

Legume Inoculants and Quality Control

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Abstract

Rhizobial inoculants have been used successfully in world agriculture for about 100 years. About 20 million ha crop and pasture legumes are inoculated in the world each year, although that figure could be increased if high-quality inoculants were available to all farmers. The characteristics of a high-quality inoculant relate to the properties of the carrier, the infective (nodulating) and effective (N₂ fixing) attributes of the rhizobial strain and the numbers of the strain and other microorganisms (contaminants) that are present in the inoculant. In countries with strict standards for inoculants such as Australia, the carrier (most commonly peat) must contain >10⁹ rhizobia/g and <10⁶ contaminants/g. Other countries demand that inoculants deliver high numbers of rhizobia to the inoculated seed, e.g. 10⁹/seed for soybean in France. Research in a number of countries has shown that the most effective inoculants are produced using a sterile carrier and are relatively fresh, i.e. <6 months old. Rhizobial inoculants lose efficacy with age. The involvement of private-sector institutions in manufacturing and marketing and public-sector in quality control and R&D appears to be the most successful model for the production and use of inoculants. The future of the inoculant industry, and its potential benefits for world agriculture, depends on improving inoculant quality, both numerically and in terms of strain effectiveness. New technologies may lead to improved inoculants in industrialised countries but the fact remains that, in many countries, the 30- and 40-year old technology has yet to be properly mastered.

LEGUME inoculation with rhizobia and bradyrhizobia is a long-established and successful practice, especially with particular crops in the more technically-advanced countries. It is difficult to access accurate figures on the global use of legume inoculants. The figures available indicate that about 2000 t inoculant is produced annually worth US\$50 million, and sufficient to inoculate 20 million ha of legumes. By far, the largest producer of inoculant is the U.S. with annual production of about 1000 t (Singleton et al. 1997).

Early attempts at inoculation were rudimentary, such as moving soil from fields growing well nodulated legumes to legume-free fields. The next step came late last century with the commercial use of pure cultures of rhizobia for inoculation (Fred et al. 1932). Since that time, the production and distribution of legume inoculants have become established industries in individual countries throughout the

world. Having said that, few farmers in the developing countries of Asia have had access to high quality inoculants or have used inoculants as a normal part of their legume culture practices. In some countries, farmers fertilise their legume crops with N, thereby losing some of the economic benefit of the legume, i.e. inputs of fixed N into the farming system. In other countries, productivity and profitability may be reduced through N deficiency. In a third group, inoculants are readily available. Countries in this group include Thailand (Kongngoen et al. 1997; Boonkerd these Proceedings), Bangladesh (Sattar et al. 1997) and Myanmar (Thein and Hein 1997).

The Need to Inoculate

Although rhizobia seem to be as widely distributed as the legumes themselves, many soils used for legume cultivation do not contain adequate numbers of highly effective rhizobia. They may be devoid of the rhizobia, they may contain low numbers of effective strains or they may contain high numbers of ineffective or partially effective strains. The question

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“When to inoculate?” is critical and has been pondered at length, although it should be stated that there are far less problems with inoculating when not needed (i.e. over-inoculating) than not using inoculants and producing N-deficient crops. Allen and Allen (1961) listed four indicators that, if positive, would necessitate inoculation:

- The absence of the same or symbiotically-related legume in the immediate past history of the land
- Poor nodulation when the same crop was grown on the land previously
- When the legume followed a non-legume in the rotation
- When the land was undergoing reclamation

The definitive indicator is numbers of rhizobia in the soil. Published data suggest a population of rhizobia of >1000/g soil is required for optimum nodulation and N₂ fixation (e.g. Thies et al. 1991; Singleton et al. 1992; Nazih and Weaver 1994). The numbers can be readily established and maintained in good quality soils through inoculation and continuing cultivation of the legume, but won't be achieved if the legume or near relatives have never been grown on the land, or if the land is severely degraded or perturbed.

Field experiments have been conducted in many countries of the world during the past 30 years to diagnose the need for inoculation. They generally involve a number of strains of rhizobia, plus uninoculated and fertiliser N controls, as treatments. At various times during the growing season, plants are harvested and assessed for nodulation and yields of biomass and grain. Such experiments are time-consuming, prompting scientists to develop rapid, laboratory-based methods for assessing the need for inoculation (e.g. Bonish 1979; Brockwell et al. 1988).

Bonish (1979) used dilutions of soil samples to inoculate clover seedlings growing in test tubes to demonstrate simultaneously the size and N₂ fixing capacity of the rhizobia in the particular soil. Brockwell et al. (1988) developed this method into a 28-day assay, which, combined with a serial-dilution, plant-infection count of numbers of rhizobia, presented the rhizobiologist with a reliable guide to the need for inoculation. Ballard and Charman (2000) used the Brockwell technique to evaluate the symbiotic N₂-fixing potential for annual medics of 28 soils from 4 regions of South Australia. They found that the soils differed substantially (up to 4-fold), that the poor soils were the most acidic and contained the lowest populations of medic rhizobia, and that 45–52% of the variation in dry matter of the test medics was related to variation in rhizobial populations. They also found soil × host specificity. This technique would appear to have relevance to other legumes and environments (countries). With

sufficient sampling and testing, it may be possible to develop more broad-scale, even nation-wide recommendations for inoculation.

Thies et al. (1991) developed simple functions to predict the need for inoculation based on numbers of rhizobia in the soil and soil nitrate levels. They then went one step further by combining GIS and soil microbiological data to predict need for inoculation on a catchment and regional basis (Thies et al. 1994).

Notwithstanding the potential value of the rapid tests, the need for the widespread and continued use of rhizobial inoculants is most strongly reinforced with traditional field-based data sets. Results from the coordinated inoculation trials of the University of Hawaii's NifTAL project indicate that the potential for inoculation to increase legume yields is great and apparently widespread across regions and species (Table 1). There are differences amongst the species in the frequency of responses. At the high end of the scale are green gram (mungbean) and soybean with 70% and 65%, respectively, of trials showing a response to inoculation. By contrast, inoculation responses were recorded for only 10 and 13% of the common bean and pigeonpea experiments.

Reasons for the pattern of responses in Table 1 include the nodulating characteristics of the host legume as well as the edaphic factors, rhizobial populations and soil nitrate. Soybean is quite specific in its rhizobial requirements, in contrast to cowpea, pigeonpea and common bean, which are considered promiscuous nodulators, i.e. will nodulate more readily with a wider range of rhizobia.

Table 1. Summary of inoculation responses of commonly-grown tropical legumes (data from Singleton et al. 1992).

| Species | No. trials | % trials with significant response to inoculation |
|-------------|------------|---|
| Green gram | 40 | 70 |
| Soybean | 40 | 65 |
| Black gram | 15 | 53 |
| Groundnut | 26 | 50 |
| Cowpea | 9 | 56 |
| Chickpea | 31 | 48 |
| Lentil | 27 | 48 |
| Leucaena | 8 | 38 |
| Pigeonpea | 8 | 13 |
| Common bean | 10 | 10 |

In the same study, analysis of 305 soil samples from 17 countries indicated that the cowpea group of rhizobia were present in high numbers (>1000/g soil) in 40% of soils, compared with only 14% of soils containing rhizobia effective on the highly-bred American soybean. It stands to reason, therefore, that

soybean would benefit more than cowpea (and pigeonpea, common bean) from inoculation.

Inoculant Technology — Carriers and Rhizobial Strains

There are a number of major texts dealing with the technology of inoculant production (see Brockwell et al. 1995). Both sterile and non-sterile peats are most commonly used as carriers for the rhizobia. Inoculants made with sterile peats are preferred, and used in countries like Australia and the U.S., because they contain up to 100-fold more rhizobia than those made with non-sterile peat. Numerous reports refer to the consistently high numbers of rhizobia, in excess of 1×10^9 per gram, that can be achieved in sterile peats. Unfortunately, because of the cost of the sterilisation (gamma irradiation) process, most of the inoculants produced in Asian countries are made with non-sterile peats. As well as such peats having lower numbers at the point of manufacture, higher death rates during storage because of the other, contaminating microorganisms can severely restrict the useful life of the inoculant.

Although peat is the most commonly-used carrier for rhizobial inoculants because of its high moisture-holding capacity and dual abilities to foster multiplication of rhizobia in the peat itself and protect the rhizobia once they are applied to the seed coat, it is by no means the only carrier tested or used. Thompson (1980) presented an imposing list of alternative inoculant carriers that included the following: coal, charcoal alone or with composted straw, mixtures of soil and compost, mixtures of soil, peat, composted bark and wheat husks (the Swedish mixture), bagasse, coir dust, composted corn cobs, filter mud, lignite, bentonite and talc. The list has since been augmented (see Brockwell et al. 1995). Keyser et al. (1992) regarded the properties of a good inoculant carrier as:

- High water holding capacity.
- Non-toxic to the rhizobia.
- Easy to sterilise by autoclaving or gamma irradiating.
- Readily available and inexpensive.
- Sufficiently adhesive for effective application to seed.
- pH buffering capacity.
- Cation- and anion-exchange capacities.

While most peats meet these criteria, the search for alternative carrier materials continues, particularly in countries that have no natural deposits of peat.

Keyser et al. (1992) also listed the following characteristics as desirable for rhizobial strains used in inoculants. They should have the ability to:

- Form nodules and fix N_2 with the target legume.
- Compete in nodule formation with populations of rhizobia already present in the soil.
- Fix N_2 with a wide range of host genotypes and across different environments.
- Form nodules and fix N_2 in the presence of soil nitrate.
- Grow in artificial media, in inoculant carrier and in the soil.
- Persist in the soil, particularly for annually regenerating legumes.
- Migrate from the initial site of inoculation.
- Colonise the soil in the absence of the legume host.
- Maintain genetic stability.
- Be compatible with agrochemicals.

They should also have as wider host range as possible, have low mortality on inoculated seed and have the ability to colonise the rhizosphere of the host plant. Strains of rhizobia used in inoculants are selected in strain trials that ideally cover the physical environments and soil types that the inoculants are to cover. This may mean a number of multi-site evaluations over a number of seasons.

The search for new inoculant strains is an ongoing process, driven by the need to extend legume cultivation into poorer-quality soils and environments, through pressure to optimise productivity, and through introductions of new legume cultivars and species. Thus, new strains are required to be more vigorously-nodulating, more effective at fixing N and/or adapted to soil and environmental constraints, such as acid soils.

Importance of Inoculant Quality — Rhizobial Numbers and Inoculant Age

Having identified the most effective rhizobial strain and the best inoculant carrier, the next imperative is to maximise the numbers of rhizobia that eventually colonise the seedling rhizosphere. Thus can be achieved by having large numbers of viable rhizobia in the inoculant itself (i.e. high-quality inoculant), using higher-than-normal rates of inoculation or by minimising the death of rhizobia between the time the seed or soil is inoculated and nodulation occurs. All three strategies have merit, although the literature attests to the advantages of using high-quality inoculants (Brockwell et al. 1995).

Just how important are rhizobial numbers? Roughley et al. (1993), in a field study of the narrow-leaved lupin, reported that increasing the numbers of rhizobia applied to the seed from 1.9×10^4 to 1.9×10^6 increased nodule number from 8 to 26/plant; nodule weight from 65 to 393 mg/plant; % plants nodulated from 89 to 98%; shoot DM from

7.8 to 9.0 t/ha and, most importantly, grain yield from 1.9 to 2.1 t/ha (i.e. a 10% increase). The responses to increasing numbers of inoculant rhizobia were almost linear through a range of just 2/seed to the highest rate of 1.9×10^6 /seed. Responses for plant nodulation and grain yield are shown in Figure 1.

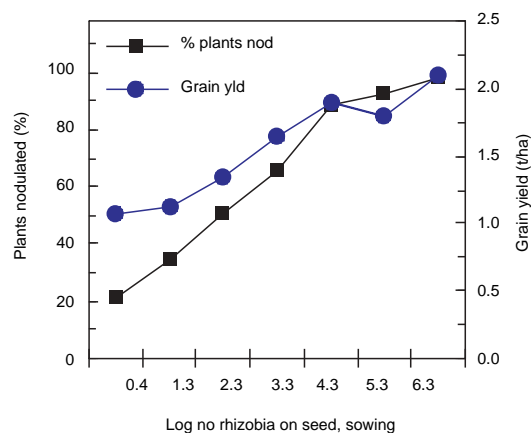


Figure 1. Relationship between numbers (\log_{10}) of rhizobia on the narrow-leaved lupin seed at sowing and % plant nodulated and grain yield (data from Roughley et al. 1993).

In similar studies of soybean, Brockwell et al. (1985, 1989) highlighted the strong, linear relationships between rhizosphere populations of rhizobia and nodulation, plant growth (shoot DM) and grain yield. Highest yields were only achieved when rhizosphere populations were $>1 \times 10^5$ /plant. Hume and Blair (1992) reported that soybean yields in land that had not grown soybean before were increased by an average of 24% when rhizobial numbers on the seed were increased from 10^5 to 10^6 .

In the narrow-leaved lupin study of Roughley and colleagues, the survival of the inoculum through the various stages of inoculation, sowing and immediate post-sowing in the soil was quantified. Results indicated that 95% of the rhizobia died between inoculation and sowing and, of those surviving, 83% died after 23 h in the soil. Thus, only 1% of the original rhizobial cells had survived the first 24 h. The authors suggested that these results reinforce the need for the highest quality inoculants coupled with inoculation and sowing procedures that aid inoculant survival.

The examples above were all from experimental systems in which the inoculant rate was varied to achieve the range of rhizobial cell numbers on the seed or in the rhizosphere. Hiltbold et al. (1980), on the other hand, examined commercial inoculants in

Alabama, USA, for quality and efficacy. In that study, rhizobial numbers in the commercial inoculants varied widely, from $<10^3$ /g to about 10^9 /g. Nodulation of the soybean was directly related to numbers, with no nodulation produced by products supplying $<10^3$ rhizobia/seed, and abundant nodulation by products providing 10^5 – 10^6 /seed (Figure 2A). Effects of inoculant quality on grain yield were similar. Yield increased linearly with increasing rhizobial numbers on the seed, in turn related to inoculant quality (Figure 2B). Products providing $<10^3$ rhizobia/seed did not yield differently from the uninoculated control. The relatively high yield of the control (2.4 t/ha) and of the poor quality inoculant treatments reflected the relatively high N status of the soil. In other situations, yields of much less would be expected.

Another important consideration, with respect to inoculant efficacy, is age of the inoculant. Non-sterile inoculants will contain large numbers of contaminants and they will depress numbers of rhizobia with time (Date and Roughley 1977). Even in sterile carriers, numbers of viable rhizobia will decrease over time, although not at the same rate as in non-sterile carriers. Boonkerd (1991) reported differences between rhizobial strains in storage characteristics and strong effects of storage temperature and peat treatment (Table 2). The report showed that storage temperature was critical with survival of the rhizobia substantially greater at 10°C than at 30°C . The pre-treatment of the peat was also critical with numbers after 12 months storage in the irradiated peats about 3–5 times those in the autoclaved peats and 10–15 times the numbers in the non-sterile peats. Such effects are important if peat inoculants are not used immediately but stored for later use. The storage effects are of less consequence if the inoculants are used within a short time of manufacture.

Currently in Australia, all peat-based rhizobial inoculants, except for the *lotononis* group, are given a 12-month expiry from the date of testing. That is fine for the pasture species, but for the crop legumes, an expiry of 15–18 months would have clear benefits for the manufacturers by allowing unsold peats (carryover stock) from the previous season to be sold at the start of the following season. Thus, a peat inoculant that was manufactured in February 2000 could still be sold during the early part of the following season, i.e. April and May 2001. There would also be a reduction in freight costs to the manufacturers with the reduction/elimination of returned product.

Research to date indicates high counts of lupin (WU425) and chickpea (CC1192) inoculants at 15–18 months, but lower counts of pea (SU303) and fababea (WSM1271). The pass rate of the standard (i.e. 1×10^9 /g peat) would have been 88 and 94% for

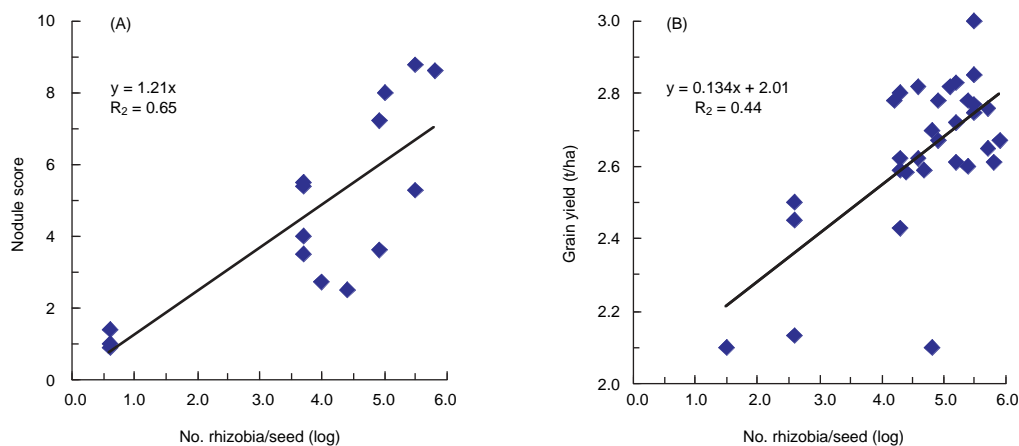


Figure 2. Relationships between the number of rhizobia on soybean seed at sowing and (A) plant nodulation, and (B) grain yield. All inoculants used in this field study in Alabama, USA, during 1976 and 1978 were commercial inoculants. Variations in rhizobial numbers on seed reflected variation in the quality of the inoculants (data from Hiltbold et al. 1980).

Table 2. Effects of storage temperature, peat treatment and rhizobial strain on numbers of viable rhizobia after 52 weeks of storage. Values are rhizobial nos/g peat ($\times 10^6$) (data from Boonkerd 1991).

| Temperature/peat | USDA110 (soybean) | THA205 (groundnut) | THA301 (mungbean) |
|------------------|----------------------|-----------------------|----------------------|
| 10°C | | | |
| irradiated | 5500 | 1230 | 2140 |
| autoclaved | 3890 | 270 | 930 |
| non-sterile | 1320 | 12 | 66 |
| 30°C | | | |
| irradiated | 11 | 830 | 1100 |
| autoclaved | 250 | 580 | 316 |
| non-sterile | 6 | 45 | 115 |

lupin and chickpea peats and just 8 and 36% for fababean and pea (Table 3). Clearly, it would be unwise to consider a 15 or 18 month expiry for the latter based on these data.

Data from two field experiments supports the above. Trials by Murdoch University's Centre for Rhizobium Studies at Gnowangerup, Western Australia, in 1999 indicated a 10% grain yield reduction using old pea and fababean inoculant. A trial by the Australian Legume Inoculant Research Unit (ALIRU) with lupin inoculant showed no effect of inoculant age on shoot yield. Further work is required to extend field evaluation of old inoculants to other groups.

Interestingly, Catroux et al. (2001) discussed effects of time of storage on numbers and efficacy of the inoculant rhizobia in some detail in a recent

review. The authors suggested that changes in rhizobial cell characteristics during long-term storage of inoculants may contribute to a loss of efficacy of the inoculant. They found that with increasing age of inoculant, the time for colony appearance on a plate and time for nodulation in tube culture increased. This suggested a decline in the fitness of the surviving bacteria. In fact, they observed increased sensitivity to desiccation and an increased number of bacteria with compromised membranes. All of this resulted in a loss of efficiency of the rhizobia with increasing inoculant age (Table 4).

Quality Control of Inoculants

Successful production of rhizobial inoculants needs to be associated with an effective, regulatory Quality

Table 3. Peat testing of Australian inoculants Groups E, F, G and N for extended expiry (data from Gemell, Hartley and Herridge, unpublished).

| Group | No peats tested | Peat counts (rhizobia × 10 ⁹ /g) | | % peats passing | |
|--------------|-----------------|--|------------|-----------------|------------|
| | | Initial | 15–18 mths | Initial | 15–18 mths |
| E — pea | 16, 10 | 2.69 | 1.18 | 100 | 36 |
| F — fababeen | 15, 11 | 2.82 | 0.63 | 100 | 8 |
| G — lupin | 24, 15 | 4.00 | 2.45 | 96 | 88 |
| N — chickpea | 23, 18 | 5.90 | 2.72 | 100 | 94 |

Table 4. Effects of time of storage on numbers and ‘efficiency’ of rhizobia in peat inoculant. Efficiency was determined by comparing the field efficacy of the inoculant with that of a fresh inoculant (data from Catroux et al. 2001).

| Storage time (years) | Inoculant | | |
|----------------------|--|--------------|---|
| | Plate count (rhizobia × 10 ⁶) | % efficiency | Actual count (rhizobia × 10 ⁶) |
| 1 | 3890 | 36 | 1400 |
| 4 | 1260 | 42 | 530 |
| 6 | 200 | 11 | 22 |
| 7 | 18 | 3 | 1 |
| 8 | 20 | 5 | 1 |

Control (QC) program. Most countries have some form of QC, which may be supported by appropriate legislation (e.g. Canada, Uruguay, France) or may be voluntary on the part of the inoculant manufacturers (e.g. Australia, Thailand, New Zealand, South Africa). The QC programs deal mainly with the quality of the strains in the inoculants and their numbers as well as the numbers of contaminating micro-organisms (Figure 3). In the U.S., regulatory control has not been considered necessary since the 1940s. However, results of independent tests published from time to time indicate that substantial proportions of the inoculants produced in the country are unsatisfactory (e.g. Hiltbold et al. 1980).

The whole question of inoculants and their use starts with quality. If the quality is poor, then everything else is irrelevant. Thompson (1992) found that 90% of inoculants sampled in India had <10⁸ viable rhizobia/g carrier and that only 3% of inoculants would have met Australian standards. All inoculants sampled were contaminated. Similar findings were reported in a NifTAL survey, i.e. half had <10⁸ viable rhizobia/g carrier (Singleton et al. 1997). The numbers of rhizobia in the inoculants were found also to be inversely related to the numbers of microbial contaminants. Most of the sampled inoculants were ostensibly produced in sterile carriers, indicating problems with production and factory-level

QC. Clearly, there is a need for regulation and enforcement of inoculant quality standards. Ideally, the inoculants should be produced in the private sector, but enforcement of standards should be independent of that, preferably in the hands of a Government Agency such as the National Department of Agriculture.

Standards of rhizobial inoculants and their regulation vary between countries. For example, standards for Canada are covered by the Fertilisers Act (Rennie 1991). Companies wanting to sell inoculants in Canada must submit registration to Agriculture Canada with data showing the product’s efficacy. Agriculture Canada inspectors randomly collect and test about 150 inoculant samples each year from factory and sales outlets. Standards state that the inoculant must provide 10³–10⁵ rhizobia/seed, depending on seed size. Rennie (1991) reported that after a decade of testing, standards of inoculants were high with an 87% pass rate (mean of years 1987–89), although the figures reported by Olsen et al. (1995) were not as positive. In 1993, they tested, as part of the Agriculture Canada program, 40 inoculants produced by three different companies. All inoculants were made using non-sterile peat. Only one of the 40 inoculants contained more rhizobia than contaminants and three contained 1000 times more contaminants than rhizobia. The remainder were in

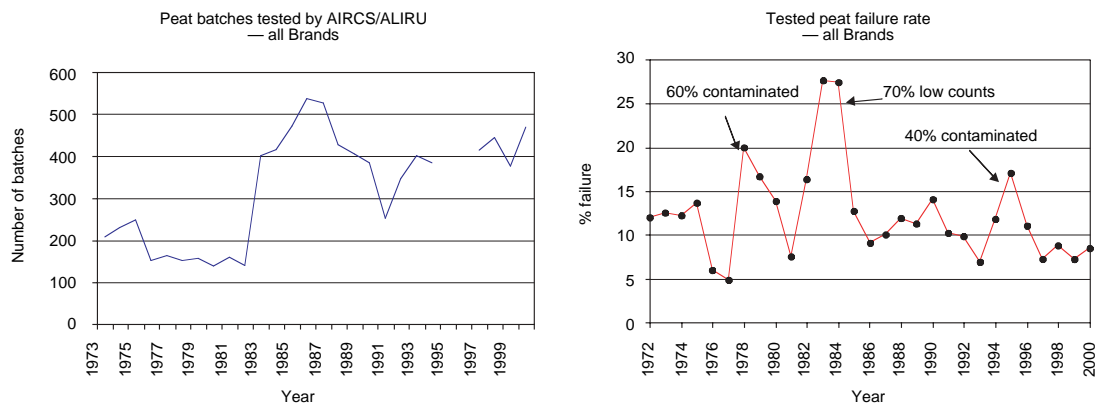


Figure 3. Numbers of batches of commercial peat inoculants (A) tested in Australia between 1973 and 2000, and (B) failure rate of the tested batches (data from Gemell, Hartley and Herridge, unpublished).

between. Rhizobial numbers were generally low. Twelve of the 40 inoculants (30%) contained $<10^8$ /g peat and 75% contained $<10^9$ /g peat. Contaminants were isolated from the inoculants of all three manufacturers that inhibited growth of clover and medic rhizobia. Human pathogens were also isolated from the inoculants.

France also has legislation covering inoculants, although standards and procedures are slightly different (Wadoux 1991). Inoculant products must be registered for sale in France. The product must have proof of efficacy and not be harmful to non-target crops, animals and humans and the environment. Thus, all inoculants are produced in sterile carriers. The inoculant must be able to deliver the equivalent of 1×10^6 rhizobia/soybean seed. Strains for particular legume species are also regulated. Inoculants are batch tested through INRA, Dijon, and certified if they pass the standard. They are retested if presented for sale in a second season.

By contrast, Thailand does not have legislation for inoculants, although there are national standards and independent testing is conducted by the Thai Department of Agriculture (Boonkerd 1991). Standards are geared to provide soybean seed with 10^5 - 10^6 rhizobia/seed. That translates into freshly-manufactured inoculant having $>10^8$ /g peat. Non-sterile carriers are used, with the proviso that the inoculants are used within a short time of manufacture.

The need for both public (e.g. Departments of Agriculture, Universities) and private sector involvement in inoculant production has been recognised for some time (Singleton et al. 1997) (Table 5). Many countries have at least rudimentary inoculant production units in the public sector institutions. These units are often established under the euphemism of

'pilot production'. Their objectives usually include establishing the technical feasibility of production, demonstrating inoculants and inoculation to farmers and raising awareness of the benefits of inoculation amongst users and policymakers.

Table 5. Roles for the public and private sectors in production and marketing of inoculants (adapted from Singleton et al. 1997).

| Public Sector | Private Sector |
|--|-------------------------------|
| Rhizobium strain evaluation | Product development |
| Rhizobium culture maintenance | Rhizobium culture maintenance |
| Applied Research | Manufacturing |
| Technical assistance | Market development |
| Training: extension, industry, farmers | Marketing/distribution |
| Quality standards | Internal QC |
| External QC | |

Few pilot facilities have gone on to spawn larger private sector operations or significantly increase market penetration. The major reasons for this may be the research, rather than commercial, focus of public institutions. The performance indicators of research are new knowledge and scientific publications leading to personal reward for the scientist. These are at odds with the performance indicators of a private sector company, i.e. good quality product, market acceptance leading to sales and profit. Ideally, the public sector, pilot production facilities should make the transition from manufacturing inoculant to providing R&D support, and perhaps external QC, as quickly as possible and leave

manufacturing in the hands of the private sector. Singleton et al. (1997) suggested appropriate roles for both sectors in the production of inoculants. Such partitioning of roles is applicable to the industry in both developed and less developed countries.

Australia's QC program

Australia provides a good example of the role the public institutions might play in QC of legume inoculants produced by the private sector. The Australian Legume Inoculants Research Unit (formerly AIRCS — Australian Inoculants Research & Control Service) is a NSW Agriculture unit, comprising the part-time services of a senior scientist and three full-time technical staff, established under an agreement of all State Departments of Agriculture. The operating and technical salaries are funded from external sources; infrastructure and the scientist's salary are provided by NSW Agriculture. ALIRU has a national mandate to ensure that Australian farmers have access to the highest quality legume (rhizobial) inoculants. Inoculants have played, and continue to play, a valuable role in Australian agriculture.

There are 39 different rhizobial inoculants produced and sold in the country, covering 100 species of legume. We estimate that Australian farmers currently inoculate about 1.5 million ha legumes annually, at a cost of US\$3 million. The benefits of inoculation are about US\$50 million annually, resulting in a benefit: cost ratio of 17:1 (assumptions on how much of the N fixed can be attributed to inoculation). The total amount N fixed by Australia's agricultural legumes is about 2 million tonnes annually, worth US\$1 billion. Almost all of that can be attributed to the residual benefits of previous inoculation. Market data from one of the two Australian manufacturers indicates that 80% of inoculants are used on grain legumes, and 20% on pasture legumes. Lupin dominates the market for legume inoculants in Australia, followed by chickpea and field pea.

Quality standards of inoculants sold in Australia are not covered by legislation and therefore there is no legal requirement that inoculants meet any standards, either for numbers of rhizobia, numbers of contaminants or for effectiveness of the rhizobia. Thus, there is little impediment to the marketing of low-quality inoculants except the now firmly-established arrangement between ALIRU, the R&D providers and funding bodies and the Australian inoculant manufacturers. The independent quality control provided by ALIRU and its predecessors, AIRCS and U-DALS (University of Sydney — Department of Agriculture Laboratory Services) has been in place now for almost 50 years and has proved extremely effective.

Genesis of ALIRU

Prior to 1953, NSW Agriculture supplied about 20,000 rhizobial inoculants to farmers annually. Private-sector production and distribution of inoculants commenced in 1953. Following widespread nodulation failures of sown legumes, U-DALS was formed as a joint undertaking between NSW Agriculture and Sydney University to provide quality control for and to trouble-shoot commercial inoculant production in Australia. In 1971, U-DALS was disbanded and replaced by the AIRCS (Australian Inoculants Research and Control Service), at the NSW Agriculture laboratories, Rydalmere. In 1973, the unit was relocated to NSW Agriculture Horticulture Research Station, Gosford. The unit was renamed ALIRU in December 2000 and remains at Gosford.

ALIRU core activities — Rhizobial inoculant testing

ALIRU tests samples from all commercially-produced batches of peat-based rhizobial inoculants for quality, i.e. purity, strain trueness, numbers (colony-forming units (cfu)) of rhizobia and contaminants, nodulating capacity and peat moisture characteristics. Five of seven packets are tested initially from each batch. If one of the five fail to reach the standard, the final two packets are tested. The number of batches peaked at 537 in 1986; the rapid increase in numbers submitted for testing between 1982 and 1986 coincided with increased areas sown to lupins and other pulses. The number of batches tested annually has now stabilised around 400. Failure rates have varied considerably during the past 30 years with the peaks mainly associated with a change in the source of peat, or changes in manufacture. In the early 1970s, high salt levels in peat used in inoculants created considerable problems until a new source of peat was found.

Strain improvement/mother culture maintenance and supply

ALIRU recommends, through its national steering committee, strains of rhizobia used in all inoculants produced and sold in Australia and supplies mother cultures of the rhizobia to the manufacturers. ALIRU also maintains elite strains for advanced stages of strain evaluation and supplies those to the national R&D program.

General requirements for strains to be used as inoculants are for broad-range effectiveness within an inoculant group, survival on seed (particularly relevant to pasture spp), ability to be cultured, and genetic stability. ALIRU protocols for strain testing require field testing, usually following extensive

screening in a glasshouse, of elite strains in well-designed and replicated experiments. Observations include nodulation, nodule occupancy (optional), dry matter/grain yield. The testing should be done for at least 2–3 years, with site replication across a range of environments and soils. Since 1996, 12 changes in the strain used in Australian inoculants have been approved by the ALIRU steering committee (Table 6).

The most recent change was to replace the current strain for the fababean/lentil group (WSM1274) with strain WSM1455. The latter increased yield of lentil by 26% (average of 5 experiments in WA) and fababean by 12% (average of 4 experiments in WA) when compared with WSM1274. In glasshouse experiments it was shown to have a broader host range than either WSM1274 or WSM1483 (highly-effective strain on fababean and lentil) and out-yielded WSM1274 by 61% on lentil and 6% on fababean (data from 10 experiments). In two field experiments in NSW, WSM1455 produced a 13% increase and 2% reduction in fababean grain yield compared with WSM1274. In Victoria, WSM1455 increased fababean yields by 4% (average of 7 experiments), relative to WSM1274. Lentil yields were identical for the two strains (9 experiments).

Table 6. Rhizobial strain changes in Australian inoculants since 1996.

| Year | Strain | Host species |
|------|---------|---------------------------|
| 1996 | WSM688 | Annual medics |
| | CB3458 | Calliandra |
| 1997 | WSM1558 | Biserrula |
| | WSM1274 | Fababean (split from pea) |
| | WSM409 | Special clovers (aerial) |
| | WSM471 | Serradella |
| | CB3481 | Caatinga stylo |
| 1998 | WSM1497 | Biserrula |
| 1999 | RRI128 | Lucerne |
| | WSM409 | Subterranean clover |
| | 5B1G | Adzuki bean |
| | CB3171 | Calliandra |
| 2001 | WSM1455 | Fababean, lentil |

Pre-inoculated seed testing

Substantial quantities of pasture legume seed are sold to farmers as pre-inoculated and custom inoculated seed. Often more than rhizobia are pelleted onto the seed. Purchase of treated rather than plain

seed is preferred by many farmers because of the convenience factor. The downside is that a substantial proportion of the product may be substandard. A 3-year survey of pre- and custom-inoculated seed in 1972–4 showed that 38 of 48 samples failed (20% pass rate) the ALIRU standard of 1000 cells/seed for species with seeds the size of lucerne and 500 cells/seed for the smaller white clover sized seeds. During 1999–2000, 42 samples of pre-inoculated lucerne, sub clover, white clover and red clover were assessed at ALIRU for rhizobial numbers at the time of receipt and for up to 16 days after receipt. Pass rates were reasonable for lucerne (62%), marginal for sub clover (43%) and very poor for white clover (7%) and red clover (0%). These data are of concern and highlight a need for research into the causes of death in the commercial processes as well as a more effective education campaign for personnel involved in pre-inoculation and sales of pre-inoculated seed.

Inoculant quality troubleshooting

In recent years, much of this has centred on the problems associated with genetic instability of rhizobial strains, variation in infectiveness and effectiveness and colony dimorphism. Strain most involved were the lucerne strain, WSM826, and the annual medic strain, WSM688.

Other activities include maintenance of a large (1700 strains) rhizobial strain collection, manufacture of special inoculants for sale (peaked during 1998/9 with >200 inoculants supplied), training, promotion and extension of inoculants and inoculation, and strain improvement (trials at 2–3 sites involving 3–5 species annually).

Conclusions

The legume inoculant industry has made and continues to make an enormous contribution to the economies of individual countries. It is a paradox that, despite almost 100 years of research and experience, many of the inoculant produced in the world today is of poor quality. Even good quality inoculants are often not used to best advantage. Brockwell et al. (1995) suggested that as much as 90% of all inoculant has no practical impact whatsoever on the productivity of the legumes for which it was used. This seems an extremely high figure which may have been used to make a point, rather than to be taken literally. Even if only 50% of the suggested 20 million ha of legumes were inoculated effectively, the economic benefit of the fixed N would be US\$0.5–1 billion annually for an outlay of US\$50 million. Brockwell et al. (1995) do go on to say that some good inoculants are produced and some of those are used properly in situations where they are

needed. In those circumstances, legume inoculation may be one of the most cost-effective of all agricultural practices.

However, Brockwell et al. (1995) were generally pessimistic about the prospects for the inoculants industry and its capacity for large-scale production of high-quality inoculants. Inoculants of the highest quality tend to be those produced by the private sector under the umbrella of an independent QC program (e.g. France, Australia). The future of the inoculant industry, and its potential benefits for world agriculture, depends on improving inoculant quality, both numerically and in terms of strain effectiveness. New technologies may lead to improved inoculants in industrialised countries but the fact remains that, in many countries, the 30 and 40-year old technology has yet to be properly mastered.

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