LEGUME INOCULANTS & NODULATION

Greg Bender & Sandy Montague
Inoculant Services Australia Pty Ltd

ABSTRACT

When a packet of legume inoculant leaves the factory it contains a minimum of 1,000 million live *Rhizobium* bacteria per gram of peat powder. Legume inoculation is the technique of placing the bacteria where they can come into contact with the legume root as it emerges from the seed at germination. The legume and the bacteria then cooperate to build root nodules inside which the bacteria extract (fix) nitrogen from the air. The death of *Rhizobium* bacteria between leaving the packet and seed germination is a major obstacle in obtaining the maximum benefit from legume inoculation. The most common causes of death are drying out of the inoculant on seed caused by delays between inoculation and sowing or sowing into dry soil, prolonged exposure to sunlight and temperatures above 30°C, or direct contact with toxic chemicals. A legume plant can obtain all its nitrogen needs from *Rhizobium* bacteria but can also use available soil nitrogen. The less nitrogen available in the soil, the more is obtained from nitrogen fixation. Hence the greatest returns from inoculation are obtained when legumes are sown into soils with low levels of available nitrogen but adequate supplies of other nutrients, suitable pH, and adequate moisture. Under good growing conditions a legume crop or pasture can fix up to 450kgN/ha/season which is stored in the plant. Legume nitrogen is removed in harvested grain, hay or straw and by grazing. The remaining plant nitrogen is returned to the soil through decomposition after the legume dies.

LEGUMES & NITROGEN FIXATION

Over 70% of the air is made up of gaseous nitrogen which is not directly available to plants. Nitrogen fixation is the process of converting inert gaseous nitrogen to a form which can be used by plants. It is a unique process that makes nitrogen the only soil nutrient that is truly sustainable, ie. renewable without adding fertiliser or recycling. All other nutrients removed from the farm (P,K,S etc) as plant or animal produce must be physically replaced if net losses are not to occur over time.

Nitrogen can be captured artificially as in the manufacture of nitrogenous fertilisers or naturally by micro organisms. Nitrogen fixation by micro organisms is the major channel for nitrogen into the Earths living systems. In agriculture, the most important nitrogen-fixing micro organisms are *Rhizobium* bacteria in a symbiotic association with legume crops and pastures (Figure 1). If a legume plant cannot obtain enough nitrogen from the soil then the plant will form root nodules containing *Rhizobium* bacteria. There are many types of *Rhizobium* bacteria, each specific to a particular legume or group of legumes. The purpose of legume inoculation is to provide high numbers of the right rhizobia around the seed at germination so that the bacteria form nodules promptly.

Rhizobium bacteria inside root nodules take up gaseous nitrogen from the air converting it into soluble forms (amino acids, ureides or ammonia) which can be used by the legume plant to make proteins for growth and seed formation. *Rhizobium* bacteria will only form nitrogen-fixing nodules on the roots of legume plants.

Air	*Rhizobium* working inside root nodules	Legume plant
$N \equiv N \rightarrow$ gaseous nitrogen	$HN=NH \rightarrow H_2N\text{-}NH_2 \rightarrow NH_3 + NH_3 \rightarrow$ ammonia	soluble fixed nitrogen taken up by the plant as amino acids, ureides or ammonia to build proteins

A well nodulated legume crop or pasture can fix up to 450kg of actual nitrogen per hectare per season if soil nitrogen levels are low. This is equivalent to over 1 tonne of urea fertiliser. However, there is a difference between adding fertiliser and using legumes for nitrogen. Biologically fixed nitrogen is locked up in the roots, stems, leaves, and seeds of the legume plant until transported off-farm as produce or slowly released to the soil through decay of plant residues. Fertiliser nitrogen is immediately available for plant uptake with the remainder often lost to the air, washed away or stored in various forms in the soil.

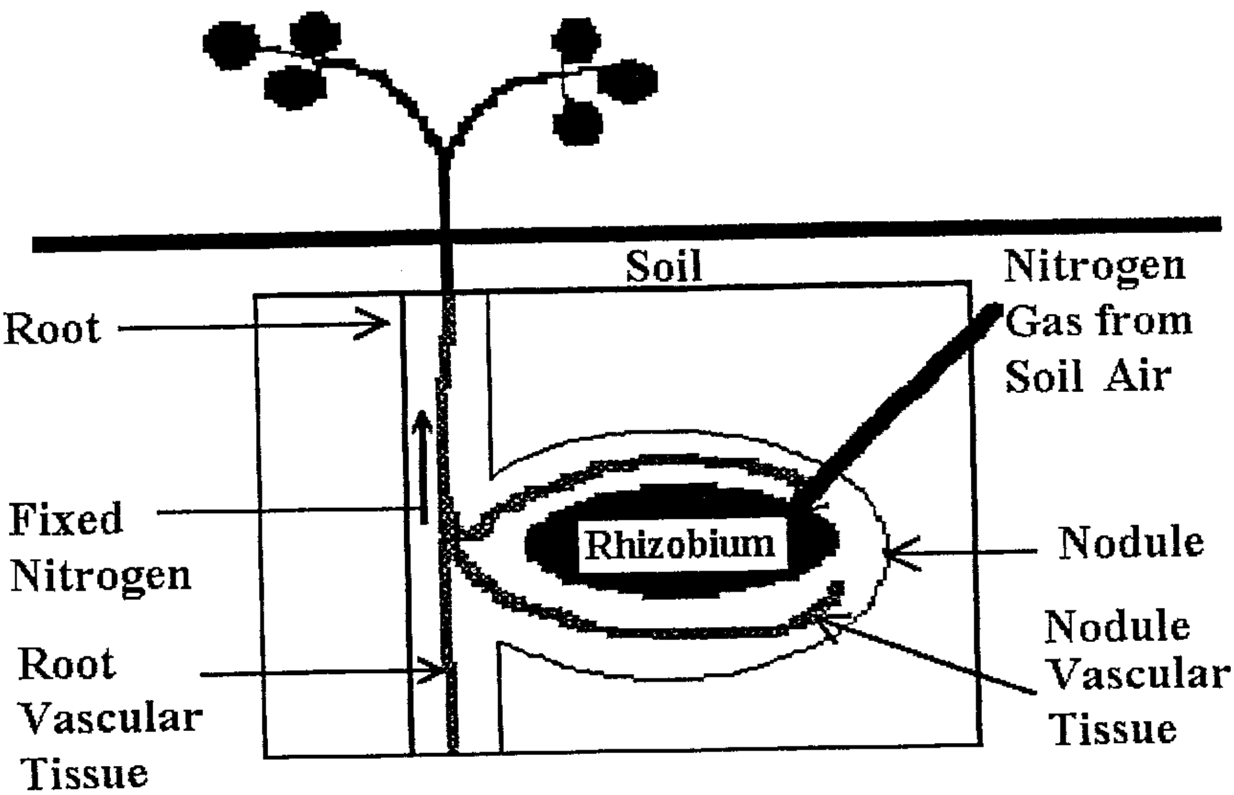


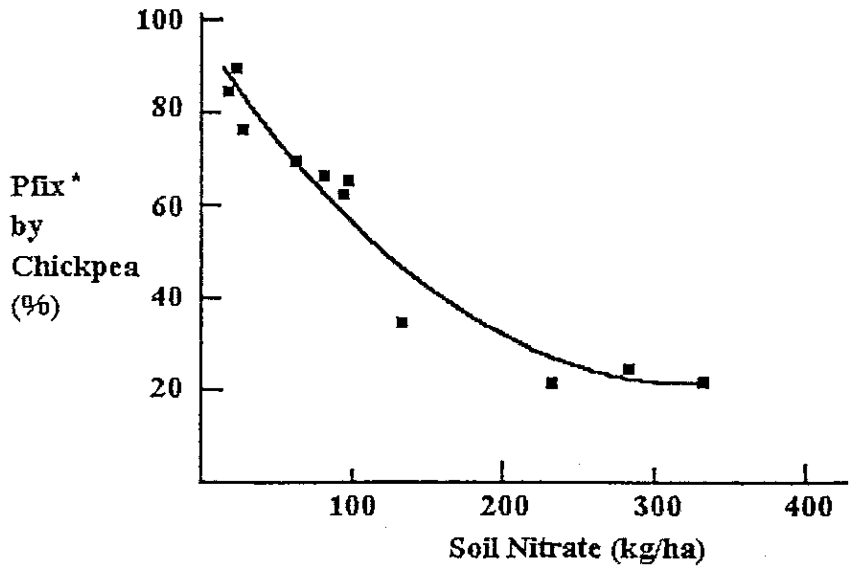
Figure 1. The symbiotic association between Legumes and Nitrogen - Fixing *Rhizobium* Bacteria

HOW MUCH NITROGEN IS FIXED BY LEGUMES

Legumes are most prized for their ability to contribute nitrogen to farming systems and remain the sole net nitrogen input for farmers that either cannot afford chemical fertilizers or wish to avoid the use of chemicals. Alternatively, farmers who wish to save money on nitrogen fertilizers can use legumes to supplement their inputs of chemical nitrogen. In less developed countries, and more recently in the West, legumes are also prized for their grains, while legume pastures remain important for wheat, meat and wool growers. In addition, there are other reasons for growing a legume. Among these are disease control, weed control, improved soil structure and timeliness, all of which contribute to improved productivity in grain, wool, meat, etc.

Before a legume plant can develop nodules and fix nitrogen it must be healthy with a good supply of nutrients and moisture. Initially, nitrogen is obtained from seed reserves and the soil before the plant can meet most of its nitrogen needs from nitrogen fixation. If soil nitrogen reserves are very low, a small quantity of nitrogen fertiliser (up to 10kgN/ha) can be beneficial for the plant if the fertilizer is kept separate from the inoculated seed. The fertiliser boosts plant growth immediately following germination, allowing quicker plant establishment but delays root nodule development. Starter nitrogen at this level is not toxic to rhizobia but adds another cost to crop production which is not necessary and does not improve the nitrogen economy of legumes.

It is vital to remember that a legume can obtain nitrogen from both soil and nitrogen fixation but has a preference for soil nitrogen while it is available (Figure 2). Consequently, the lower the level of available mineral nitrogen in the soil, the greater the amount that must be obtained from the air by nitrogen fixation (Table 1).



* Pfix is the percentage of a legumes total N reqirement met through nitrogen fixation rather than soil N.
Reference: Herridge *et al* (1993)

Figure 2. More Nitrogen is Fixed when Available Soil N is Low

LESS AVAILABLE SOIL MINERAL N = MORE FREE N FIXED FROM THE AIR

If the legume can meet its nitrogen requirements from the soil then it will not expend energy on making nodules and fixing nitrogen. This means a nett loss of soil nitrogen that is not necessary when growing a legume. A soil test is important to determine nitrogen levels. If available mineral nitrogen is high then a crop or pasture other than a legume should be planted. Once nitrogen levels are down then a legume can be used to rebuild soil nitrogen levels or extend the cropping phase without the need for a full application of nitrogenous fertiliser.

In soils with low nitrate levels, the number of live rhizobia present at germination can influence plant productivity very significantly (Table 2). Roughley *et al* (1993) reported that high numbers of rhizobia at germination can double vegetative growth and grain production for lupins over very low numbers of rhizobia at germination. Field trials carried out by researchers also reveals that the normal seed inoculation techniques practised by Australian farmers can result in the death of up to 99% of all rhizobia before germination. These practices result in nitrogen fixation levels significantly below the plants potential under good growing conditions.

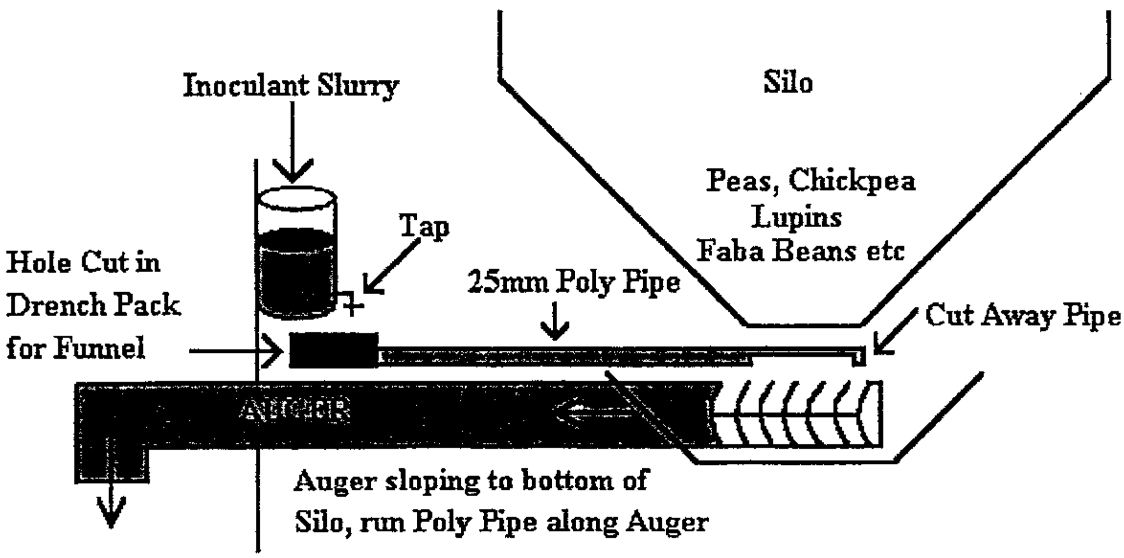


Figure 3. Procedure for Slurry Inoculation of large quantities of Legume Seed

Table 1. Estimates Of Nitrogen Fixed By Field Crops And Pastures

	Total crop nitrogen kg/ha	Amount N fixed kg/ha	% N from fixation
LUPINS			
Rutherglen VIC.	297	288	97
Wagga NSW			
Early planting	192	121	63
Late planting	159	79	50
Walpeup VIC	112	32	29
CHICKPEAS			
Roma QLD			
Low nitrate soil	114	97	85
High nitrate soil	194	33	17
Horsham VIC	120	44	37
PEAS			
Horsham VIC.	177	95	54
Walpeup VIC.	143	28	20
Rutherglen VIC.	191	177	93
SOYBEANS			
Breeza NSW			
Low nitrate soil	230	78	34
High nitrate soil	265	16	6
Kununurra WA	329	312	95
Leeton NSW			
High nitrate soil	406	139	34
Low nitrate soil	348	234	67
LUCERNE	402	288 - 369	72 - 92
SUB CLOVER [1]	4 -128	2 - 119	50 - 93
WHITE CLOVER	87 - 380	54 - 291	62 - 77
MEDIC SPP.	101 - 125	52 - 102	51 - 82

References: Peoples & Herridge Agricultural Science 24 - 29 May 1990. Peoples *et al* (1993) management of nitrogen for sustainable agricultural systems. In: Proceedings of FAO - IAEA Regional Seminar for Asia and the Pacific on Nuclear Related Methods in Soil - Plant Aspects of Sustainable Agriculture. Sri Lanka.

1 Peoples *et al* Surface application of lime and superphosphate to acid soils on growth and nitrogen fixation by subterranean clover in mix and pasture swards. Soil Biol. Biochem. (in press)

Table 2. The Effect Of *Rhizobium* Numbers On Lupin Productivity

Number of *Rhizobium* on seed.	Nodule mass 43 days mg/plant	Dry matter production 197 days kg/ha	Grain yield 226 days kg/ha
1,862,087	393	9037	2088
186,209	222	8673	1802
18,621	65	7759	1899
1,862	12	7184	1648
186	5	4754	1349
19	4	4774	1126
2	4	4663	1085

Sowing rate was 100kg/ha for Unicrop lupins in 4 randomised replicated blocks. Trifluralin (1 litre/ha) and Double Super (100kg/ha). Soil had no history of Lupin planting. Trial was done at Premer NSW

Reference: Roughley *et al* (1993) Soil Biol. Biochem. 25: 1453 - 1458.

REQUIREMENTS FOR SUCCESSFUL NODULATION

The RIGHT *Rhizobium*

Australia has no native legumes (pasture or cropping) of commercial significance. All economic legumes have been introduced and their *Rhizobium* with them. There are many different types (strains) of *Rhizobium* in soils, some native to Australia, some introduced accidentally on exotic seed and some from commercial inoculants. Most native *Rhizobium* strains are not adapted to legume crops or pastures species and will either produce no nodules or ineffective nodules.

Some legume plants are very selective (eg. lupins and chickpeas) allowing a few specific strains of *Rhizobium* to form nodules and even less to fix nitrogen for the plant. Other legumes such as cowpeas are not so fussy and allow a number of different strains to form nodules on their roots. Different *Rhizobium* strains have varying efficiencies when converting nitrogen from the air for plant growth. The most effective strains for each legume plant have been carefully selected by the Departments of Agriculture and C.S.I.R.O. over many years. There are now over 30 different strains of *Rhizobium* bacteria commercially available in Australia (Table 3).

Table 3. Legume Inoculants Commercially Available in Australia

Inoculant Group	Legumes Inoculated	Weight (kg) of Seed Treated by a Standard pack
Lucerne	all Lucerne cultivars Strand & Disc medic	**25** **50**
Medic	Barrel, Burr, Gama, Snail, Murex & all other medics	**50**
Sub Clover	Balansa clover Crimson, Cupped & Rose clover & Sub Clover	**25** **50**
White Clover	White, Alsike, Berseem, Cluster, Ball, Purple, Suckling, Strawberry & Red Clover	**25**
Shaftal Clover	Shaftal or Persian clover	**25**
Lupin	Narrow leaf, Mediterranean white, Sandplain & Yellow lupin Serradella & Slender serradella	**100** **50**
Pea	Pea, Field pea, Sweet pea, Grass pea, Common vetch, Purple vetch, Wooly pod vetch & Lentil	**100**
Faba Bean	Faba bean, Tick bean, Horse or Broad bean	**100**
Chickpea	all Chickpea cultivars	**100**
Soybean	all Soybean cultivars	**100**
Cowpea	Cowpea, Mungbean, Adzuki bean, Green & Black gram, and all *Vigna* species	**100**
Lablab	Dolichos lablab, Pigeon pea Horse & Perennial horse gram	**100** **50**

Other special inoculants are available for Arrowleaf clover, Common Bean, Birdsfoot trefoil, Centro, Desmodium, Fenugreek, Guar, Gliricidia, Joint vetch, Kenya white clover, Leucaena, Lotononis, Lotus, Peanut, Pinto peanut, Sainfoin, Siratro, Stylo, Tagasaste, Verano stylo. Inoculants for legumes not listed may be available on request. Larger packs of inoculant are available for the grain legumes lupin, pea, faba bean, chickpea and soybean. For these inoculants a MAXI pack will treat 250kg of seed and a TONNE pack will treat 1000kg of seed. Methyl cellulose for lime pelleting is available in 30gram, 150gram and 1000gram packs. Nitri-coat legume slurry inoculant protectant is available in 1000g packs for slurry inoculation.

It is possible to assess whether a legume is fixing nitrogen by carefully digging up an intact root system and examining the colour of the inside of several nodules. A pink to reddish brown colour indicates that nitrogen fixation is occurring. Nodules that are white, green or brown inside are not fixing nitrogen. It is important to examine several nodules because some may be too young (white inside) or too old (brown inside). Although a pink to reddish brown colour inside nodules is indicative of nitrogen fixation, it does not guarantee that the nodule contains the recommended commercial strain of rhizobia. If inoculation practices were below standard, the nodule may contain native soil rhizobia that are less efficient at fixing nitrogen.

The RIGHT Place & The RIGHT Time

Legume inoculation is the placement of *Rhizobium* bacteria in a position where they will be able to infect the root as it emerges from the seed at germination. In practice, inoculation usually involves the sticking of the appropriate *Rhizobium* bacteria to the legume seed before sowing. *Rhizobium* bacteria are small single cells shaped like rods about one thousandth of a millimetre long that have tails (flagella) and are motile in solution. They are mainly confined to soil and humus particles and have to passively wait for a growing root and moisture to come along. The bacteria will then congregate around the root. Consequently, the location of the RIGHT *Rhizobium* in high numbers around the seed at germination is critical.

The bacteria must be either :

1. on the seed coat at germination and "picked up" by the emerging root (seed inoculation using a slurry or lime pellet).
2. in the soil immediately surrounding the seed in high numbers (spray inoculation).
3. washed through the soil profile by irrigation and come into contact with the growing root (water run inoculation).

Keeping *Rhizobium* ALIVE on the Journey from Packet to Soil

To obtain the maximum return from legume inoculant it is essential to realize that *Rhizobium* bacteria are very delicate organisms that do not survive well outside the soil or in very dry soil. Commercial packets of inoculant contain a minimum of 1,000 million live *Rhizobium* bacteria per gram of inoculant powder. This is made possible through the use of a specially formulated peat in sealed plastic bags which contain no other microorganisms. The bacteria will survive in these bags at between 4 and 15^{o}C for at least one year and shorter periods at 30^{o}C.

Once the packet of inoculant is opened the *Rhizobium* must reach moist soil as soon as possible otherwise they start to die and the peat will become contaminated by other microorganisms. On the way from the packet to the soil there are many factors which reduce survival of the bacteria. Principal among these is drying out of the bacteria on seed. This problem is worsened by sowing into dry soil. Allowing inoculant to dry on seed is common practice because it helps sowing, however, once the inoculant has dried on the seed it is important to sow as soon as possible. Sowing the same day as inoculation is ideal with 24 hours being the maximum time allowed.

Other factors contribute to the death of *Rhizobium* on seed:

1. Temperatures above 30°C, direct sunlight or hot winds.
2. High speed mechanical mixing that mechanically damages the seed coat or heats seed.
3. Toxic chemicals contaminating slurry water such as chlorine or traces of agricultural chemicals from used containers.
4. *Rhizobium* coming into contact with toxic chemicals on the seed coat or in the soil eg. acidic superphosphate, traces of fungicides and herbicides.

Research data from the N.S.W. Dept. of Ag. (Table 4) dramatically shows how *Rhizobium* bacteria are lost during the inoculation and sowing processes. While conditions for inoculant survival were reportedly good in this trial, harsh mechanical mixing and drying probably contributed to the poor survival after inoculation. A change in the inoculation procedure can greatly improve the survival of the inoculant (Table 5). Careful handling of inoculant allows more rhizobia to survive to make more nitrogen fixing nodules on legume roots.

Table 4. Loss of Lupin Rhizobia During Inoculation & Sowing

Event	Time Elapsed (hours)	Number & % Surviving per seed
At Inoculation (0.75% methylcellulose slurry)	0	1,445,000 * (100%)
Lupins mixed in verticle feed mixer and augered into truck	1	141,200 # (9.8%)
Transported to field and augered into air seeder box (sampled from seeder box)	4.75	11,200 # (0.8%)
Planted by air seeder	4.8	6,761 # (0.5%)
Recovered from soil	22.5	1,175 # (0.1%)

* theoretical number derived from number in inoculant slurry
# Numbers determined by MPN method

Reference: Roughley *et.al*1993 Soil Biol. Biochem. 25: 1453 - 1458.

Table 5. Reduced Handling Improves the Survival of Lupin Rhizobia

Inoculation Procedure	Time	Numbers & % of Live *Rhizobium* Recovered Per Seed
At Inoculation (1% Nitri - coat)	0	1,000,000 (100%)
Unicrop lupins mixed with a slow speed mixer (auger) one pass to bags	1 hr	600,000 (60%)
Seed sampled in planter box	5 hrs	400,000 (40%)

Ambient temperature 25^{o}C, overcast conditions.All seed surface sterilised by 65^{o}C over night. Seed mixer surface sterilised and washed before use. Numbers determined by direct plate count on selective media. Unpublished data from Inoculant Services Australia Pty Ltd 1993J.

Recommendations For Keeping *Rhizobium* Alive:

1. Closely follow the instructions on the packet of inoculant.
2. Dry slurry slowly onto the seed. Avoid direct sunlight on seed and ambient temperatures over 30^{o}C if possible.
3. Sow treated seed immediately after inoculating.
4. Store treated seed in a shaded area before sowing.
5. When mixing slurry and seed together avoid harsh mechanical mixers eg. high speed augers or abrasive stock feed mixers.
6. Use clean rain water for slurry water. Wash all mixing equipment before use.
7. Avoid contact between toxic chemicals and treated seed.

RE-INOCULATING "OLD" GROUND

It has frequently been said that once a legume has been inoculated with the correct type of rhizobia then it is no longer necessary to inoculate that legume when sown into the same ground in later seasons. The truth is that a recommendation needs to be made for each case depending upon which legume is being sown, soil pH, time elapsed since inoculation was last used, previous crops or pastures grown, salinity problems and waterlogging.

Rhizobium strains introduced as commercial inoculants often do not persist because of competition from native soil microorganisms or other soil factors such as low pH. Those that do persist will be present in low numbers and/or concentrated in specific spots where old nodules have decayed from previous crops or pastures. While the persistence of these *Rhizobium* may produce nodules if the same legume is sown again, the number of nodules may be less than optimal and the nodules will be slower to form compared to using a fresh inoculation.

As a general rule, rhizobia for lucerne, medics, clovers, peas, vetch, tares and faba beans survive well in more alkaline soils. In contrast, rhizobia for lupins, serradella and soybeans survive well in more acidic soils. Salinity, waterlogging, low levels of organic matter and prolonged hot, dry periods will reduce the numbers of rhizobia in the soil. Another consideration is that new strains of improved rhizobia are routinely replacing "old" strains in commercial inoculants. In the past 4 years there have been new strains for lucerne, medic, faba bean and the pea group (group E). To introduce these strains into soils and ensure that most nodules are formed by the new strain against competition from the "old" strain which is already in the soil, legumes need to be inoculated on sowing.

A most important consideration is that maximum nodulation and nitrogen fixation require high numbers of the right rhizobia present at germination. This is especially important under good growing conditions when a lack of nitrogen must not limit a legumes productivity. The highest numbers of rhizobia are present when legumes are properly inoculated every time they are sown. In addition, sound inoculation practice ensures that most nodules are formed by the preferred inoculant strain, rather than outdated strains still present in the soil. It is also essential for inoculant rhizobia to out-compete native strains of rhizobia already present in the soil which are usually not effective on commercial legumes grown in Australia.

The recommendation from legume agronomists is that inoculation is cheap insurance against legume crop or pasture failure due to nitrogen deficiency.

HOW TO APPLY LEGUME INOCULANT

The key to successful use of legume inoculants is to remain aware that the inoculant consists of living *Rhizobium* bacteria that have very little protection when they are outside their natural environment which is the soil. The aim of legume inoculation is to place as many living rhizobia as possible around the seed at the time of germination.

When purchasing a pack of inoculant check the expiry date. The date will only be valid if the inoculant has been stored under cover away from toxic fumes and preferably at cool temperatures (between 4 and 15°C).

Packets of legume inoculant contain at least one thousand million individual *Rhizobium* cells per gram of peat powder. The weight of seed treated by a packet of inoculant will vary depending on the size of the seed to account for varying numbers of seed per kilogram (Table 6). Check the instructions on the front of the packet for the quantity of seed to be inoculated.

Table 6. Quantities of Seed Inoculated per Standard Pack * of Inoculant

SEED SIZE			
Very Small	**Small**	**Medium**	**Large**
10kg	**25kg**	**50kg**	**100kg**
Lotononis ♦Lotus	♦White Clover ♦Lucerne ♦Shaftal Clover ♦Red Clover ♦Balansa Clover Glycine	♦Sub Clover ♦Medic ♦Serradella	Lupins Faba Beans Peas Chickpea Soybean Mungbean Broadbean Vetch Cowpea Peanut LabLab

♦ Seeds recommended to be inoculated and lime pelleted when sown into acid soil

* Multiply the weight of seed by 2.5 for a maxi pack and by 10 for a tonne pack

Lime Pelleting Seed (For 1 Standard Pack)

1. Prepare a 1.5% solution of methyl cellulose by dissolving 15 grams of methyl cellulose in 200 mls of hot water, then make up to 1 litre with cold water.
2. Allow the adhesive to cool, preferably for at least 3 hours while the methyl cellulose hydrates. It must cool to below 30°C.
3. Mix one standard pack of inoculant into one litre of the cool adhesive solution and mix until a smooth slurry is formed.
4. Pour the slurry mixture over the correct weight of seed and mix until all the seeds are evenly coated. The most practical method of mixing seed is in a cement mixer or on a large tarpaulin.
5. Note: All seeds must be damp and evenly coated with moisture before applying the lime. The standard packet of inoculant may be diluted with up to 3 litres of methyl cellulose solution over the same quantity of seed to obtain better coverage. Patchy nodulation will occur from incomplete seed coverage.
6. When seeds are evenly coated add 10kg of micro fine lime all at once and continue mixing until all the seeds are evenly coated with the lime. It is important to disperse the lime quickly otherwise uneven pelleting will result and sloughing off will occur. Do not agitate for any longer than necessary. Do not use builders lime etc.
7. Plastaid (Goliath Cement Co Ltd) may be used instead of micro fine lime. Generally it makes a better seed pellet than lime.

8. Best results are obtained by sowing inoculated seed the same day as inoculation. Store before sowing for no longer than 7 days out of direct sunlight and below 30°C.

Slurry Inoculating Legume Seeds

1. Prepare a smooth peat slurry by mixing peat inoculant into cool clean water (1 litre/standard pack, 2.5 litres/maxi pack, 10 litres/ tonne pack). The water rate can be doubled for a more even coverage of seed.
2. Mix the slurry over the correct weight of seed as directed on the pack. All seeds must be coated.
3. Sow the seed as soon as possible after inoculation, preferably within 24 hours.
4. Mixing the slurry over the seed is time consuming. For small quantities a cement mixer may be used. For larger quantities of seed a simple method is to dribble the inoculant slurry into a seed auger hopper as the seed passes up the auger (Figure 3).
5. When auger inoculating large seed, minimize the agitation. Violent agitation leads to a loss of seed viability.

Spray Inoculation

Rhizobium peat cultures may be mixed with water and sprayed (dribbled) into the planting furrow as the seed is sown. The peat slurry must be filtered through a fine cloth prior to adding to the spray tank. It is important that the soil around the planted seed is inoculated. This method combines legume seed inoculation with sowing and has advantages of timeliness and efficiency for broadacre sowing.

The machinery required is a spraying system able to deliver equal quantities of water- suspended inoculant to each sowing tine. Capillary tubes of equal length must be used of 2.5mm internal diameter to deliver equal quantities behind each sowing tine to the seed furrow. Roller, flexible vane diaphragm pumps work well and are required to provide good agitation to the spray tank as well as deliver 100 l/ha of mixture. Pressure in the system should be approximately 140kPa and no greater than 170kPa. A 40 mesh (0.4mm aperture) pancake type filter should be fitted immediately after the pump to reduce the chance of blockage from peat.

Spray inoculation is not recommended by the W.A. Dept. of Ag. for broad acre crops in Western Australia.

The Advantages;

- delivers high numbers of rhizobia alive around the seed.
- delivers rhizobia directly into the soil from the packet without any drying out.
- there is no need to inoculate seed prior to sowing.
- the seed can be treated with toxic seed dressings.

The Disadvantages with Spray Inoculation;

- large volumes of water are required during seeding (100 l/ha).
- peat tends to block the spray equipment causing nitrogen-deficiency symptoms in some plants or rows.
- the method is not satisfactory with air seeders.
- it is necessary to increase the water application rate with close row spacing drills, for example, wheat drills used for sowing legumes.

For more detail on Spray Inoculation refer to Agfact P4.1.2, "Spray Inoculating Grain Legumes" First Edition. Division of Plant Industry, Canberra.

MIXING INOCULANTS WITH CHEMICALS

Maximum nodulation and nitrogen fixation requires high numbers of live rhizobia around the emerging root at germination. It follows that all factors which reduce the survival of rhizobia on their way from the packet to the soil and seed germination will also reduce nodulation and nitrogen fixation. The major killer of rhizobia is drying out on seed usually caused by the prolonged storage of inoculated seed. Direct exposure to toxic chemicals simply makes the problem worse. The solution to both drying out and exposure to toxic chemicals is to use spray inoculation to directly inject the inoculant whilst sowing. In this way rhizobia travel directly from the packet to the soil without drying out and are dispersed around the seed, not on the seed in direct contact with seed dressings. The major problem with spray inoculation is the quantity of water needed which is about 100 litres per hectare.

The number and variety of chemicals on the market and the ongoing release of new chemical products means that reliable research information on the compatibility of chemicals with legume inoculants is limited. Until more information is available, the following recommendations apply:

1. Nitrogen fertilizers are not necessary if legumes are properly inoculated.
2. Acidic superphosphate will kill rhizobia if direct contact is made. Basic or neutralized superphosphate can be mixed with inoculated seed immediately before sowing. Granulated superphosphate can be mixed with lime pelleted seed.
3. Fungicide seed dressings should be applied to the seed first and allowed to dry. Seed inoculation should then be done immediately before sowing into moist, not dry, soil. Alternatively, separate slurries can be prepared for the inoculant and seed dressing and applied separately to the seed immediately before sowing into a moist soil.
4. Make sure that the equipment used for inoculation is free from all chemical residues.

MANAGING LEGUME NITROGEN FOR ON-FARM PRODUCTIVITY

There is no doubt that legume crops and pastures can fix large quantities of nitrogen from the air if they are well nodulated by the most effective strain of *Rhizobium* bacteria. The reality is that very few farmers obtain 450 kgN/ha/season from their legumes. The most common reasons for this have been discussed in previous sections. Briefly, maximum nitrogen gains from legumes are made when they are grown in nitrogen deficient soils (with no other nutrient deficiencies) and supplied with the maximum number of the correct type of rhizobia at seed germination. Good growing conditions must also prevail.

Well nodulated pasture legumes are highly successful in building reserves of soil organic nitrogen, plant available mineral nitrogen, and in improving soil structure. Once the pasture phase is replaced by cereal cropping both nitrogen fertility and soil structure decline rapidly. For this reason alternate pasture-cropping phases are recommended.

Benefits to soil nitrogen levels are not as obvious for crop legumes because a large proportion of the nitrogen fixed is removed as harvested grain and the legume is only grown for one season at a time. The most consistent and widespread benefit of crop legumes is their reduced use of available soil nitrogen when compared to a cereal. Hence nitrogen fixation relieves the demand on soil nitrogen leaving more behind for the next crop. Some fixed nitrogen is also left behind in legume plant residue after harvest and made available after the residues decay.

In a cropping rotation it is common for a cereal to produce a greater yield after a legume than when sown after a cereal (Tables 7 & 8). The soil must supply 30-40 kgN/ha in an available form for each tonne of wheat grain produced which translates to about 200 kgN/ha required for maximum wheat yield under good growing conditions. Reported yield increases to previous legume crops are mainly in the range of 1-2 tonne/ha, representing 50-80% increases over yields in the cereal-cereal sequences. The improved yield is often attributed to a reduction in pests and/or diseases, an improvement in soil structure and an increase in the soil nitrogen levels as a consequence of nitrogen fixation by the legume. Of these benefits, nitrogen fixation is the most reproducible, frequently being equivalent to 50-100 kgN/ha as fertiliser.

Table 7. Effects of Crop Legumes on Cereal Yields, Relative to a Cereal-Cereal Sequence

Pre-crop	Increase in Cereal Yield		Fertiliser N equivalence (kg/ha)
	(tonne/ha)	(%)	
Chickpea-wheat	1.43	88	>50
Faba bean-wheat	1.25	77	>50
Lupin-wheat	1.33	82	>50
Black gram-sorghum	3.68	79	68
Green gram-sorghum	2.82	61	68

Reference: Herridge et al 1993. Legume N_2 Fixation - An Efficient Source of N for Cereal Production.
In: Proceedings of the SAO-LAEA Regional Seminar for Asia & the Pacific on Nuclear Related Methods in Soil-Plant Aspects of Sustainable Agriculture. Sri Lanka.

Table 8. Effects of 1989 Pre-Crop on Yields of Wheat in 1990

Pre-crop	Crop N (kg/ha) at Harvest	Soil nitrate (kg/ha) Harvest	Soil nitrate (kg/ha) Sowing	Wheat yield (tonne/ha)
1989	1989	1989	1990	1990
Wheat	33	26	58	1.65
Chickpea	102	59	104	3.37
Fallow	0	76	122	3.41

Field trials at Windridge in the Northern Cereal Belt of N.S.W. (Herridge *et al* 1993)

Increasing the percentage of total legume nitrogen requirements obtained from nitrogen fixation rather than from the soil is critical for getting the most out of legumes (Table 9). This means sowing legumes into soils with low levels of available mineral nitrogen. The other requirement is to remove limitations on nitrogen fixation such as poor mineral nutrition (especially P), water stress, weeds, disease, pests etc.

Table 9. Ranges in Values for P_{fix} (%) and N_2 Fixed for the Annual Crop Legumes

Legume	P_{fix} (%)	N_2 fixed (kg/ha)
Cool-season		
Chickpea	8 - 82	3 - 141
Lentil	39 - 87	10 - 192
Pea	23 - 83	17 - 330
Faba bean	59 - 92	78 - 330
Warm-season		
Soybean	0 - 95	0 - 450
Pigeon pea	6 - 88	4 - 88
Cowpea	8 - 89	9 - 125
Common bean	0 - 73	0 - 125
Ground nut	22 - 92	37 - 206

P fix is the percentage of the legumes total nitrogen requirements obtained from nitrogen fixation rather than the soil. Reference: Herridge *et al* (1993).

Examples of management practices that increase legume nitrogen fixation are presented in Table 10. Notice that the proportion of total plant nitrogen fixed from the air rather than obtained from the soil is increased by using tillage practice and crop sequence to reduce inhibition by high soil available nitrogen. Other important factors that increase nitrogen fixation are adequate plant nutrition (particularly phosphorous) and weed control. Something to be considered is the use of legume cultivars and species that can fix nitrogen without being inhibited by soil nitrogen, the advantage being that more of their nitrogen requirements are met by fixation rather than depleting soil nitrogen.

Table 10. Crop & Soil Management Practices leading to Increased Nitrogen Fixation

Management practice	Crop	Total plant N	Nitrogen fixed (kg/ha)	Increase
Inoculation				
yes	Soybean	267	149	+149
no		108	0	
Tillage practice				
no tillage	Soybean	264	232	+52
cultivation		245	180	
Crop sequence				
winter crop	Soybean	267	149	+117
winter fallow		347	32	
Time of sowing				
winter sown	Chickpea	142	115	+109
spring sown		21	6	
Pest control				
insecticide	Lentil	119	107	+23
no insecticide		98	84	
Species (Adjacent Sites)				
Large Plant	Faba Bean	220	176	+151
Small Plant	Chickpea	61	25	

Reference: Herridge *et al* (1993)

Surveys by scientists of commercial legume crops show that legumes often obtain a large proportion of their nitrogen requirements from the soil and not nitrogen fixation, and that yields are well below potential. Making more economical use of legumes to manage soil nitrogen levels will require routine measurement of the concentrations of available mineral nitrogen in the soil, the amount of nitrogen left behind in the soil after harvest and how much nitrogen is lost from the farm as harvested grain.

There are very few quick and easy ways of obtaining reliable information in the field for use in the management of legume nitrogen. The development of tools for this purpose awaits further research and innovation.

Free advice on legumes and inoculation can be obtained by contacting:

Greg Bender / Sandy Montague
Inoculant Services Australia Pty Ltd
RMB 8340
Wodonga Victoria 3691

Phone: (060) 26 4437
Fax: (060) 26 4524