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**ALOSCA[®] – A new technology to deliver rhizobia and other beneficial
microbes into broadacre agriculture**

Steve Carr¹, Angelo Loi¹, Ana Isabel Vivas-Marfisi^{1,2}, Chris Poole¹

¹ ALOSCA[®] Technologies Pty Ltd, 69 Guthrie St Osborne Park WA 6017

² Centre for *Rhizobium* Studies, Division of Science and Engineering, Murdoch University, Murdoch Drive, Murdoch, WA 6150, Australia.

www.ALOSCA.com.au, Email: steve@aglime.com.au, loiangelo@gmail.com, aivivasmarfisi@hotmail.com,
cpoole@ALOSCA.com.au

Abstract

ALOSCA[®] technology was developed to provide a more reliable and end-user friendly delivery system for rhizobia and other beneficial soil microbes. The key feature of ALOSCA[®] technology is the enhanced survival of microbes during desiccation, which leads to better survival of inoculants and ultimately greater impact on plant growth. The use of ALOSCA[®] granules eliminates the need for slurry inoculum immediately prior to planting. ALOSCA[®] granules can be mixed with either seed or fertilizer at seeding and can remain viable in the ground for extended periods. A further advantage of the ALOSCA[®] technology is the flexibility it affords when fungicide is applied to seeds without adversely affecting the rhizobia which are supplied separately in the clay based granules.

Key Words

ALOSCA[®], rhizobia, beneficial microbes, delivery technology.

Introduction

There is active research throughout the world aiming to develop growth-promoting micro organisms for application to agricultural crops. The focus of much of this research is to decrease reliance on chemical fertilisers and particularly those that supply N. The close association between oil prices and applied N costs is highly likely to result in increased reliance on atmospherically fixed N to run a profitable farming enterprise. The symbiotic association between root nodule bacteria (rhizobia) and legumes plays a significant role in the productivity of the world agriculture by reducing approximately 100 million metric tonnes of atmospheric nitrogen into ammonia and saving \$US 10 billion on N fertilizer each year (Freiberg *et al.* 1996, Herridge and Rose 2000).

Leguminous plants are generally inoculated with bacterial cultures of the genera *Rhizobium*, *Mesorhizobium*, *Bradyrhizobium* and *Sinorhizobium*. Most legumes used in agriculture are nodulated by species from these genera, by forming colonies in nodules within the roots of the legume which fix N. Peat is the most common carrier used, as it effectively maintains bacteria in a moist state. This methodology was developed in the 1950s and remains as commercial best practice today. Nevertheless, it is often limited, especially in modern farming systems. To be effective, peat needs to be applied to seed and sown immediately into moist soil. Even under optimal conditions, the death rate of cells on seed can be as high as 90% per day (Roughley, 1988) primarily because of desiccation (Vincent *et al.* 1962).

Most of Australia's agricultural legumes have been inoculated in this manner for the last 50 years, with varying success. Under ideal conditions, the use of peat-based carriers for delivery of rhizobial bacteria to legumes has been relatively effective. However, there is variation in tolerance to different levels of humidity between strains of rhizobia. The rate cells are dried plays an important role in survival, with better survival after slow drying. Changes in farming practice have been exerted by altered biological and economic pressures. A good example of the biological pressures is best exemplified by the build-up of plant pathogens. For the major Australian pulse crops lupin, chickpea, faba bean and field pea, disease pressures are such that all crops require seed-applied fungicides. This presents a management conundrum; fungicides are detrimental to the survival of rhizobia when the two are in close contact. Further, economic pressure on farmers has dictated that yields must be maximised, as yield is the greatest determinant of profitability. In dryland cropping, sowing the crop prior to winter rains to maximise water-use efficiency is useful. Weeds are controlled post-sowing, however the rapid death of rhizobia on seed sown into dry soil precludes the dry-sowing option for legumes. Inoculants applied to seeds suffer a high mortality rate under this regime. Further complications occur in seasons when early winter rains encourage the sowing of crops, but are not followed by sufficient rains to keep the soil moist.

Strategies to regulate water and oxygen gain or loss from rhizobia coated onto seed have been tried, and met with limited success particularly in the harsh mediterranean climate. The effort to separate inoculants from toxic chemicals has posed a challenging research problem. ALOSCA[®] is the first truly dry granule to be commercially released that is suitable for use in mediterranean agriculture. Current inoculation technology has been sub-optimal in changed farming systems and legume performance often suffers due to poor nodulation, especially following extended periods of dry warm weather before adequate rainfall is received. In the case of pasture improvement using legumes, farmers have a strong desire to dry sow (i.e. before the winter rainfall commences). On many large farms, this is purely a logistical decision due to time restrictions when winter rainfall commences, although there are also economical advantages (e.g. pastures commence growing as soon as rain falls, thus greater yield).

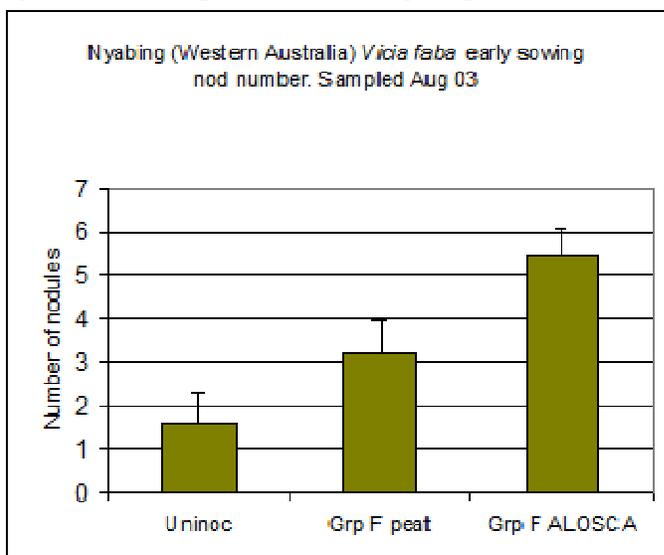
Results and Discussion

As pasture legume agronomists, Steve Carr and Angelo Loi were driven by a belief that the legume inoculation process could be transformed if a suitable medium could be found to 'house' the root nodule bacteria. In looking for a solution Carr and Loi focused on developing a carrier that could be effective in terms of agronomy, soil chemistry and rhizobiology. Having recently developed several new pasture legumes species (Howieson *et al*, 1995; Loi *et al* 2005) that require a shallow (<10mm) seeding depth, Carr and Loi were well aware that many recent cultivars have very small seeds, that are prone to poor nodulation initiation even under what would be considered favorable seeding conditions. Thus, further limitations to the peat based inoculation that had served Australian agriculture for the past 50 years. Many materials were evaluated to 'deliver rhizobia', with attention to storage and longevity under a range of conditions the key focus. Cost and commercial viability was also a determinant for material suitability.

Of all the materials tested, a unique calcium bentonite was identified as being highly suitable. Extensive and independent field-testing by staff at The Centre for *Rhizobium* Studies at

Murdoch University revealed nodulation counts on a range of legume species were comparable to those obtained with fresh peat inoculants (Figure 1).

Figure 1. Nodulation counts at Nyabing (Western Australia) on *Vicia faba* inoculated with ALOSCA granules and peat inoculant group F



Additionally, variable temperature treatments were conducted with the product subjected to daily soil surface summer temperatures ranging from 15°C to 60°C for periods up to three months. Nodulation experiments showed that even after such harsh treatment, early forms of ALOSCA® were still viable in delivering rhizobia for effective nodulation (Table 1).

Table 1. Dry matter production of Casbah biserrula grown for four weeks, and inoculated with ALOSCA granules produced and stored under different temperature regimes (eg 60/15C= simulation of temperatures on soil surface in summer) for 8 weeks prior to sowing biserrula (standard errors in parentheses)

Treatments	Shoots (g)
Uninoculated	0.014 (0.005)
Fresh peat	0.061 (0.014)
ALOSCA 8 weeks at 60/15 C	0.076 (0.024)
ALOSCA 8 weeks at 20C	0.052 (0.008)

Following early promise the field based trial program expanded and diversified. Many independent field trials were conducted by a range of research providers including CRS at Murdoch University, Ballard Seeds, Landmark and a number of legume seed producers and commercial farmers. This field program ranged from row trials through small plot trials to broad acre, farmer-sown comparisons using conventional equipment. Inoculants to satisfy the broad range of both pasture and grain legumes were made from the appropriate strains. Biserrula was often chosen because of the lack of background rhizobia in WA soils. Biserrula is a relatively new pasture legume introduced to world agriculture in 1995 (Howieson *et al.* 1995).

Trial results across Western Australia continued to be extremely encouraging, and a semi commercial 'proof of concept' release was undertaken with product to cover 10,000 hectares was done in 2004. ALOSCA[®] was subsequently commercialised in 2005 with growers in WA using the system on more than 40 000 hectares in the first season, increasing above 100 000 hectares in 2006. An Australian and international patent application on the novelty of the delivery system is currently under examination.

Features of ALOSCA[®] technology

ALOSCA[®] technology is based on a bentonite clay granule that contains high numbers of viable rhizobial cells despite being a dry product. ALOSCA[®] granules are a favourable environment for rhizobial survival and enable rhizobial respiration to proceed during desiccation. The unique clay enables rhizobia and other beneficial microbes to colonise the lattice structure of the clay and when the clay dries out during the manufacturing process, the microbes remain alive (a more detailed description will be available at the publication of the patent).

The use of ALOSCA[®] granules eliminates the requirement for legume seeds to be inoculated with the common slurry inoculum immediately prior to planting. The granules can be applied as a mix with the seed or with the fertilizer at seeding and can stay in the ground when it is dry for extended periods (many months) without losing viability. By providing granules of similar but smaller size to the seed to be inoculated, mixing and uniformity of 'flow' through seeding machinery is facilitated leading to enhanced distribution of seed and granules in the sown crop or pasture. Further, the very fine granules are able to adhere to the surface of the legume seed being sown, thus providing greater proximity between the seed and the inoculant source leading to more rapid formation of functional nodules when the plant germinates and commences growing.

An outstanding feature of ALOSCA is the capacity of the granule to release the bacteria when soil moisture conditions are adequate for seed germination and favourable for bacterial multiplication. Another important attribute is the capacity to 'preserve' the rhizobial cells. If dry conditions follow seeding, with inadequate rainfall for germination, the clay will close up around the microbes and continue maintaining viability.

The ALOSCA[®] granules have a very long shelf life (>than 6 months) because there is little decline after the initial production process, even when granules are exposed to high summer temperatures. Current peat based inoculants need to be refrigerated (4°C) to survive- ALOSCA[®] does not. This is far more practical for farmers.

Another advantage of ALOSCA[®] is the opportunity for disease control. Many crop legumes exhibit a high degree of susceptibility to a range of foliar diseases. With conventionally inoculated legumes (i.e. peat added to the seed surface), fungicides cannot be applied directly to seeds as the fungicides kill rhizobial cells as well as fungal spores. ALOSCA[®] alleviates this problem and provides a method of inoculation wherein fungicide may be applied to seeds without adversely affecting the rhizobia.

Seeds of legumes inoculated with ALOSCA[®] granules can be also coated with insecticide for controlling pest attack during the early stages of growth, which in the case of pastures like clover, is the most vulnerable stage. In this way the need to apply insecticide over whole

paddock is eliminated, saving time and money and eliminating the removal of useful insects such as bees and other natural enemies of pests such as RLEM, lucerne flea and aphids.

In addition to the delivery of root nodule bacterial strains, the granular system is a suitable carrier for a range of other soil microbes including those that are currently being investigated as potential plant growth promoting Rhizobacteria (PGPR's). There are many PGPR's that can increase yields in non-legume crops by increasing rates of germination, hormone production and root and shoot growth, and by delaying leaf senescence. Other microbes have been shown to affect plant growth by increasing nutrient solubilisation (P, N & K), production of plant growth regulators, suppression of plant pathogens by competition and the release of antibiotics or siderophores. Preliminary trials involving the use of promising PGPR's have been conducted in 2003 to 2005 using ALOSCA[®] delivery system. Early results suggest that ALOSCA[®] granules delivered these microbes in an efficacious way.

Conclusion

The vision for the ALOSCA[®] delivery system was to develop a better and user-friendly inoculant delivery system for the agricultural sector. The idea behind the system was borne out of an in depth scientific and practical understanding of a perceived deficiency within the industry, involving the viable delivery of inoculants to agriculture. Whilst the beneficial role of root nodule bacteria is well established, a practical user-friendly delivery system to capitalise on the scientific realities has not been implemented. The conceptualization of the ALOSCA[®] delivery system was from clear vision of the need to improve the prevailing methodology and enhance the efficiency and effectiveness of the inoculation process.

ALOSCA[®] represents a significant development in the delivery of root nodule bacteria to Australian agricultural systems. The nature of the granules gives to the bacteria a considerable edge over the traditional peat based system. Moreover, primary producers are no longer required to sow at ideal times to ensure survival of rhizobia, the dry granules offer new degrees of flexibility to sowing times without resulting in diminished nodulation.

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