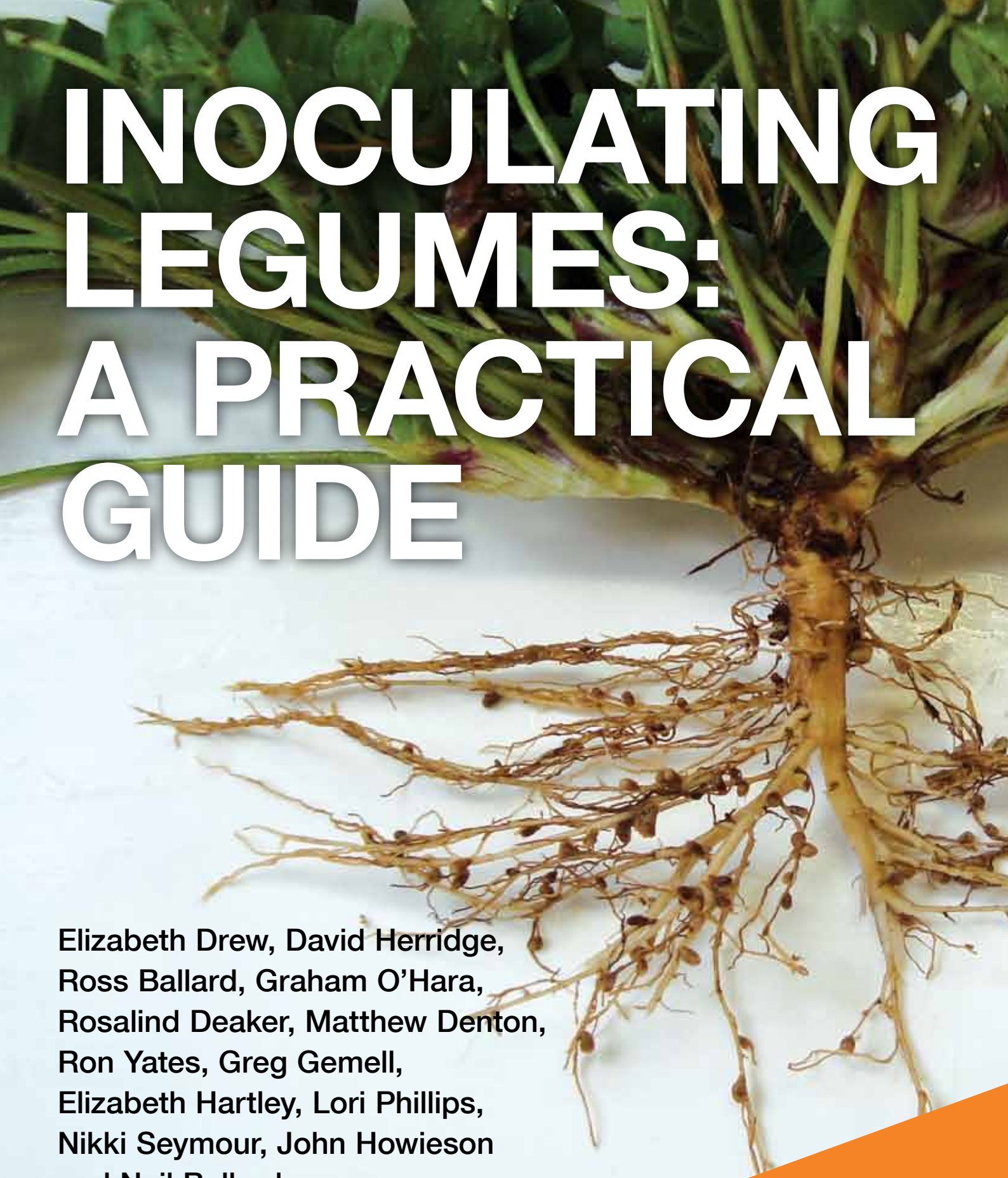
INOCULATING LEGUMES: A PRACTICAL GUIDE



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… Each year Australian growers sow inoculated legume seed on about 2.5 million hectares, equivalent to 50 per cent of the area sown to legumes. All of the nitrogen fixed annually by legumes growing on these newly sown areas together with that fixed by the 22.5 million hectares of established and regenerating legume-based pastures can be attributed to either current or past inoculation. The total amount of nitrogen fixed by the agricultural legumes is estimated at 2.7 million tonnes annually, with a nominal value for the industry of close to $4 billion annually…

CONTENTS

FOREWORD

Nitrogen (N) fixed by the soil bacteria rhizobia symbiotically with Australia's pasture and pulse legumes, has a national benefit of close to $4 billion annually. This is based on nitrogen fixation rates of about 110 kilograms of N per hectare per year, legume areas of 25 million ha and fertiliser N costed to the grower at $1.25/kg, which equates to $1.55/kg plant-available N in the soil. The price of carbon-based fossil fuels, used in the production of nitrogenous fertilisers, is expected to increase substantially in the future. As this occurs, the value of legume nitrogen fixation to Australian growers will escalate.

There is an ongoing need to ensure that Australian agriculture evolves with a reliance on legumes that are effectively nodulated and that the benefits of nitrogen fixation from legumes for farming systems are maximised.

This will not occur if legume nodulation is sub-optimal, because of one or more of the following factors:

- growers do not inoculate when they should;
- growers use inoculation practices that do not deliver sufficient rhizobia to the developing legume seedling;
- growers use inoculants of sub-optimal quality;
- legume breeding programs release cultivars that are not matched with highly effective rhizobial inoculants;
- ineffective populations of rhizobia evolve in the soil and outcompete effective inoculant rhizobia;
- inoculant rhizobia are exposed to chemical toxicities during inoculation or soon after application to the soil; and
- populations of soil rhizobia in regenerating pastures decline because the landscapes become hostile through soil salinity, acidity or for other reasons.

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

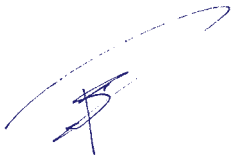
- understand the role of legumes in supplying N to agricultural production systems;
- manage legume nitrogen fixation and system N supply for maximum productivity and sustainability;
- inoculate legumes where and when appropriate;
- optimise inoculation outcomes through correct use of the inoculant product;
- 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 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).

This handbook was written by a group of Australian experts in the field of rhizobiology and nitrogen fixation from universities and state departments of agriculture and primary industries, many of whom work within the National Rhizobium Program (NRP), to address the above issues. The NRP is a GRDC R&D program, funded in three phases between 1998 and 2012, with objectives to address the science that underpins the above issues.

The major geographic focus of the handbook is the wheat-sheep belt (essentially 100% of Australia's grain production and >50% of wool production), with a minor focus on the high-rainfall belt (about 30% of Australia's wool production).

The key audiences are growers, grower groups, commercial and government advisers, agribusiness, research agronomists, legume breeders, seed pelleters, resellers and seed merchants. It is intended that material from this handbook can be extracted and used in training workshops. Workshops would need to be tailored to the particular group. For example, the material used in workshops for individual growers/grower groups may be different for seed pelleters.

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



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The preparation and publication of this handbook on the inoculation of legumes has been a long process but one that we were determined to complete. The glue that held it all together was the National Rhizobium Program (NRP), a GRDC-funded R&D program that has involved all of the authors at some point during the period 1998 to 2012. We wish to acknowledge the GRDC for their funding and, in particular, Paul Meibusch who has been a strong advocate for the NRP within the GRDC and who did all he could to ensure that this project reached fulfilment.

The inspiration was provided by Australia's legendary rhizobiologists of the 20th century whose research during the 1940s through to the 1990s laid the foundation for the inoculants industry that exists today and for many of the technologies and protocols associated with manufacture and on-farm use. In that group were Professors Jim Vincent (University of Sydney and University of NSW), Lex Parker (University of WA), John Brockwell, Fraser Bergersen, Frank Hely and Alan Gibson (CSIRO Plant Industry, Canberra), Don Norris and Dick Date (CSIRO Tropical Crops and Pastures, Brisbane), Rodney Roughley and Jack Thompson (NSW Department of Primary Industries) and David Chatel (Department of Agriculture and Food, WA).

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FREQUENTLY ASKED QUESTIONS (FAQS)

What are legume inoculants?

Legume inoculants contain live bacteria called rhizobia and should be considered as perishable products. Rhizobia are sensitive to a range of stresses (e.g. high temperature and desiccation), which decrease their viability. A more detailed description of rhizobia is provided in Chapter 2 and guidelines for handling of inoculants in Chapters 4 and 5.

What does inoculation do?

Inoculating legume seed or soil at sowing provides a large number of effective nitrogen-fixing bacteria in close proximity to the emerging legume root to optimise nodulation and nitrogen fixation.

What are inoculant groups? Why are there different groups?

Each inoculant group has a unique strain of rhizobia that is highly effective in nodulation and nitrogen fixation for a specific cluster of legumes (also known as a legume-host group). Choosing the correct inoculant group for a particular legume host (indicated by letters) is critical for good nodulation and nitrogen fixation to occur. More information and charts of inoculant groups are provided in Chapters 2, 5 and 7.

Can I test my soil for rhizobia?

No commercial test is available for determining the presence of a particular rhizobia in a soil, but paddock history can provide a guide. If the same legume was recently grown, was well-nodulated and yielded well, the soil will likely have rhizobia for that legume. Factors that affect the persistence of rhizobia in soils are examined in Chapter 3.

How do I know if I need to inoculate my crop or pasture legume?

This will depend on the legume being sown, paddock history and soil conditions. Guidelines for assessing the need for the inoculation of a major crop and pasture species are provided in Chapter 7.

Do I need to use a sticker or adhesive with the inoculant?

Stickers are used to ensure that adequate inoculant adheres to seed. Stickers are already incorporated into peat-based inoculants for crop legumes. For pasture legumes, stickers are not contained in the inoculants and should be incorporated when inoculating seed. Stickers can also improve the survival of rhizobia. Consult the inoculant package for manufacturer recommendations. Recommended stickers should always be used. The use of sugar, oils and other sticker substitutes is not recommended.

Where can I buy inoculant?

Inoculants are sold through most rural merchandising and seed companies. Commercial manufacturers are listed in the Appendix and should be able to provide information regarding availability of inoculants and the location of retail suppliers.

Does exposure to inoculants pose a risk to human health?

Rhizobia pose no known threat to human health. Peat, liquid and freeze dried formulations contain very few other organisms and so are regarded as safe to use. Although granular formulations generally contain a low proportion of dust, they do contain other soil microbes and so gloves and face masks, similar to recommendations for handling potting mixes and soils, should be used. If in doubt, consult the manufacturer recommendations on the label.

What inoculant formulation is best?

Peat inoculants are reliable and cost-effective in most situations. Other inoculants may be easier to use or better suited to specific cropping situations. The conditions that favour the use of the different formulations are summarised in Chapters 4 and 5. Look for the Green Tick Logo to be assured that the inoculant has been independently tested and satisfies Australian inoculant quality standards.

What are the benefits of inoculation?

Inoculation is essential for nodulation where the host-legume has not previously been grown. While effective rhizobia may be present in soil where a host-legume has been inoculated and grown previously, the application of a high-quality inoculant can increase the proportion of nodules formed by the selected elite inoculant strain. Nitrogen fixation benefits resulting from inoculation are described in detail in Chapter 6.

Is there any harm from over inoculation?

As long as the extra inoculant does not cause seeder blockages, there is no harm in using higher rates of inoculation. In fact, some field trials have shown benefits from increased inoculation rates, particularly in paddocks that have not grown a pulse previously.

Can inoculation rates be reduced?

This is not recommended. Insufficient numbers of rhizobia on seed or in the soil may result in inadequate nodulation. Applying the correct rate of inoculant helps ensure prompt and effective nodulation and provides good competition against other soil rhizobia that may be less effective at nitrogen fixation.

What is the benefit of lime pelleting pasture and pulse seed?

Lime pelleting helps reduce the moisture content of the seed-inoculant mix after the application of the slurry and helps prevent clumping with small seeds. It also helps to improve survival of the rhizobia particularly where the seed comes in contact with acidic fertilisers or is sown into acidic soils.

How long can I keep inoculated legume seed?

Fresh is best! Numbers of rhizobia on seed decline rapidly in the first few hours after inoculation. Rhizobial numbers on seed are highest immediately after inoculation. We recommend that farmers sow legume seed within a day of being inoculated. A significant proportion of pasture legume seed is sold preinoculated and may be have been inoculated for several months.

Should I use starter nitrogen?

In most situations, there is no reason to use starter nitrogen when sowing legumes. There may be benefits, however, for legumes growing in soils with extremely low levels of plant-available nitrogen and for the early growth of non-legume species in mixed pastures. The nodulation of legumes is suppressed by soil-mineral nitrogen.

Are there any special fertiliser needs for legumes?

Legumes need good nutrition to grow, such as the elements phosphorus and potassium. In addition to the usual nutrients needed by plants, legumes require the trace element molybdenum (Mo), which is critical for the enzyme that is responsible for nitrogen fixation. Care should be taken when selecting an appropriate Mo fertiliser as some can be toxic to rhizobia. Application of Mo fertilisers is covered in Chapter 5.

Is dry sowing of inoculated legumes OK?

Dry sowing is not ideal. Where unavoidable, the risk of nodulation failure is minimised by deep (moisture-seeking) sowing and by limiting dry sowings to paddocks where the legume has previously grown and was adequately nodulated.

How long will the inoculant survive on seed in the soil if it does not rain?

This will depend on soil conditions, planting depth, humidity, rhizobial strain (inoculant group) and inoculant formulation. Inoculants are always best delivered on seed or directly into moist soils.

Can I mix inoculated lucerne and clover together at sowing?

Yes, different pasture species can be mixed together following inoculation. The rhizobia on the inoculated seed will not usually compete with each other to form nodules. If granular inoculants are used they must be applied at the full rate for each pasture species in the mixture.

How do I assess nodulation?

Plants are best assessed for nodulation at about eight weeks after sowing. Plants should be carefully dug from the soil intact and root systems gently washed. Nodulation can be very different on different legume species but in general numerous pink nodules near the top of the root system indicates that prompt and effective nodulation has occurred. Nodule types are discussed in Chapter 2 and descriptions of nodulation for the different legume species are provided in Chapter 7.

I forgot to inoculate, what can I do?

Inoculant is best applied at sowing. It is extremely difficult to rectify a nodulation failure after sowing. The best option would be to over-sow a granular product as soon as possible in close proximity to the original sowing furrow. Responses will decline with time, as mature roots are less likely to form nodules.

Can I spray inoculant onto the top of the soil or directly onto the legume crop or pasture?

No, it is not recommended.

Are rhizobia compatible with pesticides and fertilisers?

Rhizobia are sensitive to many chemicals, fertilisers and pesticides and exposure to them should be avoided. Fertilisers are often acidic or contain elements such as zinc that are toxic to rhizobia. Where application of both pesticide and inoculant are critical to crop establishment, the use of direct soil inoculation techniques should be considered (discussed in Chapter 5). Mixing inoculated seed with fertiliser is not recommended. Even where seed is pelleted, exposure times should be minimised.

Can large packets of inoculant be resealed and used later?

Yes, if the whole packet is not used, air should be immediately expelled, the packet carefully sealed and stored in the refrigerator at 4°C. If packets are not sealed properly, the contents will dry, which may reduce rhizobial numbers and there is a risk of contamination by other microbes. Inoculants should be used as soon as possible after opening. All inoculants should be used before the expiry date.

1 INTRODUCTION

Legumes have been used as a source of food ever since humankind 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 with compatible, effective rhizobia, the group of soil organisms that infect the roots of legumes to form nitrogen-fixing root nodules.

Rhizobia live in a modified form in nodules and fix nitrogen gas (N_2) from the atmosphere. The first product of nitrogen fixation 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 arrangement 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 breaks down and disintegrates and its rhizobial content is released into the soil.

Although legumes were 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, about 40 million tonnes of nitrogen is fixed annually by 185 million hectares of crop legumes and 150 million hectares of pasture legumes. Each year in Australia, legumes are estimated to fix almost three million tonnes of nitrogen, worth $4 billion. This amount makes a substantial contribution to the estimated six million tonnes of nitrogen required annually for grain and animal production.

1.1 The practice of inoculation

Nitrogen fixation by legumes does not happen as a matter of course. Compatible, effective rhizobia must be in the soil in which the legume is growing before nodulation and nitrogen fixation can occur. When a legume is grown for the first time in a particular soil, it is highly likely that compatible, effective rhizobia will not be present. In such circumstances, the rhizobia must be supplied in highly concentrated form as inoculants.

Inoculation of legumes with rhizobia is one of the success stories of agriculture and, indeed, may be the most cost-effective of all agricultural practices. Millennia before the scientific basis of legume nitrogen fixation was understood, farmers used rudimentary means of inoculation such as the transfer of soil from paddocks growing well-nodulated legumes to others that were legume-free. As late as 1920, Australian farmers were encouraged to inoculate lucerne seed with a mixture of glue and sieved air-dried soil, the latter taken from paddocks containing well-nodulated plants of the target legume (Guthrie 1896). Inoculation of legume seeds using pure cultures of rhizobia was made possible by the groundbreaking work of German and Dutch microbiologists during the last two decades of the 19th century. Within a few years, in the marketplaces of Europe, 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.

1.2 Inoculants and inoculation of legumes in Australia

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

In 1896, the famous agricultural chemist, Frederick Guthrie, wrote about legume nitrogen 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 now, more than 100 years later, Australian farmers sow inoculated legume seed on about 2.5 million hectares, equivalent to 50 per cent of the area sown to legumes. All of the nitrogen fixed annually by legumes growing on these newly sown areas, together with that fixed by the 22.5 million hectares of established and regenerating legume-based pastures can be attributed to either current or past inoculation. The total amount of nitrogen fixed by the agricultural legumes is estimated at 2.7 million tonnes annually, with a nominal value for the industry of close to $4 billion annually (Herridge 2011).

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 putting Australia foremost in the world in inoculant development and adoption. It is timely that, in 2012, the authors, who are all involved in the discipline of rhizobiology, take time out to compile a manual that relates scientific theory to the practical aspects of the legume-rhizobia symbiosis.

1.3 This handbook

We envisage that this handbook will sit on a shelf, a desk or a counter within the reach of 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.

Names, postal and email addresses of all the contributors are provided at the front of this handbook. Users of the handbook should feel free to contact the authors directly about issues that might need clarification or elaboration. Authors will undertake to respond to all enquiries.

We hope that you enjoy the handbook and find it a valuable resource.

2 RHIZOBIA AND THE RHIZOBIA-LEGUME SYMBIOSIS

- Rhizobia are bacteria that live in the soil, on plant roots and in legume nodules.
- Rhizobia only fix nitrogen when inside a legume nodule.
- There are many species of rhizobia.
- Rhizobia species are host (legume) specific. This means different legume species require different rhizobial species to nodulate and fix nitrogen.
- Rhizobia need nutrition, water and aeration for growth.
- Rhizobia in inoculants are killed by heat (>35°C), desiccation, extremes of pH and toxic chemicals.

2.1 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. Due to their unique biological characteristic they are able to establish mutually beneficial associations with the roots of legume plants to fix atmospheric nitrogen. The availability of this fixed or reactive nitrogen can make the legume independent of soil/fertiliser nitrogen resulting in increased agricultural productivity.

This association results in the formation of specialised structures on the legume roots, known as root nodules. Within the root nodules the rhizobia absorb carbohydrate from the plant and in return fix atmospheric nitrogen 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 part of the vascular system – the same part that transports water and nutrients from the soil to the shoots. Legumes are unable to fix atmospheric nitrogen by themselves, although they can absorb mineral nitrogen from the soil. Rhizobia only fix nitrogen when inside the root nodules.

Rhizobia are microscopic single-celled organisms. They are so small, being one millionth of a metre 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.

Although all rhizobia appear very similar, they are genetically diverse and markedly different organisms. There are about 90 named species of rhizobia, and scientists are discovering and describing about 10 new species each year. Most of these new species are being discovered as scientists explore the biodiversity of our planet with the majority of new discoveries associated with native legumes not used in agriculture. Given that there are more than 18,000 species of legumes, it is not surprising that we are continually discovering new rhizobia. At present in Australian agriculture we only use as inoculants a small number of species of rhizobia that fix nitrogen with the legumes we grow. As new legume genera and species with potential for agricultural use are developed, there will be new species of rhizobia available as inoculants.

Rhizobia can have thread-like flagella that allow them to move through water films in soil and on plant roots.

Each species of rhizobia comprise 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 contain single strains of rhizobia that provide optimum nitrogen fixation with the target legume and adaptation to soils where the legume is grown.

Rhizobia can be considered to be 'probiotic' bacteria for legumes – beneficial bacteria that are not pathogenic to humans, animals or plants, and can only benefit the specific legumes they nodulate.

Rhizobia are 'probiotic' bacteria that fix nitrogen in the nodules on legumes.

2.2 Specificity of rhizobia

The relationships between particular rhizobia and particular legumes are very specific – hence different inoculants are produced for the various legumes grown in Australian agriculture.

Only specific rhizobia will nodulate and fix nitrogen 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 2.1). Different inoculation groups are nodulated by distinctly different rhizobia. For example, lupins are nodulated by the slower-growing acid-tolerant *Bradyrhizobium* spp., whereas the medics are inoculated 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 4, 5 and 7.

2.3 What do rhizobia need to prosper?

Rhizobia only exist as vegetative living cells (i.e. they cannot form survival structures like 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.

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 2.2). Rhizobia are aerobic organisms and need oxygen for respiration, just like us. Temperature also markedly affects rhizobia. Being single-celled microscopic organisms, rhizobia are always at the same temperature as their immediate surroundings. They have no insulation or ability to protect themselves from heat.

The conditions listed in Table 2.2 (substrate, air, water, pH and temperature) are what inoculant manufacturers try to optimise when they produce inoculants.

Rhizobia are killed in soil and on seed 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 2.3). These stresses must be avoided when handling inoculants to ensure a maximum number of rhizobia remain alive, and are able to colonise the soil and legume roots in sufficient number to make 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 2.4).

In soils below pH 5, aluminum 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. It is the legume that is more sensitive to salinity stress.

TABLE 2.1 Some of the legume inoculant groups used in Australian agriculture and their rhizobia (see Chapter 7 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

2.4 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 of seedling roots, the sooner root nodules develop and the rhizobia begin to fix nitrogen. A specific sequence of events and optimal conditions are required for nodulation to occur, which can be 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.

TABLE 2.2 Rhizobia are living organisms with simple needs for growth and survival.

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

TABLE 2.3 Harsh environmental conditions kill rhizobia.

High Temperatures above 35°C will kill most rhizobia	
Acidity and alkalinity	pH sensitivity of rhizobia varies (see Table 2.4)
Toxic chemicals	Fungicides, solvents, alcohols and disinfectants kill rhizobia
Inorganic chemicals	High levels of heavy metals (Zn, Cu, Co) kill rhizobia

TABLE 2.4 Sensitivity of key rhizobia to pH, where red is sensitive and green is optimal.

Rhizobia	Host legume	pH 4	pH 5	pH 6	pH 7	pH 8
Bradyrhizobium spp.	Cowpea, mungbean, 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					

An essential feature of nodule formation is the exchange of specific signal chemicals between the legume root and rhizobia. In other words, the two partners need to have a conversation with each other and 'communicate' in a language they both understand and then modify their behaviour to form a root nodule. Often, many species of rhizobia are present in the soil around legume roots but, because the rhizobia and plant are unable to communicate, there is no nodule formation.

While the rhizobia are the partner that fixes the nitrogen in this symbiosis, the legume plants generally determine the pathway of infection, and subsequently the type of root nodule that develops.

Nodule initiation can occur in three different ways:

i) via infection of the plant root hairs;
ii) via crack entry at breaks in the roots where lateral roots emerge; and
iii) between epidermal (root surface) cells.

For any specific combination of legume and rhizobia, infection will only occur by one of these processes. However, the majority of agricultural legumes grown in Australia are infected via root hairs (Table 2.5).

2.5 Root-hair infection

The rhizobia colonise the root surfaces including root hairs, and in response to chemicals released by the legume root, the rhizobia in turn manufacture specific compounds (Nod factors). These are released into the rhizosphere (area surrounding the root) and the legume responds. The rhizobia induces formation of an infection thread that grows back down the inside of the root hair, providing a channel for their entry into the root cortical cells and multiplication. The root cortical cells in the immediate region of the infection grow and divide repeatedly, ultimately forming an outgrowth (nodule) on the root. Once the rhizobia have reached these cells they are 'released' into specialised compartments where they change into bacteroids and then begin to fix nitrogen. It is important to note that infection of root hairs is most likely to occur while plants are young. Anything that affects normal root hair development may impede nodulation.

TABLE 2.5 Types of infection processes used by rhizobia to make root nodules for common legumes grown in Australian agriculture.

Legume	Infection pathway
Soybean	Root hair
Chickpea	Root hair
Pea	Root hair
Faba bean	Root hair
Clovers	Root hair
Medics	Root hair
Biserrula	Root hair
Serradella	Root hair
Lupin	Between epidermal cells
Peanut	At lateral root junctions
Stylosanthes	At lateral root junctions

For nitrogen 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), i.e. nitrogen (N_2) fixation. The enzyme requires molybdenum (Mo) to function optimally, 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 nodules, and is essential for nitrogen fixation to occur.

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

2.6 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.

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 nitrogen. Soybeans,

peanuts, serradella, lotus, navy beans, cowpeas and pigeon peas are legumes that form determinate nodules.

Indeterminate nodules can keep growing throughout the season and can remain functional to meet the nitrogen demand of the crop. These nodules can develop lobed finger-like projections to give a coralloid appearance. Internally they have distinct zones 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 nitrogen. Peas, faba beans, lentils, chickpeas, lucerne, medic, clover, biserrula and sulla are legumes that form indeterminate nodules.

2.7 Other important symbioses that fix nitrogen

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

ii) *Casuarina* are non-legume trees that can also fix nitrogen with very special and unusual soil bacteria. These bacteria are called *Frankia*. They grow as long filaments and appear more like fungi than bacteria.

2.8 Causes of poor nitrogen fixation – legume and rhizobia incompatibility

Although scientists expend a considerable amount of time and effort selecting elite strains of rhizobia, and provide these to the inoculant manufacturers for use in commercial inoculants, we cannot always control which strain of rhizobia is successful in forming the nodules on the growing legume. In many situations there are already rhizobia resident in the soil that can nodulate the legume in preference to the applied inoculant rhizobia. These resident strains may always have been present (unlikely), they may have colonised the soil after agricultural settlement (very likely), or they may have arisen from genetic changes of inoculant rhizobia after being introduced into the soil (also very likely). So, in these situations the quest to form a nodule becomes a competition between the applied inoculant rhizobia and other strains of soil rhizobia. 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 later chapters, particularly Chapters 4 and 5.

Scientists are just beginning to understand how resident strains of rhizobia evolve in the soil, and probably the best understood scenario in Australia is that of biserrula, an annual pasture legume and its inoculant rhizobia. At the time biserrula was introduced experimentally to Western Australia from the Mediterranean Basin in 1994, there were no rhizobia in Western Australian soils capable of nodulating it. All sown biserrula were inoculated with an elite strain. Within seven years we noticed that a small proportion of nodules formed on biserrula regenerating in the field 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 bacteria that were already in the soil in Western Australia, but were not biserrula rhizobia. These bacteria were able to nodulate biserrula only when they received the genes for nodulation, but they do not have all the other genes required for high levels of nitrogen fixation.

Hence, the evolution of rhizobia like these in soil can significantly impair nitrogen fixation of legumes because they can successfully out-compete the highly effective inoculant rhizobia to form nodules but, once in the nodules, cannot fix nitrogen.

The only way we have of managing this 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; such strains would be ideal for use as inoculants.

3 NUMBER AND NITROGEN FIXATION CAPACITY OF RHIZOBIA IN SOILS

- 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 a good likelihood of response to inoculation.
- Communities of rhizobia in soil tend to become more diverse with time and often less effective at fixing nitrogen, compared to commercial inoculant strains.
- Some legume species readily form less effective symbioses with soil rhizobia, while other legume species do not.
- Inoculant strains, when applied at high numbers, can compete with background soil rhizobia. This provides the opportunity to introduce effective strains.

3.1 Introduction

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

Rhizobia become established in soils in several ways. Many were introduced as high quality inoculants. Others arrived accidently 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 Chapter 1). However, because rhizobia are legume specific and their persistence is affected by soil characteristics and cultural practices, their diversity, number and nitrogen fixation capacity can vary greatly.

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

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

The legume history of the soil provides some guide. If a legume species, or others very similar to it, has not been grown in a paddock, then it is unlikely the rhizobia for that legume will be present in the soil in high numbers.

Conversely, where there has been a recent history of well-nodulated legumes in a paddock, there is a reasonable chance the rhizobia that nodulated the legume will remain in the soil.

Some extension materials suggest 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 and low clay content. Also, the communities of rhizobia that develop under legume cultivation often become less effective at fixing nitrogen over time.

3.3 How many soil rhizobia are needed for prompt nodulation?

The number of soil rhizobia needed for prompt nodulation lies somewhere between 100 and 1000 rhizobia per gram of soil.

We say this for two reasons. First, when commercial inoculants of rhizobia are applied at recommended rates, they add the equivalent of about 100 rhizobia per gram of soil to a 10 centimetre depth. This results in prompt nodulation. Second, the evidence from many field and greenhouse experiments is that there is poor nodulation once the number of rhizobia in soil is less than 100 per gram.

High numbers of rhizobia result in prompt nodulation and plants tend to have many nodules on the tap root, close to the top of the root system (Figure 3.1).

Low numbers of soil rhizobia can result in delayed nodulation and smaller numbers of nodules on the roots.

3.4 Measuring the number of rhizobia in soil

First it is necessary to point out that soils often contain several species of rhizobia. For example, it is common

to find clover, lucerne and field pea rhizobia in the same paddock, if all those legumes had been grown before.

A laboratory-based plant nodulation test is used to determine the number of rhizobia in soil. The legume of interest is inoculated with a sequence of dilutions of the collected soil (Figure 3.2). After four weeks plant growth, the number of plants with nodules in each of the different soil dilutions 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.

The test is generally used with soils collected from the top 10 centimetres of the profile, because this is where most rhizobia are concentrated and thus where most nodulation of annual legumes occurs.

Rhizobia are also found deeper in the soil profile and play an important role in nodulating annual legumes towards the end of their growth and in nodulating perennial legumes such as lucerne. These rhizobia are seldom measured.

The number of rhizobia also vary within a growing season, particularly when a legume host is grown (Figure 3.3). Numbers start to increase at the break of the season as soils become wetter and the legume host germinates. The rhizobia are stimulated to multiply in the immediate vicinity of the root (rhizosphere). They can quickly multiply to levels of 10,000 per gram of soil.

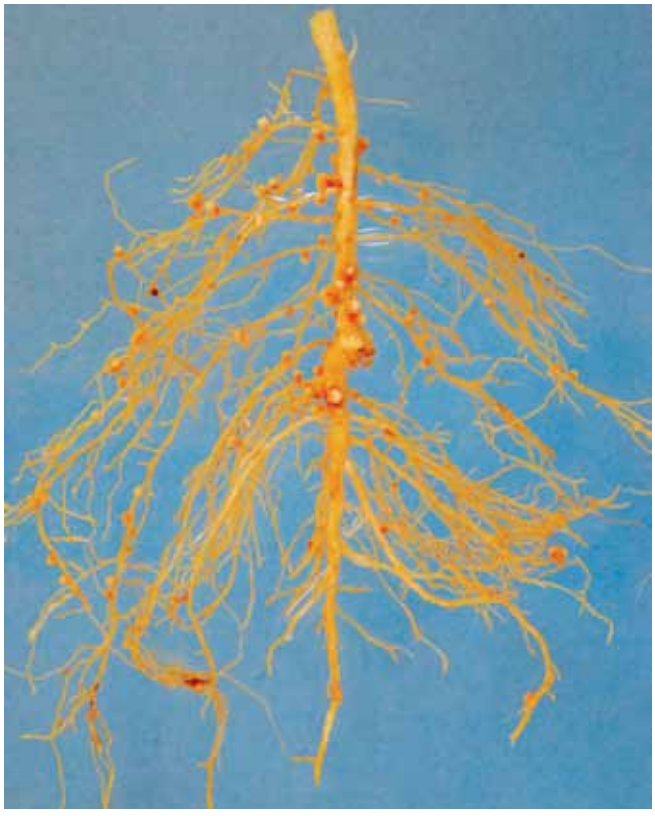
Once the rhizobia have infected the root they multiply and change into bacteroids that are able to fix nitrogen (which they cannot do in the free living form). The root cells infected with rhizobia collectively form the nodules.

When annual legumes set seed, their nodules begin to shut down as carbohydrates that provide energy to the nodules are diverted to seed development. Eventually the nodules senesce and the rhizobia are released back into the soil. Measures of rhizobial numbers at this time can exceed one million per gram of soil.

Rhizobial numbers may then decline to less than 100 per gram of soil over the next few months if soil conditions are unfavourable, or persist at a level of many thousands under more benign conditions.

Rhizobia are sensitive to desiccation and so tend to be at their lowest number at the end of hot dry summers in temperate regions. Hence, soil samples collected close to the start of the growing season provide a good conservative guide to the number of rhizobia available for legume nodulation.

FIGURE 3.1 Example of prompt and abundant nodulation on a pea root collected from a paddock containing an adequate number of rhizobia.



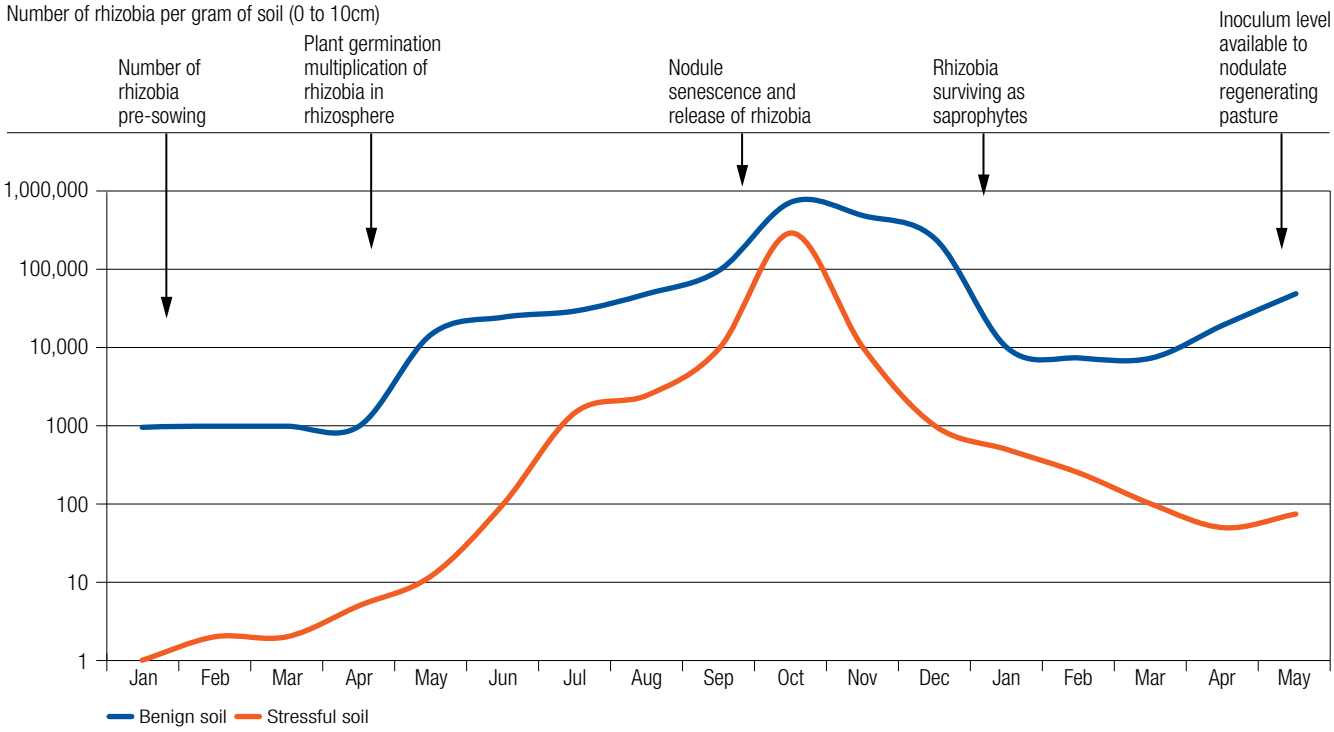
3.5 What numbers of rhizobia persist in soils?

Where soil conditions are favourable, rhizobia are able to survive in the soil for many years, even in the absence of their legume host. In this state, the rhizobia are known as saprophytes (microorganisms that live on dead or decaying organic matter). They can also live in or near the rhizospheres of non-leguminous plants and utilise their root exudates. Even so, in the absence of a legume host, numbers will progressively decrease (Figure 3.4).

Surveys of soils provide a snapshot of the number of rhizobia at a given time and reveal that many soils support large numbers of rhizobia. It is not unusual to measure more than 1000 rhizobia per gram in the top 10 centimetres of soil at the end of summer. A million rhizobia per gram have been measured in some instances. Figure 3.5 shows how the numbers of rhizobia for three pulse and two pasture legumes vary in Australian soils.

FIGURE 3.2 Method for counting rhizobia in soil. Plants are inoculated with different soil dilutions and the frequency of nodulation is measured.

FIGURE 3.3 Hypothetical scenarios of changes in the number of rhizobia through the seasonal cycle of an annual legume in southern Australia (Mediterranean climate).



Rhizobia for the pasture legumes (medic and clover) are abundant, with more than 60 per cent of soils containing 1000 or more rhizobia per gram. Large areas that grow sown, regenerating and naturalised pasture legumes (at least 25 million hectares across the country) aid the multiplication and survival of these rhizobia.

Rhizobia for the pulse legumes are less abundant. For peas, chickpeas and lupin, more than 25 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.

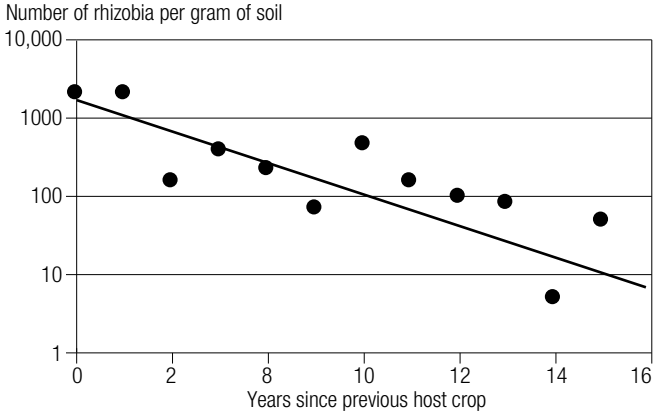
3.6 Factors affecting the survival of rhizobia in soil

Regional (local) influences can strongly affect the occurrence of rhizobia in soil. These regional effects reflect both historical differences in legumes use as well as differences in the physical and chemical characteristics of the soils.

3.6.1 Influence of host legume

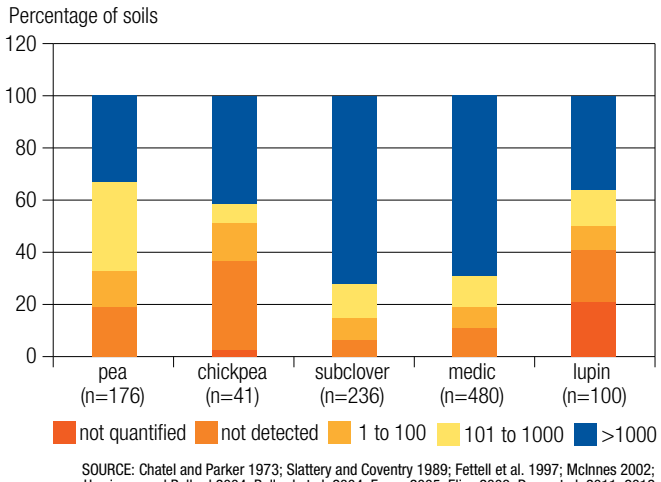
At a regional level, the more widely a legume has been grown, the more likely soils will contain the compatible rhizobia. For example, all the chickpea soils without rhizobia

FIGURE 3.4 Relationship between years of absence of the host crop and number of rhizobia in a relatively favourable soil.



SOURCE: Pea and lupin data from Evans 2005; Ballard et al. 2004; Fettell et al. 1997; Slattery and Coventry 1989; Drew et al. 2012

FIGURE 3.5 Percentage of soils classified according to number of pea, chickpea, sub-clover, medic or lupin rhizobia they contain.



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

TABLE 3.1 Optimal pH (in calcium chloride) for a range of key legumes (most acid-tolerant at top and the least acid-tolerant at the bottom) .

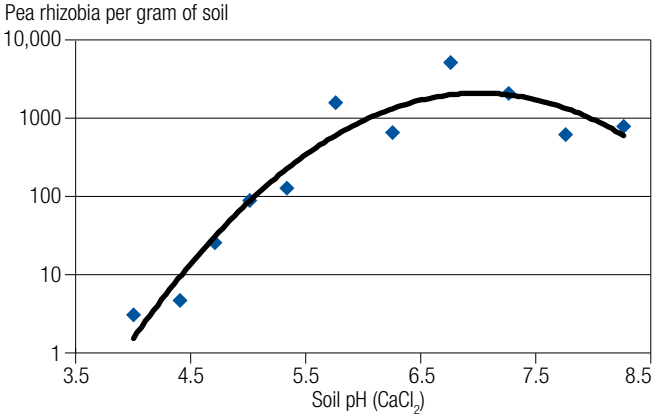
Legume species	Optimal pH range
Lupin and serradella	4.5 to 7.0
Peanut	4.5 to 7.0
Mungbean	5.0 to 7.5
Soybean	5.0 to 7.5
Subclover	5.0 to 8.0
Burr, murex, sphere medic	5.5 to 8.0
Pea/faba bean/lentil	5.5 to 8.0
Chickpea	6.0 to 8.5
Lucerne	6.0 to 8.5
Strand and barrel medic	6.5 to 8.5

shown in Figure 3.5 were from South Australia, where chickpeas are not usually grown. The remaining soils were from an area in New South Wales where they are commonly grown. Rhizobia for chickpea were abundant in most of these soils.

Pasture legume rhizobia often occur in high numbers in soils. This is likely due to the naturalisation and constant presence of subclover and medic in many soils. Even so, there are some species within the clovers and medics that do not consistently nodulate with the soil rhizobia. An example is the recently commercialised gland clover (cv. Prima). A combination of limited usage and a specific rhizobial requirement means that inoculation of this species is needed even where there are rhizobia that nodulate other annual clovers.

Such nodulation specificity is not common and cultivars within a legume species almost always behave similarly in terms of their rhizobial requirement.

FIGURE 3.6 Relationship between soil pH and the number of field pea rhizobia in soils with a history of field pea.



SOURCE: Drew et al. 2012

Soils vary widely in the number and type of rhizobia they support.

Soil properties and legume use are major factors affecting numbers of rhizobia in soil.

3.6.2 Influence of soil type

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

Soil pH is the best understood. It affects both the survival of the rhizobia and the formation of nodules. 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.

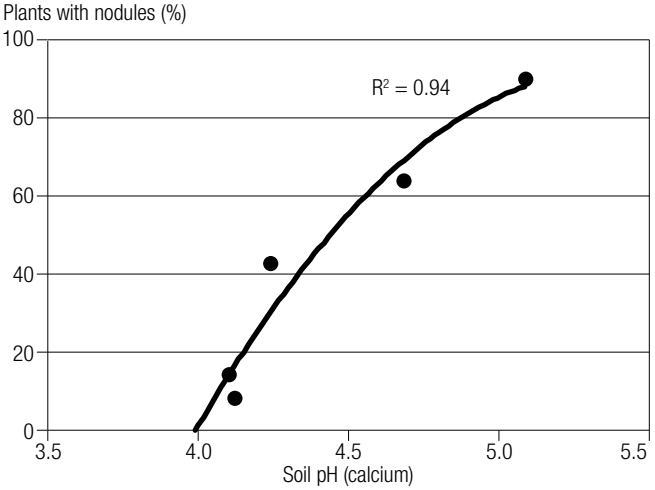
The preferred pH range of some of the more common pulse and pasture legumes is shown in Table 3.1. Narrow-leaf lupin and serradella rhizobia are highly tolerant of soil acidity. They readily form nodules at pH 4.5, but can experience nodulation problems where soil pH exceeds 7.0.

Field pea rhizobia are moderately sensitive to soil acidity. Data from several surveys of pea rhizobia across Australia have been combined in Figure 3.6 to provide a good example of the relationship between soil pH and the number of pea rhizobia in those soils. Below pH 5.5 (determined in calcium chloride), the number of rhizobia is generally less than 100 per gram of soil, the threshold below which there is a good likelihood of a response to inoculation. Hence on acidic soils, frequent inoculation is recommended for peas, faba beans and lentils.

Lucerne and its rhizobia are sensitive to soil acidity with rapid decreases in nodulation measured below pH 5.0 (Figure 3.7).

The strand and barrel medics that are assigned to the same inoculant group as lucerne (AL) are similarly sensitive

FIGURE 3.7 Correlation between soil pH (0 to 10 cm) and the percentage of one-year-old lucerne plants with nodules.



SOURCE: Unpublished data from Nigel Charman and colleagues

to soil acidity. Burr, sphere and murex medics are more tolerant of acid 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.

Soil pH affects survival of the rhizobia and the nodulation process.

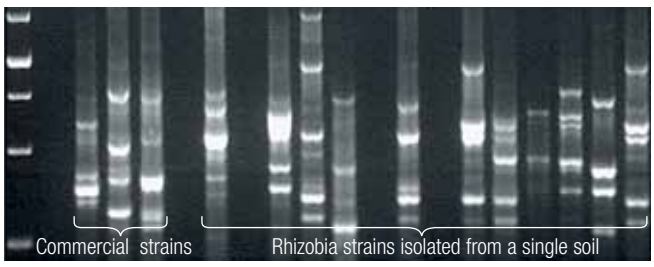
Different legume symbioses have different tolerances to extremes of soil pH.

The effects of acidity in the field are not always as obvious as shown in the lucerne example in Figure 3.7. In a subclover pasture, moderate acidity results in fewer but larger nodules. It is not until nodule mass falls below the level needed to supply the plant with adequate nitrogen that the effects of the acidity become obvious. At this point the legume content of the pasture can decline rapidly.

In some cases the acidity stresses are avoided by the rhizobia. Large numbers of rhizobia and adequate nodulation have been measured in regenerating subclover pastures, even though the pH (calcium chloride) of the bulk 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. When these soils are disturbed as a result of cropping or at pasture renovation, the number of rhizobia are reduced when they are displaced from these niches that provide protection. There is a moderate likelihood of responses to inoculation on these soils when pastures are renovated, even though nodulation constraints may not have been apparent previously.

The relationship between soil organic matter or clay content and rhizobia is less understood and has been shown to improve the survival of clover and pea rhizobia in soil. It is also 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.

FIGURE 3.8 Different strains are shown as different 'barcodes'. Many different strains can be isolated from the nodules of a single subclover plant.



3.6.3 Other factors

The extensive use of herbicides in farming systems is known to affect the legume-rhizobia symbiosis. However, their impact seems mostly detrimental to the plant, rather than to the growth, survival or effectiveness of the rhizobia. Even where rhizobia are present in high numbers, the damage to legume root systems by some herbicides (e.g. Group B herbicide residues in both acidic and alkaline soils in low-rainfall regions) can effectively halt nodulation.

Desiccation is also detrimental to the survival of rhizobia. Rhizobial numbers can decline by the end of a dry summer. Soils that experience long dry summers and are subject to higher temperatures may have fewer rhizobia, particularly where clay content is low or other soil stresses are present.

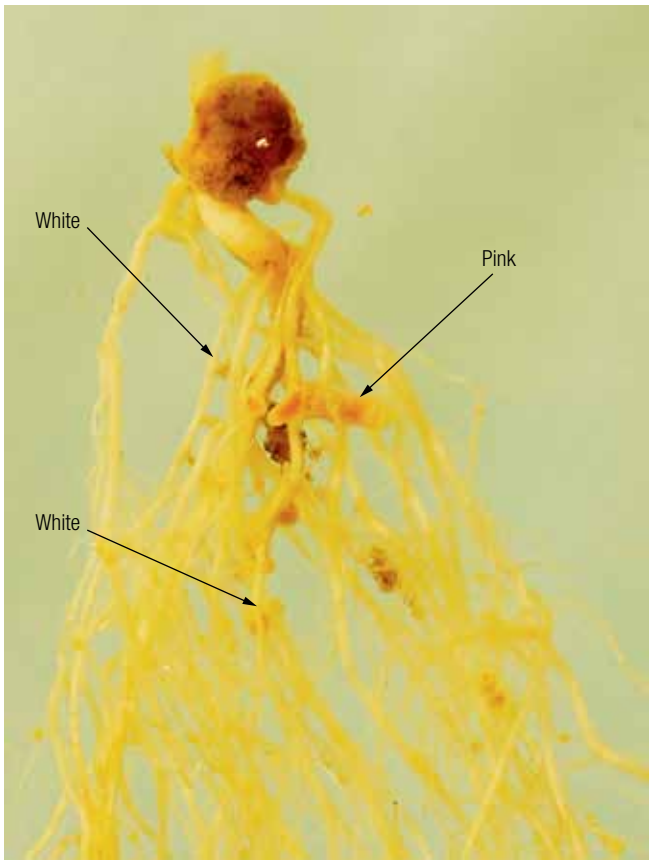
3.7 Diversity of soil rhizobia

There is nearly always more than one strain of a rhizobial species in a soil. Molecular methods make it possible to 'barcode' the strains that form nodules (Figure 3.8) and has shown that different nodules on a plant are often formed by different strains.

In some cases, more than 10 different strains of rhizobia can form nodules on a single legume plant growing in the field. Sometimes it is obvious that different strains of rhizobia occupy different nodules because the nodules differ in their appearance (Figure 3.9).

The spectrum of strains is also likely to differ from soil to soil. A common observation of strains in different soils and

FIGURE 3.9 Example of different nodule types on pea inoculated with field soil.



also within soils is that few are identified as the strains that have been used in commercial inoculants. In some instances this may simply be the result of inoculants not being used or not properly applied.

However, even where inoculants have been correctly used, the diversity of rhizobial communities in the soil tends to increase soon after legume introduction. This is often, but not always, associated with an increase in the number of less effective strains within the community.

The recent introduction of the pasture legume biserrula and its rhizobia into Australian farming systems has provided a unique opportunity to study the evolution of rhizobial communities. Studies have shown that the development of strain diversity can be rapid (years not decades) and is associated with the transfer of symbiotic genes to other members of the soil microbial community.

The presence of ineffective rhizobia is not always detrimental because the legume plant has some influence over nodulation. In some situations the plant is able to foster occupancy of its nodules by the more effective strains from within the rhizobial community. In other situations the plant can increase nodule number in order to satisfy nitrogen demand. Ineffective rhizobia are therefore most likely to become problematic where the rhizobial community is dominated by ineffective strains and where opportunities for continued nodulation are limited, as may be the case in stressed soils.

It is likely that about 50 per cent of legumes sown each year will be reliant on soil rhizobia for nodulation, because they are either not inoculated or because the inoculant rhizobia is present in low numbers on the seed (as in many preinoculated seeds, see Chapters 4 and 5). Most regenerating pastures are nodulated by existing soil rhizobia.

Even where inoculation is practiced and inoculants applied well, the soil rhizobia will compete and can form a significant proportion of nodules. It is therefore important to consider their nitrogen fixation capacity.

Communities of soil rhizobia are complex, comprising many strains.

It is common to find 10 different strains forming the nodules on a single plant.

Soil rhizobia are rarely identified as the same strains used in inoculants.

3.8 How well do the soil rhizobia fix nitrogen with legumes?

So far we have considered the number and diversity of rhizobia. Their function or capacity to fix nitrogen is just as important. Nitrogen fixation capacity is the result of the legume-rhizobia partnership, not just the rhizobia. Therefore it is possible that the same community of rhizobia may fix less or more nitrogen with different legume genotypes.

The terms effective and ineffective are commonly used to describe differences in nitrogen fixation capacity. Here, the term effective is used where the shoot weight of plants

FIGURE 3.10 Plants growing in N deficient potting media are inoculated with a suspension of soil to determine effectiveness of the rhizobia in that soil. Plant growth provides a measure of the nitrogen fixation capacity of the soil rhizobia.

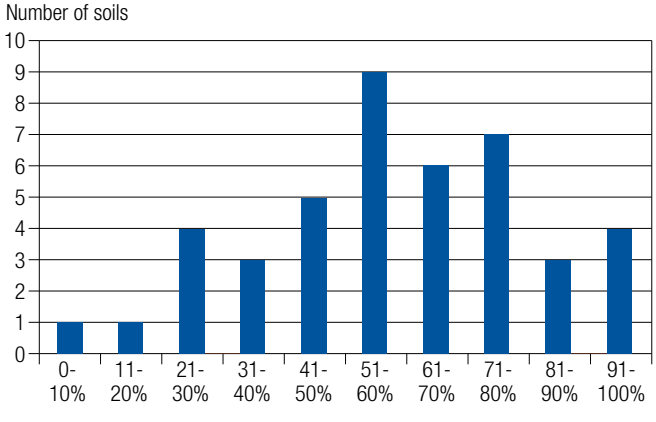
resulting from an inoculation treatment (rhizobia) is at least 75 per cent that of plants inoculated with a highly effective strain of rhizobia. Symbiotic capacity is deemed moderately effective when shoot weight is between 50 and 75 per cent and ineffective when below 50 per cent.

The effectiveness of soil rhizobia is commonly measured using a 'whole soil' inoculation method (Figure 3.10) or by inoculating plants with individual strains of rhizobia isolated from nodules.

Data for symbiotic effectiveness of soil rhizobia is 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, they are often less effective compared to the inoculant strain for the legume.

For example, the effectiveness of the symbioses formed

FIGURE 3.11 Distribution of soils according to the effectiveness of their subclover rhizobia relative to an effective inoculant strain.



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

TABLE 3.2 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
Chickpea	60	25	40	35
Yellow serradella[A]	>75	-	-	-
Subclover	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

A 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.

between subclover and the rhizobia in 43 soils ranged from eight per cent to 99 per cent of that formed between subclover and the commercial inoculant strain (WSM1325). Most commonly, the communities of soil rhizobia were 51 to 60 per cent as effective as the inoculant strain (Figure 3.11). Thirty-two per cent were classed as ineffective.

Mean nitrogen fixation capacity of soil rhizobia with a range of different temperate legumes is shown in Table 3.2. The higher prevalence of ineffective symbioses for burr medic compared to strand medic and lucerne (all *Medicago*) highlights the differences in symbiotic competence between legume species.

Among the annual clovers, symbioses tend to be similar or less effective (e.g. arrowleaf clover) compared to subclover.

For field peas the majority of rhizobial communities are classified as effective. Faba beans, lentils, vetch and lathyrus, all nodulated by the same rhizobia, are likely to be similar to field peas, since 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 is therefore difficult to predict. Generally, stressful environments exerting greater selection pressure may increase the diversity of the rhizobia at the expense of nitrogen fixation capacity.

Many soils contain rhizobia that are less effective than inoculant strains.

Some legume species are more readily compatible with a range of soil rhizobia than other legumes.

3.9 Dealing with 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. But where the soil rhizobia are not effective, high nodule occupancy by an effective inoculant strain is desirable to optimise nitrogen 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 diffusely distributed, while those applied to seed as inoculum are in close proximity to the root and able to rapidly multiply to the levels needed to achieve effective nodulation.

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 (Figure 3.12).

For example in Figure 3.12, a growth response to inoculation is only apparent in a soil containing 1000 rhizobia per gram when the number of rhizobia applied as inoculant exceeds 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.

FIGURE 3.12 Ineffective soil rhizobia (across the bottom are the log number rhizobia per gram soil) are overcome when equivalent numbers of inoculant rhizobia are applied to the seed (shown as log number per seed).

Photo: JA Ireland 1968

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

Earlier in this chapter we state that it is common where a legume species has been grown that the number of soil rhizobia can exceed 1000 rhizobia per gram. 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 thousand million (1×10^9) cells per gram peat at manufacture, seed standards are easily surpassed when recommended rates of inoculation and methods of application are followed, and the seed is promptly sown.

For the pulse legumes, where seed size is larger, the number of rhizobia applied per seed is also larger (refer to application rates in Chapter 5). For field peas 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 and nodule occupancy by inoculant strains declines with time.

While the benefits of effective strains introduced through inoculation will be important to pasture establishment, occupancy by the applied inoculant will be temporary and possibly insignificant where the pasture phase extends past a few years.

Research to manage suboptimal populations of rhizobia in soils continues. New inoculant formulations that provide competitive and stable strains of rhizobia, higher numbers of rhizobia or allow more strategic placement of the inoculant strain are being tested.

For annual pasture species that have a propensity to form ineffective symbioses with soil rhizobia, the development of varieties that can be effectively nodulated by a large proportion of soil rhizobia is being investigated to provide a long-term solution.

3.10 Concluding comments

After more than 100 years of legume cultivation, many Australian soils have developed substantial 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 often affects persistence of the rhizobia. Medic, lucerne and pea (including faba bean, lentil and vetch) symbioses are particularly sensitive to acid soils.

Where soils do support rhizobia, the communities are diverse and tend to become less effective at fixing nitrogen with time, when compared to commercial inoculant strains. The extent of ineffective symbioses formed can be modified by the host legume. Even so, symbioses between soil rhizobia and the host legume are commonly less than 50 per cent of the potential of symbiosis between the inoculant strain and host legume. It is not possible to predict the nitrogen fixing capacity of the rhizobia at a paddock level.

The good news is that inoculant strains, when applied at a high number, can compete with background soil rhizobia. This provides the opportunity to introduce effective strains in pulse crops and frequently renovated pasture systems.

Nodule occupancy by inoculant rhizobia declines with time in regenerating pastures. In these pastures there appear to be good prospects to develop ‘symbiotically promiscuous’ legumes that are better matched to the diverse communities of rhizobia that are now found in many soils.

4 RHIZOBIAL INOCULANTS – STRAINS AND QUALITY CONTROL

- Strains of rhizobia used in commercial inoculants must satisfy a number of criteria, including effectiveness at fixing nitrogen.
- Rhizobial inoculants are formulated and available in peat, clay or peat granules, liquids and as a freeze-dried powder.
- Inoculants are applied to the seed at sowing or directly to the soil in the vicinity of the seed at sowing.
- Rhizobial inoculants in Australia are subjected to independent quality testing by the Australian Inoculants Research Group (AIRG).
- 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 a number of other factors.
- Testing of inoculants and preinoculated pasture legume seed at the point-of-sale indicate high quality of inoculants but problems with often very low numbers of rhizobia on preinoculated seed.

4.1 What are legume (rhizobial) inoculants?

Inoculants for legumes are products containing commercially prepared cultures of rhizobia protected in carriers that supply large numbers of viable rhizobia for the effective nodulation of legumes. The purpose of legume inoculation is to supply selected rhizobial strains in large numbers to the roots of the legumes soon after germination, optimising the chances of effective nodulation, symbiotic nitrogen fixation and plant and grain yield, while decreasing input costs.

Inoculants in Australia contain rhizobial strains that have been selected according to the following criteria established during many years of scientific research.

4.1.1 Effectiveness of rhizobia and their legume host range

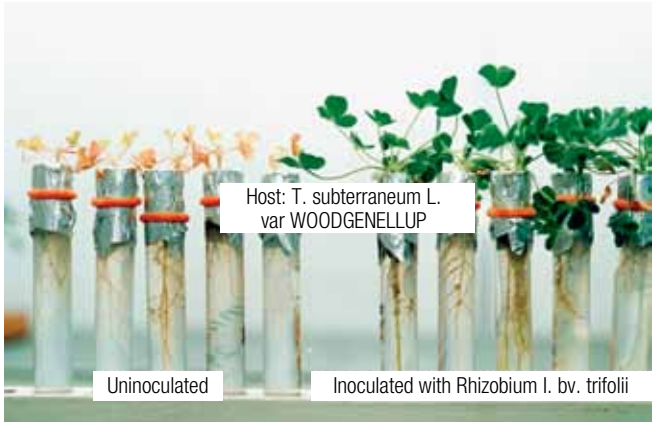
There are thousands of strains of rhizobia that can nodulate and fix nitrogen with a particular legume host. However, the amount of nitrogen fixed can vary substantially, depending on the combination of plant host and rhizobia strain. Strains that are used in commercial inoculants are the most effective at fixing nitrogen with the range of legume species/cultivars in each of the inoculant groups. Strain testing with the target legumes is conducted first in glasshouse experiments and then in field trials across the range of soil types and environments where the legumes are grown commercially.

The result of using a highly effective rhizobial strains to fix nitrogen in subterranean clover plants grown in N-deficient medium is shown in Figure 4.1.

4.1.2 Genetic stability

The strain must maintain its symbiotic capacity (nodulation and nitrogen fixation performance) and other key traits during culture, manufacture and application. Strains are tested for genetic stability throughout the selection process and annually, once they are used as commercial inoculants.

FIGURE 4.1 Growth of subterranean clover in N-free medium inoculated with a highly effective rhizobial strain.



Strains of rhizobia are selected to ensure maximum nitrogen fixation. Important criteria are:

- Effectiveness
- Host range
- Field performance
- Soil persistence
- Genetic stability
- Manufacturability
- Inoculant survival

4.1.3 Potential for scale-up production as commercial inoculants

Rhizobial strains must be able to grow and survive in large numbers in commercial inoculant formulations (manufacturability). Inoculant companies test potential commercial strains for manufacturability in their production system and suitability for growth and survival in inoculant carriers prior to commercialisation.

4.1.4 Ability to survive during inoculant application

Strains vary in their ability to survive on seed. Seed inoculation is a convenient (and the most widely used) way to introduce rhizobia into the soil at sowing. Survival on seed needs to be high and is determined by the selection process. This is particularly important for pasture rhizobia destined for application to preinoculated seed.

4.1.5 Persistence in soil in absence of host – known as 'saprophytic competence'

This trait is more important for annual pastures than for pulse legumes or perennial pastures. Growers have an opportunity to re-inoculate pulse legumes when sowing annual crops. However, sowing and inoculation tends to be less frequent for annual legume pastures as plants are typically regenerated from soil seed banks. While persistence of perennial pasture roots allows continual colonisation and survival of inoculant strains, annual pasture legume-hosts are absent during the summer months and rhizobia must therefore persist in soil between growing seasons.

4.2 Inoculant formulations

There are several different commercial inoculant formulations available to growers to allow flexibility of application (Figure 4.2).

Formulations include peat, granular, liquid and freeze dried inoculants:

(i) **Peat inoculants** are the oldest and most common form of inoculant used in Australia. They are prepared by introducing selected rhizobial strains into gamma-irradiated (sterilised) finely milled peat. The final preparation has a relatively high moisture potential when compared with other solid formulations, which, if maintained, allows survival of rhizobia for up to 18 months.

(ii) **Granular pellets or chips** are made from either peat or clay.

(iii) **Freeze-dried powder**, where a rhizobial broth culture is concentrated as a powder in a glass vial after all the water has been removed. The powder is reconstituted later on-farm.

(iv) **Liquid inoculants** are suspensions of rhizobia in a protective liquid formulation.

4.3 Application of inoculants

Application of inoculants is covered extensively in Chapter 5. Peat, freeze-dried and liquid inoculants can be applied either to seed or directly to soil. Peat inoculants should either contain, or be mixed with, a sticker or an adhesive if they are to be applied to seed before sowing. The use of a sticker ensures that the rhizobia adhere to the seed and are evenly distributed into the paddock when the seed is sown. If peat, freeze-dried or liquid inoculants are applied directly to soil, they need to be suspended in clean potable water so they can be evenly distributed over the cropping area.

Seed inoculation can be done by growers or by commercial seed coaters. Seed coaters may inoculate freshly purchased seed with peat on request from growers for sowing within a few days (custom inoculation) or prior to sale of the seed (preinoculation). Many of the small seeded pasture species (lucerne and clover) in Australia are preinoculated (Figure 4.3) providing a convenient ready-to-sow product. Preinoculated seed is generally coated with a thick pellet containing several other plant growth enhancers. Descriptions of seed preinoculation processes and microbiological quality can be found in Gemell et al. (2005), Deaker et al. (2012) and Hartley et al. (2012).

4.4 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, and this 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 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 Ourimbah. The 'National Code of Practice and Quality Trademark for Legume Microbial Inoculant Products used in Australian Crops and Pastures' can be accessed at the AIRG website (www.dpi.nsw.gov.au/research/centres/gosford/australian-inoculants-research-group).

4.5 How do we know if an inoculant is high-quality?

Since July 2010, rhizobial inoculants in Australia that have been

tested to meet strict quality standards display a registered trademark called the Green Tick Logo (Figure 4.4). The logo indicates that at the time of testing the product contained:

- the correct rhizobial strain for the target legume host;
- numbers of live rhizobia equal to or above a minimum standard; and
- zero or minimal numbers of other organisms (contaminants).

The Green Tick Logo indicates that an inoculant has been independently tested and satisfies Australian quality standards.

FIGURE 4.3 **Preinoculated lucerne seed.**

FIGURE 4.2 **Commercial inoculant formulations available for inoculating crop and pasture legumes: A – moist peat; B – peat granules (left), bentonite clay (middle), attapulgite clay (right); C – liquid inoculants; D – freeze-dried inoculants.**

A

B

C

D

The logo also indicates that labelling standards have been achieved. The 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; and
- batch number.

Inoculants will only carry the logo if a representative sample of packets from the batch has been tested.

At the date of publication of this handbook, companies which are signatories to the 'National Code of Practice: Quality Trademark for Microbial Inoculant Products used in Australian Crops and Pastures', and producing and selling inoculants that carry the Green Tick Logo are:

- Becker Underwood Pty Ltd;
- New Edge Microbials Pty Ltd; and
- Novozymes Biologicals Australia Pty Ltd (see Appendix for contact details).

4.6 Who tests inoculant quality?

Inoculant manufacturers are responsible for ensuring their product is of high quality for consumers, and they conduct a number of tests in their own laboratories. The AIRG is responsible for independent quality assessment of legume inoculants in Australia. The group is funded through service agreements with the three inoculant manufacturers that are signatories to the Code of Practice and research projects with the Grains Research and Development Corporation (GRDC), the Rural Industries Research and Development Corporation (RIRDC) and the NSW DPI. The AIRG also has collaborative support from the research community through the University of Sydney and the National *Rhizobium* Program.

The AIRG is responsible for:

- maintaining, authenticating and issuing approved rhizobial strains for commercial release to the manufacturers who comply with the national Code of Practice incorporating the Green Tick logo;
- assessing the quality of inoculants at point of manufacture for compliance to the Code of Practice and at various points through the supply chain; and
- administering and promoting the Green Tick Logo trademark.

4.7 Numerical standards

In Australia, legume inoculants displaying the Green Tick Logo must contain no less than a minimum number of rhizobia that has been 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 at an individual site will be affected to some extent by the climate 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.

FIGURE 4.4 Registered trademark for inoculants quality – the Green Tick Logo.

TABLE 4.1 Australian minimum standards for legume inoculants.

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

CFU: culture forming units; MPN: most probable number.

Standards for inoculants applied to seed have been set to achieve particular numbers depending on seed size. For large seeded legumes (e.g. soybeans), the number is 100,000 rhizobia/seed; for medium seeds (e.g. lentils), 10,000 rhizobia/seed; for small seeds (e.g. subterranean clover and lucerne), 1,000 rhizobia/seed and very small seeds (e.g. white clover), 500 rhizobia/seed.

Numerical standards for CB376 for *Lotononis bainesii* are 2×10^8 rhizobia/g moist peat (2×10^7 rhizobia/g at expiry). Standard for liquids based on a three litre bottle used to treat one tonne of seed. Standard for freeze-dried based on vial used to treat one tonne of tonne 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 4ºC. Group G is applicable to strain WU425 only.

Research with peat inoculants has been more extensive than with other formulations and so there is more confidence in quality standards for peat. Standards for all inoculant formulations are under continual review and are adjusted as new data becomes available.

In addition, peat, liquid and freeze-dried inoculants should not contain a high number of other contaminating organisms. If contaminant organisms are present within the inoculant, they should be at least 10 to 100 times lower in number than the rhizobial strain.

Non-rhizobial contaminants and moisture content of peat inoculants are effective indicators of potential shelf life and are checked routinely. If a batch of inoculant is within one month of expiry, it may be given an extended expiry of six months, provided it passes all standards when retested by the AIRG.

Standards for preinoculated seed are the same as the standards for seed listed in the footnote in Table 4.1.

4.8 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 and how it is applied.

Rhizobia are living organisms susceptible to high temperatures. It is important that inoculants are always stored according to the manufacturer's recommendations because hot temperatures (>35°C) during transportation and storage kill the rhizobia, thereby reducing their numbers in the inoculant.

Rhizobia may be exposed to detrimental conditions during inoculant delivery to the crop. Desiccation on seed, and contact with incompatible chemicals (e.g. pesticides applied to seed, nutrient residues in spray tanks and acidic superphosphate fertiliser) are major factors that can affect survival of rhizobia during application (see Chapter 5).

The careful application of high-quality inoculants to legume crops increases the chances that nodulation, nitrogen fixation and yield will be optimised.

4.9 What is the quality of inoculants and preinoculated seeds in Australia?

Shelf life of inoculants is determined by measuring the survival of rhizobia in inoculant formulations over time in the distribution chain.

Between 2005 and 2010, the AIRG conducted 23 point-of-sale surveys of inoculant and preinoculated seed quality. The surveys covered 266 towns across the Australian grainbelt.

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-based – 92 per cent;
- freeze-dried – 3 per cent; and
- granular – 5 per cent.

Each inoculant was assessed for quality and either passed or failed the standards. Pass rates between 2005 and 2010 ranged from 87 per cent to 94 per cent. There were 126 inoculant samples (eight per cent) that had numbers of rhizobia below the AIRG standard. Inoculants also failed if contamination with non-rhizobial organisms was too high.

Data obtained from monitoring survival of rhizobia on preinoculated seed has been alarming. The convenience of using pasture seed that has been preinoculated with rhizobia led to an increase in demand from growers, and the number of companies producing preinoculated seed has risen in recent years.

4.10 Quality of preinoculated seed (rhizobial numbers)

Point-of sale surveys preinoculated seed were conducted across 37 towns in the wheat/sheep belt, mainly in the eastern states. A total of 272 samples of seed of temperate and tropical legumes were obtained and tested. The majority of samples were temperate legume pasture species. Despite many attempts by various seed coaters to improve the quality of preinoculated legume seed, numbers of rhizobia on seed collected from retail outlets has not improved since the quality was assessed in an earlier survey between 1999 and 2003 (Gemell et al. 2005). Generally survival of rhizobia on lucerne seed is better than survival on clovers.

The percentage of samples of each legume species passing minimum standards between 1999 and 2003 were as follows:

- lucerne – 73 per cent;
- subterranean clover – 32 per cent;
- white clover – 3 per cent;
- red clover – 4 per cent; and
- other species – 0 per cent.

Results from 2005:

- samples passed – 5 per cent;
- rhizobia detected – 60 per cent: and
- nil rhizobia – 40 per cent.

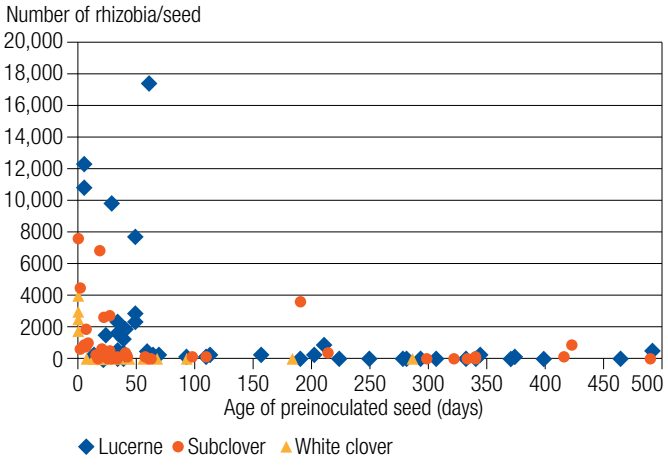
The number of rhizobia on preinoculated pasture seed products is highly variable and viability declines rapidly over time (Figure 4.5). Some of the samples meet the rhizobial numerical standards when less than 50-days-old (i.e. 50 days after inoculation) but virtually none of the older samples (i.e. >50 days) met the standards.

4.11 Non-rhizobial inoculants

Inoculants that contain potentially beneficial microorganisms other than rhizobia are also available in the market. These organisms do not produce root nodules on legumes but are marketed as enhancing plant growth in other ways.

There is scientific evidence that certain microorganisms can enhance plant growth through a range of mechanisms. Some organisms can increase root growth through the production of hormones or enzymes, theoretically improving nutrient uptake efficiency. Hormone-producing microorganisms have the potential to increase legume nodulation by rhizobia through increased root hair density

FIGURE 4.5 Survival of rhizobia on seed of different preinoculated pasture species over time. Data from the AIRG surveys of preinoculated seed, sourced at point-of-sale (resellers) during 2005-10.



where nodulation is initiated. Another potentially beneficial microbially-mediated effect is the increased availability of nutrients by solubilisation of phosphorus and sulfur and chelation of iron.

Other microorganisms have been identified for their ability to protect plants against pests and diseases. This is either by direct antagonism of the pest or disease agent or by increasing plant resistance to attack.

While the evidence for beneficial effects on plants can be demonstrated in laboratory studies, results from field application are highly variable. Little is known about environmental conditions, specificity between selected microorganisms and plant host, or numerical requirements to achieve a beneficial effect. As a result, no standards have been set for these microbial inoculants and they are not subject to quality control. However, a system is being developed to extend the trademark system to allow product differentiation on the basis of confirming manufacturers' claims of microbial identity and quantity.

As the market for microbial inoculants is not regulated in Australia, products are not restricted from sale and consumers should be aware that quality and efficacy may be variable. In the meantime, research is continuing to find more about these inoculants and how their potential may be realised.

4.12 Concluding comments

The whole question of legume inoculants and their use starts with quality. If the quality is poor, then benefits from inoculation are highly unlikely. Successful production and use of legume inoculants is often associated with an effective, regulatory quality control (QC) program that primarily focuses on the quality of the rhizobial strains in the inoculants and their numbers as well as the numbers of contaminating microorganisms. The regulatory QC 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). In other countries, such as the US, regulatory control and independent testing has been considered unnecessary, with manufacturers conducting their own internal QC.

In Australia, we are fortunate that in the early 1950s Professor Jim Vincent had the presence of mind to recognise the harmful implications of poor-quality inoculants at the farm level and to set up an independent laboratory, jointly financed by the University of Sydney and NSW Department of Agriculture, to conduct quality assessment. Additionally, the laboratory acted as a resource to assist the industry to continually improve inoculants. Now, 60 years later, the system with its clearly-stated framework has survived essentially unchanged and has become the model that other countries follow.

We readily admit that the problems remain that have plagued the industry through those 60 years, such as genetic instability of inoculant strains, peat toxicities, poor survival of some strains in peat and, particularly, on preinoculated seed. Vigilance in detecting those problems through the ongoing testing program and diligence in addressing them has meant that Australian growers are now generally supplied with high-quality product. The new Code of Practice incorporating the Green Tick Logo program should provide further support for the quest for quality inoculants.

5 INOCULATION IN PRACTICE

- Inoculation is relatively inexpensive and good insurance – always inoculate with AIRG-approved* inoculants.
- Match the correct inoculant group to each legume.
- Inoculants carry live root nodule bacteria (rhizobia), which die from exposure to sunlight, high temperatures, chemicals and freezing temperatures.
- Always use inoculants before their use-by-date has expired.
- Keep inoculants dry and cool, and reseal opened bags of inoculant. Use the resealed bags within a short time.
- Follow instructions on recommended rates of inoculation. Rates are either determined by the weight of seed (kilogram per tonne of seed) or by area (kilogram per hectare).
- Always sow freshly inoculated seed as soon as possible.
- When applying liquid or slurry inoculants, use clean, potable water and ensure the holding tank is free of toxic chemical residues.
- Do not add zinc or sodium molybdate to liquid or slurry inoculants.
- Check the product label or contact the manufacturer for compatibility of inoculants with fertilisers and seed dressings.
- Ensure inoculants remain cool in transport and do not leave inoculants or inoculated seed in the sun.

*AIRG is the Australian Inoculants Research Group, part of the NSW Department of Primary Industries.

5.1 Introduction

Inoculation is the application of root nodule bacteria (rhizobia) to a legume seed or soil in which the legume is sown. It is done to facilitate root nodulation. Improving the nodulation of a legume can increase symbiotic nitrogen fixation, crop biomass and grain yield and quality, and increase the amount of organic nitrogen contributed to the soil from legume shoot and root residues (Figures 5.1 and 5.2).

Some precautions need to be taken to ensure delivery of large numbers of rhizobia to the vicinity of the legume roots. Whichever inoculant is used, rhizobia are living organisms and their growth and survival can be reduced by coming into contact with chemicals and fertilisers, heat or freezing temperatures, sunlight, desiccation, and acidic (low pH) and highly alkaline (high pH) soil (see Chapter).

FIGURE 5.1 Aerial biomass index image of chickpea plots 12 weeks after sowing, indicating plots inoculated with Rhizobium '+', and those that are uninoculated '-'. Blue is indicative of higher biomass, yellow of low biomass and red of bare earth.

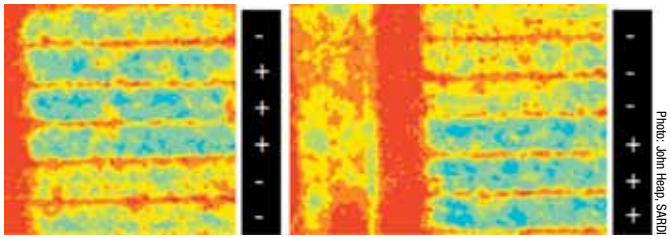


Photo: John Heap, SARDI

5.2 When is inoculation required?

When sowing legumes inoculation should always be considered due to the potential to increase nitrogen fixation and grain yield. The circumstances under which inoculation of specific legumes is required are covered in Chapter 7.

Important reasons to undertake inoculation include:

- the particular legume has not been grown in the paddock previously;
- it has been more than four years since that particular legume has been grown in the paddock;
- introduced newly selected strains with increased effectiveness and survival;
- the presence of acidic or highly alkaline soils in the paddock may limit survival of the rhizobia in the soil;
- the paddock is subjected to particularly hot, dry summers; and
- the legume has specific rhizobial requirements, e.g. lotus, biserrula, sulla.

FIGURE 5.2 The clearly beneficial effects of inoculation on the growth of serradella. Plants inoculated with effective strains of rhizobia are green and well-grown. Plants inoculated with an ineffective strain are pale and unthrifty while the uninoculated plants have, to a large extent, died.

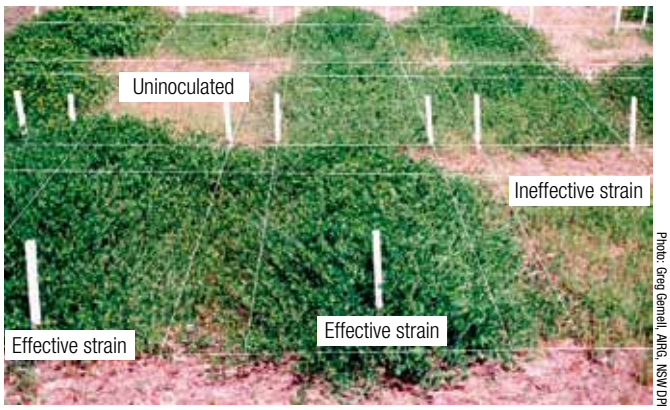


Photo: Greg Gemell, AIRG, NSW DPI

5.3 Which inoculant group should I use?

Crop and pasture legumes must be inoculated with the correct rhizobial strain for nodulation and nitrogen fixation. For example, chickpeas and field peas each require different inoculant rhizobia and will not nodulate unless the correct inoculant is used (see Table 5.1 and Chapter 7).

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

When using mixtures of different pasture legume species, each should be inoculated separately with the correct inoculant group. Once seed of each legume has been inoculated and dried off, the pasture species can be mixed together in the appropriate proportions for sowing.

5.5 What are the requirements for storing and handling inoculants?

For storage and transport of inoculants:

- always follow the manufacturer's instructions;
- keep inoculants in a cool, dry area (ideally below 10°C), except for a few inoculants for tropical/subtropical legumes, which should be stored at 20 to 25°C;
- do not freeze inoculants;
- minimise exposure to direct sunlight;
- store freeze-dried inoculants in the fridge, NOT in the freezer;
- use inoculants before their use-by-date.
- never expose inoculants to high temperatures, e.g. in a vehicle. Use an insulated box to keep them cool; and
- reseal inoculant packages after opening to reduce moisture loss and avoid contamination.

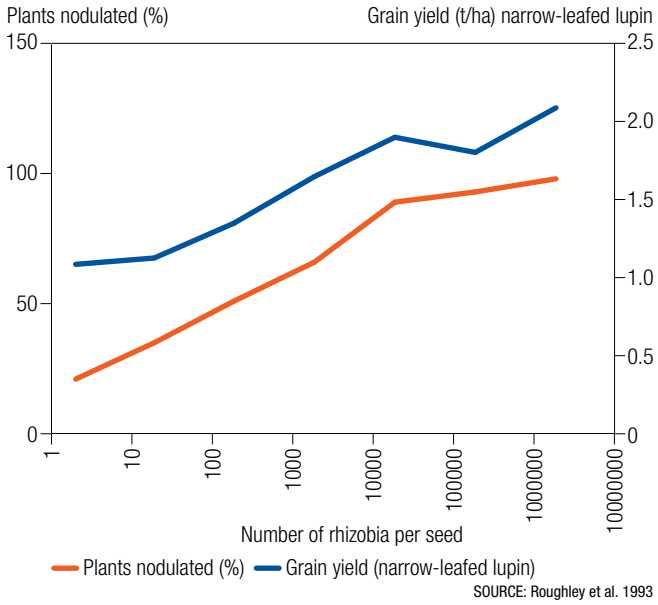
5.6 Can you use too much inoculant?

Inoculation of legumes at higher-than-recommended rates is not harmful to legume growth or production. Ensure blockages of equipment do not occur. Fewer problems result from liberal inoculation than from using inoculants at lower-than-recommended rates or not using inoculants

TABLE 5.1 Inoculant groups for some common legume species and the maximum amount of seed that should be treated by a 250 gram bag of inoculant.

Inoculant group	Common name of legume	Seed size	Maximum weight of seed treated by 250g inoculant
AL	Lucerne strand medic, melilotus, disc medic	Small	25kg
AM	Burr medic, barrel medic, snail medic, sphere medic, murex medic	Medium	50kg
B	White clover, red clover, strawberry clover, alsike clover, berseem clover, ball clover, suckling clover	Small	25kg
C	Subterranean clover, balansa clover, crimson clover, purple clover, arrowleaf clover, rose clover, gland clover, helmet clover, persian clover	Small–medium	25–50kg
E	Field pea, vetch, narbon bean, lathyrus	Large	100kg
F	Faba bean, lentil	Medium–large	50–100kg
G	Lupin	Large	100kg
H	Soybean	Large	100kg
I	Cowpea, mungbean (green and black)	Large	100kg
J	Pigeon pea, lablab, horse gram	Large	100kg
N	Chickpea	Large	100kg
P	Peanut	Large	100kg
S	French and yellow serradella	Medium	50kg
Biserrula	Biserrula	Small	10kg
Sulla	Sulla	Medium	10kg

FIGURE 5.3 Rhizobial numbers on seed at sowing and their effect.



SOURCE: Roughley et al. 1993

at all. Unnecessary inoculation represents a small cost to production, whereas poorly nodulated and N-deficient crops will cause a substantial reduction of production and profit.

5.7 How are numbers of inoculant rhizobia related to legume nodulation and yield?

Large numbers of rhizobia inoculated onto seed increase nodulation and grain yields (Figure 5.3). For pulses and grain legumes, inoculants usually contain enough rhizobia to deliver around 10^{10}–10^{11} (ten to one hundred billion) rhizobia per hectare (see Chapter 4). The recommendation for rhizobial numbers on seed at sowing when inoculated by peat slurry inoculants are 100,000 rhizobia per large seed (chickpeas, lupins) and 10,000 for smaller seeds (mungbeans, lentils). For preinoculated pasture legume seeds, the recommendations are 1000 rhizobia per medium-sized seed, such subterranean and lucerne, and 500 rhizobia per small seed, such as white clover.

5.8 Which formulation of legume inoculant should I use?

A range of different inoculant formulations are available to Australian legume growers (Table 5.2).

In selecting an inoculant formulation, consider the following characteristics:

- All inoculants are expected to work well when sown into moist soils, where rhizobial survival should be optimal.
- The cost of inoculants is influenced by such factors as the cost of production, the cost of freight and rate of application. Peat inoculants are considered both the highest quality and the least expensive option.
- Soil-applied inoculants (i.e. granular and liquids applied in-furrow) allow the separation of the inoculant from potentially harmful seed applications such as fungicides, insecticides and trace elements.
- Granular and in-furrow application of liquid inoculants have increased in popularity due to their ease-of-use. Granules are particularly attractive for large sowings of pasture legumes (i.e. more than one tonne of seed). Although the application of peat slurry to seed during busy seeding times is often viewed as inconvenient, it remains the most popular form of inoculation.
- Granular inoculants contain fewer rhizobia per gram than peat and need to be applied at higher rates and cost more per hectare.
- Liquid inoculants should be used immediately after dilution. Freeze-dried inoculant should be sown within five hours after application to seed. Peat slurry inoculant should be sown within 24 hours of application to seed. Granular inoculants can be stored for up to six months after manufacture.
- Current recommendations are that to ensure rhizobial survival, inoculated legume seed should not be sown into dry soil. In particular, freeze-dried and liquid inoculants should only be applied to moist seedbeds. Note that some manufacturers do recommend application into dry soil.
- Preinoculated pasture seed is seen as very convenient but varies in quality, with the number of rhizobia on seed at the point of purchase sometimes inadequate (see Chapter 4). Preinoculated seed coatings can add significant cost to pasture seed.

5.9 Peat inoculants

Peat inoculants are cost-effective and reliable, and the most commonly used formulation. These inoculants consist of finely ground peat with a single strain of rhizobia. The rhizobia are grown by the inoculant manufacturers to high concentrations in a nutrient broth in large fermenters, and then injected into packets containing sterilised peat. The rhizobia multiply further in numbers in the peat. Packets from selected batches are independently tested by the Australian Inoculants Research Group (AIRG), and only batches that reach the stringent standards carry the Green Tick Logo (see Chapter 4). Each packet has a use-by-date, which should be adhered to.

TABLE 5.2 Inoculant formulations available to Australian growers.

Inoculant formulation	Composition
Peat	High organic matter soil, milled and irradiated, with rhizobia added in a nutrient suspension
Freeze dried	Concentrated pure cells of rhizobia following extraction of water under vacuum
Granular	Clay or peat granules impregnated with rhizobia
Liquid	Suspension of rhizobia in a protective nutrient solution
Preinoculated seed	Seed coated with polymers and peat inoculant

PEAT INOCULANTS

- Peat-based inoculants are usually applied as a slurry to the seed coat so that rhizobia are in direct contact with the seed. They can also be applied as a liquid directly to the soil, usually with water rates of 50 to 100 litres per hectare.
- Seed inoculated with peat slurry is best sown on the day of inoculation to maximise the number of live rhizobia delivered with the seed to the soil.
- Peat inoculants are highly effective when sowing seed into moist soil.
- Aerial or dry sowing peat-inoculated seed should be avoided where possible, as rapid death of rhizobia may result in sub-optimal nodulation.
- Packet size of inoculant varies depending on the supplier, with smaller inoculants bags (250 grams) usually provided for pastures and larger bags (up to 2.5 kilograms) often provided for grain legumes. It is important to inoculate correctly to ensure that sufficient rhizobia are present on seed to provide effective nodulation.
- Use clean, potable water where possible in the process of inoculation.
- Always use clean equipment for mixing (e.g. do not mix in herbicide drums).
- Ensure adhesive solutions are cool before adding the inoculant.

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. Simply sprinkling the peat into the seed box is not recommended as this results in poor contact between the rhizobia and the seed and may lead to patchy and inconsistent nodulation.

5.9.1 Preparation, water quality and application of peat slurries to seed

The inoculant is mixed with clean water and sometimes an adhesive to form a slurry. Adhesive solutions are used to improve the contact of inoculant with seed and to protect the rhizobia from desiccation. Most peat inoculants for grain legumes already include an adhesive in the peat and only water is required to create the slurry. In contrast, peat inoculants for pasture legumes usually do not contain adhesive and the peat slurry is made using an adhesive solution prepared separately.

The use of rainwater or preferably drinking (potable) water is recommended for the preparation of all slurries.

FIGURE 5.4 Peat inoculant is easily seen on faba beans (top left) and peas (lower left) when compared with uninoculated seeds (right).

It is important that the pH of the water is checked and is between 5.5 and 7.0 or rapid death of the rhizobia will probably result. It is critical to avoid 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.

5.9.2 Preparation of adhesive solution for pasture legumes

Adhesive solutions or 'stickers' such as Seedstik™ are often used where the seed is to be lime pelleted. To prepare one litre
of Seedstik™ adhesive solution:

- for a solution of 20 per cent, sprinkle 200 grams of the granulated powder into 200 millilitres of hot (~80°C) water, stirring vigorously until the powder is dispersed;
- slowly add 800mL of cold water while still stirring vigorously, until an even gel is produced;
- sticker is best prepared the day before inoculation. Sticker should be used within three days; and
- periodically stir the solution until fully dissolved. Cool the solution to less than 30°C before use. Thoroughly stir the solution prior to use. Combine peat inoculant and sticker together for immediate application to seed.

Less concentrated adhesive solutions (refer to the manufacturer's instructions) may be used when seed is not lime pelleted. 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 that adhesives be used that are recommended for use with legume inoculants.

5.9.3 Application of the slurry to seed

The slurry is mixed with the seeds using a concrete mixer, shovelling on a cement floor, or by using a rotary coater, on-the-go applicator or auger to provide even coverage of the seed (Figure 5.4). 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. Slurry must be applied in a calibrated flow to ensure consistent distribution across the seed lot.

Inoculated seed should be sown as soon as possible, ideally on the same day as inoculation. For grain legume inoculants already containing adhesive, a 2.5kg packet when mixed with water will provide sufficient rhizobia for 1000kg of a larger seeded grain legume e.g. lupin or 500kg of a medium size grain legume e.g. lentils (see manufacturer's instructions on packet label for exact amounts of seed and water).

5.9.4 Field inoculation

Peat is made up into a slurry as per manufacturer directions in a clean drum and mixed well (Figure 5.5A). The slurry is ideally pumped rather than poured from the container (Figure 5.5B) into the path of seed going up the slow moving, flighted auger (Figure 5.5C). Inoculated seed is augered into the grain/grouper bins and transported to the planter/airseeder in the paddock (Figure 5.5D). Freeze-dried inoculum can be applied to seed in the same way as peat slurry and as per the manufacturer's instruction. Inoculant rates on seed are given on inoculant packets and should be applied to the correct weight of seed. Volumes needed may vary according to pumping rates and auger speeds. If seed is transferred with a tabulator or conveyer auger, a mixing ladder will be needed to enhance inoculant distribution on the seed.

5.9.5 Lime pelleting of pasture legumes

Pasture seed is often coated with fine lime immediately after the application of the peat slurry to help dry the seed and to prevent clumping (Figures 5.6 and 5.7). Liming also protects rhizobia against acid soils and acidic fertilisers, such as superphosphate.

Lime pelleting may improve survival of the rhizobia when delays between inoculation and sowing are unavoidable. It also reduces the clumping of seed from the slurry mix and forms a seed pellet favourable for easy flow in the sowing process. However, lime pelleting is not required when sowing podded seed such as serradella or soft-seeded sulla as the seed pod absorbs the slurry and does not affect flowability.

Grain legumes are not lime pelleted. Similarly, tropical pastures legumes (except *Leucaena leucocephala*) should not be lime pelleted because it has been reported to kill the applied rhizobia. Most temperate pasture legume seeds, i.e. those grown in the southern and western grain regions,

FIGURE 5.5 Peat inoculant made into a slurry in a drum (A); slurry pumped out of the container (B); slurry pumped from the container into the path of the seed going up the auger (C); and inoculated seed is augered into the bins and transported to the planter/airseeder (D).


A

B


C


D

FIGURE 5.6 **Peat slurry inoculant being added to biserrula (left) and then coated with lime (right) while being mixed.**

should be lime pelleted using fine lime (calcium carbonate) following inoculation with the peat slurry and adhesive. Slaked, hydrated lime and builder's lime are too alkaline and will kill the rhizobia and should not be used. Keep in mind that the pellet can increase the weight of the seed substantially, so that sowing rates may need to be adjusted.

To lime pellet pasture seed:

- pour the mixture of peat slurry and sticker over the seed and mix in a rotating drum (concrete mixer) until seeds are evenly coated;
- immediately add the appropriate amount of very fine lime (such as Seed Cote™, Microfine® or Omyacarb®) in one step to the rotating seed, and roll for one to three minutes; and
- allow pelleted seed to dry in a cool place out of direct sunlight.

PLEASE NOTE: Preparation of a small trial batch is always recommended, particularly if the process is being undertaken for the first time.

Good quality pelleted seed is:

- evenly coated with the lime (see Figure 5.8); 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;
- pasty with the seed surface showing, the result of too much adhesive. This may be rectified by adding more lime;
- clumped together, the result of too much adhesive or inadequate mixing prior to adding lime; or
- hard, glossy or smooth resulting from too little lime, or too much mixing after adding the lime.

5.9.6 Using peat inoculants for liquid injection

Inoculum, suspended in potable water, is injected into the seed furrow in a band. Peat is mixed into dilute slurry or placed into a porous bag (calico bag or fine muslin, cheesecloth or nylon stocking) before adding to the tractor-mounted water tank. Peat inoculants are finely milled products and readily disperse in water. Despite this, the use of a fine filter, such as a stocking, is encouraged to ensure that any extraneous material does not block the liquid injection system. The liquid inoculum is made by mixing the required amount of peat inoculant, for a specific amount of seed, into water. For example, if one large (1.2 kg) packet of peat inoculates 500kg of seed then at a seeding rate of 100kg/ha the liquid (300–500L) should be injected over 5ha.

FIGURE 5.7 **Subterranean clover uninoculated (left) and inoculated and lime pelleted (right).**

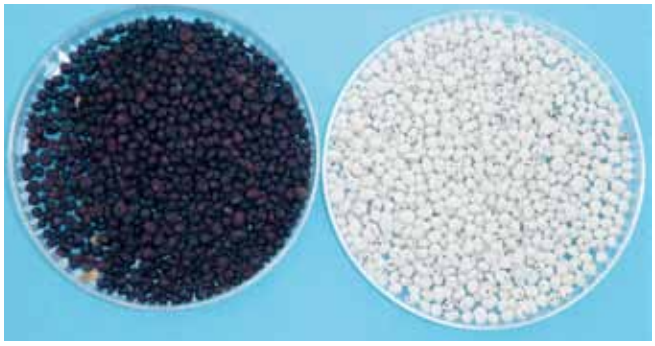


FIGURE 5.8 **Three different batches of lime pelleted clover seed inoculated with a group C slurry mix. The seeds on the left display insufficient lime or uneven mixing, the seeds on the right (clumpy) show too much sticker. The seeds in the centre indicate an even amount of mixing and adequate lime addition.**

For more details on applying liquid inoculants see Sections 5.11 and 5.12.

5.10 Freeze-dried inoculants

Inoculants containing freeze-dried rhizobia are available as powders in 30g glass vials (Figure 5.9). They become active when the powder is reconstituted with liquid. The product comes with a protective polymer in a separate bottle, which assists survival of the rhizobia. A vial will treat between 25 and 500kg of seed, depending on the legume species. These products allow for liquid injection of inoculants into the seeding furrow or seed can be coated immediately prior to sowing. Treated seeds need to be sown into moist soil within five hours of application. Contact with pesticides and fungicides must be avoided. Do not freeze this product.

5.10.1 How do I apply freeze-dried inoculant?

Remove cap and rubber bung from the glass vial, add potable water, replace bung and shake until all powder is dissolved. For liquid injection into the seeding furrow, add the vial of inoculant solution to 2L of cool water containing the protective polymer, supplied by manufacturer of the freeze-dried product. Add this solution to the spray tank and deliver 50 to 100L of clean water per hectare into the furrow during sowing. It is important to ensure that the protective agent is added to the tank mix, prior to the addition of the freeze-dried rhizobia.

To coat seed, add dissolved solution from the vial into 2.5L of water (containing protective polymer). Apply to the seed until evenly coated and allow to dry before sowing.

5.11 Liquid inoculants

Liquid inoculants should only be used where the seedbed is moist. Liquid injection of inoculant into furrows is increasing in practice, due to the relative ease of applying liquid inoculants to broad acre crops. It is very important that the tanks on spray rigs and seeders be thoroughly clean of residues, which can be toxic to rhizobia. The concentrated inoculant should be diluted with good-quality, clean water of neutral pH before application. Diluted inoculant should be delivered to the sowing furrows at rates of 50 to 100L/ha. Inject liquid inoculant immediately or within six hours.

FIGURE 5.9 Concentrated rhizobia in a freeze-dried formulation which can be applied to legume seed or to injected into the soil at sowing.

DO NOT MIX PEAT, FREEZE DRIED AND LIQUID INOCULANTS WITH:

- chemicals containing high levels of zinc, copper or mercury;
- fertilisers and seed dressings containing sodium molybdate, zinc, manganese and molybdenum;
- fungicides such as Sumisclex® or Rovral®
- herbicides such as MCPA, 2,4-D and Dinoseb; ot
- insecticides containing endosulfan, dimethoate, omethoate, or carbofuran.

5.12 Applying inoculants by water injection

Water injection methods can use peat, freeze-dried or liquid forms of inoculum. The inoculants are diluted with water in tanks mounted on tractors (Figure 5.10) and applied through spray lines attached behind each planting tyne/boot (Figure 5.11). Agitators and in-line filters may be necessary, particularly for peat-based inoculum. Rates of inoculum need to be calculated for planting rates (ilograms of seed per hectare) and water volumes able to be carried. Typically application rates are 50 to 100L/ha.

5.13 Granular inoculants

Granular inoculants can simplify the delivery of rhizobia to the legume. For most granular inoculants, a third seeding box is required as mixing with seed or fertiliser is not recommended. The technology is an alternative to the standard peat slurry on seed and can provide greater flexibility and practical solutions in sowing operations. The physical separation of rhizobia from the seed also allows insecticides and fungicides to be applied to the seed, which may otherwise kill the rhizobia.

5.13.1 Types of granules

Granular inoculants can be manufactured from prilled peat, clay (bentonite or attapulgite) or a mixture of peat and clay and vary in appearance and characteristics such as particle size and uniformity of particle size (Figure 5.12). Granules should be stored in a dry, cool area away from direct sunlight. Clay-based granules can be stored for up to six months after manufacture without refrigeration.

Peat-based granules should be sown with the seed into moist soil. Clay-based granules have been promoted as being more reliable when dry sown. However, it is important to note that dry sowing may reduce nodulation and that the outcome may vary with soil moisture, soil temperature and the time between inoculation and crop emergence.

FIGURE 5.10 **Different configurations of water tanks mounted to tractors in order to apply inoculants by water injection in sowing furrows.**

GRANULAR INOCULANTS

- Granules should be drilled into the furrow with the seed to ensure rhizobia are placed in close proximity to the emerging legume root.
- Preferably granules should be applied from a third box separated from seed and fertiliser.
- Granules can be added to the seed box; however, differences in particle size may lead to settling and uneven delivery of inoculant and seed.

A common feature of granular inoculants is that they have fewer rhizobia per gram than the peats used for slurry inoculation. They must be applied at higher rates to achieve similar levels of nodulation. Granules are typically applied at 5 to 10kg/ha when sowing on 18cm row spacings, depending on manufacturer, the strain and number of rhizobia per gram of product. Lower rates of attapulgite and peat granules can be used with wide row spacings according to manufacturers' guidelines e.g. if row spacings are doubled, the application of inoculant can be halved (Table 5.3). However, bentonite clay granules are recommended to be sown at a rate of 8 to 10kg/ha no matter what row spacing is used at sowing. When sowing mixtures of pasture legumes, the full rate of granular inoculant per hectare for each pasture inoculant group must be used.

Granular products differ in their ability to be mixed with seed or fertiliser, and manufacturers' recommendations should always be followed. In general, excessive auguring should be avoided to ensure that the particle size is maintained and to minimise dust. Granules are best distributed through a third sowing box, rather than mixed with seed because differences in granule and seed size may result in separation or settling and uneven distribution of both granules and seed.

Contact of granular inoculants with moisture during seeding operations should be avoided and they should not be stored in the seeder boxes overnight because some products can absorb moisture, stick together and cause blockages in seeding equipment.

FIGURE 5.11 **Spray lines attached behind each planting tyne/boot dispense inoculants by water injection.**

5.14 Preinoculated and custom-inoculated seed

Some seed companies sell pasture seeds that contain rhizobia as part of a specialised seed coating process. The coating may include insecticides, fungicides and micro-nutrients. This has provided more flexibility with problems such as sowing delays. It is advisable to sow as soon as possible after the seed coating treatment.

The main use of preinoculated seed is for pasture species, particularly lucerne and annual medics, because the rhizobia for these species survive well in this form.

FIGURE 5.12 Examples of attapulgite clay granules (left), peat granules (middle) and bentonite clay granules (right) used to deliver rhizobia to grain and pasture legumes.

PREINOCULATED SEED

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.

Testing of preinoculated seed samples collected from retail outlets has indicated that many samples did not meet the AIRG standard for numbers of rhizobia on the seed (see Chapter 4).

5.15 Are there compatibility issues between seed-applied inoculants and fertilisers, chemicals and pesticides?

As rhizobia are living organisms, it is very important that inoculants are kept away from toxic substances that will reduce their viability, such as fertilisers, fungicides, insecticides and herbicides. Inoculated seed should not come in direct contact with fertiliser because it will kill the rhizobia through desiccation and exposure to acidity. Certain pesticides can also have an impact on rhizobial survival and nodulation.

There are three major factors to be considered:

- **Are the chemicals acidic in solution?** Most rhizobia are sensitive to solutions with pH values below 5.0 or above 7.5.

TABLE 5.3 The influence of row spacing on application rates for the three different types of granular inoculants.

Row spacing (cm)	Attapulgite clay granule rate (kg/ha)	Peat granule rate (kg/ha)	Bentonite clay granule rate (kg/ha)
18	6.0	5.6	8–10
20	5.3	4.9	8–10
23	4.6	4.4	8–10
25	4.2	3.9	8–10
28	3.8	3.6	8–10
31	3.5	3.3	8–10
33	3.2	3.0	8–10
36	3.0	2.8	8–10
38	2.8	2.6	8–10

- **Do the preparations contain toxic chemicals?** Metals such as mercury, copper and zinc are harmful. Effects of other active ingredients may be difficult to predict.
- **Is there prolonged direct contact between the substance and inoculated seed?** Direct contact between the inoculated seed and other substances should be avoided at all times. If contact is made, and for only a short period the effect may be reduced.

5.15.1 Fertiliser compatibility

Superphosphate and related products are acidic and toxic to rhizobia when in direct contact, and contact between seed and fertiliser should be avoided even if the seed has been lime pelleted.

Inoculated seed should not be sown or be in contact with any fertiliser except lime, dolomite or gypsum. If contact cannot be avoided, lime pellet the seed first and do not store it mixed in with the fertiliser — sow immediately.

5.15.2 Adding molybdenum at inoculation

Low molybdenum (Mo) in the soil can cause a reduction in the nodulation and nitrogen fixation of a legume crop, particularly in soils with a low pH (<6.0). Adding Mo to seed is more cost-effective and ensures even distribution of Mo in the paddock. However, sodium molybdate is toxic to rhizobia and should not be applied to inoculated seed. Use either molybdenum trioxide (66 per cent Mo) or ammonium molybdate (54 per cent Mo) for seed application.

When sowing pasture legumes in molybdenum-deficient soils, 50g of Mo is required per hectare, equivalent to that supplied in 250kg/ha 0.02% Mo superphosphate. Then, every four to five years, 25g of Mo per hectare should be applied as a maintenance dressing (e.g. 125kg/ha of 0.02% Mo superphosphate). In some areas, responses to larger quantities of molybdenum have occurred. Check local recommendations.

5.15.3 Fungicide compatibility

Seed-applied fungicides are marketed specifically for the purpose of killing or inhibiting the growth of disease-causing fungi and are considered preventative. Seed-applied fungicides (sometimes called pickles) can reduce the survival of rhizobia on seed. Table 5.4 indicates the compatibility of rhizobia with various seed-applied fungicides. Note that rhizobial survival is dependent on the period of time that the inoculant is in contact with the seed-applied fungicides prior to sowing. Recent tests have shown that Metalaxyl and Metalaxyl-M, when applied to inoculated seed, contribute to a reduction in the number of rhizobia, and therefore should be used strictly in accordance with the manufacturer's instructions.

5.15.4 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 within one day of treatment for subterranean clover and murex medic, or within six

days of treatment for other species such as white clover, serradella, lucerne and barrel medic.
- Dimethoate can harm rhizobia.
- Follow label instructions carefully.

5.15.5 Herbicide compatibility

Rhizobia are relatively tolerant of herbicide concentrations recommended for field use. Because of the differences in susceptibility between the host and their rhizobia, it is difficult to make accurate assessments of the general impact of herbicides and additives on all legumes and rhizobia, and the specific impacts on plant growth, nodulation and nitrogen fixation. However, recommendations have been made that rhizobia are killed by the herbicides MCPA, Dinoseb and 2,4-D. Recently it has also become evident that application of residual sulfonylurea-based chemicals are affecting the production of pasture legumes. Research is ongoing to clarify our understanding of these interactions.

5.16 Dry sowing of inoculated legume seed

Dry sowing of inoculated seed is not recommended where the legume is being sown in the paddock for the first time or where soil conditions are hostile to survival of the rhizobia. In paddocks with frequent use of the same legume and where effective nodulation was recently observed, the risk of nodulation failure resulting from dry sowing is greatly reduced.

5.17 Formulations of inoculants containing co-inoculants

With co-inoculants, an additional microorganism is applied with the rhizobia. A range of co-inoculants have recently been introduced to the market. Some have had the extra microorganism added during manufacture of the peat or granular inoculant, but sometimes the extra microorganism is supplied separately. These organisms include strains of *Bacillus subtilis* and *Penicillium bilaii*, added in addition to a rhizobial inoculant. The mode of action varies according to the particular microbe co-inoculated with the rhizobia; these co-inoculants are marketed as increasing root growth, nodulation, phosphorus uptake, or reduce the incidence of pathogens affecting root growth. Advice from the individual manufacturer should be sought.

5.18 Concluding comments

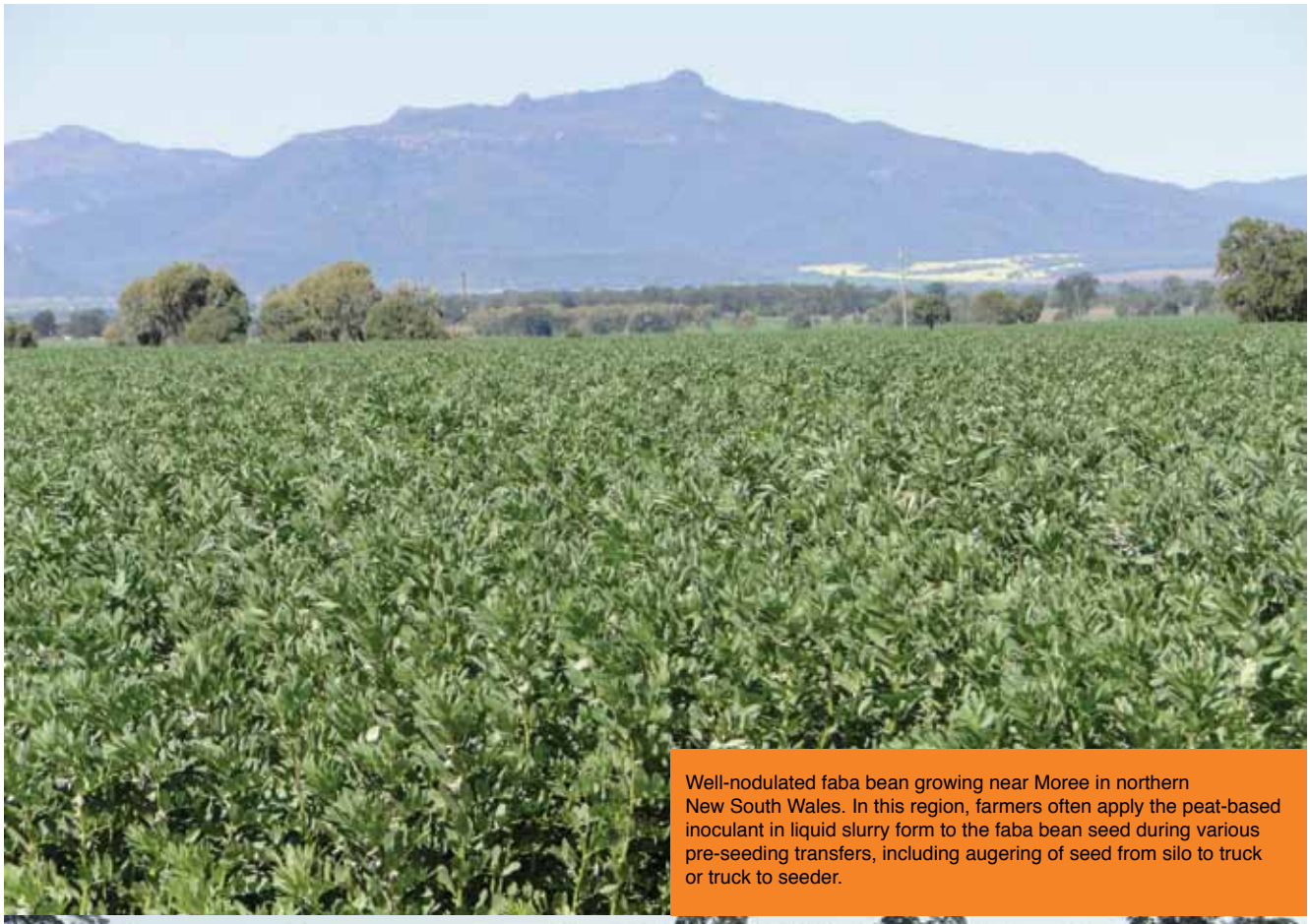
This chapter has highlighted the **dos** and **don'ts** when inoculating legume seed to achieve effective root nodulation. Rhizobia are living organisms and their survival can be severely reduced when this is not kept in mind. When handling inoculants remember that many things are toxic to rhizobia such as direct contact with chemicals and fertilisers, high or freezing temperatures, sunlight, desiccation, and acidic (low pH) and highly alkaline (high pH) soil.

Legumes must be inoculated with the correct rhizobia strain (inoculant group) for maximum benefit. In Australia, the inoculant rhizobia are currently available in different carriers: peat, freeze-dried powders, granules and as preinoculated seed. The shelf life of these products varies from several weeks in the case of some preinoculated seeds to three years for the freeze-dried powder. The cost of inoculation can vary depending on the product. Peat is the cheapest form of inoculant to purchase but there are additional application costs in time and labour to consider. The more expensive options can be easier to use and offer flexibility at sowing.

Although inoculation of legumes can be perceived as a difficult exercise, by following some simple instructions and precautions you can ensure delivery of large numbers of the commercial inoculum to the target legume roots. Successful inoculation should improve nodulation, resulting in increased symbiotic nitrogen fixation and yield of the legume, and ultimately produce more yield and higher grain quality in the following non-leguminous crops.

TABLE 5.4 Compatibility of different rhizobia groups with seed-applied fungicides. Information sourced from commercial product information guides (Becker Underwood and Novozymes)

Inoculant group / crop	Fungicide type	Planting window of inoculated seed
E – pea, vetch	P-Pickle T	6 hours
	Gaucho® 600 FL	4 hours
F – faba bean, lentil	Gaucho® 600 FL	24 hours
	P-Pickle T	24 hours
	Thiram	Compatibility not known
G – lupin	Rovral	6 hours
	Thiram	24 hours
H – soybean	not compatible with seed dressings	
N – chickpea	P-Pickle T	6 hours
	Thiram	6 hours
	Apron® XL 350 6 hours	6 hours
	Gaucho® 600 FL	6 hours
P – peanut	not compatible with seed dressings	



Well-nodulated faba bean growing near Moree in northern New South Wales. In this region, farmers often apply the peat-based inoculant in liquid slurry form to the faba bean seed during various pre-seeding transfers, including augering of seed from silo to truck or truck to seeder.

High-yielding inoculated soybean ready for harvest, grown in rotation with sugarcane in north-coastal New South Wales. Growers inoculate soybeans using mainly peat and liquid inoculant formulations and are looking to capture the benefits of the soybean by reducing amounts of fertiliser N applied to the subsequent cane.

PHOTOS: DAVID HERRIDGE

6 LEGUME NITROGEN FIXATION AND ROTATIONAL BENEFITS

- Legume–rhizobia symbioses fix approximately 2.7 million tonnes nitrogen (N) annually in Australian agricultural systems, with a nominal value of about $4 billion.
- At the paddock scale legumes fix, on average, about 110 kilograms of N per hectare annually. The range is large, from close to zero to more than 400kg N/ha.
- The amount of nitrogen fixed increases as potential legume dry matter yield (biomass) increases, but is reduced by high levels of soil nitrate.
- The actual amount of nitrogen fixed in any one paddock varies with the species of legume, site and season and the applied agronomic management.
- The fixed nitrogen is used by the legume itself for growth.
- The legume residues left in the soil after the grain is harvested or the grazed/cut pasture legume phase is terminated represent, upon decomposition, a potent source of plant-available nitrogen for subsequent cereal and oilseed crops.
- Cereals grown after legumes generally out-yield cereals grown after non-leguminous crops. The extra yield is mostly due to the higher levels of soil nitrate following the legumes but will also include other factors such as a disease-break effect.
- Depending on the circumstances, the economic benefits of including legumes in crop production systems can be substantial.

6.1 Introduction

Grain and pasture legumes are valued components of Australian agricultural production systems. More than a century ago J.L. Thompson (1895) summarised their worth in rotations 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.

Nothing much has changed. Growers still grow legumes as rotation crops because it helps to spread risk and manage weeds, pests and diseases in the production system. 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 largely used by the legume for growth. In return, the legume provides the rhizobia with nutrients, energy and habitat.

The principal beneficiary of nitrogen (N) fixation is the legume itself. It is self-sufficient in N, it can grow in essentially any soil without inputs of fertiliser N. The amount of N fixed is influenced by the type 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 6.1). The mineral

FIGURE 6.1 Cycling of N through the legume phase of a rotation to the following cereal crop.

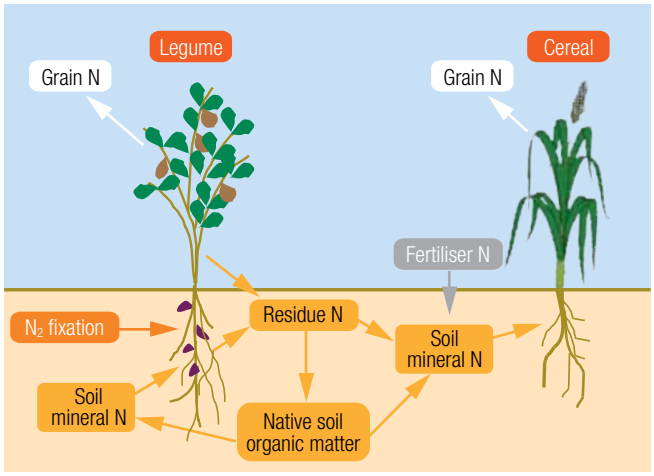


TABLE 6.1 Annual contribution of symbiotically (legume) fixed nitrogen.

	Globally	Australia
Amount N fixed (million tonnes)	40	2.7
N fertiliser equivalent (million tonnes)	50	3.4
Economic value (billion dollars)	63	4.3

N released from these residues as they decompose is taken up by the following cereal (or oilseed) crop in the rotation. Legumes have a role in supplying nitrogen to the farming system following their harvest.

This chapter examines legume N fixation within global and Australian contexts, the drivers of legume N fixation and how they might be managed and, finally, the benefits of legumes and legume N in our agricultural systems.

6.2 Legume nitrogen fixation – globally and on Australian farms

Agricultural legumes fix a lot of N. Globally, there are 185 million hectares of crop legumes and more than 100 million hectares of pasture and fodder legumes and they fix about 40 million tonnes of N every year (Herridge et al. 2008). This represents a significant saving of fertiliser N that would otherwise need to be applied and has substantial positive economic and environmental consequences.

6.2.1 Economic consequences

Almost all the fixed N is available for use by the growing legume. If we compare this to an approximate 80 per cent conversion of fertiliser N into plant N, then the 40 million tonnes of biologically fixed N has a fertiliser-N equivalence of 50 million tonnes. This represents more than 50 per cent of current global inputs of nitrogenous fertilisers. The nominal annual value of this fixed N is about $60 billion, assuming a cost of fertiliser N of $1.25/kg.

The situation for Australian agriculture is equally impressive. The 23 million hectares of legume-based pastures are estimated to fix about 2.5 million tonnes of N every year. Nitrogen fixation by the crop legumes is estimated at approximately 0.2 million tonnes annually. Using the same assumptions above, the economic value of the N fixed by legumes in Australia's agricultural systems is close to $4 billion annually.

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

Table 6.1 summarises the economic contributions of nitrogen fixation by legumes in agriculture.

6.2.2 Environmental consequences

Legume nitrogen fixation is basically a solar driven process. Plants use solar energy to convert atmospheric carbon dioxide (CO_2) to carbohydrates. Some carbohydrates are transferred to the nodules, where they are used by rhizobia as an energy source.

By way of contrast, industrial N fixation, which is used to produce nitrogenous fertilisers, requires high temperatures and pressures and the expenditure of large amounts of fossil fuels. The transport and application of the N fertilisers are also energy demanding and nitrous oxide, a potent greenhouse gas, is often emitted from soils following application of nitrogenous fertiliser. All these processes result in large amounts of greenhouse gas emissions. Current estimates suggest that 10 tonnes CO_2 equivalents are emitted per tonne of N fertiliser used.

While biological nitrogen fixation will not replace the need for N fertilisers in agriculture, legume-based rotations can significantly reduce the amounts used.

6.3 Comparing nitrogen fixation by the different crop and pasture legumes

Not all legumes have the same capacity for nitrogen fixation. There are inherent differences among the commonly grown legumes. External factors, such as how much water they receive from rainfall and irrigation, also impact N fixation.

6.3.1 How much nitrogen do crop legumes fix?

Table 6.2 lists the major crop legumes grown by Australian growers. The numbers in the table are averages, derived from many studies. They provide an overview of crop legume N fixation only – the values are not representative of all paddocks sown to these crops. The amounts of N fixed by individual crops will reflect environmental and management effects.

The second column shows the percentage of crop N derived from nitrogen fixation (per cent of N fixed) for each of those crops. Clearly, navy beans have a weak capacity for N

TABLE 6.2 Estimates of the amounts of N fixed annually by crop legumes in Australia.

Legume	% N fixed	Shoot dry matter (t/ha)	Shoot N (kg/ ha)	Root N (kg/ha)	Total crop N (kg/ ha)	Total N fixed[1] (kg/ha)
Lupin	75	5.0	125	51	176	130
Pea	66	4.8	115	47	162	105
Faba bean	65	4.3	122	50	172	110
Lentil	60	2.6	68	28	96	58
Soybean	48	10.8	250	123	373	180
Chickpea	41	5.0	85	85	170	70
Peanut	36	6.8	190	78	268	95
Mungbean	31	3.5	77	32	109	34
Navy bean	20	4.2	105	43	148	30

1 Total N fixed = per cent N fixed x total crop N; Data sourced primarily from Unkovich et al. 2010.

fixation, fixing only 20 per cent of its requirements for N. At the other end of the scale are faba beans and lupin, which both have strong capacities for N fixation.

For all crops, the remaining N requirements have to be supplied from soil and/or fertiliser sources.

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

The percentage of legume N derived from nitrogen fixation is only part of the story. The total amount of N fixed per hectare is also strongly influenced by the size of the crop (i.e. the more biomass the crop produces, the more it potentially fixes).

Crops such as soybeans, faba beans and peanuts often produce large amounts of biomass because they tend to be irrigated or are grown in high-rainfall areas. Other crops such as mungbeans and lentils are low-yielding crops often grown under water-limited conditions. Both root and shoot N contribute to the total amount of N fixed by a crop. Root N, listed in the fifth column of Table 6.2, is substantial for all crops, and in particular for chickpeas and soybeans.

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 is very important. These N inputs are the basis for the legume effect on the improvement of soil-N fertility and yields of subsequent crops. When all of these factors are taken into account, soybeans, lupin, and faba beans fix the most N on an area basis. The low estimate for navy beans reflects its low efficiency of N fixation coupled with the fact that all commercial crops are fertilised with N.

6.3.2 How much N do pasture legumes fix?

All pasture legumes have a relatively strong capacity for N fixation, as shown in Table 6.3. As with the crop legumes, a major factor affecting the amounts of N fixed by the different pasture legumes is their production of biomass. The annual clovers, for example, typically produce twice the biomass as the annual medics and fix nearly twice as much N.

The majority of legume-based pastures in Australia are dominated by subterranean clover and the annual medics and, to some degree, lucerne. Therefore, the overall value for nitrogen fixation by pasture legumes across the whole of the country would likely be approximately 110 to 120kg N/ha annually.

6.3.3 How much N will legumes fix in my paddock?

The N fixation data for crop and pasture legumes in Tables 6.2 and 6.3 were derived from very large amounts of data across a range of sites. As stated above, these values are intended to provide a broad picture of the average amounts of N fixed by the major crop and pasture legumes in Australian agriculture.

The actual amounts of N fixed by legumes in specific paddocks will vary enormously with site, season, and management by the grower. In the next section, we look at some of the management effects on legume nitrogen fixation.

6.4 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 lower the soil nitrate and the greater the biomass produced, the greater the amount of N fixed.

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, stress-free growing conditions, including low soil nitrate.

6.5 Soil nitrate suppresses legume nitrogen fixation

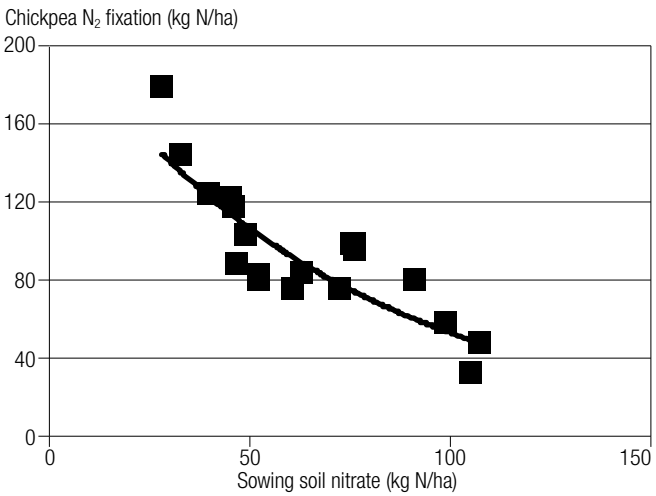
Soil nitrate is a potent inhibitor of legume nodulation and nitrogen fixation. At low soil nitrate (i.e. less than 50kg N/ha in the top metre or so of soil), the legume reliance on nitrogen fixation (% N fixed) is generally high. As soil nitrate increases, legume nodulation and nitrogen fixation become more and more suppressed. Eventually, at very high soil

TABLE 6.3 Estimates of the amount of N fixed annually by the pasture legumes in Australia.

Legume	%N fixed	Shoot DM (t/ha)	Total crop N (kg/ha)	Total N fixed (kg/ha)
Annual clovers	60	5.8	234	140
Subterranean clover	81	2.8	150	120
Annual medics	74	2.6	110	80
Perennial clovers	72	4.0	180	130
Lucerne	60	4.4	298	180

Values for annual shoot dry matter (DM) production are taken from Unkovich et al. 2010, and are aggregated from 240 individual values. Note that vetch is not included.

FIGURE 6.2 Impact of soil nitrate on chickpea nitrogen fixation in northern NSW.



SOURCE: Unpublished data of WL Felton, H Marcellos, DF Herridge, GD Schwenke and MB Peoples

nitrate (more than 200kg N/ha), nodulation and nitrogen fixation will be close to zero. Figure 6.2 illustrates that the impact of soil nitrate on chickpea crops in northern NSW, where nitrate levels greater than 40kg N/ha had a suppressive effect on nitrogen fixation.

The actual amount of soil nitrate that will inhibit legume nodulation and nitrogen fixation in a specific 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 and long pre-crop fallows all increase soil nitrate levels.

Low soil nitrate leads to greater N_2 fixation activity.

6.6 What are the best management practices to improve legume growth and nitrogen fixation?

Apart from inoculating the legume seed with the appropriate rhizobia (see Chapter 5), optimising basic agronomy (best management practice) is the key to legume productivity and therefore 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. It also means optimising nutrient inputs, reducing acidity with lime, and managing weeds, disease and insects.

6.6.1 Tillage practices

One management option for cropping that has gained popularity in recent years is no-tillage. No-tillage may lead to increased soil water and decreased soil nitrate accumulation during the pre-crop fallow and in-crop.

Studies in northern NSW show a positive effect of no-tillage on productivity and nitrogen fixation of chickpeas (Table 6.4). No-till plots had more soil water at sowing and less nitrate-N than the cultivated soils. As a result, chickpea biomass, grain yields and nitrogen fixation increased.

However, under no-tillage non-legume crops (e.g. cereals, oilseeds) additional fertiliser N may be required to supplement the reduced soil nitrate.

6.6.2 Sowing practices

The N fixation potential of legumes may be maximised by sowing on time and at the appropriate density.

Sowing on time takes full advantage of growing-season rainfall and temperatures. Studies with field peas in Victoria and southern NSW showed that N fixation increased from 64kg N/ha to 180kg N/ha by planting earlier (Figure 6.3).

The use of narrow row spacing and/or high plant density can improve N fixation. Increasing lucerne density from 5 to 40 plants/m^2 more than doubled crop biomass and nitrogen fixation in lucerne-based pastures in south-eastern Australia (Figure 6.4). Scientists in northern NSW also found that N fixation of faba beans and chickpeas increased with higher plant densities (Schwenke et al. 1998).

6.6.3 General soil conditions

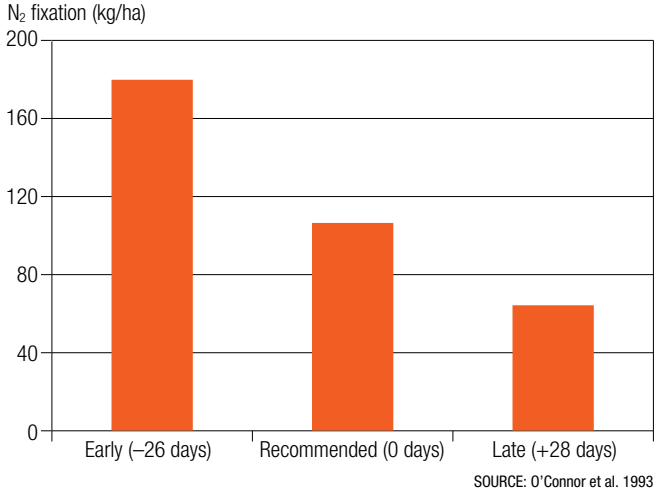
Soil acidity and phosphorus (P) deficiency are common constraints to legume N fixation. Soils that are very acidic or very alkaline may result in reduced N fixation.

TABLE 6.4 Effects of tillage on soil water and nitrate at sowing, and on chickpea growth, grain yield and nitrogen fixation.

	No till	Cultivated
Sowing soil water (mm)	144	109
Sowing soil nitrate (kg N/ha)	71	86
Shoot dry matter (t/ha)	5.4	4.7
Grain yield (t/ha)	2.01	1.83
% N fixed	55	44
Crop N fixed (kg/ha)	107	75

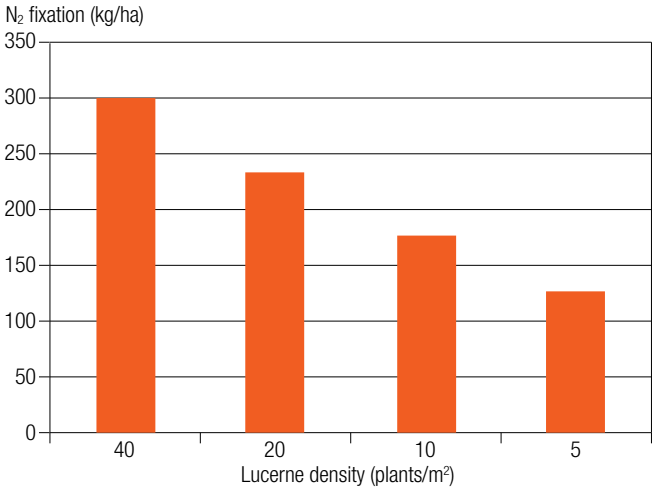
Data are the means of 21 site/years of experiments
SOURCE: Unpublished data of WL Felton, H Marcellos, DF Herridge, GD Schwenke and MB Peoples

FIGURE 6.3 The early-sown pea had the highest rates of nitrogen fixation.



SOURCE: O'Connor et al. 1993

FIGURE 6.4 Increasing legume density increases nitrogen fixation.



SOURCE: Peoples et al. 1998

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 average N fixation increases of 100 per cent (Figure 6.5).

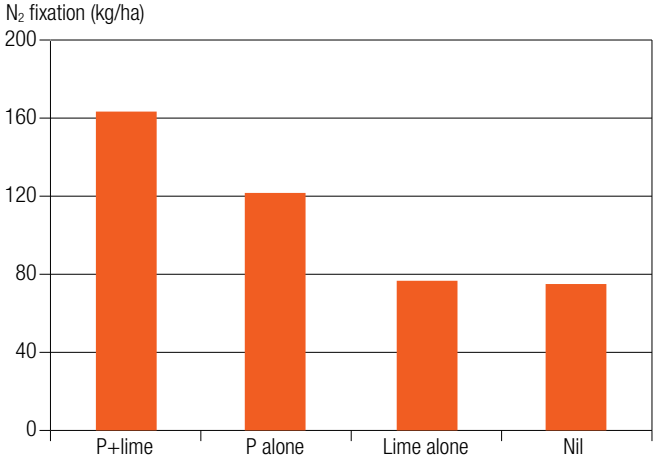
Increasing soil pH with lime application decreases the availability of aluminium and manganese. At elevated concentrations, both of these metals are toxic to legume roots and rhizobia (Peoples et al. 1995).

Not all plant species react similarly. Different species, and even different cultivars, of legumes may have different tolerances to 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 in southern Australia showed that lupin fixed approximately 80 per cent more N than field peas in acidic soils. However, in alkaline soils the field peas fixed more N. The results were due to changes in both crop biomass and N fixation rates (Evans et al. 1989).

Other soil constraints to N fixation include salinity, sodicity, and nutrient toxicities and deficiencies. Such constraints must be addressed if potential legume biomass production is to be realised.

FIGURE 6.5 Optimising plant nutrition increases nitrogen fixation.



SOURCE: Peoples et al. 1998

Optimise agronomic practices to maximise nitrogen fixation.

Research has also established that nitrogen-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).

6.7 What are the nitrogen and rotational benefits of crop legumes?

As previously stated, N fixation provides 'free' N to the legume eliminating the need for additional inputs of fertiliser N. This is only the first part of the story. Incorporating legumes into a cropping system also provides rotational benefits. Rotational benefits include an N benefit and a biological benefit. Both factors often lead to significantly increased yields of subsequent crops.

A substantial body of research examining the rotational benefits of N-fixing crop legumes and legume pasture leys in Australia's wheat production systems has now been published. These include, among others, the Sustainable Agriculture Through Wheat and Good Legumes (SATWAGL) experiments at Wagga Wagga, NSW (Heenan and Chan 1992) and the Tarlee, SA, pasture and pulse rotations (Schultz 1995).

6.7.1 What is the N benefit?

The N benefit of a legume phase of a rotation comprises the mineral N conserved in the soil during growth of the legume and the addition of N-rich residues following legume harvest.

Since legumes fix a percentage of their required N, they use less of the available soil N. The residual soil N that is not used is normally carried over to the subsequent cropping year.

Legumes also produce N-rich residues, which decompose in the soil after the pulse crop is harvested or pasture senesces. The mineral N released during decomposition is then available to be taken up by the following crop (see Figure 6.1, page 41).

6.7.2 How do the N-rich legume residues contribute to the N benefit?

To determine how much N-rich legume residues contribute to the N benefit, we need to examine crop, residue and soil N values for the two years of a legume-cereal rotation. Table 6.5 shows factors affecting the N balances of chickpea/wheat and wheat/wheat rotations in northern NSW.

In the first year of the sequence, all crops were grown in a soil with moderate nitrate at sowing (67kg N/ha). Chickpeas fixed 135kg N/ha and produced far more residue-N than both wheat crops. The chickpea residues were also N-enriched (lower carbon (C):N ratio) compared to the wheat residues.

The low C:N ratio of the chickpea residues means that mineral N (ammonium and nitrate) was released into the soil during microbial decomposition, resulting in net mineralisation.

C:N ratio of plant residues	The ratio of carbon to nitrogen in the plant residues. The carbon content of plant materials is fairly constant at about 40 per cent but the N content varies considerably, from about 0.4 per cent to 3.0 per cent. The C:N ratio thus varies accordingly from 100:1 to 13:1.
Net mineralisation and immobilisation	The decomposition of residues by the soil microbes will either result in the release of mineral N into the soil (net mineralisation) or tie up mineral N (immobilisation). Net mineralisation is associated with residues with C:N ratios of less than 30 and immobilisation with those with C:N ratios of more than 30.

In order to decompose higher C:N wheat residues, microbes must use soil mineral N. The use of this N results in net N immobilisation of the mineral N into microbial biomass within the soil.

Thus, we can estimate that the chickpea residues released 16kg mineral N/ha into the soil during the six-to-seven-month summer fallow, compared to 21 to 22kg N/ha immobilised by the wheat residues during the same period.

In Table 6.5, at crop sowing time in Year 2 at the end of the summer fallow, nitrate in the soil following chickpeas was much higher than following wheat crops. As a result, grain yields and grain N were higher after chickpeas.

The data clearly shows 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.

Legumes produce residues with a higher N concentration compared to cereals.

6.7.3 What is the biological benefit?

The biological benefit is largely related to the break-crop effect of the legume phase on soil and stubble-borne diseases of cereals.

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 can enhance plant health and help to minimize the impact of the disease.

Host-specific diseases such as take-all (*Gaeumannomyces graminis*) and crown rot (*Fusarium* spp.) can usually be managed using legumes (and crops such as canola) as a break crop. 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.

For example, a SA study showed that seminal root infection in wheat by take-all was three per cent following a legume, compared to eight per cent following wheat (King 1984).

Similarly, the data from northern NSW in Table 6.6 shows substantially less crown rot in wheat after chickpeas than following wheat. There were associated yield increases as all crops were well-fertilised with N, so the increased yield after chickpea was not related to an N benefit from the chickpea.

In general, the non-N biological benefit of legumes to grain yield in the following crop may range from negligible to more than 2t/ha.

TABLE 6.5 Explaining the N and yield benefits of a chickpea-wheat rotation compared with unfertilised or N-fertilised wheat-only sequences.

	Chickpea/ wheat (0 N)	Wheat (0 N)/ wheat (0 N)	Wheat (100kg/ ha N)/ wheat (0 N)
Year 1 (chickpea or wheat)	Chickpea	Wheat	Wheat
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
Residue N (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	Wheat	Wheat
Sowing soil nitrate (kg N/ha, 1.2 \m depth)	102	53	74
Wheat grain yield (t/ha)	2.8	1.7	1.8
Wheat grain N (kg/ha)	55	30	33

TABLE 6.6 Biological break benefit.

Rotation	Crown rot incidence (%)	Yield (t/ha)
Wheat/wheat	20–27	2.7
Chickpea/wheat	15	3.0

Source: Kirkegaard et al., 2004

6.7.4 How does a legume break the cereal pathogen cycle?

The numbers of the pathogen in the soil decrease when the host (cereal crop) is not present. A study of different sites in south-eastern Australia showed lower numbers of the crown-rot fungi in soils after legume-based rotations than continuous cereals (Evans et al. 2010).

Similarly, cereal cyst nematode (*Heterodera avenae* Woll.) populations in SA and Victoria decreased to almost undetectable levels following two years of peas or fallow. In comparison, numbers after two years of resistant wheat were four eggs per gram of soil, and 15 eggs per gram of soil after susceptible wheat (Table 6.7).

6.7.5 What are the yield benefits of crop legumes in rotation?

Pulses and other legumes are usually grown in rotation with cereals. The benefits to the system are measured in terms of increased soil total and plant-available (nitrate) N, and grain N and yield of the subsequent cereal crop, all relative to a cereal/cereal sequence.

Studies on different cropping systems in different regions of Australia have typically found that cereals grown after crop legumes commonly yield an additional 0.5 to 1.5 t/ha grain compared with cereals grown after cereals without fertiliser N.

To generate equivalent yields in the cereal-cereal

TABLE 6.7 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

sequence, 40 to 100kg fertiliser N/ha would need to be applied.

For example, 167 experiments were conducted in WA between 1974 and 2007 to examine the rotational benefit of the narrow-leafed lupin and field peas on subsequent wheat crops (Seymour et al. 2012). Over all experiments, the rotational benefit of lupin was 0.6 tonnes of wheat grain/ha and for pea was 0.45t/ha. For both, the benefit was a combination of extra N plus disease-break (principally take-all).

It is worth noting that the benefits were more substantial in the high-rainfall areas that produced higher-yielding lupin crops, and with the more recent trials (Table 6.8). The larger benefits during the 1990s were likely related to improved agronomy (weed management etc.) of both the lupin and wheat crops. Significant benefits (0.4t wheat grain/ha) persisted into a second wheat crop.

Rotation trials at different sites in Victoria and NSW show consistent increases in wheat grain yield following both faba bean and lupin crops (Figure 6.6). The yield benefit of the legume rotation was equivalent to fertilising with 80kg N/ha.

Rotation trials in the grain-growing regions of northern NSW and southern Queensland showed that both yield and grain protein of wheat increased substantially following chickpea, compared to the wheat/wheat sequence. A summary of the results is presented in Table 6.9.

The yield benefit of chickpea was equivalent to fertilising with 75 to 150 kg N/ha. The major factor in the increased wheat yields was soil nitrate. In NSW, there was, on average, an additional 35kg nitrate-N/ha in the 1.2m profile after chickpeas compared with the continuous wheat.

TABLE 6.8 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.

Lupin grain yield / years of experiments	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

Cereals grown after crop legumes commonly out yield cereals grown after cereals.

Comparable benefits were established in the grainbelts of southern NSW and Victoria, where wheat yield and grain protein were greater in legume/wheat rotations than in wheat-wheat rotations (Figure 6.7).

In a number of cases, yield increases were more than 200 per cent. On average, wheat after lupin yielded an additional 0.9 t grain/ha and wheat after pea yielded an additional 0.7 t/ha, compared with wheat after wheat.

Again, increased plant available N was the main factor governing yield increases. Plant available N increased by 54 per cent following lupin and 61 per cent following peas.

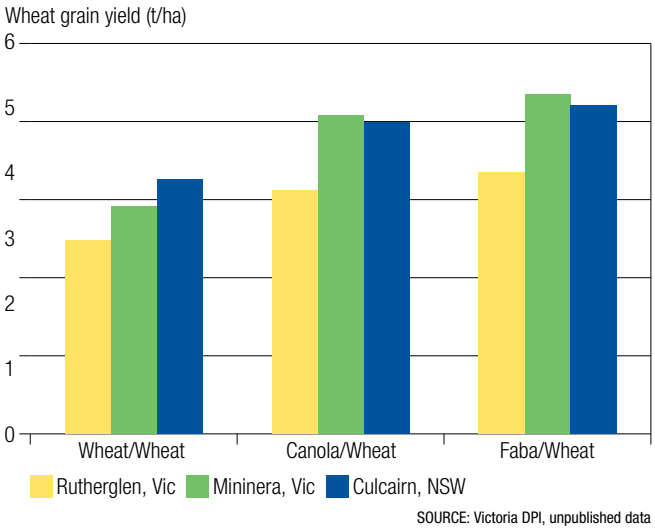
6.7.6 How does the inclusion of a crop legume in the rotation impact on the overall profitability of the system?

Crop legume/cereal rotations often show improved gross margins compared with the cereal/cereal sequences. When the gross margins for the crop sequences in Table 6.5 were calculated, the chickpea/wheat rotations were far more profitable (Table 6.10).

After Year 1, there was not a lot of difference between the gross margins of chickpea and the N-fertilised wheat. In Year 2 however, wheat after chickpea had gross margins more than double those of the wheat/wheat sequences. The least profitable sequence was the unfertilised wheat followed by unfertilised wheat. Over the two years of the sequences, the chickpea/wheat rotation had a gross margin that was 50 to 90 per cent greater than those of the continuous wheats.

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

FIGURE 6.6 Rotation crop impact on wheat grain yield at three Victorian Department of Primary Industries' sites.



SOURCE: Victoria DPI, unpublished data

TABLE 6.9 Benefits of chickpeas on yield and grain protein of the following wheat crop.

Sites / rotations	No fertiliser N		+ fertiliser N (75–150kg/ha)	
	Yield (t/ha)	% protein	Yield (t/ha)	% protein
Wheat after wheat	2.1	11.2	2.7	13.2
Wheat after chickpea	2.8	12.2	2.9	13.8

Data sourced from Lucy et al. 2005, representing the summary of a decade of rotations in the northern grainbelt of NSW.

6.7.7 How long does the rotational benefit last?

The rotational benefits of crop legumes for following cereal crops last for one to two seasons, depending on particular circumstances. A study of six sites in northern NSW showed an average yield benefit following chickpea of 46 per cent (3.2t/ha for wheat after chickpea versus 2.2 t/ha for wheat after wheat; Marcellos et al. 1993). For five of the six sites in this study, there were no effects of the chickpeas on yields of a second wheat crop. In WA, on the other hand, the benefit of the narrow-leafed lupin lasted into a second wheat crop, likely through disease-break effects (Seymour et al. 2012).

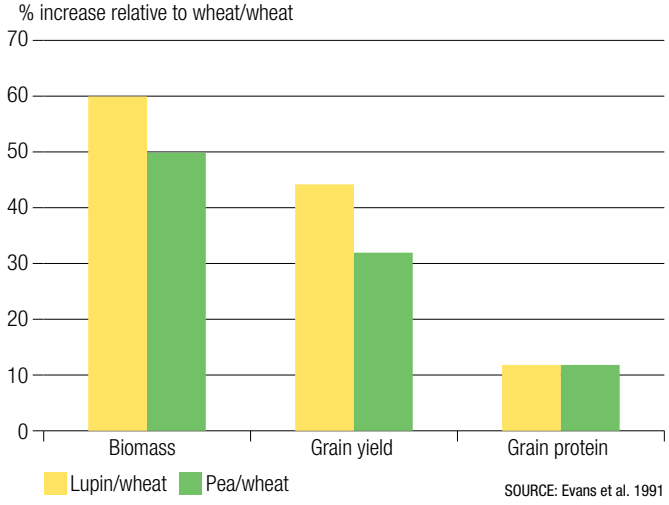
6.8 What are the benefits of pasture legume rotations?

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

Pasture legume leys also benefit soil N and soil structure. These benefits can be derived from single or multi-year pasture leys. When the pasture is moved into crop production, these benefits enhance productivity of subsequent cereal 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/ha per year. Higher levels of soil total N were maintained during more than nine years of following wheat cropping (Figure 6.8).

FIGURE 6.7 Average percentage increase in wheat yields and grain proteins for wheat following either lupin or peas, relative to wheat following wheat. Values are averages from 18 experiments.



SOURCE: Evans et al. 1991

TABLE 6.10 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 (0 N)	Wheat (0 N)/ wheat (0 N)	Wheat (100 kg/ ha N)/wheat (0 N)
Year 1	Chickpea	Wheat	Wheat
Grain yield (t/ha)	2.3	2.3	3.2
Grain ($)[1]	920	575	800
Cost of production ($)[2]	465	270	400
Gross margin ($)	455	305	400
Year 2 (wheat only)	Wheat	Wheat	Wheat
Grain yield (t/ha)	2.8	1.7	1.8
Grain ($)	700	425	450
Cost of production ($)	270	270	270
Gross margin ($)	430	155	180
2-year gross margin ($)	885	460	580

Yields taken from Table 6.5 and are the means of no-tillage and cultivated treatments at two sites in northern NSW (source: unpublished data of WL Felton, H Marcellos, DF Herridge and GD Schwenke).
1 Chickpea at $400/t; wheat at $250/t; 2 NSW DPI figures

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

Comparable benefits were found on a red earth soil, where the lucerne pasture added about 110kg N/ha per year.

Additional studies in the Tamworth region showed the positive impact of pasture legume leys on nitrate-N and subsequent wheat yields (Table 6.11).

Grazed pasture leys accumulated 290 to 854kg of biomass-N per hectare during three years of growth. Following the pasture phase, up to 215kg of nitrate-N/ha became available for crop growth. By comparison, nitrate levels were 15kg/ha in the adjacent continuous wheat plots.

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

The long-term benefits resulted in savings on N fertiliser inputs, as shown in Table 6.12.

Single–year pasture leys 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 crops, 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, longer growth periods, and

TABLE 6.11 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

TABLE 6.12 Savings in fertiliser N (kg/ha) from the three-year legume pasture leys at NSW Department of Primary Industries, Tamworth.

Previous crop/pasture	Wheat crop 1	Wheat crop 2	Wheat crop 3	Average 3 wheat crops
Lucerne	45*	120	65	80
Clover	>100	60	45	70
Annual medic	70	30	25	45

Data from long-term rotation experiments on black and red soils during 1988–93.
* low because of the soil drying effect of the lucerne ley.

greater nitrogen fixation efficiency.

An additional benefit of pasture legumes is the impact the extra organic N can have on soil structure. Figure 6.9 clearly shows the positive effect of pasture leys on aggregate stability of a red-earth soil in the Victorian grainbelt. Aggregate stability declined once wheat cropping recommenced.

The effect of organic N 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 grainbelts was built around sequences of pasture leys and cereals. As agricultural land used for cropping continues to lose organic matter and structural integrity, the role of pasture leys in restoring organic fertility and productivity may need to be expanded.

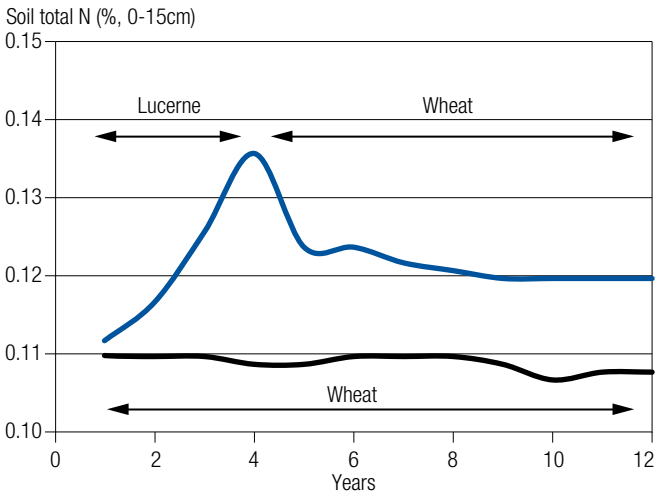
Legume pasture leys have a positive impact on soil structure as well as soil fertility.

6.9 Concluding comments

Legumes have been used as a source of food ever since humankind 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

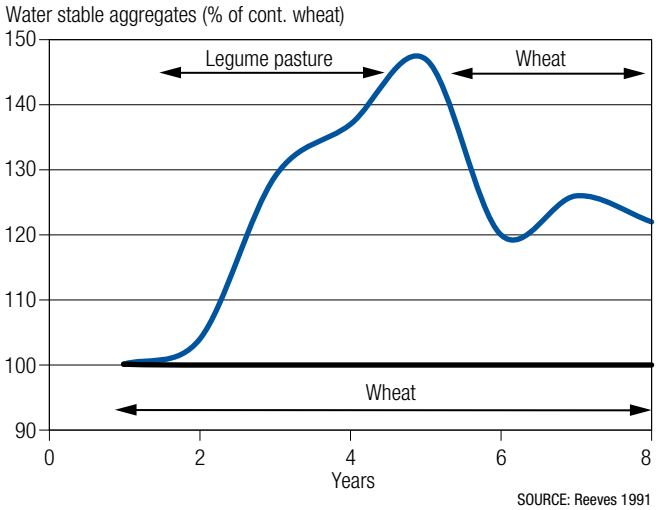
FIGURE 6.8 Build-up of soil total N under a well-managed, intensively grazed lucerne pasture on a black earth at Tamworth and the subsequent run-down during wheat cropping. Soil N levels under the wheat monoculture are shown also.



A 0.01 per cent increment in soil N to 15cm depth is equivalent to 180kg N/ha.

SOURCE: Holford 1981

FIGURE 6.9 Positive effects of pasture leys on aggregate stability of a red earth at Rutherglen, Victoria.



SOURCE: Reeves 1991

that cereals, their mainstay crops, were healthier and higher yielding when grown after a legume break-crop.

Nothing much has changed. Growers still grow legumes as rotation crops because of the N benefits and because it helps them to spread risk and manage weeds, pests and diseases in the production system, and improve soil health.

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

Optimising legume yields within any system can only be achieved through best management practice in agronomy where production is not constrained by soil 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 well-conducted 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 mechanisms of the rhizobia-legume symbiosis, and explored management decisions regarding when and how to inoculate.

7 LEGUME INOCULATION FACT SHEETS

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

7.1 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 glanuliferum*
		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 daisycarpa*
		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	*Neontonia wightii*
		Butterfly pea	*Clitoria ternatea*
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.

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)

7.2 CHICKPEA inoculation fact sheet

Chickpea	Strain: CC1192 (Group N)
Cicer arietinum	*Mesorhizobium ciceri*

Legume use and rhizobia distribution

Chickpea plantings have been steadily increasing over the past decade to an annual total of more than 500,000 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.

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 inoculum can be applied in-furrow when planting using a water-injection system. Granular inoculum 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 nitrogen.

Nodulation

Nodules are indeterminate and often multi-lobed (see Figure 7.1).

For chickpeas, 10 to 30 nodules per plant is satisfactory after about eight weeks of plant growth.

Likelihood of response to inoculation for sown chickpea	
HIGH	• Chickpeas not previously grown.
MODERATE	• Previous chickpea crop was grown more than four years ago (recommended pulse rotation); OR • legume nodulation or growth below expectation.
LOW	• Recent history of well nodulated chickpea crop in past two years.

FIGURE 7.1 Roots of deep-sown chickpea plants showing multi-lobed nodules particularly around the crown (i.e. site of inoculation).

7.3 FIELD PEA, VETCH, FABA BEAN and LENTIL inoculation fact sheet

Field pea and vetch	Strain: SU303 (group E)
Pisum sativum Vicia species	*Rhizobium leguminosarum* bv. *viciae*
Fababean, broad bean and lentil	Strain: WSM1455 (group F)
Vicia faba *Lens culinaris*	*Rhizobium leguminosarum* bv. *viciae*

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, particularly, pea and vetch cultivation. Present combined sowings of pea, faba bean and lentil are about 600,000 hectares per year. Spread and survival of the rhizobia has also been assisted by vetch, which is broadly naturalised and also sown as a forage/green manure crop.

Although the rhizobia have been widely distributed, their moderate sensitivity to soil acidity means they sometimes occur at levels below what is needed for optimal nodulation.

Inoculation Method

Inoculation usually occurs by pouring or spraying a slurry of peat inoculant over seed during transfer (augering) prior to sowing. Peat, granule and freeze-dried inoculant formulations can also be used.

Key considerations

Two inoculant strains are provided for these legumes to optimise nitrogen 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 moderately sensitive to soil acidity. Their number may be sub-optimal or absent where soil pH is less than 6.0, 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 contain insufficient rhizobia to maximise pea nodulation.

For up to 30 per cent of soils, effectiveness of the rhizobia with field pea ranges from 50 to 70 per cent of the commercial inoculant strain and therefore many field pea crops may benefit from inoculation. Grossly ineffective symbioses are rarely observed.

Crops such as faba beans that have potential to produce a lot of dry matter have a higher demand for nitrogen and therefore may be more responsive to inoculation than field pea.

Nodulation

More than 100 pink nodules per plant are often observed after eight to 10 weeks plant growth in loam-clay soils. For lighter textured soils 20 nodules per plant is deemed satisfactory (see Figure 7.2).

Likelihood of response to inoculation for sown pea, faba bean, lentil and vetch	
HIGH	• Soils with pH($CaCl_2$) below 6.0 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 (recommended pulse rotation); 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.

FIGURE 7.2 Well-nodulated roots of field pea (left) and faba bean (right).

7.4 LUPIN and SERRADELLA inoculation fact sheet

Lupin	Strain: WU425 or WSM471 (group G)
Lupinus species Narrow-leafed, white, yellow and sand-plain	*Bradyrhizobium* spp. lupinus
Serradella	**Strain: WSM471 or WU425 (group S)**
Ornithopus species Yellow, pink, hybrid, slender and birdsfoot	

Legume use and rhizobia distribution

Legumes in the groups 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.

Inoculation method

Lupin is usually inoculated by pouring or spraying a slurry of peat inoculant over seed during transfers (augering) prior to sowing. Peat, granular and freeze-dried inoculant formulations are also used.

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 5). 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

Since late 2006, 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 highly tolerant of soil acidity but some instances of inadequate number in soil after fours years 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 nitrogen, 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 serradella more than 20 pink nodules per plant is satisfactory after eight to 10 weeks plant growth. 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 7.3).

Likelihood of 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. • 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.

FIGURE 7.3 Examples of well-nodulated serradella (left) and lupin (right).

7.5 PEANUT inoculation fact sheet

Peanut (or groundnut)	Strain: NC92 (group P)
Arachis hypogaea	*Bradyrhizobium* spp.

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 and northern WA. One third of production is rain-fed and two thirds is irrigated, with respective average yields of 2 and 5t/ha.

Inoculation method

Water injection of peat or freeze-dried inoculum is recommended to eliminate damage to the seed from applying a slurry coating. Alternatively, granular inoculum can be dispensed with the seed at planting.

Key considerations

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

Nodulation

Peanuts can form many nodules (i.e. more than 100/plant). It is virtually impossible to state the number of nodules per plant after eight to 10 weeks of plant growth that might be considered satisfactory (See Figure 7.4).

Likelihood of 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 7.4 Photo of well-nodulated peanut.

7.6 MUNGBEAN and COWPEA inoculation fact sheet

Mungbean	Strain: CB1015 (group I)
Vigna radiata, V. mungo	*Bradyrhizobium* spp.
Cowpea	
Vigna unguiculata	

Legume use and rhizobia distribution

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

Inoculation method

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

Key considerations

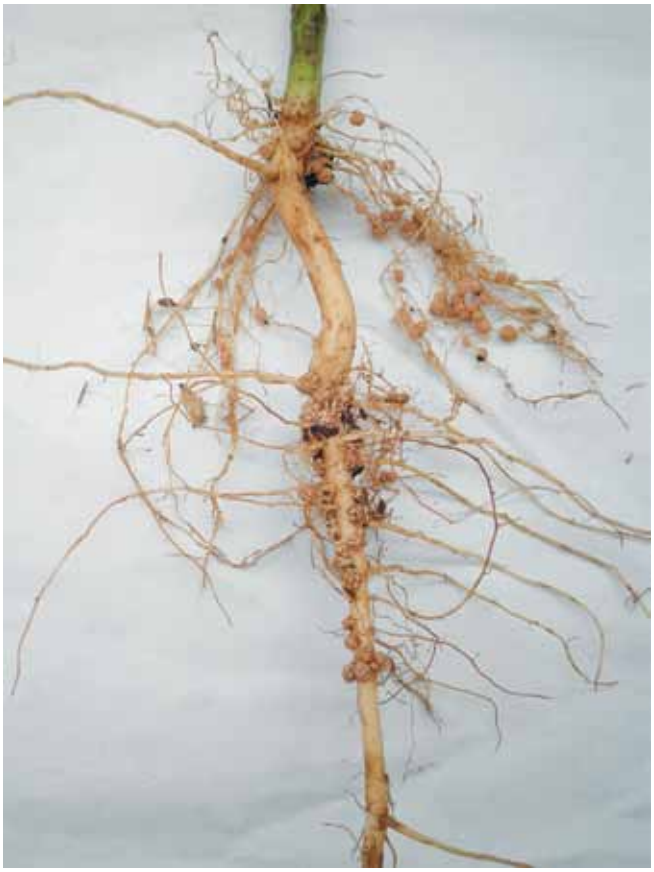
Soil nitrate may depress nodulation and nitrogen fixation of mungbean, even at relatively low mineral nitrogen supply.

Nodulation

For mungbean and cowpea, more than 20 nodules per plant is satisfactory after eight to 10 weeks of plant growth (see Figure 7.5).

Likelihood of 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.

FIGURE 7.5 Well-nodulated mungbean from field.



7.7 SOYBEAN inoculation fact sheet

Soybean	Strain: CB1809 (Group H)
Glycine max	*Bradyrhizobium japonicum*

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 granular and freeze-dried forms. Seed can be coated with either the peat or freeze-dried inoculant formulations as slurries just prior to planting, and are commonly applied to the seeds during transfer (augering). Alternatively, peat or freeze-dried inoculum can be applied in-furrow when planting using a water-injection system or granular inoculum can be dispensed with the seed at planting.

Key considerations

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 fix as much as 300 to 400kg N/ha. Soybean is specific in its requirement for rhizobia. Soybean will not nodulate with the same range of naturalised soil rhizobia as mungbean or cowpea. Therefore, good agronomy and good inoculation practice are necessary to achieve yield and nitrogen fixation potentials.

Nodulation

For soybeans more than 20 nodules per plant is satisfactory after eight to 10 weeks of plant growth (see Figure 7.6).

Likelihood of 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.

FIGURE 7.6 Well-nodulated soybean roots dug from soil when plants were mid-flowering.

7.8 ANNUAL CLOVERS inoculation fact sheet

Annual Clovers	Strain: WSM1325 (group C)
Trifolium species	*Rhizobium leguminosarum* bv. *trifolii*
Subterranean, balansa, Persian, bladder arrowleaf, rose, gland, crimson, purple, bladder, cupped, helmet and berseem.	

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 of neutral to acid soils in southern Australia. Many non-sown clover species that have naturalised extensively have assisted the widespread proliferation of clover nodulating rhizobia.

Inoculation method

Inoculation is mostly done with the application of a slurry of peat followed by pelleting with fine lime or other suitable product. Large sowings of bladder clover in WA and NSW has resulted in granular inoculants being used.

The availability of preinoculated seed has increased. However, survival of the rhizobia is often poor and therefore freshly inoculated (coated) seed is preferred.

Granule and freeze-dried inoculant formulations are available.

Key considerations

The majority of Australian soils with a history of growing annual or perennial clovers contain clover nodulating rhizobia. Effectiveness of the naturalised soil rhizobia with subclover is often sub-optimal, averaging 50 per cent of the commercial inoculant strain. Inoculation will help overcome sub-optimal symbioses in short-term 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.

Clover symbioses are reasonably tolerant of low soil pH, but ideally soil pH should be greater than 5.5. 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

50–100 pink nodules per plant after eight week's growth indicates good nodulation of subclover (see Figure 7.7).

Likelihood of 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 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 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.

FIGURE 7.7 Well-nodulated subterranean clover. Plant grown in greenhouse (left) and plant from field (right).

7.9 ANNUAL MEDICS inoculation fact sheet

Annual Medics	Strain: WSM1115 (group AM)
Medicago species (except strand and disc) Barrel, burr, snail, murex, sphere and gama	*Sinorhizobium* medicae

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 the1930s 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 or other suitable product. Granule 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) will contain medic-nodulating rhizobia.

Effectiveness of the naturalised soil rhizobia is often sub-optimal, averaging 50 per cent of the commercial inoculant strain. Inoculation will help overcome sub-optimal symbioses in short-term pastures.

Mildly acidic soils (pH 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 nitrogen with some medic species in this group.

Nodulation

10-20 pink nodules per plant after eight week's growth indicates good nodulation of annual medics (see Figure 7.8).

Likelihood of response to inoculation for sown annual medics	
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 development of sub-optimal populations of rhizobia. Mean effectiveness of soil rhizobia with burr medic estimated to be 30 per cent. High numbers 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 or alkaline pH and a recent history of vigorous medic growth and good nodulation

FIGURE 7.8 Well-nodulated medic plants grown in greenhouse (left) and field (right).

7.10 BISERRULA inoculation fact sheet

Biserrula (special)	Strain: WSM1497
Biserrula pelecinus	*Mesorhizobium* spp.

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. Approximately 90 per cent of plantings occur in WA.

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 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.

The inoculant strain WSM1497 persists in low pH soils based on observations of good nodulation on regenerating plants five years after introduction of the inoculant strain.

Nodulation

At least five large (>5mm) and 10 small nodules per plant after eight week's growth indicates good nodulation of biserrula (see Figure 7.9).

Likelihood of response to inoculation for sown biserrula	
HIGH	• Biserrula host 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.

FIGURE 7.9 **Well-nodulated biserrula.**

7.11 LOTUS inoculation fact sheet

Lotus (group D)	Strain: CC82
	Strain: SU343 (Special)
Lotus pedunculatus (syn *uliginosus* *Lotus corniculatus*	*Mesorhizobium* spp.

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 seed. Freeze-dried products are 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 of soil acidity.

Nodulation

Expected nodulation after eight to10 weeks is considered to be more than 30 pink nodules per plant. (see Figure 7.10).

Likelihood of 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 good nodulation.

*Good nodulation of lotus at eight weeks after planting is considered to be more than 15 pink nodules

FIGURE 7.10 Example of well-nodulated lotus plant.

7.12 LUCERNE, MELILOTUS (albus), STRAND and DISC MEDICS inoculation fact sheet

Lucerne, Melilotus (albus)	Strain: RRI128 (group AL)
Strand and disc medic	
Medicago sativa, *Medicago littoralis* *Medicago tornata* *Melilotus albus*	*Sinorhizobium meliloti*

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 sown and naturalised medics do not commonly occur. Soils in these areas are unlikely to support suitable rhizobia for lucerne.

Inoculation method

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

Key considerations

Inoculation is always recommended for lucerne because establishment of 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 acid soils.

Where soil pH is less than 6.0, soils will often contain no suitable rhizobia and will be highly responsive to inoculation.

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. 10 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

FIGURE 7.11 **Well-nodulated lucerne grown in (A) greenhouse and (B) field; and (C) strand medic.**

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. 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.

Nodules tend to rapidly develop lobed or coral type structures (see Figure 7.11).

Likelihood of 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 and a recent history of vigorous medic growth and good nodulation.

7.13 PERENNIAL CLOVERS inoculation fact sheet

Perennial clovers	Strain: TA1 (group B)
	Strain: CC283b (Caucasian clover only)
Trifolium species White, strawberry, red, talish, alsike and caucasian	*Rhizobium leguminosarum* bv. *trifolii*

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 sown and non-sown clover species that have naturalised in the areas where perennial clovers are grown 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.

FIGURE 7.12 Well-nodulated white clover showing an abundance of nodules on the tap root and close to the crown of the plants.

Clover symbioses are reasonably tolerant of low soil pH, but ideally soil pH should be greater than 5.5. 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 7.12).

Likelihood of 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 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.

7.14 SULLA inoculation fact sheet

Sulla (special)	Strain: WSM1592
Hedysarum coronarium	*Rhizobium sullae*

Legume use and rhizobia distribution

Sulla is comparatively new to Australian agriculture, having 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 or other suitable product. Seed sold through retail outlets may be preinoculated.

Key considerations

Sulla tends to be a 'shy' nodulator and young seedlings quickly develop nitrogen 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 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, four large (>5 mm) nodules per plant is satisfactory after eight to 10 weeks of plant growth (see Figure 7.13).

Likelihood of 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 and a recent history (past two years) of sulla with good* nodulation.

* Good nodulation of sulla at eight weeks after planting is considered to be more than four large (>5mm) pink nodules.

FIGURE 7.13 Well-nodulated sulla plant.

APPENDIX: LEGUME INOCULANT MANUFACTURERS IN AUSTRALIA

Company: Becker Underwood Australia and Asia
Address: 1205 Old Pacific Hwy, Somersby, NSW, 2250
Phone: 1800 558 399 02 4340 2246
Fax: 02 4340 2243
Email: info.au@beckerunderwood.com
Web: www2.beckerunderwood.com/en/home

Company: New Edge Microbials Pty Ltd
Address: 951 Garland Avenue, Albury, NSW, 2640
Phone : 02 6025 0044
Fax: 02 6040 0237
Email: newedge@microbials.com.au
Web: www.microbials.com.au

Company: Novozymes Biologicals Australia Pty Ltd
Address: Lot 1, Bush's Lane, Bendigo, Victoria, 3550
Phone: 03 5443 6331
Fax: 03 5441 6611
Email: rgv@novozymes.com (Rob Velthuis, General Manager)
Web: www.bioag.novozymes.com

Company: ALOSCA Technologies Pty. Ltd.
Address: Unit 1/ 50 Atwell Street, Landsdale, WA, 6065
Phone: 08 6305 0123
Fax: 08 6305 0112
Email: cpoole@alosca.com.au (Chris Poole)
Web: www.alosca.com.au

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