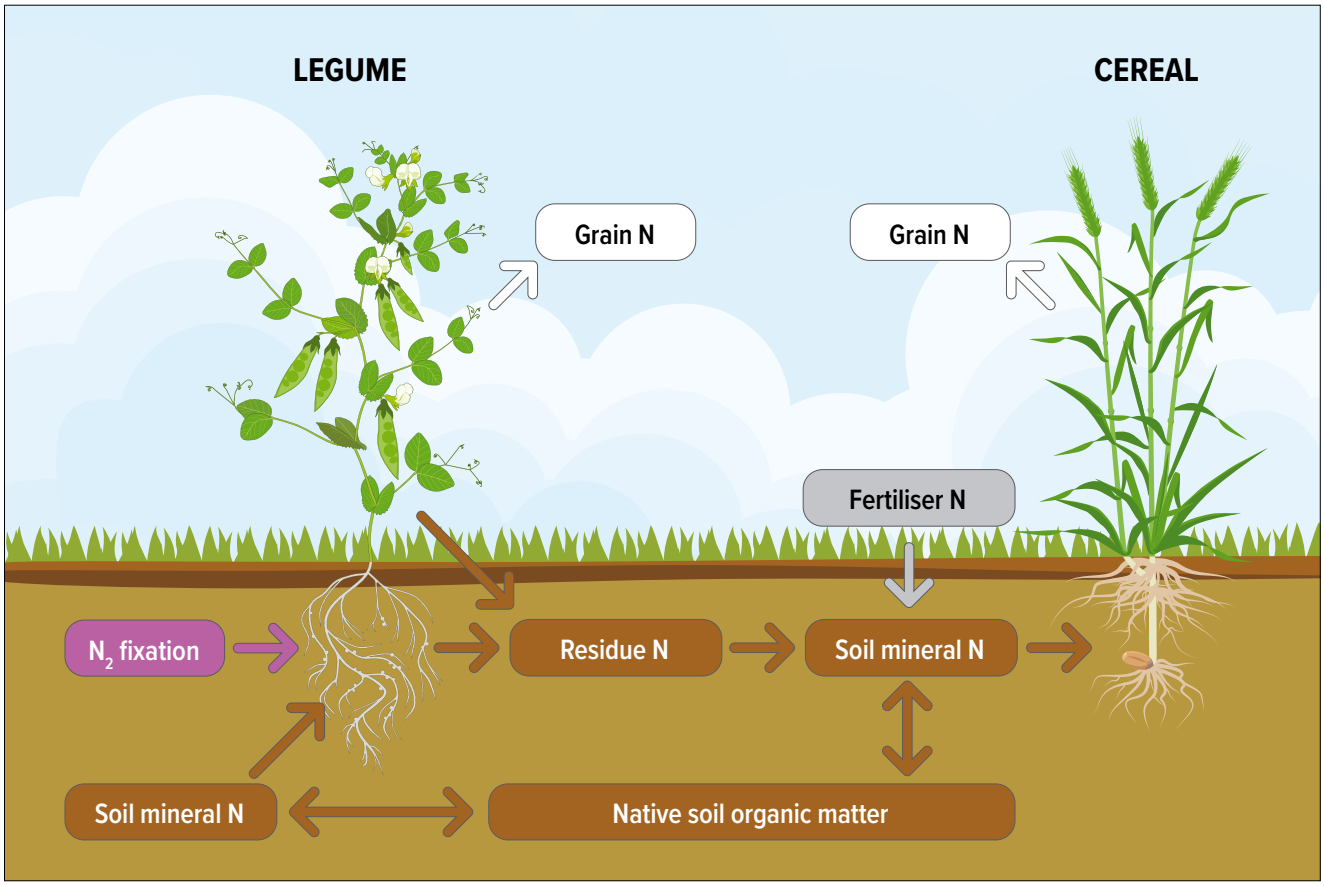


Photo: N Moore, NSW DPI.

KEY POINTS

- Legume-rhizobia symbioses fix approximately 3.5 million tonnes of nitrogen (N) annually in managed crop and pasture systems in Australia, with a nominal value of about $3.5 billion.
- At the paddock scale legumes fix, on average, about 70 kilograms of N per hectare annually. This can range from close to zero to almost 600kg N/ha.
- Legume-rhizobia symbioses also fix N in the 287 million hectares of unmanaged grass and native species rangelands, estimated to be about 1 million tonnes.
- The amount of N fixed increases as legume dry matter yield (biomass) increases; the percentage of legume N derived from fixation and therefore the amount of N fixed, however, is reduced by high levels of soil nitrate.
- Nitrogen fixation in any one paddock varies with the species of legume, site and season (rainfall), and the agronomic management by the farmer.
- Agronomic practices that favour high rates of N fixation include inoculation, maintaining good stubble cover, no-tillage, ameliorating soil acidity and nutrient deficiencies, optimising plant populations including use of narrow row spacing, avoiding residual herbicide damage and managing weeds, insects and diseases.
- The fixed N is used by the legume itself for growth. Legume residues left in the soil after the grain is harvested or the grazed/cut pasture terminated represent a valuable source of N for subsequent cereal and oilseed crops, which can be up to 100kg N/ha.

FIGURE 7-1. Cycling of nitrogen in a legume – cereal rotation



7.1 Introduction

Grain and pasture legumes are valued components of Australian agriculture. More than a century ago J.L. Thompson (1895) summarised their worth as contributing to: more economical use of manures; more economical use of nutrients in the soil; improved distribution of labour on the farm; improved weed control; improved soil conditions through the benefits of deep-rooted and 'air feeding crops'; improved productivity of following cereal crops; improved management of plant pathogens and insects; improved management of livestock and spread of economic risk.

More than a century later and the points above remain valid. Farmers still cultivate legumes in cropping and pasture systems because it helps to reduce fertiliser costs and manage weeds, pests and diseases. A number of the pulses (food legumes) are also valuable crops in their own right, attracting high prices for good-quality grain. Arguably, the major enduring value of legumes relates to their ability to form a mutually beneficial (symbiotic) association with rhizobia, a soil bacterium. This symbiotic association starts when rhizobia infect the roots of the legume and form nodules. In the nodules, the rhizobia convert gaseous atmospheric nitrogen (N_2) into ammonia (NH_3), which is then used by the legume for growth. In return, the legume provides the rhizobia with nutrients, energy and habitat.

The principal beneficiary of N fixation is the legume itself. It is for the most part self-sufficient in N and can grow in essentially any soil without inputs of fertiliser N. The amount of N fixed depends on the species of legume, its health and yield, soil nitrate levels and a range of environmental factors. The legume also produces N-rich residues that remain in the soil after the crop is harvested (Figure 7-1). The mineral N released from these residues as they decompose is available to the following cereal (or oilseed) crop in the rotation, which can be grown with minimal fertiliser N inputs. Legumes have a role in supplying N to the farming system and reducing reliance on N fertilisers. This scenario applies to both annual crop systems as well as legume pasture ley-crop systems. In permanent legume-based pastures, the fixed N sustains land productivity and, as a consequence, livestock production capacity of the pasture.

This chapter examines legume N fixation within global and Australian contexts, the drivers of legume N fixation and how they might be managed. The benefits of legumes to the economic viability and productivity of our agricultural systems are explored in more detail in the next chapter.

7.2 Legume nitrogen fixation – globally and on Australian farms

Agricultural legumes fix a lot of N. Globally, there are 250 million hectares of crop legumes and more than 200 million hectares of managed pasture and fodder legumes which together fix about 50 million tonnes of N annually.

Economic consequences

Table 7-1 summarises the economic contributions of nitrogen fixation by legumes in agriculture.

TABLE 7-1. Annual contribution of symbiotically (legume) fixed nitrogen.

	Globally	Australia
Amount N fixed (million tonnes)[1]	50	3.5
N fertiliser equivalent (million tonnes)	63	4.4
Economic value (billion dollars)	50	3.5

[1] Statistical data from FAOSTAT (2021a, b) on areas and production of crop legumes plus areas of different land use categories were combined with empirically-based estimates of legume N fixation to determine the total amounts of legume N fixed globally and for Australia

The N fixed by the agricultural legumes represents a significant saving of nitrogenous fertiliser that would otherwise need to be applied and has substantial positive economic and environmental consequences. Almost all the fixed N is available for use by the growing legume. If we compare this to a conservative 80 per cent conversion of fertiliser N into plant nitrogen, then the 50 million tonnes of biologically fixed N is equivalent to 63 million tonnes of nitrogenous fertiliser. The nominal annual value of the fixed legume N is about $50 billion, assuming a cost of fertiliser N of $0.76/kg.

The situation for Australian agriculture is equally impressive. The 2.8 million hectares of crop legumes and 47 million hectares of managed, legume-based pastures are estimated to fix about 3.5 million tonnes of N annually. Using the same assumptions above, the economic value of the N fixed by legumes in Australia's agricultural systems is about $3.5 billion annually.

The incorporation of legumes into rotations helps reduce reliance on high-cost fertiliser N.

Environmental consequences

Legume N fixation is basically powered by renewable energy. Plants use sunlight to convert atmospheric carbon dioxide to carbohydrates, and some of the carbohydrates are transported to the nodules, where they are used by the rhizobia contained in the nodules as an energy source for nitrogen N.

Nitrogen fixing legumes may have a more benign environmental footprint than grasses and cereal and oilseed crops reliant on N fertilisers, particularly for categories such as non-renewable energy demand, greenhouse gas emissions and eutrophication potential. The grasses and non-legume crops rely on soil N and manufactured fertiliser N for growth. Production of the various types of nitrogenous fertilisers requires high temperatures and pressures and the consumption of large amounts of fossil fuels. Transport and application of the fertilisers also require energy and emissions of nitrous oxide, a potent greenhouse gas, are often greater from soils following their application compared to N derived from legume residues.

All these processes result in large amounts of greenhouse gas emissions which contribute to global warming and climate change. Current estimates for Australia indicate that about five tonnes of carbon dioxide are emitted per tonne of fertiliser N used on-farm (Schwenke *et al.* 2018).

While biological N fixation may not completely replace the need for nitrogenous fertilisers in agriculture, legume-based pastures and rotations can significantly reduce the amounts used.

7.3 Comparing nitrogen fixation by different crop and pasture legumes

There are inherent differences among legumes in how much N they fix. External factors, such as rainfall, also impact N fixation.

How much nitrogen do crop legumes fix?

Table 7-2 (page 78) lists the major crop legumes grown by Australian farmers. The coefficients used to estimate total crop N fixed for the whole of Australia and on a per hectare basis were derived from more than 400 Australian and international studies. These estimates are averages only, and don't tell us how much might be fixed by a crop legume in an individual paddock or in any one year. The amounts of N fixed by individual crops will reflect environmental and management effects.

What we can say is that the total amount of N fixed by each species across the whole country largely reflects the total area sown and legume productivity. The average amount fixed per hectare also reflects to some degree the inherent capacity of each legume to fix N. Clearly, faba beans and lupins derive more of their N from fixation than other crops. The other key factor affecting N fixation is crop productivity, with the more vigorous crops like soybean, normally cultivated under irrigation or in the higher rainfall (coastal) zones, fixing more N.

In summary, we can say that lupin, faba beans and soybean are the most efficient N fixing crop legumes in Australia, fixing a total about 27kg N per tonne of shoot biomass. Chickpeas, lentils, field peas and mungbean are in a second group, fixing about 23kg N per tonne of shoot biomass. These values include the fixed N contained in the roots.

For all crops, the percentage of the N in the crop that is derived from N fixation is less than 75 per cent, with the remaining N supplied from the soil and fertilisers. How the crops are managed can affect the relative importance of these three sources.

The total amount of N fixed by a legume is determined by its N fixation capacity and dry matter production.

The more N that is fixed by the legume, the greater the inputs of N rich residues into the cropping system. In this context, the N contained in and associated with the roots and nodules is just as important as the N in the above-ground stubble. All of that N forms the basis for the legume effect on improved soil N fertility and yields of subsequent crops (see next chapter on rotational benefits of legumes).

How much nitrogen do pasture legumes fix?

Accurate accounting of the N fixed by grazed pastures in Australia is extremely challenging. The main reason that it is so problematic is the lack of statistical data on Australia-wide pasture areas, productivity and the amount of legume in those pastures. Notwithstanding the challenges and uncertainties, a set of recent estimates is presented in Table 7-3 (page 79). The pastures are defined as either managed lucerne and annual legume-based pastures or unmanaged grass or native species rangeland pastures.

There are an estimated 46.6 million hectares of the managed, legume-based pastures (ABS 2019), producing an average 4.6 tonnes of shoot biomass per hectare annually, 41 per cent of which is legume, and fixing a total (including root N) of 3.2 million tonnes of N. That works out to be, on average, 69kg N per hectare.

For the unmanaged rangelands, there is an estimated 287 million hectares producing an average 0.8 tonnes of shoot biomass per hectare annually, just 11 per cent of which is legume, and fixing a total of 0.96 million tonnes of N, equivalent to 3.4kg N per hectare.

The data in Table 7-2 and Table 7-3 (page 73) suggest that the crop and pasture legumes together fix about 4.5 million tonnes of N annually across 335 million hectares agricultural land. This is an estimate only and one that is constrained by a lack of accurate data on areas and intensity of legumes in both managed and unmanaged pastures. Angus and Grace (2017), in a recent review of N in Australian agriculture, estimated that legumes fix about 4.5 million tonnes of N annually across 450 million hectares of agricultural land. These values are very similar to the ones presented in Tables 7-2 and 7-3, even though the methods and raw data used to calculate the values were quite different.

These estimates of pasture productivity and N fixation are averages across the whole country and, as with the crop legumes, don't inform us of what might be happening in individual paddocks. Published reviews of pasture legumes in Australia, such as Peoples *et al.* (2012) indicate that N fixation in pastures containing either subterranean clover, annual medics, lucerne or white clover can fix between 3 and 300kg per hectare for the annual species and 4 and 570kg per hectare for the perennial lucerne and white clover. Those values were derived from a total of 230 observations.

The Peoples *et al.* (2012) review also indicates that the annual species have a slightly higher reliance on N fixation for growth, with averages of 81 per cent for subterranean clover and 74 per cent for the annual medics, compared with 60 per cent for lucerne and 68 per cent for white clover.

As with the crop legumes, a major factor affecting amounts of N fixed is their production of biomass which, in turn, is largely driven by rainfall. Other major factors affecting pasture productivity and legume N fixation are plant nutrient supply, particularly phosphorus and sulfur (Hackney *et al.* 2019), and soil acidity (Peoples *et al.* 2012). Soil acidity is a widespread constraint of both pasture legumes and pulses, with an estimated 50 million hectares of agricultural land having an acidic surface soil, i.e. pH_{CaCl_2} less than 5.5, and 24 million hectares also having an acidic subsoil (Dolling 2001, McKenzie *et al.* 2017).

How much N will legumes fix in my paddock?

The N fixation values for crop and pasture legumes in Tables 7-2 and 7-3 were derived from very large amounts of data across a range of sites. As stated above, these values are intended to provide more of a broad account of N fixation by agricultural legumes in Australia than what might be happening on a particular farm with particular management. In the next section, we look at some of the management effects on legume N fixation.

TABLE 7-2. Estimates of the amounts of N fixed in 2017/18 by crop legumes in Australia.

Legume	Area sown (hectares)[1]	Grain production (tonnes)[1]	Grain yield (t/ha)[1]	% crop N from fixation[2]	Crop N fixed (tonnes)[3]	Crop N fixed (kg/ha)[4]
Chickpea	1,075,100	998,200	0.93	62	88,660	82
Lupins	612,000	714,250	1.17	74	69,850	114
Lentils	418,500	542,800	1.30	62	39,850	95
Faba beans	313,100	415,600	1.33	74	36,160	115
Field peas	291,490	317,200	1.09	62	24,610	84
Mungbean	100,000	87,000	0.87	62	7,270	73
Soybean	37,000	63,000	1.70	61	4,730	128

[1] Statistical data for 2017/18 from ABARES (2020); [2] Values for % crop N fixed were aggregated from 3,100 observations in 225 studies across the globe (MB Peoples and DF Herridge, personal communication); [3] For each species, crop N fixed = % crop N from fixation x total crop N, the latter determined by dividing grain production (3rd column) by harvest index to determine above-ground biomass, then multiplying above-ground biomass by its N concentration before accounting for root N using a below-ground factor. Aggregated values from an additional 3,550 observations in 200 studies were used in these calculations (DF Herridge and MB Peoples, personal communication; Herridge et al. 2008; Unkovich et al. 2010); [4] Calculated by dividing column 6 by column 2

7.4 How does crop and soil management affect legume nitrogen fixation?

The amount of N fixed by legumes essentially depends on how well the legume grows and the level of nitrate in the soil. The greater the biomass produced, the greater the amount of N fixed, while high soil nitrate levels suppress the need for N fixation.

In the Australian environment, legume growth is most strongly determined by the amount of water that the crop or pasture can access. Management practices can be optimised to maximise water use and provide the legume with ideal growing conditions, including low soil nitrate to maximise N fixation.

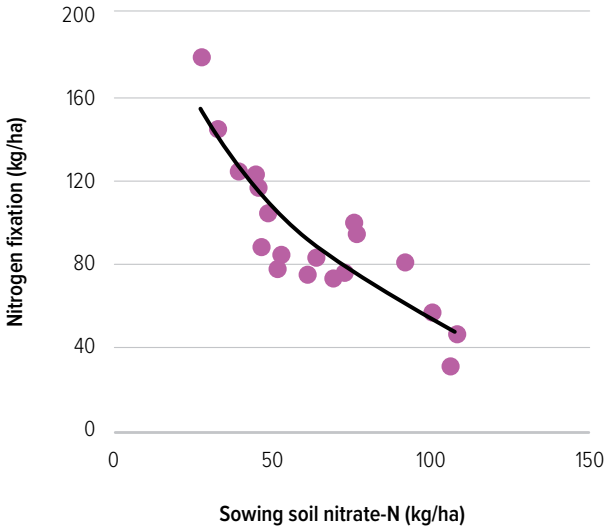
Soil nitrate suppresses legume nitrogen fixation

Soil nitrate is a potent inhibitor of legume nodulation and N fixation. This is because rhizobia in nodules require a supply of carbohydrates for the symbiotic N fixation process, and it is less costly for the legume to take up nitrate from the soil if available, than relying on N fixation. At low soil nitrate (i.e. less than 50kg N/ha in the top metre of soil), the legume reliance on N fixation is generally high. As soil nitrate levels increase, legume nodulation and N fixation become more and more suppressed. Eventually, at very high soil nitrate (more than 200kg N/ha), nodulation and N fixation will be close to zero. Figure 7-2, illustrates the suppressive effect of different soil nitrate levels on chickpea N fixation.

The actual amount of soil nitrate that will inhibit legume nodulation and N fixation in any single paddock will vary with the legume species and environmental conditions. Nitrogen fixation of faba beans, for example, is far less prone to the suppressive effects of soil nitrate, compared with crops such as chickpeas and field peas.

Aggressive cultivation, heavy use of nitrogenous fertilisers, long and wet pre-crop fallow periods and a high proportion of legumes in the rotation can all increase soil nitrate levels.

FIGURE 7-2. Impact of soil nitrate on chickpea nitrogen fixation in northern NSW



Source: Herridge *et al.* 1998

Herbicide impacts on nitrogen fixation

It is well recognised that some herbicide residues and in-crop applications, particularly those in group 2 (B) (Imidazolinones and Sulfonylureas) and group 4 (I) (e.g. Clopryalid) can significantly reduce the growth, N fixation and yield of some legume crops (Dear and Sandral 1999, Hollaway *et al.* 2006, Drew et al 2007). This is predominantly due to the susceptibility of the plant to the herbicide which in turn impacts on the symbiosis, and less often, a result of direct impact on the rhizobia (Anderson *et al.* 2004, Farquharson 2010). Crop damage resulting from herbicides tend to be exacerbated in low rainfall cropping zones, particularly on alkaline soil types where residues of many herbicides break down slowly.

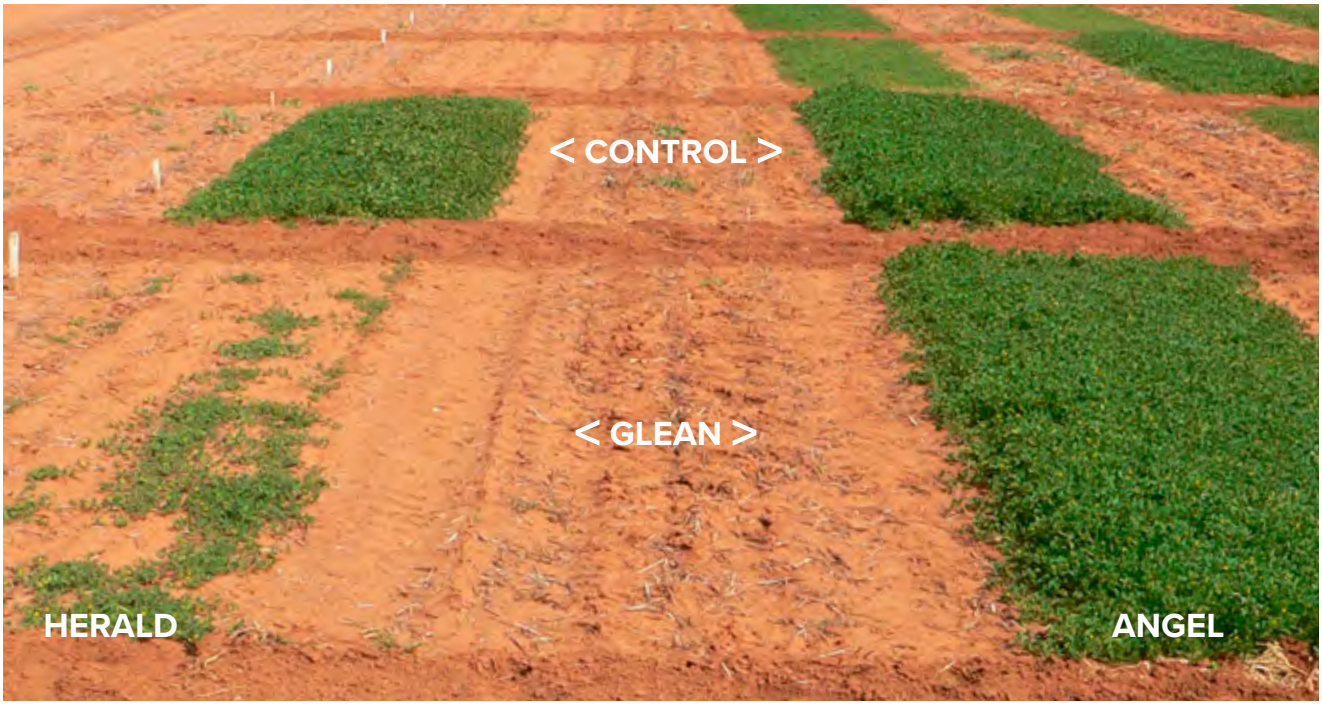
TABLE 7-3. Estimates of the amount of N fixed annually by pasture legumes in Australia.

Pasture type	Area pasture (hectares)[1]	Annual total pasture production (t/ha)[2]	Annual pasture legume production (t/ha)[3]	Total N fixed (tonnes)[4]	N fixed (kg/ha)[5]
Lucerne, improved annual legume-based pastures	46,600,000	4.6	1.9	3,200,000	69
Rangelands, grass, native species-based pastures	287,000,000	0.8	0.1	960,000	3.4

[1] Statistical data from the 2017-18 Agricultural Commodities (ABS 2019); [2] Values for annual pasture production modelled using an algorithm describing the relationship between either annual rainfall (for lucerne and perennial legume based pastures) or seasonal rainfall (for annual clover, annual medic and other annual legume based pastures) and pasture biomass production; [3] Derived using averages of 41% legume in managed lucerne and annual legume based pastures and 11% legume in unmanaged rangelands pastures. These values in turn derived from Donald (2012); [4] Calculated by multiplying total legume production (tonnes) by the amount of fixed N in the legume; 0.037 (% of fixed N in biomass) for lucerne, perennial legume pastures and 0.035 for annual legume pastures (Angus and Peoples 2012); [5] Calculated by dividing column 5 by column 2.

FIGURE 7-3. Benefit of legume tolerance to SU herbicides. The tolerant medic cultivar Angel (right) is largely unaffected by the presence of residues of Glean (4g/ha at front of picture) compared to the intolerant medic Herald (left). Angel medic was the first legume with SU herbicide tolerance released in Australia.

Photo: Jeff Hill, SARDI.



In the last ten years, there have been significant advancements in plant breeding for herbicide tolerant legumes including medic (Oldach *et al.* 2008), lentil (McMurray *et al.* 2019) and faba bean (Mao *et al.* 2019), with tolerant commercial cultivars now widely grown in Australia (Figure 7-3). As they are developed, cultivars are assessed for good nodulation and N fixation capacity. Figure 7-4 shows the benefits to N fixation from using group 2 (B) Imidazolinone tolerant lentil (PBA Hallmark XT) compared to the susceptible lentil (PBA Jumbo2), where herbicide residues are present. More generally, herbicide labels and legume plant back times should always be followed to reduce the chance of legume crop damage and N fixation penalties.

7.5 Management practices to improve legume growth and nitrogen fixation

Apart from inoculating the legume seed with the appropriate rhizobia (see Chapter 2), optimising basic agronomy (best management practice) is the key to improving legume productivity and, in turn, N fixation. This means maintaining a good cover of stubble on the soil surface in the pre-crop fallow, sowing on time and establishing the appropriate plant density, along with optimising nutrient inputs, reducing acidity or other soil constraints, and managing weeds, disease and insects.

FIGURE 7-4. Impact of post sowing pre-emergence herbicide application of Imazamox (33g/L)+Imazapyr (15g/L) applied at 750g/ha to two lentil cultivars; PBA Jumbo 2 and PBA Hallmark XT (Imidazolinone tolerant). Trial was conducted at Hart, SA in 2019. Bars with the different letters indicate significance at P<0.05. (Farquharson and Ballard, unpublished).

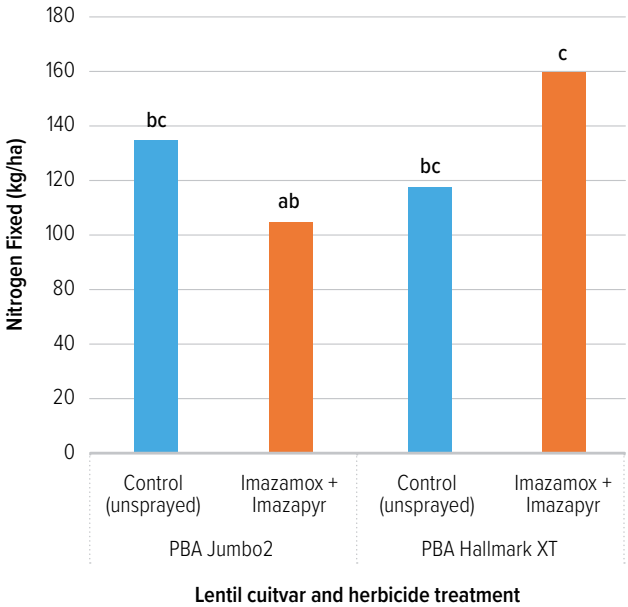


Photo: Liz Farquharson, SARDI.

Tillage practices

One management practice that has become almost universal in recent years in the grains industry is the reduction or elimination of soil cultivation. No-tillage has a number of advantages over cultivation, including greater flexibility in sowing, reduced soil losses from erosion and improved surface and sub-surface soil structure. Potential benefits of no-tillage are increased soil water and decreased soil nitrate accumulation during the pre-crop fallow and in-crop.

Experiments in the NSW Department of Primary Industries farming systems program in northern NSW during the 1980s and 1990s highlighted the positive effect of no-tillage on productivity and N fixation of chickpeas (Felton *et al.* 1998; Marcellos *et al.* 1998; Herridge *et al.* 1998) (Table 7-4). No-tillage plots had 32 per cent more soil water at sowing and 18 per cent less soil nitrate than the cultivated plots. As a result, chickpea biomass increased by 15 per cent, grain yields by 10 per cent and N fixed by 43 per cent.

In recent years, strategic deep tillage has overcome high strength subsoils especially on sands, which improves root exploration and extraction of subsoil water and nutrients. These benefits are also relevant to legume growth and N fixation.

TABLE 7-4. Effects of tillage on soil water and nitrate at sowing, and on chickpea growth, grain yield and nitrogen fixation.

	Cultivated	No tillage	No-tillage as % of cultivated
Sowing soil water (mm)	109	144	+32
Sowing soil nitrate (kg N/ha)	86	71	-18
Shoot dry matter (t/ha)	4.7	5.4	+15
Grain yield (t/ha)	1.83	2.01	+10
% crop N from fixation	44	55	+25
Crop N fixed (kg/ha)	75	107	+43

Values are the means of 21 site/years of experiments conducted in northern NSW during the 1980s and 1990s.

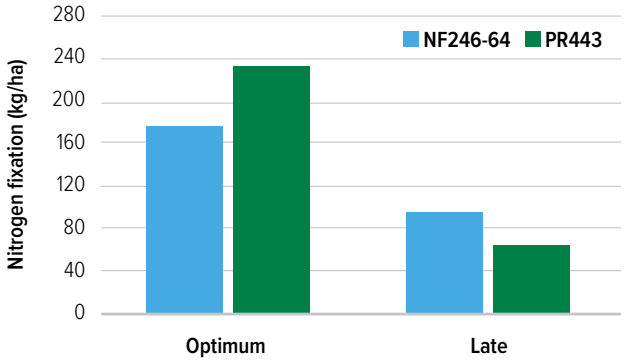
Sowing practices

Legume N fixation can be improved by sowing on time and at the appropriate density. Sowing on time optimises the day length requirements of the crop as well as taking advantage of growing-season rainfall and more favourable temperatures. Studies with two soybean lines in northern NSW showed that planting late reduced N fixation by an average of 60 per cent compared with planting at the optimum time (Figure 7-5).

The use of narrow row spacing and/or high plant density can improve N fixation. Nitrogen fixation of chickpeas at two sites in south-eastern Qld was increased by about 50 per cent as row spacing was reduced from 1.0 metre to 25cm (Figure 7-6).

Scientists in northern NSW had previously reported that N fixation of faba beans and chickpeas was higher with high plant densities and narrow rows (Schwenke *et al.* 1998). The situation was similar for the pasture legumes with N fixation increasing from 130 to 300kg N per hectare as lucerne density in the pasture increased from 5 to 40 plants per square metre (Peoples *et al.* 1998). In western and southern Australia, early establishment of pulse crops (e.g. April) generally increases biomass and N fixation.

FIGURE 7-5. Soybean lines (NF246-64 and PR443) sown at the optimum time fixed more nitrogen than when sown late.



Source: Seymour *et al.* (2016)

General soil conditions

Soil acidity and phosphorus (P) and sulfur (S) deficiencies are major constraints to legume nitrogen fixation (Peoples *et al.* 2012; Hackney *et al.* 2019).

In a three-year study of subterranean clover pastures in south-eastern Australia, applying lime and P fertiliser increased total yields and N fixation. The two amendments together were more effective than either one alone, resulting in an average N fixation increase of 100 per cent (Figure 7-7).

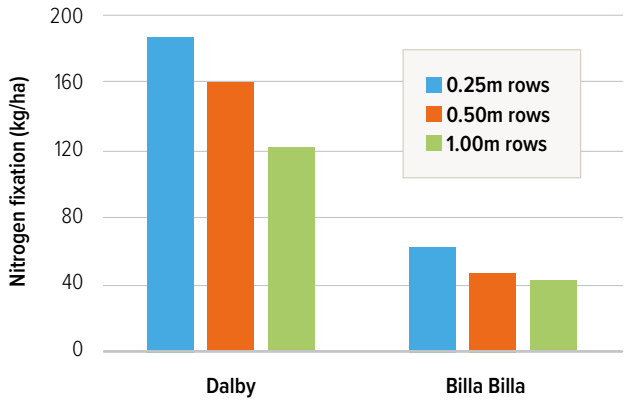
Increasing soil pH with lime application decreases the availability of aluminium and manganese. At elevated concentrations, both of these metals are toxic to legume roots and rhizobia (Peoples *et al.* 1995). Readers are directed to Peoples *et al.* (2012) for more comprehensive information on the positive effects of liming acidic soils on rhizobial numbers, legume nodulation and N fixation.

Different species, and even different cultivars, of legumes may have different tolerances to hostile soil conditions. A legume that fixes a lot of N under one set of conditions may not perform as well under another set of conditions. For example, a study by Evans *et al.* (1989) in southern Australia showed that lupins fixed approximately 80 per cent more N than field peas in acidic soils. However, in alkaline soils the outcome was reversed with lupins fixing about 35 per cent less N than field peas.

Other soil constraints to legume N fixation include water repellency, salinity, sodicity, nutrient toxicities and deficiencies and residual herbicide residues (Peoples *et al.* 2012; Burns *et al.* 2020). These constraints must be addressed if potential legume biomass production and N fixation are to be realised.

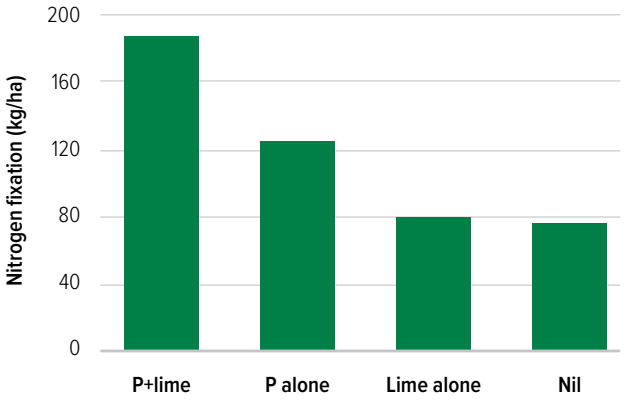
Research has also established that N-fixing legumes may have additional nutritional requirements, compared with plants that do not fix N. For example, nodulated legumes have higher requirements for calcium, boron and molybdenum (O'Hara *et al.* 1988).

FIGURE 7-6. Chickpea nitrogen fixation declined as row spacing increased.



Source: Seymour *et al.* (2016)

FIGURE 7-7. Optimising plant nutrition increases nitrogen fixation of sub clover pastures.



Source: Peoples *et al.* (1995)

8 | Legumes in rotations

KEY POINTS

- The legume residues left in the soil after the grain is harvested or the grazed/cut pasture legume phase is terminated represent a valuable source of plant-available nitrogen (N) for subsequent cereal and oilseed crops.
- Cereals grown after annual crop and pasture legumes generally out-yield cereals grown after either cereal or oilseed crops. The extra yield is mostly due to the higher levels of plant-available soil N following the legumes but may also include other factors such as a disease-break effect and carry-over of unused water.
- The average soil mineral-N benefit from pulses is about 40kg/ha and this benefit can last into a second cereal crop, although at a reduced level.
- Analysis of a large data set from Australia and overseas indicates the rotational benefit of the crop legumes for cereal grain yield is, on average, 1.2t/ha for the first cereal crop and 0.7t/ha for the second crop.
- Depending on yields obtained and grain prices, the economic benefits of including the annual crop legumes in cereal rotations can be substantial.
- The pasture legumes, usually in the form of grazed pasture leys, provide even greater and longer lasting soil N and structural benefits, and increased cereal yields.
- The rotational benefits for cereals of both annual crop legumes and pasture legumes are related to the productivity of the legume – the bigger the crop or pasture, the greater the benefit.

FIGURE 8-1. Aerial photograph of the rotation crops, including both pulse and pasture legumes, at the main site of the Northern Farming Systems project, Pampas, Eastern Darling Downs, Qld. The project involves collaboration between GRDC, CSIRO, the Qld Department of Agriculture & Fisheries and the NSW Department of Primary Industries.

Photo: Lindsay Bell, CSIRO.

8.1 Introduction

Nitrogen fixation provides 'free' N to the legume, eliminates the need for additional inputs of fertiliser N for the legume and reduces the uptake of soil N. This is only the first part of the story. Incorporating legumes into a cropping system also provides rotational benefits, which include the N benefit and a soil health benefit. Both factors usually lead to significantly increased yields of subsequent crops.

A substantial body of research has been undertaken in Australia examining and refining crop and pasture legume rotations for different regions and production systems. This research includes medium to long-term rotations at Wagga Wagga, NSW (Heenan and Chan 1992), Tarlee, SA (Schultz 1995), Tamworth, north-eastern NSW (Holford *et al.* 1998), Warra, south-eastern Qld (Dalal *et al.* 1995), the Moree region, north-eastern NSW (Felton *et al.* 1998) and Jambin, central Qld (Cox *et al.* 2010). In five of the six studies, crops were grown using both conventional cultivation and no-tillage as part of the development of conservation agriculture during the 1980s and 1990s. An excellent account of the evolution of conservation agriculture in Australia can be found in Pratley and Kirkegaard (2019).

More recently, the traditional rotation experiments have been largely replaced by the farming systems model in which farmers participate in the design and implementation of the research, the crop and pasture sequences are flexible and varied and multi-year crop sequences are evaluated in both agronomic and economic terms. Examples are the Crop Sequence Initiative (Peoples *et al.* 2015, 2017), the Southern Farming Systems project (Kirkegaard *et al.* 2021) and the Northern Farming Systems project (Bell *et al.* 2017; Erbacher *et al.* 2020).

Collectively, this research has generated important data on the N dynamics of those rotations related to N fixation by the legumes and to the benefits of the fixed N to soil and subsequent crops.

The previous chapter focused on N fixation by legumes and how optimising basic agronomy and nutrition would not only improve amounts of N fixed but also productivity of the legume crop or pasture. In legume-based rotations, the next phase usually involves a cereal crop (Figure 7-1, page 76).

In this chapter, we will use examples from some of the studies mentioned above to highlight how the N fixing legumes deliver additional mineral N to the following cereal crop and the yield and economic benefits that result from the extra N. We will also examine other rotation effects of legumes, in particular the biological benefits.

8.2 Rotational benefits of crop legumes

Angus *et al.* (2015) published a comprehensive review of the yield benefit of break crops in cropping systems dominated by cereals. Their main conclusion, based on more than 900 comparisons, was that oats grown prior to wheat improved wheat grain yields by an average of 0.5t/ha, canola improved yield of the following wheat by 0.8t/ha with the crop legumes providing the largest average benefit of 1.2t/ha compared to wheat following wheat. The average yield benefit of a canola break crop for the subsequent wheat crop was 0.16t/ha, compared to a yield increase of 0.72t/ha in the subsequent year following crop legumes. The average two-year wheat yield benefits were about 1.0t/ha following canola and 2.0t/ha following crop legumes.

Despite the strong evidence that break crops enhance the productivity of the following two crops, some Australian farmers remain committed to growing cereal, either in continuous monoculture or with the occasional break crop or fallow. Our cropping statistics bear this out with wheat accounting for 47 per cent of the 23 million hectares cropped in 2017/18 and barley accounting for an additional 18 per cent. These two cereals alone account for about 65 per cent of Australia's crop area and this lack of crop diversity on Australian farms was a major prompt for the ongoing investment by the grains industry in projects such as CSP00146 'Crop Sequence Initiative' (Peoples *et al.* 2015, 2017) to increase adoption of more diverse crop sequences.

The bigger the legume crop, the greater the benefit

Angus *et al.* (2015) highlighted the positive relationship between the yield (productivity) of the legume and the size of the benefit for the succeeding cereal or oilseed crop. In other words, the bigger the legume, the greater the benefit. Similarly, Seymour *et al.* (2012) had previously summarised this effect using data from 167 experiments conducted in WA between 1974 and 2007. Across all WA experiments, the rotational benefit of lupin was 0.6t/ha of wheat grain tonnes/ha and for field pea was 0.45 tonnes/ha. For both, the benefit was a combination of extra N plus a disease-break, principally against take-all (*Gaeumannomyces graminis*).

The benefits of the lupins on the yield of following wheat crops were correlated with lupin yield, with more substantial yields in the high-rainfall areas, and with the more recent trials (Table 8-1) during the 1990s related to improved agronomy of both the lupin and wheat crops. Significant benefits (0.4t/ha wheat grain) persisted into a second wheat crop after the lupins.

TABLE 8-1. Effects of size of the lupin crop (lupin grain yield) and year of study on rotational benefits of the narrow-leafed lupin on wheat grains yields in WA (167 trials).

Lupin grain yield / years of experiment	Increase in wheat grain yield following lupin (t/ha)
0.5–1.0t/ha	0.5
1.0–1.5t/ha	0.7
> 1.5t/ha	0.9
1974–80	0.4
1981–90	0.5
1991–97	1.0

In the following section, we present case studies involving chickpea-wheat rotations in northern NSW and a variety of crop legume-wheat rotations in south-eastern Australia to highlight the importance of the N benefit in the rotation and, depending on the relative gross margins of the legumes and cereals, the potential economic benefits.

Chickpea in northern NSW and southern Qld

The NSW Department of Primary Industries rotation experiments, North Star (south of Goodiwindi), provide an example of how the N-rich chickpea residues remaining in the soil after grain harvest boost mineral N levels in the soil compared to two wheat crops (with and without 100kg fertiliser N/ha) (Table 8-2, page 86). The extra soil N was then taken up by the following wheat crop.

Soil nitrate levels down to 1.2m depth were moderate at sowing in the first phase of the rotation at 68kg N/ha. Chickpeas fixed 135kg N per hectare and produced a grain yield of 2.3t/ha. The non-fertilised wheat also produced 2.3t grain/ha, while the wheat fertilised with 100kg N/ha produced 3.2t/ha. Soil nitrate levels at harvest time were higher under chickpeas (54kg N/ha) than under the wheat crops (32-35kg N/ha) indicating that the chickpeas used less of the soil mineral N than the wheat.

The chickpeas produced far more residue N than both wheat crops (133kg N/ha versus 20-55kg N/ha, measured after harvest). The chickpea residues also had a higher concentration of N, resulting in a lower carbon to nitrogen (C:N) ratio compared to the wheat residues (25:1 versus 44-50:1).

The low C:N ratio of the chickpea residues meant that mineral N was released into the soil from microbial decomposition during the summer fallow – termed net mineralisation. Net mineralisation (transfer of N to plant available forms) is associated with residues with C:N ratios of less than 30:1.

In order to decompose the higher C:N wheat residues, microbes needed to use soil mineral N. The use of this soil N is termed immobilisation.

The chickpea residues were estimated to release 16kg mineral N/ha into the soil during the seven-month summer fallow, compared to 21-22kg N/ha immobilised by the wheat residues during the same period.

Decomposition of the chickpea and wheat residues during the summer fallow meant that at sowing in Year 2, soil nitrate following chickpeas at 100kg N/ha was much higher than following both the unfertilised and N-fertilised wheat crops (51-53kg N/ha). An extra 35-40kg/ha mineral N in the soil profile at sowing in Year 2 was derived from the mineralisation of native soil organic matter during the winter/spring growing season. Grain yield was much higher after chickpeas (2.8t/ha) than after the wheat crops (1.7-1.8t/ha).

These results from northern NSW clearly show the benefits of chickpea in wheat rotations and the key role the residues have in determining how much plant-available N will be in the soil at the time of sowing the next crop. Both the amount and the concentration of N in those residues (described by the C:N ratios in the example) are critical.

TABLE 8-2. Explaining the N and yield benefits of a chickpea-wheat rotation compared with unfertilised or N-fertilised wheat-wheat sequences[1].

	Chickpea/wheat	Wheat/wheat	Wheat/wheat
Year 1 (chickpea or wheat)	**Chickpeas – No N**	**Wheat – No N**	**Wheat – 100N/kg**
Sowing soil nitrate (kg N/ha, 1.2m depth)	67	67	67
Fertiliser N applied (kg N/ha)	0	0	100
Grain yield (t/ha)	2.3	2.3	3.2
Total crop N (kg/ha)	205	55	115
Crop N fixed (kg/ha)	135	0	0
Soil nitrate at grain harvest (kg N/ha, 1.2m depth)	54	35	32
Residue N remaining in the soil (kg/ha)	133	20	55
Residue C:N	25:1	50:1	44:1
Estimated mineralisation (+) or immobilisation (–) (kg N/ha)	+16	–22	–21
Year 2 (wheat only)	**Wheat – No N**	**Wheat – No N**	**Wheat – No N**
Sowing soil nitrate (kg N/ha, 1.2m depth)	100	51	53
Wheat grain yield (t/ha)	2.8	1.7	1.8

[1] *Values are the means of no-tillage and cultivated treatments at two sites at North Star, northern NSW (source: Herridge et al. 1995; Felton et al. 1998; Marcellos et al. 1998)*

The data presented in Table 8-2 are representative of a large body of research on chickpea-wheat rotations conducted in northern NSW and southern Qld during the 1980s and 1990s (Lucy *et al.* 2005). The data are also consistent with the meta-analysis of Angus *et al.* (2015) which considered 300 comparisons of legume-wheat and wheat-wheat sequences from 40 Australian studies and 34 studies from overseas. The legumes included chickpeas, lupins, field peas, faba beans and lentils.

Profitability of chickpea-wheat rotations and wheat monocultures

Given the yield advantages and reduced fertiliser N requirements of cereals following legumes, it is not surprising that crop legume-cereal rotations often show higher gross margins than the cereal-cereal sequences especially when pulse prices are high. When the gross margins for the crop sequences in Table 8-2 were calculated, the chickpea-wheat rotations were far more profitable (Table 8-3, page 87).

The high gross margin of chickpeas in Year 1 ($860/ha) reflected the higher-than-average yield and the premium price for chickpea compared to wheat. In Year 2 when unfertilised wheat was sown in all plots, the 60 per cent increase in yield of wheat following chickpeas compared to the wheat following wheat translated into a more than doubling of gross margins for that year. Over the full two years of the sequences, the gross margin of the chickpea/wheat rotation was $680 to $820/ha higher than those of the continuous wheats.

Legume-wheat rotations can be more than twice as profitable as wheat-wheat rotations.

Pulse-wheat rotations in south-eastern Australia

In parts of south-eastern Australia where lentil is particularly well adapted (i.e. Yorke Peninsula SA and Wimmera Victoria), even when lentil prices are moderate, the gross margin from wheat and other crops struggles to compete with lentil for experienced growers with suitable soil types. When the lentil price is moderate to high, it is often included in the rotation every 2-3 years, and wheat becomes the break crop for lentil.

Results are presented from farmer participatory studies in south-eastern Australia in which 13 of the 16 sites were farmers' crops. The remaining three sites were on research stations (Peoples *et al.* 2015, 2017). Soil mineral N levels for the on-farm research reported by Peoples *et al.* (2015, 2017) were, on average, 25 to 74kg/ha higher after the legumes than after N-fertilised wheat (Figure 8-2, page 87). The largest soil N benefit was from faba beans (74kg N/ha) and the smallest from lentils (25kg N/ha). Data from commercial soil testing in SA indicate similarly higher levels after the legumes. The absolute values were not as great as the on-farm research values because the commercial testing was done to just 0.6m depth, compared with 1.2m for the majority of the on-farm research sites. For the commercial testing, the overall average soil mineral N benefit across the 1300 paddocks was 25kg/ha, compared with 35kg/ha for the 26 legume crops from the on-farm research sites.

At 15 of the on-farm research sites, wheat grain yields after N-fertilised wheat or the legumes were compared. Results indicated that the average wheat yields were 0.9t/ha higher after the legumes than after wheat.

What these results and those involving chickpeas in the north indicate is that wheat production can benefit substantially by including crop legumes in the cropping sequence with much of the benefit resulting from the additional soil mineral N following breakdown of the legume residues. However, there is more to the rotation effect.

Additional benefits of legumes in rotation

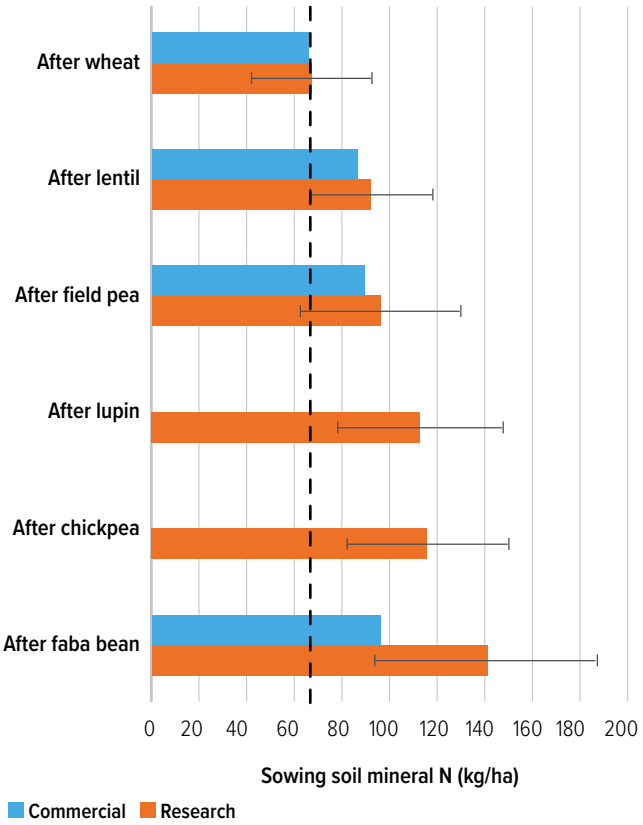
As mentioned above, the rotational benefit of legumes extends beyond N additions, including the ability to manage

TABLE 8-3. Simple gross margin analysis of the N and yield benefits of a chickpea-wheat rotation compared with unfertilised or N-fertilised wheat-only sequences

	Chickpea/wheat	Wheat/wheat	Wheat/wheat
Year 1	Chickpeas – No N	Wheat – No N	Wheat – 100N/kg
Grain yield (t/ha)[1]	2.3	2.3	3.2
Grain ($)[2]	1360	690	960
Cost of production ($)[3]	500	320	480
Gross margin ($)	860	370	480
Year 2 (wheat only)	**Wheat – No N**	**Wheat – No N**	**Wheat – No N**
Grain yield (t/ha)	2.8	1.7	1.8
Grain ($)	840	510	540
Cost of production ($)	320	320	320
Gross margin ($)	520	190	220
2-year gross margin ($)	1380	560	700

[1]Yields from Table 8.2 and are the means of no-tillage and cultivated treatments at two sites in northern NSW; [2]NSW DPI Weekly Commodity Report March 2021 (dpi.nsw.gov.au/agriculture/commodity-report) chickpea price at $590/t; wheat at $300/t; [3] SAGIT Farm Gross Margin and Enterprise Planning Guide 2019 grdc.com.au/FarmGrossMarginGuide

FIGURE 8-2. Soil mineral N levels are increased after a legume crop; 'Commercial' data from 1300 paddocks in SA (sourced from Allan Mayfield Consulting, Holmes Farm Consulting and McAg Consulting and presented in Peoples *et al.* 2015). 'Research' data from 43 paddocks across south eastern Australia (Peoples *et al.* 2015, 2017). Horizontal lines associated with the 'Research' data indicate standard deviations. The length of the histogram bars beyond the dotted line denotes the soil mineral N increase relative to wheat.



grass weeds through different herbicide chemistries, the conservation of soil moisture and management of cereal root diseases. The latter largely relates to the suppressive effect of the break crop on soil- and stubble-borne diseases of cereals. Major cereal diseases in the northern grains region are crown rot (*Fusarium* spp.), common root rot (*Bipolaris sorokiniana*), root-lesion nematode (*Pratylenchus* spp.) and yellow leaf spot (*Pyrenophora tritici-repentis*). In the southern and western grains region, diseases such as take-all, *Rhizoctonia*, damping-off caused by *Pythium* and cereal cyst nematode (*Heterodera avenae*) dominate.

The biological benefit is largely due to the legumes reducing inoculum levels of cereal (mainly wheat) pathogens in the soil. In wheat monocultures, those pathogens would normally carry-over from one crop to the next, firmly attached to stubble or existing in the soil (see Angus *et al.* 2015 for additional discussion of possible mechanisms involved in the control of root diseases).

For example, cereal cyst nematode populations in SA and Victoria decreased to almost undetectable levels following two years of field peas or fallow (Fisher and Hancock 1991). In comparison, numbers after two years of resistant wheat were four eggs per gram of soil, and potentially yield limiting 15 eggs per gram of soil after susceptible wheat (Table 8-4).

TABLE 8-4. Population changes of cereal cyst nematode under different rotational regimes.

	Nematode eggs/gram soil		
	Initial	1984	1985
Wheat (Resistant)	40	9	4
Wheat (Susceptible)	33	19	15
Field pea	43	8	0.1
Fallow	38	6	0.3

Source: Fisher and Hancock, 1991

Photo: Morgan McCallum, SARDI.

The benefit depends on the nature of the disease. Diseases with a broad host range, such as *Rhizoctonia solani*, are not effectively controlled by legume rotations. However, the increased available soil N following legumes can enhance plant health and help to minimise the impact of the disease.

Diseases with a more restricted host range such as take-all and crown rot can usually be managed using legumes (and crops such as canola) as a break crop. In one study in northern NSW, crown rot incidence was reduced by about 40 per cent in the chickpea-wheat sequence compared with the wheat-wheat sequence. The grain yield benefit for the wheat from the disease break was estimated to be 0.3t/ha (Kirkegaard *et al.* 2004).

Control of grassy weeds will still be important. Crop legumes are generally more effective than pasture legumes because the latter tend to be part of a mixed legume-grass sward with the grasses acting as disease carriers, except where pasture leys are managed to remove the grass component.

In summary, the biological benefit of legumes to the following wheat crop may range from negligible to more than 2.0t/ha of grain.

8.3 Rotational benefits of pasture legumes

Pasture legumes provide high-quality feed for grazing animals. Therefore, a major benefit of pasture legumes is the contribution made to productivity of the whole pasture, which flows through to animal production.

Pasture legume leys also benefit soil N, soil organic matter content and soil structure. These benefits can be derived from single or multi-year pasture leys. When the pasture is terminated and the land used for cropping, these benefits enhance productivity of subsequent cereal (and oilseed) crops grown on the same land.

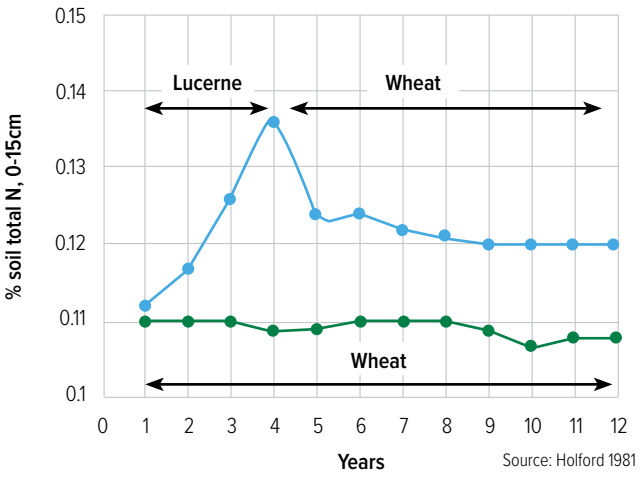
Research at Tamworth in northern NSW clearly illustrated the benefit of legume-based pasture leys on soil total N. The well-managed, intensively grazed lucerne pasture on a black earth added about 140kg N per hectare per year. Higher levels of soil total (organic) N were maintained during more than nine years of following wheat cropping (Figure 8-3).

Legume-pasture leys increase soil organic N and enhance productivity of subsequent crops.

Comparable benefits were found on a red earth soil, where the lucerne pasture added about 110kg N per hectare per year. Pasture and subsequent wheat yield data from the same study highlighted the positive impact of pasture legume leys on soil nitrate-N and grain yields of subsequent wheat crops (Table 8-5, page 89).

Depending on soil type (black earth or red earth), the grazed pasture leys produced between 9 to 27t/ha shoot biomass and 250 to 960kg/ha of biomass N during three years of growth. The black soil was about 30 per cent more productive than the red soil.

FIGURE 8-3. Build-up of soil N under a well-managed, intensively-grazed lucerne pasture on a black-earth at Tamworth, NSW, and run-down during the subsequent nine years of wheat cropping (blue). Soil N levels under the wheat monoculture are shown also (green).



Source: Holford 1981

TABLE 8-5. Summary of data from pasture ley rotation experiments at NSW Department of Primary Industries, Tamworth.

Previous crop/ pasture ley	Years duration	Shoot biomass dry matter (t/ha)	Shoot biomass N (kg/ha)	Nitrate-N at sowing[1] (kg/ha)	Wheat grain yield[2] (t/ha)	Wheat grain protein[2] (%)
Lucerne	3	24.7	854	215	2.9	12.7
Clover	3	12.7	425	150	2.8	10.4
Annual medic	3	10.8	290	110	2.2	9.5
Wheat	1	3.3	37	15	1.1	9.6

Data sourced from Holford and Crocker 1997 and Holford et al. 1998. Data are the means of six replicates and averaged over two soil types (black and red).
[1] Nitrate-N levels to 1.2m at sowing in the first year after the pasture ley or after continuous wheat
[2] Averaged over three years

At the time of sowing of the first wheat crop after the pasture phase, as much as 215kg of nitrate-N per hectare had accumulated in the soil potentially available for the wheat crop. By comparison, nitrate levels were just 15kg/ha in the adjacent continuous wheat plots.

Increased grain yields and protein in the three wheat crops following the pasture leys reflected the substantial production of pasture legume N and the mineralisation of the legume residues into soil mineral N. The benefits of the pasture leys were still apparent after three years of wheat crops, particularly for lucerne pastures.

The relationship between production of legume biomass during the three years of the leys and levels of soil nitrate N at sowing of the three succeeding wheat crops was strong. At the time of sowing of the first wheat crop, an average of 7.2kg nitrate N had been released into the soil for each tonne of pasture legume dry matter.

During the wheat phase of the rotations, between 42 and 330kg/ha additional N was taken up by the three wheat crops (including N in the wheat roots) following the pasture leys which equated to an average of 11kg wheat N per tonne legume biomass (Figure 8-4, page 90).

In summary, the greatest benefit for soil nitrate-N, wheat grain yields and grain proteins were strongly related to the amount of legume biomass produced, and the N fixed, during the preceding 3-year pasture ley. Such cause-and-effect relationships between pasture productivity and residual benefits for subsequent cropping have been promoted for many years by the CSIRO working in close collaboration with the NSW Department of Primary Industries and others in southern Australia (see Peoples *et al.* 2004, 2015).

Single-year pasture rotations are also excellent for increasing soil nitrate and enhancing wheat production. Research on one-year lucerne and annual medic leys at Warra in southern Queensland demonstrated that soil nitrate following the legume ley increased by as much as 180 per cent compared to that following wheat (Weston *et al.* 2002).

In those trials, the higher soil-water use by lucerne meant that the additional soil nitrate following lucerne did not translate into higher yields of the following wheat crop, but the extra nitrate meant far higher grain protein (13.1 per cent) than for continuous wheat (9.7 per cent). Pasture legumes typically provide greater soil N increases than crop legumes. This difference is related to greater biomass return to the system, greater recycling of N through the grazing animals back to the soil, longer growth periods, and greater N fixation efficiency (Peoples and Baldock 2001).

Soil structure can also benefit from pasture legumes. Figure 8-5 clearly shows the positive effect of pasture leys on aggregate stability of a red-earth soil in the Victorian grain belt, especially after the third year of pasture. Aggregate stability declined once wheat cropping recommenced.

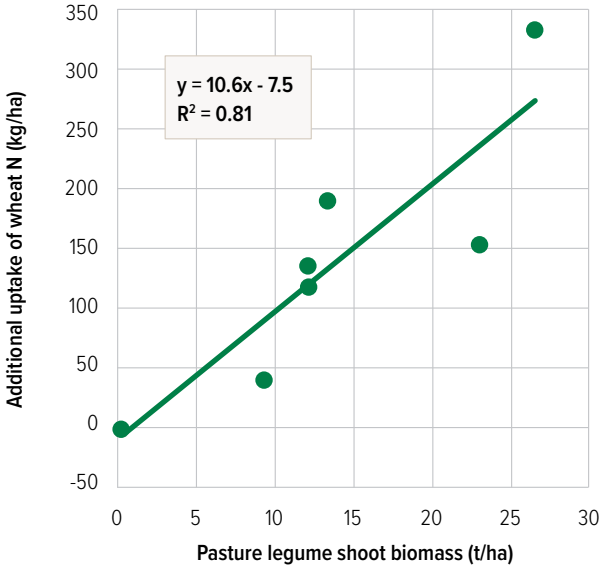
The effect of the residue biomass and N of pastures on soil structure varies with the type of clay and the clay content of the soil (Russell 1987). With vertosols (black earths high in clay content), there is little relationship between soil organic matter and structure.

On the other hand, loss of organic matter can have serious negative effects on structure of soils of less than 30 per cent clay (e.g. red-brown earths), or with high proportions of sand and silt (e.g. sands, sandy loams).

Much of the agriculture in Australia's southern and western grain belts involves sequences of pasture leys and cereals. As agricultural land used for cropping continues to lose organic matter and structural integrity, the role of pasture rotations in restoring organic fertility and productivity may need to be expanded.

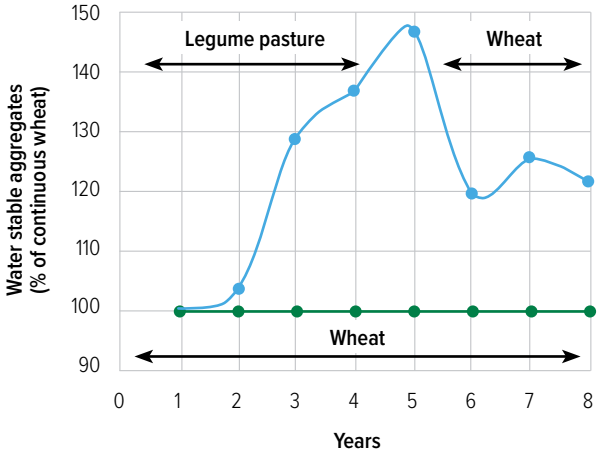
Legume pasture leys have a positive impact on soil structure as well as soil fertility.

FIGURE 8-4. Relationship between production of pasture legume biomass during the three-year leys and the additional uptake of nitrogen by the three wheat crops following termination of the leys at Tamworth, NSW. Data points are the means of black-earth and red-earth soils.



Source: Holford and Crocker 1997 and Holford *et al.* 1998.

FIGURE 8-5. Positive effects of pasture leys on aggregate stability of a red earth at Rutherglen, Vic. Once wheat cropping commenced, aggregate stability declined. (Reeves 1991)



8.4 Concluding comments

Legumes have been used as a source of food ever since humankind first tilled the soil many thousands of years ago. From those very early times, legumes were recognised as soil improvers. Growers today grow legumes in rotation with wheat and other cereals and oilseeds because of the N benefit and because it helps them to spread risk and manage weeds, pests and diseases in the production system, and improve soil health.

In this and the previous chapter, we have tried to flesh out the nature of legume N fixation and the rotational benefits of legumes by summarising some of the extensive research data on the topics. We have also provided examples of how legume N fixation and yields might be optimised through crop and pasture management.

Optimising legume yields and benefits within any system can only be achieved through best management practice in agronomy where production is not limited by soil constraints and nutrient deficiencies, poor agronomy, insects, disease, weeds or nutrients. Once this is achieved, further yield gains may be made through using elite, high-yielding varieties that are well-adapted to the location.

Nodulation must also be optimised, either through inoculation or by growing the legume in soils that are known to contain high numbers of effective, compatible rhizobia. Previous chapters in this handbook examined the rhizobia-legume symbiosis in detail and explored management decisions regarding when and how to inoculate.

9 | Legume inoculation fact sheets

In this Chapter, we present the full list of rhizobial strains that are available for use by Australian farmers, followed by a series of Fact Sheets for inoculating the more widely-grown legumes.

Photo: Bridget Penna, AgCommunicators.

9.1 List of rhizobial strains used in Australian inoculants

Inoculant group	Rhizobial strain	Legume common name	Legume botanical name
AL	RRI128	Lucerne or alfalfa	*Medicago sativa*
		Strand medic	*Medicago littoralis*
		Melilotus	*Melilotus albus*
		Disc medic	*Medicago tornata*
AM	WSM1115	Barrel medic	*Medicago truncatula*
		Burr medic	*Medicago polymorpha*
		Snail medic	*Medicago scutellata*
		Sphere medic	*Medicago sphaerocarpus*
		Gama medic	*Medicago rugosa*
		Murex	*Medicago murex*
B	TA1	White clover	*Trifolium repens*
		Red clover	*Trifolium pratense*
		Strawberry clover	*Trifolium fragiferum*
		Alsike clover	*Trifolium hybridum*
		Talish clover	*Trifolium tumens*
		Berseem, Egyptian clover	*Trifolium alexandrinum*
		Cluster or ball clover	*Trifolium glomeratum*
		Suckling clover	*Trifolium dubium*
C	WSM1325	Subterranean clover	*Trifolium subterraneum*
		Balansa clover	*Trifolium michelianum*
		Bladder clover	*Trifolium spumosum*
		Crimson clover	*Trifolium incarnatum*
		Purple clover	*Trifolium purpureum*
		Arrowleaf clover	*Trifolium vesiculosum*
		Rose clover	*Trifolium hirtum*
		Gland clover	*Trifolium glanduliferum*
		Helmet clover	*Trifolium clypeatum*
		Persian or shaftal clover	*Trifolium resupinatum*
D	CC829	Lotus	*Lotus pedunculatus*
E	SU303 or WSM1455	Pea, field pea	*Pisum sativum*
		Tares or common vetch	*Vicia sativa*
		Woolly pod vetch	*Vicia dasycarpa*
		Grass pea	*Lathyrus sativus*
		Bitter vetch	*Vicia ervilia*
		Narbon bean	*Vicia narbonensis*
		Lathyrus	*Lathyrus cicera*
F	WSM1455	Faba, tick or broad bean	*Vicia faba*
		Lentil	*Lens culinaris*
G	WU425 or WSM471	Narrow-leaf lupin	*Lupinus angustifolius*
		Mediterranean white lupin	*Lupinus albus*
		Yellow lupin	*Lupinus luteus*
		Sandplain lupin	*Lupinus cosentinii*
H	CB1809	Soybean	*Glycine max*
I	CB1015	Cowpea	*Vigna unguiculata*
		Mungbean (green gram)	*Vigna radiata*
		Mungbean (black gram)	*Vigna mungo*
J	CB1024	Pigeon pea	*Cajanus cajan*
		Lablab, hyacinth bean	*Lablab pupureus*
		Horse gram, biflorus	*Macrotyloma uniflorum*
		Perennial horse gram	*Macrotyloma axillare*
L	CB376	Lotononis	*Lotononis bainesii*
M	CB756	Velvet bean, banana bean	*Mucuna deeringiana*
		Siratro	*Macroptilium atropurpureum*
		Phasey bean	*Macroptilium lathyroides*
		Puero, tropical kudzu	*Pueraria phaseoloides*
		Calopo	*Calopogonium mucunoides*
		Glycine	*Neonotonia wightii*
		Butterfly pea	*Clitoria ternatea*

Inoculant group	Rhizobial strain	Legume common name	Legume botanical name
N	CC1192	Chickpea (desi and kabuli)	*Cicer arietinum*
P	NC92	Peanut or groundnut	*Arachis hypogaea*
S	WSM471 or WU425	Yellow serradella	*Ornithopus compressus*
		Slender serradella	*Ornithopus pinnatus*
		Pink serradella	*Ornithopus sativus*
		Hybrid serradella	*Ornithopus compressus X sativus*
		Birdsfoot	*Ornithopus perpusillus*
SPECIAL			
	CB82	Fine stem stylo	*Stylosanthes guianensis* var. *intermedia*
		Stylo	*Stylosanthes guianensis* var. *guianensis*
		Townsville stylo	*Stylosanthes humilis*
		Shrubby stylo	*Stylosanthes viscosa*
	CB1923	Centro	*Centrosema pubescens*
		Centurion	*Centrosema pascuorum*
	CIAT3101	Pinto peanut	*Arachis pintoi*
	CB627	Desmodium	*Desmodium intortum*
	CB3126	Desmanthus	*Desmanthus virgatus*
	CB3060	Leucaena	*Leucaena leucocephala*
	CB1650	Caribbean stylo (verano)	*Stylosanthes hamata*
	CC1502	Tree lucerne or tagasaste	*Chamaecytisus palmensis*
	CB2312	Bargoo jointvetch	*Aeschynomene falcata*
	WSM1592	Sulla	*Hedysarum coronarium*
	CC283b	Caucasian clover, kura clover	*Trifolium ambiguum*
	CB3035	Guar or cluster bean	*Cyamopsis tetragonoloba*
	SU277	Fenugreek	*Trigonella foenum-graecum*
	CB3481	Caatinga stylo	*Stylosanthes seabrana*
	SU343	Lotus	*Lotus corniculatus*
	WSM1497	Biserrula	*Biserrula pelecinus*
	CB3171	Calliandra	*Calliandra spp.*
	CC1099	Sainfoin	*Onobrychis viciifolia*
	CC511	French or common bean	*Phaseolus vulgaris*
		Lima bean, butter bean	*Phaseolus lunatus*
		Scarlet runner bean fire bean	*Phaseolus coccineus*
	CB1717	Burgundy bean	*Macroptilium bracteatum*
	5G1B	Adzuki bean	*Vigna angularis*
	CB2312	Jointvetch	*Aeschynomene americana*
	CB3090	Gliricidia	*Gliricidia spp.*
	SRDI554	Messina	*Melilotus siculus*
	SRDI736	Lucerne on acidic soils (pH< 5.0)	*Medicago sativa*
	WSM4083	Tedera	*Bituminaria bituminosa* var. *albomarginata*

The fact sheets are arranged in the following order:

Grain legumes (pulses and oilseed legumes)

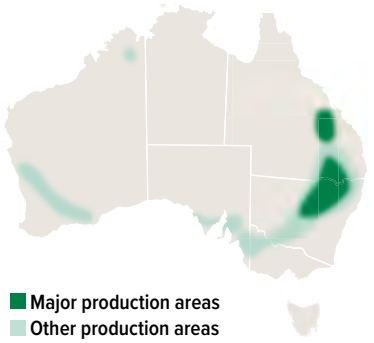
- Chickpea (group N)
- Field pea, vetch (group E) and faba bean, lentil (group F)
- Lupin and serradella (groups G and S)
- Peanut (group P)
- Mungbean and cowpea (group I)
- Soybean (group H)

Pasture legumes

- Annual clovers (group C)
- Annual medics (group AM)
- Biserrula (Special biserrula)
- Lotus (group D and special lotus)
- Lucerne, strand and disc medic (group AL)
- Perennial clovers (group B)
- Serradella (groups G and S; see serradella with lupin above)
- Sulla (special sulla)
- Messina
- Tedera

CHICKPEA INOCULATION

Inoculant group	Rhizobial strain	Legume common name	Legume botanical name
N	CC1192: *Mesorhizobium ciceri*	Chickpea (desi and kabuli)	*Cicer arietinum*



LEGUME USE AND RHIZOBIA DISTRIBUTION

Chickpea plantings total more than 600,000 hectares throughout Australia. About 90 per cent of these areas are in New South Wales and Queensland. Chickpea rhizobia are generally present in soils where chickpea has been recently grown, although numbers can vary substantially with soil type and environment. Levels of rhizobia for legumes in the N inoculation group can be measured using a DNA test*.

INOCULATION METHOD

Peat inoculants applied to the seed remains the most commonly used method of inoculation for chickpea. Some inoculant is also applied as granular and freeze-dried formulations. Seed can be coated with either the peat or freeze-dried inoculant formulations as slurries just prior to planting or during transfer (augering). Alternatively, peat or freeze-dried inoculant can be applied in-furrow when planting using a water-injection system. Granular inoculant can be dispensed into the seed row with the seed at planting.

KEY CONSIDERATIONS

Where chickpea has not been grown before, inoculation is required to achieve good nodulation. Even where background populations of rhizobia are present, inoculation may be worthwhile because the background rhizobia are often not as effective at fixing N. Where seed applied fungicides are used rhizobia inoculant is best separated from the seed. Rhizobial survival and nodulation are decreased where the soil pH_{CaCl_2} is less than 5.5.

NODULATION

Nodules are indeterminate and often multi-lobed (see Figure 9-1). For chickpeas, 10 larger or 30 smaller nodules per plant is satisfactory after about eight weeks of plant growth.

LIKELY RESPONSE TO INOCULATION FOR SOWN CHICKPEA

HIGH — Chickpeas not previously grown.

MODERATE — Previous chickpea crop was grown more than four years ago OR

Legume nodulation or growth below expectation.

LOW — Loam or clay soils with neutral or alkaline pH and recent history of well nodulated chickpea crop in past two years.

Photo: Ross Ballard, SARDI.

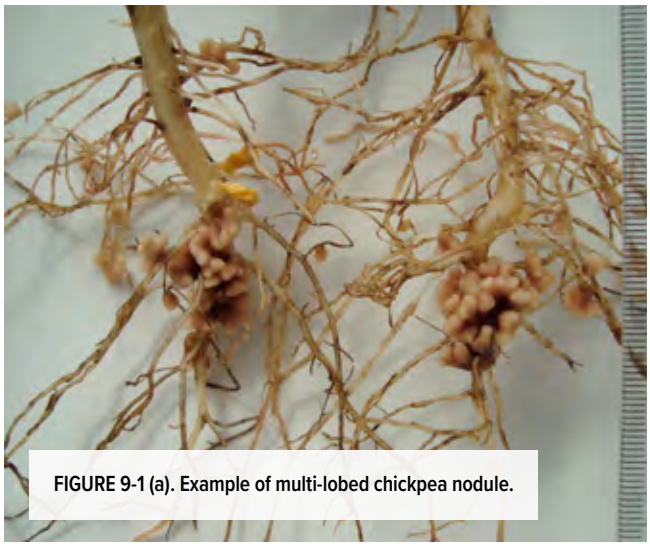


FIGURE 9-1 (a). Example of multi-lobed chickpea nodule.

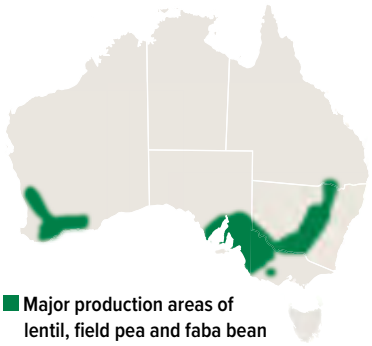
FIGURE 9-1 (b). Example of good nodulation of chickpea.

Photo: Andrew Heath.

*For PREDICTA®B accredited agronomists: rootdisease.aweb.net.au
Further information is available at pir.sa.gov.au/research/services/molecular_diagnostics/predicta_b
also see: Ballard *et al.* 2020.

FIELD PEA, VETCH, FABA BEAN AND LENTIL INOCULATION

Inoculant group	Rhizobial strain	Legume common name	Legume botanical name
E	SU303 or WSM1455: *Rhizobium leguminosarum* bv. *viciae*	Field pea	*Pisum sativum* *Vicia species*
F	WSM1455: *Rhizobium leguminosarum* bv. *viciae*	Faba bean, broad bean and lentil	*Vicia faba* *Lens culinaris*



Major production areas of lentil, field pea and faba bean

LEGUME USE AND RHIZOBIA DISTRIBUTION

The same species of rhizobia can nodulate legumes in inoculant groups E and F. The rhizobia have been widely distributed following decades of field pea and vetch cultivation. Present combined sowings of field pea, faba bean and lentil is almost 800,000 hectares per year, most of which is in South Australia and Victoria. Spread and survival of the rhizobia in these states has also been assisted by vetch which is grown as forage, and tares which are naturalised in some areas.

Although the rhizobia have been widely distributed, their sensitivity to soil acidity (<pH_{CaCl_2} 5.5) means they sometimes occur at levels below what is needed for optimal nodulation. Levels of rhizobia for legumes in the E/F inoculation group can be measured using a DNA test*.

INOCULATION METHOD

Peat inoculants applied to the seed remains the most commonly used method of inoculation for this group. Some inoculant is also applied as granular and freeze-dried formulations. Seed can be coated with either the peat or freeze-dried inoculant formulations as slurries just prior to planting or during transfer (augering). Alternatively, peat or freeze-dried inoculant can be applied in-furrow when planting using a water-injection system. Granular inoculant can be dispensed into the seed row with the seed at planting.

KEY CONSIDERATIONS

Two inoculant strains are provided for these legumes to optimise N fixation potential of the different legume hosts. For this reason, only group F should be used on faba beans and lentils. Group E (SU303) is preferred for field peas, but group F (WSM1455) can be used in its place as it is only marginally less effective.

Rhizobia for these legumes are sensitive to soil acidity. Their number may be sub-optimal or absent where soil pH is less than 5.5, even where there has been a recent history of legume host. About 20 per cent of soils in South Australia and Victoria and 60 per cent of soils in Western Australia with a history of these crops contain insufficient rhizobia to maximise the nodulation of legumes in the E/F inoculation group.

Where seed applied fungicides are used, rhizobia inoculant is best separated from the seed.

NODULATION

More than 50 pink nodules per plant is considered satisfactory after eight to 10 weeks plant growth on most soil types. (see Figure 9-2, page 96). It could equally be argued that on lighter textured soils that are lower in N, that more nodulation is needed/possible, in the absence of other constraints.

LIKELY RESPONSE TO INOCULATION FOR SOWN PEA, FABA BEAN, LENTIL AND VETCH

HIGH

Soils with pH_{CaCl_2} below 5.5 and high summer soil temperatures (>35°C for 40 days) OR

Legume host (pea, faba bean, lentil, vetch) not previously grown.

MODERATE

No legume host (pea, faba bean, lentil, vetch) in previous four years OR

Prior 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.

*For PREDICTA®B accredited agronomists: rootdisease.aweb.net.au
Further information is available at pir.sa.gov.au/research/services/molecular_diagnostics/predicta_b
also see: Ballard *et al.* 2020.

FIELD PEA, VETCH, FABA BEAN AND LENTIL INOCULATION, CONT...

Photos: Liz Farquharson, SARDI.

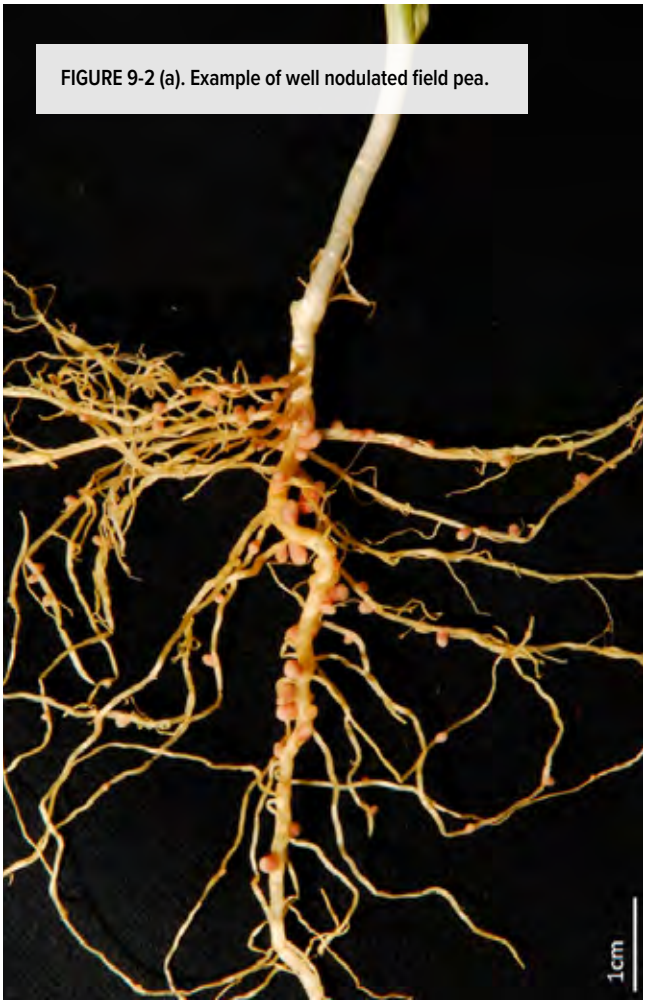
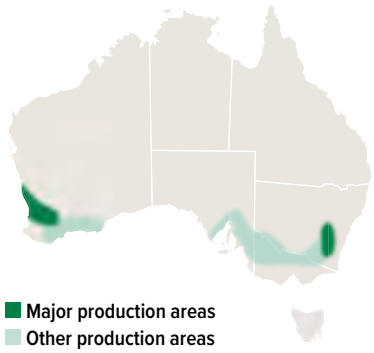


FIGURE 9-2 (a). Example of well nodulated field pea.

FIGURE 9-2 (b). Example of well nodulated faba bean.

LUPIN AND SERRADELLA INOCULATION

Inoculant group	Rhizobial strain	Legume common name	Legume botanical name
G	WU425 or WSM471: *Bradyrhizobium* spp. *lupini*	Lupin (Narrow-leafed, white, yellow and sand-plain)	*Lupinus* species
S	WSM471 or WU425 *Bradyrhizobium* spp. *lupini*	Serradella (Yellow, pink, hybrid, slender and birdsfoot)	*Ornithopus* species



LEGUME USE AND RHIZOBIA DISTRIBUTION

Legumes in the G and S inoculation groups are nodulated by the same species of rhizobia (i.e. *Bradyrhizobium* spp). Commercial plantings of serradella began in the 1950s while significant plantings of lupin commenced in the 1970s. Both legumes are adapted to acidic to neutral sandy soils and are therefore widely grown in WA where they have been sown on several million hectares. The rhizobia tend to be persistent where the legume has been grown, but remain confined to those areas because, unlike the clovers and medics, an array of legume species that host the rhizobia have not dispersed and naturalised in Australian soils. Levels of rhizobia for legumes in the G/S inoculation group can be measured using a DNA test*.

INOCULATION METHOD

Peat inoculants applied to the seed remains the most commonly used method of inoculation for lupin. Some inoculant is also applied as granular and freeze-dried formulations. Seed can be coated with either the peat or freeze-dried inoculant formulations as slurries just prior to planting or during transfer (augering). Alternatively, peat or freeze-dried inoculant can be applied in-furrow when planting using a water-injection system. Granular inoculant can be dispensed into the seed row with the seed at planting.

Inoculation of serradella is mostly done with the application of a slurry of peat. Where podded serradella is being inoculated, adjustments to liquid volumes are required to ensure even distribution and survival of inoculant and the manufacturer's instructions should be carefully followed (see Chapter 2). Granular inoculant in furrow can also be used. Lime pelleting has been shown to be advantageous to rhizobial survival and serradella nodulation in eastern Australia, even though serradella rhizobia are naturally acid tolerant. Lime pelleting of serradella is recommended in all states except WA.

KEY CONSIDERATIONS

Two inoculant groups are available and can be used for both lupin and serradella. They are group G, containing strain WU425, or group S, containing strain WSM471.

Rhizobia for these legumes are tolerant of soil acidity but some instances of inadequate numbers in soil after four years of legume absence have been recorded. Top-up inoculation may be worthwhile where the host crop has been absent four or more years.

As these legumes are often grown on very sandy soils that are acutely deficient in available N, nodulation failure can result in total crop or pasture failure. Where there is no previous history of lupin or serradella, inoculation is essential.

NODULATION

For lupin, nodules can be difficult to count, but the collar region (top of root system) is typically covered by nodule material in well nodulated plants (see Figure 9-3, page 98). Lupin roots can have a pink colouration inside the root that is unrelated to nodulation and nitrogen fixation.

For serradella more than 20 pink nodules per plant is satisfactory after eight to 10 weeks plant growth.

LIKELY RESPONSE TO INOCULATION FOR SOWN LUPIN AND SERRADELLA

HIGH

Lupin or serradella not previously grown in paddock.

MODERATE

No legume host in past four years OR

Previous host crop not inoculated and legume growth or nodulation below expectation.

LOW

Sowing in the north and central regions of the Western Australian wheat/sheep belt OR

Recent history (past four years) of vigorous lupin/serradella growth and good nodulation.

***For PREDICTA®B accredited agronomists:** rootdisease.aweb.net.au
Further information is available at pir.sa.gov.au/research/services/molecular_diagnostics/predicta_b
also see: Ballard *et al.* 2020.

LUPIN AND SERRADELLA INOCULATION, CONT...

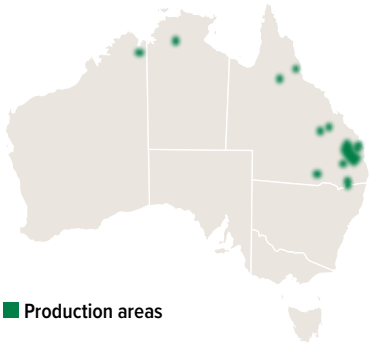
Photos: Ross Ballard, SARDI.

FIGURE 9-3 (a). Examples of well nodulated lupin.

FIGURE 9-3 (b). Example of well nodulated serradella.

PEANUT INOCULATION

Inoculant group	Rhizobial strain	Legume common name	Legume botanical name
P	NC92: *Bradyrhizobium* spp.	Peanut (or groundnut)	*Arachis hypogaea*



LEGUME USE AND RHIZOBIA DISTRIBUTION

Australian growers produce about 40,000 tonnes of peanuts annually from about 15,000 hectares. More than 90 per cent of these are grown in Queensland with a few growers also in northern NSW, NT and northern WA. One third of production is rain-fed and two thirds is irrigated.

INOCULATION METHOD

Water injection of peat or freeze-dried inoculant is recommended to eliminate damage to the seed from applying a slurry coating.

KEY CONSIDERATIONS

Inoculation every season is recommended to maximise yields as native or background rhizobia compete strongly with the inoculant strain for root infection but are not as effective at fixing N.

NODULATION

Peanuts form many nodules (i.e. more than 100/plant). It is not possible to state the number of nodules per plant after eight to 10 weeks of plant growth that might be considered satisfactory (See Figure 9-4).

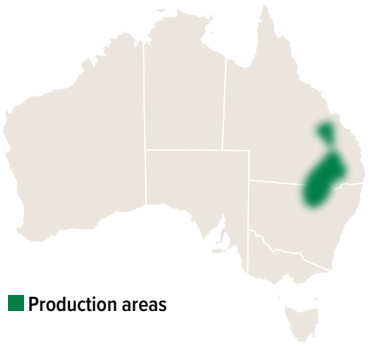
LIKELY RESPONSE TO INOCULATION FOR SOWN PEANUT

HIGH	Peanut not previously grown.
MODERATE	Most other situations due to likely presence of poorly effective rhizobia.
LOW	Recent and/or intensive cultivation of peanut

FIGURE 9-4. Example of well nodulated peanut.

Photo: Nikki Seymour, QDAF.

MUNGBEAN AND COWPEA INOCULATION



Inoculant group	Rhizobial strain	Legume common name	Legume botanical name
I	CB1015: *Bradyrhizobium* spp.	Mungbean	*Vigna radiata, V. mungo*
		Cowpea	*Vigna unguiculata*

LEGUME USE AND RHIZOBIA DISTRIBUTION

Mungbeans are the more widely grown legume in this inoculant group. Most are grown in southern and central Queensland and northern NSW.

INOCULATION METHOD

Peat inoculants applied to the seed remains the most commonly used method of inoculation for mungbean. Some inoculant is also applied as freeze-dried formulations. Seed can be coated with either the peat or freeze-dried inoculant formulations as slurries just prior to planting or during transfer (augering). Alternatively, peat or freeze-dried inoculant can be applied in-furrow when planting using a water-injection system.

KEY CONSIDERATIONS

Soil nitrate-N may depress nodulation and N fixation of mungbean, even at relatively low levels (>20kg N/ha).

NODULATION

For mungbean and cowpea, more than 20 nodules per plant is satisfactory after six to eight weeks of plant growth (see Figure 9-5).

LIKELY RESPONSE TO INOCULATION FOR SOWN MUNGBEAN AND COWPEA

HIGH — **No previous mungbean, cowpea or other related *Vigna* species.**

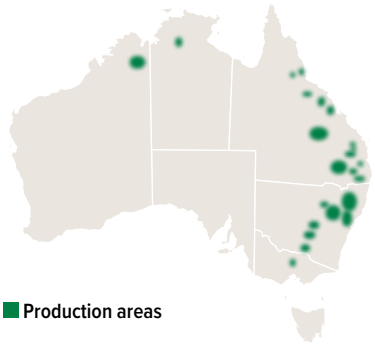
MODERATE — **Most other situations due to likely presence of poorly effective rhizobia.**

LOW — **Recent and/or intensive cultivation of mungbean or cowpea.**

Photo: Nikki Seymour, QDAF.

FIGURE 9-5. Example of well nodulated mungbean.

SOYBEAN INOCULATION



Inoculant group	Rhizobial strain	Legume common name	Legume botanical name
H	CB1809: *Bradyrhizobium japonicum*	Soybean	*Glycine max*

LEGUME USE AND RHIZOBIA DISTRIBUTION

Soybean is grown in areas of adequate-to-high summer rainfall or where irrigation is possible. This includes a wide area from northern Queensland, along the coastal sugar belt and in central Queensland, to the Darling Downs, into the NSW coastal hinterland and to inland cropping regions of southern NSW and Victoria. They are also grown in the northern irrigation areas of WA.

INOCULATION METHOD

Peat inoculants applied to the seed remain the most commonly used method of inoculation for this legume. Inoculant is also available in liquid and freeze-dried forms. Seed can be coated with either the peat, liquid or freeze-dried inoculant formulations as slurries just prior to planting, and are commonly applied to the seeds during transfer (augering). Alternatively, peat, liquid or freeze-dried inoculant can be applied in-furrow when planting using a water-injection system can be dispensed with the seed at planting.

KEY CONSIDERATIONS

Soybean is specific in its requirement for rhizobia. Soybean will not nodulate with the same range of naturalised soil rhizobia as mungbean or cowpea. When grown with irrigation or under high-rainfall conditions, soybeans can produce considerable shoot biomass (seven to eight tonnes per hectare) and grain yield (four tonnes per hectare) and therefore has a high N demand in excess of 300 to 400kg N/ha. Therefore, good agronomy and good inoculation practice are necessary to achieve yield and N fixation potentials.

NODULATION

For soybeans more than 20 nodules per plant is satisfactory after eight to 10 weeks of plant growth (see Figure 9-6).

LIKELY RESPONSE TO INOCULATION FOR SOWN SOYBEAN

HIGH	No previous soybean crop. Highly alkaline or highly acidic soils.
MODERATE	Soybean cultivated in paddock more than three to five years ago.
LOW	Recent and/or intensive cultivation of soybean.

Photo: Nikki Seymour, QDAF.

FIGURE 9-6 (a). Example of well nodulated soybean.

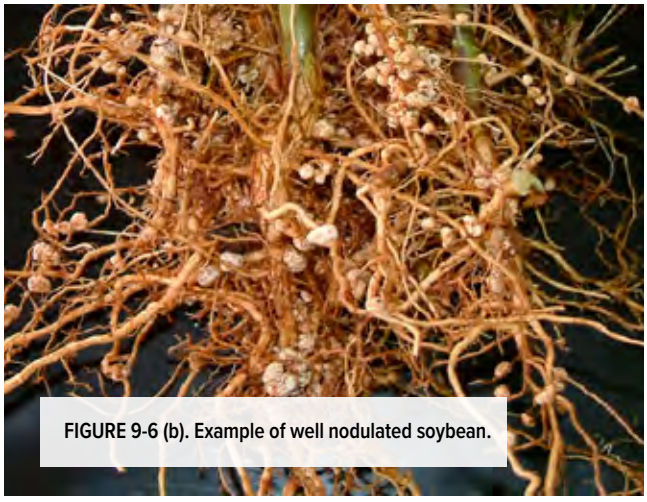


FIGURE 9-6 (b). Example of well nodulated soybean.

Photo: David Herridge, UNE.

ANNUAL CLOVERS INOCULATION

Inoculant group	Rhizobial strain	Legume common name	Legume botanical name
C	WSM1325: *Rhizobium leguminosarum* bv. *trifolii*	Annual Clovers (Subterranean, balansa, persian, arrowleaf, rose, gland, crimson, purple, bladder, cupped and helmet)	*Trifolium* species

Photo: Andrew Craig.

LEGUME USE AND RHIZOBIA DISTRIBUTION

Subterranean clover is the most widely sown legume in this group. It is sown on about 300,000 hectares annually and occurs on more than 10 million hectares across southern Australia. Many non-sown clover species that have naturalised extensively have assisted the widespread proliferation of clover nodulating rhizobia.

INOCULATION METHOD

Peat inoculant applied to the seed has been widely used to inoculate annual clovers, followed by pelleting with fine lime. The availability of preinoculated (pelleted) seed has increased. However, survival of the rhizobia is often poor and therefore freshly inoculated seed is preferred. Granular and freeze-dried inoculant formulations are available.

KEY CONSIDERATIONS

The majority of Australian soils with a history of growing clovers contain clover nodulating rhizobia. Effectiveness of the naturalised soil rhizobia with sub clover is often sub-optimal, averaging 50 per cent of the commercial inoculant strain. Inoculation may help overcome sub-optimal symbioses to assist the establishment of sown pastures.

Some annual clover species, notably gland, bladder and arrowleaf clovers, are less compatible with naturalised soil rhizobia and inoculation is considered essential to ensure adequate establishment. High numbers of rhizobia on sown seed will compete with soil rhizobia at sowing but potency will diminish after several seasons.

Clover symbioses are reasonably tolerant of low soil pH, but ideally soil pH_{CaCl_2} should be greater than 5.0. Background soil rhizobia should not be relied upon in very low pH soils, even where good nodulation is observed in the pasture before renovation. Disruption of background rhizobia from soil micro-sites during pasture renovation may result in their death with the site becoming responsive to inoculation.

NODULATION

More than 50 pink nodules per plant after eight weeks growth indicates good nodulation of sub clover (see Figure 9-7, page 103).

LIKELY RESPONSE TO INOCULATION FOR SOWN ANNUAL CLOVERS

HIGH

- **Gland, bladder and arrowleaf clovers should always be inoculated.**
- **All annual clovers where there is no history of clover having been grown.**
- **Soils with pH_{CaCl_2} below 5.0 and where there is tillage at pasture renovation.**

MODERATE

- **No clover host in past four years and soil pH_{CaCl_2} below 5.5 OR**
- **Clover present, but growth or nodulation below expectation.**

LOW

- **Soils with neutral or alkaline pH and a recent history of good clover growth and nodulation.**

ANNUAL CLOVERS INOCULATION, CONT...

FIGURE 9-7. Example of well nodulated sub clover.

Photo: Ross Ballard, SARDI.

ANNUAL MEDICS INOCULATION

Photo: David Peck, SARDI.

Inoculant group	Rhizobial strain	Legume common name	Legume botanical name
AM	WSM1115: *Sinorhizobium medicae*	Annual Medics (barrel, burr, snail, murex, sphere and gama)	*Medicago* species (except strand and disc)

LEGUME USE AND RHIZOBIA DISTRIBUTION

The diverse medic species in this inoculation group are grown in the medium-to-low-rainfall cropping regions where soils are neutral to alkaline and not subject to waterlogging. They have been grown extensively since the 1930s and therefore their rhizobia are also widely distributed.

INOCULATION METHOD

Inoculation is mostly done with the application of a slurry of peat followed by pelleting with fine lime. The availability of preinoculated (pelleted) seed has increased. However, survival of the rhizobia is often poor and therefore freshly inoculated seed is preferred. Granular and freeze-dried inoculant formulations are available.

KEY CONSIDERATIONS

The majority of Australian soils that are neutral or alkaline in pH and have a history of growing annual medic (both sown and naturalised species) contain medic-nodulating rhizobia.

Effectiveness of the naturalised soil rhizobia is often sub-optimal, averaging 50 per cent of the commercial inoculant strain. Inoculation may help overcome sub-optimal symbioses to assist the establishment of sown pastures. High numbers of rhizobia on sown seed will compete with soil rhizobia at sowing but potency will diminish after several seasons.

Mildly acidic soils (pH_{CaCl_2} 5.0 to 6.0) where the more acid tolerant species, namely burr, murex and sphere medic are grown, often contain insufficient rhizobia for good nodulation at establishment.

The group AL inoculant should not be used as a substitute because the inoculant strain (RRI128) is less effective at fixing N with most medic species in this group.

NODULATION

10-20 pink nodules per plant after eight weeks growth indicates good nodulation of annual medics (see Figure 9-8).

LIKELY RESPONSE TO INOCULATION FOR SOWN ANNUAL MEDIC

HIGH

Burr, sphere and murex medic sown on soils with pH_{CaCl_2} below 6.0 OR

No presence or history of sown or naturalised medic.

MODERATE

Medic present, but growth or nodulation below expectation. May be associated with the presence of sub-optimal populations of rhizobia.

LOW

Loam or clay soils with neutral or alkaline pH_{CaCl_2} and a recent history of vigorous medic growth and good nodulation

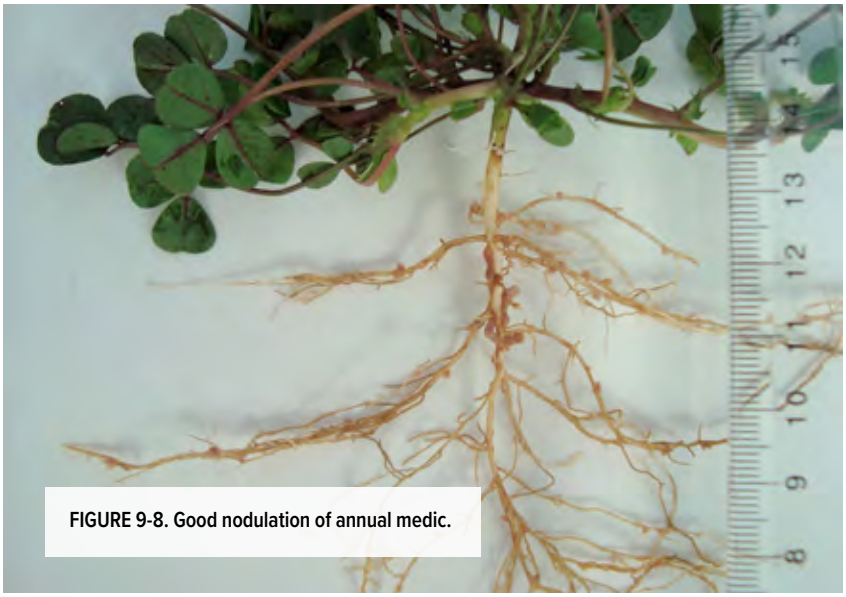


Photo: Ross Ballard, SARDI.

FIGURE 9-8. Good nodulation of annual medic.

BISERRULA INOCULATION

Inoculant group	Rhizobial strain	Legume common name	Legume botanical name
SPECIAL	WSM1497: *Mesorhizobium ciceri* bv. *biserrulae*	Biserrula	*Biserrula pelecinus*

Photo: Ashlea Webster, AIRG, NSWDPI.

LEGUME USE AND RHIZOBIA DISTRIBUTION

A relatively new annual pasture legume with the first cultivar Casbah registered in 2001. It is presently grown on about 100,000 hectares, mainly in mixed-farming areas. Plantings have mostly been in WA, but are expanding in NSW.

INOCULATION METHOD

The two common methods of inoculation are peat-slurry lime pelleted seed or seed sown with granular inoculant. Increased inoculation rates (above recommended rates) of one 250g packet of inoculant for 10kg of seed are recommended.

KEY CONSIDERATIONS

Because biserrula and its rhizobia are relatively new to Australian agriculture it is essential to inoculate if the legume has not been recently grown in the paddock. Biserrula and their associated rhizobia are very specific. The plant does not nodulate with the rhizobia associated with other indigenous or cultivated legumes.

NODULATION

At least five large (>5mm) and 10 small nodules per plant after eight weeks growth indicates good nodulation of biserrula (see Figure 9-9).

LIKELY RESPONSE TO INOCULATION FOR SOWN BISERRULA

HIGH — Biserrula host has not been previously grown.

MODERATE — No biserrula in past four years OR Last host crop not inoculated or lacked 'good' nodulation near top of root system.

LOW — Loam or clay soils with neutral or alkaline pH and a recent history (past two years) of host crop with good nodulation.

Photo: Ross Ballard, SARDI.

FIGURE 9-9. Example of well nodulated biserrula.

LOTUS INOCULATION

Inoculant group	Rhizobial strain	Legume common name	Legume botanical name
D	CC829: *Bradyrhizobium* sp.	Greater lotus	*Lotus pedunculatus (syn uliginosus)*
SPECIAL	SU343: *Mesorhizobium loti*	Birdsfoot trefoil	*Lotus corniculatus*

Photo: Ronald Yates, DPIRD.

LEGUME USE AND RHIZOBIA DISTRIBUTION

The use of these perennial pasture legumes is largely restricted to permanent pastures in the medium to high rainfall districts of eastern Australia and their rhizobia will be similarly restricted in their distribution. Although there are some naturalised species of lotus, they occur in low numbers and are unlikely to maintain rhizobia in sufficient number to negate the need for inoculation.

INOCULATION METHOD

Inoculation is mostly done with the application of a slurry of peat followed by pelleting with fine lime or other suitable product. One packet of peat inoculant (250g) will inoculate 10kg of seed. Freeze-dried products are also available.

KEY CONSIDERATIONS

A different inoculant strain is provided for each species of lotus, recognising that they have different rhizobial needs. *Lotus pedunculatus* is particularly specific in its rhizobial need. The two inoculant strains should not be interchanged. The rhizobia have moderate tolerance to soil acidity.

NODULATION

Good nodulation after eight to 10 weeks is considered to be more than 30 pink nodules per plant (see Figure 9-10).

LIKELY RESPONSE TO INOCULATION FOR SOWN LOTUS

HIGH — Lotus not previously grown.

MODERATE — No lotus in past four years OR

Prior lotus pasture not inoculated or lacked good nodulation near top of root system.

LOW — Loam soils with neutral pH and a recent history (past two years) of lotus with adequate nodulation.

Photo: Ronald Yates, DPIRD.

FIGURE 9-10. Example of well nodulated lotus.

LUCERNE, MELILOTUS (albus), STRAND AND DISC MEDIC INOCULATION

Inoculant group	Rhizobial strain	Legume common name	Legume botanical name
AL	RRI128: *Sinorhizobium meliloti*	Lucerne or alfalfa, Strand medic, Melilotus, Disc medic	*Medicago sativa, Medicago littoralis, Medicago tornata, Melilotus albus*
SPECIAL	SRDI736: *Sinorhizobium meliloti*	Lucerne on acidic soils (pH< 5.0)	*Medicago sativa*

Photo: Ross Ballard, SARDI.

LEGUME USE AND RHIZOBIA DISTRIBUTION

About 300,000 hectares of lucerne are sown annually, with stands persisting on three to five million hectares. It is most widely grown in NSW and least grown in WA, where summer rainfall is scarce.

By comparison the area sown annually to strand and disc medic is less than 20,000 hectares. However, established pastures of strand medic persist over wide areas of SA's Eyre Peninsula and the Mallee region bordering SA and Victoria. Medic sowings are generally aimed at renovation of pastures in these areas, which support large populations of rhizobia which are able to nodulate both medic and lucerne.

Lucerne is also often sown in permanent pasture areas where naturalised medics do not commonly occur. Soils in these areas are unlikely to support suitable rhizobia for lucerne.

INOCULATION METHOD

Peat, granular and freeze-dried inoculant formulations are available. Most seed sold through retail outlets is preinoculated.

KEY CONSIDERATIONS

Inoculation is always recommended for lucerne because good plant density at sowing is critical to long-term production and cannot be recovered if compromised nodulation leads to poor establishment.

Most lucerne seed is sold preinoculated. Seed should not be used where the period since inoculation exceeds six months, even if it has been stored under cool dry conditions. Seed that exceeds this expiry period should be re-inoculated.

The lucerne and medic symbioses are very sensitive to low pH. Coating the inoculated seed with fine lime is advisable to provide protection from acidic fertilisers and aid establishment in acidic soils.

Where soil pH_{CaCl_2} is less than 6.0, soils will often contain no suitable rhizobia and will be highly responsive to inoculation. Responses to inoculation in the SA/Vic Mallee are commonly observed. Inoculation in this region is likely to be beneficial, even if there has been a recent history of medic.

Inoculant strain SRDI736 is produced as a special inoculant for use on lucerne on soils between pH_{CaCl_2} 4.5 and 5.0. It has been selected and tested on SARDI 7 Series 2 lucerne, so no warranty of its performance with other lucerne cultivars is possible. Lucerne seed inoculated with SRDI736 should be lime pelleted and sown within 3 days after inoculation to maximise nodulation potential in acidic soils.

For lucerne being sown in soils above pH_{CaCl_2} 5.0, rhizobia strain RRI128 remains the preferred strain.

The group AM inoculant should not be used as a substitute for AL because the inoculant AM strain (WSM1115) is less effective at fixing nitrogen with lucerne, strand and disc medic.

NODULATION

Young lucerne plants should have at least five pink nodules per plant at eight to 10 weeks after sowing. Ten to 15 nodules are ideal at this time.

For mature lucerne plants where tap root development has occurred, nodules may be restricted to the finer lateral roots and to a depth of 30cm in the soil. Nodules on mature lucerne are therefore easily detached and difficult to find.

The strand medics are sometimes referred to as shy nodulators due to the low number of nodules commonly observed on their roots (Figure 9-11). This is a characteristic of the plant and so the presence of five nodules at eight to 10 weeks after sowing is regarded as satisfactory.

LIKELY RESPONSE TO INOCULATION FOR SOWN LUCERNE, STRAND & DISC MEDIC

HIGH

- Lucerne should always be inoculated at sowing.
- Soils with pH_{CaCl_2} below 6.0.
- No history or presence of sown or naturalised medic.

MODERATE

Medic present, but growth or nodulation below expectation. Maybe associated with development of sub-optimal populations of medic rhizobia in the soil. High number of rhizobia on sown seed will compete with soil rhizobia at sowing but potency will diminish after several seasons.

LOW

Loam or clay soils with neutral to alkaline pH_{CaCl_2} and a recent history of vigorous medic growth and good nodulation.

LUCERNE, MELILOTUS (albus), STRAND AND DISC MEDIC INOCULATION, CONT...

Photos: Ross Ballard, SARDI.

FIGURE 9-11 (a). Examples of well nodulated lucerne seedlings.

FIGURE 9-11 (b). Roots of 18-month-old lucerne plant.

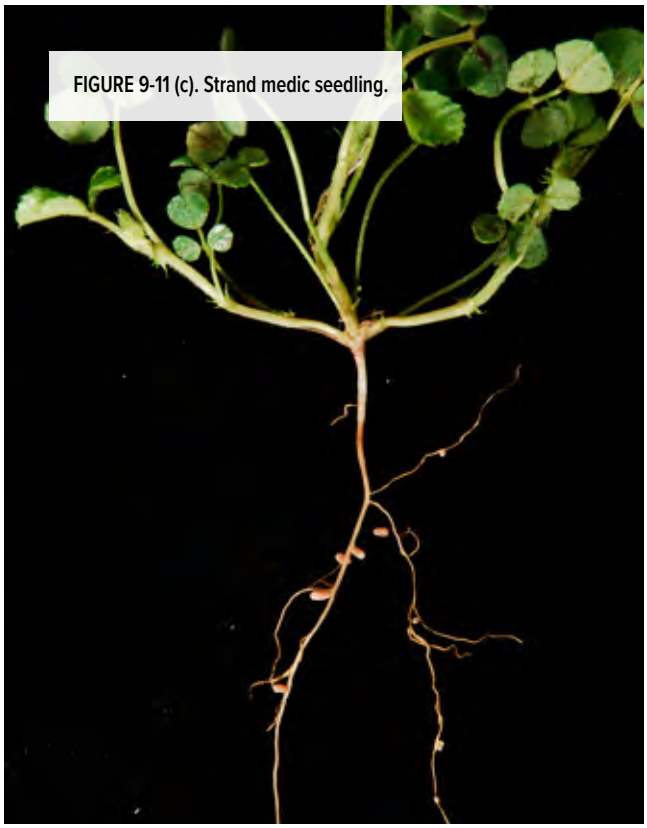


FIGURE 9-11 (c). Strand medic seedling.

PERENNIAL CLOVERS INOCULATION

Inoculant group	Rhizobial strain	Legume common name	Legume botanical name
B	TA1: *Rhizobium leguminosarum* bv. *trifolii*	Perennial clovers (White, strawberry, red, talish, alsike, berseem, Egyptian, cluster or ball and suckling)	*Trifolium* species
SPECIAL	CC283b: *Rhizobium leguminosarum* bv. *trifolii*	Caucasian clover, kura clover	*Trifolium ambiguum*

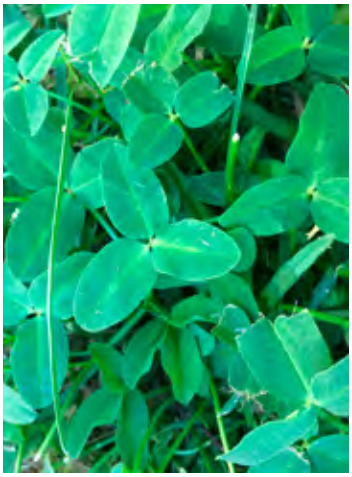


Photo: Ross Ballard, SARDI.

LEGUME USE AND RHIZOBIA DISTRIBUTION

White clover is the most widely sown legume in this group.
It is grown on more than five million hectares, generally in high-rainfall (>700mm) coastal areas and cooler tableland districts or elsewhere where irrigation is available. Many annual clover species that have naturalised in the areas where perennial clovers are grown and have assisted the widespread proliferation of clover nodulating rhizobia.

INOCULATION METHOD

Peat and freeze-dried inoculant formulations are available. Most seed sold through retail outlets is preinoculated.

KEY CONSIDERATIONS

The majority of Australian soils with a history of growing annual or perennial clovers contain clover nodulating rhizobia, but their effectiveness is often sub-optimal.

Inoculation will help overcome sub-optimal symbioses and can be important to ensure that the early growth of smaller seeded perennial legumes is vigorous.

Clover symbioses are reasonably tolerant of low soil pH, but ideally soil pH_{CaCl_2} should be greater than 5.0. Background soil rhizobia should not be relied upon in very low pH soils, even where good nodulation is observed in the pasture before renovation. Disruption of background rhizobia from soil micro-sites during pasture renovation may result in their death, resulting in the site becoming responsive to inoculation.

Most perennial clover seed is sold preinoculated. Survival time of rhizobia strain TA1 on seed is less than for other rhizobia. Seed should not be used where the period since inoculation exceeds two weeks, even if it has been stored under cool dry conditions. Seed that exceeds this expiry period should be re-inoculated. Freshly inoculated seed is preferred.

Seed size of many perennial clovers is small and inoculation rate needs to be adjusted accordingly. For white clover, the standard 250g packet of peat inoculant is recommended for the inoculation of 25kg of seed.

The group C inoculant (WSM1325) for annual clovers should not be used as a substitute for the group B inoculant (TA1). Nitrogen fixation by the perennial clovers is significantly better with strain TA1.

NODULATION

Young clover plants should have at least 10 pink nodules per plant at eight to 10 weeks after sowing (see Figure 9-12, page 110).

LIKELY RESPONSE TO INOCULATION FOR SOWN PERENNIAL CLOVERS

HIGH

- Caucasian clover should always be inoculated.
- All perennial clovers where there is no history of clover having grown.
- Soils with pH_{CaCl_2} below 5.0 and where there is tillage at pasture renovation.

MODERATE

- No clover host in past four years and soil pH_{CaCl_2} below 5.5.
- Clover present, but growth or nodulation below expectation. May be associated with development of sub-optimal populations of soil rhizobia. High numbers of rhizobia on sown seed will compete with soil rhizobia at sowing but potency will diminish after several seasons.

LOW

- Soils with neutral or alkaline pH and a recent history of good clover growth and nodulation.

PERENNIAL CLOVERS INOCULATION, CONT...

Photo: Greg Gemell.

FIGURE 9-12. Example of well nodulated white clover showing an abundance of nodules on the tap root and close to the crown of the plant.

SULLA INOCULATION

Inoculant group	Rhizobial strain	Legume common name	Legume botanical name
SPECIAL	WSM1592: *Rhizobium sullae*	Sulla	*Hedysarum coronarium*

Photo: GRDC.

LEGUME USE AND RHIZOBIA DISTRIBUTION

Sulla is comparatively new to Australian agriculture and has only been sown on about 10,000 hectares annually since 2007. It is suited to moderate to high rainfall zones (400 to 1000mm) and soils with pH_{CaCl_2} in the range 5.5 to 8.0, but prefers alkaline soils. It is essential to inoculate sulla as their associated rhizobia are very specific and the species rarely nodulates with background rhizobia in the soil.

INOCULATION METHOD

Inoculation is mostly done with the application of a slurry of peat followed by pelleting with fine lime. Seed sold through retail outlets may be preinoculated. Fresh inoculation is strongly preferred.

KEY CONSIDERATIONS

Sulla tends to be a shy nodulator and young seedlings quickly develop N deficiency symptoms where nodulation is inadequate. Higher rates of inoculation can be used to ensure adequate nodulation. One packet of peat inoculant (250g) should be used to inoculate 10kg of seed.

In preinoculated seed, the rhizobia have a very short shelf life and so seed is best sown as soon as possible after inoculation.

NODULATION

For sulla, five or more large (>5 mm) nodules per plant is satisfactory after eight to 10 weeks of plant growth (see Figure 9-13).

LIKELY RESPONSE TO INOCULATION FOR SOWN SULLA

HIGH

Sulla not previously grown OR

Soils with pH_{CaCl_2} below 6.0.

MODERATE

No sulla in past four years OR

Growth or nodulation of previous crop below expectation.

LOW

Loam or clay soils with neutral or alkaline pH_{CaCl_2} and a recent history (past two years) of sulla with good nodulation.

FIGURE 9-13 (a). Examples of good sulla nodulation.

FIGURE 9-13 (b). Examples of good sulla nodulation.

Photo: Ronald Yates, DPIRD.

MESSINA INOCULATION

Inoculant group	Rhizobial strain	Legume common name	Legume botanical name
SPECIAL	SRDI554: *Sinorhizobium medicae*	Messina	*Melilotus siculus*

Photo: Amanda Pearce, SARDI.

LEGUME USE AND RHIZOBIA DISTRIBUTION

Messina is comparatively new to Australian agriculture and has only been sown on about 20,000ha since 2017. It was developed for salt-land pastures and has high levels of salinity and waterlogging tolerance. Related to the annual medics, it prefers alkaline soils and should not be grown on soils below pH_{CaCl_2} 5.8.

It is essential to inoculate messina because the saline areas where it is grown generally lack suitable rhizobia. Inoculant strain SRDI554 has been selected for its ability to survive in saline soils, which is critical for nodulation in regenerating messina pastures.

INOCULATION METHOD

Inoculation should be undertaken using a slurry of peat inoculant followed by pelleting with fine lime. Fresh inoculation immediately before sowing is recommended to maximise nodulation potential. Peat is the only inoculant formulation available.

KEY CONSIDERATIONS

Messina must be inoculated with inoculant strain SRDI554. Messina is an annual legume which regenerates from seed banks each year, after the initial establishment year. Nodulation of regenerating messina pastures will only be satisfactory where inoculant strain SRDI554 has been introduced because other rhizobia strains survive less well in saline soils.

Messina and its rhizobia prefer alkaline soils. Seed should be pelleted with fine lime after the application of inoculant. Messina should not be sown on soils below pH_{CaCl_2} 5.8.

Although messina is related the annual medics, group AM and AL inoculant rhizobia for medics should not be used as a substitute for messina inoculant strain SRDI554. The AM and AL strains do not survive saline soil conditions and the AL strain forms an ineffective symbiosis with messina.

NODULATION

For messina, more than 20 nodules per plant is satisfactory after eight to 10 weeks of plant growth (see Figure 9-14).

LIKELY RESPONSE TO INOCULATION FOR MESSINA

HIGH

Messina not previously grown OR

Soils with pH_{CaCl_2} below 6.0, salinity >8 dS/m

MODERATE

No messina in past four years OR

Growth or nodulation of previous messina below expectation.

LOW

Loam or clay soils with neutral or alkaline pH_{CaCl_2}, low salinity levels, and a recent history (past two years) of messina or medics in AM inoculation group with good nodulation.

FIGURE 9-14. Example of well nodulated messina showing an abundance of nodules on the tap root and close to the crown of the plant.

Photo: Ross Ballard, SARDI.

TEDERA INOCULATION

Inoculant group	Rhizobial strain	Legume common name	Legume botanical name
SPECIAL	WSM4083: *Mesorhizobium ciceri*	Tedera	*Bituminaria bituminosa* var. *albomarginata*

Photo: Peter Maloney DPIRD.

LEGUME USE AND RHIZOBIA DISTRIBUTION

Tedera is new to Australian agriculture. It is a perennial forage legume native to the Canary Islands, with the first cultivar Lanza released in 2018.

Inoculation is always recommended for two reasons. Firstly, it is very unlikely that Australian soils will contain suitable rhizobia. Secondly, inoculation is important because good plant density at sowing is critical to the long-term production of perennial legumes and cannot be recovered if compromised nodulation leads to poor establishment.

Tedera is best suited to well drained soils with $pH_{CaCl_2} \geq 4.8$. It is suited to a range of soil textures from sands to clay.

INOCULATION METHOD

Inoculation should be undertaken using a slurry of peat inoculant followed by pelleting with fine lime. Fresh inoculation immediately before sowing is recommended to maximise nodulation potential. Peat is the only inoculant formulation available.

KEY CONSIDERATIONS

Tedera must be inoculated with inoculant strain WSM4083.

NODULATION

Seedlings (3 months old) should have 5 to 10 nodules per plant.

LIKELY RESPONSE TO INOCULATION FOR TEDERA

HIGH | **Tedera is a new species to agriculture in Australia and should always be inoculated.**

FIGURE 9-15a. Nodulation of young Tedera plants.

Photo: Daniel Real, DPIRD.

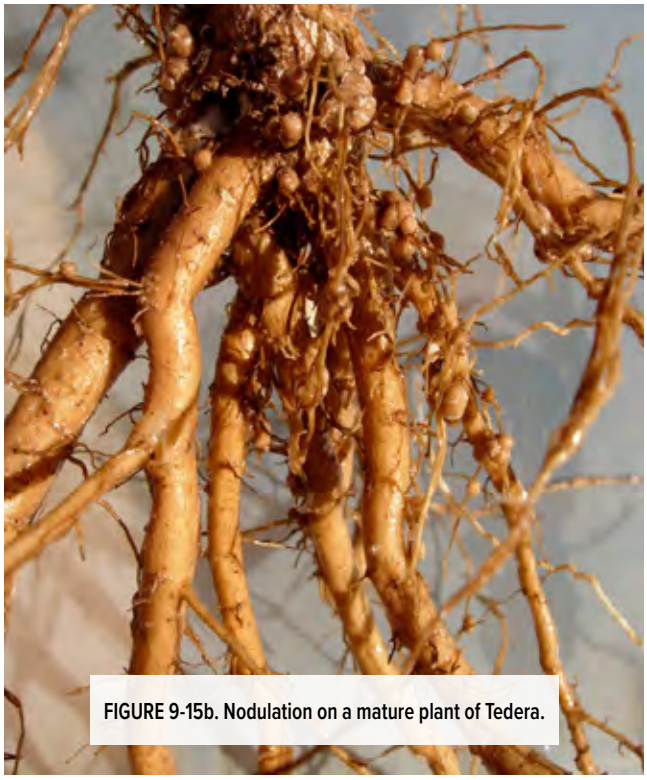


FIGURE 9-15b. Nodulation on a mature plant of Tedera.

Photo: Ronald Yates, DAFWA.

10 | Useful resources

PUBLICATION: Inoculating Legumes: The Back Pocket Guide
grdc.com.au/GRDC-BPG-Inoculating Legumes

PUBLICATION: GRDC Tips and Tactics - Legumes and nitrogen fixation
grdc.com.au/tt-legume-n-fixation

PUBLICATION: Legumes in Acidic Soils – Maximising production potential in south eastern Australia
grdc.com.au/legumes-in-acidic-soils

PUBLICATION: A Nitrogen Reference Manual for the Southern Cropping Region
grdc.com.au/a-nitrogen-reference-manual-for-the-southern-cropping-region

FACT SHEET: Doubling inoculant rates
grdc.com.au/doubling-inoculant-rates-fact-sheet

FACT SHEET: Inoculating Legumes in Acidic Soils
grdc.com.au/inoculating-legumes-in-acidic-soils

WEBSITE: Australian Inoculants Research Group
www.dpi.nsw.gov.au/agriculture/soils/australian-inoculants-researchgroup

WEBSITE: GRDC Acid Soils SA
acidsoilssa.com.au

PODCAST: The lowdown on subsurface acidity
https://grdc.com.au/news-and-media/audio/podcast/the-lowdown-on-subsurface-acidity

PODCAST: Introduction and pre-sowing inoculation
player.whooshkaa.com/episode?id=848146

PODCAST: Dry sowing
player.whooshkaa.com/episode?id=848145

PODCAST: Acid soils
player.whooshkaa.com/episode?id=848144

PODCAST: In-season assessment
player.whooshkaa.com/episode?id=848143

PODCAST: Introducing pulses to acid soils
https://grdc.com.au/news-and-media/audio/podcast/introducing-pulses-to-acid-soils

VIDEO: Optimising pulse nodulation in low pH soils
youtu.be/R8CZ5rLUgig

VIDEO: Boosting on farm nitrogen fixation in pulses – Michael Moodie
youtu.be/QqI8H3lSpg0

VIDEO: Boosting on farm nitrogen fixation in pulses | Pulse inoculation and fungicide use
youtu.be/MNGae9HhA0A

VIDEO: GCTV17 - Legume Nodulation - field sampling
youtu.be/bfnBsEM64t0

VIDEO: GCTV17 - Legume Nodulation - sample preparation
youtu.be/0VL7ClY-K9w

VIDEO: GCTV17 - Legume Nodulation - sample scoring
youtu.be/Nd303SFITDk

11 | Appendix:
Legume inoculant manufacturers in Australia

Photo: GRDC.

BASF Australia Ltd
Address: 1205 Old Pacific Hwy, Somersby, NSW, 2250
Phone: 1800 803 440, 02 4340 2246
Fax: 02 4340 2243
Email: agro-ANZ@basf.com
Web: crop-solutions.basf.com.au/

New Edge Microbials Pty Ltd
Address: 951 Garland Avenue, Albury, NSW, 2640
Phone: 02 6025 0044
Email: newedge@microbials.com.au
Web: microbials.com.au

Bayer CropScience
Address: Level 1, 8 Redfern Rd, Hawthorn East, Vic 3123
Phone: 1800 804 479
Web: crop.bayer.com.au

ALOSCA Technologies Pty. Ltd.
Address: Unit 1/ 50 Atwell Street, Landsdale, WA, 6065
Phone: 08 6305 0123
Fax: 08 6311 7390
Web: alosca.com.au

Green Microbes Australia Pty Ltd
Address: Unit 5 42 Sam Street, Forbes, NSW 2871
Phone: 02 6851 2788, 0435 207 783
Email: contact@greenmicrobes.com.au
Web: greenmicrobes.com.au

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