INOCULATING LEGUMES



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Rhizobia

- Rhizobia are living micro-organisms (also called root-nodule bacteria)
- Nodules on legume roots contain rhizobia, which fix nitrogen from the air
- Each type of legume is nodulated by a specific type of rhizobia, which are identified as belonging to different "Inoculant Groups"
- Rhizobia in inoculants do not survive well in extremes of temperature (over 30°C and at freezing temperatures) or drying conditions that lead to desiccation
- Different legume-rhizobia associations will tolerate different soil conditions. Generally rhizobia preferences are similar to those of the legume host
 - medic, pea, faba bean and chickpea rhizobia prefer neutral to alkaline soils (pH CaCl $_2$ 6.0 7.5)
 - lupin rhizobia tolerate acid soils (pH ${\rm CaCl}_{\rm 2}$ less than 6.0)

Non-nodulated soybean (right front) and well-nodulated soybean (left rear)

Inoculation benefits

- Inoculated legumes will fix 25kg of nitrogen per tonne of legume shoot dry matter, on average
- Low soil nitrate levels, good nodulation and agronomic practices that promote high legume production all increase N inputs from N fixation
- Decomposing legume residues are a good source of slow-release nitrogen for a following crop
- Economic benefits of legumes in crop production systems can be substantial, both from N fixation and the disease-break effect
- For example: Inoculated faba beans in western Victoria yielded 2.7t/ha (1 t/ha more than the uninoculated crop) and added 180kg/ha of extra fixed nitrogen to the soil





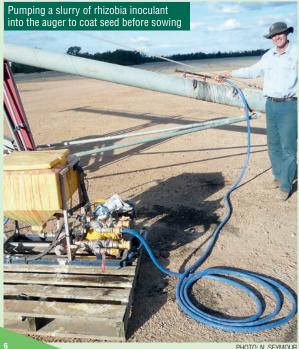
* Australian Inoculants Research Group

Getting inoculation right

- Use quality inoculants (the Green Tick logo is a trademark of AIRG* approval)
- Match the correct inoculant group to each legume
- Inoculants contain LIVE bacteria: make sure they are kept in moderate temperatures (less than 30°C, not frozen) away from sunlight and chemicals
- Sow freshly inoculated seed as soon as possible and definitely within 24 hours of inoculation
- Use clean potable water for dilution when using liquids or slurries, and make sure holding tanks are free from chemical and fertiliser residues
- Many pesticides, mineral and organic fertilisers are toxic to rhizobia and should never be mixed with rhizobia
- Rhizobia can be compatible with seed pickles or dressings for a limited time prior to sowing (see *Inoculating legumes: a practical guide* (2012) for manufacturer's guidelines and Table 5.4 in Resource #1). Always apply the seed dressing first and allow it to fully dry before applying the rhizobia as a second process
- Always use inoculants before their expiry date
- Reseal opened bags of peat inoculant and use them within 2 weeks of first opening the bag

USING DIFFERENT INOCULANT FORMULATIONS

	PEAT	FREEZE-DRIED	GRANULAR
DESCRIPTION	Finely ground sterilised peat containing a high density of rhizobia	Powder containing a very high density of rhizobia	Granules of peat or clay or a mixture; contain a lower number of rhizobia per gram
STORAGE	Winter legume inoculants – refrigerate at 4°C; summer legume inoculants – store in cool, dry place	Refrigerate at 4°C DO NOT FREEZE	Store in a cool and dry place away from direct sunlight
COMMON APPLICATION	Mix with clean water to make a slurry, apply direct to seed (except for peanut). Can also be used in furrow	Reconstitute with clean water and add protective compound. The liquid suspension is applied direct to seed (except for peanut) or can be injected into the furrow	Granules are delivered in furrow at sowing. DO NOT allow granules to become moist during seeding as some products can cause blockages
USING Additives	If used, ensure adhesive solutions are cooled before rhizobia are added. Generally NOT COMPATIBLE with mineral and organic fertilisers and pesticides; check manufacturer's guidelines	Generally NOT COMPATIBLE with mineral and organic fertilisers and pesticides; check manufacturer's guidelines	Check inoculant manufacturer's compatibility guidelines
SOWING	Best sown on day of coating into moist soil	Sow treated seed into moist soil within 5 hours of application	A third seeding box should be used to keep the granular formulation separate from fertilisers and pickled seed



Seed coating in practice

- Rates of application, i.e. volumes and weights of formulation, water and seed, are given on inoculant packets
- Peat formulation is made into a slurry using clean potable water in a clean drum and mixing well
- For pasture seed, an adhesive is often added to the slurry
- NOTE: Avoid fertiliser and pesticide residues and saline water
- Always grade seed first to remove pod debris and fine grain dust. which can block seeders.
- Freshly prepared slurry is pumped from the drum (or poured) into the path of grain legume seed going up a slow-moving flighted auger into a grain bin
- Pasture seed, being small, can be coated in a concrete mixer or by mixing with a shovel on a concrete floor
- Most temperate pasture seed is best coated with fine lime (builders' and slaked lime should be avoided)
- Freeze-dried inoculant can be applied in the same way as peat slurry, as per manufacturer's instructions
- Allow slurry-treated seed to dry before filling air-seeders to prevent 'bridging' in the tank

Assessment of nodulation

- A well-nodulated plant has nodules on the crown (where the root meets the shoot) and on the tap root and lateral roots
- Take a few plants from each of several locations in the paddock, to cover paddock variability
- Carefully dig up plants with root systems intact and gently wash roots (e.g. in a bucket of water) to remove soil

Cut nodules open: pink-coloured tissue indicates active N fixation
 Desired numbers of nodules per plant at 8-10 weeks old are given in the sections of the guide for individual legume species
 Assessment of nodulation for chickpea: 0-1 = inadequate 2-3 = adequate 4-5 = good





PHOTO: N. SEYMOUR

Troubleshooting nodulation failure

- Indications of poor nodulation are yellowing young leaves, yellow and/or stunted patches of plants, and lack of nodules on root systems
- Nodulation failure is difficult to remedy, except by adding inorganic nitrogen, which can be costly

- Possible other remedies (if done immediately):
 - In flood or sprinkler-irrigated fields, add slurry or liquid inoculant to the irrigation water
 - Over-sow a granular inoculant close to the original sowing furrow



Chickpea Cicer arietinum INOCULANT GROUP N (STRAIN CC1192)

CHICKPEA CROPS AND RHIZOBIA

- Chickpea has a very specific rhizobia requirement
- Rhizobia generally absent outside main growing areas

CHICKPEA INOCULATION

- Peat formulation: as slurry to seed (most common) or in furrow
- Freeze-dried formulation: as slurry to seed or liquid in furrow
- Granular formulation: in furrow at sowing

ASSESSMENT OF NODULATION

After 8 weeks, 10 – 30 pink nodules per plant are satisfactory

HIGH	Chickpea not previously grown
MODERATE	Previous inoculated chickpea crop more than 4 years ago,
	or recent crop performed below expectation
LOW	Well-nodulated chickpea crop in past 2 years



Field Pea, Vetch Pisum sativum, Vicia species **INOCULANT GROUP E (STRAIN SU303)** (GROUP F IS ALSO SUITABLE FOR PEA)

PEA AND VETCH CROPS AND RHIZOBIA

- Where not previously grown, inoculate with rhizobia for effective nodulation and nitrogen fixation
- Group E rhizobia are moderately sensitive to soil acidity

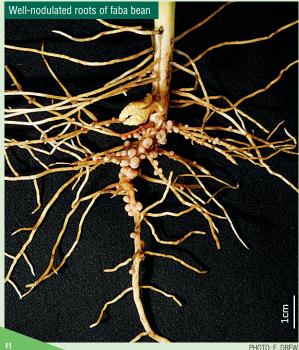
PEA AND VETCH INOCULATION

- Peat formulation: as slurry to seed (most common) or in furrow
- Freeze-dried formulation: as slurry to seed or liquid in furrow
- Granular formulation: in furrow at sowing

ASSESSMENT OF NODULATION

After 8 weeks, 100 pink nodules per plant (heavier textured soils) and 20 nodules per plant (lighter soils) are satisfactory

HIGH	Crop not previously grown, or soils with pH (CaCl ₂) below 6.0
	and high summer temperatures (over 35°C for 40 days)
MODERATE	Previous inoculated pea, vetch (or bean) crop more than
	4 years ago, or recent pea/vetch crop nodulated poorly and
	performed below expectation
LOW	Soils loam or clay, neutral to alkaline pH, and recent
	well-nodulated host crop



Faba Bean, Broad Bean, Lentil Vicia faba. Lens culinaris **INOCULANT GROUP F** (STRAIN WSM1455)

BEAN AND LENTIL CROPS AND RHIZOBIA

- Where not previously grown, inoculate with rhizobia for effective nodulation and nitrogen fixation
- Group F rhizobia are moderately sensitive to soil acidity

BEAN AND LENTIL INOCULATION

- Peat formulation: as slurry to seed (most common) or in furrow
- Freeze-dried formulation: as slurry to seed or liquid in furrow
- Granular formulation: in furrow at sowing

ASSESSMENT OF NODULATION

After 8 weeks, 100 pink nodules per plant (heavier textured soils) and 20 nodules per plant (lighter soils) are satisfactory

- HIGH Crop not previously grown, or soils with pH (CaCl₂) below 6.0 and high summer temperatures (over 35°C for 40 days) **MODERATE** Previous inoculated bean or lentil crop more than 4 years ago, or recent bean/lentil crop nodulated poorly and performed below expectation LOW Soils loam or clay, neutral to alkaline pH, and recent well-nodulated host crop



Lupin and Serradella Lupinus and Ornithopus species **INOCULANT GROUPS G** (LUPIN STRAIN WU425) S (SERRADELLA STRAIN WSM471)

LUPIN AND SERBADELLA CROPS AND RHIZOBIA

- Groups G and S both nodulate lupin and serradella
- Inoculation is essential where lupin or serradella have not been grown, because sandy soils are often acutely N-deficient

LUPIN AND SERRADELLA INOCULATION

- Peat formulation as slurry to seed (most common) or in furrow
- Lime pelleting of serradella recommended in all states except WA
- Inoculating podded serradella: adjust liquid volumes to ensure even distribution; follow manufacturer's instructions carefully

ASSESSMENT OF NODULATION AFTER 8 WEEKS

- Lupin: crown region (top of root system) covered with nodules
- Serradella: more than 20 pink nodules per plant are satisfactory

- HIGH No previous lupin or serradella grown in paddock **MODERATE** More than 4 years since growing inoculated legume host,
 - or recent crop performed below expectation
- LOW In the north and central regions of the WA wheat/sheep belt OR vigorous lupin/serradella growth and good nodulation in past 4 years



Peanut or groundnut Arachis hypogaea INOCULANT GROUP P (STRAIN NC92)

PEANUT CROPS AND RHIZOBIA

Main growing area: Queensland, with some growers in northern NSW and northern WA

PEANUT INOCULATION

PHOTO: N. SEYMOUR

- Recommend water injection of peat or freeze-dried inoculum to prevent damage to seed from slurry coating
- Granular inoculum dispensed with seed at planting

ASSESSMENT OF NODULATION

- Peanut can form many nodules, i.e. more than 100/plant
- Satisfactory number of nodules per plant 8-10 weeks after sowing: not possible to stipulate

LIKELIHOOD OF CROP RESPONSE TO INOCULATION HIGH Peanut not previously grown

 High
 Peanut not previously grown

 MODERATE
 Where there is a history of poor nodulation

 LOW
 Recent and/or intensive peanut cultivation



Mungbean and Cowpea Vigna radiata (green gram), V. mungo (black gram) and V. unguiculata INOCULANT GROUP I (STRAIN CB1015)

MUNGBEAN, COWPEA CROPS AND RHIZOBIA

Where not previously grown, inoculate with rhizobia for effective nodulation and nitrogen fixation

MUNGBEAN AND COWPEA INOCULATION

Peat formulation: as slurry to seed (most common) or in furrow
 Freeze-dried formulation: as slurry to seed or liquid in furrow
 Granular formulation: in furrow at sowing

ASSESSMENT OF NODULATION

After 8 weeks, more than 20 pink nodules per plant are satisfactory

LIKELIHOOD OF CROP RESPONSE TO INOCULATION

- HIGH No previous mungbean, cowpea or other related *Vigna* species grown
- MODERATE Previous inoculated crop more than 4 years ago, or recent crop performed below expectation
- LOW Recent and/or intensive mungbean or cowpea cultivation

PHOTO: G. CUMMING



Soybean Glycine max **INOCULANT GROUP H** (STRAIN CB1809)

SOYBEAN CROPS AND RHIZOBIA

- Soybean specifically requires Group H rhizobia and will not nodulate with the same rhizobia as mungbean or cowpea
- Good agronomy and inoculation practice are needed for good yield and nitrogen fixation

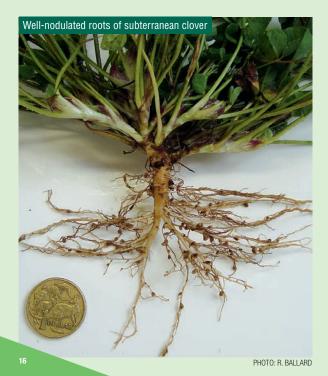
SOYBEAN INOCULATION

- Peat formulation: as slurry to seed (most common) or in furrow
- Freeze-dried formulation: as slurry to seed or liquid in furrow
- Granular formulation: in furrow at sowing

ASSESSMENT OF NODULATION

After 8 weeks, more than 20 pink nodules per plant are satisfactory

HIGH	Soybean not previously grown, or very alkaline or very acid soils
MODERATE	Previous inoculated soybean crop more than 4 years ag
LOW	Recent and/or intensive cultivation of soybean



Annual clovers *Trifolium* species

(SUBTERRANEAN, BALANSA, PERSIAN, BLADDER, ARROWLEAF, ROSE, GLAND, CRIMSON, PURPLE, CUPPED AND HELMET)

INOCULANT GROUP C (STRAIN WSM1325)

ANNUAL CLOVERS AND RHIZOBIA

Inoculation is essential for gland, bladder and arrow-leaf clovers and recommended for all other annual clovers

ANNUAL CLOVER INOCULATION

- Most commonly applied as a slurry of peat followed by pelleting with fine lime or other suitable product
- All inoculant formulation types can be used
- Seed often purchased already inoculated. Check time from inoculation not more than six weeks. Freshly inoculated seed is best

ASSESSMENT OF NODULATION

■ Good: 50 – 100 pink nodules per plant after 8 weeks of growth

LIKELIHOOD OF CROP RESPONSE TO INOCULATION

MODERATE No annual clover host in past 4 years and soil pH (CaCl.) below 5.5; annual clover present, but growth or nodulation below expectation

LOW Soils with neutral or alkaline pH and recent history of good clover growth and nodulation



Annual medics Medicago species (except strand and disc) INOCULANT GROUP AM (STRAIN WSM1115)

ANNUAL MEDICS AND RHIZOBIA

- Inoculation always recommended for burr, murex and sphere medic, sown into slightly acidic soils (pH CaCl, below 6.0)
- DO NOT use Group AL inoculant because it is less effective at fixing nitrogen with some medic species in this group

ANNUAL MEDIC INOCULATION

- Most commonly applied as a slurry of peat followed by pelleting with fine lime or other suitable product
- Granular and freeze-dried inoculant formulations are available
- Seed often purchased already inoculated. Check time from inoculation. Freshly inoculated seed is best

ASSESSMENT OF NODULATION

■ Good: 10 – 20 pink nodules per plant after 8 weeks of growth

LIKELIHOOD OF CROP RESPONSE TO INOCULATION

 HIGH
 Burr, sphere and murex medic on soils with pH (CaCl₂) below
 6.0 OR no history of sown or naturalised medic

 MODERATE
 Medic present, but growth or nodulation below expectation

 LOW
 Neutral to alkaline loam or clay soils with recent vigorous medic growth and good nodulation



Biserrula *Biserrula pelecinus* **INOCULANT GROUP BISERRULA SPECIAL (STRAIN WSM1497)**

BISERBULA AND RHIZOBIA

- An annual pasture legume, grown since 2001, mostly in WA
- Biserrula has a very specific rhizobia requirement
- It does not nodulate with rhizobia associated with other indigenous or cultivated legumes
- It is essential to inoculate if *Biserrula* has not been recently grown

BISERRULA INOCULATION

- Peat-slurry lime pelleted seed or seed sown with granular inoculant
- Higher inoculation rates (above recommended rates),

e.g. one 250g packet of inoculant for 10kg seed is recommended

ASSESSMENT OF NODULATION

Good: at least 5 large (>5mm) and 10 small nodules per plant after 8 weeks of growth

HIGH	Biserrula host not previously grown
MODERATE	No Biserrula in past 4 years OR last Biserrula pasture not
	inoculated or lacked 'good' nodulation near top of root system
LOW	Neutral to alkaline loam or clay soils and recent
	(past 2 years) host crop with good nodulation



Lotus Lotus pedunculatus (syn. uliginosus) INOCULANT GROUP D (STRAIN CC829) Lotus corniculatus (Birdsfoot trefoil) INOCULANT GROUP LOTUS SPECIAL (STRAIN SU343)

LOTUS AND RHIZOBIA

- NOTE: A different strain of rhizobia is needed for each species of Lotus
- Perennial Lotus pastures are mostly in medium to high rainfall areas of eastern Australia; their rhizobia have a similar distribution
- Lotus rhizobia are moderately tolerant of soil acidity

LOTUS INOCULATION

PHOTO: R. YATES

- Most commonly applied as 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 formulations are available

ASSESSMENT OF NODULATION

Good: more than 30 pink nodules per plant after 8 weeks of growth

LIKELIHOOD OF CROP RESPONSE TO INOCULATION

HIGH	Lotus host not previously grown
MODERATE	No Lotus in past 4 years OR last Lotus pasture not inoculated or
LOW	lacked 'good' nodulation near top of root system Loam soils with neutral pH and a recent history (past 2 years) of <i>Lotus</i> with good nodulation

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Lucerne, strand and disc medics, Melilotus albus Medicago sativa, M. littoralis, M. tornata **INOCULANT GROUP AL (STRAIN RRI128)**

LUCERNE, MEDIC AND MELILOTUS ALBUS PASTURES AND RHIZOBIA

- Inoculation is always recommended for good lucerne establishment
- DO NOT USE Group AM inoculant because it is less effective at fixing nitrogen with lucerne, strand and disc medic

RHIZOBIA INOCULATION

- Most lucerne seed is sold preinoculated; if it is more than six months since inoculation, the seed should be reinoculated
- Due to nodulation sensitivity to low pH, coat inoculated seed with lime
- One packet of peat inoculant (250g) will inoculate 25kg seed

ASSESSMENT OF NODULI ATION

Lucerne: at least 5 pink nodules (ideally 10-15) per plant at 8 weeks Strand medics often form few nodules: 5 nodules at 8 weeks are satisfactory

LIKELIHOOD OF CROP RESPONSE TO INOCULATION

Always inoculate lucerne at sowing; soils with HIGH pH (CaCl₂) less than 6.0; no sown or naturalised medic MODERATE Medic present, but growth or nodulation below expectation LOW Neutral to alkaline loam or clay soils with recent vigorous medic growth and good nodulation

Well-nodulated white clover: abundant nodules on the tap root and close to the crown

Perennial clovers Trifolium species

(WHITE, STRAWBERRY, RED, TALISH, ALSIKE AND CAUCASIAN)

INOCULANT GROUP B (STRAIN TA1) EXCEPT FOR CAUCASIAN CLOVER SPECIAL (STRAIN CC283B)

PERENNIAL CLOVERS AND RHIZOBIA

Inoculation assists vigorous early growth of small-seeded perennial legumes

- For good nodulation, soil pH (CaCl₂) should ideally be greater than 5.5
- DO NOT USE Group C inoculant (WSM1325); nitrogen fixation by perennial clovers is significantly better with the Group B inoculant strain TA1

PERENNIAL CLOVER INOCULATION

- Most perennial clover seed is sold preinoculated; if it is more than two weeks since inoculation, the seed should be reinoculated
- For white clover, use 250g packet of peat inoculant to inoculate 25kg of seed; adjust the inoculation rate for small-seeded species

ASSESSMENT OF NODULATION

Good: at least 10 pink nodules per plant after 8 weeks of growth

LIKELIHOOD OF CROP RESPONSE TO INOCULATION

clover growth and nodulation

- **HIGH** Always inoculate Caucasian clover; no previous perennial clover grown in paddock; soils with pH (CaCl₂) below 5.0; where soil is tilled at pasture renovation
- MODERATE
 No perennial clover host in past 4 years and soil pH below 5.5; perennial clover present, but growth or nodulation below expectation

 LOW
 Soils with neutral or alkaline pH and recent history of good



Sulla Sulla coronaria INOCULANT GROUP SULLA SPECIAL (STRAIN WSM 1592)

SULLA AND RHIZOBIA

- It is essential to inoculate Sulla as it has very specific rhizobia requirements; Sulla rarely nodulates with background soil rhizobia
- Seedlings quickly develop nitrogen deficiency symptoms where nodulation is inadequate

SULLA INOCULATION

PHOTO: L. MASTERS

- Most commonly applied as a slurry of peat followed by pelleting with fine lime or other suitable product
- Higher inoculation rates (above recommended rates) of one 250g packet of inoculant for 10kg seed are recommended
- Seed sold through retail outlets may be preinoculated; due to its very short shelf life, sow as soon as possible after inoculation

ASSESSMENT OF NODULATION

Good: 4 large (>5 mm) nodules per plant after 8 weeks of growth

- HIGH Sulla not previously grown OR soils with pH (CaCl₂) below 6.0
- MODERATE No Sulla in past 4 years OR growth or nodulation of previous Sulla pasture below expectation
- LOW Neutral or alkaline loam or clay soils and recent (past 2 years) Sulla host with good nodulation

INOCULANT GROUPS FOR COMMON LEGUME SPECIES AND THE MAXIMUM AMOUNT OF SEED THAT SHOULD BE TREATED BY A 250-GRAM BAG OF PEAT INOCULANT

INOCULANT GROUP	COMMON NAME OF LEGUME	SEED SIZE	MAXIMUM WEIGHT OF SEED TREATED BY 250- GRAM PEAT INOCULANT
AL	Lucerne, strand and disc medics, Melilotus albus	Small	25kg
AM	Burr medic, barrel medic, snail medic, sphere medic, murex medic	Medium	50kg
В	White clover, red clover, strawberry clover, alsike clover, talish clover	Small	25kg
С	Subterranean clover, balansa clover, crimson clover, purple clover, arrowleaf clover, rose clover, gland clover, helmet clover, Persian clover	Small-medium	25–50kg
D	Greater lotus	Small	10kg
E	Field pea, vetch, narbon bean, lathyrus	Large	100kg
F	Lentil, faba bean, broad bean	Medium–large	50–100kg
G	Lupin	Large	100kg
Н	Soybean	Large	100kg
1	Cowpea, mungbean (green and black)	Large	100kg
N	Chickpea	Large	100kg
Р	Peanut or groundnut	Large	100kg
S	French and yellow serradella	Medium	50kg
Biserrula	Biserrula	Small	10kg
Lotus	Birdsfoot Trefoil	Small	10kg
Sulla	Sulla	Medium	10kg

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Grains

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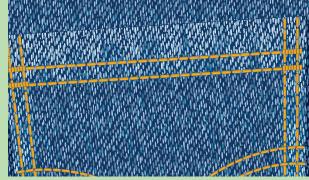
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USEFUL RESOURCES:

- Inoculating Legumes: a practical guide (2012) Free, online www.grdc.com.au/GRDC-Booklet-InoculatingLegumes
- 2. Fact Sheet: Rhizobial inoculants 2013 Free, online www.grdc.com.au/GRDC-FS-RhizobialInoculants
- www.agwine.adelaide.edu.au/farming/legumes-nitrogen/legume_inoculation
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