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D L. Chatel
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Nitrogen fixation and inoculation by lupins

By D. L. Chatel, Principal Plant Pathologist and I. C. Rowland, Research Officer

The benefits of lupins as a grain legume crop are clear:
- They increase the amount of protein produced from each hectare of crop;
- They build up ‘fixed’ nitrogen to promote the growth of subsequent non-legume crops or pastures; and
- They provide grazing value through high quality stubbles.

A major disadvantage, in the eyes of many farmers is the irksome task of inoculating the seed with rhizobia—a treatment that minimises the risk of nodulation failure. Such a failure can mean that the crop may produce little, if any, of its own nitrogen.

This article briefly explains the reasoning behind seed inoculation. If growers have a better understanding of the biology of the system, they will be able to more easily assess their need to inoculate and know what, where, when and how to inoculate.

Principles behind inoculation

All plants require nitrogen for growth. Despite the fact that the air animals and plants breathe contains nearly 80 per cent nitrogen gas it is not in a form that makes it available for plant growth. Fortunately many agricultural plants are legumes...a group of plants that can convert gaseous nitrogen from the atmosphere into a form the plant can use. Because they have this ability, properly-functioning legumes do not require either soil nitrogen or fertiliser nitrogen to produce a productive pasture or crop.

The conversion process is known as nitrogen fixation. It occurs in nodules on the plant’s roots. The nodules are outgrowths of the roots containing very specific bacteria (rhizobia) that interact with the nodule tissue and ‘fix’ the nitrogen.

■ An uninoculated plot lags behind in this trial.
gas which enters the nodules from the air in the soil. The fixed nitrogen is immediately available for the growth of the host plant. Later, most of it is incorporated into the soil as plant and animal residues. The nitrogen-containing protein in these residues is not available for growth until soil as plant microbes decompose them. As this happens much of the nitrogen is converted into nitrate and ammonium forms of nitrogen, both of which can be used readily by most plants.

Various estimates have been made of the amounts of nitrogen fixed by legumes. Generally the better a legume grows the more nitrogen it fixes. Species of clover and medics have been estimated to fix from 50 to 200 kg nitrogen/ha/year. This is equivalent to 150 to 600 kg of ammonium nitrate (Agran 34.0) or 100 to 400 kg of urea fertiliser. However, because legume nitrogen is released over a long period it is really of more value to plants than an equivalent amount of fertiliser nitrogen.

Unfortunately legumes do not always nodulate in all the soils in which they are planted. If rhizobia are present, they may not be capable of nodulating the legume the farmer wishes to grow. Even if they can nodulate the plant, they may not be very effective, that is, they may not produce much nitrogen. If fully effective there may not be enough of them to promote prompt and adequate nodulation. For such reasons the farmer often must introduce the right rhizobia to the soil by adding them to the seed (inoculation) when the pasture or crop is sown.

Bacteria are classified, as are plants, into genera and species.

The rhizobia for medics will only nodulate the medics and other closely related genera such as Melilotus and Trigonella. The same general rule holds for all other genera. Even within the broad general groups, specificities can have a marked effect on the usefulness of commercial inoculants for legumes used in agriculture.

There is more than one inoculant available for some plant groups. Within the clovers there are two, one for white clover, strawberry and red clover (Group B) and one for all subterranean, crimson, rose and cupped clovers (Group C). There are similar special groups for medics (Group A, Rugosa Group), lotus and soybeans.

There are no clearly separate groups for the different lupin species. The commercial inoculant available in Australia effectively nodulates all lupins and serratellias used in Australian agriculture.

Cereal responses to lupins

The amount of nitrogen, fixed by a lupin crop, that becomes available to succeeding cereal crops varies with soil type, temperature, amount and distribution of rainfall and the success of the lupin crop.

Preliminary experiments comparing cereals grown after lupins or wheat showed that cereal grain yields could be increased by an average of 30 per cent in crops following a year of grain lupins. The trial results indicated a continuous cropping system of grain lupins alternating with wheat could be possible. Further support for such an intensive system comes from the permanent rotation experiment on the Esperance Downs Research Station. In the seven years from 1975 to 1981, total grain yields of lupins and cereals were similar in a continuous cropping system in which lupins alternated with cereals, to those in rotations including two or four years of subterranean clover-based pasture. This was a response to the combined effects of lupins maintaining soil fertility and herbicide control of annual ryegrass—which otherwise would have built up rapidly under continuous cropping.

Recent trials at West Binnu, more typical of the State’s main lupin growing areas, compared a lupin: wheat rotation with continuous wheat cropping. The 1979 lupin crop yielded 2.5 tonnes of seed per hectare. About 40 kg/ha of fertiliser nitrogen was required to equal the value of the lupins. The response to fertiliser nitrogen of wheat-after-lupins roughly paralleled that of wheat-after-wheat, indicating that some factor other than a simple nitrogen response was involved. On the deep sand section of the trial much of the fertiliser nitrogen applied at seeding would have leached out of the growing-root zone, whereas the nitrogen released from the lupin residues by microorganisms would have been available in small amounts over a longer period. Although there is evidence that including lupins in a rotation can lead to a marked reduction in cereal disease, there were no obvious differences in disease infection or weed density between the 1980 wheat crops.

A local trial at West Arrino (Rowland, unpublished data) showed that the cereal grown after a year of lupins gained 23 kg/ha of nitrogen from the soil and only a further 9 kg/ha from breakdown of lupin residues. This was equal to the cereal yield from about 30 kg/ha of fertiliser nitrogen applied to a second wheat crop.

Rhizobia from native legumes

There are no known lupins or serratellias native to Western Australia. Therefore we assume that, as is the case with clovers and medics, lupins and their rhizobia have been introduced since European style development in the 1930s. However rhizobia from many of our native legumes are capable of nodulating certain lupin species—a phenomenon which does not occur with clovers and medics.

From the late 1950s to early 1960s R. L. Lange at the Institute of Agriculture, University of Western Australia exhaustively surveyed the native legumes of the South West Province and isolated pure cultures of rhizobia from the nodules of some 85 species. He assessed their ability, in glasshouse tests to nodulate a range of plants of agricultural interest. Seventy six of them nodulated green beans, 71 nodulated the sandplain lupin (*L. cosentini), formerly *L. digitatus*) and 47 nodulated white lupins (*L. albus*). None of the 85 nodulated narrow leafed lupins (*L. angustifolius*), sweet yellow lupin (*L. luteus*), serratellias (*O. saxifraga*, and *O. compressus*), subterranean clover (*Trifolium subterraneum*), barrel medic (*Medicago truncatula* or peas (*Pisum arvense*). Although 71 of the
85 isolates nodulated the sandplain lupin, they were not equally effective. Some were highly effective, some partly effective and some were totally ineffective. The most effective ones were as good as the standard strain for lupins; they were from *Jacsonia furcellata*, *Isotropis cuneifolia* (lamb poison), and *Actus preissii*.

The most interesting observation from Lange’s work was that none of the rhizobia from native legumes nodulated the narrow-leaved lupin (*Lupinus angustifolius*)—the species to which most of the recently developed crop lupins belong.

Thus growers should beware of the widespread assumption that lupins do not require inoculation when sown on land recently cleared of vegetation containing native legumes. Scientific evidence and farmer/agronomist observation clearly shows that the sandplain lupin can grow much better than can the narrow-leaved lupins or the sweet yellow lupin (*L. luteus*) without inoculation. Many people familiar with lupins can point to some successful sowings of non-inoculated narrow-leaved lupins. It is rare to find all plants without nodules from non-inoculated seed sown in newly-cleared areas well away from lupin or serradella sowings. It is likely that the chance nodulation comes from “lupin” rhizobia introduced as contaminants in wind, dust or on implements however, some could be native rhizobia, missed by Lange, or native rhizobia that have been modified to suit narrow-leaved lupins after association with sandplain lupins.

**Responses to inoculation**

The need for lupin inoculation depends on the numbers and distribution of suitable rhizobia in the soil. There could be prospects of competition from rhizobia already there but we are not aware of any such problems yet.

Some indications of the likely response to inoculation can be gauged from a knowledge of cropping history, vegetation, host and type of rhizobia present or needed. However, the simplest way is to compare inoculated and non-inoculated treatments using the best available inoculant and host combination. The subsequent growth and nodulation will indicate the likely response, particularly if the trials are conducted in conditions representative of expected large scale field sowings.

Since 1980 the Western Australian Department of Agriculture has conducted 62 such experiments with inoculation on sweet lupins, mainly on the *L. angustifolius* varieties Unihwhite, Uniharvest and Unicrop. Most were drill-sown in commercial conditions. Of the 55 trials examined for nodulation, 45 had nodulated better after inoculation. There were few naturally-occurring rhizobia at these sites rather than big numbers of ineffective rhizobia.

**Crop yields**

It is more difficult to demonstrate a response to inoculation in terms of yield rather than nodulation. Forty-seven of the 62 trials were harvested for seed; 24 gave higher yields when inoculated, with responses ranging from 100 to 1500 kg/ha.

Eleven of the 24 responsive sites were on newly cleared land; the remainder were on old land new to lupins, and were either the first, second or third crops after clover.

Good spring growth does not necessarily indicate good seed yield. In some instances, short, yellow, poorly nodulated plants yielded as much seed as well nodulated plants that grew very well early in the season. The usual reasons for these differences include moisture shortage at the end of the season, loss of leaves by the more vigorous plants before they can contribute to pod fill, and premature cessation of nitrogen fixation.

One should not become too complacent about the need for inoculation when poor early growth eventually recovers to give a “reasonable” seed yield. Early, effective nodulation usually results in greener, larger and healthier plants with obvious advantages in coping with weeds and disease. Also high vegetative yields are almost certainly reflected in higher soil nitrogen build-up, and in improved stubble value.

Despite the fact that about 80 to 90 per cent of the lupins produced in Western Australia are grown north of Badgingarra, there have been very few reports of nodulation failure in those regions on the North-Midland and Eradu sandplain.

Satisfactory nodulation in the region results from a combination of three related factors:

- The suitability of the rhizobia from the native vegetation for the sandplain lupin.
- The use of the sandplain lupin as a pioneer species in the sandplain country and
- The relatively long history of lupins-growing in the area which would have resulted in the widespread distribution of rhizobia by natural means.

Even so, many farmers inoculate—particularly when they sow lupins in a paddock for the first time.
The history of lupin growing in other areas such as Wongan Hills, Esperance, Katanning, Williams, Wagin, Narrogin, Merredin, Lake Grace and Albany is much shorter. Also, much of this land has been cleared for many years so it is likely that most native rhizobia suitable for lupins would have disappeared by now. There is no simple soil test to assess the need for inoculation. Therefore, until experiments and commercial experience show otherwise, farmers would be well advised to inoculate all lupin sowings in those areas. They also should take care with sowing in the infertile deep sands like those found in the Lancelin area. It was there that lupin crops were found to respond to a soil application of cobalt—but only when the seed contained little cobalt, and when it was not inoculated. Cobalt is not an essential element for plant growth, however it is required by rhizobia. The symptoms of cobalt deficiency are the same as those of nitrogen deficiency.

**Methods of inoculation**

Commercial inoculants normally consist of a pure culture of rhizobia in a suitable carrier. In Australia they are prepared by injecting the rhizobia, with the nutrient broth they are growing in, into sterilised powdered peat. The rhizobia do not form spores, nor have they any resting or protecting phase in their life cycle. Therefore they must be protected from harsh chemical and physical environments. The finely ground peat provides some protection in the period between manufacture and use. The inoculum survives over periods up to six months after release by the manufacturer if it is stored under cool conditions.

Four methods of inoculation are used in Western Australia.

**Dust inoculation**—The dust inoculum is dusted on the seed. This is a wasteful method because relatively few rhizobia stay on the seed. It is not recommended.

**Water slurry**—The inoculum is made into a paste with water and mixed with the seed. This method has the same disadvantages as the dust method. Water slurry inoculation is not recommended, but if it is used, the treated seed must be sown into moist soil immediately after inoculation.

**Gum slurry**—The inoculum is made into a paste with weak gum solution. This method is recommended if pelleting is not necessary. The gum promotes better retention of the inoculum on the seed. Also it has the added advantage of slowing the death rate of the inoculum.

**Pelleting**—Lime pelleting protects the sensitive rhizobia from the effects of acid superphosphate—particularly for pasture legumes—when the seed is to be mixed with the fertiliser before sowing. The gum slurry system is used, except that a larger volume of a stronger gum solution is needed. Then the wetted seed is mixed with a finely powdered substance, usually lime. The adhesive used for sticking the pellet on the seed improves the rhizobia’s survival, irrespective of the pelleting substance.

Liquid and granular inoculants are also used in some countries in specialised circumstances. They are not likely to be used for large-scale lupin sowings as in Western Australia, unless special problems arise . . . for example inoculating a crop and treating the seed with a pesticide.

Already farmers have developed an auger-bin system for inoculating large quantities of lupin seed. The inoculum gum mix is dripped onto seed as it travels along an auger from a storage bin to a large field bin. The system is cheap and fast. Its main disadvantage is the difficulty of controlling the rate of inoculation.

Most trials conducted since 1980 included comparisons between gum slurry and lime pelleted inoculation. Usually there are no differences in the results. Lime pelleting gave better results on a few new land sowings, apparently because the lime provided protection against the copper mixed into the superphosphate.

**Survival of rhizobia on inoculated seed**

Inoculant rhizobia do not store well once on the seed. Ideally, treated seed should be sown into moist soil immediately after inoculation. Fortunately the nature of the adhesive and the high quality of Australian inoculants help enough rhizobia to survive some delay between inoculation and germination. The adhesive slows desiccation and the Australian inoculants contain high densities of rhizobia. Their numbers can decrease to 1 per cent within four weeks of sowing into dry soil.

There always has been concern that high rates of superphosphate drilled with inoculated seed may harm inoculant bacteria. An experiment was conducted to assess how varying rates of superphosphate drilled with inoculated seed, both gum slurry and lime pellet inoculated, affected noduleation and seed yield.

None of the superphosphate rates killed all the inoculant rhizobia. There was no difference between lime pellet and gum slurry inoculation.

The effect of manganese sulphate on nodulation, when drilled with the seed, was studied in a similar trial. It had no effect on nodulation, therefore it is safe to drill inoculated lupin seed with manganese-superphosphate.

Farmnote 55/80 describes lupin seed inoculation.

**Concluding comment**

It is difficult to predict the need for inoculation on a paddock scale and impractical to conduct hundreds of response experiments. Therefore the Department offers the following general recommendation.

Lupins should be inoculated when land is sown to them for the first time. It is cheap if the farmer does it himself, and it is a worthwhile insurance.

Farmers should order their inoculum several months before seeding so that suppliers can ensure it is available when required. Inoculum is best stored at 4°C in a refrigerator and should not be kept after the expiry date.