NITROGEN FIXATION BY LUPINS IN WESTERN AUSTRALIA: WHICH MICROBES ARE RESPONSIBLE, FROM WHERE DID THEY ORIGINATE, AND CAN WE INTERCEDE?

Professor John Howieson and Dr G.W. O'Hara

Centre for Rhizobium Studies, Murdoch University, Murdoch WA 6151

Corresponding author's email: j.howieson@murdoch.edu.au

ABSTRACT

Lupins first appeared in Western Australia (WA) as weeds in the early 19th century, probably introduced accidentally in forage for animals. The first recorded inoculation experiments with lupins in WA were conducted by Adams and Riches in the 1920s. In these experiments, it was revealed that inoculation was essential for *L. angustifolius* and *L. luteus*, but not for *L. cosentinii*. In the 1960s, Lange and Parker demonstrated that root-nodule bacteria (rnb) associated with WA native legumes were not symbionts of the former species of lupin, but were of the latter, and in this era inoculation responses in lupin trials were significant. Commercial-quality inoculant strains were hence developed. The lupin industry expanded rapidly in WA in the 1970s, and by the early 1980s D.L. Chatel had demonstrated that inoculation responses were rare. Nitrogen (N) fixed by lupin crops met > 75% of the cereal N demand in crops grown after lupins. It was assumed that the inoculant strains had colonised the WA wheatbelt. But had they? Which organisms actually nodulated *L. cosentinii*? Why are cereals grown in rotation with lupins in the modern era fertilised heavily with inorganic N? Can lupin N fixation in the 21st C be increased by inoculation with superior strains or new carriers or other potentiating bacteria? These questions will be addressed in this manuscript.

KEYWORDS

inoculation, nodule occupancy, *Bradyrhizobium,* nitrogen fixation, phylogeny

INTRODUCTION

As for all commercial Agricultural legumes in Australia, lupins were introduced to this continent, and like many, the manner of their introduction was originally accidental (Gladstones, 1998). The understanding of the primary role of root-nodule bacteria in nitrogen fixation with legumes was not revealed until late in the 19th century, about the same time as these accidentally introduced legumes began to colonise many infertile soils in Australia. This manuscript outlines our current understanding of how lupins managed to nodulate when originally introduced to Australia, the organisms nodulating commercial crops presently, the current status of nitrogen fixation in this symbiosis and our attempts to improve the nodulation and N fixation in commercial lupin crops.

THE EARLY YEARS OF LUPIN CULTIVATION

Adams and Riches (1930) noted that lupins, predominantly *L. angustifolius* and *L. luteus*, were absolutely dependent upon inoculation for vigorous growth. However, it was also apparent to workers of that era that these species sometimes nodulated poorly (presumably because of inferior inoculation technology) in regions where *L. cosentinii* (then *L. digitatus*) readily established (Cass-Smith and Pittman, 1939). The question was often raised as to whether the latter species could nodulate with bacteria (hereafter rhizobia) associated with the indigenous legumes. Given that the lupins arrived to WA accidentally, this was a reasonable question to ask, bearing in mind that the science of rnb and inoculation practices was then quite rudimentary. It must have been tempting to speculate that the introduced legumes nodulated with the rhizobia associated with the indigenous legumes.

These field observations and questions led to an intensive investigation of the cross-inoculation characteristics of lupins in relation to rhizobia from indigenous WA legumes by workers in the laboratory of C.A. Parker, notably R.T. Lange. As the taxonomy of rhizobia at that time was wholly based upon the cross-nodulation concepts (Vincent) this was the only practical experimental means for investigating the question of the origin of the lupin rhizobia. Lange and Parker (1960) proved that a high level of specificity existed in lupin nodulation. They could not easily reconcile the differences between their careful glasshouse experiments and field observations, which they interpreted as indications of nodulation by indigenous bacteria. These suspicions were fuelled by some ineffective nodulation of *Jacksonia, Gastrolobium* and *Kennedya* by isolate D25 (and perhaps 24) from *L. cosentinii* (Lange, 1961). We see later some possible explanations for this apparent cross-over.

IN J.A. Palta and J.B. Berger (eds). 2008 'Lupins for Health and Wealth' Proceedings of the 12th International Lupin Conference, 14-18 Sept. 2008, Fremantle, Western Australia. International Lupin Association, Canterbury, New Zealand. ISBN 0-86476-153-8.

INOCULATION RESPONSES IN LUPIN SOWINGS POST 1980

Despite the early knowledge that lupin absolutely required inoculation, so vigorous was the colonisation of sandplain soils by the lupin and serradella nodulating rhizobia, that by 1980 the experiments of D.L. Chatel and colleagues demonstrated only rare responses to inoculation. These responses tended to occur in the eastern wheatbelt far from the coast, or on duplex soils in the Great Southern region of WA not traditionally cropped to lupin.

UNDERSTANDING LUPIN NODULE OCCUPANCY

The molecular era provided rhizobiologists with new tools to investigate nodule occupancy. These tools were not immediately applied to lupin rhizobiology, because reports of failure in nitrogen fixation of lupins were very rare. The symbiosis had proven to be robust on the acid sands where the lupin industry had strongly developed. Because of excellent responses to wheat after lupins it was assumed that the symbiosis was essentially highly effective and it was (perhaps optimistically) hoped that the nodule occupants were related closely to the commercial inoculant strains. However, in a PCR-RAPD based survey of lupin crops conducted by CRS and DAFWA scientists in 2005, it was revealed that the commercial inoculant strains (e.g. WU425, WSM471) were completely absent from nodules. This was not altogether a shock, as previously McInnes (2002) had surveyed serradella nodules in WA and returned a similar finding. However, the complete absence of commercial inocula in nodules was intriguing. We naturally then asked questions of the origin of these strains, with the following explanations being most likely. The genotypes were:

- Escaped research isolates.
- Native rhizobia that had acquired symbiotic genes from lupin inoculants.
- Native rhizobia able to nodulate lupins as suggested by Lange (1962), originally in low number, that had increased in population size in the presence of agricultural hosts.
- Specific lupin bacteria that had arrived by accident and colonised the WA soils in parallel with the lupin industry.

The solution was not obvious, remembering that Lange (1961) had provided some evidence of cross-nodulation between native and introduced legumes, and that McInnes (2002) had recently provided evidence that isolates from *Daviesia* and *Mirbelia* could nodulate serradella.

THE ANSWER TO OUR QUESTION

Polish scientist Tomasz Stepkowski had become interested in the phylogeny of *Bradyrhizobium* (the taxonomic name for the lupin nodulating organisms) during the 1990s and had developed a number of analyses based upon both housekeeping and symbiotic genes. He met several of the Australian *Rhizobium* scientists at the 2004 European Nitrogen Fixation Conference and soon organised an exchange of some 40 isolates. Application of his molecular phylogenetic techniques demonstrated unequivocally that the nodule isolates from WA lupin and serradella plants, including several early *L. cosentinii* isolates from the lab of CA Parker (W72, WU140, WU8), belonged to a clade of Bradyrhizobia of exclusively European origin. Using the nodulation genes *nodA nodZ nolL*, the housekeeping genes *dnaK, recA, glnII, atpD* and the 16S-23S rRNA ribosomal intergenic spacer, Stepkowski et al (2005) demonstrated with substantial clarity that the lupin nodulating clade was distinct from Bradyrhizobia isolated from Australian native legumes. Included in the European clade were Australian inoculant strains WU426 and WSM471.

So, after nearly 100 years of speculation and investigation, the origin of the lupin nodulating organisms in WA was clarified. This knowledge led to new questions being asked. Were the nodule organisms as effective as the inoculant strains at nitrogen fixation? If not, could they be displaced by superior strains? Was there any benefit in inoculating?

N FIXATION IN LUPINS AND RESPONSE TO INOCULATION IN THE 21ST CENTURY

A CRS study of the strains collected in the 2005 survey revealed the isolates differed by some 50% in nitrogen fixing ability (Fig. 1); strain WSM4024 was identified as a nodule occupant with greater than average nitrogen fixing ability (arrowed Fig. 1). Experiments were undertaken to see if we could influence lupin nodulation by inoculation, and whether this led to increased growth or nitrogen fixation. These are important questions as the apparent value of N fixed by lupins has decreased over the last 20 years. Whereas Perry *et al.* (1998) demonstrated that wheat grown after lupins required only approximately 50 kg of fertiliser N for maximum yield, farmers on the same soils today routinely apply more than 100 kg of N to cereals grown after lupins. Has there been some decrease in the level of N fixed by lupins and available to subsequent crops?

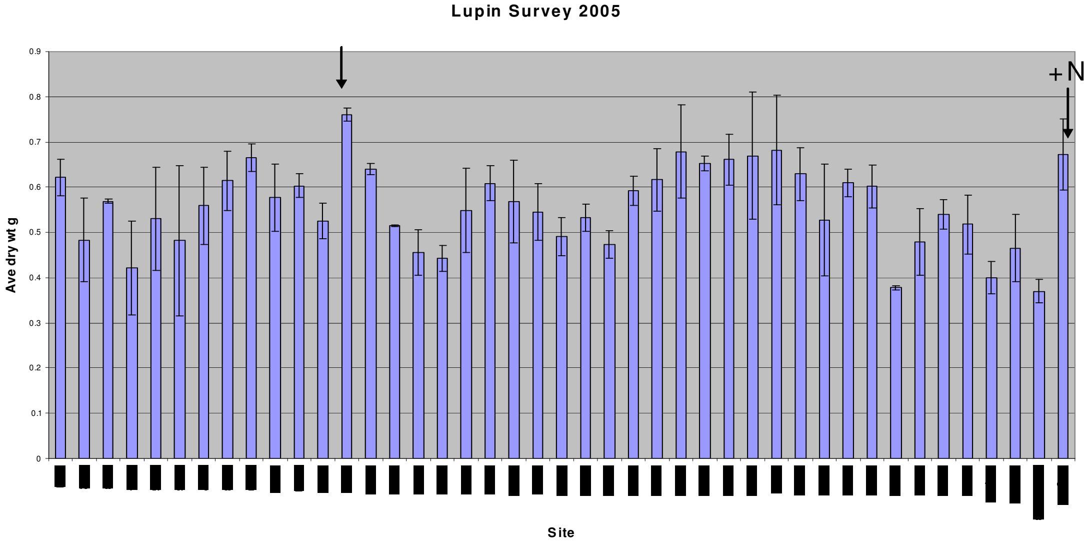

Fig. 1. N fixation by isolates obtained from the 2005 survey of WA wheatbelt lupin fields with *L. angustifolius* grown under glasshouse conditions. Arrowed is strain WSM4024 (left) and the +N control (right).

Inoculation experiments by CRS and DAFWA through 2005-2008 have repeatedly shown trends towards increased early nodulation following inoculation, even on soils with a rich history of lupin cultivation. This effect has been maximised in the presence of a Nodule Increasing Bacteria (NIB) added to the inoculants or independently to inoculated seed (Fig. 2). Although we have experienced a run of years with below-average rainfall, increased early nodulation has occasionally correlated with an increase in seed yield or grain protein (Fig. 3).

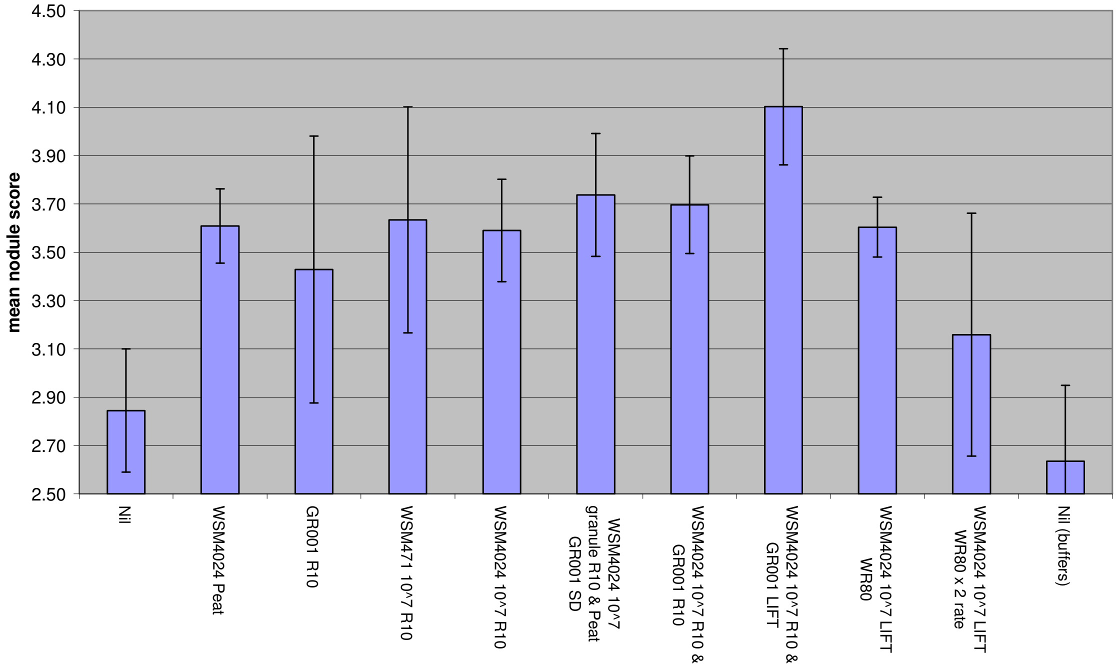

Fig. 2. Response in early nodulation to inoculation of lupins grown on Mingenew sandplain with high N fixing strain WSM4024 in association with a Nodule Increasing Bacteria (GR001) delivered as a liquid in furrow (LIFT) coinoculant.

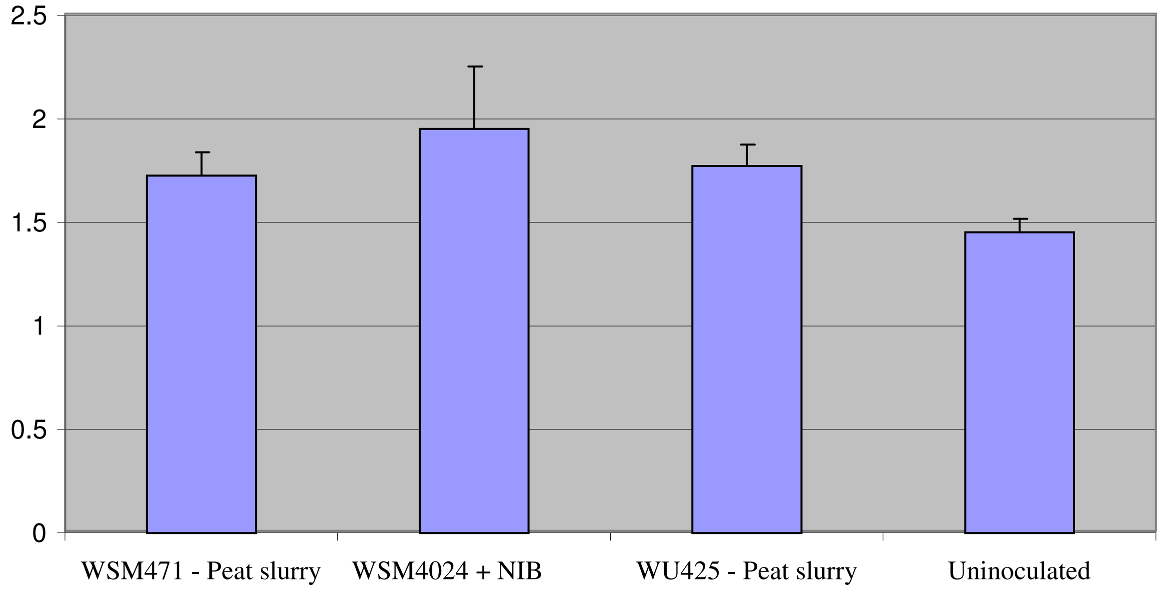

Fig. 3. A 25% response to lupin yield following inoculation with commercial strains WSM471 or WU425, relative to new strain WSM4024 in association with a nodule inducing PGPR (NIB) at Eradu in the dry year of 2006. Data are seed yield (kg)/plot.

CONCLUSIONS

The 100 year mystery surrounding the origins of strains nodulating Lupin crops in WA has been solved by application of molecular phylogenetic tools to identify nodule isolates. Nodulation is overwhelmingly by the ancestors of strains that, like lupins themselves, arrived accidentally to Australia from Europe. This knowledge has precipitated a new investigation of whether inoculation with elite strains is necessary to maximise nitrogen fixation in lupin crops. Evidence suggests that superior strains can increase the early nodulation of lupins, leading to increased yield from improved nitrogen fixation. This is an important outcome in an agricultural setting where an analysis of the carbon footprint of farming shows that nitrogen fertiliser is the single greatest contributor to greenhouse gases from cereal farming.

REFERENCES

Adams, A.B. and J.H. Riches. 1930. Root nodules on Lupins. J. Dept. Agric. West. Aust. 2: 556.

Cass-Smith, W.P. and H.A.J. Pittman. 1939. The effect of inoculation on the growth of field peas. Lucerne, lupins and tick beans. J. Dept. Agric. West. Aust. 16: 73-79.

Gladstones, J.S. 1998. Distribution, origin, taxonomy, history and importance. In J.S. Gladstones, C.A. Atkins and J. Hamblin (Eds.), Lupins as Crop Plants: Biology, Production, and Utilisation (pp. 1-37). Cambridge: CABI Publishing.

Perry, M.W., M. Dracup, P. Nelson, R. Jarvis, I. Rowland and R.J. French. 1998. Agronomy and Farming Systems. In: J.S. Gladstones, C.A. Atkins and J. Hamblin (Eds.), Lupins as Crop Plants: Biology, Production, and Utilisation (pp. 291-338). Cambridge: CABI Publishing.

Stepkowski, T., L. Moulin, A. Krzyzanska, A. McInnes, I.J. Law and J.G. Howieson. 2005. European origin of *Bradyrhizobium* populations infecting lupins and serradella in soils of Western Australia and South Africa. Applied and Environmental Microbiology 71: 7041-7052.

Lange, R.T. and C.A. Parker. 1960. The symbiotic performance of lupin bacteria under glasshouse and field conditions. Plant Soil 13: 137-46.

Lange, R.T. 1961. Nodule bacteria associated with indigenous *Leguminosae* of south-western Australia. Journal of General Microbiology 26: 251.

McInnes, A. 2002. *Bradyrhizobium* spp. associated with serradella in Western Australia. PhD thesis, Soil Science and Plant Nutrition, UWA.