Australian Inoculants Research Group

Rhizobial Inoculation

Improved quality and productivity in Australia's variable climate: Pasture Legumes

Pasture legumes are used to provide high-quality, nutritional feed for direct grazing or conserved hay and silage, often as a part of a mixed pasture. They contribute to enhancing quality and yield of companion pasture grasses, follow-on crops or pastures and overall soil health, as they play an essential role in nitrogen (N) supply through their mutually symbiotic association with soil bacteria called rhizobia.

Why **is relationship between pasture legumes and rhizobia beneficial?**

Rhizobia infect the roots of pasture legumes during seed germination, then multiply rapidly to form root nodules. They are dependent on the host for water, nutrients and energy. In exchange, they supply N in a form that can be readily taken-up by the developing plant to boost growth. This conversion of gaseous atmospheric N (N_2) into ammonia (NH_3) is called N fixation. As well as the legume pasture itself, some N is returned to the soil via the grazing animal or by breakdown of organic matter, feeding the soil N pool (mineralised N) and improving soil structure. A well nodulated pasture legume leaves an enduring legacy of N for follow-on crops or pastures in a rotation system, used to optimise animal production and cereal or grain yield.

There is variation across pasture legumes in N fixation capabilities, but generally the more biomass produced, the more N fixed. Therefore, environmental factors that impact upon plant vigour and rhizobia strain persistence, such as soil moisture or soil acidity constraints, will also affect the rate of N fixation.

N inputs from well nodulated legumes vary dependent on the pasture species/cultivar, growing conditions (soil/climate) and management. For example, subterranean clover pastures can add anywhere from 39-81 kg N/ha/year whilst northern NSW trials on lucerne demonstrated contributions of up to 140 kg N/ha/year and further beneficial soil N, yield and protein impacts over subsequent years of wheat cropping (GRDC, 2014).

Any short-fall in a pasture N budget has to be supplied from soil mineralised or fertiliser N sources. Conversely, if there is an over-supply of N from these sources, rhizobia are less active and nodulation and N fixation becomes suppressed.

When **is inoculation needed to ensure a beneficial relationship between pasture legumes and rhizobia?**

The purpose of inoculation is to supply selected elite rhizobial strains in large numbers to the roots of the legumes soon after germination to optimise effective nodulation, symbiotic N fixation and pasture yield, while decreasing input costs. Although Australian soils have developed substantial populations of rhizobia, the suitable strain for the selected pasture legume/s may be absent from the soil if the species or cultivar has not previously been grown or where long-term rhizobial survival may be impacted by certain soil constraints such as acidity or prolonged drying. Each individual legume in a mixed pasture system must be inoculated with the correct rhizobial strain for nodulation and N fixation.

Inoculant products are specifically manufactured to contain live, unique rhizobia that are highly effective in nodulation and N fixation for a specific host group of legumes. When applied in sufficiently high numbers, inoculant strains will successfully out-compete established soil rhizobia. While the benefits of effective strains introduced through inoculation are important to pasture establishment, occupancy by the applied inoculant may be temporary and possibly insignificant where the pasture phase extends past a few years. This is dependent on the legume species (GRDC, 2014).

The use of pre-inoculated pasture seed is common across Australia today. Whilst convenient, use of these seed products does come with a level of uncertainty regarding rhizobia efficacy.

Inoculate bare seed or re-inoculate pre-coated seed when:

- the particular legume has not been grown in the paddock previously, or it has been longer than 4 years since the legume was grown
- there are newly introduced advanced strains with increased effectiveness and survival than those used in the paddock in the past
- the pre-inoculated seed has a batch date greater than five weeks prior to the date of intended use
- the presence of acidic (<5.5 pH_{Ca}) or highly alkaline soils in the paddock may limit survival of the rhizobia in the soil

- the paddock is subjected to particularly prolonged hot, dry conditions
- the legume has specific rhizobial requirements, e.g. check clover cultivars.

The Australian Inoculants Research Group (AIRG) is responsible for maintaining approved rhizobial strains for commercial release to manufacturers. The Rhizobial Inoculant Strains Table is updated annually and can be accessed directly from the website. It is good practice to check the recommended rhizobial strain for the legume host group during planning and prior to product purchase. Each host group is identified by a dedicated letter on product packaging.

What product is best for the beneficial relationship between pasture legumes and rhizobia?

Successful production and use of inoculants start with quality and concentration of the right rhizobial strain in the product. Quality assurance is provided by the AIRG's independent Green Tick Program that tests manufactured batches of inoculant products against stringent rhizobial viability and product quality criteria, as well as contaminant and moisture thresholds.

Standards for legume inoculants are based on scientific research that has defined the number of rhizobia required for adequate nodulation. Whilst testing is undertaken at the point of manufacture, the way any inoculant product is transported and stored along the supply-chain affects the viability of the living rhizobia. Site specific environmental conditions, such as climate, extreme weather events, soil moisture and soil pH have an influence on the type and rate of inoculant best used and the effectiveness of inoculation.

Pre-inoculated seed

Legume pasture seed is predominantly available from resellers with a pre-inoculated coating in Australia. Although considered convenient, these products are generally not the most reliable way to support a beneficial relationship between a pasture legume and rhizobia. They are more effective for certain rhizobia species, particularly those associated with lucerne and annual medics, whilst rhizobia populations associated with clovers, serradella, biserrula and sulla rapidly decline in this form (GRDC, 2014). There are currently no coated seed products that are tested by the AIRG that meet the standards of the Green Tick program.

In the manufacturing process, pre-inoculated seed products are also coated with other additives, such as insecticides, fungicides, lime and micro-nutrients (e.g. Molybdenum). Although these additives are designed to be beneficial to nodulation and plant development, the duration of rhizobia exposure can be problematic, compromising viability to the point where insufficient live rhizobia may remain at the time of sale (Hartley, 2012). The result is ineffective nodulation of the legume or an inability for the introduced rhizobia to compete against possibly ineffective background soil rhizobia. Whilst manufacturers mostly label products with a shelf-life of between six to twelve months on these products, best nodulation success results from seed that has been sown within five weeks of the batch date (Hartley, 2012; Gemmel, 2005).

Care must be taken to ensure inoculation with the appropriate inoculant host group for each legume species used in a mixed pasture. Pre-inoculated seed of one pasture species will not address the rhizobia requirements of other non-coated or compromised pre-coated pasture species.

Recent research undertaken by NSW DPI (Rigg et.al, 2021) found that cross-nodulation of clover cultivars is increasingly common. This is when a less-effective rhizobia strain may nodulate a legume within the mix rather than the recommended strain, resulting in poor nitrogen fixation. For best results, it is recommended to select compatible mixes of clover cultivars that have the same host group. These tips apply whether purchasing pre-inoculated, custom-inoculated (by local retailer) or self-inoculating seed.

For pre-inoculated pasture legume seeds, the recommendations are 1000 rhizobia per medium sized seed, such as subterranean clover and lucerne, and 500 rhizobia per small seed, such as white clover (GRDC, 2014).

Inoculating bare or pre-inoculated seed

Where bare legume pasture seed is available, or the efficacy of pre-inoculated seed may have been compromised, on-farm or custom-inoculation using a **peat inoculant** slurry, within 24 hours prior to sowing, is the recommended practice to optimise nodulation. Each legume pasture species should be separately inoculated, dried and then mixed with other species in preparation for sowing.

The following products are also critical:

Adhesive solutions are used to improve contact between inoculant and seed, as well as protect rhizobia from desiccation. They need to be prepared separately using potable (non-chlorinated) water and then combined with the peat inoculant, prior to lime pelleting (if required). It is important that adhesives have been tested to support rhizobia survival and avoid toxicity.

Lime pelleting is when the pasture seed is coated in a fine lime, calcium carbonate ($CaCO_3$), to help dry the seed, avoid clumping and protect the rhizobia against acid soils and acid fertilisers (i.e. phosphorous (P)). It is not necessary for podded (i.e. seradella), soft seeded (i.e. sulla) or tropical pasture legumes. There are exceptions to the general rule for tropical pastures so it is necessary to seek advice. Good pelleted seed should be evenly coated with lime and firm when dry and rolled between the fingers (NSW DPI, 2017).

Molybdenum (Mo) added directly to seed at inoculation is cost effective and results in good distribution in the paddock (NSW DPI, 2017). Use either molybdenum trioxide or ammonium molybdate as these sources provide higher levels of pure Mo and are not toxic to rhizobia. *Do not* use Sodium molybdate as it is toxic to rhizobia!

Whilst yet to be comprehensively trialled, there is also evidence of good nodulation results with **granule** inoculants applied at double the recommended rate directly into the seed furrow.

How is the beneficial relationship between pasture legumes and rhizobia best managed?

There are factors that may compromise high numbers and effectiveness of rhizobia along the supply-chain, effecting pasture legume nodulation and therefore field efficacy.

Rhizobia inoculant products should be treated with consideration for the following:

- *Good planning and soil testing 8-12 months prior to pasture legume sowing:*
    - match pasture legumes and rhizobia to the specific soil conditions;
    - optimise pH for growth by applying lime ameliorants if the soil is acidic (<5.5 pH_{Ca}) (Hackney et. al. 2019);
    - ensure adequate nutrients to support functioning of the host pasture legume and rhizobia – review your nutrient strategy, especially P, sulfur (S) and Mo. (NSW DPI, *Eight steps to successful perennial pasture establishment,* undated); and
    - organise with your retailer to access *fresh* pre-coated seed and/or the *correct*, *fresh* inoculant product for each pasture legume to be self or custom inoculated, *immediately prior* to the planned sowing date. Also pre-order additional products required for pelleting seed.
- *Rhizobia are living organisms susceptible to high temperatures:* It is important that inoculants or pre-inoculated seed are transported and stored away from direct sunlight and in temperatures less than 25°C for short periods. Peat products are best refrigerated at 4°C.
- *Read the expiry date before use:* Only use a product that is within date. A well-stored peat product will have a shelf-life of 12 months for most strains.
- *Read the batch date for pre-inoculated seed:* Only use a product that is within five weeks of the batch date. If available stock is beyond this optimal period, re-inoculate.
- *Fresh is best:* Sow seed into the ground as soon as possible after it is inoculated to maximise the potential for nodulation. Sow peat inoculated seed within 24 hours into moist soils.
- *Rhizobia are sensitive to chemical residues:* Use clean equipment and potable (non-chlorinated) water for mixing and application.
- *Add P, sulfur (S) and Mo if soil tests demonstrate sub-optimal levels.* These can be added at sowing, however, Mo is most effective if applied directly to seed during the inoculation process.
- *Trace elements, fertilisers and pesticides are toxic to rhizobia:* Don't mix inoculant directly with these. Where application of both pesticide and inoculant are critical to establishment, the use of direct soil inoculation techniques should be considered.
- *Rhizobia survival on seed is reduced by fungicides:* Apply rhizobia last and limit exposure time between inoculation and sowing to <5 hours.
- *Opened inoculant product can be stored for short periods:* Air needs to be immediately expelled, the packet sealed and refrigerated at 4°C. Use as soon as possible and never beyond the use by date.

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

Review the success of the beneficial relationship between grain legumes and rhizobia

Nodulation should not be assumed. Assess the effectiveness of the rhizobial inoculant, and the practices used, by checking the degree of nodulation from 8 to 10 weeks. In general, numerous pink nodules near the top of the root system indicate that effective nodulation has occurred.

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