Inoculants and Nitrogen Fixation of Legumes in Vietnam

Proceedings of a workshop held in Hanoi, Vietnam 17–18 February 2001

Editor: D. Herridge

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ACIAR Proceedings 109e
(printed version published in 2002)

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Herridge, D. (ed.) 2002. Inoculants and Nitrogen Fixation of Legumes in Vietnam. ACIAR Proceeding 109e
www.aciar.gov.au/publications

ISBN 1 86320 335 4

Editorial management: PK Editorial Services, Brisbane, Australia
Typesetting and layout: Sun Photoset Pty Ltd, Brisbane, Australia

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Preface

ACIAR Project LWR2/98/27 'Increasing yields and nitrogen fixation of soybean, groundnut and mungbean in Vietnam through rhizobial inoculation' commenced in April 1999 with the broad mission of laying the foundation of a program to produce sufficient high-quality rhizobial inoculants to satisfy Vietnam's expanding legume industry.

Specific objectives of LWR2/98/27 were to:

- Quantify N_2 fixation by legumes in farmers' fields in Vietnam using natural ^{15}N abundance and xylem ureide methods and to identify farmer management practices that either depress or contribute to N_2 fixation.
- Evaluate the quality of inoculants, currently produced in Vietnam.
- Complete multi-location inoculation experiments to determine the most effective strains for each species and benefits of inoculation in terms of yield and N_2 fixation.
- Enhance the capacity in Vietnam to produce and distribute high quality rhizobial inoculants through research and training and infrastructure development.
- Lay the foundation for a development project to produce large volumes of high quality rhizobial inoculants in Vietnam.

It was our intention that, at the end of the project, the potential benefits of rhizobial inoculation in Vietnam would have been quantified and the capacity of inoculant R&D and quality control (QC) in the country enhanced. The long-term aim, however, was to develop a large-scale, efficient inoculant production facility, preferably in the hands of the private sector, with technical inputs, such as the R&D and QC, from the Government research institutes and Universities. We recognised that this may only be achieved through a larger, long-term funded project. Thus, this ACIAR small project was instigated as a precursor to that larger project, providing technical information and scientific capacity as well as momentum.

A technical workshop at the Vietnam Agricultural Science Institute (VASI) in Hanoi during 17–18 October, 2001, was the trigger to collate, interpret and publish outputs of the LWR2/98/27 experimental program and to plan a next phase of the Vietnam inoculants program. The workshop in Hanoi attracted about 30 participants, mainly country scientists associated with LWR2/98/27, VASI Micobiology Department personnel and scientists from the Hanoi University. Dr Paul Singleton, NifTAL-University of Hawaii, Dr Nantakorn Boonkerd, Suranaree University, Thailand, Dr Graham O'Hara, CRS-Murdoch University, Australia, and Ms Jo Slattery, NRE Rutherglen, Australia, participated as did Dr Tony Fischer, ACIAR Land and Water program manager.

Seventeen technical papers were presented during the two days of the workshop, covering applied and fundamental issues of rhizobial and non-rhizobial inoculant production and application. A number of the papers clearly outlined the substantial economic benefits from replacing fertiliser N with rhizobial inoculation in legume cultivation in Vietnam. Vietnam cultivates about 700,000 ha of legumes annually, equally distributed between the north and the south of the country. Production is about 700,000 t, worth A$350 million. None of the crops are inoculated and all are fertilised with 30–150 kg N/ha at a cost to the farmers of A$50–60 million annually. Results from LWR2/98/27 inoculation experiments in the Mekong Delta and in the Eastern region in the south of the country indicate this to be an unnecessary cost, and one that could be substantially reduced if fertilising with N was replaced by inoculation. The cost of the latter would be in the order of A$2 million annually.

In the 23 experiments, profitability was increased by as much as A$760/ha through inoculation and substantially reduced fertiliser N inputs. Average grain yield responses

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for groundnut, relative to the −N and +N uninoculated controls, were 12%, and 7% (8 experiments), although responses in the very acidic Mekong delta averaged 42% (relative to −N control) and 28% (relative to the +N control). For the 13 soybean experiments, the average response was 19%, relative to the −N control. Individual experiments showed grain yield responses of 40–50%. Yields of the inoculated and +N plots were almost identical.

It was also clear that for inoculation to become a reality, high-quality inoculants would need to be as readily available in the market place as fertiliser N and farmers would need to be educated in their use, just as they are educated about using fertilisers and chemicals. Not every legume crop would need to be inoculated. In many of the established legume areas in the country, the naturalised rhizobia already present in the soil would be adequate. In other areas, however, inoculation would have clear economic benefits.

The workshop ended very positively with consensus among the group that a new coordinated program would be developed encompassing large-scale inoculant production in both the north and south of Vietnam, and associated research, extension, policy development and evaluation. The program would likely involve the key institutes in Vietnam currently doing rhizobiology research and international collaborators from the U.S., Thailand and Australia. Funding would be sought from international agencies. The expectation is that Vietnam can mimic the success story of Thailand, where inoculant production increased from 30,000 units in 1980 to >1 million units 13 years later. This is the challenge.

David Herridge
Leader, LWR2/98/27

Legume N and Inoculants: Global and Vietnamese Perspectives

David Herridge[1]

Abstract

The grain and oilseed legumes fix about $US8.5 billion worth of N annually and provide almost 20% of the N required for global grain and oilseed production. Much of this N results from either current inoculation of the legumes with rhizobia or from inoculation of previous crops which has left a population of rhizobia in the soil. Vietnamese farmers do not inoculate their legume crops; rather they apply fertiliser N at rates of 50–150 kg N/ha. Substituting fertiliser N with inoculant could save them US$25–30 million annually. Developing the capacity in the country to produce large volumes of high-quality inoculants requires a committed, focussed co-ordinated national program with adequate funding incorporating research and training and involving both private- and public-sector interests to cover manufacture, marketing and quality control. The challenge is to couple together the international and national expertise, resources and funding in such a program.

LEGUME N_2 fixation plays a key role in world crop production. About 100 million t N, valued at $US50 billion, is required annually for the production of the world's grain and oilseed crops (Table 1). Of this amount, N_2 fixation by the oilseed legumes, soybean and groundnut, and by pulses supplies almost 20% (17 million t N). With N fertiliser costing about $US0.50/kg, this is equivalent to a saving of $US8.5 billion. Much of the 26 million t N derived from soil sources could also have originated from legume residues.

Legumes can only fix N if they are nodulated by effective, compatible rhizobia. In many soils, populations of naturalised rhizobia are present in sufficient numbers to nodulate the sown legume. In other situations, there may be only low numbers of rhizobia in the soil or they may be entirely absent. Under these conditions, introduction of highly-effective rhizobia at the time of sowing of the legume will usually result in sufficient N being fixed by the crop to fulfil its requirements for growth.

During the past 100 years, rhizobial inoculation of legumes has proved to be one of the most cost-effective of all agricultural practices, and has played a major role in establishing soybean, groundnut and the pulses as major global crops. Soybean is now grown on about 70 million ha world-wide and has been estimated to fix about 11 million t N annually, worth US$5.5 billion. It is likely that only 10–15 million ha (i.e. 14–21% of the total) are inoculated annually. However, virtually all of the 11 million t N currently fixed by soybean results from either past or current inoculation. This is because soybean, for the most part, is grown on land that initially did not contain the soybean rhizobia.

Vietnam cultivates about 700,000 ha of legumes annually, none of which are inoculated and all of which are fertilised with N at rates of 50–150 kg N/ha. This represents a cost to the farmer of US$25–30 million annually. It would appear to be an unnecessary cost, and one that could be substantially reduced if the practice of applying fertiliser N was replaced by the practice of inoculation. The cost of the latter would be in the order of US$1 million annually.

For inoculation to become a reality, inoculants would need to be as readily available in the market place as fertiliser N and farmers would need to be educated in their use, just as they are educated about using fertilisers and chemicals. Not every legume crop would need to be inoculated. In many of the established legume areas in the country, the naturalised rhizobia already present in the soil would be

[1] NSW Agriculture, Centre for Crop Improvement, Tamworth, NSW 2340, Australia

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Table 1. Global statistics of crop areas and production in 1998 and estimates of N required and supplied from various sources (FAO 1996, 1998)

Crop	Area (million ha)	Production (million t)	N required (million t)[a]	N supplied (million t)		
				Fertiliser[b]	Soil[c]	N_2 fixation[d]
Cereals	670	2,055	62	60	17	0
Oilseeds	92	117	5	6	0.5	0
Soybean	71	156	19	0.5	7.5	11
Groundnut	24	31	3	0.5	0.5	2
Pulses	68	57	5	0.5	0.5	4
Total	925	2,416	94	67.5	26	17

[a] Amounts N required to produce each tonne grain were as follows: cereal: 30 kg N/t; oilseed: 40 kg N/t; soybean: 120 kg N/t; groundnut: 100 kg N/t; pulses: 80 kg N/t.
[b] FAO statistics
[c] Calculated by difference, assuming 75% fertiliser-N used in crop growth
[d] Assume average % Ndfa for: soybean: 60%; groundnut: 70%; pulses: 80%

adequate. In other areas, however, inoculation would have clear economic benefits.

Lessons from Other Countries

The first lesson worth noting is that broad-scale adoption of legume inoculation by farmers will only come with local production of high-quality inoculants. The experience in Asia is that an initial infusion of external funds and technical support are needed to achieve this, e.g. Thailand through USAID, Bangladesh through the International Atomic Energy Agency (IAEA), Myanmar partially through USAID.

The other lesson is that inoculant manufacturing has frequently been created in concert with the large-scale introduction of exotic, rhizobia-specific legumes. For example, inoculant markets developed concurrently with the development of the soybean industries in the U.S., Brazil, Indonesia and Thailand, with the pea and lentil industries of Canada, with chickpea in Turkey and with sub clover in Australia. Dramatic responses to legume inoculation in these systems facilitated market development. Such new crop introductions tend to be highly responsive to inoculation, not only because the specific rhizobia tend to be either low in numbers or absent in the soil, but also because there is usually a simultaneous introduction of other production inputs that increase yield, and therefore N_2 fixation, potential. This synergy between inoculant and other management inputs can play a major role in gaining farmer acceptance of inoculation.

Potential for Expansion of Inoculation in Vietnam

Legume inoculation could expand substantially in Vietnam, even without projected increases in legume production. The practice is inexpensive, requires only a small amount of technical expertise and there is low economic and environmental risk associated with its production and use.

The most probable limitations to expansion relate both to the inoculants themselves and to the alternative source of N for legume crops—fertiliser N. First, reasons relating to the inoculants:

- lack of awareness by farmers and extension workers of the benefits of inoculation, which includes masking of the benefits of inoculation by other yield-limiting factors, such as P and K;
- lack of confidence by the farmers in inoculant quality or efficacy, resulting from past experience with poor-quality inoculants;
- lack of appropriate methods of application;
- the difficulty for inoculant manufacturers to develop markets composed of small holders operating at low input, subsistence level;
- manufacture, marketing and distribution problems that restrict availability of inoculants;
- lack of private sector involvement in inoculant production. It is really only the private sector that could produce and successfully distribute the large volumes of inoculant required for a possible market of 200,000–300,000 ha of soybean, groundnut and other pulses.

Inoculants have also been out-competed in the marketplace by fertiliser N. It is difficult to obtain

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accurate, definitive information as to whether the fertiliser N is subsidised or not in Vietnam. However, it appears the price to the farmer may be 20–30% below market price. Thus:

- successful promotion and marketing of fertiliser N as a more effective and profitable source of N for legume crops.

What is needed to overcome the above constraints to inoculant production and use in Vietnam? The following are suggested:

- a committed, focussed and coordinated national legume inoculants-N_2 fixation program with adequate funding;
- a person or persons to champion the technology; interestingly, in many countries that have successfully embraced rhizobial inoculation, much of the success can be traced to individuals;. examples are Vincent, Date and Roughley (Australia), Freire (Brazil), Labandera (Uruguay), Burton (USA), and Boonkerd (Thailand);
- external funding to provide the necessary training and help with setting up of pilot production facilities in Vietnam;
- training in inoculant production techniques and QC programs; the latter is particularly critical—inoculants will only be accepted by farmers and used over the long-term if they are of high quality and are efficacious;
- R&D on rhizobial strains, carriers, methods of production and application for local conditions;
- field demonstrations of effects of inoculation on legume yields; better-planned experiments that show the benefits of inoculation in a total package that may include other key nutrients, e.g. P and K; such experiments are necessary for extension purposes and help to create the demand for inoculants;
- private-sector involvement in inoculant production; this will only occur if the market is sufficiently large and can sustain the viability of private companies; therefore, private sector involvement should be expected after a period of market development and growth.

Increasing inoculant use in Vietnam will require a sustained effort to produce quality product and, importantly, to extend the technology to farmers. Extension programs require information on the value, application and marketing of inoculants.

Singleton (1989) reported that extension specialists in Indonesia had little or no knowledge of N_2 fixation and were unable to recognise nodulation failures in farmers' fields. Hall and Clark (1995) showed that, despite the relatively successful extension program in Thailand, up to 42% of farmers in some regions still viewed inoculants as materials to improve seed germination. The latter program was relatively successful, however, with inoculant production increasing about 30-fold in a 10-year period and the percentage of soybean crops inoculated increasing from 30% in 1986 to 50% three years later. The key to that success was that inoculants were part of a total legume-production package.

Conclusions

Countries such as Thailand have shown that it is possible to develop large-scale capacity for inoculant production and distribution, provided the market is clearly defined and farmer education is coupled to the production and distribution. In Vietnam, surveys of farmer legume crops and inoculation experiments during the past two years indicate almost zero use of inoculants but large potential for improved productivity and profitability with their use. What remains now is to consider these results and to determine a future course of action. It might be to vigorously pursue the means to expand inoculant production, marketing and QC in the country or, on the other hand, it might be to accept the current situation. Whatever the decision, it should be based on clear economic benefits.

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Nitrogen Fixation of Soybean and Groundnut in the Mekong Delta, Vietnam

Nguyen Huu Hiep[1], Cao Ngoc Diep[1] and David F. Herridge[2]

Abstract

Rice is the major crop of the Mekong Delta (MD) of Vietnam with legumes, groundnut (*Arachis hypogaea*) and soybean (*Glycine max*) cultivated in sequence with the rice in some areas. As part of a country-wide study to determine the current use and potential benefits of rhizobial inoculation in Vietnam, we conducted surveys of commercial groundnut and soybean crops and six inoculation experiments in the MD. The surveys were conducted in winter-spring 1999–2000 and summer-autumn 2000 (groundnut in Long An province) and spring-summer 2000 and 2001 (soybean in Dong Thap, Vinh Long and Can Tho provinces). All farmer crops in the surveys were nodulated and fixed N (means of 50% Ndfa for groundnut and 70% for soybean), even though none of the crops were inoculated. All crops were fertilised with N at high rates (range 35–165 kg/ha, with averages of 70 kg/ha (groundnut) and 108 kg/ha (soybean)). The six inoculation/fertiliser experiments showed that both legumes responded to inoculation by producing more abundant nodulation and higher pod or grain yields. Pod yield benefits were as much as 1.5 t/ha for groundnut and 0.5–0.6 t/ha for soybean. For both species, the optimum fertiliser mixture plus inoculation produced the greatest economic returns. Farmers could clearly improve profitability by reducing fertiliser N inputs from the current rates of 50–150 kg/ha to 'starter' rates of <20 kg/ha and inoculating with high-quality rhizobial inoculants.

THE MEKONG DELTA (MD) occupies 2.9 million ha (12% of the Vietnam's total land area) and is one of the two principal areas of rice production of Vietnam. The weather in the MD is determined by two seasons—the rainy season from May to October followed by the dry season from November to April. Annual rainfall is 1200–2400 mm and mean temperatures are high (25–29ºC). About 35% of the MD is alluvial soil, covering 1.1 million ha along the rivers with most of the remainder acid sulfate clay soil (1.6 million ha). Both the acid and alluvial soils are deficient in phosphorus (P) since P generally reacts with aluminium and iron under low pH conditions and forms insoluble compounds (Thanh et al. 1997).

Paddy rice dominates the MD with an average yield of 4.1 t/ha. Soybean is grown on 9100 ha with an average yield of 2.2 t/ha and groundnut is similarly cultivated on 9900 ha with an average yield of 1.5 t/ha (General Statistical Office 2000). Paddy rice is either monocultured or rotated with legumes (soy-bean, groundnut, mungbean), vegetables, or other cereals (sweet potato, corn). To obtain the high rice and other crop yields, large amounts of fertiliser N are applied, e.g. 100–150 kg N/ha for rice, 100–120 kg N/ha for soybean and 80–100 kg N/ha for groundnut. Soybean and groundnut have been cultivated in the MD in rice rotations in order to improve soil N fertility and soil structure. Several cultural techniques have been developed to produce high soybean yields in MD (Duong et al. 1984a,b, 1992; Hiep and Huy 1999).

We report in this paper results of surveys of farmers' crops of soybean in the Dong Thap, Can Tho and Vinh Long provinces of the MD and groundnut in the Long An province. The aims of the surveys were to quantify N_2 fixation activity of the commercial crops and to identify factors either contributing to (e.g. rhizobial inoculation, optimum fertiliser management) or depressing N_2 fixation and yield (e.g. poor agronomy). Of particular importance was data on farmer use of and atitudes to rhizobial inoculants. The second part of this paper reports

[1] Biotechnology Research and Development Institute, Can Tho University, Can Tho province, Vietnam
[2] NSW Agriculture, TCCI, Tamworth, NSW, 2340 Australia

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results of four field experiments involving soybean and two experiments with groundnut, all in the MD, to assess the effects of inoculation and fertiliser N on nodulation, N_2 fixation and yield of the two species.

Materials and Methods

Surveys

The groundnut surveys were conducted during winter-spring 1999–2000 (W-S) and summer-autumn 2000 (S-A) in the Duc Hoa district, Long An province, about 85 km from Ho Chi Minh City. Ten farmers' fields were involved in each survey. The soybean surveys were in spring-summer 2000 (20 fields) and spring-summer 2001 in the Dong Thap, Vinh Long and Can Tho provinces.

Data were collected on cultural practices (fertiliser applications, plant cultivars, cropping sequences), soil (pH, organic C, soil type (% sand, % silt, % clay) and plant traits (nodulation scores, shoot dry matter (DM), grain and pod yield, shoot N and $\delta^{15}N$). Non N_2-fixing reference plants (weeds) were collected for $\delta^{15}N$ analysis. Procedures for soil and plant sampling and analysis and for %Ndfa estimations can be found in Lien Hoa et al. (these Proceedings). The B values for groundnut and soybean were –1.87‰ and –2.50‰, respectively (Peoples et al. 1989).

On-farm experiments

Two on-farm inoculation experiments involving groundnut were conducted in Long An province during winter-spring 2000–2001. These were followed by four soybean inoculation experiments during spring-summer 2001 in Dong thap and Vinh Long provinces. Treatments, common to all experiments, were as follows:

1. Farmers' practice without fertiliser N (FP0N);
2. Farmers' practice with fertiliser N (FP+N);
3. Farmers' practice without N + inoculation with foreign strain (NC92 for groundnut and CB1809 for soybean) (FP+NC92, FP+CB1809);
4. Farmers' practice without N + inoculation with local strain (FP+local strain);
5. Optimum fertiliser formula + inoculation with foreign strain (OP+NC92, OP+CB1809).

Peat-based inoculants were applied at sowing as seed coatings for both soybean and groundnut. The experimental design was a randomised complete block with 4 replications. Plot size was 4 m × 5 m; plants were spaced at 10 cm intervals in 40 cm rows (soybean) or at 20 cm intervals in 20 cm rows (groundnut).

Measurements were made of soil parameters, pH, %C, total N, total P_2O_5, available P, and exchangeable K. Crop parameters measured were nodulation score, nodule dry weight, shoot (soybean) DM, N and $\delta^{15}N$, pod yield (groundnut) and grain yield (soybean).

Results and Discussion

Farmers' field surveys

Soil, agronomic and plant data

Data from the farmer crop surveys of groundnut and soybean are summarised in Tables 2 and 3. The groundnut fields were acidic (means of 4.68 and 4.76 for the W-S and S-A surveys, respectively) (Table 2). Organic C levels were low, although variable. The soils were sandy loams, with very little clay (<12%) and were characterised as having poor nutrient retention and being prone to leaching. As a consequence of these traits, the farmers routinely apply large quantities of coconut ash, which also acts as an effective source of K (Duong et al. 1992). Average rates of application were 3.9 and 4.4 t/ha for the two seasons, with individual fields receiving as much as 6 t/ha.

Inorganic fertiliser applications involved N, P and K with farmers applying 30–70% more to the W-S crops than to those grown in S-A. Winter-spring is the main groundnut season and higher yields are expected. Thus, higher inputs of nutrients are required. Additionally, farmers consider that the residues of the W-S groundnut would supply some of the nutrients of the following S-A crop. Rates of N, P and K applied to individual fields varied substantially, i.e. >10-fold. For example, N rates varied between 35 and 165 kg/ha with averages of 82 kg N/ha for the W-S season and 58 kg N/ha for S-A.

Table 1. Fertilisers applied in groundnut and soybean inoculation experiments (kg/ha).

Treatments	Groundnut	Soybean
1	2000 coconut ash	$0N + 40P_2O_5 + 30K_2O$
2	2000 coconut ash + 100N	$100N + 40P_2O_5 + 30K_2O$
3	2000 coconut ash	$0N + 40P_2O_5 + 30K_2O$
4	2000 coconut ash	$0N + 40P_2O_5 + 30K_2O$
5	$20N + 40P_2O_5 + 60K_2O + 200CaCO_3$	$20N + 40P_2O_5 + 30K_2O$

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Table 2. A summary of data from the farmer' field surveys of winter-spring 1999–2000 and summer-autumn 2000 groundnut in Long An province of the Mekong Delta, Vietnam.

Data	Factor	Winter-spring 1999/2000		Summer-autumn 2000	
		Mean	Range	Mean	Range
Soil	pH (soil:water, 1:2.5)	4.68	4.29–5.23	4.76	4.47–5.42
	Organic C (%)	0.88	0.56–1.20	0.76	0.46–1.16
	Sand (%)	58	36–68	67	53–80
	Silt (%)	36	26–54	29	20–43
	Clay (%)	6	2–11	4	0.6–12
Agronomy	Crop area (m^2)	5000	1500–10,000	2830	1000–7000
	N applied (kg/ha)	82	40–165	58	35–80
	P_2O_5 applied (kg/ha)	118	20–200	90	50–130
	K_2O applied (kg/ha)	73	15–200	43	0–80
	Coconut ash (kg/ha)	3900	0–6000	4350	3250–5000
	Cropping system	R-R-G/R-G		R-G-G	
Plant	Shoot DM (t/ha)	5.54	4.38–6.30	5.65	4.26–8.42
	Nodules score (0-3)	2.3	1.9–2.6	2.0	1.2–2.4
	Potential pod yield (t/ha)	2.72	2.50–3.30	1.74	1.20–3.00
N budget	Crop N (kg/ha)	218	177–260	168	119–258
	%Ndfa	53	40–71	47	12–71
	Crop N fixed (kg/ha)	116	88–155	77	21–153
Economic analysis	Input (VND)	12,051,805		11787945	
	Output (VND)	15,719,600		10753600	
	Benefit (VND)	3,667,795		–1034345	

R = Rice, G = Groundnut
Price 1 kg groundnut = 5200 VND (April 2000); ($US1 = 14,500 VND); VND : Vietnamese dong

Table 3. A summary of data from the farmers' field surveys of spring-summer 2000 (20 fields) and spring-summer 2001 (10 fields) in Dong Thap, Can Tho and Vinh Long provinces of the Mekong Delta, Vietnam.

Data	Factor	Spring-summer 2000		Spring-summer 2001	
		Mean	Range	Mean	Range
Soil	pH (soil:water, 1:2.5)	4.45	4.14–4.84		
	Organic C (%)	1.68	1.08–2.19		
	Sand (%)	2	0.4–5		
	Silt (%)	56	42–69		
	Clay (%)	42	26–57		
Agronomy	Crop area (m^2)	3620	1500–10,000		
	N applied (kg/ha)	108	80–150		
	P_2O_5 applied (kg/ha)	51	30–80		
	K_2O applied (kg/ha)	28	0–40		
	Coconut ash (kg/ha)	0	0		
	Cropping system	R-S-R			
Plant	Shoot DM (t/ha)	6.50	5.15–8.13		
	Nodules score (0-3)	1.4	0.8–2.4		
	Potential grain yield (t/ha)	2.38	1.50–3.00		
N budget	Crop N (kg/ha)	326	266–413		
	%Ndfa	70	50–92	70	51–92
	Crop N fixed (kg/ha)	232	152–377		
Economic analysis	Input (VND)	7,002,747			
	Output (VND)	7,538,240			
	Benefit (VND)	535,493			

R = Rice, S = Soybean
Price 1 kg soybean = 3200 VND (May 2000)

Shoot DM yields were similar for the two seasons, with individual crops varying about 2-fold (4.3–8.4 t/ha). Pod yields were about 60% higher for the W-S crops than for those in S-A. Even though none of the crops were inoculated, all were nodulated.

The soybean fields were also acidic, but were quite different from the groundnut fields in physical composition (Table 3). They had much higher clay levels (average of 42%) and very little sand (<5%). Consequently, organic matter levels were about double those of the groundnut soils. Coconut ash was not used on soybean. With the chemical fertilisers, N rates were higher than used on groundnut (average 108 kg N/ha), but P and K rates were lower. Shoot DM and grain yield were, on average, 6.5 and 2.4 t/ha, respectively.

None of the soybean crops were inoculated either. Nodulation scores ranged about 3-fold and were slightly inferior overall to the groundnut nodulation. It appears that the rhizobia for both species survives the flooding and associated anaerobic conditions, even though there may be some loss of numbers (e.g. Rupela et al. 1987; Wood and Myers 1987). Generally, the nodules on the roots of both species were small and white and, rather than being clustered near to the crown, were scattered throughout the root system. This suggests that they were not highly effective.

Nitrogen fixation and N balance

Nitrogen fixation was quantified using the natural ^{15}N abundance method. The $\delta^{15}N$ values of the groundnut and soybean crops, together with their non N_2-fixing reference plant values, are presented in Figure 1. The $\delta^{15}N$ values of the reference plants were similar for both surveys, with ranges 2.51–9.36‰ for the groundnut fields and 0.88–11.08‰ for soybean. Mean values were 4.79‰ (groundnut reference) and 3.60‰ (soybean reference). For the legumes themselves, mean values were 1.41‰ (groundnut) and –0.80‰ (soybean). Differences between the $\delta^{15}N$ values of the legumes and reference plants were, for the most part, 3.0–4.0‰.

Average %Ndfa values were 50% for groundnut and 70% for soybean, although there was substantial variation in the values for each species (Tables 2 and 3). With groundnut, most crops fixed 40–60% of their N requirements and none fixed >80% (Figure 2A). Soybean %Ndfa values, on the other hand, were mainly in the 60–80% range with 20% of the crops fixing 80–100% of N requirements (Figure 3A). Crop N fixed reflected the %Ndfa distributions. With groundnut, the majority of crops fixed 50–150 kg N/ha (Figure 2B), while the majority of soybean crops fixed 200–300 kg N/ha (Figure 3B). Average values for crop N fixed were 116 kh/ha (groundnut) and 232 kg/ha (soybean).

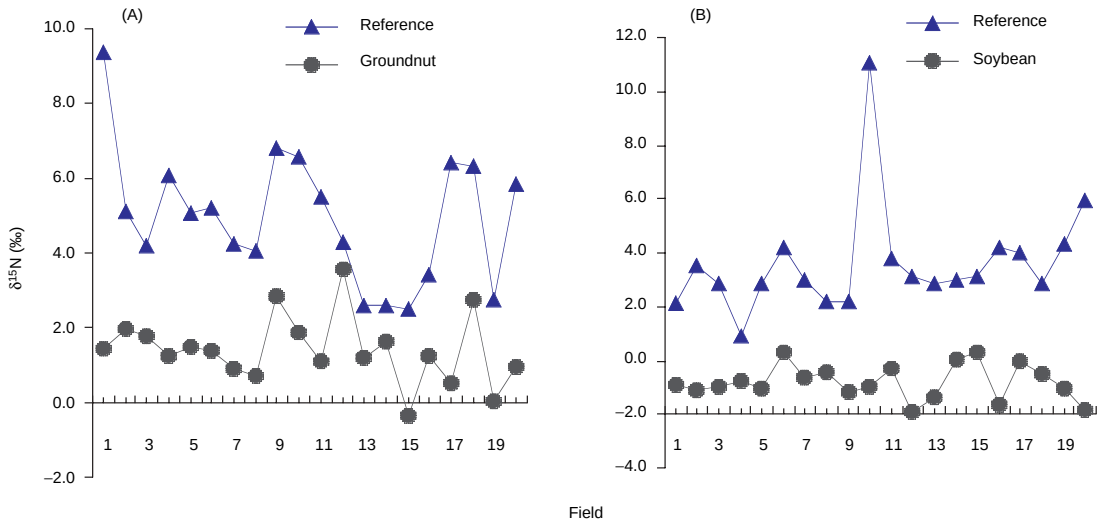

Figure 1. $\delta^{15}N$ values of (A) groundnut and reference weeds and (B) soybean and reference weeds from farmers' fields in the surveys of legume N_2 fixation in the Mekong Delta of Vietnam. The groundnut surveys were conducted in winter-spring 1999–2000 and summer-autumn 2000 groundnut in Long An province; the soybean surveys in spring-summer 2000 in Dong Thap, Can Tho and Vinh Long provinces.

Factors affecting N_2 fixation

Correlation analysis of the various soil and plant parameters in Tables 2 and 3 indicated, for both species, crop N fixed was highly correlated with %Ndfa (data not shown) and shoot N (Figure 4). Unlike other surveys of legume N_2 fixation in Vietnam (see Lien Hoa et al., Tien et al., and Hong and Herridge, these Proceedings), there were no soil or plant parameters that were correlated with N_2 fixation.

Economic analysis

The economic analyses of the two seasons' groundnut crops provided an interesting contrast. For the winter-spring crops, the benefit was, on average, 3,667,800 VND/ha (about $US250), compared to a net loss of 1,034,350 VND/ha (USD71) for the summer-autumn crops. The largest contributing factor to the differences in profitability was pod yield (see Table 2). For soybean, both input costs and grain prices were less than for groundnut. The average benefit was 535,500 VND, equivalent to $US37.

Inoculation experiments

Soil, agronomic and plant data

The two groundnut inoculation experiments were conducted in low organic matter, acidic (pH 5.49–5.71) sandy loam soils. Major nutrients

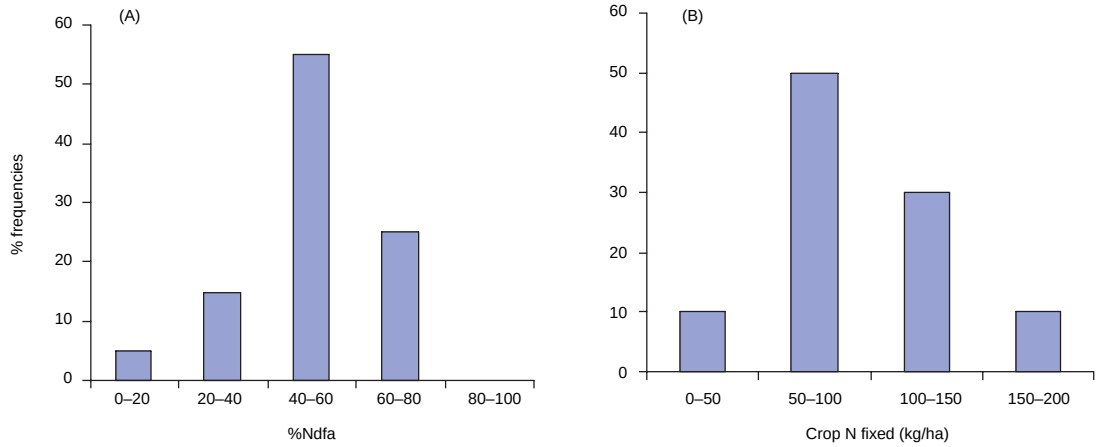

Figure 2. Frequency histograms of %Ndfa and crop N fixed of farmers' fields of winter-spring 1999–2000 and summer-autumn 2000 groundnut in Long An province of the Mekong Delta. There were 20 fields in the surveys.

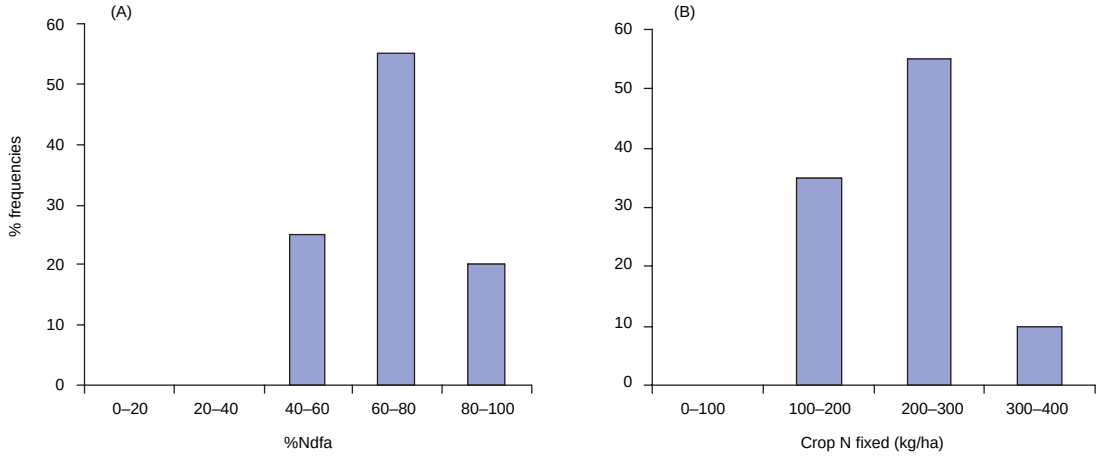

Figure 3. Frequency histograms of %Ndfa and crop N fixed of farmers' fields of soybean surveyed in spring-summer 2000 in Dong Thap, Can Tho and Vinh Long provinces of the Mekong Delta. There were 20 fields in the surveys.

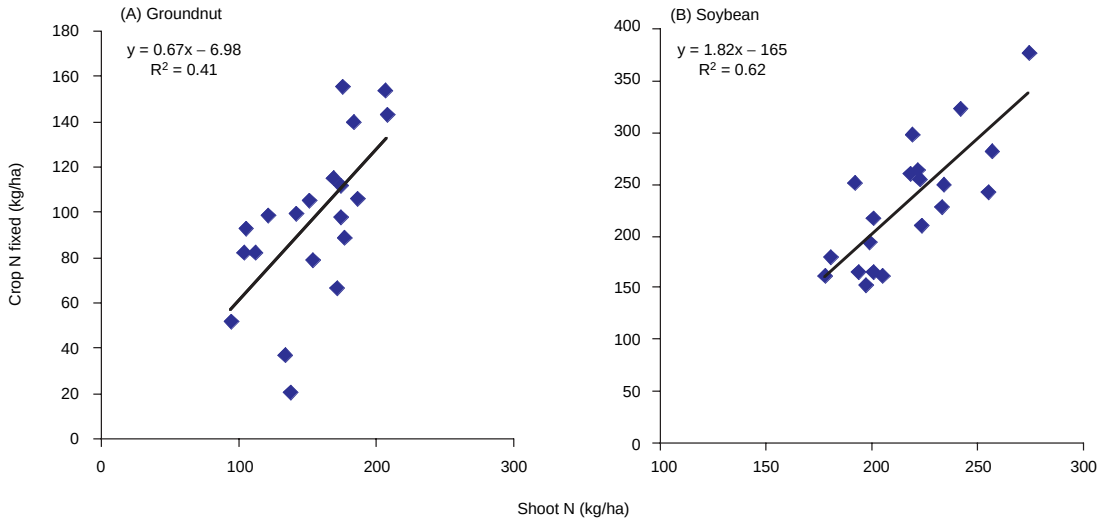

Figure 4. Relationships between crop growth (shoot N) and N_2 fixation (crop N fixed) of (A) groundnut and (B) soybean in surveyed farmers' fields in the Mekong Delta of Vietnam. The groundnut surveys were conducted in winter-spring 1999–2000 and summer-autumn 2000 groundnut in Long An province; the soybean surveys in spring-summer 2000 in Dong Thap, Can Tho and Vinh Long provinces

(N, P and K) were low as was CEC (data not shown). The four soybean inoculation experiments, on the other hand, were conducted in acidic clay loams (pH 4.58–5.76). Total N and P were average but exchangeable K was low. CEC was higher than in the groundnut soils.

Data on plant nodulation from all 6 experiments are presented in Tables 4 and 5. Groundnut responded to inoculation at both sites with no apparent difference between the local strain and the imported strain, NC92.

Results were similar for soybean, except that nodules were entirely absent from the uninoculated treatments (1 and 2) at two of the four sites. Again, there was no difference in nodulating ability between the local strain and the imported strain, CB1809. The groundnut and soybean plants in Treatments 1 and 2 that were nodulated (sites Long An 1 and 2, Dong Thap 1 and 3) did not have large nodules near to the crown region as with Treatments 3–5; rather the nodules were small and white and scattered along the entire root.

Nodule mass, assessed for soybean only, reflected the difference in size of the nodules formed by the indigenous rhizobia in the uninoculated plots (Treatments 1 and 2) and those formed by the inoculant rhizobia (Treatments 3–5) (Table 5). At Dong Thap 3, for example, the inoculated plants in

Table 4. Effects of rhizobial inoculation and fertiliser application on nodulation scores of groundnut at two field sites in Long An province and soybean at 4 sites in Vinh Long and Dong Thap provinces of the Mekong Delta of Vietnam. The experiments were conducted during winter-spring 2000–2001 and spring-summer 2001.

Treatments	Long An 1 (GN)[A]	Long An 2 (GN)	Vinh Long (SB)[B]	Dong Thap 1 (SB)	Dong Thap 2 (SB)	Dong Thap 3 (SB)
1. FP0N-inoc	2.0 a	1.6 a	0.0 a	1.0 a	0.0 a	1.0 a
2. FP+N-inoc	1.9 a	1.9 a	0.0 a	1.0 a	0.0 a	0.8 a
3. FP+NC92/CB1809	2.9 b	2.6 b	2.0 b	2.0 b	2.8 b	2.0 b
4. FP+local strain	2.9 b	2.5 b	1.8 b	2.0 b	2.8 b	2.0 b
5. OP+NC92/CB1809	2.9 b	2.9 b	2.0 b	2.0 b	3.0 b	2.0 b
CV (%)	5.5	14	37	13.5	16	14.4

[A]GN = groundnut; [B]SB = soybean
Means followed by the same letter in the same column were not significantly different (P>0.05)

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Treatments 3–5 had 20–40 times the nodule mass as the uninoculated Treatment 1 and 2 plants.

The enhanced nodulation of inoculated groundnut was reflected in higher pod yields (Table 6). Overall, Treatment 5 (optimum fertiliser + inoculation) produced the highest yield (average of 5 t/ha). The yield was 1.5 t/ha (42%) greater than that of Treatment 1 (0N, no inoculation) and 1.1 t/ha (28%) greater than Treatment 2 (100N, no inoculation). Treatments 3 and 4 were intermediate between Treatment 5 and the two uninoculated treatments.

Soybean grain yields also responded to inoculation. However, Treatment 2 (+fertiliser N) performed relatively better than it had with groundnut. The responses to inoculation (Treatments 3–5 *versus* Treatment 1) and fertiliser N (Treatment 2 *versus* Treatment 1) were consistent for the four experiments. Yield benefits were 0.51–0.64 t/ha (26–33%).

Economic analysis

Economic analysis of the data indicated very large differences between groundnut and soybean in returns to the farmers (Tables 7 and 8). Even though costs of production for groundnut were about 50% higher than for soybean, this was more than offset by the 75% higher yields and 67% higher prices for groundnut.

Table 5. Effects of rhizobial inoculation and fertiliser application on nodule weights (mg/plant) of soybean at 4 sites in Vinh Long and Dong Thap provinces of the Mekong Delta of Vietnam. The experiments were conducted during spring-summer 2001.

Treatments	Vinh Long (SB)[A]	Dong Thap 1 (SB)	Dong Thap 2 (SB)	Dong Thap 3 (SB)
1. FP0N-inoc	0 a	29 a	0 a	5 a
2. FP+N-inoc	0 a	25 a	0 a	8 a
3. FP+CB1809	174 b	221 c	23 b	192 b
4. FP+local strain	140 b	183 bc	21 b	133 b
5. OP+CB1809	215 b	158 b	27 b	158 b
CV (%)	58	30	31	45

[A]SB = soybean
Means followed by the same letter in the same column were not significantly different (P>0.05)

Table 6. Effects of rhizobial inoculation and fertiliser application on pod/grain yields (t/ha) of groundnut at two field sites in Long An province and soybean at 4 sites in Vinh Long and Dong Thap provinces of the Mekong Delta of Vietnam. The experiments were conducted during winter-spring 2000–2001 and spring-summer 2001.

Treatments	Long An 1 (GN)[A]	Long An 2 (GN)	Vinh Long (SB)[B]	Dong Thap 1 (SB)	Dong Thap 2 (SB)	Dong Thap 3 (SB)
1. FP0N-inoc	3.90 a	3.13 a	3.32 a	2.37 a	0.81 a	1.34 a
2. FP+N-inoc	4.31 a	3.48 bc	4.35 b	3.12 b	1.18 b	1.73 b
3. FP+NC92/CB1809	4.55 b	3.83 cd	4.20 b	3.04 b	1.29 b	1.62 b
4. FP+local strain	5.56 b	3.83 cd	4.16 b	2.75 ab	1.26 b	1.70 b
5. OP+NC92/CB1809	5.76 b	4.23 d	4.33 b	3.07 b	1.30 b	1.70 b
CV (%)	11	3	5	9	7	8

[A]GN = groundnut; [B]SB = soybean
Means followed by the same letter in the same column were not significantly different (P>0.05)

Table 7. Economic analysis of inoculation and fertiliser treatments in two field experiments in Long An province, Mekong Delta, involving groundnut. The experiments were conducted during winter-spring 2000–2001.

Treatments	Pod yield (t/ha)	Fertiliser input (VND)	Total input (VND)	Output (VND)	Benefit (VND)
1. FP0N-inoc	3.52	975,000	9,475,000	15,840,000	6,365,000
2. FP+N-inoc	3.90	1,520,000	10,020,000	17,550,000	7,530,000
3. FP+NC92	4.20	1,005,000	9,475,000	18,900,000	9,425,000
4. FP+local strain	4.70	1,005,000	9,475,000	21,150,000	11,675,000
5. OP+NC92	5.00	739,000	9,209,000	22,500,000	13,291,000

Price of 1kg of groundnut (May 2001) = 4500 VND ($US1 = 14,800 VND)

Table 8. Economic analysis of inoculation and fertiliser treatments in four soybean field experiments in Vinh Long and Dong Thap provinces, Mekong Delta. The experiments were conducted during spring-summer 2001.

Treatments	Grain yield (t/ha)	Fertiliser input (VND)	Total input (VND)	Output (VND)	Benefit (VND)
1. FP0N-inoc	1.96	450,000	6,365,000	5,292,000	–1,073,000
2. FP+N-inoc	2.60	949,000	6,864,000	7,020,000	156,000
3. FP+CB1809	2.54	480,000	6,395,000	6,858,000	463,000
4. FP+local strain	2.47	480,000	6,395,000	6,669,000	274,000
5. OP+CB1809	2.60	589,000	6,504,000	7,020,000	516,000

Price of 1kg of soybean (May 2001) = 2700 VND ($US1 = 14,800 VND)

For groundnut, best returns were from Treatment 5. It was almost double that of Treatment 1 and 75% higher than Treatment 2. Results were much the same for soybean with Treatment 5>Treatments 3 and 4>Treatment 2>Treatment 1. Although Treatment 2 yielded well, the high cost of fertiliser N substantially reduced profitability.

Attitude to the use of inoculants

Farmer interviews in both the groundnut and soybean-growing areas revealed that they knew a little about inoculants, but encountered difficulty in purchasing them. They were eager to use inoculants on groundnut and saw the economic benefits (through reduced fertiliser and labour costs) of replacing coconut ash with the optimum fertiliser mixture. With soybean, farmers preferred to used N fertiliser because it was cheap, easy to buy and apply and the effects were quick and clearly evident within a few days of application.

With the six inoculation/fertiliser experiments, many of the nearby farmers came to study the plots and discuss the optimum fertiliser formulae. In particular, they picked up on the fact that P and K were just as important to apply as N. Farmers were surprised when shown the large nodules of the inoculated plants of both groundnut and soybean compared to the small nodules formed by indigenous rhizobia.

At the four soybean sites, we also discussed with farmers about the positive effects of the nodules for subsequent crops, through improved soil structure and soil fertility.

Conclusion

The surveys of farmers' crops of groundnut and soybean indicated that they were not inoculated with rhizobia. Nonetheless, all 40 crops were nodulated and all fixed N. Inputs were high, particularly fertiliser N inputs and economic analysis of the 20 soybean fields and 10 summer-autumn groundnut fields indicated either low or negative returns. Only the winter-spring groundnut showed good levels of profitability.

The six inoculation/fertiliser experiments showed that both groundnut and soybean responded to inoculation by producing more abundant nodulation and higher pod or grain yields. For both species, the optimum fertiliser mixture plus inoculation produced the greatest economic returns. Farmers could clearly improve profitability by reducing fertiliser N inputs from the current rates of 50–150 kg/ha to 'starter' rates of <20 kg/ha and inoculating with high-quality rhizobial inoculants.

Acknowledgments

We would like to express our deep gratitude to the Australian Centre for International Agriculture Research (ACIAR) for part-funding of this work through Project 9827 'Increasing yields and nitrogen fixation of soybean, groundnut, and mungbean in Vietnam through rhizobial inoculation'. We thank Dr Mark Peoples, CSIRO Plant Industry, Australia, for ^{15}N analysis.

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N_2 Fixation of Groundnut in the Eastern Region of South Vietnam

Nguyen Thi Lien Hoa[1], Tran Yen Thao[1], Phan Lieu[1] and David Herridge[2]

Abstract

To determine the need for and potential benefits of rhizobial inoculation of groundnut (*Arachis hypogaea*) in the southeastern region of Vietnam, surveys of 40 farmers' fields and 6 inoculation experiments were conducted during winter-spring (1999–2000), summer (2000) and winter-spring (2000–2001) in the Tay Ninh province and Ho Chi Minh City area. All 40 farmer crops in the survey were nodulated, although none had been inoculated. As well, all crops were fertilised with N, at an average rate of 50 kg/ha (range of 10–120 kg/ha). The percentage of crop N derived from N_2 fixation (%Ndfa) varied in the range 24–72%, with an overall average of 46%. Cop N fixed ranged 49–205 kg/ha (average 106 kg/ha). Factors enhancing crop N fixed were plant population, soil organic fertility and fertiliser P applied. Plant population was also highly correlated with pod yield. The six inoculation experiments indicated both inoculation and fertiliser N application to be unnecessary because they did not enhance nodulation (in the case of inoculation) or pod yield (inoculation and fertiliser N). Both surveys and inoculation experiments were conducted in established groundnut areas. In new groundnut land, we would expect a response to inoculation and inoculation would be required. In the absence of inoculation, the farmer would have to supply the crop with substantial amounts of fertiliser N or suffer a yield decline because of N deficiency. Surveys of farmers indicated an almost total lack of knowledge of inoculants but a willingness to use them if they were shown to provide a yield benefit. They also saw that information about inoculants would need to be provided to them and to the extension officers.

THE EASTERN region of the South of Vietnam (ERS) comprises 2.3 million ha. The region is located in the tropical monsoon climatic zone, with high solar radiation (>130 Kcal/cm^2/year), high mean temperatures (26–27°C), and high precipitation (1800–2000 mm/year). The ERS is dominated by 744,650 ha (32% of total) of grey soils (Acrisols), which are characterised as acidic, eroded, light texture and low fertility (Lieu 1992). Paddy rice is the main crop of the region with 542,800 ha yielding an average of 3.1 tonnes/ha. Groundnut (*Arachis hypogaea*) is grown on 50,500 ha with an average yield of 1.6 tonnes/ha and soybean (*Glycine max*) grown on 13,700 ha with an average yield of 0.8 tonnes/ha (General Statistical Office 2000). Paddy is either grown as a monoculture or rotated with legumes (groundnut, soybean, mungbean (*Vigna radiata*)), vegetables and other annual crops. In the ERS, farmers commonly apply N-fertiliser to groundnut, at rates of about 50 kg N/ha, a practice that represents an unnecessary expense because of the capacity of groundnut to fix its own N.

We report a 2-season survey of N_2 fixation of groundnut in farmers' fields and multi-locational inoculation experiments in ERS. These surveys and experiments were part of a coordinated project, funded by the Australian Centre for International Agricultural Research (ACIAR) in Vietnam. The objectives of the survey were to determine current levels of N_2 fixation in farmers' fields and the cultural practices that either enhance (e.g. inoculation, effective weed and insect management) or suppress N_2 fixation (e.g. fertiliser N, poor agronomy). The inoculation experiments built on the outcomes of the survey to determine the need for and assess the biological and economic benefits of inoculation.

[1] Oil Plant Institute of Vietnam, 171–175 Ham Nghi St., District 1, Ho Chi Minh City, Vietnam
[2] NSW Agriculture, TCCI, Tamworth, NSW 2340, Australia

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edited by D. Herridge
ACIAR Proceedings 109e
(printed version published in 2002)

Materials and Methods

Surveys

The surveys were conducted during two seasons, the first in winter-spring 1999–2000 (WSG 1999–2000) and the second in summer 2000 (SG 2000). Both involved 20 farmers' fields, and both were in the ERS, in Tay Ninh province and in the outer suburbs of Ho Chi Minh City. All sites were within 100 km of Ho Chi Minh City. Groundnut is the main legume grown in these areas and usually rotated with summer or autumn rice.

On-farm experiments

There were six experiments conducted in farmers' fields in Tay Ninh province and HCM City's suburb during winter-spring 2000–01 (WS 2000–2001). Three of the sites represented the groundnut-rice-rice (G-R-R) cropping system and three sites represented the groundnut-groundnut-rice (G-G-R) system.

Treatments were as follows:

1. Farmer's practice, but without fertiliser N (FP0N).
2. Farmer's practice, including fertiliser N (FP+N).
3. Farmer's practice (–N) + inoculation with local rhizobial strain (FP+local strain).
4. Farmer's practice (–N) + inoculation with NC92 strain (FP+NC92).
5. Optimum fertiliser except N (–N) + inoculation with local strain (OF+local strain).

Additional details of site names and fertiliser application rates including N rates are in Table 1. Fertiliser inputs using farmers' practices were different among the sites. The chemical fertilisers that were used commonly were urea, single superphosphate and compound N-P-K. Fertiliser N applications were in the range 27–70 kg/ha, compared with 30–136 kg P_2O_5/ha and 15–85 kg K_2O/ha. Ash and lime inputs also varied with lime absent from the Gia Loc site. The optimum fertiliser used at all 6 locations was $80P_2O_5 + 100K_2O$ + 300lime, all in kg/ha.

The experiments were a randomised complete block design with 4 replications. Plots were 2 m × 10 m (i.e. 20 m^2). Seed was inoculated at sowing using peat-based inoculum.

Data collection

For each field in the survey, information on the following was gathered from the farmers: previous cropping history, fertiliser applications and sowing details. Measurements were made of a number of soil parameters—pH, %C, total %N, available, P and K_2O, and crop parameters—nodulation score,

Table 1. Treatments and site details of the 6 inoculation experiments, conducted in the ERS region during winter-spring 2000–2001.

Treatment	Trang Bang	Gia Loc	Phuoc Thanh	Bau Don	Trung Lap Thuong	Tan An Hoi
1	FP[A]: 95 P_2O_5[B] 85 K_2O 2250 ash 300 lime	FP: 40 P_2O_5 30 K_2O 2250 ash	FP: 72 P_2O_5 56 K_2O 1650 ash 250 lime	FP: 57 P_2O_5 76 K_2O 1800 ash 280 lime	FP: 136 P_2O_5 74 K_2O 3000 ash 500 lime	FP: 30 P_2O_5 15 K_2O 3000 ash 300 lime
2	FP + 63N[C]	FP + 70N	FP + 36N	FP + 32N	FP + 27N	FP + 30N
3	FP + Local strain	FP + Local strain	FP + Local strain	FP + Local strain	FP + Local strain	FP + Local strain
4	FP + NC92 strain	FP + NC92 strain	FP + NC92 strain	FP + NC92 strain	FP + NC92 strain	FP + NC92 strain
5	OF[D] + Local strain	OF + Local strain	OF + Local strain	OF + Local strain	OF + Local strain	OF + Local strain

[A] FP – farmer practice
[B] all fertiliser values expressed as kg/ha
[C] kg/ha fertiliser N
[D] optimum fertiliser

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edited by D. Herridge
ACIAR Proceedings 109e
(printed version published in 2002)

maximum shoot dry matter (DM), %N and $\delta^{15}N$, non N_2-fixing reference plant (weed) $\delta^{15}N$, and potential and actual pod yield.

For each of the six inoculation experiments, the following parameters were measured: soil pH, %C, total %N, available N, P and K_2O, and nodule score, total crop DM, N and $\delta^{15}N$, pod yield and pod N.

For both surveys and inoculation experiments, inputs were costed and used to construct economic budgets.

Soil sampling and analysis

Before sowing of the surveyed farmer crops and the inoculation experiments, soil samples were taken from each field (4 cores to a depth of 15 cm then bulked). For the inoculation experiments, the soils were also sampled to 60 cm depth, in 4 sections 0–15 cm, 15–30 cm, 30–45 cm and 45–60 cm, and analysed for nitrate. Soil pH was measured using a pH meter, and organic C by the Walkley-Black method. Available N was extracted by 2M KCl and total (%)N analysed by Kjeldahl digestion. Olsen available P was extracted using sodium bicarbonate, then determined colorimetrically. Available K_2O was extracted using 1N ammonium acetate and determined by flame photometry.

Plant sampling and analysis

At late flowering (40 days after sowing (DAS)), groups of 10 plants were sampled from each of the 4 sampling areas in each field (40 plants/field) for nodulation assessment (score 0: no nodules; score 1: <10 nodules; score 2: 10–30 nodules; score 3: >30 nodules). At maximum crop DM (physiological maturity stage, 80–83 DAS), all plants in a 0.5 × 0.5 m quadrat were pulled, counted and weighed immediately. Weeds were also harvested from each sampling area to use as non N_2-fixing references for ^{15}N analysis of N_2 fixation. All samples were then oven dried at 80°C for 48 h. The dry samples were then ground and sent to CSIRO, Canberra, Australia, for analysing %N and $\delta^{15}N$. The pods were finely ground and analysed for %N using Kjeldahl digestion.

N_2 fixation

The finely ground (<0.1 mm) legume and weed samples were analysed for %N and ‰^{15}N using an automated N and C analyser/mass spectrometer (ANCA-SL/20-20 stable isotope mass spectrometer, Europa Scientific, Crewe, UK). In the determinations of N_2 fixation, shoot ^{15}N values were expressed with reference to air N_2 as follows:

$$‰\ ^{15}N = 1000\ (R_{sample} - R_{air})/R_{air} \qquad (1)$$

where R is the ratio mass 29/mass 28. The proportion of plant N derived from atmosphere (%Ndfa) was then calculated:

$$\%Ndfa = 100\ (x–y)/(x–z) \qquad (2)$$

where 'x' is the ‰ ^{15}N of shoots of the weeds deriving all N from the soil; 'y' is the ‰ ^{15}N of sampled groundnut shoots; and 'z' is the ‰ ^{15}N of groundnut receiving all N from N_2 fixation. The value for 'z' was –1.87‰ (Peoples et al. 1989).

Statistical analysis

Data were subjected to correlation and simple and multivariate regression analysis and to analysis of variance using Statgrapics Vers. 7.0 and Excel 2000.

Results and Discussion

Farmers' fields surveys

Climatic conditions

In 1999, the rainy season in Ho Chi Minh City ended very late; monthly rainfall for November (414 mm) was the highest of the year and effectively delayed sowing the WSG 99/00 crops. In 2000, high rainfall (about 500 mm) in May and June flooded many of the SG fields when they were almost ready for harvesting. The high mean temperatures during March and April and high radiation were very convenient for harvesting WSG.

Soil, agronomic and plant data

Summaries of the soil, agronomic, plant, N, and input-output data for the 40 farmers' crops from the WSG and SG surveys are presented in Table 2. Soils in the farmers' fields in both surveys were acidic and generally low in %C and total N. However, there was considerable variation in both %C (range 0.21–2.02%) and %N (range 0.02–0.24%), reflecting differences in inherent soil quality, i.e. the black sandy-loams were higher in organic fertility than the true grey sands. Available P varied about 10-fold, in the range 13–124 ppm. Available K_2O was generally low and also variable (range 22–126 ppm). Potassium has been recognised as limiting groundnut yields on the grey soils of the ERS. For this reason, farmers apply plant ash to groundnut (Lien Hoa, 1999).

Rates of fertiliser N, P, K and ash varied substantially between fields and between seasons. All of the fields were fertilised with NPK, but many of the fields, particularly in the summer season, were not given ash. Generally, fields were given more fertiliser in the winter/spring season because farmers considered that some of the nutrients would be carried over as unused fertiliser and as crop residues to be used by the following summer groundnut crop.

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Table 2. A summary of data (mean, range of values) from farmers' fields of winter-spring groundnut (WSG), surveyed in 1999–2000 and summer groundnut (SG), surveyed in 2000. Each of the two surveys comprised 20 crops.

Data	Factor	Winter-spring groundnut 1999–2000		Summer groundnut 2000	
		Mean	Range	Mean	Range
Soil[A]	pH (soil:water, 1:2)	5.34	4.52–6.06	5.39	4.78–6.43
	Organic C (%)	0.62	0.21–1.78	0.54	0.22–2.02
	Total %N	0.06	0.03–0.10	0.05	0.02–0.24
	Available P (ppm)	32	13–88	65	36–124
	Available K_2O (ppm)	48	22–100	79	44–126
Agronomy	Area of crop (m^2)	1980	500–3000	2880	1000–6000
	Age of field (years)	>30	20–40	>30	25–40
	N application (kg/ha)	55	30–115	45	10–120
	P_2O_5 application (kg/ha)	104	37–220	61	18–120
	K_2O application (kg/ha)	59	15–120	44	14–115
	Ash application (t/ha)	2.1	0.0–3.8	0.93	0.0–3.8
	Cropping system	G-R-R		G-G-R	
Plant	Plant density (plant m^2)	34	24–40	27	22–32
	Total dry matter (t/ha)	6.62	4.52–9.16	7.01	5.57–9.55
	Nodule score (0–3)	2.3	1.5–2.8	2.2	0.8–3.0
	Actual pod yield (t/ha)[B]	2.47	0.83–3.72	1.98	1.69–2.28
	Potential pod yield (t/ha)[C]	3.12	2.08–5.18	2.19	1.69–2.65
N budget	Crop N (kg/ha) (Shoot N × 1.25)	248	172–337	216	168–292
	%Ndfa	47	28–72	44	24–71
	Crop N fixed (kg/ha)	117	54–205	95	49–152
Economic analysis	Input (VND)	7,100,000		4,410,000	
	Output (VND)	11,393,000		9,245,000	
	Benefit (VND)	4,293,000		4,835,000	

[A] Soil measurements were made on samples from 0–15 cm
[B] Actual pod yield was the harvestable yield
[C] Potential pod yield included all the groundnut pods of the plant, hand-sampled from the quadrat.

Plant density was slightly greater for the WSG than for the SG, because there was more individual plant branching with the latter in the wet and warm summer environment. Yields of DM were similar between the two seasons (averages of 6.6 and 7.0 t/ha). Actual pod yield, as reported by the farmers, were 10–20% lower than the yields measured in the harvested quadrats (potential yields). Yields were also different between the two seasons with the highest yields in the winter/spring season. For SG, many of the pods were small and not filled. Although SG followed the upland WSG, the nodule ratings were similar to those of WSG, which had followed two crops of lowland rice. This reinforces the notion that rhizobia can survive in flooded soils in sufficient numbers for nodulation of a sown crop, even though there may have been some loss of numbers in the anaerobic conditions (Rupela et al. 1987; Wood and Myers 1987)

N_2 fixation

The $\delta^{15}N$ values of the non N_2-fixing reference plants (weeds) for the WSG survey were in the range 1.6–14.9‰, with a mean 5.0‰. By comparison, $\delta^{15}N$ values of the groundnut shoots ranged 0.4–5.0‰, with a mean 1.4‰. For the SG survey, the range for the weeds was 2.4–9.7‰, with a mean 5.2‰, and for the groundnut was 0.4–4.1‰, with a mean 2.0‰. Differences between the $\delta^{15}N$ values of the groundnut plants and corresponding reference weeds were generally large enough to have confidence in the methodology. The differences ranged 0.7–13.2‰ for WSG and 0.6–8.3‰ for SG. For the WSG, 73% of the comparisons had differences in $\delta^{15}N$ values >2‰; for the SG, the figure was 75%.

The means and ranges for %Ndfa for the two surveys were almost identical (Table 2) and were similar to the values (range 33–95%, mean of 58%)

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reported by Maskey et al. (2001) for groundnut in Nepal. Crop N fixed was slightly higher for the WSG (54–205 kg N/ha, mean of 117 kg N/ha) than for the SG (49–152 kg N/ha, mean of 95 kg N/ha). The slightly higher %Ndfa and %N values more than offset the greater biomass DM of the SG (7.01 versus 6.62 t/ha).

Data from the two surveys on %Ndfa and crop N fixed were combined and presented as frequency distributions (Figure 1). All crops had %Ndfa values in the range 20–80%, with the majority fixing 40–60% of their requirements for N (Figure 1A). This translated into the majority of crops fixing 50–150 kg N/ha (Figure 1B). Only a crop fixed >200 kg N/ha. These values were also similar to those reported by Maskey et al. (2001) for groundnut in Nepal.

Relationship among agronomic and N_2 fixation parameters

Correlation among the measured soil, plant, fertiliser and N parameters of the 40 groundnut fields indicated that the amount of N fixed was strongly associated with yield (DM and N) and %Ndfa (P<0.001) (Table 3). This is not surprising and confirms the well-accepted rule that legume N_2 fixation is determined by growth of the plant (N demand) and by the proportion of that demand that is met by either N_2 fixation or soil mineral N. Our results also confirm results of previous surveys of both winter and summer legumes in Nepal (Maskey et al. 2001) and Pakistan (Shah et al. 1997; Aslam et al. 1997).

The major factor contributing to crop N yield appears to have been plant density (r = 0.61; P<0.001) (Table 3). Soil organic fertility (C and N) and fertiliser P were positively correlated with crop N fixed, essentially through association with %Ndfa.

Figure 2 highlights the relationships between crop N and %Ndfa and crop N fixed. The fact that there was no association of crop N and %Ndfa (r = –0.02; n.s.) suggests that fixed N and soil N were complementary in providing for the N requirements of the groundnut crops. The data also suggest that N_2

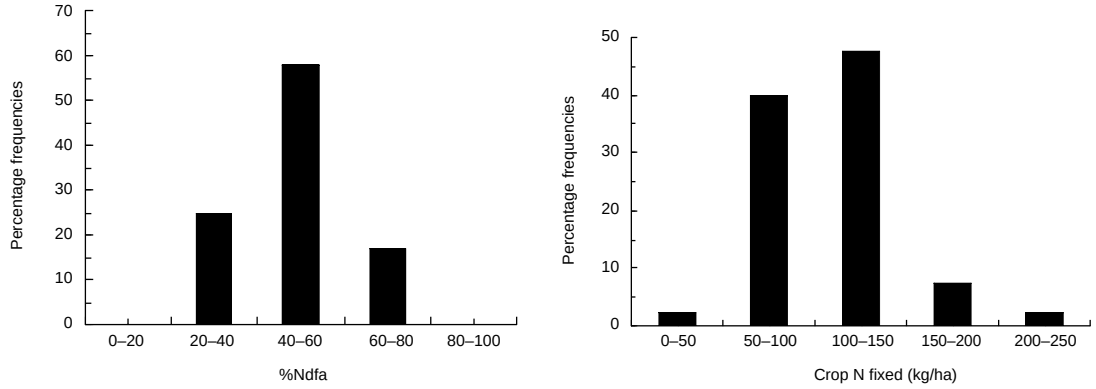

Figure 1. Frequency histograms of %Ndfa and crop N fixed of farmers' fields of winter-spring groundnut (WSG), surveyed in 1999–2000 and summer groundnut (SG), surveyed in 2000. Each of the two surveys comprised 20 crops.

Table 3. Correlation matrix of N2 fixation with soil, fertiliser and plant measures from a total of 40 farmers' fields of winter-spring groundnut (WSG), surveyed in 1999–2000 and summer groundnut (SG), surveyed in 2000.

	Soil organic C (%)	Soil total N (%)	Fertiliser P (kg/ha)	Plant density (m^{-2})	Crop N (kg/ha)	%Ndfa
Soil total N	**0.83**					
Fertiliser P	–0.24	–0.17				
Plant density	0.24	0.08	**0.32**			
Crop N	0.28	0.14	0.15	**0.61**		
%Ndfa	0.22	**0.34**	**0.36**	0.12	–0.02	
Crop N fixed	**0.34**	**0.36**	**0.41**	**0.45**	**0.57**	**0.80**

Values in bold are significant at either the 5% level (>0.31), 1% level (>0.40) or 0.1% level (>0.50)
Degrees of freedom = 38

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fixation was effectively suppressed by soil mineral N with no effect, either positive or negative, on yield. We would have to conclude that the high applications of fertiliser N (up to 120 kg N/ha) would have been the contributing factor to this.

The relationships between plant density and crop N and fertiliser P rate and %Ndfa are highlighted in Figure 3. Even though the density range was relatively small (22–40 plants/m^2), the impact of the higher densities, i.e. 30–40 plants/m^2, was apparent. Optimising plant densities would appear to be one of the more straight-foward and simple agronomic practices that farmers could apply to not only ensure high levels of N_2 fixation, but also high pod yields. The correlation coefficient between plant density and potential (quadrat-harvested) pod yield was 0.69 (P<0.001).

Strangely, neither soil available P or fertiliser P rate was significantly correlated with crop growth (crop DM, N) or pod yields. Instead, fertiliser P was significantly correlated with %Ndfa (Figure 3B).

Economic analysis

The economic benefit of groundnut production was assessed for the crops of the survey, by accounting for all input costs and groundnut prices. Mean input

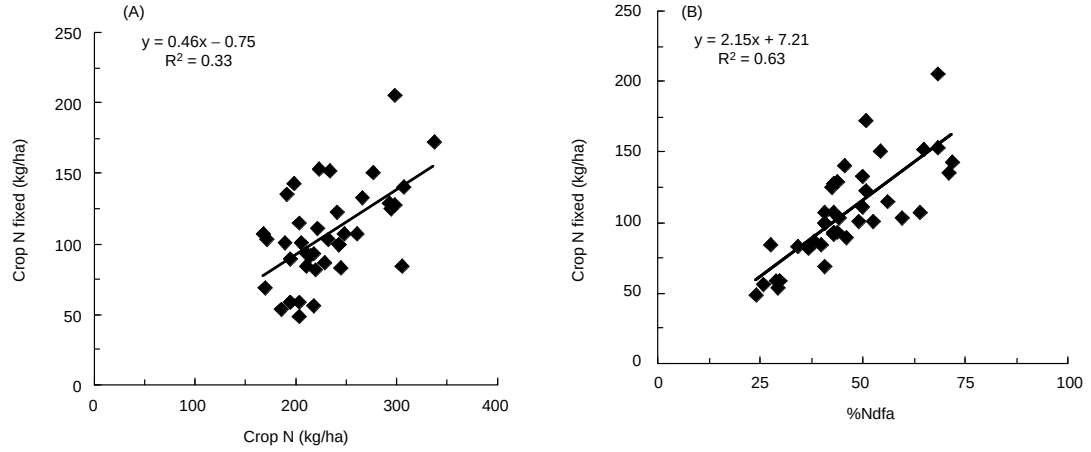

Figure 2. Relationships between (A) crop N and crop N fixed and (B) %Ndfa and crop N fixed. Data from the 40 farmers' groundnut crops from the 2 surveys (winter-spring 1999–2000 and summer 2000) were combined.

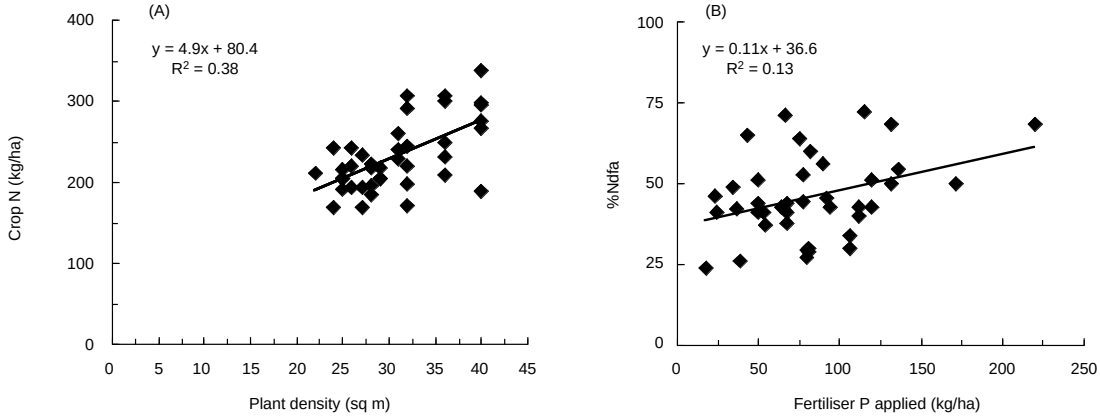

Figure 3. Relationships between (A) plant density and crop N and (B) fertiliser P applied and crop N fixed. Data from the 40 farmers' groundnut crops from the 2 surveys (winter-spring 1999–2000 and summer 2000) were combined.

costs for the WSG were almost double those of SG (Table 1). Thus, although the mean gross income of WSG was higher than for SG (VND11.4 million versus 9.2 million), the mean benefit was lower. This is unusual. In Normally, the benefit of WSG would be expected to be double that of SG.

Inoculation experiments

Soil, agronomic and plant data

Soils at the 6 experimental sites ranged from acidic (pH of 5.32) to near-neutral (pH of 6.56) (Table 4). Organic C and N contents were very low (< 0.6% and 0.08%, respectively). Available P varied from low (8 ppm) to medium (35 ppm), available K_2O varied between very low (20–40 ppm) and low (72 ppm), whilst mineral N was very low at all sites. The latter was to be expected because the previous crop in all cases was flooded rice (Ladha and Kundu (1997).

There were no responses to inoculation at 5 of the 6 sites (Table 5). At the Trang Bang site, there was a small but significant decrease in nodulation for treatment 2, which contains fertiliser N. At all sites, nodulation scores were high with many of the treatments scoring a maximum of 3 out of 3. It should be noted at this stage that the 6 experimental sites were located in areas that have been used for groundnut production for a number of years. In such areas, populations of infective and effective groundnut rhizobia would have become established in the soil. In fact, populations of >3.5 cowpea rhizobia g/soil were counted in rice-groundnut production soils of the ERS (Lien Hoa et al. 1997). In areas new to groundnut, on the other hand, substantial responses to inoculation would be expected. In inoculation experiments conducted in the region during 1986–1991, average yield responses to inoculation were 18% (Song Be province), 32% (Ho Chi Minh City), 17% (Long An province) and 7% (Tay Ninh province) (Lien Hoa et al. 1997). In experiments in the north of the country during 1993–95, pod yield increases with inoculation were in the range 0–19%, with an average of 7%. Other experiments have similarly demonstrated the need for inoculation on new groundnut land. In a rhizobial strain trial in Long An province in 1992/3, pod yields were increased by as much as 31% with a multi-strain inoculant. In an associated trial, the best inoculation treatment increased pod yield by 50%.

Table 4. Chemical properties of the soil from the 6 inoculation experimental sites in Tay Ninh province and the outskirts of Ho Chi Minh City.

Location	Soil pH (1:2, H_2O)	Organic C (%)	Total N (%)	Nitrate-N (ppm)[A]	Available P (ppm)	Available K_2O (ppm)
Trang Bang	5.32	0.47	0.07	1.6	8	20
Gia Loc	5.76	0.45	0.07	1.9	24	22
PhuocThanh	5.69	0.46	0.05	1.6	35	72
Bau Don	5.35	0.52	0.04	1.2	18	24
Trung Lap	6.56	0.58	0.07	1.9	10	40
Thuong Tan An Hoi	6.50	0.58	0.07	1.4	19	24

[A] Mean value, 0–60 cm

Table 5. The effect of rhizobial inoculation and fertiliser application on nodulation scores of groundnut at 6 sites in Tay Ninh province and the outskirts of Ho Chi Minh City. The experiments were conducted in winter-spring 2000–2001.

Treatment	Trang Bang	Gia Loc	Phuoc Thanh	Bau Don	T.L. Thuong	T.A. Hoi
1. FP+ ON –inoc	2.8 bc	3.0	2.9	3.0	3.0	3.0
2. FP+ N	2.7 c	2.8	2.7	2.9	3.0	3.0
3. FP+ Local strain	3.0 a	3.0	2.9	3.0	3.0	3.0
4. FP+ NC 92 strain	2.9 ab	3.0	3.0	3.0	3.0	3.0
5. OF + Local strain	2.95 ab	2.9	2.9	3.0	3.0	3.0
SEM[A]	0.05	0.09	0.14	0.02		

[A] Standard error of the mean

There were no inoculation and fertiliser effects on pod yields (Table 6). This was in spite of the fact that 2 weeks after sowing, plants at the 6 sites in all of the 0N plots (i.e. treatments 1, 3, 4 and 5) appeared yellow and N deficient. The yellowness was short-lived and had disappeared within another 2 weeks as N_2 fixation began to supply the plants with N. Starter N is commonly applied to legumes in the tropics because of the N hunger phase during early crop growth. It is likely, however, that such a practice has little beneficial effect on pod yields.

Results of our experiments would suggest that there was adequate N supplied to the crops through N_2 fixation, irrespective of whether they were inoculated with a local groundnut strain or the highly-effective NC92, or not inoculated. These results also suggest that farmer practice for fertiliser application was as effective as the optimum mixture of fertiliser, recommended by Oil Plant Institute. The pod yields varied substantially between sites (Table 6) because of differences in crop management and, particularly, because of the weather. Pod yields varied between 1.03 t/ha (Tan An Hoi) and 4.89 t/ha (Bau Don). At the 2 low-yielding sites, Trung Lap Thuong and Tan An Hoi, heavy rain after sowing and during crop development compacted the soil and severely restricted root development. Many pods at these sites were also rotted and immature because of the excessive water.

Economic analysis

Economic analysis of the inoculation/fertiliser treatments at 4 of the 6 sites indicated negligable differences in profitability for the 4 farmer practice teatments (i.e. treatments 1–4) (Table 7). The optimum fertiliser treatment was about 13% more profitable than the 4 farmer practice teatments because of lower costs for fertiliser inputs. It should be noted that the yield levels of 3.4–3.5 t/ha of winter spring season were more than double the average yields of 1.6 t/ha for groundnut in the ERS (General Statistical Office, 2000). Thus, the gross margin benefits of VND7.4–8.4 million/ha in our experiments would be expected to be substantially less for many commercial crops.

Table 6. The effect of rhizobial inoculation and fertiliser application on pod yield (t/ha) of groundnut at 6 sites in Tay Ninh province and the outskirts of Ho Chi Minh City. The experiments were conducted in winter-spring 2000–2001.

Treatment	Trang Bang	Gia Loc	Phuoc Thanh	Bau Don	T.L. Thuong	T.A. Hoi
1[A]	3.78	2.38	2.53	4.83	1.62	1.03
2	3.67	2.28	2.90	4.96	1.61	0.93
3	3.76	2.43	2.61	4.84	1.56	1.14
4	3.48	2.39	2.82	4.80	1.61	1.03
5	3.97	2.29	2.56	5.00	1.59	1.02
SEM	0.12	0.05	0.13	0.10	0.07	0.10

Treatments were: 1. FP+ ON –inoc; 2. FP+ N; 3. FP+ Local strain; 4. FP+ NC 92 strain; 5. OF + Local strain

Table 7. Economic analysis of effects of rhizobial inoculation and fertiliser application on pod yields of groundnut at sites in Tay Ninh province and the outskirts of Ho Chi Minh City. The experiments were conducted in winter-spring 2000–2001. (Mean values from 4 sites; the 2 sites that suffered water logging damage Trung Lap Thuong and Tan An Hoi were not included.)

Treatment	Pod yield (t ha^{-1})	Fertilizer input (VND)	Total input (VND)	Output (VND)	Benefit (Gross margin) (VND)
1. FP+ ON (control)	3.38	1,986,900	10,174,800	17,576,000	7,401,200
2. FP+ N	3.45	2,260,800	10,435,500	17,940,000	7,504,500
3. FP+ Local strain	3.41	2,017,800	10,217,500	17,732,000	7,514,500
4. FP+ NC 92 strain	3.37	2,017,800	10,205,000	17,524,000	7,319,000
5. OF+ Local strain	3.46	1,378,000	9,590,100	17,992,000	8,401,900

Price of 1 kg groundnut (March 2001): 5200 VND ($US1 = 14,600 VND)
VND = Vietnamese dong

Attitudes of farmers to the use of inoculants

Interviews were conducted among the 40 farmers of the surveys on their knowledge of and attitudes to rhizobial inoculants. Only one of the 40 farmers knew about inoculants and that was because the Oil Plants Institute had on-farm rhizobial trials in his field during WS 1995/96. Most of the other farmers were not aware of inoculants because they had not been told about them and they were not available in the market. All 100% of the farmers said that they would use inoculant for groundnut if it made their groundnut yield better and the product was available.

We also interviewed the six farmers on whose land the inoculation trials were conducted. They were interviewed after the treatment effects became apparent. They said that they will reduce rates of fertiliser N in the future or not use it al all for groundnut because they saw clearly no benefit of the fertiliser N in the trials. They also recognised that it would be easier for extension officers to recommend such a practice change to progressive farmers and that it would be very difficult to persuade the conservative farmers to change. With respect to inoculation, they said that the effect of inoculation on pod yield was not clear, because pod yield of inoculated and uninoculated plots were the same. Thus, they considered it difficult to recommend that farmers use inoculants for groundnut. They did think, however, that farmers would apply inoculants to groundnut if the yield benefits could be shown and if the appropriate information was made available to them and to the extension officers.

Conclusions

The surveys of 40 commercial groundnut crops in the Tay Ninh province and Ho Chi Minh City area indicated that farmers do use rhizobial inoculants, instead they apply moderate to high rates of fertiliser N (average of about 50 kg/ha). All crops in the survey were nodulated, although some not as well as others. The 6 inoculation experiments, conducted in the same areas, indicated both inoculation and fertiliser N application to be unnecessary because they did not enhance nodulation (in the case of inoculation) or pod yield (inoculation and fertiliser N). On the other hand, high plant densities, high organic matter content of the soil and high rates of fertiliser P all enhanced N_2 fixation. High plant densities were also associated with high pod yields.

The areas in which both surveys and inoculation experiments were conducted were established groundnut areas. It is clear that infective and effective groundnut rhizobia have colonised the soils here. In new groundnut land, we would expect a response to inoculation. In such areas, inoculation would be required. In the absence of inoculation, the farmer would have to supply the crop with substantial amounts of fertiliser N or suffer a yield decline because of N deficiency. Surveys of farmers indicated an almost total lack of knowledge of inoculants but a willingness to use them if they were shown to provide a yield benefit. They also saw that information about inoculants would need to be provided to them and to the extension officers.

Acknowledgments

We would like to express our gratitude to the Australian Centre for International Agriculture Research (ACIAR) for part-funding of this work through Project 9827 'Increasing yields and N_2 fixation of soybean, groundnut, and mungbean in Vietnam through rhizobial inoculation'. We thank Dr Mark Peoples, CSIRO Plant Industry, Australia, for ^{15}N analysis.

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Rhizobial Inoculation and N_2 Fixation of Soybean and Mungbean in the Eastern Region of South Vietnam

Ha Huu Tien[1], Tran Minh Hien[1], Mai Thanh Son[2] and David Herridge[3]

Abstract

Increasing yields and economical efficiency of grain legumes in the Eastern region of South Vietnam (ERS) remains a priority of governmental strategy on rural development. We report a 5-year program at the Institute of Agricultural Science of South Vietnam (IAS) to address the issue. Initially (1996–1998) cultivar, rhizobial strain and fertiliser N and P effects on yield and N_2 fixation of soybean in glasshouse experiments and in the field in the Dong Nai province of the ERS were investigated. During 1999–2001, surveys of farmers' crops of soybean (*Glycine max*) and mungbean (*Vigna radiata*) were conducted to determine current inoculation and cultural practices and levels of nodulation and N_2 fixation in commercial crops. Six field experiments were conducted during autumn 2000 to determine fertiliser and rhizobial inoculation effects on nodulation, N_2 fixation and yield of soybean and mungbean. Rhizobial inoculation had little effect on the yield and N_2 fixation of soybean and mungbean. The %Ndfa values were in the range 30–56%. Our activities were, for the most part, conducted in established soybean and mungbean areas. Small responses were recorded in areas that were new to the legumes (up to 10% increase in grain yield). Even so, the uninoculated plants were nodulated indicating that naturalised rhizobia were in these soils, perhaps resulting from long ago cultivation of the legumes, or from natural contamination of the soil via water and wind movement. Both legumes responded to small starter doses of fertiliser N (20–40 kg N/ha). Surveys of commercial crops of soybean and mungbean indicated large variations in soil quality, cultural practices (particularly fertiliser rates) and crop growth. The N_2 fixation data showed generally high levels of symbiotic dependence (Ndfa ranges of 56–89% for soybean and 45–76% for mungbean) and a moderate level of variation. Our results appear to vindicate the fact that farmers do not inoculate soybean and mungbean in the Dong Nai and Tay Ninh provinces, and that they apply fertiliser N.

THE GOVERNMENT of Vietnam acknowledged many years ago the importance of grain legumes and their roles as human food, animal feeds and benefit to the soil. Soybean (*Glycine max*) was considered a major source of feed for an expanding livestock industry with a projected demand for 800,000 t soybean annually by the year 2000. The potential for expansion as a human food was also recognised, particularly to alleviate malnutrition in mountainous rural communities. The aim of the Government's Development Plan for 1995–2000 was to increase productivity to at least 1.5 t/ha, and to expand the area sown, particularly in the upland regions of the North and the Eastern region of South Vietnam, to achieve an annual production of 500,000 t. Soybean was the third priority for upland crop research in Vietnam after rice (*Oryza sativa*) and maize (*Zea mays*).

In l994, the area sown to soybean in Vietnam was 132,000 ha, with an average yield of 0.94 t/ha. Total production was 124,500 t. The current figures are 102,000 ha, yielding 1 t/ha and total production of 102,000 t. The major soybean-producing regions of the country are the North Mountainous and Midland region, the Red River Delta region, the South Eastern region and the Mekong Delta. Nationally, soybean yields are low and unstable compared to neighbouring countries. This level production has not satisfied national demand.

The Eastern region of South Vietnam (ERS) is suitable for short-duration industrial crops and boasts the biggest grain legume growing area of South

[1] Institute of Agricultural Science of South Vietnam, Ho Chi Minh City, Vietnam
[2] Atomic Research Centre of Ho Chi Minh City, Vietnam
[3] NSW Agriculture, TCCI, Tamworth, NSW 2340, Australia

Inoculants and Nitrogen Fixation of Legumes in Vietnam
edited by D. Herridge
ACIAR Proceedings 109e
(printed version published in 2002)

Vietnam. The ERS has a monsoonal climate, with the wet season from May to November and the dry season from December to April. The highest rainfall occurs in September-October (450–500 mm/month). The area sown to soybean in this region is about 26,400 ha, mainly on red soils of Dong Nai Province. This represents about 27% of the country's total area of soybean, and about 65% of national production. Nevertheless, average yields of soybean in Dong Nai remain low at 0.69 t/ha. In the ERS, dark brown soils cover about 100,000 ha. These soils are among the best of South Vietnam. They have good chemical and physical properties because they have developed from young volcanic materials. The main food crop grown on these soils is maize. Soybean and mungbean (*Vigna radiata*) together are cultivated as subsidiary crops (intercropped or relayed)in the Dong Nai province on about 50,000 ha annually.

As is the case in other parts of the world, farmers prefer to grow high-yielding cereals like maize and rice, particularly if fertiliser N is readily available and relatively inexpensive. Even so, soybean and mungbean may still provide an economic alternative to at least some of the cereal production in regions such as the Dong Nai province provided they are high yielding and robust in terms of pest and disease resistance. For farmers to replace part of their cereal-production with legumes, they must be convinced of the benefits. For the legumes, the benefits would need to include an attractive market value and marketability of the grain, and a positive impact of growing the crop on associated crops and on the soil itself. Clearly, cultivars would need to be made available to farmers that fulfil these requirements and appropriate technologies, such as the provision of effective rhizobial inoculants, and cultural practices developed.

We report a 5-year program at the Institute of Agricultural Science of South Vietnam (IAS) to address the issues outlined above. The first part of the program (1996–8) investigated cultivar, rhizobial strain and fertiliser N and P effects on yield and N_2 fixation of soybean in glasshouse experiments and in the field in the Dong Nai province of the ERS. During 1999–2001, surveys of farmers' crops of both soybean and mungbean were conducted to determine current inoculation and cultural practices and levels of nodulation and N_2 fixation in commercial crops. In autumn 2000, a further six field experiments were conducted to determine the benefits of rhizobial inoculation on nodulation, N_2 fixation and yield of soybean and mungbean.

Materials and Methods

Cultivar, rhizobial strain and fertiliser N and P effects on soybean and mungbean yield and N_2 fixation

Cultivars, glasshouse

Cultivars of soybean were DT84, MTD120, MTD176, G87-5, MTD455-3, QT95, Nam Vang, MTD6, MTD22, HL2, and HL90. The experiment was conducted in autumn 1996. The plants were grown in basins filled with a 50:50 mixture of sand and red soil (Luvisol), composed of 54% clay, 13% sand, 1.1% organic C, 0.148% N, 0,085% total P, 6.1 ppm available P and a pH (water) of 5.42. To determine the percentage of plant N derived from N_2 fixation (%Ndfa), the ^{15}N isotope dilution method was used. Upland rice and non-nodulating soybean (M129) were used as non N_2-fixing references. Thus, ^{15}N was incorporated into each basin at a rate of 15 mg ^{15}N/kg soil. Plants were harvested at physiological maturity for shoot dry matter (DM), %N and ^{15}N.

Cultivars, field

Soybean cultivars HL92, HL2, QT95, MTD176, and Nam Vang were tested in the field in autumn 1997. Experimental design was a randomised complete block with 4 replicates. Fertiliser was applied as 40N — $60P_2O_5$ — $50K_2O$. Individual plots were 24 m^2, containing an 8 m^2 sub-plot for N application. Soybean M129 and upland rice were again used as non N_2-fixing references. Plants were harvested at maturity for grain yield and shoot dry matter (DM), %N and ^{15}N.

Fertiliser N, field

Treatments were 2 soybean cultivars, HL2 and MTD176, and 3 rates of fertiliser N, 20, 40 and 60 kg/ha. The experimental design was a randomised split-plot with 4 replicates. The experiment was conducted in the autumn 1997. Main plots and ^{15}N sub-plots were as described above, as were sampling procedures.

Rhizobial strain

Treatments were 3 soybean cultivars, HL2, Nam Vang and MTD176, and 3 different strains of rhizobia (*Bradyrhizobium japonicum*) OR, G49 and USDA110. The experimental design was a randomised split-plot with 4 replicates. The experiment was conducted in the autumn 1997. Main plots and ^{15}N sub-plots were as described above, as were sampling procedures.

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Phosphorus rate

Treatments were 2 soybean cultivars, HL2 and MTD176, and 3 rates of P — 0, 30 and 60 kg P_2O_5/ha. The experimental design was a randomised split-plot with 4 replicates. The experiment was conducted in the autumn 1997. Main plots and ^{15}N subplots were as described above, as were sampling procedures. Total P content was determined by the Murphy-Riley method

Rhizobial inoculation and fertiliser N effects on different soil types in different seasons

Effects of rhizobial inoculation and fertiliser N on soybean and mungbean yield and N_2 fixation were determined in field experiments in the Dong Nai and Tay Ninh provinces of ERS during 1998–2000. In 1998, the experiment was conducted on a Luvisol that had been used for soybean production. Treatments were ± inoculation, 2 soybean cultivars, HL2 and MTD176, and 3 rates of N — 0, 20 and 40 kg/ha. Experimental design was a split plot with 4 replicates. Nodulation, DM production and grain yield were assessed.

In 1999, the experiment was conducted on a Luvisol that was new to soybean production. Treatments were ± inoculation, 1 soybean cultivar, MTD176, and 3 rates of N — 0, 20 and 40 kg/ha. Experimental design was a split plot with 4 replicates. Nodulation and grain yield were assessed.

The 2000 experiments were conducted at 6 sites (5 in Dong Nai and 1 in Tay Ninh), details of which are given in Table 1. Soybean was sown at sites 1, 4 and 5 and mungbean sown at sites 2, 3 and 6. Sowing dates ranged between 17 July and 16 August 2000. Experimental design was a randomised complete block with 4 replicates. Individual plots were 21 m^2. Treatments were as follows:

1. Farmer's practice, including fertiliser N (FP+N)
2. Farmer's practice, but without fertiliser N (FP0N)
3. Farmer's practice (–N) + inoculation with local rhizobial strain (USDA110 for soybean and 049 for mungbean) (FP+local)
4. Farmer's practice (–N) + inoculation with CB1809 strain for soybean (FP+CB1809) and CB1015 for mungbean (FP+CB1015)
5. Optimum fertiliser except N (–N) + inoculation with CB1809 strain for soybean (OP+CB1809) and CB1015 for mungbean (OP+CB1015)

Fertiliser N applications were (N-P-K in kg/ha) 30–40–60 (farmer's practice) and 0–60–60 (optimum fertiliser). Plants were assessed at flowering for nodulation, at physiological maturity for DM (for %N and $\delta^{15}N$ analysis) and at maturity for grain yield.

Surveys of farmers' fields of mungbean and soybean in the Dong Nai and Tay Ninh provinces of the ERS — 2000

During 2000, 20 farmers' crops of soybean and mungbean were surveyed for various soil and plant measures including N_2 fixation and fertiliser and cultural practices that might have an impact on yield and N_2 fixation. The 10 soybean crops were in the Dinh Quan, Xuan Loc and Thong Nhat districts of Dong Nai province. The 10 mungbean crops were in the Xuan Loc and Thong Nhat districts of Dong Nai province and the Go Dau district of Tay Ninh. In these areas soybean is principally grown as an autumn crop and mungbean as a summer crop. The preceding crop in 19 of the 20 fields was maize.

The soil in each field was sampled at sowing to a depth of 10–15 cm from 5 randomly-selected points and analysed for organic C, total N, available P and available K. These same sampling points were used later for plant and grain sampling. At 40 days after sowing (DAS) for mungbean and 50 DAS for soybean, 5-plant samples from each of the 5 sampling points were used for assessment of nodulation. At physiological maturity (60 DAS for mungbean and 70 DAS for soybean), 5-plant samples and associated weeds (usually grasses) were taken for DM, %N and $\delta^{15}N$ analysis. Grain yield was estimated from the real yield that farmers obtained on their entire field. Information on land cultivation history and cultural practices including fertiliser inputs were gathered from each of the participating farmers.

Table 1. Chemical properties of the soils from the 6 inoculation experimental sites in Dong Nai and Tay Ninh provinces of ERS of Vietnam, conducted during autumn 2000.

No	Farmer name	Legume	Soil type	Organic C (%)	Total N (%)	P_2O_5 (ppm)	K_20 (ppm)	PH (water)
1	Lyu Caem Voong	Soybean	Fe Luvisol	1.76	0.16	74	107	5.05
4	Traan Thea Huong	Soybean	Ferralsol	1.56	0.12	18	178	5.46
5	Buoi Duy Duong	Soybean	Ch Luvisol	1.36	0.17	63	609	6.07
2	Phaim Tho Loan	Mungbean	Fe Luvisol	1.02	0.11	26	304	5.65
3	Traan Vane Cu	Mungbean	Acrisol	0.34	0.05	84	30	4.93
6	Traan Thea Huong	Mungbean	Ferralsol	1.97	0.16	25	272	6.10

Fe – ferric; Ch – chromic

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Results and Discussion

Cultivar, rhizobial strain and fertiliser N and P effects on soybean yield and N_2 fixation — 1996–99 glasshouse and field experiments

The %Ndfa values were high for all soybean cultivars in this glasshouse pot experiment, even though the values were slightly different according to which species was used as the non N_2-fixing reference (Table 2). There did not appear to be any relationship between shoot DM and %Ndfa.

In the subsequent field experiment, %Ndfa values were much lower (range 32–43% with non-nodulating soybean as the reference and 28–38% with rice as the reference) (Table 3). As with the glasshouse trial, soybean cultivar differences were minor, although both MTD176 and HL2 performed well.

Effects of fertiliser N on yields and N_2 fixation of the same two cultivars, MTD176 and HL2, were examined on a Luvisol in the ERS of Vietnam (Table 4). Shoot DM, N and N fixed increased with increasing N rate for MTD176 but not for HL2. The %Ndfa values were similar overall to those of the previous field experiment, i.e. about 40%. Grain yields were not affected by fertiliser N.

Three strains of *B. japonicum*, OR, G49 and USDA110, were tested on three cultivars, Nam Vang, HL2 and MTD176. The strain used for many years in the U.S. as both an inoculant strain and in research, USDA110, was the best overall (Table 5). It produced an average of 10% more shoot DM than strain G49 and 17% more than strain OR. Similarly, %Ndfa values were 16 and 23% higher for USDA110 than for G49 and OR, respectively.

There were no responses to applied P in this experiment, either in terms of shoot DM, grain yield, %Ndfa or shoot N fixed (Table 6). It should be noted here that the %Ndfa values of 45–48% were very consistent with values in the three previous field experiments.

Table 2. Nitrogen fixation data for 11 cultivars of soybean, grown in basins in a glasshouse filled with a 50:50 mixture of a red soil (Luvisol) and sand during 1996.

Cultivar	Shoot DM (g/plant)	%Ndfa	
		M129 reference	Upland rice reference
DT84	6.6	70	80
MTD120	7.1	70	80
MTD176	9.7	69	79
HL2	9.0	68	79
QT95	7.2	65	77
Nam Vang	9.4	65	77
MTD6	8.8	64	76
MTD22	7.4	64	76
MTD455-3	7.8	63	76
G87-1	7.6	62	75
HL90	6.9	62	75

Table 3. Yields and N2 fixation of soybean cultivars in the field in Dong Nai province of ERS in 1996.

Cultivar	Shoot DM (t/ha)	Shoot N (kg/ha)	Grain yield (t/ha)	%Ndfa	
				M129 reference	Upland rice ref.
MTD176	5.05	142	1.42	43	38
HL2	4.47	124	1.71	39	38
G87-5	4.84	126	1.47	38	33
Nam Vang	4.61	109	1.30	30	29
HL92	3.84	126	1.42	32	28

Table 4. Cultivar and fertiliser N effects on shoot DM, grain yield and N2 fixation of soybean grown in the field in Dong Nai province of the ERS of Vietnam during 1997.

Cultivar	Fertiliser N (kg/ha)	Shoot DM (t/ha)	Shoot N (kg/ha)	Grain yield (t/ha)	%Ndfa	Shoot N fixed (kg/ha)
HL2	20	4.30	120	1.90	44	52
	40	4.40	124	2.02	45	58
	60	4.50	126	1.99	39	53
MTD176	20	4.56	127	1.75	40	50
	40	5.40	143	1.82	42	60
	60	5.80	152	1.88	45	68

Table 5. Cultivar and rhizobial strain effects on shoot DM yield and N2 fixation of soybean grown on a Luvisol in Dong Nai province of the ERS of Vietnam during 1998.

Cultivar	Rhizobial strain	Shoot DM (g/plant)	%Ndfa
Nam Vang	OR	13.5	41
	G49	13.5	43
	USDA110	15.7	48
HL2	OR	15.2	45
	G49	16.4	52
	USDA110	16.9	56
MTD176	OR	12.4	39
	G49	13.5	40
	USDA110	15.5	52

In an experiment in an established soybean area on a Luvisol, there was no effect of inoculation (Table 7). Populations of *B japonicum* were found to be 1×10^3/g soil. The early effect of fertiliser N on shoot DM was not sustained. There was no effect of fertiliser N on grain yield.

The inoculation × fertiliser N experiment was repeated in the following year (1999), but this time in an area that was new to soybean. In this experiment, there were responses to inoculation with a 72% increase in nodule numbers and 26% increase in nodule weight (Table 8). Increases in shoot DM and grain yield due to inoculation were 6% and 10%, respectively. There were large benefits of even small amounts of fertiliser N (i.e. 20 kg N/ha). Responses were 94% for grain yield and about 70% for shoot DM. Such responses may have been mediated

Table 6. Cultivar and fertiliser P effects on shoot DM, grain yield and N2 fixation of soybean grown on a Luvisol in Dong Nai province of the ERS of Vietnam during 1998.

Cultivar	Fertiliser P_2O_5 (kg/ha)	Shoot DM (t/ha)	Grain yield (t/ha)	%Ndfa	Shoot N fixed (kg/ha)
HL2	0	8.23	1.48	48	57
	30	8.53	1.61	46	60
	60	8.54	1.75	46	58
MTD176	0	7.13	1.11	45	60
	30	7.46	1.29	45	58
	60	7.48	1.30	45	60

Table 7. Inoculation and fertiliser N effects on nodulation, shoot DM and grain yield of soybean grown in an established soybean area on a Luvisol in Dong Nai province of the ERS of Vietnam during 1998.

Inoculation	Fertiliser N (kg/ha)	Nodulation (no/plant)	Shoot DM (t/ha)	Grain yield (t/ha)
+	0	18	7.2	1.15
	20	14	11.0	1.14
	40	11	11.7	1.30
–	0	20	7.3	1.30
	20	18	11.5	1.19
	40	15	11.9	1.38

Table 8. Inoculation and fertiliser N effects on nodulation, shoot DM and grain yield of soybean grown in a new soybean area on a Luvisol in Dong Nai province of the ERS of Vietnam during 1999.

Inoculation/ fertiliser N	Nodulation (no/plant)	Nodulation (g fresh wt/plant)	Shoot DM (t/ha)	Grain yield (t/ha)
+ inoc	19	1.44	7.85	1.20
– inoc	11	1.14	7.43	1.09
0N	11	0.78	5.68	0.70
20N	17	1.57	9.65	1.36
40N	17	1.51	9.27	1.37

through beneficial effects of the fertiliser N on plant establishment and early growth.

Rhizobial strain and fertiliser N effects on soybean and mungbean yield and N_2 fixation — 2000 field experiments

The six field experiments conducted in the Dong Nai (five sites) and Tay Ninh (one site) provinces during autumn 2000 had the same matrix of inoculation and fertiliser treatments as experiments conducted in Tay Ninh province and Ho Chi Minh City region on groundnut (Lien Hoa et al. this proceedings) and on groundnut and soybean in the Mekong R. delta (Hiep et al. these Proceedings).

The data for soybean are presented in Tables 9 and 10. Uninoculated plants of soybean were well nodulated at the three sites, although at sites 4 and 5, there appeared to be a small response to inoculation (Table 9).

There were small but consistent responses to inoculation for shoot DM and grain yield (4–7% for shoot DM and 5–9% for grain yield, mean values for the 3 sites) (Table 10). There was no consistent difference between the two strains, USDA110 and CB1809, and no effect of optimum fertiliser. The best treatment overall, however, was farmer's practice (+fertiliser N, –inoculation). For the three sites, this treatment produced, on average, 21% more shoot DM and 18% more grain yield than the control (farmer's practice –fertiliser N, – inoculation).

The fertiliser N rate for treatment 1 was 40 kg N/ha, sufficient to boost seedling and vegetative growth of the soybean, but not sufficient to meet a substantial part of the N demand of the crop (about 200–300 kg N/ha). It is conceivable that the fertiliser N stimulated plant establishment and early growth and may have even improved nodulation through positive effects on seedling root development.

The fact that responses to inoculation were very small and that uninoculated plants were well nodulated indicates that these areas have established populations of *B. japonicum* in the soils. This is not surprising as soybean has been grown in the Dong

Table 9. The effect of rhizobial inoculation and fertiliser application on nodulation of soybean grown at 3 sites in Dong Nai province of the ERS of Vietnam during autumn 2000.

	Nodule number/plant			Nodule mass (g/5 plants)		
Cultivar	Site 1 Fe Luvisol	Site 4 Ferralsol	Site 5 Ch Luvisol	Site 1 Fe Luvisol	Site 4 Ferralsol	Site 5 Ch Luvisol
FP +N –inoc	22	15	19	1.42	0.59	n.d.
FP –N –inoc	19	17	12	1.16	0.86	n.d.
FP –N +USDA110	25	26	18	1.31	1.19	n.d.
FP –N +CB1809	28	22	18	1.31	0.97	n.d.
OF –N +CB1809	26	21	19	1.28	0.98	n.d.
LSD (P = 0.05)	n.s.	8	4	n.s.	0.40	

FP – farmer practice; OF – optimum fertiliser
n.d. not done

Table 10. The effect of rhizobial inoculation and fertiliser application on shoot DM and grain yield of soybean grown in the field in Dong Nai province of the ERS of Vietnam during autumn 2000.

	Shoot DM (t/ha)			Grain yield (t/ha)		
Cultivar	Site 1 Fe Luvisol	Site 4 Ferralsol	Site 5 Ch Luvisol	Site 1 Fe Luvisol	Site 4 Ferralsol	Site 5 Ch Luvisol
FP +N –inoc	5.30	7.60	9.05	1.13	1.54	1.34
FP –N –inoc	4.80	5.35	7.90	0.90	1.33	1.18
FP –N +USDA110	5.23	6.25	7.38	0.99	1.32	1.35
FP –N +CB1809	4.90	5.55	8.40	0.98	1.36	1.37
OF –N +CB1809	5.08	5.83	8.50	0.98	1.35	1.26
LSD (P = 0.05)	0.36	1.35	1.10	0.16	0.19	0.09

FP – farmer practice; OF – optimum fertiliser

Nai province and associated areas for a long time. Such a lack of response to rhizobial inoculation would not necessarily be expected in areas that had never been used for soybean production.

Effects of inoculation on mungbean were very similar to those already described for soybean. Essentially, inoculation did not affect mungbean nodulation at the three field sites (Table 11). All treatments were well nodulation. Treatment effects on mungbean shoot and grain yields were also very similar to soybean (Table 12). The best treatment overall was the farmer's practice plus fertiliser N, which produced an average of 18% more shoot DM and 11% more grain yield than the control (FP-N-inoc). Responses to inoculation were small and inconsistent with strain O49 slightly better than CB1015. As with soybean, these experiments were conducted in established mungbean areas and logically the soil would have contained large established populations of effective mungbean rhizobia.

Surveys of farmers' fields of mungbean and soybean in the Dong Nai and Tay Ninh provinces of the ERS — 2000

During 2000, surveys were conducted of 10 farmer soybean and 10 farmer mungbean crops. Seventeen of the 20 fields were in the Dong Nai province of the ERS and the other three in Tay Ninh. The principal objectives of the survey were to quantify nodulation and N_2 fixation of the commercial crops and to determine cultural practices that might be contributing to high levels of symbiotic activity (e.g. inoculation, P and K fertiliser, good agronomy) or, on the other hand, depressing symbiotic activity (high rates of fertiliser N, poor agronomy).

A summary of the available data is presented in Table 13. None of the crops were inoculated and all were fertilised with N. Data on the soil, plant and cultural practices showed substantial variation between fields, particularly soil available P and K

Table 11. The effect of rhizobial inoculation and fertiliser application on nodulation of mungbean grown in the field in Dong Nai and Tay Ninh provinces of the ERS of Vietnam during autumn 2000.

	Nodule number/plant			Nodule mass (g/5 plants)		
Cultivar	Site 2 Fe Luvisol	Site 6 Ferralsol	Site 3 Acrisol	Site 2 Fe Luvisol	Site 6 Ferralsol	Site 3 Acrisol
FP +N –inoc	24	2.6[A]	13.2	0.17	n.d.	n.d.
FP –N –inoc	35	2.5	15.7	0.31	n.d.	n.d.
FP –N +O49	29	2.7	14.2	0.24	n.d.	n.d.
FP –N +CB1015	34	2.5	16.7	0.28	n.d.	n.d.
OF –N +CB1015	35	2.7	15.7	0.28	n.d.	n.d.
LSD (P = 0.05)	n.s.	n.s.	2.3	n.s.		

[A]Nodule score on 0 to 3 basis
FP – farmer practice; OF – optimum fertiliser
n.d. not done

Table 12. The effect of rhizobial inoculation and fertiliser application on shoot DM and grain yield of mungbean grown in the field in Dong Nai and Tay Ninh provinces of the ERS of Vietnam during autumn 2000.

	Shoot DM (t/ha)			Grain yield (t/ha)		
Cultivar	Site 2 Fe Luvisol	Site 6 Ferralsol	Site 3 Acrisol	Site 2 Fe Luvisol	Site 6 Ferralsol	Site 3 Acrisol
FP +N –inoc	7.20	2.88	9.14	1.70	0.83	0.90
FP –N –inoc	6.10	2.40	7.78	1.32	0.81	0.94
FP –N +O49	5.83	2.83	9.47	1.45	0.89	0.89
FP –N +CB1015	5.95	2.33	8.36	1.48	0.80	0.90
OF –N +CB1015	6.00	2.25	7.91	1.35	0.69	0.85
LSD (P = 0.05)	1.23	n.s.	1.14	n.s.	n.s.	n.s.

FP – farmer practice; OF – optimum fertiliser

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Table 13. A summary of data (mean, range of values) from farmers' fields of autumn-sown soybean and mungbean crops, surveyed in 2000. There were 10 fields for each species.

Data	Factor	Soybean		Mungbean	
		Mean	Range	Mean	Range
Soil[A]	pH (soil:water, 1:2)	5.86	4.74–7.32	5.85	4.60–7.34
	Organic C (%)	1.74	1.23–2.17	1.22	0.54–1.69
	Total %N	0.04	0.01–0.17	0.03	0.01–0.13
	Available P_2O (ppm)	77	1–203	61	6–182
	Available K_2O (ppm)	279	68–761	147	34–415
Agronomy	Years growing soybean/mungbean	21	10–30	11	2–22
	N application (kg/ha)	34	25–40	36	30–45
	P_2O_5 application (kg/ha)	26	0–40	38	30–45
	K_2O application (kg/ha)	33	0–60	42	30–60
Plant	Plant density (plant/m)	39	35–43	35	31–41
	Shoot dry matter (t/ha)	7.07	3.5–10.0	4.86	3.4–6.3
	Nodule score (0–3)	1.9	1.0–3.0	2.4	2.0–3.0
	Grain yield (t/ha)	1.04	0.8–1.4	1.14	1.0–1.5
	%Ndfa	71	56–89	65	45–76

[A] Soil measurements were made on samples from 0–15cm

and P and K fertiliser application rates. Plant density, shoot DM and nodulation varied up to 3-fold. The %Ndfa values were in the range 56–89% for soybean and 45–76% for mungbean. Mean values were 71% (soybean) and 65% (mungbean). Crop N fixed could not be determined because there were no data for %N of shoots at maximum biomass. Fertiliser rates were similar for the two species. The soybean crops produced more shoot DM, but tended to have poorer nodulation than mungbean. Grain yields were similar for the two species.

Conclusions

The data from experiments and surveys in the Dong Nai and Tay Ninh provinces of the ERS of Vietnam indicated that rhizobial inoculation had little effect on the yield and N_2 fixation of soybean and mungbean. Our activities were, for the most part, conducted in established soybean and mungbean areas. Small responses were recorded in areas that were new to the legumes (up to 10% increase in grain yield). Even so, the uninoculated plants were nodulated indicating that naturalised rhizobia were in these soils, perhaps resulting from long ago cultivation of the legumes, or from natural contamination of the soil via water and wind movement. Both legumes responded to small starter doses of fertiliser N (20–40 kg N/ha).

Results appear to vindicate the fact that farmers do not inoculate soybean and mungbean in the Dong Nai and Tay Ninh provinces, and that they apply fertiliser N. Surveys of commercial crops of soybean and mungbean indicated large variations in soil quality, cultural practices (particularly fertiliser rates) and crop growth. The N_2 fixation data showed generally high levels of symbiotic dependence and a moderate level of variation.

Acknowledgments

We would like to thank all people and organizations ACIAR, MARD for the financial support and assistance during the two years of the project. We wish to express our special gratitude to Professor Pham Van Bien, Director of IAS; Dr Nguyen Huu Hiep (Vietnamese project leader of ACIAR PN9827). Furthermore, thanks to all staff members of the Fertilizer and Soil Department, and the Grain Legumes Department of IAS who always supported us in helpful discussions.

The Impact of Background Rhizobial Populations on Inoculation Response

Jo Slattery[1] and David Pearce[1]

Abstract

Agronomic programs that target the introduction of legumes into different agro-ecosystems focus initially on the selection of legumes that have the ability to tolerate edaphic constraints that include disease resistance, water stress and tolerance to salinity, acidity and sodicity. Within such selection programs the interaction of the legume with soil *Rhizobium* has largely been ignored. In order to maximise agricultural production, specific programs must link strain selection with plant development together with an understanding of the impact of background populations.

PASTURE and crop legumes have been used extensively in agriculture over the past century in Australia, mainly for maintaining soil fertility. These agricultural soils are often constrained in their ability to sustain productive farming systems due to factors associated with low fertility, sodicity, salinity and extremes of acidity and alkalinity. These same attributes can also have a negative impact on the legume-*Rhizobium* symbiotic relationship reducing the ability of rhizobia to form nodules with optimal N_2-fixing capacity, thus impeding the continued success of legumes in Australian agricultural systems.

Considerable variation in the soil populations of *Rhizobium* spp has been found in soils throughout Australia, with the range varying from less than 10 to in excess of 10^6 *Rhizobium* bacteria g/m soil (Gibson et al. 1975; Slattery et al. 1999), generally with an average soil population above 1×10^4 *Rhizobium* bacteria g/m soil. The size of the soil population is dependent on field history, location of sampling, soil characteristics and the presence of a host plant.

Where there are low (<50 *Rhizobium* bacteria g/m soil) naturalised populations of rhizobia specific to a target legume, the introduction of new strains by seed inoculation is normally successful. On the other hand, inoculation into soils where naturalised rhizobial populations are high (>10^3 *Rhizobium* bacteria g/m soil) introduction of new strains can be difficult and often unsuccessful (Thies et al. 1991; Brockwell et al. 1995).

The Australian continent provides a large landmass that constitutes a wide spectrum of climatic conditions and soil environments, in which to maintain a viable agricultural industry. Soil types in the pulse growing regions of southeastern Australia vary from alkaline (NW Victoria and much of South Australia), neutral acidic soils (Wimmera, central and southern Victoria) to highly acidic soils (NE Victoria and southern NSW). This wide variation in soil type has serious implications for *Rhizobium* survival, *Rhizobium* effectiveness and the need for re-inoculation of following pulse crops.

Techniques used for the development of elite inoculant strains have been discussed in an accompanying workshop paper (Slattery and Pearce 2001). The final criteria adopted for the selection of *Rhizobium* strains are the assessment of germplasm for edaphic adaptation and field performance (Howieson et al. 2000; Slattery and Pearce 2001). The capacity of inoculant strains to colonise soils in sufficient quantity to provide effective nodulation is very much dependant on the soil type. Thus, a thorough understanding of the presence of background populations will enable recommendations to be made regarding the need for re-inoculation of pulse legumes.

In this paper, we study the effect of the soil environment on the ability of rhizobia to form nodules and the need for rhizobial inoculation when introducing pulse legumes into different soil environments. A comprehensive soil survey across the pulse legume growing regions of Victoria provide a critical

[1] Rutherglen Research Institute, Department of Natural Resources and Environment, Rutherglen, Victoria 3685, Australia

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(printed version published in 2002)

analysis of the soil chemical and background rhizobial populations under different soil environments. Once the rhizobial status is defined, criteria are needed to identify the requirement for the re-inoculation of pulse crops and background rhizobial survival across these diverse soil types.

Materials and Methods

Field survey and soil sampling

Rhizobial populations were monitored and assessed for a range of pulse/crop/pastures. Fifty paddocks were sampled during August 1997, over a wide geographical range for the pulse legume growing regions of Victoria (Rutherglen, Dookie, Elmore, Charlton, Birchip, Horsham and Walpeup regions) (Table 1). *Rhizobium* survival, soil classification and soil chemical characteristics of each paddock were also measured. Ten soil cores (10 cm × 2.5 cm diameter) were collected aseptically (Slattery and Coventry 1993) from each paddock, bulked, thoroughly mixed, passed through a 2 mm sieve to remove stones and large pieces of undecomposed organic matter, then stored at room temperature before chemical and rhizobia analysis.

Table 1. Number of paddocks and regional location of sites used in the legume field survey.

Regional location	Number of paddocks sampled
Birchip, Southern Mallee, NW Victoria	11
Walpeup, Northern Mallee, NW Victoria	10
Horsham, Woomera, NW Victoria	7
Elmore, Central Victoria	7
Charlton, NC Victoria	5
Dookie, NE Victoria	7
Rutherglen, NE Victoria	3

Evaluation of nitrogen-fixing effectiveness

Air-dried soil samples collected from each site were used to estimate the symbiotic effectiveness of rhizobial populations. Extreme care was taken in the collection of soil to avoid the sampling of soil from around the plant rhizosphere. The timing of sampling (late August) coincided with seasonal conditions for that year; in particular, the likelihood of an abrupt early ending to the growing season was a key factor in early sampling. The symbiotic effectiveness was tested with 6 legumes—field pea (*Pisum sativum*), faba bean (*Vicia faba*), lentil (*Lens culinaris*), vetch (*Vicia sativa*), chickpea (*Cicer arietinum*) and lupin (*Lupinus angustifolius*)—as the plant hosts using the whole soil inoculation method (Brockwell et al. 1988). This method uses soil rhizobial populations as a means of assessing the N_2– fixation potential of that soil. Test plants were grown in a semi-sterilised system (washed sand:vermiculite mixture, moistened with N-free nutrient salt solution (Slattery and Pearce 2001)). In this system, the shoots are exposed to the atmosphere while the roots are grown under aseptic microbiological conditions.

Test plant seeds were surface sterilised, germinated on 2% water agar plates at 22ºC, prior to planting in 3 cm square seedling tubes. Five days after planting, each seedling was inoculated with a 1 mL soil suspension (10 g soil in 90 mL N-free nutrient salt solution) containing about 10^7 cells of each isolate. The commercial rhizobial strains were used as the positive controls for each species, whilst the uninoculated treatments were either supplemented with nitrogen or without nitrogen. The plants were grown for 4 weeks in a glasshouse (range 12–25ºC), with the moisture content of each plant maintained with either sterile distilled water or N-free nutrient solution. All plants were harvested, the roots examined for nodulation, and dry matter (DM) from the whole plant tops was measured after oven drying at 70ºC.

Soil chemical analysis

Soil pH_{Ca} (0.01M $CaCl_2$) and soil pH_W 1:5 soil:extractant was measured using an automated system, while Olsen P (0.5 M $NaHCO_3$) 1:100 soil:extractant, extractable K (0.5 M $NaHCO_3$) 1:100 soil:extractant, total N (Kjeldahl), mineral N (2 M KCL—Kjeldahl) and organic C were determined by standard procedures (Slattery et al. 1999). Soils were identified according to the Australian soil classification (Isbell 1996).

Experimental sites

In order to make recommendations for farmers on the need for re-inoculation of subsequent legume crops additional treatments (+/– inoculation) were imposed across farmer paddocks. Inoculation response trials for 6 legumes, field pea, faba bean, lentil, vetch, chickpea and lupin, were established at 6 sites in 2000 across the pulse growing regions of northern Victoria. Site establishment and maintenance details are similar to those methods outlined in Slattery and Coventry (1999). At each site, the commercial inoculant was introduced as one of the inoculation treatments in the form of a peat inocula.

Site management and sowing

Preparation, sowing and maintenance of each site were carried out throughout the growing season.

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When needed annual grasses and broadleaf weeds were controlled with the appropriate chemicals according to registered recommendations.

Field plant sampling

Individual plants were collected 10–12 weeks after sowing for nodulation and plant dry matter measurements. Plant roots were scored for nodulation on a 0–5 scale, based on the nodule number, size, position, distribution and pigmentation of effective nodules on the crown and lateral roots (modification of Corbin et al., 1977). Plant material (tops) was dried in a forced-draught oven at 70°C for 48 hour, then weighed.

Grain yields

Grain seed yield was determined by mechanical harvesting of the entire plot.

Results and Discussion

Evaluation of nitrogen fixing effectiveness

In general, each survey site could by categorised into 7 groups based on soil type and location of site. In this study, we investigated the ability of background rhizobial populations to infect commercially significant cool-season pulse crop species, namely *Rhizobium leguminosarum* bv *viciae*, *Bradyrhizobium* sp. and *Mesorhizobium cicer*.

Results from this soil survey have shown that there was considerable variation in the presence (or survival) of root-nodule bacteria at individual sites within a location or between rhizobial species (Table 2 and Table 3). On some soil types rhizobial strains are present that are capable of infecting the host legume and forming nodules, but at other sites rhizobia are not present in the soil and hence no nodules are formed.

Of the 50 paddocks surveyed 33% of the paddocks had sufficient background populations of *Rhizobium leguminosarum* bv *viciae* for infection of the faba bean host, 54% for lentils, 55% for field pea, and 66% had sufficient populations for infection of the vetch host plant (Table 2). At 17 sites, no *Rhizobium leguminosarum* bv *viciae* populations were detected on either host plant identifying the importance of understanding your paddock history when sowing a legume crop. The presence of chickpea rhizobia, *Mesorhizobium cicer* was very low with only 7% of paddocks surveyed having sufficient background populations for the infection of chickpea plants. In the case of *Bradyrhizobium* sp, 38% of paddocks had background populations with numbers sufficiently high enough for effective nodulation of the lupin plant.

When the data from the survey paddocks were assessed across each location the presence of background root-nodule bacteria could be related to soil type and pH. The major *Rhizobium leguminosarum* bv *viciae* pulse growing regions in Victoria are the Birchip and Horsham regions. Background rhizobial populations in these regions are present and in the past, rhizobial inoculation has not been recommended, however this survey suggests that 30% of faba bean legume crops have inadequate nodulation leading to a reduction in crop yields. The presence of background chickpea rhizobia was lower in the Birchip regions (10%) compared to a value of 67% in the Horsham region (Table 3).

Rhizobium leguminosarum bv *viciae* populations at the Elmore, Dookie and Rutherglen locations are extremely low (Table 3). Soil pH_{Ca} for these sites are also low (Table 5), suggesting a strong correlation between soil pH and rhizobial persistence (Figure 1). Poor rhizobial persistence indicates a need for inoculation of pulse legumes when sowing into acidic soils. In contrast to this for the alkaline soils there are usually high background rhizobial populations and the inoculation of pulses is not always necessary. Pulse production in Australia is increasing rapidly as farmers appreciate the financial and rotational benefits that pulses provide (Siddique and Sykes 1997) but when sowing pulse legumes the response of pulse production to inoculation must be clearly

Table 2. Mean values for background root-nodule bacteria encountered in a soil survey of paddocks (n=50) in the pulse growing regions of Victoria using the whole soil inoculation technique as the method of assessment.

	Host plant					
	Field pea	Faba bean	Lentil	Vetch	Chickpea	Lupin
Mean value (n=50)	55[A]	33	54	66	7	38

[A]Number refers to the % of paddocks exhibiting infective soil root-nodule bacteria as recorded by the effective nodulation of the host plants.

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Table 3. Presence of soil root-nodule bacteria collected from 50 paddocks across 7 locations in the pulse growing regions of Victoria using the whole soil inoculation technique as the method of assessment.

		Host					
Location	Soil classification	Field pea	Faba bean	Lentil	Vetch	Chickpea	Lupin
Birchip	Calcarosol	100[A]	67	100	100	10	33
Walpeup	Calcarosol	71	0	56	78	0	14
Horsham	Sodosol	86	71	100	100	67	13
Elmore	Dermosol	0	0	0	14	0	57
Charlton	Dermosol	67	100	40	80	0	33
Dookie	Ferrosol	14	0	14	14	0	71
Rutherglen	Kurosols	0	0	0	0	0	33

[A]Number refers to the % of paddocks exhibiting infective soil root-nodule bacteria as recorded by the effective nodulation of the host plants.

understood. To further increase pulse production, inoculation of the crop is essential especially where the naturalised rhizobial populations are low.

With the exception of the Horsham location, the extremely low *Mesorhizobium cicer* population again highlights the need for inoculation when chickpea crops are introduced into a paddock (Table 3). The chickpea-*Rhizobium* symbiosis is highly specific and extensive studies have demonstrated the uniqueness of the chickpea rhizobia with 99% of the chickpea isolates nodulating only the original host plant and not species belonging to the Fabaceae and Mimosaceae families (Gaur and Sen 1979).

Bradyrhizobium sp populations were present in all soil types and varied between 12 and 71% across the different locations (Table 3). With the exception of the Dookie location where 71% of sites had sufficient population numbers, inoculation of future lupin crops is necessary at the other locations. Nonetheless, *Bradyrhizobium lupini* does not occur naturally

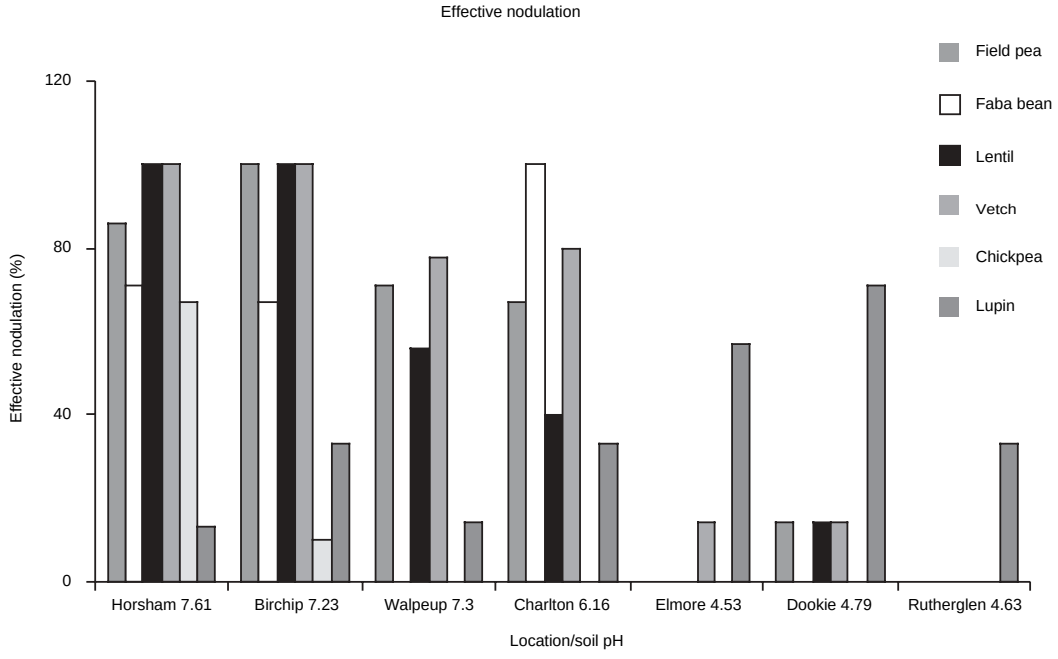

Figure 1. Relationship between effective nodulation of six legume species on soil pH_{Ca} at seven locations.

in the soils of north-east Victoria, and it is essential that lupin seed be inoculated when first growing lupins (Slattery and Coventry 1989).

Soil chemical parameters

Values for the means and range of concentration of soil properties at the Birchip sites are shown in Table 4. Soil pH for the 8 paddocks ranged from 6.83 to 8.83, with 63% of the sites being highly alkaline (pH_W>8.0). Soil pH_{Ca} for the 8 paddocks ranged from 6.06 to 8.03. Soil organic C ranged from 0.40 to 1.62%; the mean value of 1.1% is fairly low and can be explained by intense cereal rotation preventing a build up of organic matter. The mean total soil N value is low (0.06%), indicating potential for improvement in crop yields, if nitrogen were supplied, in fact 63% of the sites had a total soil N value <0.06%. There was a ten fold difference in maximum and minimum values of extractable P, with 75% of sites having a low Olsen P value of <17 mg/kg and would benefit from additional applied superphosphate. With the exception of one property the soils at the Birchip sites were classified as Calcarosols derived from limestone with calcium carbonate throughout the profile (Isbell 1996).

Table 4. Mean values and ranges for soil properties (n=8) encountered in a survey of the Birchip pulse growing regions of Victoria.

Soil Properties	Mean	Min. of range	Max. of range
pH_w	8.09	6.83	8.83
pH_{Ca}	7.23	6.06	8.03
Organic carbon (%)	1.15	0.40	1.62
Total N (%)	0.06	0.01	0.09
Olsen P (mg P/kg)	15.6	4.5	46.6
Extractable K (mg K/kg)	420	168	618

Values for the means and range of concentration of soil properties at the Charlton, Dookie, Elmore, Horsham and Rutherglen sites vary according to the region and the different soil types (data not shown). A summary showing soils classification and soil pH_{Ca} for the 7 sites identified the similarities and differences between soil types and regions (Table 5). Soil pH_{Ca} at the Charlton and Elmore sites has declined compared with previous data with pH_{Ca} values ranging from 5.78 to 6.77 at Charlton and from 4.41 to 4.71 at the Elmore sites. The major soil classification for the Charlton and Elmore sites is a Dermosol; these soils tend to form surface crusting if not managed appropriately (Isbell 1996). Soil pH_{Ca} in the Dookie region were lower than for the other regions with pH_{Ca} ranging from 4.42 to 5.29 and soils classified as Ferrosols, containing a high iron content and a distinctive chocolate brown colour (Isbell 1996).

Table 5. Summary of soil pH and soil classification data across 7 locations in the pulse growing regions of Victoria.

Location	Soil classification	Mean pH_{Ca}	Min. of range	Max. of range
Birchip	Calcarosol	7.23	6.06	8.03
Walpeup	Calcarosol	7.30	5.93	8.05
Horsham	Sodosol	7.61	7.40	7.70
Elmore	Dermosol	4.53	4.43	4.71
Charlton	Dermosol	6.16	5.78	6.77
Dookie	Ferrosol	4.79	4.42	5.29
Rutherglen	Kurosols	4.63	4.21	4.92

Results from this survey have shown that soil factors influence the legume-*Rhizobium* symbiotic relationship (Figure 1). The growth and survival of *Rhizobium* spp in soil environments can be affected by a combination of factors including acidity (including toxicities of Al), salinity, alkalinity (including high concentrations of Ca and B), soil temperature, moisture, fertility (including nutrient deficiencies), and soil structure. Much of the area affected by soil acidity is naturally acidic, though agricultural practice is further acidifying the soil. Acidity factors (high Al, low Ca and low PO_4^-) have a direct impact on either rhizobia growth and persistence, or nodule initiation and N_2-fixation effectiveness (Coventry and Evans 1989). On acid soils the populations of rhizobia can be low in subterranean clover-based pasture (Coventry and Hirth 1992) or poorly nodulated in medic-based pasture (Howieson and Ewing 1986).

Soil acidity limits *Rhizobium* survival and persistence in soils, and the subsequent root colonisation, infection and nodule activity (Brockwell et al. 1991). The correct soil pH is crucial for the survival of *Rhizobium* spp, and in adverse soil pH environment strains of rhizobia differs in their ability to infect the host plant (Brockwell et al. 1995).

This soil survey identified considerable variation in both soil type and the survival of effective *Rhizobium* strains. However, we need to understand the implications that these factors have for farmers regarding the need for re-inoculation when sowing into a paddock with a previous history of legumes. If there is sufficient effective rhizobia, is there a need to re-inoculate? This question is especially important in Australian systems where intensified cropping is often accompanied by an increased reliance on chemicals for weed, pest and disease control; these chemicals are used as seed dressings or in pre- and post-emergent crop situations. To answer

the question of re-inoculation of subsequent legume crops additional treatments (+/– inoculation) were imposed on these same farmer paddocks.

Farmer inoculation response trials

Following the assessment of survey data, a selection of paddocks were sown with inoculated and non-inoculated legumes in 2000. These trials were established to verify *Rhizobium* survival in the soils and then make recommendation tor farmers regarding the need for re-inoculation of a paddock based on soil type and background *Rhizobium* populations. Inoculation trials were sown at six sites across the Victorian Mallee (alkaline soils, high background populations), southern Mallee to north-east Victoria (acidic soils, low background populations). Selection criteria for each site was based on soil type, soil pH, location and rainfall (Table 6).

Table 6. Location, soil pH and background rhizobial populations for farmer inoculation response trials (2000).

Site location	Soil type	Soil pH_{Ca}	Background populations
Bakers-Rutherglen, NE Vic	Kurosols	4.60	Low
Ferriers-Birchip, NW Vic	Calcarosol	7.60	High
Smith-Birchip, NW Vic	Calcarosol	7.78	High
Corbetts-Walpeup, NW Vic	Calcarosol	8.80	High
Nihill-Walpeup, NW Vic	Calcarosol	7.29	High
Pohlner-Walpeup, NW Vic	Calcarosol	7.16	High

On the acidic kurosol clay soils at Rutherglen the nodulation derived from the background rhizobial population varied between plant species (Table 7). For lupins, there was a limited response to inoculation, which supports the survey findings for the 6 district paddocks in that 33% of the paddocks had background *Bradyrhizobium lupini* in numbers sufficient for effective nodulation of lupins. Adequate nodulation occurred in the nil treatment of field pea, lentil and vetch. However, with inoculation nodulation and grain yields were further increased thus highlighting the need, and additional benefits of inoculation when growing pulse legumes in these acidic soils.

Visible growth differences were observed late in the growing season for lentils. Nonetheless, these differences could not be related to increased grain yields as the farmers' sheep grazed the lentil crop prior to harvest. Inoculation of faba bean was vital in this paddock as shown by the increase in yield from 0.34 t/ha for the nil treatment to 4.4 t/ha after inoculation. Even though background rhizobial populations were present the response to inoculation was enormous. This farmer routinely inoculates his legume seed but now understands the reasons why it is necessary. Poor persistence of *Rhizobium leguminosarum* bv *viciae* in acidic soils was also demonstrated and was shown by a low nodulation score and poor plant growth, however in another study some strains were found to be more tolerant of acidic soils than other strains examined (Carter et al. 1995). Frey and Blum (1994) also give an example of soil pH affecting *Rhizobium* survival and legume growth.

A visual response to inoculation was also obtained for the chickpea crop with an increase in yields from 0.47 to 2.37 t/ha. In general, chickpea crops are not

Table 7. Nodule number, nodule score, shoot dry weight (g/pl), root dry weight (g/pl) and grain yield (t/ha) for the farmer inoculation response trial at the Rutherglen site (2000).

Legume	Rhizobia Strain	Nodule no./plant	Nodule score	Root DM (g/plant)	Top DM (g/plant)	Yield (t/ha)
Lupin	Nil	16.4	2.64	0.63	1.80	0.71
	WU425	19.9	3.18	0.50	1.91	0.98
Faba bean	Nil	10.2	2.64	1.39	4.33	0.34
	WSM1274	34.5	4.21	4.21	18.50	4.41
Field pea	Nil	43.1	4.07	0.55	6.28	2.15
	SU303	53.7	4.27	0.46	7.43	2.87
Lentil	Nil	10.1	2.21	0.11	0.89	0.15[A]
	WSM1274	21.6	3.19	0.11	0.86	0.12[A]
Vetch	Nil	33.6	3.45	0.66	5.44	7.87[B]
	SU303	64.1	4.81	0.51	7.09	8.07[B]
Chickpea	Nil	1.62	0.62	0.88	3.13	0.47
	CC1192	23.5	4.02	2.20	3.78	2.37

[A] Crop grazed by sheep, reduced grain yield
[B] Vetch dry matter t/ha, vetch cut prior to grain harvest

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usually grown on acidic soils, especially where the soil pH is only 4.6. However, when the growing seasonal rainfall is below average and crop disease is controlled, chickpea yields of 2.37 t/ha are an economic option for farmers in northeast Victoria. The chickpea-*Rhizobium* symbiosis is highly specific and, as a consequence, rhizobial survival in the soil and growth in the rhizosphere is most important. Rai (1991) in a study involving the symbiosis between chickpea and *Rhizobium leguminosarum* bv *cicer* strains for adaptation to acidic soils demonstrated that only 5% of the strains examined were found to be suitable for nodulation and growth in these strongly acidic soil environments.

On the alkaline calcarosol soils, the response to inoculation was not visually evident. At the Ferrier-Birchip site (Table 8), background populations under lupins were low; inoculation significantly increased nodulation and improved grain yields. However, as a consequence of the high background populations of *Rhizobium leguminosarum* bv *viciae* inoculation did not improve the nodulation of faba bean, field pea, lentil or vetch. Inoculation with SU303 improved the yields compared to the nil treatment. In the case of lentils, grains yields for WSM1455 and WSM1483 were higher than WSM1274. For chickpeas, background populations were low and inoculation significantly increased nodulation and spring dry matter production. The low district growing season rainfall is able to explain the low grain yields at this site.

The inoculation response trials provided us with field evidence that supports the field survey data. Soil pH, paddock history, disease control and rainfall all contribute to successful crops and grain yields. The presence of background rhizobia is not always sufficient in itself, to ensure optimal N_2-fixing capacity in the host legume as the effectiveness of strains to fix N within naturalised populations can vary considerably. It is usually accepted that it is difficult to introduce superior strains (by inoculation) when there is a high background population of indigenous and well-adapted rhizobia, and nodules occupied by the inoculant strain may decline in the years following inoculation (Unkovich and Pate 1998; Slattery and Coventry 1999. Soil surveys in high pH areas of southeast Australia show that the lack of effective *Rhizobium* may limit the performance of annual medics (Ballard and Charman 1996; Slattery et al. 1999). In these areas, the naturalised medic *Rhizobium* populations are generally high (>10^4 *Rhizobium* bacteria g/m soil), but the annual medic species frequently do not achieve an effective symbiosis with the naturalised rhizobia (Ballard and Charman 1996; Slattery et al. 1999). The reasons for this poor effectiveness of naturalised medic populations are uncertain at this stage but may be related to soil chemical characteristics at high pH. It is clear that to obtain optimum N_2-fixation for a range of

Table 8. Nodule number, nodule score, shoot dry weight (g/pl), root dry weight (g/pl) and grain yield (t/ha) for the farmer inoculation response trial at the Ferriers-Birchip site (2000).

Legume	Rhizobia Strain	Nodule no./plant	Nodule score	Root DM (g/plant)	Top DM (g/plant)	Yield (t/ha)
Lupin	Nil	0.1	0.10	0.33	1.80	0.74
	WU425	6.1	2.37	0.40	1.62	0.81
Faba bean	Nil	23.6	4.31	0.99	6.01	0.26
	WSM1274	37.9	4.25	0.78	5.15	0.59
	SU303	25.5	4.02	0.92	5.61	0.73
	RRI294	20.2	3.69	0.65	4.09	0.27
	RRI339	20.2	3.95	0.89	5.48	0.46
Field pea	Nil	30.8	3.95	0.40	6.57	0.78
	SU303	24.1	4.15	0.28	6.19	0.92
	RRI294	22.4	4.18	0.28	6.57	0.81
	RRI339	22.3	4.04	0.23	5.92	0.81
Lentil	Nil	24.1	4.24	0.09	1.03	0.83
	WSM1274	18.8	3.57	0.10	1.09	1.01
	WSM1455	24.6	4.02	0.09	1.03	1.17
	WSM1483	21.0	3.51	0.09	1.00	1.35
Vetch	Nil	20.5	4.19	0.17	2.40	1.76
	SU303	13.5	3.36	0.12	2.14	1.81
Chickpea	Nil	0.19	0.19	0.23	1.30	NH
	CC1192	4.00	2.02	0.26	1.61	NH

NH– not harvested, cut and removed due to Ascochyta damage.

soil environments the soil chemical status and background rhizobial populations must first be investigated. Only then can rational conclusions be made regarding the effectiveness of new strains for specific soil conditions.

Conclusion

Cropping systems in Australia are intensifying with longer periods of the crop phase used in a rotation. Associated with this intensification is the higher input of chemicals, increased reliance on N-fertiliser, less tillage and more flexibility in sowing management. Within these changed systems there still exists a need for productive pulse crops, not only as economic crops in there own right, but to provide options for weed and disease management. However, these pulse crops are reliant on optimal nitrogen fixation and it is unlikely that intensified cropping systems will be sustainable in the long term without periods of highly productive legume-based pastures. Pasture input may be required for weed management, organic matter conservation and in some situations for managing a rising subsoil watertable. Pasture quality for either livestock or forage enterprises is dependent on a significant and productive legume component within the pasture and will also require optimal nitrogen fixation to achieve maximum production. In association with the improvement in legume productivity and subsequent improvements in soil fertility, there is an on-going need to develop more effective rhizobial microflora.

Acknowledgments

We acknowledge the financial support of the Grains Research and Development Corporation (GRDC) to undertake this work. The financial support provided to the senior author by the Australian Centre for International Agriculture Research (ACIAR) to attend this workshop is gratefully appreciated.

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Potential for Legume Inoculation in Vietnam

Pham Van Toan[1]

Abstract

Legume crops have a long history of cultivation in Vietnam and play an important role in Vietnam agricultural systems. In recent years, the nation's legume crop scientists have expended considerable effort to access and learn from advanced cultivation technologies available in the world, and to develop and to disseminate to Vietnam's farmers yield-enhancing new varieties and sustainable technologies. The average groundnut yield increased from 13 quintals/ha in 1995 to 15 quintals/ha in 2000 and from 7.9 quintals/ha in 1995 to 10.4 quintals/ha in 2000 for soybean. However, legume yields in Vietnam remain low compared to those of other Asian countries, where soil and climatic conditions are similar. Research, development and dissemination of advanced cultivation technology to achieve high legume yields and decreased production cost are the wish of all Vietnamese agricultural scientists and farmers. Legume inoculation is one of the ways to meet these challenges. This paper discusses the status of legume inoculation research and development in Vietnam as well as the potential for legume production in Vietnam.

Status of Legume Inoculation Research and Development in Vietnam

VIETNAM is predominantly an agricultural country with more than 7 million ha of agricultural land, so the demand for the fertiliser is very high. For the past 15 years, the mineral fertiliser supply has been continuously increasing by 7% of nitrogen (N) fertiliser, 8% of phosphorus (P) fertiliser and 12% of potassium (K) fertiliser (Table 1). This tendency will continue in the coming time. At present, the chemical factories can produce only 20% of N and 80% of P fertiliser demand. The government has to balance this by importation, which every year costs nearly US$500,000 (Table 2). To improve this situation, Vietnam has planned to expand existing factory capacity, while stimulating research, production and the utilisation of biofertilisers. The research on rhizobium symbiosis has been carried out in Vietnam for more than 20 years. There are 5 scientific organisations working on it (Table 3) with more than 50 scientists. They are capable of carrying out different research on rhizobium inoculant development, with main activities as follows:

- Collection, isolation and preservation of Rhizobium strains;
- Testing N-fixing activity of isolated Rhizobien by ARA or ^{15}N method;
- Developing Rhizobium inoculant for groundnut and soybean in different combination (single R.strain, multi R.strains or mix of Rhizobium and other N- fixing or P-solubilising microorganisms...);
- Field trials and demonstrations on the effect of Rhizobium inoculant on the legume growth and yield in different legume cultivation zones (mountain and midland, Red River delta, Middle Vietnam, Southeast Vietnam and Cuulong delta).

Rhizobium inoculant can increase pod yield of groundnut 13.8–17.5% in North and Middle and 22% in South Vietnam (Table 4, 5 and 6). In combination with an N fertiliser dose of 30–40 kg/ha Rhizobium inoculant has the same yield as an N-fertiliser dose of 60–90 kg/ha. The effect of Rhizobium inoculant is especially high in poor soil and new land under legume cultivation. The benefit of Rhizobium inoculant can be estimated at about 400,000–500,000 VND/ha. Rhizobium inoculant is very useful and benefits farmers. Because of limited funds and lack of technical equipment, it could be produced only at laboratory scale during the time of the project. At present, Vietnam has no company producing Rhizobium inoculant, so that the demand for the Rhizobium inoculant cannot be met here.

[1] Vietnam Agricultural Science Institute, Thanh Tri District, Hanoi, Vietnam

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edited by D. Herridge
ACIAR Proceedings 109e
(printed version published in 2002)

Table 1. Fertiliser utilisation in Vietnam (1000 t).

Kind of fertiliser	Year				
	1981	1985	1990	1995	2000
Nitrogen	175.7	267.0	358.8	506.0	690.0
Phosphorus	38.4	49.0	82.6	185.0	260.0
Potassium	22.0	22.1	27.8	31.6	166.0

Source: Bui Huy Hien (pers. comm.)

Table 2. Fertiliser importation in Vietnam.

1997		1998		1999	
Quantity (1000 t)	Cost in 1000$US	Quantity (1000 t)	Cost in 1000$US	Quantity (1000 t)	Cost in 1000 $US
2,458.373	424,946	3,454.213	474.697	2720	400.00

Source: Tu Kien 2000

Table 3. Research organisations working on legume inoculation in Vietnam.

Name of research organisation	Main activities
1. Vietnam Agricultural Science Institute (VASI)	Coordination of Rhizobium inoculant projects from 1982–1989 National centre for agricultural microorganisms Collection and preservation R&D of production technology of N-fixing and P-solubilising inoculants transfer technology to production companies
2. Agricultural University Hanoi	Rhizobium inoculant development for soy bean and carrying out the field trials
3. Research Institute of forestry	Rhizobium inoculant for forestry trees
4. Institute of R&D of biotechnology, CT University	R&D of Rh.inoculant for soy bean
5. Oil plant research institute	Field trials of Rhizobium inoculants for legumes
6. Agricultural Institute of South Vietnam	Rhizobium inoculant for groundnut and soybean

Table 4. Effect of Rhizobium inoculant on grain yield of groundnut in North and Middle Vietnam.

Soil	Fertiliser	Grain yield (t/ha)		Increasing to control	
		Control	Inoculation	t/ha	%
Infertile soil	NPK 30:60:60, FYM 5 tonnes	19.72	22.72	3.0	115.2
Fertile soil	NPK 30:60:60, FYM 5 tonnes	23.1	26.31	3.21	113.8
Feralit soil	NPK 30:60:60, FYM 5 tonnes	15.76	18.53	3.76	117.5

Source: Ngo The Dan et al. 2000

Table 5. Effect of Rhizobium inoculant on grain yield of groundnut in South Vietnam.

Cropping system	Fertiliser	Grain yield (t/ha)		Increasing to control	
		Control	Inoculation	T/ha	%
New cultivation	NPK 30:60:60, FYM:5t Lime:5t	15.6	17.8	2.2	114
Intercropped Rice-groundnut	NPK 30:60:60, FYM:5t Lime:5t	5.0	6.6	1.6	131
Intercropped Rice-groundnut	NPK 30:60:60, FYM:5t Lime:5t	6.1	6.5	0.,4	106
Intercropped Vegetable-groundnut	100 kg SA, 70 kg KCl, 150 kg Coconut ash	14.1	16.95	2.85	120
Intercropped Vegetable-groundnut	NPK 30:60:60, FYM:5t Lime:500kg	14.7	16.3	1.7	111
Intercropped Vegetable-groundnut	NP:20:60, FYM:3t Lime:400kg	—	—	—	138
Intercropped Vegetable-groundnut	NPK 30:60:40, FYM:3t Lime:100kg	22.0	24.6	2.6	112

Source: Ngo The Dan et al. 2000

Table 6. Effect of Rh.inoculant on grain yield of groundnut fertilising different mineral N doses.

Fertilise	Pod/plant	Thickseed pod/plant	Pod yield (t/ha)
NPK 30:60:60, FYM 8t, lime 400kg	15.5	7.0	18.61
NPK 30:60:60, FYM 8t, lime 400kg +Inoculant	16.9	7.5	20.50
NPK 60:60:60, FYM 8t, lime 400kg +Inoculant	16.9	7.2	18.50
NPK 90:60:60, FYM 8t, lime 400kg +Inoculant	18.2	6.9	19.11

Source: Ngo The Dan et al. 2000

Legume Production in Vietnam

Legumes are traditional crops cultivated in Vietnam and play an important role in Vietnam's agricultural system. To enhance development of legume production, different policies are applied:

- To increase legume production areas and cultivation seasons to meet the increasing demand on legumes for internal consumption and exportation.
- To establish intensive legume cultivated zones in combination with the exploitation of local land available for legume cultivation.
- To stimulate the application of new technologies to legume cultivation, harvesting and processing.

The current status of legume production in Vietnam is shown in Tables 7 and 8. The dates indicates that the production of soybean and groundnut, the main legume crops growing in Vietnam has increased continuously in the past 5 years, caused by expanding the cultivated area and increasing the legume yield. Based on the soil type, climatic conditions and production tradition, there are 6 legume cultivated zones in the country. Legume growth area and yield in different zones are different, but they have the same tendency to increase from time to time. However, legume yield in Vietnam is still low compared to China or other Asian countries, where natural and climatic conditions are similar.

Table 7. Vietnam legume production in 1990–1998.

Legumes	Parameters	1990	1995	1998
Groundnut	Area (1000 ha)	201.4	259.9	269.4
	Yield (quintals/ha)	10.6	12.9	14.3
	Total production (1000 tons)	213.1	334.5	386.0
Soybean	Area (1000 ha)	110.0	121.1	127.8
	Yield (quintals/ha)	7.9	10.4	11.1
	Total production (1000 tons)	86.6	125.5	141.3
Other	Area (1000 ha)	133.0	187.5	221.5
	Yield (quintals/ha)	5.8	6.8	6.5
	Total production (1000 tons)	77.1	127.7	144.1

Source: Hoang Minh Tam 2001

Table 8. Legume production in different cultivated zones (1998).

No	Cultivated zones	Groundnut			Soybean			Other legumes		
		Area ***	Yield **	Production *	Area ***	Yield **	Production *	Area ***	Yield **	Production *
1	North mountains and midland	42.2	10.3	43.6	54.6	8.6	46.4	27.8	6.1	17.0
2	Red river delta	23.5	15.1	35.6	24.5	13.5	33.0	10.0	7.7	7.7
3	North middle Vietnam	71.1	13.1	93.4	3.3	10.0	3.3	30.8	3.4	10.5
4	Coastal area of south middle Vietnam	28.9	13.0	37.5	3.6	15.3	5.5	14.4	8.0	11.5
5	High land of Tay Nguyen	18.6	10.2	18.9	12.0	8.8	10.6	39.7	5.1	20.3
6	Southeast Vietnam	68.8	18.2	125.0	15.3	8.4	12.8	77.4	5.7	44.3
7	Cuu Long delta	16.3	19.6	32.0	14.5	20.5	29.7	21.4	15.5	33.2
8	Total	269	14.3	386.0	127.8	11.1	141.3	221.5	6.5	144.1

*: Area in 1000 ha **: Yield in quintals/ha ***: Production in 1000 tonnes/year
Source: Hoang Minh Tam 2001

Constrains of legume production in Vietnam are follows:

- The old policy concentrated only on increasing important food and industrial crop production and paid no attention to developing traditional crops like legumes, so that the production of legumes cannot meet the consumer or export demand.
- The perception of farmers on legume production is limited. They are not ready to cultivate legumes instead main food crops. Legumes can be grown only in the legume special zones or intercropped with other crops.
- High legume production costs and unstable markets are other reasons for the limited production of legumes in Vietnam.

At present, a program to develop legumes production in Vietnam has been established. Its purposes are:

- to increase legume production to meet the demand of nutrition for humans and for livestock production;
- to increase legume yield and decrease production costs by the application of new technologies and advances in legume cultivation, harvesting and processing.

The main program activities concentrate on:

- Increasing legume cultivation areaóthe production area will expand from 250,000 ha in 2001 to 330,000 ha in 2005 for groundnut and from 130,000 ha in 2001 to 500,000 ha in 2005 for soybean by establishing intensive legumes production zones, by rotation or intercropping with industrial crops (Tables 9 and 10).
- Increasing legumes yield and decreasing legume production costóto increase legume yield, a new system for seed selection, processing and distribution is being established. In parallel, solutions to the problems of cultivation season, plan densities, fertiliser, irrigation, disease control and extension will be sought. The Vietnam Agricultural Science Institute has been nominated by the Vietnamese

government as the coordinator of two projects on improving soybean and groundnut production in Vietnam during the period 2001–2005. The total production will attain nearly 1,000,000 tonnes of soybean and 700,000 tonnes of groundnut in 2005. At the same time, the average yield of soybean and groundnut will reach 18 quintals/ha (Table 11).

Conclusion

Vietnam is an agricultural country with comfortable conditions for legume cultivation, but at present legume production cannot meet internal consumer demand. The Government of Vietnam will expand legume cultivation areas in the next 5 to 10 years to increase legume production as human food, animal feed and to improve the soil. At present, legume production costs in Vietnam are high, caused by low yield. It is necessary to find a way to improve this situation. Rhizobium symbiosis is very beneficial for crops. Even though N-fixing research began in Vietnam more than 30 years ago, Rhizobium inoculant application is very limited.

In all of the country, no company is currently producing Rhizobium inoculant. A system of Rhizobium inoculant development, production, quality control, extension and distribution should be established to contribute to expanding legume production in Vietnam

Table 9. Expanding legume production area in Vietnam (ha).

Year	Groundnut	Soybean
2001	250,000	130,000
2005	330,000	500,000
2010	—	700,000

Source: Hoang Minh Tam 2001

Table 10. Soybean cultivation area in different production zones in Vietnam (1000 ha).

Production zones	Total area	Two rice cropped land	Once Rice cropped land	Soybean special land	Inter cropped land	Rotation land
North mountains and midland	140	120	–	20	–	
Red river delta	150	4	42	83	18	3
North middle Vietnam	30	5	–	20	–	5
Coastal area of south middle Vietnam	30	–	–	25	–	5
High land of Tay Nguyen	110	–	–	97	10	3
Southeast Vietnam	85	9	1	60	11	4
Cuu Long delta	115	100	20	25	–	10
Total	700	238	63	330	39	30

Source: Hoang Minh Tam 2001

Table 11. Plan for legume production in Vietnam.

Year	Soybean		Groundnut	
	Yield (quintal/ha)	Total production (1000 t/year)	Yield (quintal/ha)	Total production (1000 t/year)
2001	12.5	187.50	15.00	375.00
2005	18.0	902.00	18.00	700.00

Source: Hoang Minh Tam 2001

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Development and Evaluation of Liquid Inoculants

Paul Singleton[1], Harold Keyser[1] and Eve Sande[1]

Abstract

Changes in the scale and planting equipment used on large soybean farm operations in North America have forced inoculant manufacturers to develop alternatives to peat based inoculants that are too cumbersome for large-scale field application and tend to plug precision air seeders used on large farms. A liquid inoculant formulation with good field performance characteristics that uses low cost materials and are easily attainable by small producers could overcome many problems associated with processing solid carriers. The field performance was evaluated of two prototype formulations with a network of 27 collaborators in sixteen countries including three in Vietnam to compare the liquid formulations to local inoculant products and to sterilised Canadian peat-based inoculant.

LEGUME inoculation is an old practice in many agricultural systems. Because of excellent performance characteristics, ground, neutralised peat (humus) has become the standard carrier material for the manufacture of inoculant in North America, Europe and Australia.

Large, easily accessible deposits of true peat are rare, especially in the tropics. This has led to research into alternative carrier materials such as lignite, charcoal, coir dust, and compost of various origins, sugarcane filter mud, bagasse, soils mixed with various organic amendments, clay, expanded mica (horticulture vermiculite) and liquid formulations with various additives. Most materials, however, have not demonstrated performance characteristics equivalent to peat based inoculant products.

Changes in the scale and planting equipment used on large soybean farm operations in North America have forced inoculant manufacturers to develop alternatives to peat based inoculants. Their focus has been directed toward liquid inoculant formulations for several reasons. Peat based inoculants are too cumbersome for large-scale field application and tend to plug precision air seeders used on large farms. Liquid formulations have an in that they can be applied to seed as it passes through seed augers on the way to the planting machinery.

Liquid formulations may have advantages for smaller scale agricultural systems in the tropics and their local inoculant manufacturer. First, most inoculant producers in the tropics do not have easy access to quality peat deposits. Even when they do, mining, drying, grinding and neutralising peat to a standard consistency is expensive and requires a considerable capital investment in equipment for small production operations. Other materials, such as composted wastes may be more available but are even more difficult to process to consistent characteristics. A liquid inoculant formulation with good field performance characteristics that uses low cost materials and are easily attainable by small producers could overcome many problems associated with processing solid carriers.

We organised a project that aimed to develop and evaluate a liquid inoculant formulation that 1) was low in cost, 2) used readily available, non-perishable materials, 3) could be grown under normal fermentation conditions, 4) supported high cell numbers in storage, and 5) promoted nodulation better than local inoculant products and equivalent to the best quality peat carrier materials.

We took a multifaceted approach to the problem including 1) identifying compounds that detoxified seed exudates and promoted cell survival upon rapid desiccation after application to seed, 2) strain selection for survival and superior BNF capacity, 3) preconditioning cells to withstand stress after

[1] University of Hawaii NifTAL Project, Department of Tropical Plant and Soil Science, University of Hawaii at Manoa, 1000 Holomua Rd, Paia, HI 96779 USA niftal@hawaii.edu

Inoculants and Nitrogen Fixation of Legumes in Vietnam
edited by D. Herridge
ACIAR Proceedings 109e
(printed version published in 2002)

inoculation. We believed that these effects may be additive in creating a high performance product.

Our project had several facets. First, we developed laboratory assays for *B. japonicum* survival after application to seed that correlated with inoculant performance in soil. This allowed us to screen compounds and strains that had a likelihood of enhancing inoculant performance in the field. We then evaluated prototype formulations under long term storage conditions and evaluated conditions in fermentors that enhance cell growth. Finally, we evaluated the field performance of two prototype formulations with a network of 27 collaborators in sixteen countries including our three colleagues in Vietnam to compare the liquid formulations to local inoculant products and to sterilised Canadian peat based inoculant.

Materials and Methods

Laboratory assay for testing inoculant survival on seed: materials and methods

A liquid culture of *Bradyrhizobium japonicum* SEMIA 5019 in late log phase was used to inoculate seed. Williams soybean seed were inoculated in batches of 120 seeds with 60 µL liquid inoculant or 0.06 g peat. Peat inoculated seeds were pre-coated w/75 µL gum arabic sticker solution (40 g gum arabic in 100 mL H_2O, heated in microwave). Seeds were coated in sterile 50 mL beakers with a glass stir rod. Immediately following inoculation seeds were poured into petri dishes which were then placed in sealed containers above a saturated solution of $CuCl_2.2H_2O$ in a 26°C incubator. This produces a 68% relative humidity a seed moisture content of 0.13 g H_2O/g seed. At various times from inoculation, seed were removed from the incubator and poured into a 250 flask containing 120 mL sterile diluent (0.85% NaCl, 0.01% Tween 80). The flask was shaken for 5 min on a Burrell wrist action shaker set at maximum speed, followed by 5 min of ultrasound exposure in a Bronson ultrasonic cleaner containing water up to the level of diluent.

Three steps of serial, ten-fold dilutions in saline diluent were prepared, with 100 µL aliquots from each of the 10^{-1} to 10^{-3} dilutions spread on G6 agar plates. The plates were incubated at 28°C for 5–7 days, and plates with 30–300 colonies were chosen for counting

Samples were replicated, and duplicate plate counts made from each dilution level. YM, AG or other suitable media can be substituted for G6 in the inoculant broth or agar media. In our experience the G6 medium supports the most rapid growth.

Evaluation of inoculant performance in green house pot trials

Plant culture

All experiments were conducted in soil from the Kula Agricultural Park, Maui, Hawaii. The soil, a Torroxic Haplustall, had a pH of 7.5, organic C of 1.3% organic N of 0.3%, and clay, silt and sand fractions of 42%, 43% and 14% respectively. Soil was mined to a depth of 25 cm, passed through a screen with mesh dimensions of 0.64 × 0.64 cm. Liquid stocks of KH_2PO_4 and $MgSO_4.7H_2O$ were sprayed onto the air-dried soil (15 g H_2O/g soil) as it rotated in a concrete mixer. These stocks provided 128 mg K, 100 mg P, 20 mg Mg, 27 mg S per kg soil. A liquid micro-nutrient mix (Hawaiian Horticultural Mix — Monterrey Chemical) was also sprayed onto the soil at the rate of 0.5 mL/kg soil to provide 7.5 mg Fe, 2.5 mg Zn, 2.3 mg Mn, 1.75 mg B, 0.75 mg Mg, 0.2 mg Mo and 0.15 mg Cu per kg soil.

Plastic pots (3.75 L) were filled with 2.6 kg air-dried soil. A circular depression (6.4 cm dia.) was made to a depth of 0.5 cm in the soil with the cross section of a PVC pipe. Four inoculated seeds were planted in this depression. Seeds were immediately covered with an additional 350 g of soil and the soil was pressed onto the seed to uniformly cover with 2.5 cm of soil. Pots were planted by block. The entire planting procedure took between 20–60 min. Thermometers were placed into the several pots at a depth of 2.5 cm. Soil temperatures were recorded at 1400–1430 h and at 0630–0700 h.

Except for control treatments, which were watered immediately at planting, the soil remained dry for 48 h when irrigation was applied to all pots simultaneously via a drip irrigation system. A drip irrigation emitter was placed at the center of each pot and 30 mL of de-ionised water was applied to each pot followed by 50 mL at 12 min intervals until 380 mL had been applied. This watering schedule simulated a 1.5 cm/h rainfall event. Pots were brought up to 36% moisture over a period of days and then maintained at 36–37% moisture until harvest at 30–33 days after planting.

Shoots were cut at soil level, dried at 70°C and weighed. Nodules were removed from roots, counted, dried at 70°C and weighed. Uninoculated controls remained non-nodulated.

Seed inoculation

Manufacture of the inoculant followed procedures above. Williams soybean seed (‘Belts95’, USDA, Beltsville MD) was inoculated in lots of 48 g (approximately 250 seeds) with 240 µL liquid inoculant or 0.24 g peat. Peat inoculated seeds were

pre-coated w/720 µL gum arabic sticker solution (40 g gum arabic in 100 mL H_2O, melted in microwave). Seeds were coated in sterile 250 mL beakers with a glass stir rod and approximately half the seed removed to a sterile petri-dish for enumeration and storage at 68% R.H. and 26°C. The remaining half was placed into an insulated box with ice and removed to the greenhouse. Inoculated seed were kept in the chilled box except when used for planting.

Yield measures were then correlated with estimates of cell survival at various times form inoculation.

Seed exudate assay

Extraction of seed exudates

Seed exudates were extracted from 100 unsterilised Williams soybean seed ('Belts97', USDA, Beltsville MD) in a sterile flask with 100 mL sterile de-ionised H_2O and aeration on a platform shaker at 200 r/min. After extraction for four hours the seeds were rinsed with sufficient sterile H_2O to return the volume of extract to 100 mL. The extract was filter sterilised (0.2 µM) and 10 mL aliquots placed into sterile plastic tubes, frozen at –20°C for 2 h, and lyophilised over 42 h on a Super Modulyo freezer dryer (Edwards). Extracts were held in a desiccator in a freezer until use.

Growth of bacterial cells

B. japonicum TAL 102 were grown to stationary phase in 250 mL flasks on a 200 r/min shaker at 25°C in 50 mL of either YMB or YMB with PVP (polyvinylpyrrolidone) K30 at 20 g/L.

Inoculant cultures were serially diluted to 10^{-3} in the appropriate media and one mL of the 10^{-3} dilution (equivalent to the number of cells inoculated onto 10 seeds) was placed into a tube containing the lyophilised seed exudate derived from 10 seeds and into an empty tube for the control. The cultures were vortexed well to mix and held on a 200 r/min shaker at room temperature for 24 h. Serial dilution was resumed and the 10^{-5} to 10^{-7} dilutions were spread plated (100 µL) on AG plates.

Testing shelf life of liquid inoculants

Bradyrhizobia strain

SEMIA 5019 was used in all media. The sources for inoculation into media were early stationary phase cultures (population densities $>10^9$ cells/mL) of YM, G5, G6 or G6+3g $CaCO_3$/L. Samples from these sources were used undiluted or diluted, as described below.

Treatments

Different media (see Table 2. for formulations) were evaluated for ability to sustain high densities of viable cells over a 180 day period at a constant temperature of 25°C. The media tested were the following:

1. YM undiluted.
2. YM cells washed (shaking + centrifugation) 3 times in saline, final re-suspension in distilled water.
3. G5 undiluted.
4. G6 undiluted.
5. G6 diluted 1:5 in 25 g PVP/L.
6. G6 diluted 1:5 in 15 mL glycerol/L.
7. G6 diluted 1:5 in 400 µM Fe-EDTA.
8. G6 diluted 1:5 in 25 g PVP/L + 15 mL glycerol/L + 400uM Fe-EDTA.
9. G6+3g $CaCO_3$/L undiluted.
10. Commercial liquid undiluted.

Experimental units consisted of 100 mL volume in a 250 mL flask fitted with a foam stopper (for air exchange). Dilutions were prepared by addition of 20 mL of the inocula source to 80 mL of the sterile diluent. All diluents were prepared with de-ionised water. Treatments had five replicates.

Storage conditions

Treatments were kept in a gravity convection incubator at a constant temperature of 25°C. Incubator humidity was not controlled.

Sampling

Two replicates of each treatment were sampled for viable population density and survival on seed (see above for procedure) at 48 h at 30, 60, 90 and 180 days after placement in the incubator. Diluted samples were spread on G6 agar medium. The commercial liquid inoculant treatment was added after initiation of the experiment, and was sampled at 16, 106 and 180 days after incubation. A peat control was used but became too contaminated to count after 30 days of storage.

Field trials

Twenty seven collaborators from 16 countries tested the liquid formulations under their local conditions. There were two sets of field trials. The first compared the G5 medium with B. japonicum strain SEMIA 5019 with a local inoculant product and an Uninoculated control. Collaborators added other treatments as they wished but that data is not included in this presentation. The second set of trials compared the G5 and G6 + PVP formulations to a local inoculant product and a sterile Canadian peat

based inoculant with Semia 5019. A minimum data set was specified that included seed yield, total seed n, nodule weight/ha and nodule number/ha.

Collaborators manufactured the liquid formulations and peat inoculant with materials provided by NifTAL. Aggultination and a rapid enzyme immunoabsorbant assay quality control kits were provided for SEMIA 5019. Samples of local inoculant products were evaluated for quality at NifTAL.

Growth of liquid formulations in aerated fermentors

The fermentor set up consisted of a 2.0 L borosilicate screw top bottle (Corning brand) containing 1.5 L of liquid media. Each 2.0 L vessel had an 18 mm dia. hole drilled into the side and near the bottom of the vessel to accommodate a tapered (17 mm dia at the small end) red rubber septum. The septum port was used for sterile sampling of the media. Each 2.0 L bottle was connected to the same style 250 mL bottle by silicone tubing. The second bottle was a reservoir for any foam generated by the culture that filled the head space of the 2.0 L bottle.

The centres of the both bottles' plastic screw caps were drilled out. An 8.0 mm thick silicone gasket with two holes was fit to the inside of each bottle cap. One hole in the silicone gasket of the 2.0 L bottle was fit with a 7 mm i.d. polypropylene air sparger tube that extended to 2 cm of the bottom, of the bottle. A flexible silicone tube connected the sparger tube to a 0.2 uM Millipore filter that sterilised air entering the 2.0 L vessel. The other hole in the gasket of the 2.0 L bottle was fit with a 7 mm i.d. silicone tube (flush with the gasket surface) that also connected to the gasket of the 250 mL bottle. Air exited the 250 mL bottle through another silicone tube that terminated in a 50 mL plastic syringe body filled with two foam plugs. It is important when using the submicron filters on the air supply that the air exit filtration system has significantly less air flow resistance. This avoids the possibility of back siphoning broth into the air supply filter when the air flow is interrupted.

The fermentor assemblies were connected to an air delivery manifold via a brass needle valve to control air flow. Air flow was monitored at the air outlet of each assembly with a glass tube air flow meter. Back pressure in the air manifold was maintained at 2.0 psi. Assemblies were placed on a heat mat with the thermocouple of the controller placed into a dummy vessel that was also aerated. Temperatures were monitored with a thermocouple pressed against the glass of the 2.0 L vessel with a 10 mm thick piece of latex rubber. Temperatures measured this way were within 0.2°C of temperatures measured by immersing the thermocouple into the liquid.

Results and Discussion

Survival of *B. japonicum* in commercial liquid inoculant formulations after application to seed.

We evaluated several commercial formulations for their ability to support survival of *B. japonicum* USDA 110 inoculated onto soybean seed. Figure 1 compares the number of viable cells remaining on seed over a 30-day period for four commercial liquid formulations, gamma irradiated Australian peat or cells suspended in water.

Results demonstrate the large variation in performance that is due to formulation and the apparent advantage of high quality peat carriers over most existing liquid formulations. Some formulations are no better that cells suspended in water. The initial inoculation level of this batch of peat inoculant was almost one log lower than Commercial 1 formulation. Despite this poor start, the peat inoculant still supported as many cells on the seed within a few days after inoculation as Commercial 1. Commercial 1 is a proprietary product and we have no knowledge of its formulation. These results indicate there is substantial room to improve liquid inoculant formulation.

Laboratory assay for testing efficacy of inoculant formulations

Table 1 shows the correlation between survival of *B. japonicum* after application to seed under standardised laboratory conditions (68% R.H. and 26°C) and performance of the inoculant in potted soil. The correlation between survival in the laboratory assay and nodulation performance in the potted soil improved with storage time. Control pots that were watered immediately after planting or controls that were inoculated with 10^9 cells/seed (injected with a

Table 1. Correlation between number of *B. japonicum* cells surviving on soybean seed in the laboratory at 26°C and 68% R.H. and nodulation and growth of soybean planted into dry potted soil.

Time from inoculation (h)	Nodule dry weight	Shoot ery weight
	R	
0	0.72*	0.70
2	0.76*	0.70
4	0.92***	0.84**
24	0.83**	0.76*
48	0.86**	0.77*

*** P≤.01; ** .05≥P>.01; *.1≥ P >.05; Pots irrigated 48 h after planting; Pots reached maximum temperatures of 48°C for 2 d before irrigation and 38°C for next 7 d; harvest 33 DAP

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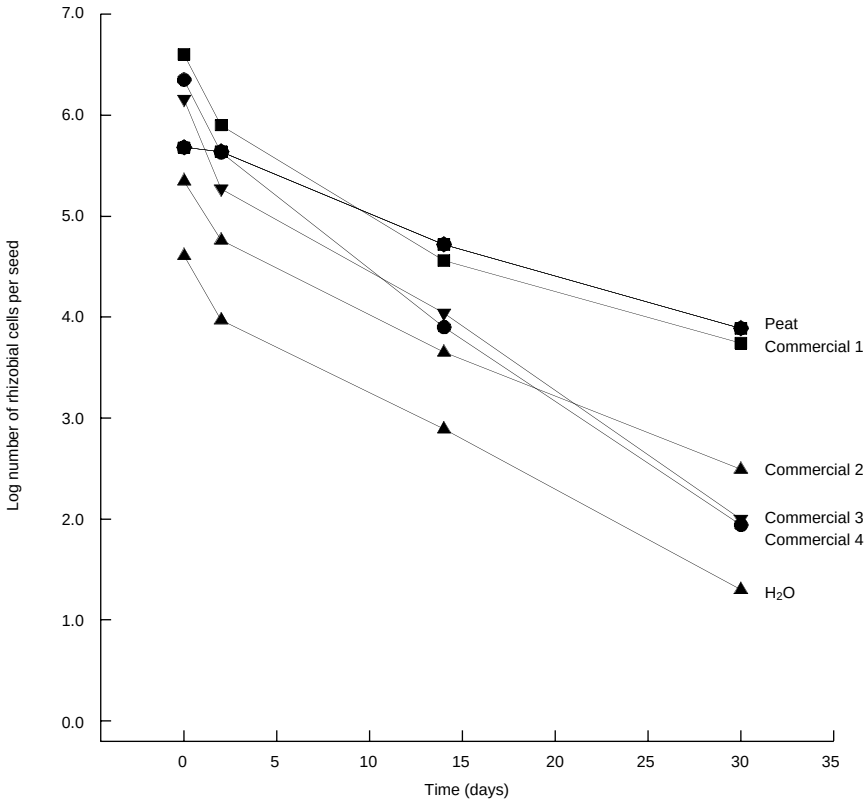

Figure 1. Survival of *Bradyrhizobium japonicum* in several commercial liquid inoculants and Australian peat and deionized water after application to soybean seed.

pipette into the soil) after the 48 h stress period consistently had greater nodulation than any treatment with delayed watering (data not shown). Since we exposed the inoculated seed to severe stress in the dry potted soil by withholding water for 48 hours following planting and allowing the soil to reach temperature maximums of 45–48°C we believe that differences in nodulation by different formulations and strains reflect the number of cells surviving until root emergence from the seed

We selected a 48 h laboratory storage period for all evaluations of the effect of materials, formulations and strains on cell survival after application to seed.

Effect of amendments on *B. japonicum* growth in liquid media and survival after application to seed

Reasoning behind selection of some test compounds

Several compounds were tested for their ability to promote survival of *B. japonicum* after inoculation. Selections were made to counter various stresses the cell may encounter after application to seed. We have not examined the interactions between compounds on cell survival. Synergistic interactions may be a highly productive area of research to improve the performance of liquid formulations.

1. Polyvinylpyrrolidone — PVP may bind toxic compounds present in seed exudates that are mobilised during inoculation and seed germination. PVP has a high water-binding capacity and appears to slow drying of the inoculant after application. PVP solution tends to coalesce into ridges on the seed coat as it dries, perhaps providing a thicker layer of protection than some other compounds. Its sticky consistency may also enhance cell and inoculant adherence to seed.

2. FeEDTA — One report in the literature hypothesised that seed-released compounds bind iron in the yeast extract, making it unavailable to cells. Supplementary iron may, therefore, replace Fe bound by seed exudates.

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3. Glycerol — Glycerol is a carbon source for rhizobia. Glycerol also has a high water-binding capacity and may protect cells from the effects of desiccation by slowing the drying rate. Its flow characteristics appear to promote rapid and even coating of seeds.

4. Trehalose — This compound is widely reported to enhance cell tolerance to desiccation and to osmotic and temperature stress. Trehalose acts by stabilising both enzymes and cell membranes, and is a compatible osmoticum as well. Trehalose is readily manufactured by *Bradyrhizobium* given the correct conditions. We found that additions of trehalose to the growth media had no effect on survival of *B. japonicum* applied to seed.

5. Glucose — Glucose promotes the production of the storage product glycogen. In the absence of glycogen, osmotically stressed cells are reported to release their supplies of trehalose, an undesirable outcome. Glucose also enhances exopolysaccharide production, which could protect cells during the rapid drying they experience at inoculation. Glucose had no apparent effect on cell survival.

6. Mannitol — Mannitol has many potential roles in a liquid inoculant. It is a carbon source for all strains of *Bradyrhizobium*, and in conjunction with arabinose it is reported to support the greatest production of trehalose in *B. japonicum* TAL 102. Mannitol is reported to protect cells during the rapid drying they experience at inoculation both as a solute and in enhancing polysaccharide production. When the mannitol was removed from the media survival of *B. japonicum* was adversely affected. Its effect on survival appears to be maximum at approximately 1.0 g/L. Formulations such as G6 +PVP (see Table 2 for formulation and Figures 6 and 7) with no mannitol had excellent survival characteristics.

Effects of FeEDTA and PVP on survival of *B. japonicum* after application to seed

FeEDTA (Figure 2) and PVP K30 (Figure 3) are examples of additives that improved survival of *B. japonicum* on soybean seed. PVP and FeEDTA appear to enhance survival of *B. japonicum*, and the promotion is concentration-dependent. The data for glycerol is less clear.

There was a significant (r^2 >.93) linear relationship between FeEDTA concentration and the proportion of viable cells remaining on soybean seed at 2 h and 48 h after inoculation. While high concentrations of FeEDTA seem to promote survival on seed, we have some evidence that concentrations exceeding 400 μM reduce survival in the inoculant formulation. This sensitivity in the media may be strain dependent.

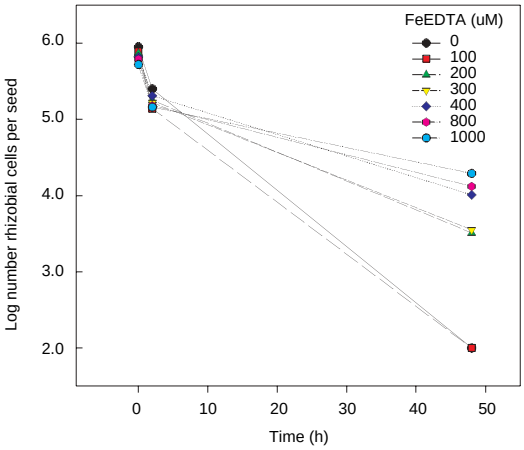

Figure 2. Survival of *B. japonicum* on seed when 100 to 100 µM FeEDTA is added to YMB media.

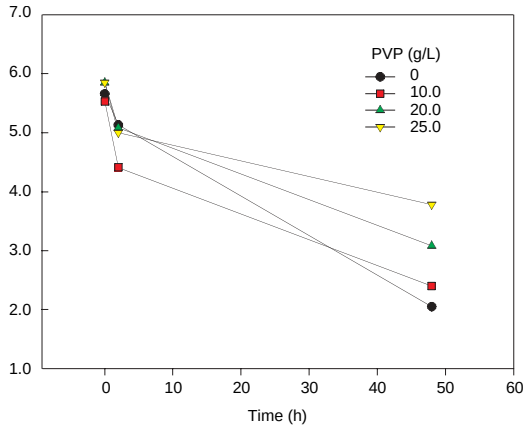

Figure 3. Survival of *B. japonicum* on seed when 0 to 25 g/L PVP K30 is added to YMB media.

Similarly, increasing amounts of PVP K30 in the media increased survival on the seed by 100-fold 48 hours after inoculation. In a parallel experiment we showed water extracts of soybean seed inhibited growth of *B. japonicum* (TAL 102) on agar plates unless the inoculant contained 20 g/L PVP. In that qualitative assay cells spread onto YMB agar plates layered with a filter sterilised water extract from soybean seed failed to grow unless PVP was incorporated (see Materials and Methods for details of the assay).

Effects of amendments on *B. japonicum* growth in YMB culture media

Amendments that proved to be promising at promoting cell survival on seed were evaluated for their

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effects on cell growth in broth. Figure 4 shows the effect that some additives to YMB broth have on growth of *B. japonicum*. Only 8.0 mL/L glycerol had a large negative effect, slowing growth and reducing the final cell number compared to YMB medium.

Survival differences between strains

Several *B. japonicum* were grown in a fourth generation formulation (G4 media — see Table 2 for composition) and applied to soybean seed. Surviving cells measured at inoculation (where the inoculant was removed from seed within 1 min of application) and at 4 h and 48 h later. At 48 h from inoculation, the number of viable cells per seed for the liquid formulation ranged from log = 2.4 to log = 5.0 (Figure 5). The peat inoculant (TAL 102 in gamma irradiated Canadian peat) supported survival better than any strain in the liquid formulation.

Table 2. Formulation of liquid inoculant prototypes tested (g/L unless otherwise stated).

	YMB*	G1	G2	G4	G5	G6+ pvp
Mannitol	1.0	10.0	1.0	1.0	1.0	
K_2HPO_4	0.5	0.5	0.5	0.5	0.5	0.8
$MgSO_4?7H_2O$	0.2	0.2	0.2	0.2	0.2	0.5
NaCl	0.1	0.1	0.1	0.1	0.1	0.1
Yeast extract	0.5	1.0	1.0	1.0	1.0	2.0
Glucose		1.0	1.0	1.0	1.0	
Arabinose			1.0	0.5	0.5	
PVP-40**		20.0	20.0	20.0	20.0	20.0
Trehalose		10 mM	2 mM			
FeEDTA		200 µM	200 µM	400 µM	200 µM	
$CaCl_2$						0.14
$FeCl_3$						8.4 mg***
NH_4Cl						0.6
NaOH						0.16****
Glycerol		1.0 mL	1.0 mL	4.0 mL	4.0 mL	12.0 mL

* Controls in this work used YMB media modified from Vincent, 1970 by incorporating 1 g/L mannitol rather than 10 g/L. ** PVP av. mol. wt. = 40,000, K value (viscosity) = 26–35. Add the pvp last and shake or agitate media vigorously to dissolve. PVP can cause foaming when forced air is used to aerate culture. ***Add 8.4 mL of a stock solution containing 1.0 g $FeCl_3$/L. **** Add 4.0 mL of a 1.0 M NaOH stock solution (40 g NaOH/L). The final pH will be approximately 6.8.

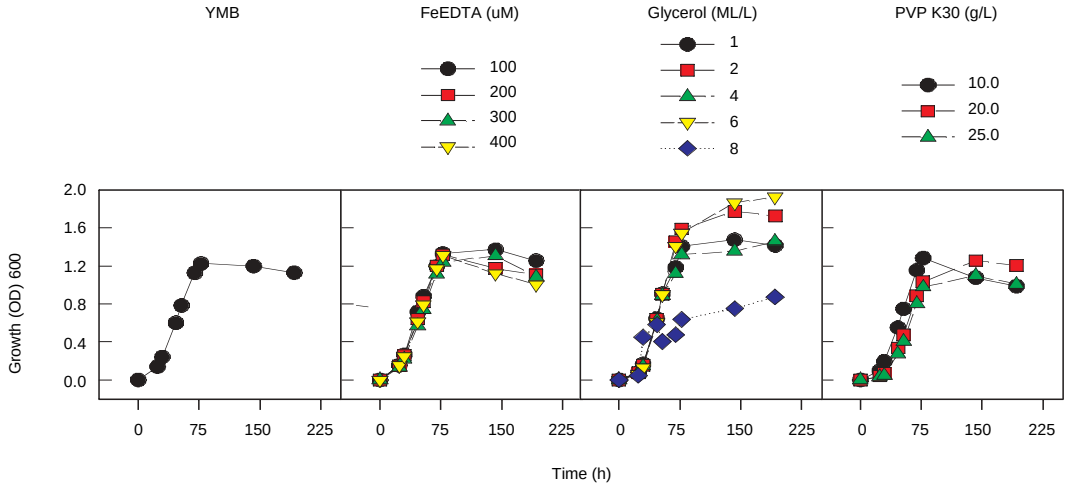

Figure 4. Growth rate of *B. japonicum* in YMB amended with different levels of protective compounds

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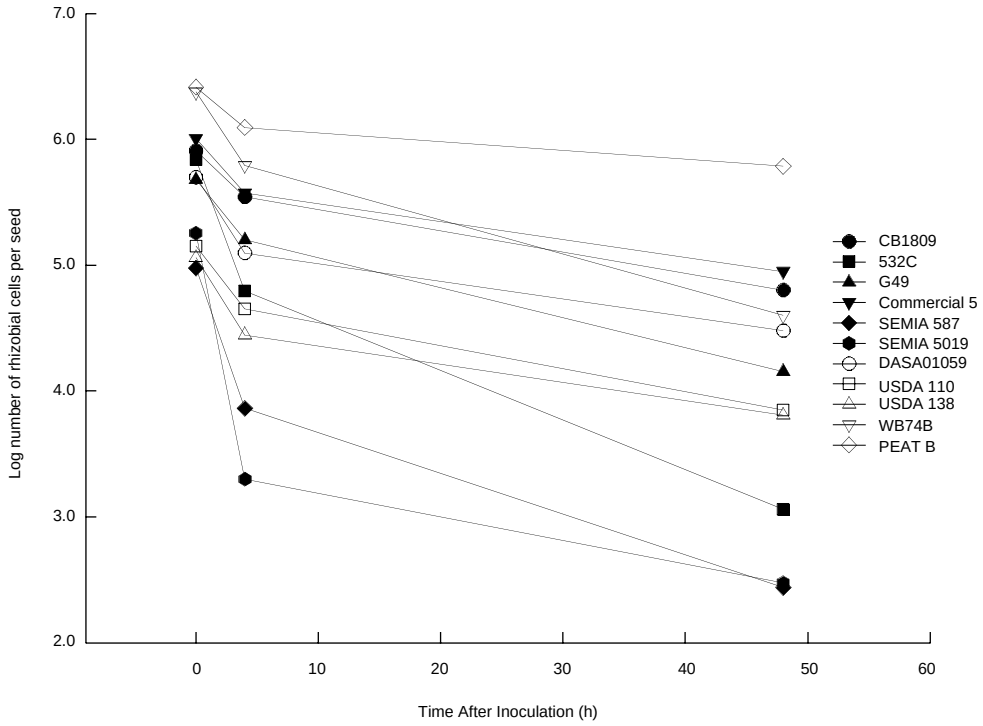

Figure 5. Effect of *Bradyrhizobium japonicum* strain on survival on seeds. All strains were tested in NifTAL's G4 inoculant, with the exceptions of peat and Commercial 5 product.

Interactions

Two factors determined the number of viable cells remaining on seed 48 h after inoculation: 1) differences in cell numbers in the inoculant, and 2) the rate of cell death. The most important factor is the rate of cell death. Original populations in the liquid inoculant formulations ranged from 2.8×10^8 to 2.4×10^9/mL. Cell number in the inoculant explained only 57% of the variance between strains for the number of cells surviving at initial inoculation (t=0). By 4 h and 48 h after inoculation the number of cells in the inoculant explained only 15% and 14% of the differences in viable cells remaining on the seed.

The rate with which viable cells declined differed between strains and formulation. The rate of cell death in Australian peat (with gum arabic as a sticker) was less than in the liquid formulations. Despite the same number of cells on the seed as peat at T=0, the Commercial 5 liquid formulation exhibited very rapid cell death. Some strains such as 532C, SEMIA 587 and SEMIA 5019 in the G4 media also survived very poorly compared to other strains.

When the same set of strains was tested in G5 media results were very different (data not shown). Strains that performed poorly in G4 media (e.g. SEMIA 5019, SEMIA 587) survived better than the others when grown in G5 media. It appears there is a large interaction between strain and media components in determining cell numbers in the inoculant and their degree of survival after inoculation onto seed.

Selecting strains for survival on seed may be a very productive way of increasing performance of liquid inoculant. Research to improve survival characteristics of liquid inoculants must, however, tailor the formulation to the individual strain.

Physiological pre-conditioning of cells to tolerate stress

Several research papers have shown that when bacteria are either starved for carbon or exposed to oxidative stress (H_2O_2 for example) they become more tolerant to many harmful environmental conditions (low pH, desiccation, salinity, temperature etc.). There is evidence that cells exposed to stress generate signal molecules that induce stress response genes in other cells. It may be possible, therefore, to produce inoculant with cells that are pre-conditioned

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to better tolerate the stresses they encounter after application to seed.

We tested whether inoculant age influenced cell survival after seed coating. It was hypothesised that with age, cells may run out of carbon or other metabolites that will pre-condition them to tolerate environmental stress.

The G2 media enhanced survival in the culture compared to the YMB media (data not shown) over a 40 day period. The G2 media also promoted even greater relative survival of cells than YMB after their application to seed. Aging the culture, however, did not enhance survival of cells after application to seed. The regression of cell survival in the G2 media after inoculation on culture age is essentially horizontal.

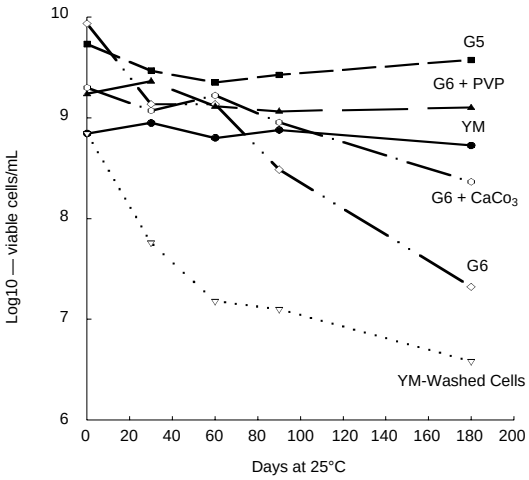
Our attempts to pre-condition cells of *B. japonicum* with H_2O_2 met with mixed success. There was a fine line between applying enough oxidative stress to elicit cell survival mechanisms and killing the cells.

Shelf life of liquid inoculant formulations

The quality of a legume inoculant at the farm is determined by how many viable cells are in the inoculant and how well they survive after application to the seed. The most consistent feedback we receive from inoculant producers is concern about the shelf life of liquid inoculants. It is a common perception that rhizobia and bradyrhizobia do not survive well in liquid media especially when stored without refrigeration. To address this concern we tested the long-term survival of several liquid media stored at 25°C for 180 days. In addition, we evaluated whether long storage periods affected the ability of cells to survive after application to seed.

We evaluated the shelf life of a) G5 medium, b) a glycerol based medium, G6 (see Table 2 for formulations), c) G6 medium diluted with solutions of eight individual and combinations of additives that seem to promote cell survival after application to seed, d) a commercial liquid product, and e) a control consisting of washed cells suspended in water.

After 180 days of storage the number of viable cells remained nearly constant for the G6 medium with 20 g polyvinylpyrrolidone (pvp)/L, yeast mannitol medium (YM) and the G5 medium (Figure 6). To simplify data presentation, Figures 6 and 7 only show results from a few treatments. When glycerol, FeEDTA or gylcerol+FeEDTA+pvp were added to the G6 medium survival was poor (data not shown) and cell numbers declined to levels approaching the washed cells suspended in de-ionised water. The pH of the G6, G6 + pvp and the G5 media after 180 days in storage were 4.46, 3.77, and 6.66 respectively. The final pH of these stored media was similar to the pH at the end of cell growth in the fermentor.



G6 media was diluted with additives at a ratio of 1.5 (media additive solution) before being placed into storage.

Figure 6. Survival of B. japonicum SEMIA 5019 in liquid media stored at 25°C.

Research has shown that carbon or nitrogen starvation at the beginning of the stationary growth phase creates stress resistance in rhizobia cells. It may be that when glycerol is added in the stationary growth phase as we did, the stress resistance was not generated. The G6 and the G5 medium survived better than the commercial liquid product tested even though the original number of cells in the G6 medium was less. Without pvp, the G6 medium performed poorly. The population declined to less than 1.0% of the original population.

Both the G5 and G6 + pvp liquid media enhanced survival of cells after inoculation onto seed (Figure 7). There was 10 times the number of cells surviving on the seed compared to YM medium and 100 times the number compared to G6 without additives or G6 with additives other than pvp (not all data shown). The better performance of the YM medium compared to G6 with no additives is surprising since the main difference between the two media is the carbon source. Several papers have shown that the production of exo-polysaccharides protects cells from desiccation. It is well known that significant polysaccharide production occurs in YM medium. Our observations suggest little polysaccharide production takes place in glycerol based media. It may be that the performance differences between the G6 and G5 media are related to differences in polysaccharide production. This could be a fruitful area of investigation if the effect of pvp and polysaccharides on cell survival is synergistic.

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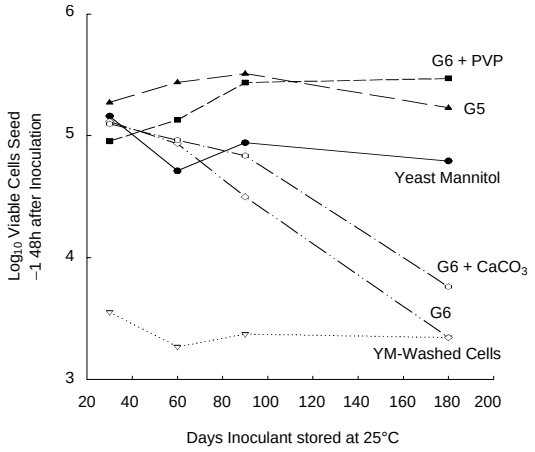

Figure 7. Number of viable cells of B. japonicum SEMIA 5019 on seed 48 h after inoculation.

Fermentor conditions and cell growth of glycerol based media

Comments from many recipients of our first report indicated interest in a medium that could produce high numbers of cells but was less expensive to formulate than the G5 medium. The main objections to the G5 medium revolved around the need for arabinose and mannitol. We evaluated several media for *B. japonicum* and found that formulas relying on glycerol as a carbon source met these requirements.

Glycerol based media are not new. Lorda and Balatti (1996) describe the growth characteristics of *B. japonicum* in glycerol based liquid media under various environmental conditions. They showed more rapid growth in a 'balanced' medium that used 10 mL of glycerol as a substitute for mannitol. Populations in the glycerol sometimes reached densities in excess of 1×10^{10} cells/mL. We conducted several experiments with a glycerol-based medium to evaluate cell production with different strains and under different conditions. Results that follow are based on 1.5 L batch cultures grown in 2.0 L glass fermentors that are aerated with filtered sterilised air delivered to the fermentors by a oil-less air pump.

Growth of B. japonicum in G6, G5 and YM media

Data in Figure 8 compares the growth of *B. japonicum* strain SEMIA 5019 in several media. Media temperatures ranged between 22°C and 24°C and aeration was approximately 2.0 L air/L media/min. Cell production was higher in the G5 and G6 media (both contain glycerol) compared to the YM medium. Adding $CaCO_3$ to the G6 medium to buffer the pH had an adverse effect on growth. In several other tests cell populations in the G6 medium reached 3×10^{10} cells/mL. From our experience, however, the populations reached in Figure 9 are more the norm in media containing glycerol.

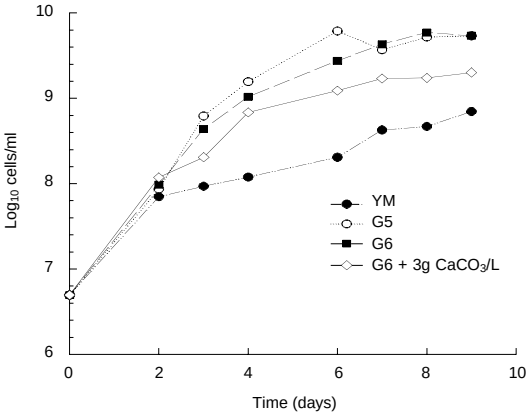

Figure 8. Growth of Semia 5019 in different liquid media.

Growth of inoculant strains in G6 medium

We evaluated several inoculant quality strains for their ability to grow in the G6 medium (Figure 9). All *B. japonicum* strains tested grew well in these medium reaching populations greater than 10^9 cells/mL five days after inoculation. The SEMIA 5019 and SEMIA 587 strains reached slightly higher populations than the other *B. japonicum* strains. There was a rapid decline in pH as the cultures approached the stationary phase of growth. The pH of all cultures but SEMIA 587 declined to 4.0 and the rate of pH decline in SEMIA 5019 culture was slightly less than the non-SEMIA strains. Increasing the ratio of NO_3 to NH_4 in the medium or buffering it with K_2HPO_4 will stabilise pH at a higher level. The pH if the medium had little effect on growth and shelf life of in the liquid inoculant formulations. We measured the pH of the formulations used in the shelf life study and found the G6 medium remained at low pH during the entire trial.

We evaluated whether buffering the culture against pH decline and adding additional nutrients would increase cell production (data not shown). There was no effect of buffering the medium against pH decline nor adding additional yeast extract or carbon source on cell production. The highest cell production (1×10^{10} cells/mL) was recorded in the G6 medium and G6 plus 6 mL glycerol/L medium. The final pH of these treatments was 3.3 compared to a pH of 7.0 or above in cultures buffered with K_2HPO_4. In comparison, the buffered cultures

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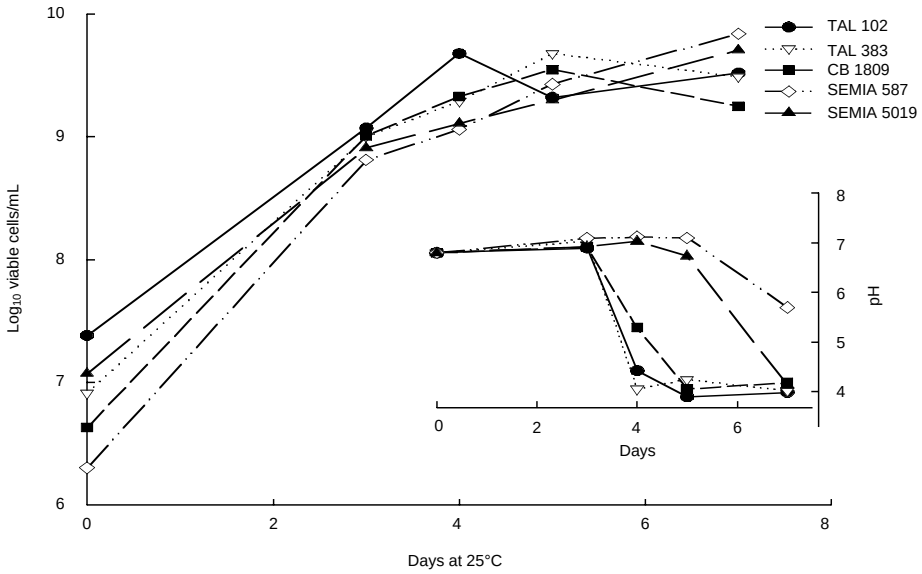


Figure 9. Growth of Inoculant Quality Strains of *B. japonicum* in G6 Medium.

reached cell pop of 5–6 × 10^9 cells/mL, a half log lower than the non-buffered cultures.

It appears pH has little effect on several strains of *B. japonicum* growth and survival in the liquid media we tested.

Air flow rate

We grew SEMIA 5019 in the G5 media (contains 20 g/L pvp) under six air-flow rates ranging from 0.050 to 1.000 L air/L media/min. Cultures were grown at temperatures between 29.7˚C and 30.7˚C. The small differences in broth temperature were negatively correlated with air-flow rate (r = 0.93).

There was a significant (r = 0.91) positive linear correlation between the flow rate and cell number reached after eight days of growth (Figure 10). Cell numbers in the 1.0 L air/L media/min treatment reached 1.04 × 10^{10} cells/mL compared to only 1.97 × 10^9 cells/mL with 0.050 L air/L media/min. Cultures growing with the three highest air-flow rates reached a stationary growth phase after three days. Cell numbers continued to increase in the lowest air flow rate (0.05 L air/L broth/min until the experiment ended at nine days from inoculation. We cannot determine from this test whether this extremely low flow rate would have reached numbers equivalent to the other treatments with additional time.

It appears that there is only a small response to greater aeration and mixing in the fermentor when air flow rates are above 0.25 L air/L media/min. The effect of flow rates below 0.25 L air/L media/min was attenuated with time. Regardless of flow rate, however, all the cultures eventually produced copious amounts of foam that filled the fermentor head space as cell numbers exceeded 10^9/mL.

Cell growth in fermentors with forced air and in flasks with rotary shaking

Many small and medium sized inoculant production facilities rely on shake cultures for mass culturing cells. The method requires less equipment investment and is more reliable We compared growth rates in cultures grown in G5 and G6 media with forced air (1.0 L air/L media/min) in 2.0 L fermentors and in 250 mL flasks placed on a rotary shaker (200 r/min; 2.54 cm orbit). Figure 11 shows that slightly higher cell numbers were attained in the flasks agitated on a rotary shaker than when aerated with forced air.

Response to inoculation with the liquid inoculant in multi-location field trials

Comparing G5 to local inoculant products

Our goal was to determine whether the G5 liquid formulation had field performance characteristics equivalent or better than local products being sold to farmers around the world. In a second set of trials we compared the G5 and G6+PVP formulations to local products and to sterilised Canadian peat based inoculant. Collaborators from sixteen countries including

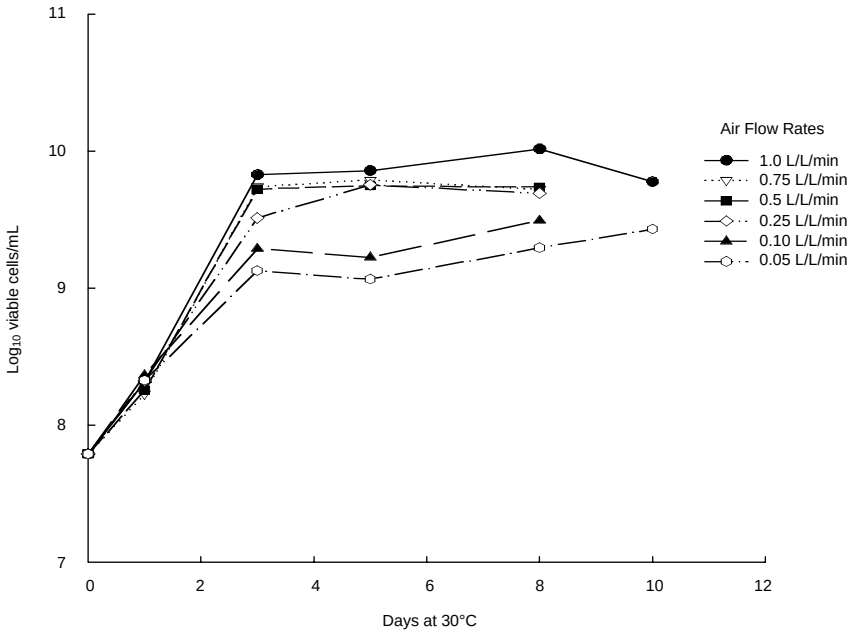
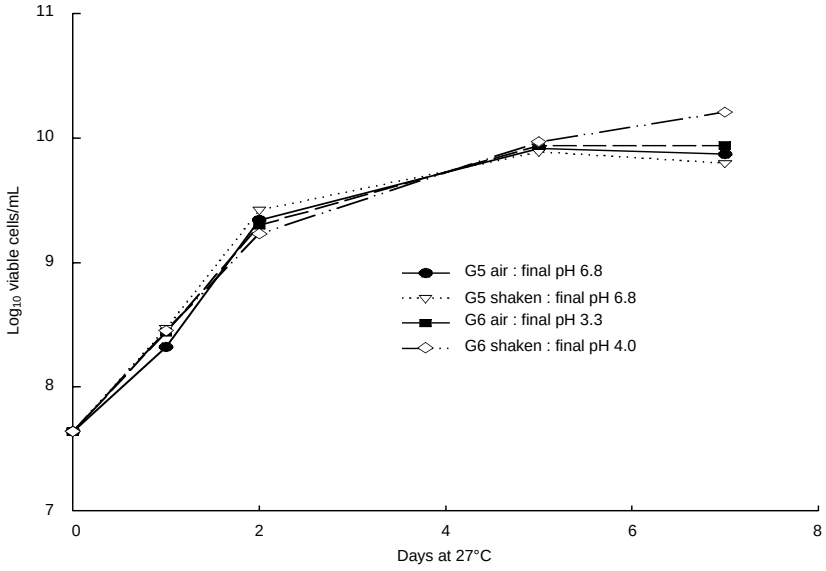


Figure 10. Growth of SEMIA 5019 in G5 Medium with different air flow rates.



Air = 1.0L air/L culture/m in continuously provided by a pump.
Shaken = culture continuously shaken at 200 RPM in an incubator-shaker with a gyrotory, 1 inch circular orbit.

Figure 11. Growth of *B. japonicum* strain SEMIA 5019 in aerated and in shaken media.

Vietnam (Drs Hiep, Toan and Yen Thao) agreed to conduct trials.

Table 3 shows the frequency distribution and relative response to inoculation by G5 compared to the uninoculated control and local inoculant products. The number of observations for the four performance indicators varies due to data collection problems at some sites. There are no field data included from trials where our tests indicted there were no bradyrhizobia in the local inoculant.

The G5 liquid inoculant increased crop seed yield above local products more than 68% of the time producing an average seed yield increase of 6%. Nodule number increased an average of 20% in 77% of the trials when G5 inoculant was compared to local inoculants. Increased nodule number indicates the G5 inoculant probably supported more viable cells between seed inoculation and the time roots emerged and colonisation and infection by the inoculant began. That nodule number response to inoculation is relatively higher than measures of seed yield is not surprising. Many factors other than survival of cells in the inoculant and consequent root infection by the inoculant can attenuate the yield response.

Field evaluation of G5 and G6+PVP formulations with local inoculant products and sterilised peat based inoculant

As a result of comments from producers desiring liquid formulations without mannitol and arabinose we developed and tested the survival characteristics of a glycerol based medium with PVP (G6+PVP). Field tests of G6+PVP along with an earlier formulation that relies on mannitol and arabinose as primary carbon sources (G5) which was extensively tested in earlier field trials form the basis of these trails.

Tables 4–5 present a summary of 29 comparisons (the data set for these trials is not yet complete) of two liquid formulations (G5 and G6) to an un-inoculated control and inoculated treatment that used a sterile peat-based carrier provided to each collaborator. The peat carrier is a commercial product used in the U.S. and Canada and is manufactured by neutralising finely ground (100% to pass 300 mesh) and then sterilising it through electron beam-irradiation. This material and process is regarded as a carrier with high field performance.

Table 4. Mean seed yield and nodulation of liquid formulations (G5 and G6) and a sterile peat-based formulation

Response indicator	Uninoculated control	Formulation		
		G5	G6+PVP	Peat
Seed Yield (kg/ha)	1318	2078	2050	1933
Nodule wt (kg/ha)	20.3	80.7	87.3	82.8
Nodule no. (millions/ha)	2.5	7.9	8.4	7.2

N=29 sites X strain combinations except for Peat treatment N= 28

The liquid and peat based product used the same strains. All collaborators used SEMIA 5019; some also evaluated performance of other well-known inoculant strains. The frequency of response to the two liquid products compared to the control and the peat carrier was the same regardless of strain used so this feature is not distinguished in the data details presented. Data for tissue N-analysis is also not complete so it has not been included in the presentation of results.

The relative frequency of response to inoculation for all measured indicators was at or near 1.0 for both liquid formulations when compared to the un-inoculated controls (Table 5). Seventy nine

Table 3. Field performance summary of G5 inoculant formulation compared to uninoculated control and local inoculant products

	Response of G5 inoculant above:			
	Control		Local inoculant	
Response indicator (n)	Relative frequency	Percent increase	Relative frequency	Percent increase
		%		
Seed Yield[1] (n=37)	97	90	68	6
Total Seed N (26)	96	112	50	3
Nodule no. (n=39)	97	>1000	77	20
Nodule wt. (n=42)	97	>1000	71	14

[1] Tabulated results do not include results of sites 1a – 1d since flawed genetic compatibility between SEMIA 5019 (USDA 74 sero-group) in the G5 formula and the local cultivar generated rhizobiatoxins causing chlorosis in early growth.

Table 5. Relative frequency of increase in seed yield and nodulation from inoculation with NifTAL's liquid formulations (G5 and G6) compared to the control and sterile peat-based formulation.

Response indicator	Relative frequency of G5 response above:		Relative frequency of G6+PVP response above:	
	Control	Peat	Control	Peat
Seed Yield (kg/ha)	1.0	.61	.97	.61
Nodule wt (kg/ha)	1.00	.32	.97	.46
Nodule no. (millions/ha)	1.00	.46	.97	.43

N=29 sites X strain combinations except comparison to peat N=28

percent of the comparisons between G5 and control (n=29) and G6 and the control (n=29) were statistically significant at $P \leq 0.05$. Average liquid inoculation responses were greater than 700 kg seed/ha, 60 kg nodule tissue/ha and 5 million nodules/ha compared to the control. This result is consistent with 47 earlier trials with G5.

When liquid inoculation was compared to the peat carrier seed yield increased 61% of the time (average increase greater than 100 kg/ha) but the frequency of a nodulation response indicators (number/ha and weight/ha) were less than 0.5 for both G5 and G6. The average increase in nodulation indicators for both liquids was slightly greater than the peat except for G5 nodule weight.

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Inoculation Responses of Soybean and Liquid Inoculants as an Alternative to Peat-Based Inoculants

Tran Yen Thao[1], Paul W. Singleton[2] and David Herridge[3]

Abstract

Field experiments were conducted in acrisol, ferrasol and fluvisol soils in the Easthern region and Mekong delta of the south of Vietnam to evaluate nodulation and yield of 'promiscuous' soybean lines from Africa in the presence and absence of inoculation, and the efficacy of liquid formulations as an alternative to peat-based inoculants. Both the 'promiscuous' lines and the liquid inoculants represented technologies that might have relevance for the farmer of Vietnam. Notwithstanding individual treatment effects, responses to rhizobial inoculation were observed in each of the six experiments of this study. Responses on the acrisol and alluvial soils were large, up to 40-fold increases in nodulation and 87% and 51% increases in shoot DM and grain yield, respectively. All four sites were new to soybean. Inoculation responses were even recorded at the ferrasol sites, both of which were on old soybean land. In the two experiments for which we had N fixation data, inoculation increased total N fixed by as much as 400%. Although successful in Africa, the 'promiscuous' lines did not nodulate well in the absence of inoculation and showed substantial increases in nodulation and yield when inoculated. Responses were similar to the responses of the local cultivars. The second technology related to media formulation used to produce the inoculant rhizobia and to deliver them to the legume seed. The different growth formulations (G5, G6 and YEM) were similarly effective as were the two methods of delivery, peat and liquid. Future utilisation of liquid inoculants in Vietnam will depend to a large extent on demonstrated benefits in the manufacturing process and in inoculant distribution. Farmer acceptance will most likely be the ultimate determinant of their future.

TECHNOLOGY that has been developed in the technically-advanced countries may not be immediately appropriate to the smallholder farmers in the less-developed countries. Rhizobial inoculation of legumes is one such technology. It is not the price of the product that is the problem; rather the problems relate to quality and availability in the market place.

In Vietnam, the current production and distribution of inoculants may only be sufficient for about 1000 ha, far less than the quantity required to inoculate the 700,000 ha of legumes currently grown each year in the country. Vietnamese farmers fertilise their legume crops with N instead of inoculating, a practice that adds US$25–30 million annually to their cost of production and to Vietnam's import account. Inoculation, on the other hand, would cost the farmers only about US$1 million annually and they would be supporting a local industry.

Responses to inoculation in research experiments in the country show clearly that inoculation is justified, at least in soil that has never grown the legume before or has not grown the legume for many years (Tran Phuoc Duong et al. 1984; Cao Ngoc Diep et al. 1996; Pham Thi Phuong Lan et al. 1999; Tran Yen Thao 1997, 2001). It is also beneficial in soils that are marginal for survival of the rhizobia, e.g. very acid soils. What is needed is research to develop inoculant-production technology that is relevant to the needs to of the country an to extend the message to farmers that inoculating rather than fertilising legumes with N should lead to greater profitability. In addition to enhancing the country's inoculant technology, other strategies should be explored that might reduce legume cropping dependence on either fertiliser N or inoculants.

[1] Oil Plant Institute of Vietnam, 171–175 Ham Nghi St., District 1, Ho Chi Minh City, VN
[2] Department of Tropical Plant and Soil Science, University of Hawaii at Manoa, Paia, HI 96779 USA
[3] NSW Agriculture, TCCI, Tamworth, NSW 2340, Australia

Inoculants and Nitrogen Fixation of Legumes in Vietnam
edited by D. Herridge
ACIAR Proceedings 109e
(printed version published in 2002)

In this paper, we present results from research that has started to examine both of these options. We compared 'promiscuous' nodulating soybean (*Glycine max*) from the International Institute for Tropical Agricture (IITA) with local cultivars in a single field experiment Tay Ninh province in the south of the country. We evaluated all cultivars for ability to nodulate without inoculation and nodulation and yield responses to inoculation. In addition, in a series of experiments on three major soil types in the south, we examined both liquid and traditional peat-based inoculants of *Bradyrhizobium japonicum* produced using different culture medium formulations. The liquid inoculants may have particular relevance to Vietnam because of their relative ease of production and distribution.

Materials and Methods

Evaluation of 'promiscuous' cultivars of soybean

Field site

The experiment was conducted at Trang Bang, Tay Ninh Province, in the Eastern Region of the south (ERS) of Vietnam on an acrisol. The most common cropping system there is groundnut–groundnut–rice. The land has never before grown soybean.

Experimental treatments, cultural practices and sowing

Treatments were 7 cultivars as main plots and +/– inoculation as subplots. The cultivars were HL2 and MTD–176, 2 commercial soybean cultivars in Vietnam, Nam Vang, a local cultivar, and 4 soybean lines from IITA, Nigeria, supplied by Dr Andrew James (CSIRO, Australia). They were considered as 'promiscuous' soybean that nodulate abundantly and effectively with naturalised rhizobia in most soils of Nigeria. Days to maturity were as follows: 80–85 days (HL2), 85–90 days for MTD–176 and Nam Vang, 90–100 days for TGX1437–1D and TGX1447-3D, and 100–110 days for TGX1440 and TGX1448–2E.

The 14 treatments were arranged in randomised complete blocks, replicated 4 times, with the main factor (7 cultivars), split for inoculation treatment (2). Each plot measured 9 m × 5 rows wide. Rows were spaced 40 cm apart.

Phosphate (60 kg P_2O_5/ha), potash (90 kg K_2O_5/ha), and lime (500 kg/ha) were incorporated into the soil to 10–15 cm depth on the day before sowing. Fertiliser N was not applied to any of the plots. The soybean seed was sown in April 2001. Three seeds were placed in holes 30 cm apart along the rows. Seeds in the plus inoculation treatments were inoculated, using a liquid formulation at a rate of 10 mL/kg seed, with *B. japonicum* strain SEMIA 5019, supplied by the University of Hawaii NifTAL Project. The plants were thinned to two/planting hole after 10 days of growth. During the experiment period, plots were kept weed-free, and pesticides were applied when necessary

Sampling

Plants were assessed for nodulation 55 days after sowing (DAS) in the case of MTD–176, HL2, Nam Vang, TGX1437–1D and TGX1447–3D, and at 75 DAS for TGX1440 and TGX1448–2E. Nodules were counted, oven dried at 65°C, weighed.

Shoot samples were collected 10 days before final grain harvest. Plants were cut at the soil surface, oven dried at 65°C, weighed, ground and send to CSIRO Plant Industry, Canberra, Australia, for ^{15}N analysis. At crop maturity, the grain was harvested, oven dried at 65°C, weighed, analysed for total N by Kjeldahl analysis.

Statistical analysis

All measured and calculated variables were subjected to analysis of variance using MSTATC. Comparisons between treatment means were made by the Duncan Multiple Range Test.

Evaluation of inoculant formulations

Field sites

The experiments were conducted in the ERS of Vietnam, one in Cu Chi, Ho Chi Minh City, on an acrisol (Phuoc Thanh site) and two in Dong Nai province (Vinh Cuu and Tan Phu sites) on ferrasols. The fourth site was in the Mekong Delta on an fluvisol soil (Vinh Long site). At Phuoc Thanh and Vinh Long, the land was new to soybean. At both sites, the soybean experiment followed rice. At Vinh Cuu, soybean was grown every year after rice. At Tan Phu, soybean had been intercropped with maize in the past; in the year before the experiment, only maize was grown by the farmer.

An experiment to examine rates of liquid inoculation was conducted at Tan An Hoi (Cu Chi) on an acrisol. Soybean has never been grown there before. Unfortunately, plants were damaged by the bad weather at pod filling. Only nodule data were collected.

Other experiments, damaged by bad weather to the point that data could not be collected, were on ferrasols at Hung Loc (>5 years without soybean) and at Dinh Quan (farmers routinely intercrop soybean and maize).

Bradyrhizobium culture and inoculant preparation

The rhizobial strain used was *B. japonicum* SEMIA 5019, supplied by the University of Hawaii, NifTAL Project. The culture was maintained on yeast-extract mannitol agar (YEM) and stored at 4°C. The inoculant cultures were prepared in yeast mannitol broth or liquid inoculant formulations, G5 and G6. The G5 formulation contained per litre: 1 g mannitol, 0.5 g K_2HPO_4, 0.2 g $MgSO_4 .7H_2O$, 0.1 g NaCl, 1 g yeast extract, 1 g glucose, 0.5 g arabinose, 20 g PVP K30, 0.073 g FeEDTA, and 4.0 ml glycerol. G6 consisted of 0.8 g K_2HPO_4, 0.49 g $MgSO_4$, 0.1 g NaCl, 0.14 g $CaCl_2$, 8.4 mL/l of a $FeCl_3$ solution (1g/L), 12 mL glycerol, 0.6 g NH_4Cl, 2 g yeast extract, 4 mL of 1.0 M NaOH solution, and 20 g PVP. When the broths in the 3 different media were sufficiently grown (4–5 days), they were injected aseptically into autoclaved, sterile polythene bags of peat (peat-based inoculants) or into sterile polythene bags (liquid inoculants). For peat inoculants made using G5 or G6 media, PVP was not included.

Two other inoculants, one liquid and one peat-based, were also used. They were from from the QUIMICA Company, Argentina. The peat inoculant was a commercial product but the liquid one was for experimental purposes only. All inoculants were applied direct onto the seed at a rate of 10 mL/kg seed for liquid inoculants and 10 g/kg seed for peat-based inoculants.

Experimental treatments, cultural practices and sowing

In all experiments, a randomised complete block design with 4 replications was used. The soybean cultivar was MTD-176, supplied by University of Can Tho (growth duration 85–90 days). Inoculated treatments consisted of liquid formulations G5 and G6, peat inoculants based on YEM and peat inoculants made from G5 and G6 media without PVP. There were two control treatments, uninoculated without N and uninoculated with 40 or 60 kg N/ha.

In the experiment at the Tan An Hoi site, the treatments were different rates of liquid inoculant – 5, 10, 15, 20 and 40 mL/kg seed, the 2 uninoculated controls and commercially-prepared peat inoculant and experimentally prepared liquid inoculant from Argentina.

Sampling

Nodulation was assessed 55–60 days after sowing, except at the Vinh Cuu site where nodules were sampled 35 days after sowing. Nodules were counted, oven dried at 65°C and weighed. Biomass and grain yields were assessed at 80 and 90 DAS, respectively. Plants were cut at the soil surface, oven dried and weighed, ground and sent to CSIRO Plant Industry, Australia, for ^{15}N analysis. After grain was oven dried to constant weight, samples were sent to the University of Hawaii NifTAL for total N analysis.

Statistical analysis

All measured and calculated variables were subjected to analysis of variance using MSTAT. Comparisons between treatment means were made by the Duncan Multiple Range Test.

Economic analysis

Economic analysis of inoculation/uninoculation (with and without N fertiliser application) treatments at 4 sites was done. The calculation was based on output and input and data of inoculation treatment was on average of 5 inoculant formulations. The total input was consisted of fertiliser input including P, K, Mg, microfertilisers, lime for all treatments, urea for uninoculation plus N fertiliser treatment, inoculant cost for inoculation treatments (50,000 VND/ha), and other managements such as pesticides, weed control, irrigation for all treatments. Extra labour for harvest due to yield increase of inoculation treatments was also included.

Results and Discussion

Evaluation of 'promiscuous' cultivars of soybean

Nodulation of both local and 'promiscuous' IITA cultivars showed large responses to inoculation (Table 1). Plants were poorly nodulated without inoculation; nodule number was in the range 1–6/plant and nodule weight 16–69 mg/plant. There were no differences between the local cultivars and the 'promiscuous' cultivars. When inoculated, soybean nodule number was increased to 8–54/plant and nodule weight to 91–946 mg/plant. With such a large range, it is not surprising that the interaction of cultivar and inoculation was highly significant ($P<0.01$). Cultivars that performed best were the 'promiscuous' TGX1440 and TGX1448-2E. Next best were local cultivars MTD-176 and Nam Vang. The least responsive of the cultivars was 'promiscuous' line TGX1447-1D.

Inoculation effects on shoot and grain yields of the 7 cultivars are shown in Table 2. Shoot DM yields were 2.4–5.1 t/ha without inoculation, increasing to 2.6–7.3 t/ha with inoculation. Grain yields followed similar trends, i.e. 0.45–1.38 without inoculation and 0.48–1.81 with inoculation. Grain yields were particularly low for the non-adapted IITA lines, averaging just 0.64 t/ha (uninoculated)

and 0.77 t/ha (inoculated). By comparison, yields of the local cultivars were 1.11 t/ha (uninoculated) and 1.38 t/ha (inoculated). The most responsive cultivars (i.e. 29–43% for shoot DM and 25–37% for grain yield) were the 'promiscuous' TGX1440 and TGX1448-2E and local cultivar MTD-176. The 'promiscuous' TGX1447-1D was again the least responsive; increases due to inoculation were just 9% for shoot DM and 7% for grain yield

There was a moderately high and significant ($P<0.01$) correlation between nodulation and shoot DM, suggesting that nodulation played a key role in plant growth. On the other hand, variation in nodulation amongst the cultivars may reflect their different capacities for growth under the environmental and edaphic conditions of this experiment.

The TGX lines express high levels of nodulation without inoculation in West Africa and in other parts of Africa. The fact that none of the four lines in this experiment nodulated well in the absence of inoculation suggests that compatible, effective cowpea-type rhizobia, present in the soils of Africa, are absent from this part of Vietnam. In the field in which the experiment was conducted, there would have been abundant cowpea rhizobia in the soil because the farmer grows well-nodulated groundnut every year without inoculation. However, MNP counts using soybean or related species were not done to determine the number of rhizobia infective and effective for soybean. Such counts would most likely have confirmed an absence of compatible soybean rhizobia. 'Promiscuous' cultivars still represent a highly attractive and appropriate technology for the small-holder farmers of Vietnam, as they do in Africa. However, it seems that such cultivars, adapted to the soils of Vietnam, will need to be identified from other germplasm sources, if indeed they exist.

Table 1. Nodulation of local and 'promiscuous' cultivars of soybean in the field in Tay Ninh province in the south of Vietnam during spring-autumn 2001.

Cultivar	Nodule no/plant		Nodule wt/plant (mg)	
	Uninoculated	Inoculated	Uninoculated	Inoculated
Local cultivars				
MTD-176	2	38	25	671
HL92	2	15	16	249
Nam Vang	2	30	22	503
'Promiscuous' cultivars				
TGX1447-1D	1	8	22	91
TGX1437-3D	1	16	29	118
TGX1440	5	52	59	852
TGX1448-2E	6	54	69	946
LSD (P = 0.05)	5		67	

Table 2. Yields of shoot DM and grain of local and 'promiscuous' cultivars of soybean in the field in Tay Ninh province in the south of Vietnam during spring-autumn 2001.

Cultivar	Shoot DM (t/ha)			Grain yield (t/ha)		
	Uninoculated	Inoculated	% response	Uninoculated	Inoculated	% response
Local						
MTD-176	4.08	5.67	+39	1.38	1.81	+31
HL92	3.32	4.09	+23	0.72	0.93	+29
Nam Vang	4.23	5.05	+19	1.24	1.41	+14
'Promiscuous'						
TGX1447-1D	2.39	2.60	+9	0.45	0.48	+7
TGX1437-3D	3.53	4.21	+19	0.70	0.78	+11
TGX1440	4.55	5.86	+29	0.67	0.92	+37
TGX1448-2E	5.09	7.26	+43	0.72	0.90	+25
LSD (P = 0.05)	0.40			0.10		

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Evaluation of inoculant formulations

Experiments on acrisols

There were large, i.e. about 10-fold, nodulation responses to both peat-based and liquid inoculants as shown in Table 3. Shoot dry matters also responded strongly to inoculation, with increases of 42–68%. Grain yield were improved by 17–34%, The G6 formulation was slightly superior to the G5 formulation, producing 30–50% more nodule mass and numbers and 7–9% more shoot biomass and grain. The liquid and peat-based formulations were essentially identical in their efficacy.

The %Ndfa values were 18% for uninoculated plants and in 35–51% for the inoculated plants (Table 4). Again, the G6-formulated inoculants preformed better than the G5 inoculants and there were no differences between liquid and peat inoculants. The differences in shoot N contents and %Ndfa values between inoculated and uninoculated plots compounded to generate even larger differences in crop N fixed. It was 33 kg N/ha for uninoculated and 110–217 kg N/ha (average of 177 kg N/ha) for the inoculated. In the case of the latter, the net N balances (fixed N – grain N harvested) were very positive, ranging from just 7 kg N/ha for G5-liquid inoculant to 96 kg N/ha for G6-peat inoculant.

Strong responses to inoculation were recorded in an experiment to examine the efficacy of the liquid inoculants used at different rates (Table 5). The results showed that there was no effect of rate in the range 5–40 mL liquid inoculant/kg seed and there were no differences between the local products and the commercial products from Argentina. As stated previously, yield data was not obtained from this experiment because of extensive storm damage. It is likely though, that the very large nodulation responses to inoculation would have meant large responses in DM and grain yields.

Experiment on alluvial soil

Although this land has not been planted soybean before, root nodules were abundant in the uninoculated plots (Table 6). Even so, there were strong responses to inoculation – 3–4 fold increase for nodulation, 55–87% increase for shoot DM and 36–51%

Table 3. Effects of inoculant formulations on nodulation, shoot biomass and grain yield of soybean, grown on an acrisol at Phuoc Thanh (Cu Chi) in the south of Vietnam. The land had never grown soybean.

Treatment	Nodule no./ plant	Nodule wt/ plant (mg)	Shoot DM (t/ha)	Grain yield (t/ha)
1. Uninoculated, 0N	1	25	5.0	1.67
2. Uninoculated, 40N	1	6	6.1	1.81
3. Liquid inoculant G5	12	280	7.1	1.96
4. Liquid inoculant G6	20	401	8.4	2.12
5. Peat inoculant using G5	16	351	7.9	2.00
6. Peat inoculant using G6	21	414	7.5	2.18
7. Peat inoculant using YMB	21	353	8.1	2.24
LSD (P = 0.05)	3	36	0.8	0.20

Table 4. Effects of inoculant formulations on shoot and grain N and N2 fixation of soybean, grown on an acrisol at Phuoc Thanh (Cu Chi) in the south of Vietnam. The land had never grown soybean.

Treatment	Shoot N (kg/ha)	Grain N (kg/ha)	%Ndfa	Crop N fixed[A] (kg/ha)
1. Uninoculated, 0N	124	71	18	33
2. Uninoculated, 40N	126	87	*	*
3. Liquid inoculant G5	209	103	35	110
4. Liquid inoculant G6	283	114	48	204
5. Peat inoculant using G5	276	111	41	170
6. Peat inoculant using G6	284	122	51	217
7. Peat inoculant using YMB	262	110	47	184
LSD (P = 0.05)	54	13	9	60

[A]Crop N = shoot N × 1.5, to account for below-ground N

Table 5. Effects of different rates of liquid inoculants on nodulation of soybean, grown on an acrisol at Tan An Hoi (Cu Chi) in the south of Vietnam. The land had never grown soybean.

Treatment	Nodule no./plant	Nodule wt (mg/plant)
Uninoculated, 0N	3	18
Uninoculated, 40N	1	9
G6 liquid inoculant, 5 ml/kg seed	44	902
G6 liquid inoculant, 10 ml/kg seed	42	864
G6 liquid inoculant, 15 ml/kg seed	41	858
G6 liquid inoculant, 20 ml/kg seed	40	872
G6 liquid inoculant, 40 ml/kg seed	42	967
Experimental liquid inoculant from Argentina, 5 ml/kg seed	48	1003
Commercial peat inoculant from Argentina, 5 g/kg seed	40	870
LSD (P = 0.05)	10	162

Table 6. Effects of inoculant formulations on nodulation, shoot biomass and grain yield of soybean, grown on a fluvisol soil at Vinh Long in the Mekong Delta in the south of Vietnam. The land had never grown soybean.

Treatment	Nodule No./ plant	Nodule wt (mg/plant)	Shoot DM (t/ha)	Grain yield (t/ha)
1. Uninoculated, 0N	31	391	3.22	1.06
2. Uninoculated, 60N	15	250	4.71	1.39
3. Liquid inoculant G5	122	1119	5.53	1.44
4. Liquid inoculant G6	141	1039	4.99	1.49
5. Peat inoculant using G5	118	1047	5.80	1.48
6. Peat inoculant using G6	93	1046	6.03	1.60
7. Peat inoculant using YMB	115	1094	4.95	1.43
LSD (P = 0.05)	28	256	1.23	0.37

increase for grain yield. There were no differences between the G5 and G6 formulations and no differences between liquid and peat-based inoculants.

The %Ndfa values were similar for the uninoculated-ON control (71%) and the five inoculation treatments (average of 74%) (Table 7). The uninoculated-6ON control %Ndfa value was substantially less at 29%. Estimated crop N fixed was 118 kg N/ha for the uninoculated-ON control and 51 kg N/ha for the uninoculated-6ON control. It would seem that the fertiliser N effectively replaced fixed N in the crop, but did little to add to total crop N (estimated at 167 and 176 kg N/ha, for the two control plots). The inoculated plants fixed 192–228 kg N/ha (average of 212 kg N/ha) compared with the 118 kg N/ha fixed by the uninoculated (0N) control.

Experiments on ferrasols

Inoculation effects at the two ferrasol sites were consistent with the other sites, even though soybean had been grown on this land previously (Table 8). Nodulation responses were about 3-fold at tan Phu and only marginal, about 50%, at Vinh Cuu. This was not surprising and would have reflected the more intensive soybean cultivation at Vinh Cuu. Responses in shoot DM were 64–75% (Tan Phu). There was no shoot biomass data for Vinh Cuu. Grain yield responses were 0–16% at Tan Phu and 12% at Vinh Cuu. There were no differences between inoculant formulations or inoculant types.

Economic analysis

There were differences in profitability for the inoculation and unioculation treatments (Table 9). It was 91% more profitable in inoculation treatment than the uninoculation one because of grain yield increase. The benefit that farmers gained from the inoculation was 19% higher when they applied urea. This benefit came from the lower costs of fertiliser input (no urea was used) and grain yield increase.The benefit from the inoculation was 1.454.000 VND per ha.

The economic value of inoculation would be more considerable if we see it in term of benefits for soil N

Table 7. Effects of inoculant formulations on N2 fixation of soybean, grown on a fluvisol soil at Vinh Long in the Mekong Delta in the south of Vietnam. The land had never grown soybean.

Treatment	Shoot N (kg/ha)	%Ndfa	Crop N fixed (kg/ha)[A]
1. Uninoculated, 0N	111	71	118
2. Uninoculated, 60N	117	29	51
3. Liquid inoculant G5	170	75	192
4. Liquid inoculant G6	184	78	215
5. Peat inoculant using G5	204	72	220
6. Peat inoculant using G6	227	67	228
7. Peat inoculant using YMB	175	78	205
LSD (P = 0.05)	68	25	88

[A]Crop N = shoot N × 1.5, to account for below-ground N

Table 8. Effects of inoculant formulations on nodulation, shoot biomass and grain yield of soybean, grown on ferrasols at two sites in Dong Nai province in the south of Vietnam. Soybean had been grown at both sites previously.

Treatment	Tan Phu				Vinh Cuu		
	Nod.no /plant	Nodule wt. (mg/plant)	Shoot DM (t/ha)	Grain yield (t/ha)	Nod.no /plant	Nodule wt. (mg/plant)	Grain yield (t/ha)
1. Uninoculed, 0N	8	91	4.31	1.83	27	184	1.73
2. Uninoculated, 60N	5	63	7.41	1.90	14	135	2.21
3. Liquid inoculant G5	21	245	7.41	2.06	34	262	1.95
4. Liquid inoculant G6	18	214	7.44	2.12	*	*	*
5. Peat inoculant using G5	18	234	7.07	2.12	*	*	*
6. Peat inoculant using G6	20	258	7.22	1.82	*	*	*
7. Peat inoculant using YMB	23	252	7.54	1.96	36	260	1.96
LSD (P = 0.05)	5	53	1.4	0.34	8	27	0.28

Table 9. Economic analysis of effects of inoculation and uninoculation applications on grain yield of soybean (mean values were from 4 sites).

Treatment	Grain yield (t/ha)	Fertiliser input (VND)	Total input (VND)	Output (VND)	Benefit (Gross margin) (VND)	Benefit from N fertiliser or inoculation
1. Uninoculation without urea application	1,57	2,134,000	6,252,000	7,850,000	1,598,000	
2. Inoculation with urea application	1,83	2,396,250	6,592,000	9,150,000	2,558,000	960,000
3. Inoculation	1,89	2,184,000	6,398,000	9,450,000	3,052,000	1,454,000

Price of 1kg soybean grain (March 2001): 5000 VND (1US$ = 14,600 VND)
VND: Vietnamese dong

fertility and resultant increased productivity of subsequent crops as we discussed previously in the part of results and discussions.

Conclusions

Responses to rhizobial inoculation were observed in each of the six experiments of this study. Responses on the acrisol and alluvial soils were large, up to 40-fold increases in nodulation and 87% and 51% increases in shoot DM and grain yield, respectively. All four sites were new to soybean. Inoculation responses were even recorded at the ferrasol sites, both of which were on old soybean land. In the two experiments for which we had N fixation data, inoculation increased total N fixed by as much as 400%.

The purpose of this study was not to examine effects of inoculation per se, rather to examine two different technologies that might have relevance for the farmer of Vietnam.

The first technology was the 'promiscuous' nodulation trait that has been effectively exploited in soybean cultivation in parts of Africa. Thus, we evaluated four lines from the IITA breeding program, together with three local cultivars. Although successful in Africa, the IITA lines did not nodulate well in the absence of inoculation and showed substantial increases in nodulation and yield when inoculated. Responses were similar to the responses of the local cultivars.

The second technology related to media formulation used to produce the inoculant rhizobia and to deliver them to the legume seed. The different growth formulations (G5, G6 and YEM) were similarly effective as were the two methods of delivery, peat and liquid. Future utilisation of liquid inoculants in Vietnam will depend largely on demonstrated benefits in the manufacturing process and in inoculant distribution. Farmer acceptance will most likely be the ultimate determinant of their future.

Acknowledgments

We wish to thank Dr Mark People and his assistants (CSIRO) for ^{15}N analysis; Dr Andrew James (CSIRO) for 'promiscuous' soybean line supply. Dr C. Bonfiglio for inoculant supply through his Quimica Company, Argentina. The NifTAL and ACIAR projects provided funds that enabled the work to be undertaken.

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Selection of Strains of Root Nodule Bacteria to Improve Inoculant Performance and Increase Legume Productivity in Stressful Environments

Graham O'Hara[1], Ron Yates[1] and John Howieson[1]

Abstract

Pulse, pasture and grain legumes are important for maintaining productivity in many agricultural systems. The formation of effective nitrogen-fixing symbioses between legumes and root nodule bacteria is essential for many legumes in agriculture. In many situations, soil inoculation with effective strains of root nodule bacteria is required for maximising legume yields. A key strategy to enhance the performance of inoculants to improve productivity of legumes is the selection of elite strains with improved characteristics such as greater nitrogen fixing ability, ability to survive stressful edaphic conditions, and greater competitive ability. The approach used with success at the Centre for *Rhizobium* Studies to identify, characterise and select strains of root nodule bacteria for use in Australian agriculture includes a four-phase glasshouse and field-based program.

LEGUMES are well recognised for the important roles they have in maintaining productivity in agricultural systems (Giller and Wilson 1991; O'Hara 1998; Graham and Vance 2000). Pulse, pasture and grain legumes are grown because they can form nitrogen-fixing symbioses with soil inhabiting root nodule bacteria (Vance 1997), and because their use in rotation systems can help control pests, disease and weeds (Howieson et al. 2000b). As agriculture continues to develop there are new roles emerging for legumes in the new farming systems such as the continued expansion of pulse crops onto infertile more stressful soils, and the exploration for new genera and species of pasture and forage legumes with deep-rooting habits for control of soil moisture to combat salinity (Howieson et al. 2000b).

The successful use of legumes in these new roles in agriculture will be dependent upon appropriate attention to the formation of effective symbioses with root nodule bacteria. An essential component for increasing the use of legumes is the integration of plant breeding and cultivar development with appropriate research leading to the selection of elite strains of root nodule bacteria. In addition the benefits of this research for farmers will be increased through advances in the application of appropriate inoculation technologies to deliver the new strains of bacteria to the soil with enhanced survival (Smith 1992; Stephens and Rask 2000).

This paper outlines the approach used at the Centre for *Rhizobium* Studies (CRS) to select, from a genepool of natural isolates, improved inoculant quality strains of root nodule bacteria for increasing productivity of pulse, pasture and forage legumes in Mediterranean environments in Australian agriculture. The approaches, techniques and procedures outlined here have been successfully used to select a range of new inoculant strains for both established and new legumes being grown in Australia (Howieson et al. 2000a). The principle theme we address here is that in order to maximise productivity in legume-based agriculture there is an essential requirement for matching of root nodule bacteria to both host legumes and soil conditions. The paper also discusses some of the present and future challenges for strain selection in root nodule bacteria and introduces some novel uses for this technology in agriculture.

Is There a Need for Inoculation?

Before embarking on a lengthy program to select inoculant strains of root nodule bacteria it is essential

[1] Centre for *Rhizobium* Studies, Division of Science and Engineering, Murdoch University, Murdoch, WA, 6150 Australia

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edited by D. Herridge
ACIAR Proceedings 109e
(printed version published in 2002)

that there is a clear understanding of whether there is a need to inoculate. Demonstration of whether there is a need for inoculation is a relatively simple task, but it is sometimes not always undertaken correctly because producers do not have the appropriate background skills to adequately interpret the results (Date 2000). In essence, simple experiments using three treatments (uninoculated, inoculated and fertiliser nitrogen) can be done to answer the question whether there is a need for inoculation (Brockwell and Bottomley 1995; Brockwell et al. 1995, Date 2000).

The root nodule bacteria are a diverse group of ubiquitous soil inhabiting Gram-negative bacteria (O'Hara 2001), and it is very likely that all agricultural soils contain some bacteria capable of nodulating some legumes. However, the particular root nodule bacteria present in any given soil may not be able to nodulate legumes being grown as crops or pastures, or if they can they do not form an effective symbiosis that contributes nitrogen to the host legume and to the farming system. This is quite common for the situation where new legumes are being introduced to new lands, as is currently occuring in many areas of the world.

A key feature of the symbiotic relationship between root nodule bacteria and legumes is the very high degree of specificity shown for effective nodulation of a particular host legume by a strain/species of root nodule bacteria (see below). This specificity operates at both the nodulation and nitrogen fixation levels of the symbiosis, and is a function of the exchange of specific chemical signals between the two partners (Denarie et al. 1992; Perret et al. 2000). The specificity is controlled by the fact that the chemicals can only be produced if the organisms contain the information for the synthesis of the chemicals on their genes. Thus the development and function of the symbiosis between legumes and their associated root nodule bacteria is controlled at a molecular level, and the development of effective nitrogen-fixing symbioses is conditional on both partners containing appropriate sets of genes (Phillips et al. 1997; Schlaman et al. 1998; Perret et al. 2000).

Three general scenarios are possible when a new legume is introduced to new lands (Howieson et al. 2000c). One scenario is that the uninoculated legume may form abundant effective nodules indicating the presence in the soil of a large population of effective root nodule bacteria. In this case a plant breeding program to select a genotype able to nodulate effectively with the indigenous root nodule bacteria would be the simplest approach for successful introduction of the legume. Secondly, there is the less frequently encountered situation where no nodules are formed on uninoculated legumes. This result indicates there is very little or in some cases no background population of root nodule bacteria in the soil that are able to nodulate the particular host legume. More often, uninoculated legumes form variably effective nodules indicating the presence of a population of ineffective or variably effective root nodule bacteria. In the latter two scenarios the clear solution to ensure success with the legume crop or pasture is to select an effective, competitive inoculant strain of root nodule bacteria adapted to the soil conditions.

Selection of Improved Strains of Root Nodule Bacteria

The abundant diversity clearly present in soil populations of root nodule bacteria provides a large resource of natural germplasm to screen for desired characteristics (Sadowsky and Graham 1998; Dilworth et al. 2001). Useful variation in many of the appropriate characteristics required in inoculum strains would seem to exist in this natural pool of soil root nodule bacteria.

What characteristics are needed?

An essential desired characteristic for inoculum strains of root nodule bacteria is highly effective nitrogen-fixation with the intended host species, and in some instances there is a requirement for the strain to effectively nodulate a wide range of host legume species (see below). Other beneficial characteristics include stress tolerance, competitive ability against the indigenous strains, genetic stability and satisfactory growth and survival during procedures for manufacture of inoculum (Howieson et al 2000b).

How can we screen for them in RNB?

The current techniques used at the Centre for *Rhizobium* Studies to select appropriate strains of root nodule bacteria require a combination of glasshouse and field work (Howieson et al. 2000a, b). At present screening and selection of root nodule bacteria in the laboratory is not possible nor worthwhile to attempt. The criteria for successful laboratory screening for the key characteristics, such as acid tolerance, are not known (Dilworth et al. 2001), and we still need to necessarily include the host plant and soil environment in any screening program.

The strain selection program developed at the Centre for *Rhizobium* Studies has four principle phases. Firstly, there is germplasm acquisition, isolation of strains and their storage and maintenance in a stable secure environment. Often isolates of root nodule bacteria are obtained from the centres of origin of the host legume and/or stressful soils. At the Centre for *Rhizobium* Studies strains of root nodule bacteria are stored as lyophilised cultures,

and in deep frozen suspensions in 20% v/v glycerol. The second phase involves glasshouse experiments to authenticate isolates as root nodule bacteria and screen for effective nitrogen fixation. A pool of effective strains are then taken to the field for assessment of their adaptation to the edaphic environment where they are intended to be used. These field trials are conducted over two or three years to determine the capacity for the strains to survive in, and colonise, the soil. There is often considerable variation between effective strains in their performance in the field (Figure 1). The final phase involves validation of strain performance using a smaller number of strains in larger scale rotation trials in farming systems, often carried out independently by researchers in different locations. Even at this stage, there may be significant variation in strain performance (Figure 2). The outcome from our use of this process is the provision of robust, effective strains of root nodule bacteria for the inoculum industry in Australia (Herridge et al. these Proceedings).

Current Challenges for Selection of Inoculant Strains of Root Nodule Bacteria

Two current challenges in Australian agriculture for the selection of improved inoculant quality strains of root nodule bacteria are being addressed by research

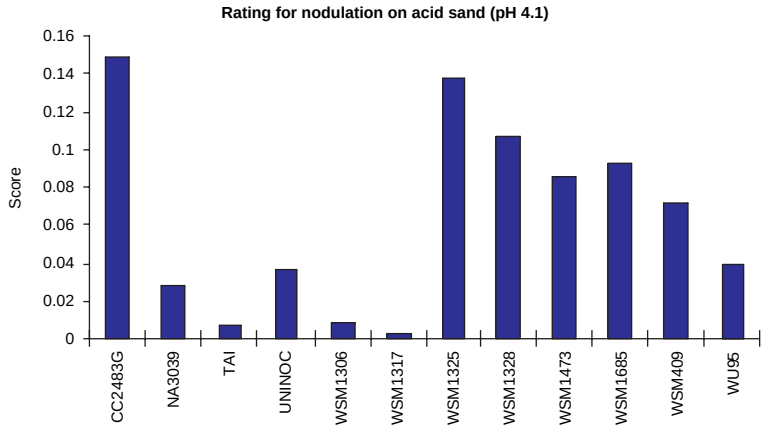

Figure 1. Nodulation (nodule score per plant) for uninoculated *Trifolium subterraneum* sown into an acid sand (pH 4.1) 11–20 cm distant from the placement of strains of *Rhizobium leguminosarum* bv. *trifolii* added to the soil as inoculum 12 months previously.

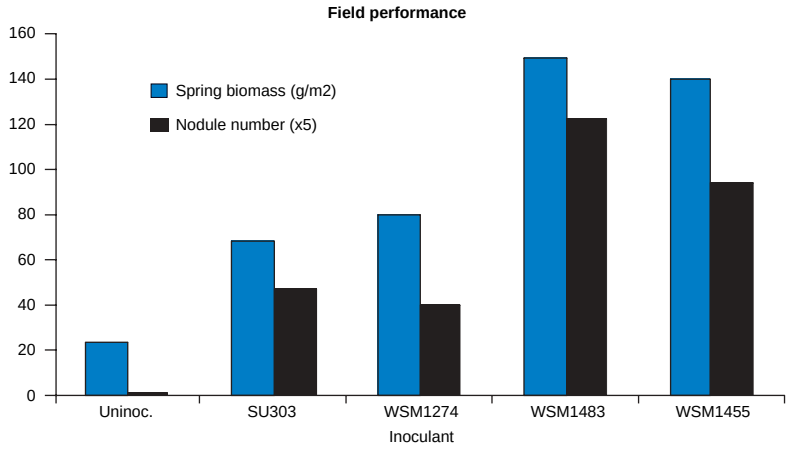

Figure 2. Field performance of potential inoculant strains for *Lens culinaris* in a three-year rotation trial on an acidic loam (pH 4.9).

at the Centre for *Rhizobium* Studies. Firstly, there is the considerable challenge of the need for a single inoculant strain to effectively nodulate a broad range of legume hosts (Howieson et al. 2000b). One example of this 'host range problem' where it appears possible to be successful in obtaining a single inoculant strain effective across a broad host range is with the need to find a strain of *Rhizobium leguminosarum* bv. *viciae* to nodulate species of *Lens*, *Lathyrus*, *Vicia* and *Pisum*. It does seem that it will be possible to achieve this aim for these pulse legumes (Figure 3, Table 1).

A second example of a challenge in this area where there is no solution at present, and the difficulties seem greater here, is with the new annual and perennial *Trifolium* spp. currently being investigated for introduction in agricultural systems in Mediterranean regions of southern Australia (Table 2). The issue in this situation is that strains of *R. leguminosarum* bv. *trifolii* effective on annual species of *Trifolium* are not effective on perennial species of *Trifolium*, and vice versa. The challenge, therefore, is to obtain a strain of *R. leguminosarum* bv. *trifolii* that is effective on the new annual and perennial *Trifolium* spp. Comprehensive ecological studies of the interactions between legumes and their microsymbionts in both natural and managed ecosystems are required to address this issue. Few genera of the herbaceous legumes grown in agriculture for pasture and forage contain both annual and perennial species. In the Mediterranean-type regions of the world these genera include *Lotus*, *Medicago* and *Trifolium*. Many species of the key genus *Trifolium* are important in agricultural systems and components of the flora of natural systems. In addition, this genus is a focus for research programs in Australia,

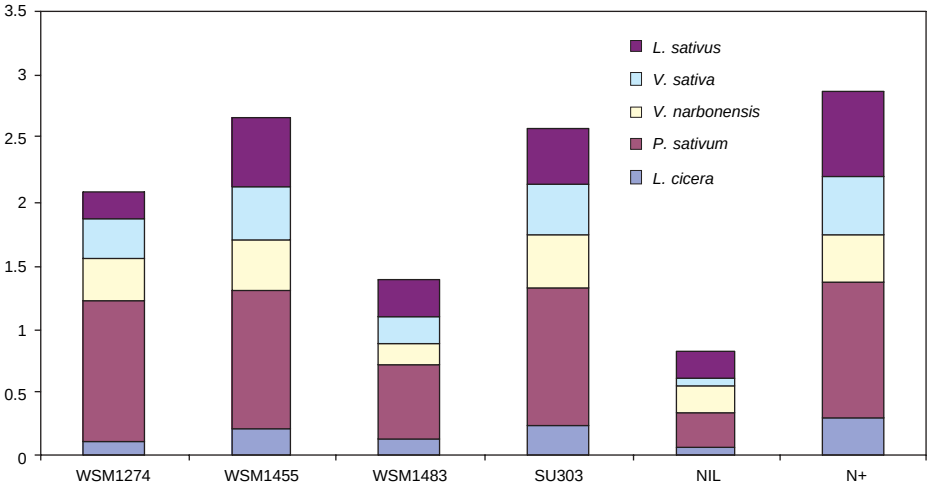

Figure 3. Response of cool season temperate pulse legumes to inoculation with strains of *Rhizobium leguminosarum* bv. *Viciae.*

Table 1. Diversity of symbiotic interactions between a range of cool season temperate pulse legumes and potential inoculants strains of *Rhizobium leguminosarun* by *viciae*.

	WSM1274	WSM1455	WSM1483	SU303
Lathyrus cicera	O	E	O	E
Pisum sativum	E	E	O	E
Vicia narbonensis	E	E	I	E
Vicia sativa	O	E	O	E
Lathyrus sativus	O	E	O	E

E = effective nodulation
O = partial effective nodulation
I = ineffective nodulation

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Table 2. Diversity of symbiotic interations between annual and perennial species of *Trifolium* and strains of *Rhizobium leguminosarum* by *trifolii* from annual (WSM409) and perennial clovers (WSM2011, WSM2010, WSM2012, WSM 2013, WSM2014 and WSM 2016).

	WSM409	WSM2011	WSM2010	WSM2012	WSM2013	WSM2014	WSM2016
T. subterraneum	E	X	X	X	X	X	X
T. africanum	X	O	O	O	E	O	E
T. usamburense	X	E	E	E	E	E	O
T. burchellianum	O	E	O	E	E	E	O
T. crytopodium	O	N	E	E	E	E	X
T. cherangiensis	O	X	X	X	O	O	N
T. polymorphum	O	N	X	X	N	X	E
T. matti roliamun	O	E	X	E	O	O	O
T. decorum	O	O	O	O	O	X	E
T. rueppellianum	O	O	O	E	O	E	E
T. simense	O	E	O	E	E	O	E
T. steudneii	O	E	O	O	O	O	O

E = effective nodulation
X = no nodulation
O = ineffective nodulation
N = no data

Uruguay and countries of the Mediterranean basin to develop new species of annual and perennial pasture legumes for rotation-based systems for sustainable agriculture. The objective of the current research program is to increase our understanding of symbiotic interactions of this genus as a model to develop strategies to improve the management of symbiotic interactions between legumes and their root nodule bacteria to extend, rather than restrict, their use in agriculture.

Secondly, there is the clear need for strains with enhanced survival and increased effectiveness under edaphic stress. That is the producers require strains with the ability to survive in stressful soils in sufficient numbers to provide a population able to give good nodulation under stresses such as pH, temperature and competition from less effective indigenous and naturalised strains. This latter problem of the inoculant strain having to compete with other root nodule bacteria in the soil is a significant problem in many areas, not the least in many soils in the tropics and sub-tropics (Barran and Bromfield 1997).

One component of the research program at the Centre for *Rhizobium* Studies to address the problem of stress tolerance in root nodule bacteria is currently focussed on the well recognised acid sensitivity of the *Medicago – Sinorhizobium* symbiosis. At present the research is aimed at improving our understanding of the molecular and symbiotic mechanisms of acid-sensivity (Dilworth et al. 2001, Cheng et al. 2001), with a view to eventually being able to improve the acid tolerance of the symbiosis in both lucerne (*M. sativa*) and annual *Medicago* spp.

Future Areas for Research and Development in the Use of Inoculants

One area where there clearly may be beneficial improvements in the use of inoculants in the future is to increase the survival of the bacteria during inoculation on seed, and into soil, using developments in new formulations, granular inoculants, and seed coating techniques that may protect the bacteria from environmental stress and physically keep them apart from toxic chemicals such as fungicides.

A second area of research where we see potential for further developments is to use root nodule bacteria as model rhizosphere-inhabiting bacteria to deliver other products to both legumes and other crops. This approach will require appropriate use of molecular knowledge and techniques to transfer into root nodule bacteria useful genes coding for products that will benefit legumes. Two examples of this approach are the insertion of Bt-toxin genes for control of insect pests that attack legume roots and nodules, and the transfer of genes coding for plant growth hormones. In effect, this approach uses the root nodule bacteria, as successful colonizers of the soil and rhizosphere, to be vehicles to deliver useful products to improve legume productivity. A third area with wider application is to use the knowledge and techniques developed with root nodule bacteria to successfully investigate and deliver other useful microbes to soils and crops, for example PGPR's and microbial agents for biological control of pests, diseases and weeds. Research into these areas is currently underway in several research groups

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throughout the world and it is anticipated that this research will provide beneficial outcomes resulting in improved sustainability and productivity in agricultural systems.

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Effects of Rhizobial Inoculation and Inorganic Nitrogen Fertiliser on Vegetable Soybean (*Glycine max* (L.) Merr.) Cultivated on Alluvial Soil of Cantho province (Mekong Delta) using ^{15}N Isotope Dilution Technique

Cao Ngoc Diep[1], Vo Huy Dang[2], Nguyen Van Ngau[3], Mai Thanh Son[4] and Tran Phuoc Duong[1]

Abstract

^{15}N techniques was used to evaluate the effects of N fertiliser and rhizobial inoculant on yield, quality of pod and N_2 fixation by vegetable soybean (*Glycine max* (L.) Merr.) cultivated on alluvial soil in Mekong Delta, Vietnam. The increasing application rates of N fertiliser reduced amount of fixed N. High N rates (100 and 150 kg N/ha) increased nitrate concentration in soybean seeds but decreased N total and organic matter in the soil after soybean plants were harvested. Application of small N-fertiliser (25 kg N/ha) in combination with rhizobial inoculation seemed to be an appropriate cultivation practice for vegetable soybean on alluvial soil of the Mekong Delta.

SOYBEAN (*Glycine max* (L.) Merr.) is the most important grain legume crop in the world in terms of total production and international trade. In the 1995–1996 crop year, world production was about 124 million tonnes. Soybean seeds contain from 18% to 23% oil and from about 38% to 44% protein (Hymowizt et al. 1998). Many different foods were developed from the soybean seed as miso (soy paste), shoyu (soy sauce), tofu (soy curd), soymilk and tempeh (a fermented cake-like product).

While most soybean cultivars are grown for their high protein, high oil seed, a few are commercially grown as a vegetable (Yinbo et al. 1997). Vegetable soybean is harvested fresh during late reproductive growth (after the R6 and before the R7 growth stage) (Fehr et al. 1971) while the pod is still green and seeds have developed to fill 80–90% of the pod. Vegetable soybean has a sweet and nutty flavor, is rich in protein, oil, phosphorus, calcium, iron, vitamins B1 and B2 and has the highest net protein utilisation value of all soybean products (Shanmugasnudaram et al. 1989). It is harvested and sold as fresh or frozen pods or shelled beans.

[1] Biotechnology R&D Institute, Cantho University
[2] College of Biology, HCMC National University, HCMC
[3] Agriculture Service of Can Tho province
[4] Centre of Nuclear Technique, HCMC

While the use of green soybean was popular in the USA in the 1930s and 1940s as a valuable protein source, it is now predominatly eaten as a snack food with beer or as a stir-fried vegetable throughout Asia (Yinbo et al. 1997). Japan is the major producer, importer and consumer of vegetable soybean. Japan produces between 100,000 and 120,000 metric tons of green soybean each year; however, the annual demand is 160,000 to 170,000 metric tons. The gap is met by importing frozen soybean from Taiwan, China, Thailand and Vietnam. The export of vegatable soybean to Japan is currently worth between US$100–110 million per annum (Lumpkin and Konovsky 1991; Shanmugasundaram, 1996).

Vietnam is one of the many countries which exported frozen vegetable soybean to Japan; many companies contracted local farmers to grow vegetable soybean for export. To maximise yield and pods size (marketed pod), farmers often applied high rates of nitrogen fertiliser in the vegetable soybean cultivation. Symbiosis nitrogen fixing system in soybean could be exploited to maximise grain yield as well.

This paper reports a field investigation using a ^{15}N isotope dilution technique to evaluate the importance of nitrogen fixation of rhizobial inoculated soybean.

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Materials and Methods

Experimental design

The trial was conducted at the Can Tho University experimental station, Can Tho city, in the Mekong Delta, Vietnam, between January 18, 1999 to March 24, 1999. Total N of 20 cm top soil was 0.2226%, organic matter was 5.09%; 16.3 mg P_2O_5/100 g soil as exchangeable P and 0.31 meq/100 g soil as exchangeable K. Daily mean temperatures ranged from 23 to 32°C in the dry season.

Vegetable soybean cultivar VL-3 was supplied by CAFATEX Company. Weeds were cleaned by knife and moved out of the experiment area, no-tillage, soybean seeds were sown with 6 m rows spaced at 40 cm and interrows spaced at 10 cm in rows. Before sowing, seeds were inoculated with a mixture of rhizobial inoculant (*Bradyrhizobium japonicum* USDA110 and VNR71 strains (supplied by Biotechnology R&D Institute, Cantho University) combined with sterile peat [rate of 10 kg/ha]) and rice husk ash to fill the holes containing two seeds. The soil surface was covered with a layer of rice straw to limit water evaporation and the experiment plots were irrigated twice each day with a watering-can.

The experiment design was a randomized complete block with four replications of four treatments. The four nitrogen fertiliser were as follows:

- Treatment A: [25 kg N/ha total] applied at 10 days after sowing [DAS] with inoculation.
- Treatment B: [50 kg N/ha total] applied 25 kg N/ha at 10 DAS and 25 kg N/ha at 30 DAS with inoculation.
- Treatment C: [100 kg N/ha total] applied 25 kg N/ha at 10 DAS, 20 DAS, 30 DAS and 40 DAS without inoculation.
- Treatment D: [150 kg N/ha total] applied 50 kg N/ha at 10 DAS, 20 DAS and 30 DAS without inoculation.

Plot size was 24 m^2 (4 × 6 m) with two parts in each plot. The first part (2 × 6 m) consisted 2 subplots [0.8 × 1.2 m in each subplot]. One was sown with soybean cultivar VL-3 and another was reference crop with a soybean cultivar mutant [cultivar M.129] (non-nodulating cultivar) (Figure 1) [Treatment A received 25 kg N/ha as ^{15}N-urea with 6% atom ^{15}N excess; Treatment B received 50 kg N/ha with 4% atom ^{15}N excess; Treatment C received 100 kg N/ha with 2% atom ^{15}N excess and Treatment D received 150 kg N/ha with 1.0% atm ^{15}N excess]. The second part (2 × 6 m) was planted for recording yield component and grain yields, shoot yields. Basal phosphorus and potassium applications were applied at the rate of 60 kg P_2O_5/ha as super phosphate (15% P_2O_5) and 30 kg K_2O as KCl (60 kg K_2O). N fertiliser was urea (46% N).

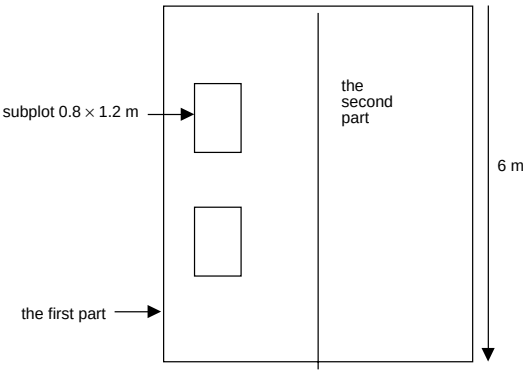

Figure 1. Plot design.

Sampling

Plant height, yield component were recorded before the harvested stage (58 DAS). All of soybean plants in the first part were taken, One kg fresh shoot and one kg fresh pod were dried at 75°C in 48 hours to constant weight, weighed, ground to < 1 mm size and analysed for total N and ^{15}N. Total N and ^{15}N to ^{14}N ratio were determined by a block spectrophotometer at a CSIRO laboratory in Australia. The percentage of nitrogen derived from fertiliser, soil, air (% Ndff, % Ndfs, % Ndfa) and N_2 fixation in plant samples were calculated following the A-value method (Fried and Broeshart 1975). All plants in the second part were harvested to calculate fresh shoot and fresh pod per ha (Yield consisted of dry shoot and dry pod).

After harvesting, soil samples were taken to measure N total (micro-Kjeldahl method) and organic matter (Walkley-Black method). Nitrate concentration in seed was determined following Bremner and Mulvaney method (1982).

An analysis of variance was done on data obtained from each parameter of field experiment. Treatment means were processed by LSD 0.05.

Results

Effects of N levels and rhizobial inoculant on nodulation and yield

Increasing levels of N application reduced nodulation of vegetable soybean (Figure 2). Pod/plant and green pod yields were the highest at 25 kg N/ha (treatment A); application of 100 kg N/ha (treatment C) produced the lowest pod number and pod yield while pod yields decreased slightly at 50 kg N/ha (treatment B). Pod yields of treatment D and treatment A did not differ significantly (Table 1). However, these double treatments (treatment A and B) produced

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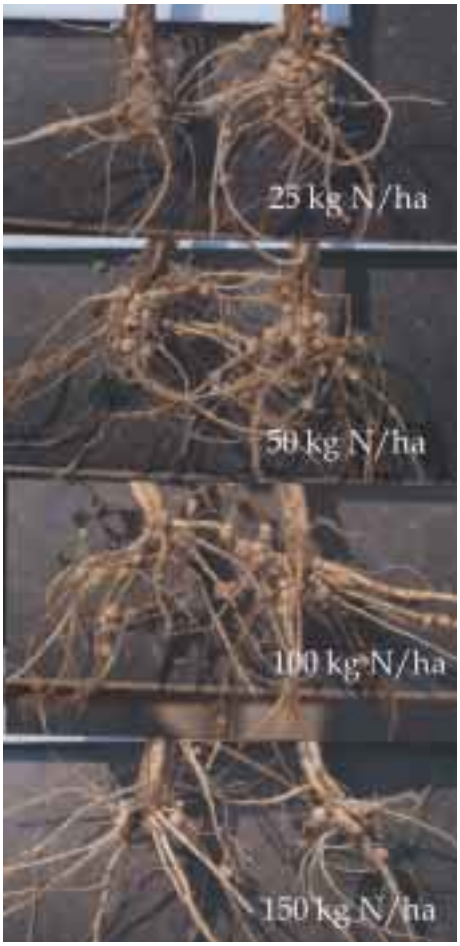

Figure 2. Effects of N fertiliser levels on nodulation of vegetable soybean.

significantly higher organic matter and total N of top soil as compared to high rate nitrogen application without rhizobial inoculation (Table 3).

No difference of DW of shoot + pod (biomass) between treatments but N total (%) of biomass of treatment A and C had the highest values which correspond to the highest N-crop yields (Table 2). Increasing N levels from 25 to 150 kg N/ha increased the amount of nitrogen derived from fertiliser (% N dff) but reduced (although not significant at p = 0.05) the quantity of fixed N (% N derived from air) and N derived from soil (Figure 3). Hence, high N application rates reduced biological nitrogen fixation and soil nitrogen uptake. Low application rate of chemical fertiliser (treatment A) stimulated high uptake of soil nitrogen and biological nitrogen fixation.

Applications of the high N levels (100 kg N/ha and 150 kg N/ha) increased nitrate concentration in soybean seeds (Figure 4) lowered organic matter and total soil N (Table 3). Combination of low levels of nitrogen application (25 and 50 kg N/ha) and rhizobial inoculation did not change significantly N total of top soil.

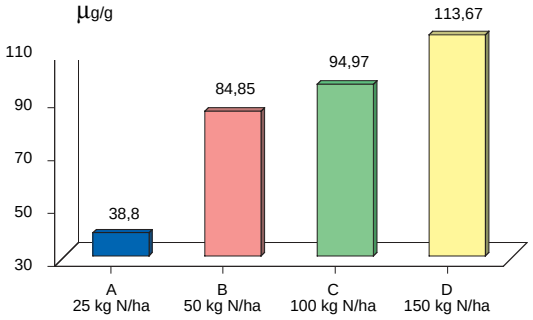

Figure 4. Effects of N fertiliser and rhizobial inoculant on nitrate concentration of soybean seed.

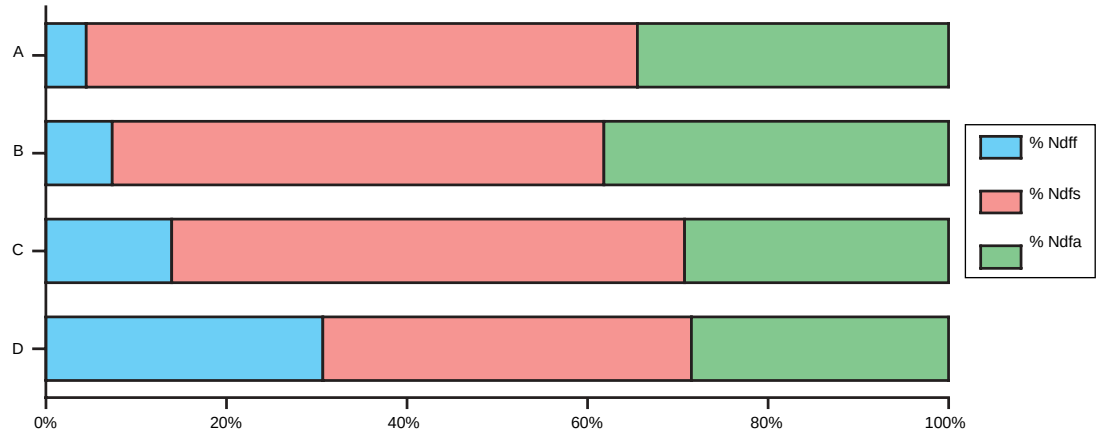

Figure 3. Effects of N fertiliser and rhizobial inoculant on ratio derived from fertiliser, soil and air (%D dff, % Ddfs, %D dfa).

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Table 1. The effects of rhizobial inoculant and N fertiliser on yield components and pod yields by vegetable soybean cultivated on alluvial soil.

Treatment	First pod * number/plant	Second pod ** number/plant	Fresh shoot kg/ha	Green pod yield kg/ha
A (25 kg N/ha)	15.06	8.00	9520	9478
B (50 kg N/ha)	14.31	8.56	9225	9060
C (100 kg N/ha)	11.68	8.68	9022	8715
D (150 kg N/ha)	14.12	8.02	10702	9480
LSD 0.05	2.36	n.s	983	541

* First pod/plant = pods have two or three seeds
** Second pod/plant = pods have one seed

Table 2. The effects of rhizobial inoculant and N fertiliser on yield and N_2 fixation by vegetable soybean cultivated on alluvial soil.

Treatment	DW of shoot+pod	N total in biomass	N-crop yield	^{15}N atom excess	N derived from fertiliser	N derived from soil	N derived from air
	(kg N/ha)	(%)	(kg N/ha)	(%)		(kg N/ha)	
A (25 N/ha)	6958	4.414	307.2	0.26913	13.82	187.35	106.07
B (50 N/ha)	6551	4.002	262.3	0.29392	19.32	140.49	102.38
C (100 N/ha)	6916	4.456	307.1	0.27852	41.89	172.63	92.57
D (150 N/ha)	7291	3.485	255.1	0.30662	78.37	104.72	72.05
LSD(p=0.05)	n.s	0.733	46.8	n.s	18.5	51.27	n.s

Table 3. The effects of N fertiliser and rhizobial inoculant on organic matter and N total of top soil after vegetable soybean harvested.

Treatment	Organic matter (%)	N total (%)
Treatment A (25 kg N/ha with inoculation)	5.311	0.2461
Treatment B (50 kg N/ha with inoculation)	5.114	0.2292
Treatment C (100 kg N/ha without inoculation)	3.826	0.2033
Treatment D (150 kg N/ha without inoculation)	3.655	0.1761
LSD (p=0.05)	0,368	0.0041

Discussion

Positive effects of N-fertiliser applications to soybean have been observed in some field investigations (Norhayati et al. 1988; Takahashi et al. 1991) but not in others (Herridge and Brockwell, 1988; Ying et al. 1992). Many factors for variations such as growth conditions, management practices, initial level of soil fertility, native rhizobia population or the timing of N application could be cited (Peoples et al. 1995).

De Mooy et al. (1973) and Watanabe et al. (1986) have suggested that N applied prior to reproduction can influence yield through improvements in plant growth. It has been shown that application during the early period of development (10 DAS) of small amount of chemical N fertiliser to inoculated soybean cultivated in the Mekong Delta soil stimulated nodulation and produced high rate (equivalent to 94.5 kg N/ha) of biological nitrogen fixation (Duong et al. 2000), high rate of N application up to 100 kg N/ha did not significantly increase biological fixation (Ngau 1998). In the other hand, a high rate of chemical fertiliser increased nitrate concentration in soybean seeds, hence reduced quality of soybean.

Conclusion

Application of about 25 kg N/ha together with rhizobial inoculation seemed to be an appropriate cultivation practice for vegetable soybean cultivated in this type of soil in the Mekong Delta.

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Development of Elite Inoculant *Rhizobium* Strains in Southeastern Australia

Jo Slattery[1] and David Pearce[1]

Abstract

Matching rhizobial strains to host legumes is the most important factor in maximising the productivity of Australian pulse and pasture legumes. This paper reports on a system that we have developed for the selection of elite inoculant strains. This system involves the collection of *Rhizobium* germplasm, screening their nitrogen fixation capability and assessing their adaptation to different soil environments. Soil factors that influence the legume-*Rhizobium* symbiotic relationship include extremes in soil pH (highly acidic or alkaline), salinity, and high soil temperatures in crop and pastoral soils in southern Australia. These soil conditions limit the establishment of *Rhizobium* populations introduced as seed inoculum. Therefore, a process has been developed, and is discussed in this paper, that better aligns strain selection with plant development.

FARMING in Australia is continually changing because of intensification, higher inputs and the quest for increased production as well as adapting to specific soil and climatic conditions. With intensification of cropping, the need to maximise the benefits of productive pastures and crop legumes in rotation with crops is recognised for long-term farm sustainability. To achieve these benefits, the legume must have effective root rhizosphere associations. Given the poor fertility of many Australian soils, there is a need to select appropriate plant-*Rhizobium* symbioses for Australian environments. Agriculturally productive legumes and their root-nodule bacteria have evolved outside of Australia. Now, more than ever, the need for legumes and root-nodule bacteria adapted to the Australian environment is critical.

Rhizobial strains suitable as inoculants must have the following characteristics: able to colonise the soil and to tolerate environmental stresses, able to compete with populations of background rhizobia to form nodules, able to form effective nodules which fix N_2, and to have no deleterious effects on non-target hosts (Brockwell et al. 1995; Howieson 1999). Strains of rhizobia differ widely in their ability to survive nodulate and fix nitrogen in soil environments; thus the selection of rhizobia with specific symbiotic and competitive attributes suited to a range of soil environments must assume a high priority.

Naturally occurring *Rhizobium* populations often occur in high numbers in soil, and can compete strongly with introduced *Rhizobium* inoculant. However, their ability to fix nitrogen is generally poor. The introduction of new legume genera to Australian farming systems usually requires the identification of new and specific inoculant *Rhizobium* strains not found in Australian soils, but necessary for optimum N fixation.

Strain selection focuses on root nodule bacteria that offer a broad-host range for nodule formation and nitrogen fixation, as well as adaptation to the soil niche of the host legumes. The task of selecting rhizobial strains that match host legumes can be a long and complex process, and includes the following steps:

1. Collection, isolation and maintenance of the *Rhizobium* germplasm.
2. Authentication and screening of the rhizobial isolates for genetic compatibility and nitrogen fixation.
3. Assessment of the *Rhizobium* germplasm for edaphic adaptation and *in situ* performance (Howieson et al. 2000a).

Much progress has been made in the selection and commercial release (or future release) of elite

[1] Rutherglen Research Institute, Dept of Natural Resources and Environment, Rutherglen, Victoria 3685, Australia

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edited by D. Herridge
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(printed version published in 2002)

Rhizobium strains with the ability to tolerate soil and environmental stresses. Some examples of highly effective strains with traits for acid tolerance are RRI128 for lucerne, WSM409 for aerial clovers (Howieson 1999; Watkin et al. 2000) and WSM1455 for lentils (Howieson et al. 2000b). Considerable gaps still exist in the availability of elite strains for alkaline, sodic and saline soils in Australia where most of the broadacre cereal production occurs.

This paper reports on the methodology used to select and match elite inoculant strains of Rhizobium spp to host legumes for southern Australia. In particular the differences in selection processes used for different Rhizobium spp will be highlighted and an attempt will be made to show that rhizobia selected from different soil environments can be used as inoculum sources.

Materials and Methods

Collection of field isolates

Germplasm acquisition involves the collection of legume roots growing in edaphic geographical regions. Pulse legumes were collected from 12 paddocks with high background populations of rhizobia within the pulse growing regions of NW Victoria. Plant root systems were carefully excavated, the soil removed and stored at –20°C prior to the isolation of the rhizobia bacteria.

Isolation of rhizobial isolates

Nodules were selected on the basis of size, colour and position relative to the crown from each plant. Rhizobia were isolated using the nodule squash technique (Gault et al. 1973) following surface sterilisation in 3% calcium hypochlorite; colonies were maintained on Yeast Extract Mannitol Agar (YMA) (Vincent 1970). Isolates were subcultured onto YMA slopes, incubated at 28°C for 7 days, and either stored at 4°C for short-term (1–2 years) storage or vacuum dried for long-term (indefinite) storage in glass ampoules.

Reference strains

Cultures of *Rhizobium* strains WSM1274 and SU303 were obtained from the Australian Legumes Inoculants Research Unit (previously AIRCS, Gosford, NSW, Australia) for comparison with the field isolates.

Glasshouse evaluation

Glasshouse screening was undertaken in a temperature-controlled glasshouse using a sterile sand culture system. Disinfected plastic pots (19 cm diam.) were filled with 50:50 mixture of coarse, washed packing sand and vermiculite (steam sterilised at 121°C/1 h) and saturated with ultrapure water prior to the sowing of seed.

Four seeds were planted into each pot to a depth of 3.0 cm. Seven days after sowing the emerged seedlings were inoculated with 1 mL of a 5-day-old YMB culture (Vincent 1970). Each rhizobia isolate was replicated three times and the moisture content of each pot maintained with a N-free nutrient solution (Broughton and Dilworth 1971) (weekly) and watered (twice weekly). Air temperature was maintained below 25°C. Potassium nitrate (0.05%) was applied to the + Nitrogen treatment pots at the time of inoculation and at weekly intervals thereafter.

All plants were harvested 8–10 weeks after sowing, the roots scored for nodulation (Corbin et al. 1973), and the tops dried at 70°C and weighed. Capacity of each isolate to fix N was assessed against the DM yields of the inoculated plants grown with and without KNO_3, as well as against those grown with the commercial inoculant strains WSM1274, SU303, and CC1192.

Evaluation of nitrogen-fixing effectiveness

During 1998 and 1999, more than 180 Rhizobium strains were screened in the glasshouse. Most strains were isolated from NW Victoria and form part of the Rutherglen collection (RRIV). An additional 18 strains from the Western Australian (WSM) and Canberra (CC) collections were included in the screening. Vetch (Vicia sativa) was used as the host plant. The 19 best strains from the Rutherglen collection were further screened on 5 pulse legumes — field pea (Pisum sativum), faba bean (Vicia faba), lentil (Lens culinaris), vetch (Vicia sativa) and chickpea (Cicer arietinum). Additional screening of interstate strains over a range of soil pHs was undertaken using narbon beans (Vicia narbonensis) as the host plant.

Field evaluation

The third stage in the development of rhizobial inoculants is the evaluation of rhizobial inoculants in the free-living saprophytic state. Elite rhizobial strains and rhizobial strains from the glasshouse experiments exhibiting optimal nitrogen fixing capacity were evaluated under field conditions on a range of soil types with different soil pHs, chemical characteristics and background rhizobial populations. Each rhizobia strain was evaluated on one or more of the following legumes — field pea, faba bean, lentil, vetch, and chickpea. Site establishment and maintenance details were similar to those outlined in Slattery and Coventry (1999).

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Preparation of rhizobial peat inoculants

Rhizobial strains were introduced into the soil at each site as peat inocula. Peat is also used by the two Australian Rhizobium manufacturers as the carrier medium for commercial inoculants. This same aseptic procedure was adopted in our laboratory for the preparation of Rhizobium, Sinorhizobium and Bradyrhizobium inoculants (Slattery and Coventry 1999).

Site management and sowing

Preparation, sowing and maintenance of each site were carried out throughout the growing season. When needed annual grasses and broadleaf weeds were controlled with the appropriate chemicals.

Plant sampling

Individual plants were collected 10–12 weeks after sowing for nodulation and plant dry matter measurements. Plant roots were scored for nodulation on a 0–5 scale, based on the nodule number, size, position, distribution and pigmentation of effective nodules on the crown and lateral roots (modification of Corbin et al. 1977). Plant material (tops) were dried in a forced-draught oven at 70°C for 48 hours and weighed.

Grain yields

Grain seed yield was determined by mechanical harvesting the entire plot.

Results and Discussion

Glasshouse inoculation responses — *Vicia sativa*

The capacity of the top 19 RRI field isolates to fix nitrogen relative to *Rhizobium leguminosarum* bv *vicia* strain SU303 and WSM1274, ranged between 109% and 135% (data not presented). This initial screening on one host species alone (vetch) increased our capacity to handle more rhizobial strains. Once strains were identified for a high capacity to fix nitrogen on vetch, the second stage of our screening process examined their potential on a broad range of most species, namely field pea, faba bean, lentils and vetch.

Glasshouse inoculation response — *Vicia sativa, Vicia faba, Pisum sativum, Lens culinaris*

Inoculation responses varied between *Rhizobium* strains, and to a lesser extent between legume species. With the exception of strains RRI322 and RRI231, all strains nodulated at least one plant species, with more than half the strains nodulating all four plant hosts (Figure 1). Nodulation of faba beans ranged from non-infective, with a score of 0, to highly effective with a nodule score of 4.5. The nodulation scores for field pea, vetch and to a lesser extent lentil, were similar to that obtained for faba bean (Table 1).

Nodulation scores and shoot dry matter response to inoculation of the 19 *Rhizobium* strains showed considerable variation between strains. Large host-strain interactions were evident in the nodule scores (Figure 1) and plant yields (Figure 2) on *V. sativa*, *V. faba*, *P. sativum* and *L. culinaris* (Figure 1). Rhizobial strains RRI303, 298, 240, 366, 339, 294, 224 and 317 effectively nodulated and increased DM yields relative to the uninoculated control, of faba bean, field pea, lentil and vetch (with RRI303 not tested on vetch in Figure 1) suggesting that these strains are worthy of further field testing. The performances of the remaining strains (poor nodulation and lower DM yields) suggested that these strains have highly specific host-strain requirements and would not nodulate a wide enough range of pulse legumes to be suitable as a commercial inoculant. Similar studies on host-strain interactions using *P. sativum*, *L. culinaris* and *V. faba* showed that some of the most effective strains on *P. sativum* were relatively poor at N_2-fixation on *V. faba* and *L. culinaris* (Howieson et al. 2000a). Nonetheless, similar to our findings they were able to select strains that fixed nitrogen on all three pulse legumes.

Table 1. Mean values and ranges for root nodule scores[A] (0–5 scale) of four pulse legumes when separately inoculated with 18–19 strains of root nodule bacteria under controlled conditions.

Legume species	No. of *Rhizobium* strains	Mean value	Min. of range	Max. of range
Faba bean	19	2.35	0.00	4.50
Field pea	19	3.09	0.00	5.00
Lentil	19	1.50	0.00	3.58
Vetch	18	2.58	0.00	5.00

[A] Nodule score (1–5), modification of method by Corbin et al. (1977)

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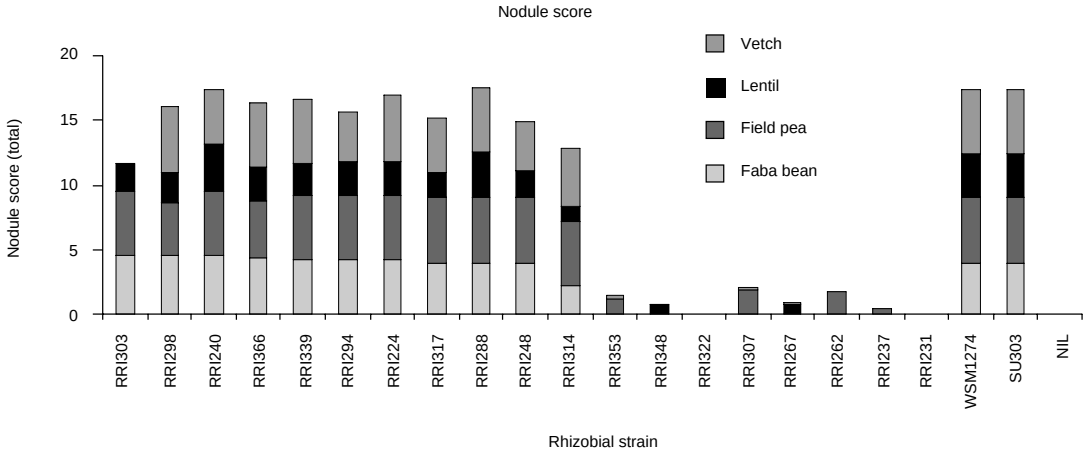

Figure 1. Total nodule score produced by four legume species (faba bean, field pea, lentil and vetch) when inoculated separately with 19 strains of root nodule bacteria under controlled conditions

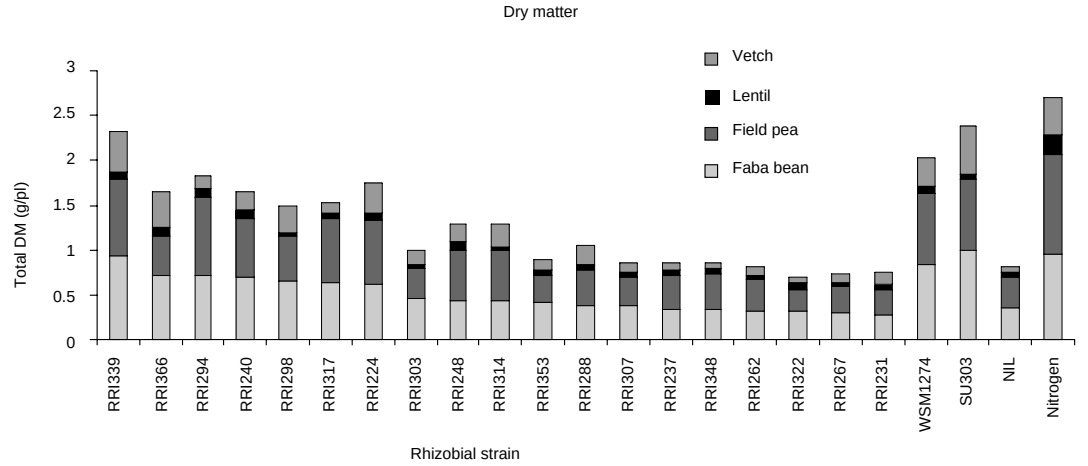

Figure 2. Total shoot dry weights (g/pl) of four legume species (faba bean, field pea, lentil and vetch) when inoculated separately with 19 strains of root nodule bacteria under controlled conditions.

Glasshouse inoculation response — *Vicia narbonensis*

Narbon bean has been identified as a promising grain legume for low rainfall (250–400 mm) environments with adoption by the Australian grains industry dependent on its marketability and acceptance by the stockfeed industry and its on-farm usage (Siddique et al. 1996). Linking rhizobial evaluation programs with the breeding program for narbon bean is equally important. Rhizobial strain selection focuses on selecting strains with a broad host range for nitrogen fixation, and adapted to the soil requirements of the host plant (Howieson et al. 2000a). Strain selection for narbon beans showed that both nodulation and DM shoot responses varied between strains and the level of effectiveness of some strains was classified as highly effective, having relative high nodule scores (data not presented) and dry matter yields (Figure 3). Other strains were ineffective, as shown by their low nodule scores and poorer dry matter yields.

The rhizobial strain that produced the highest DM yield was strain WSM1529, followed by strains CC328, WSM1521 and WSM1455. The least productive strains in terms of DM production were

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strains CC9002, WSM1469 and WSM1483. In this experiment the performance of SU303 (the commercial strain for narbon bean) was sub-optimal, highlighting the importance of matching rhizobial strains to the host legume and the need for ongoing evaluation of rhizobial strains.

A second *Rhizobium* strain pH experiment addressed the agronomic suitability of the narbon bean plant across the pH range of 5 to 9 (Figure 4). In this experiment, nodulation and DM shoot responses varied between strains and pH values. The rhizobial strain that produced the highest DM yield

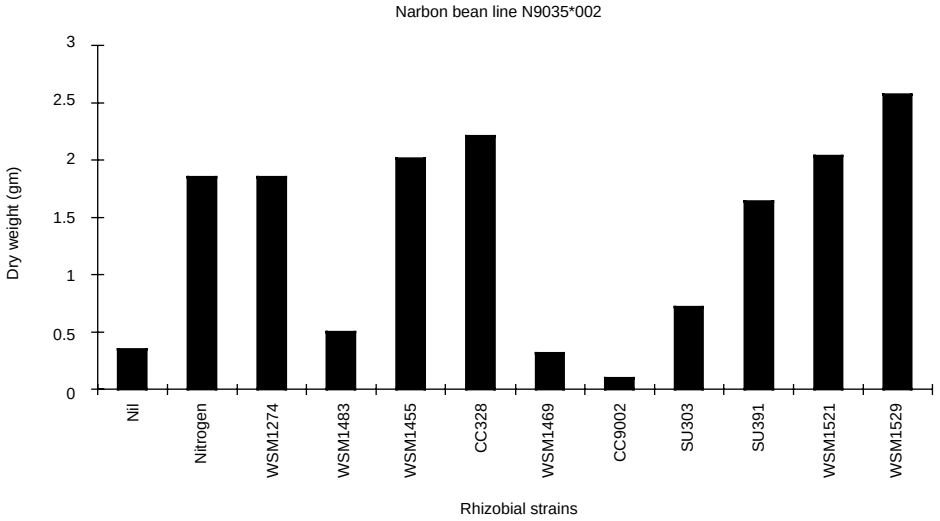

Figure 3. Dry matter productions of the narbon bean line N9035*002 inoculated separately with 10 rhizobial strains.

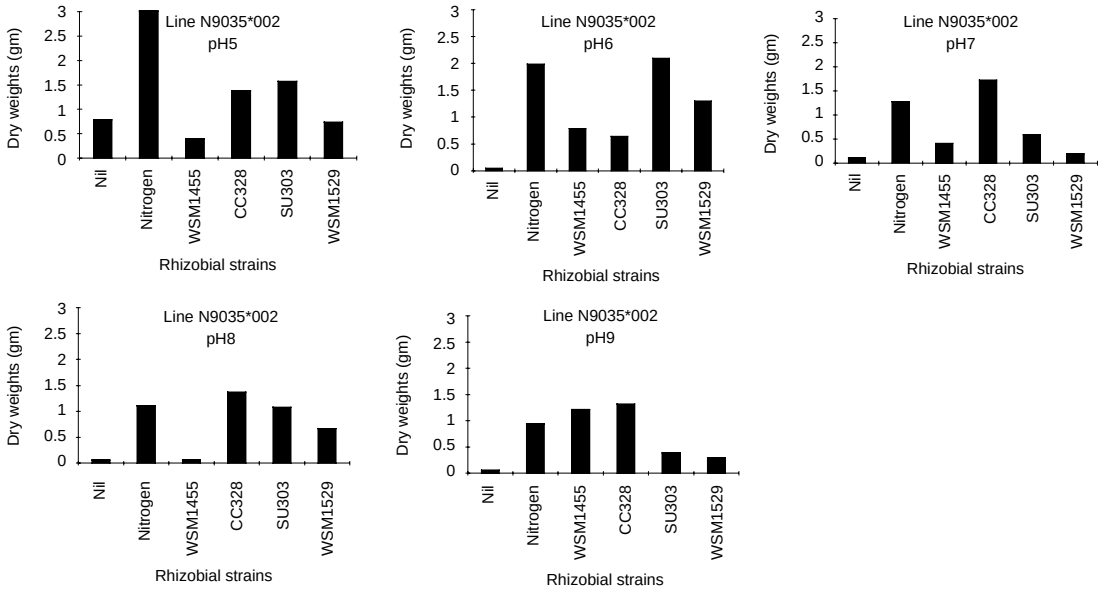

Figure 4. Dry matter production of the narbon bean line N9035*002 inoculated with four rhizobial strains and grown over the pH ranges of 5 to 9 units showing the symbiotic variability of rhizobial strains.

was strain SU303 at pH 5 and pH 6, strain CC328 at pH 7, 8 and 9, while strain WSM1529 only performed well at pH 6 and strain WSM1455 at pH 9. However, these glasshouse results must be verified in the field. In glasshouse experiments, environmental conditions (moisture, temperature or light) are well controlled and extrapolation of glasshouse findings to the field should not be made. The glasshouse experiment suggested that strain SU303 is suitable as an inoculant for acidic soils whilst strain CC328 would be more suitable as an inoculant for alkaline soils.

Legume responses to inoculation in the field

An important characteristic for the selection and evaluation of rhizobia is to ensure that not only are the rhizobia matched with the legume host, but also to the soil conditions. Many farmers in southern Australia may over time grow a range of pulse legumes in the same paddock and strains that compromise N_2 fixation of other commercially important species that may be sown in later seasons, should not be selected.

This is especially important in strain selection when the specificity of the rhizobial strain is considered. Selection of rhizobia with broad range specificity, as shown by those strains exhibiting high nodulation and DM across the four legume species, has an enormous impact on the economics of commercial grain production.

The comprehensive glasshouse screening with four legumes (faba bean, field pea, lentils and vetch) identified elite *Rhizobium* strains suitable for field evaluation at a range of sites across SE Australia from 1998 to 2001. Elite strains from the Rutherglen (RRIV), Western Australia (WSM) and Canberra (CC) collections were evaluated in field studies which focussed on the selection of strains suited to a wide range of soils, highly acidic (pH_{Ca}<4.5) soils with no background rhizobial populations to alkaline soils (pH_{Ca}>9) with diverse background populations. The selection of each site was also based on soil type, soil pH, location and rainfall (Table 2).

Strong correlations between the glasshouse and field results were not always evident. The 1998 field study showed that the performances of some highly-effective strains in the glasshouse could not be duplicated in the field. Such findings highlight the importance of repeated evaluation of strains in the glasshouse and the field under different soil (e.g. pH) and climatic conditions.

Elite *Rhizobium* strains evaluated in the field on 4–5 lines of field pea, faba bean, lentil, vetch or narbon bean have shown some differences in plant performance between different pulse lines. The Rutherglen site (1999) had low rhizobial background populations as shown by poor nodulation of the lentils in the nil treatment (Figure 5). There was considerable variation in nodulation between strains, with some strains performing no better than the uninoculated controls. Strain CC9002 out performed all other strains in terms of nodulation and produced good grain yields. Poor nodulation was often an indicator of the grain yield responses to inoculation in the field as treatments with poor nodulation generally produced low grain yields.

Table 2. Location, soil pH and background rhizobial populations of rhizobial inoculation trials in the years 1998–2001.

Year	Location	Soil pH_{Ca}	Background population
1998	Rutherglen, NE Victoria	4.3	Low
	Speed, NW Victoria	7.7	High
1999	Rutherglen, NE Victoria	4.7	Low
	Walpeup, NW Victoria	6.0	High
	Penshurst, SW Victoria	4.8	Low
	Streatham, SW Victoria	5.0	Low
	Horsham, NW Victoria	8.5	High
	Wagga Wagga, southern NSW	4.9	Low
2000	Rutherglen, NE Victoria	4.7	Low
	Temora, southern NSW	4.4	Low
2001	Rutherglen, NE Victoria	4.7	Low
	Temora, southern NSW	4.4	Low

The expansion of pulse cropping into new, non-traditional pulse growing regions, such as the acidic soils of southern Australia, requires the selection of appropriate germplasm of both legume and root-nodule bacteria. In recent years SW Victoria has emerged as a new cropping region for the growth of pulse crops, especially faba beans. Much of this region has had a long history of livestock grazing enterprises on pastures based on subterranean clover.

Background rhizobial populations were low at the Penshurst site (Figure 6). Poor nodulation in the Nil rhizobia treatments highlights the need for, and importance of inoculation. Ascot, Fiesta and Manifest are commercial faba bean cultivars selected for the more traditional cropping soils and are now being evaluated on the marginal acidic soils of southern Australia. For each cultivar, rhizobial strain WSM1274 was generally more poorly nodulated and yield less grain than the other three strains.

The growth and survival of *Rhizobium* spp in soil is influenced by a number of soil properties including pH, salinity, soil temperature, moisture, fertility and structure (Slattery et al. 2001). Soil acidity is a major cation imbalance affecting about 90 million hectares of agriculturally productive land in Australia. Acidity factors can have a direct impact on either rhizobial growth and persistence, or nodule initiation and nitrogen fixation effectiveness. Therefore, any rhizobial selection process must also include the screening for acid-tolerance.

The inclusion of legumes into agriculture enhances the productivity and potential sustainability of farming systems (Siddique and Sykes 1997). The benefits include improved nitrogen fixation, high protein, high-value cash crops, disease breaks and reduced growth of weed species. Excessive rainfall in winter and spring can cause waterlogging, affecting soils in the higher rainfall (550–750 mm) marginal pulse growing regions of NE and SW Victoria. If waterlogged soils can be better managed, pulse yields can be high yielding; for example, in 1999 yields were high at the Rutherglen site (Table 3), with lentils up to 3.39 t/ha, suggesting that pulse crops may have a potential in NE Victoria. In contrast however, in 2000, with additional winter rainfall (25% more wet days) than 1999, combined with colder temperatures, the grain yields for lentils and narbon beans were greatly reduced, and that of field peas and faba beans were reduced to a lesser extent.

Table 3. Impact of seasonal conditions on pulse grain yields (t/ha) at Rutherglen (NE Victoria).

	1999	2000
Lentils	2.95–3.39	0.25–0.50
Faba beans	3.23–4.38	1.58–2.72
Field peas	2.87–3.69	1.55–2.36
Vetch	1.67–2.20	1.93–2.08
Narbon beans	1.76–3.16	0.36–0.72

Conclusion

The development of elite inoculant rhizobial strains can be a long and slow process. Strains of rhizobia with a commercial potential are intensively evaluated across a broad range of hosts both in the glasshouse and in the field. Before strains can be recommended as inoculants, they must be screened for genetic stability, satisfactory growth and survival under inoculant manufacturing conditions and for broader edaphic adaptation across Australia. Once elite strains have been identified, the Australian Legume Inoculants Research Unit at Gosford, NSW, assesses their commercial potential.

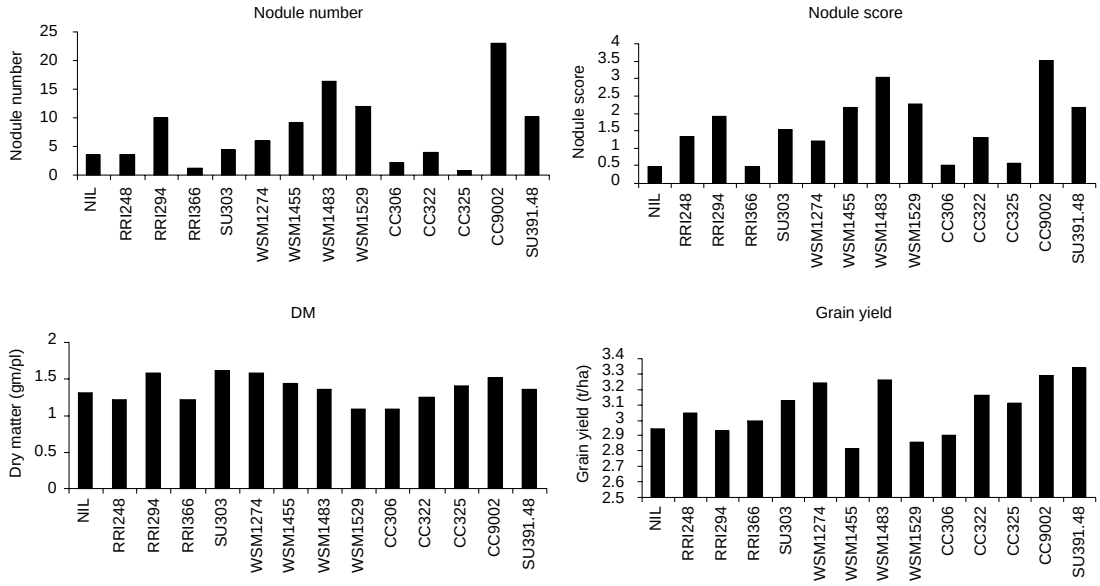

Figure 5. Nodulation, shoot dry matter and grain yield responses of lentils when inoculated separately with 13 strains of *Rhizobium leguminosarum* bv *viceae* and sown into an acidic soil at Rutherglen in NE Victoria (1999).

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ACIAR Proceedings 109e
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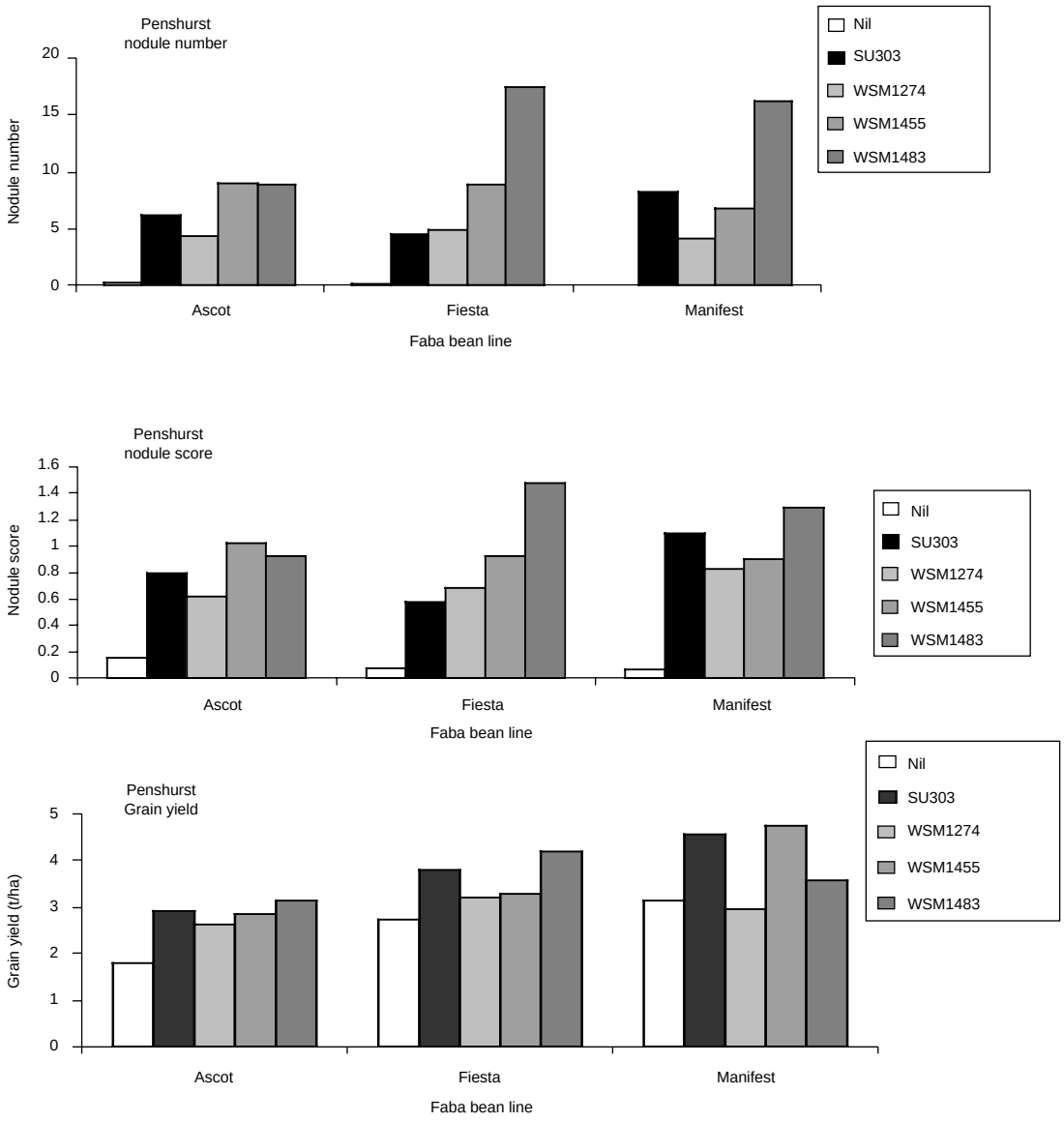

Figure 6. Nodulation and grain yield responses for three faba bean varieties when inoculated separately with four strains of *Rhizobium leguminosarum* bv *viceae* and sown into an acidic soil at Penshurst in SW Victoria (1999).

Acknowledgments

We acknowledge the financial support of the Grains Research and Development Corporation (GRDC) to undertake this work. The financial support provided to the senior author by the Australian Centre for International Agriculture Research (ACIAR) to attend this workshop is gratefully appreciated.

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Development of Inoculant Production and Utilisation in Thailand

Nantakorn Boonkerd[1]

Abstract

The development of the legume inoculant industry in Thailand was started by the public sector in 1976 under a USAID loan. The main objective of the program was to promote the use of inoculant in order to increase important legume products such as soybean, groundnut and mungbean to meet domestic demand. Most of the inoculant production was used in the government-supported programs. With regard to the potential of the inoculant industry, more on-farm research and demonstrations on the use of inoculant should be conducted in order to convince farmers of the benefits of inoculant use, the Government should stop providing free inoculant to farmers and the price of inoculant should be determined by its cost of production. Research on inoculant production technology should be conducted in order to obtain a higher quality product.

INOCULANT research has been conducted in Thailand for a long period. It was reported that the first symposium was held in Bangkok during November 1975, to review rhizobial research in Thailand. Most of the research during that period was conducted by the Bacteriology and Soil Microbiology Branch of the Division of Plant Pathology, Department of Agriculture, Khon Kaen University, and the Applied Scientific Research Corporation of Thailand. Emphasis had been placed on the physiological characterisation of rhizobia, particularly soybean rhizobia, in different environments. Other legumes, such as mungbean and groundnut, were of minor importance.

In December 1975, the Department of Agricultural Extension (DOAE) initiated the Seed Multiplication Project to promote the use of high quality seeds to farmers. In this project, USAID offered a low interest rate loan of 79.8 million baht (US$4 million) for the construction of Seed Centres in Phitsanulok, Chiangmai, Nakhon Ratsima and Chainat. The establishment of the Seed Project generated a huge demand for legume inoculant to go with the foundation seed and commercial seed to be distributed to the farmers. As a result, USAID experts were sent to Thailand in January, 1976, to conduct a feasibility study for the construction of an inoculant production plant at the Department of Agriculture laboratories in Bangkhen, Bangkok. A loan of US$ 0.75 million was guaranteed for the purchase of necessary equipment. The Thai government provided all construction facilities and staff. Production of inoculant in the early stages was behind schedule due to delays in the purchasing of equipment from the U.S. Equipment delivery was completed in 1983.

[1] School of Biotechnology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand

Training in Rhizobial Inoculant Technology

Training in rhizobial inoculant technology was one of the functions under the Memorandum of Understanding between the Thai DOA and the USAID-funded NifTAL Project, University of Hawaii. The Research Corporation of the University of Hawaii (RCHU) established a model Biological Nitrogen Fixation Resource Centre (BNF Resource Centre) at the DOA inoculant production facility, Bangkok in 1983. The main objective was to provide research support and training in inoculant technology for Southeast Asia and other developing countries.

Following the planning workshop of the leaders of the national BNF programs in the region to formulate future activities of the centre, training workshops in inoculant technology by BNF specialists were implemented frequently. From 1983 to 1993, a total of 165 scientists participated in these training workshop activities (Table 1).

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edited by D. Herridge
ACIAR Proceedings 109e
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Table 1. International workshop and training in Rhizibial Inoculant Technology during 1983–1993 classified by participated countries.

Participated country	Number of participants
Thailand	46
India	29
Indonesia	24
Nepal	9
Bangladesh	8
China	8
Philippines	8
Sri Lanka	7
Vietnam	6
Malaysia	5
Pakistan	5
Lao PDR	2
Australia	1
Belgium	1
Burundi	1
Kenya	1
Uganda	1
Honduras	1
Korea	1
Singapore	1
Total	165

As well as the international training, the BNF Resource Centre, Bangkok, also provided inoculant training for local extension workers of the DOAE, DAO staff and instructors of agricultural colleges. This group comprised about 40 participants per year since 1984. Finally, the Resource Centre assisted the private sector to produce inoculants for commercial purpose.

Research on Rhizobial Inoculants in Thailand

Research in Thailand on biological nitrogen fixation of the legume-rhizobium symbiosis has been conducted extensively since the world oil crisis of the 1970s. A group of DOA research scientists worked hard to promote the use of rhizobial inoculants as an alternative to the more expensive N-fertiliser. Supporting the rhizobial inoculant production plant was research by the Thai DOA and other institutes concerning inoculant production, application and its improvement.

Rhizobial strain selection

So far, there are many recommended soybean groundnut and mungbean cultivars to meet the needs of the farmers in various environmental situations. Therefore, rhizobial strain selection suitable for each legume cultivar is the first important role for inoculant production because of the specificity of the nodule formation is governed by genes or depends upon genetic inheritance.

To produce inoculant for each recommended cultivar of soybean, groundnut and mungbean, several strains and isolates of rhizobia were firstly screened for their capability in nodulation and N_2-fixation in the glasshouse. After that, each selected strain was produced as inoculum and tested for its capability in nodulation, N_2-fixation and yield response in several fields. Results of most experiments showed that for each legume cultivar there was no extremely significant difference among selected rhizobial strains. Table 2 shows Thai recommended soybean, groundnut and mungbean cultivars and their compat-

Table 2. Recommended soybean, groundnut and mungbean cultivars with their selected rhizobial strains in Thailand.

Legume	Cultivar	Rhizobial strain
Soybean	SJ 1, SJ 2, SJ 4 and SJ 5 Nakorn Sawan 1 (NW 1) Chaing Mai 60 (CM 60)	THA 5, THA 7, THA 1, 2, TAL 102 (USDA 110) USDA 122
Groundnut	Lampang Sukhothai 38 (SK 38) Tainan 9 Khon Kaen 60–1 (KK-1) Khon Kaen 60-2 (KK-2) Khon Kaen 60-3 (KK-3)	THA 201, THA 205, TAL 1000, NC 92
Mungbean	U-Thong 1 (UT 1) Chainat 36 (CN 36) Chainat 60 (CN 60) Kampangsaen 1 (KPS 1) Kampangsaen 1 (KPS 1) PSU 1	THA 301, THA 302, THA 305

Source: Soil Microbiology Research Group, Soil Science Division, DOA

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ible rhizobial strains for inoculant production. These rhizobia are distributed on request for private inoculant production also.

Inoculant carrier selection

Selection of the inoculant carrier is the second concern for rhizobial inoculant production. At the very beginning, we produced inoculant using ground and sieved compost and coconut shell dust as carriers. At present, most of the inoculant produced in Thailand uses peat dust as the carrier. But liquid inoculant is being considered to replace the need for solid carriers.

Patiyuth et al. (1985) compared the use of lignite and peat as carriers. They concluded that survival of rhizobia was better in peat carrier. Using peat-lignite mixed enhanced rhizobial survival better than using lignite alone. The number of rhizobia in carriers stored cold (10ºC) was higher than when stored at room temperature (Table 3) after long periods of incubation. The results of this experiment were useful for setting time schedules of inoculant production for farmers.

Further study on production and application of liquid inoculant is in progress. A Thai DOA researcher and another from the private sector are now testing its efficiency and feasibility. This kind of inoculant is said to survive longer with 10–100 times higher the number of survival cells than that of the peat-based inoculum, and the cost of production is about 4–5 times less (Susewee 1994). Thananusonth et al. (1994) produced liquid inoculum of *Bradyrhizobium japonicum*, tested it in the field, but found no significantly different response of soybean compared to the use of peat-based inoculant of the same strain.

Inoculant application

Production of highly effective inoculant will be wasteful if application methods and its value cannot be accepted by farmers. There are many diverse situations such as environmental stresses affecting the benefits from inoculant application. Therefore, several experiments were carried out in the field to overcome these problems.

Rate of application

Studies on the rate of inoculant application were carried out to set up standards in Thailand. Boonkerd et al. (1974) tested the effects of five inoculum rates with soybean at three locations (Table 4). They found that increasing inoculum rates up to 100 and 1000 times over normal (10^6 cells of rhizobia per seed) significantly increased nodulation and seed yield of soybean when grown in the field were essentially free of soybean rhizobia, low pH and low fertility (Roi-Et). But when the soil was amended by liming, inoculation at the normal rate was necessary to achieve fair nodulation and increased yield of soybean. In fields where soil fertility was high enough (Hua-Hin and Chainat) an inoculation rate higher than normal was not necessary.

Rungratanakasin et al. (1984) studied a suitable number of rhizobia for inoculating groundnut at three locations. Results of experiments (Table 5) showed that inoculating the groundnut cultivar Tainan with 10^5, 10^6, 10^7 and 10^8 cells per seed (THA 205-peat based inocula) produced no significant difference on nodule fresh weight and pod yield. A slightly significant difference occurred only between inoculated and uninoculated treatments.

From those experiments, standards set up in Thailand were that, under field conditions, an inoculated seed should receive 10^5 to 10^6 cells of rhizobia (Boonkerd 1991). Thus, an acceptable quality inoculant must contain at least 5×10^7 cells of rhizobia per gram (peat-based inoculum).

Table 3. Log_{10} number of viable cells of rhizobia using peat and lignite as carriers stored in room and cold temperature after various of incubation, Patiyuth et al. 1985.

Days after inoculation	Carriers	Room temperature			Cold (10ºC) temperature		
		USDA 110	USDA 122	THA 7	USDA 110	USDA 122	THA 7
0	Peat	8.948	9.120	9.208	8.948	9.120	9.208
	Lignite	8.500	8.566	8.650	8.500	8.566	8.650
15	Peat	8.909	9.257	9.024	8.982	9.109	9.150
	Lignite	7.738	8.142	8.240	8.150	8.309	8.560
30	Peat	8.914	9.148	9.002	9.060	9.146	9.135
	Lignite	7.574	7.556	7.478	8.236	8.356	8.548
60	Peat	8.700	9.096	8.990	9.086	9.090	9.112
	Lignite	7.204	7.346	7.327	8.168	8.463	8.654

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Table 4. Responses of soybean to inoculation rates.

Inoculation rate	Roi-Et			Hua-Hin			Chainat		
	Seed yield (kg/rai)	Nodule number (/pl)	Nodule mass (mg/pl)	Seed yield (kg/rai)	Nodule number (/pl)	Nodule mass (mg/pl)	Seed yield (kg/rai)	Nodule number (/pl)	Nodule mass (mg/pl)
1 x	105	12	310	223	9	230	217	10	230
10 x	150	19	570	229	8	160	223	12	240
100 x	205	23	800	254	9	210	228	18	340
1000 x	113	18	630	215	11	240	235	15	250
0 (Uninoc)	51	<1	20	203	4	245	245	8	160
1 x + Lime	269	44	950	226	6	130	217	8	200
10 x + Lime	301	49	910	235	9	130	202	13	290
100 x + Lime	343	64	1290	239	9	180	217	15	250
1000 x + Lime	369	87	1730	244	8	100	213	16	290
0 + Lime	207	4	250	231	6	80	213	10	210

Table 5. Responses of groundnut to inoculum sizes at three locations.

Inoculum size	Nakornratchasima		Mahasarakarm		Chainat	
	Nodule fr. wt (mh/pl)	Dry pod (kg/rai)	Nodule fr. wt (mh/pl)	Dry pod (kg/rai)	Nodule fr. wt (mh/pl)	Dry pod (kg/rai)
0	30 b	212 b	1000	393	1400 bc	308 b
10^5	550 a	353 a	1100	379	1600 b	332 ab
10^6	390 a	368 a	1400	374	1200 c	354 ab
10^7	570 a	360 a	1200	400	2700 ab	378 a
10^8	510 a	335 a	1500	364	3400 a	378 a
C.V. (%)	31.8	19.6	26.1	9.6	28.6	9.2

Method of inoculant application

Kotepong et al. (1986) studied the number of rhizobia retained on soybean seed after inoculation with peat inoculum using synthetic glue, gum arabic, vegetable oil, tapioca starch 5% boiled, 30% sugar syrup and water as stickers. Results (Table 6) showed that these stickers could retain 1.15×10^6 to 8.4×10^5 cells of rhizobia a seed. Toomsaen et al. (1986) mixed inoculant with soil then inoculated the seeds in shallow furrows or inoculated the seed by pouring peat inoculant solution on to the seeds. This produced better yield than using 40% gum arabic and 40% sugar syrup as stickers. This result supported the finding of Rungratanakasin (1985) who used the same methods to study rhizobial inoculation techniques for peanut.

Rungratanakasin (1986) reported that inoculation with calcium carbonate, gypsum and rock phosphate as seed pelletting increased nodulation and nitrogenase activity over the uninoculated but not seed yield of groundnut. Furthermore, post inoculation can be carried out by pouring inoculum dilution to the rows of soybean as late as two weeks after sowing (Table 7).

Extension Program to Promote Rhizobial Inoculants in Thailand

Most rhizobial legume inoculant extension work in Thailand has been conducted by Department of Agricultural Extension (DOAE) while Department of Agriculture (DOA) is responsible for inoculant production and multi-disciplinary research. The rhizobial promotion includes the training of extension workers at the BNFRC, on-farm trial activities in specific localities to provide information to extension specialists and farmers, and organising field days to demonstrate the results and profitability of the inoculant. (Chanaseni 1991).

Distribution of the rhizobial inoculant during the early stages of production (1977–1981) was

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performed by DOAE and Market Organisation for Farmers (MFO) as shown in Table 8. In 1982, DOAE started a Seed Exchange Program in order to boost soybean production in Thailand. This 5-year program focused on soybean expansion and improvement of production efficiency. Participating

Table 6. Number of rhizobial cells per seed retained at different time after inoculation (Kotepong et al. 1986).

Adhesive agent	Concentration % (w/v)	Time after inoculation (hr)			
		0	2	4	6
Synthetic glue	100	5.30×10^5	1.45×10^6	8.40×10^5	4.55×10^5
Gum arabic	40	1.55×10^5	3.00×10^5	2.75×10^5	1.45×10^5
Vegetable oil	100	2.45×10^4	2.35×10^4	2.10×10^4	1.40×10^4
Tapioca starch solution	5	7.40×10^4	2.29×10^5	5.80×10^4	4.30×10^4
Sugar syrup	30	8.80×10^4	8.00×10^4	6.10×10^4	6.10×10^4
Water	100	2.40×10^4	1.55×10^4	1.45×10^4	1.15×10^4

Table 7. Effect of post inoculation on nodulation and yield of soybean at two locations (Rungratanakasin et al. 1983).

Inoculation days after sowing	Khon Kaen			Shachoengsao		
	Nodule no. (/pl)	Nodule wt. (g/pl)	Yield (kg/rai)	Nodule no. (/pl)	Nodule wt. (g/pl)	Yield (kg/rai)
0	52 ab	1.8 a	264 a	16 ab	1.9 a	330 ab
5	46 ab	1.3 ab	262 a	12 ab	1.2 ab	357 a
10	35 b	1.2 ab	258 a	11 bc	0.7 b	291 abc
15	49 ab	1.2 ab	235 ab	28 a	0.7 b	275 bc
20	59 a	1.0 b	230 ab	3 bc	0.1 c	226 c
Uninoc.	0	0	111 c	0 c	0 c	249 c

Table 8. Rhizobium production by the Thai Department of Agriculture (DOA), and distribution through three sectors: 1) Thai Department of Agricultural Extension (DOAE), 2) Private Sector (PS), and Marketing of Farmer Organisation (MFO), from 1977–1990.

	Tonnes inoculant		Bag's inoculant distributed			Total quantities of inoculant distributed	
Year	Producted	Used	DOAE	PS	MFO	Bags	Value
1977	5.00	3.36	6,950	—	9,865	16,815	6,467
1978	10.59	11.55	17,523	—	40,430	57,753	22,213
1979	7.42	5.77	22,296	—	6,548	28,844	11,094
1980	4.92	5.64	16,761	—	11,429	28,190	10,842
1981	7.48	7.36	26,649	—	10,164	36,813	14,159
1982	6.58	6.64	23,877	8,584	3,763	33,224	12,778
1983	14.36	13.15	34,557	30,079	1,104	65,740	25,285
1984	36.16	33.79	112,073	56,885	—	168,985	64,994
1985	48.77	46.51	157,323	75,264	—	232,577	89,453
1986	78.00	74.78	285,796	88,115	—	373,911	143,812
1987	81.63	79.79	248,595	150,378	—	398,973	153,451
1988	140.70	136.23	593,941	90,237	—	681,178	261,992
1989	134.27	125.30	557,527	68,996	—	626,523	240,970
1990	126.35	117.67	557,772	30,578	—	588,350	226,288
1991	73.78	72.30	338,006	23,500	—	361,506	129,453
1992	98.44	92.81	445,454	18,621	—	464,075	203,448

[1] One bag contains 200 gm inoculant at a cost of 10 baht ($US 0.40)

farmers were allowed to exchange their local variety seed for selected varieties produced by Government Seed Centres at a 1:1 ratio. Participating farmers were encouraged to buy a 200 g bag of inoculant for every 10 kg of exchanged seed at B10 per bag (B25 = US$1) which enable them to grow 1 rai (6.25 rai = 1 hectare). Production and distribution of inoculant expanded rapidly from 33,200 bags to 373,900 bags or more than ten times because of this program. In order to promote the use of inoculant, DOAE also developed cooperation with the private sector, especially with local dealers close to the cultivated areas to sell inoculant to remote farmers who may need to purchase only small quantities.

A Soybean Marketing and Production Development Project was initiated in 1987 following the Seed Exchange Program. This 5-year project was slightly different from the first program. Farmers were allowed to purchase superior variety seed at B10 per kilogram instead of the normal price of B15, provided they also purchased a bag of inoculant at the normal price of B10. To promote the use of inoculant, DOAE also provided a mobile unit to attract local farmers with training activities, slide presentations on-farm demonstration, field days and inoculant sales.

A similar program was also initiated for groundnut in 1987. Since the use of inoculant on groundnut was not common, farmers were given a bag of inoculant free of charge for a purchase of one kilogram of groundnut seed at B10.

A Soybean Joint Venture Project was conducted between 1989 and 1990. Under this project, the private sector also produced inoculant and distributed approximately 100,000 bags a year to the farmers who received credit from the BAAC to purchase agricultural material such as seed, fertiliser, rhizobial inoculants and pesticide from a certain contracted private company. A major component of the project was an agreement that the contracted company had to purchase farm produce from the farmers at a guaranteed price to ensure a stable market. It was reported that in 1990 the use of rhizobial inoculant produced by DOA were as follows: 477,333 bags for soybean (95.5 t) 72,746 bags for groundnut (14.5 t) and approximately 38,000 bags for mungbean and other leguminous crops.

The Soybean Joint Venture Project was extended from 1990 to 1996 in other to increase soybean production. In 1993, DOAE distributed 3598 tonnes of good quality seed to participating farmers in 33 provinces at B2.0 per kg plus free rhizobial inoculant.

In conclusion, the use of rhizobial inoculants in Thailand increased gradually because of several government programs. A monitoring and evaluation report on the use of inoculant by DOAE showed that in the major growing area the number of inoculant users increased from 30% in 1986–87 to 51% in 1989–90 for soybean and from 9.8% to 22% for groundnut during the same period, as shown in Table 9. However, the percentage of inoculant users will increase further as the government promotion programs continue.

Emergence of the Private Sector

Unlike other farm inputs, rhizobial inoculant required a certain level of production technology. Furthermore, market demand was uncertain. Therefore, it took 10 years before the first private firm entered the market in 1988. Since rhizobial inoculant was rather new to the farmers, marketing of the product was the major problem of the producers. Besides, storage of rhizobium at room temperature for a long period reduced the quality of the product. It was reported that there were four private firms producing rhizobial inoculant in 1989. At present, there are only two firms remaining. The estimated production of rhizobial inoculant by the private sector from 1988–1993 is shown in Table 10.

It can be observed that production of rhizobial inoculant by the private sector fluctuated from 200,000 to 300,000 bags depending upon the demand from the public sector in the government supported programs as mentioned earlier. At present, both firms are producing much below their capacities.

The sale of rhizobial inoculant to government supported programs was based on a bidding process. The wholesale price was around B7 per bag and the government program sold to the participating farmers at the normal market price of B10 per bag at the beginning of the Seed Exchange Program. However, at present, it is given free of charge.

Table 9. Monitoring and evaluation report (MER) on use of rhizobia inoculants (DOAE 1990).

	Percentage of crops inoculated with rhizobia			
Crops	1986–87	1987–88	1988–89	1989–90
Soybean	30.3	44.8	52.4	50.9
Groundnut	9.8	12.3	17.4	22.0

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Another marketing channel for privately produced rhizobial inoculants was through the provincial wholesalers located in the important legume producing province. The wholesalers then distributed inoculants to retailers in their own provinces. The wholesale price was around B6–7 per bag and the retail price was normally fixed at B10 per bag. The difference between privately produced inoculant and the DOA product was that the private firm used sterile peat at 100 grams per bag instead of 200 grams of non-sterile as produced by DOA. However, both sizes were recommended for use with 10 kg of seed to plant 1 rai of land (0.16 hectare).

Growth of Rhizobial Inoculant Production and Price

As mentioned earlier, soybean rhizobial inoculant has played an important role in the development of the inoculant in Thailand. At present, about 90% of the inoculant produced in Thailand is aimed at increasing soybean production. Table 11 shows that production of soybean inoculant increased from 7400 bags in 1980 to 1.0 million bags in 1993, a growth rate of 199%. For groundnut and mungbean, even though the rates of growth are high, the share of production was small, especially in 1993.

Nonetheless, the use of rhizobial inoculant in Thailand depends heavily on the promotion of the public sector. At present, where government policies have been geared towards an increase in soybean production for domestic requirements and groundnut and mungbean as substitute crops of paddy in the dry season, it is expected that there will be a strong demand of rhizobial inoculant, especially through the government-supported programs. With regard to inoculant price, there is no evidence that the retail price will change from B10 per bag. However, private producers complained that it was unfair that the participating farmers in the government-supported program received free inoculant for the purchase of quality seed while other farmers had to pay for the inoculant.

Table 10. Production of rhizobial producted by the private sector classified by type of legume. Unit: bag (100 gram).

Year	Soybean	Groundnut	Mungbean	Total
1988	200,000	—	—	200,000
1989	221,924	25,592	7,764	255,280
1990	310,521	43,329	13,676	367,526
1991	192,180	21,215	10,705	224,100
1992	118,512	213,746	9,382	341,640
1993	248,700	13,013	7,038	268,750

Table 11. Growth of rhizobial inoculant produced by DOA and the private sector classified by types of legumes. Unit: bag (200 g).

Year	Soybean	Groundnut	Mungbean	Others	Total
1980	7,400	12,920	7,318	552	28,190
1981	5,950	20,020	10,756	87	36,813
1982	5,184	13,380	5,907	8,456	32,927
1983	39,162	13,979	7,932	4,667	65,740
1984	97,469	30,482	48,893	2,114	178,958
1985	108,586	21,251	97,115	5,635	232,587
1986	217,988	66,699	83,763	5,461	373,911
1987	347,961	21,849	16,251	12,876	398,937
1988	778,502	46,850	51,626	4,200	881,178
1989	709,110	96,082	72,366	4,245	881,803
1990	787,853	116,074	49,137	2,810	955,876
1991	476,666	66,817	41,169	945	585,597
1992	396,790	275,762	132,675	488	805,715
1993	1,032,899	59,053	34,049	432	1,126,433
Total	5,011,520	861,218	658,957	52,968	6,584,665
Growth rate (%)	199	280	270	−7.8	287

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Benefit of Legume Inoculants

Physical impact of rhizobial inoculant in Thailand

As in the other countries, rhizobial inoculant has shown a great potential for increasing legume crop yield. However, research on rhizobial inoculant used has been conducted repeatedly in several legume-growing areas in Thailand to confirm the result of the experiments in various soil types of both growing seasons. The main objective of the research on rhizobial inoculant has been on the effect on legume crop yield, return on investment and the nitrogen replacement by rhizobial inoculant.

Table 12 shows the physical impact of soybean rhizobial inoculant in both wet and dry seasons. The average yield revealed a difference of about 40 kg/ha of soybean grain with the use of rhizobial inoculant over the control plot in both seasons. The experiment on the use of rhizobial with P_2O_5 and K_2O at 56.25 and 37.5 kg/ha also showed a favorable result over N:P:K of 75-56-37 at a difference of 130 kg/ha in both seasons.

Table 12. Physical impact of rhizobial inoculant in wet and dry season.

Treatment	Yield (kg/ha)	
	Wet season	Dry season
Control	803	982
Rhizobium	1228	1377
R + (P − K) (56.25 + 37.5)	1352	1559
N:P:K (75:56:37)	1224	1426

N replaced by R (6.25 bags)

Wet season 1224 kg = 75 kg N

$$1352 \text{ kg} = \frac{75 \times 1352}{1224} = 82.8 \text{ kg N} = 180 \text{ kg of Urea}$$

Dry season

$$= \frac{75 \times 1559}{1426} = 89.0 \text{ kg N} = 178.3 \text{ kg of Urea}$$

Average both seasons = 179.2 kg of urea

Nitrogen replacement was calculated in term of urea fertiliser. It was found that 1250 g of inoculant can replace about 179.2 kg of urea fertiliser in both growing seasons or one bag of inoculant for 28.6 kg of urea.

The use of rhizobial inoculant on groundnut and mungbean also showed a positive response. Groundnut yield response to cowpea rhizobium of Tainan 9, the most common cultivar, showed an additional increase of 396.9 kg/ha and 126.9 kg/ha for mungbean rhizobial inoculant, as shown in Table 13.

Table 13. Physical impact of rhizobial inoculant used on groundnut and mungbean yield. Unit: kg/ha.

Treatment	Groundnut	Mungbean
Control	1412.5	1055.0
Rhizobium	1809.4	1181.9
Difference	396.9	126.9

Economic Impact of Rhizobial Inoculant in Thailand

The economic impact of rhizobial inoculant was derived from the physical impact by estimating costs and returns from the use of rhizobial inoculant. Table 14 shows the economic impact on soybean in wet and dry seasons. The use of rhizobial inoculant alone without adding chemical fertiliser on soybean in wet and dry seasons showed the highest net benefit over the control plot at $126.7 and $144.2 per ha respectively. This was mainly due to the high cost of chemical fertiliser.

The economic impacts of rhizobial inoculant used on groundnut and mungbean are also shown in Table 13. Net benefit of using rhizobial inoculants on groundnut and mungbean over the control plots were $91.5 and $36.2 per rai respectively. The use of rhizobial inoculant with additional chemical fertiliser did not reveal higher physical and economic return and was therefore negligible.

Estimated Cumulative Impact of Inoculant at National Level

The cumulative impact of inoculant, calculated from the additional benefit of inoculant in soybean, groundnut and mungbean from 1980–93, is:

$$Abt = Rit\ dYi\ Pit;$$

Where Abt = Accumulative benefit in year t;
Rit = Quantity of inoculant used on legume I in year t;
DYi = Yield increase from inoculant use on legume I;
Pit = Prince of legume I in year t;
Vit = Value of inoculant use on legume I in year t.

The estimated benefit from inoculants classified by types of legumes during 1980–93 was shown in Table 14. The total benefit of soybean inoculant from 1980–93 was US$100.2 million or more than 80% of the total benefit.

For the economic benefit of inoculant in substitution of nitrogen fertiliser, only soybean inoculant was used in the calculation. Price of urea fertiliser (Table 16) was used to estimate the value of N-fertiliser as shown in Table 16. The total value of N-fertiliser replaced by rhizobial inoculant was US$25.9 million.

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Table 14. Economic impact of soybean rhizobial inoculant in wet and dry seasons. Unit: US$/ha.

Treatment	Wet season			Dry season		
	Revenue	Additional cost	Net benefit over control	Revenue	Additional cost	Net benefit over control
Control	244.1	0	0	364.9	0	0
Rhizobial	373.3	2.5	126.7	511.7	2.5	144.3
R + (P – K) (56 – 37)	411.0	57.2	109.7	579.3	71.5	142.9
N:P:K (75:56:37)	372.1	101.5	26.5	529.9	100.0	65.0

For dry season:
Soybean price at $ 0.37/kg
Urea (46-0-0) at $ 0.19 kg. T.S.P at $0.35 kg, KCl at $ 0.18/kg
Fertiliser application at $15/ha, rhizobium at 2.5/ha
For wet season :
Soybean price at 7.60 B./kg
Urea (46-0-0) at 4.90 B./kg, T.S.P at 9.65 B./kg, KCl at 4.90 B./kg

Table 15. Economic impact of rhizobium inoculant used on groundnut and mungbean. Unit: f baht/ha.

Treatment	Groundnut			Mungbean		
	Revenue[1]	Additional cost	Net benefit over control	Revenue[2]	Additional cost	Net benefit over control
Control	351	0	0	322.5	0	0
Rhizobium	445	2.5	91.5	361.2	2.5	36.2

[1] Farm gate price of groundnut = $ 0.25 per kg
[2] Farm gate price of mungbean = $ 0.31 per kg

Table 16. Cumulative impact of rhizobial inoculant classified by types of legume during 1980–93. Unit: million US$.

Year	Soybean	Groundnut	Mungbean	Total
1980	0.1	0.3	0.04	0.44
1981	0.1	0.3	0.06	0.46
1982	0.1	0.2	0.03	0.33
1983	0.6	0.3	0.04	0.94
1984	1.5	0.4	0.26	2.16
1985	1.7	0.4	0.50	2.60
1986	3.5	0.8	0.40	4.70
1987	7.3	0.4	0.10	7.80
1988	17.3	0.9	0.36	18.56
1989	13.6	2.0	0.37	15.97
1990	15.2	2.4	0.25	17.85
1991	9.8	1.5	0.34	11.64
1992	8.1	5.9	1.20	15.20
1993	21.3	1.2	0.25	22.75
Total	100.2	17.0	4.20	121.40

Conclusions and Recommendations

The development of the legume inoculant industry started by the public sector in 1976 under the USAID loan in accordance with the establishment of Seed Centres in Thailand. The main objective of the program was to promote the use of inoculant in order to increase important legume products such as soybean, groundnut and mungbean to meet domestic demand. Most of the inoculant production was used in the government-supported programs.

Research on inoculant has long been conducted by many government agencies prior to the construction of the plant. After the construction, research and demonstrations have been conducted in various locations in Thailand. After the establishment of the Biological Nitrogen Fixation Resource Centre (BNF Resource Centre) at DOA in 1983, it was used as an international training centre for training in inoculant technologies for scientists from all over the world.

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With regard to the potential of the inoculant industry, the following recommendations should be considered.

1. More on-farm research and demonstrations on the use of inoculant should be conducted in order to convince farmers of the benefits of inoculant use.
2. Government should stop providing free inoculant to farmers and the price of inoculant should be determined by its cost of production.
3. Research on inoculant production technology should be conducted in order to obtain a high quality product.

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Legume Inoculants and Quality Control

David Herridge[1], Greg Gemell[2], Elizabeth Hartley[2]

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 (N_2 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 $>10^9$ rhizobia/g and $<10^6$ contaminants/g. Other countries demand that inoculants deliver high numbers of rhizobia to the inoculated seed, e.g. 10^6/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

[1] NSW Agriculture, Centre for Crop Improvement, Tamworth, NSW 2340, Australia
[2] NSW Agriculture, Horticultural Research and Advisory Station, Gosford, NSW 2250, Australia

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edited by D. Herridge
ACIAR Proceedings 109e
(printed version published in 2002)

"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 N_2 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 N_2 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 N_2-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. trails	% 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

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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-leafed 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

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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.

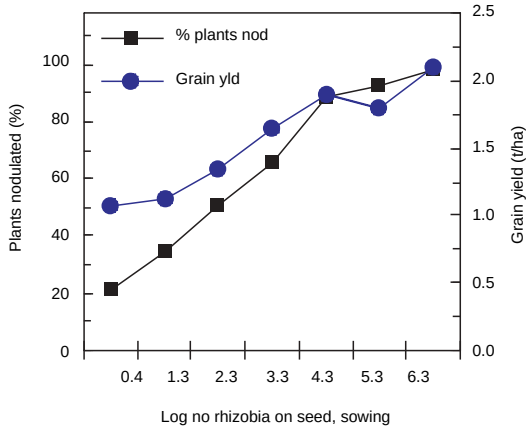

Figure 1. Relationship between numbers (log_{10}) of rhizobia on the narrow-leafed lupin seed at sowing and % plant nodulated and grain yield (data from Roughley et al. 1993).

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 × 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-leafed 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 fababean (WSM1271). The pass rate of the standard (i.e. 1 × 10^9/g peat) would have been 88 and 94% for

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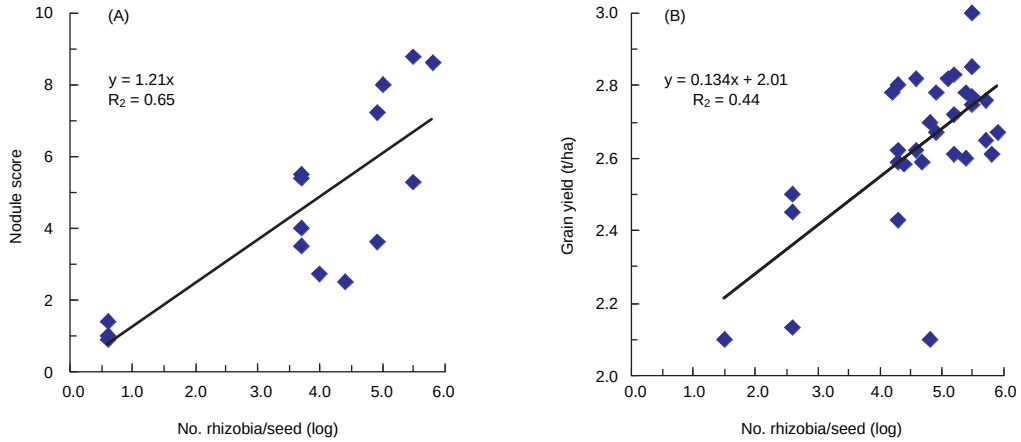

Figure 2. Relationships between the number of rhizobia on soybean seed at sowing and (A) plant nodulation, and (B) grain yield. All inoculants used in this field study in Alabama, USA, during 1976 and 1978 were commercial inoculants. Variations in rhizobial numbers on seed reflected variation in the 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 (× 106) (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 comprimised 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 — fababean	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 $<10^8$ 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 $<10^8$ 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 10^3–10^5 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

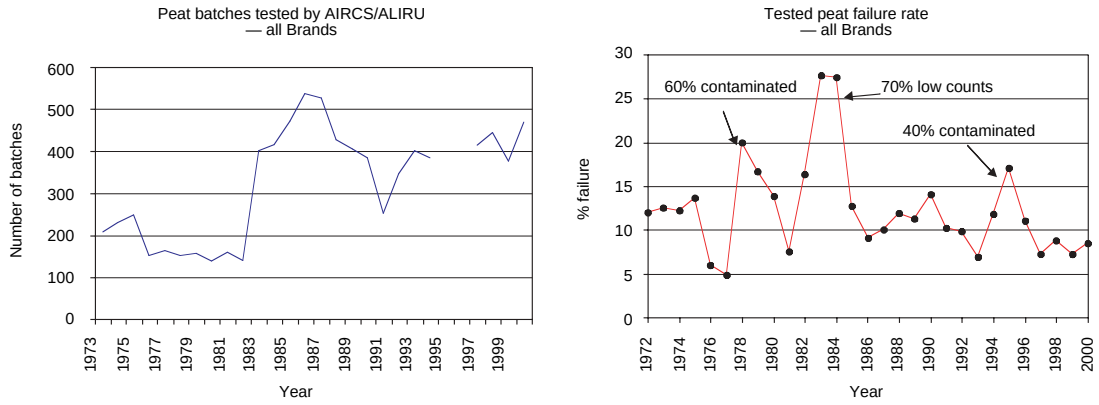

Figure 3. Numbers of batches of commercial peat inoculants (A) tested in Australia between 1973 and 2000, and (B) failure rate of the tested batches (data from Gemell, Hartley and Herridge, unpublished).

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

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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 receival and for up to 16 days after receival. 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

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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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List of Participants

Dr Nguyen Xuan Thanh
Department of Soil Management
University of Agriculture 1, Hanoi
VIETNAM
Email: ngxthanh123@yahoo.com

Prof. Pham Van Bien
Director of Institute of Agricultural Science of South Vietnam (IAS)
Ho Chi Minh City
121-Nguyen Binh Khiem St.
District 1, Ho Chi Minh City
VIETNAM
Email: pvbien@hcmc.netnam.vn

Prof. Phan Lieu
Oil Plant Institute
171–175 Ham Nghi St.
District 1, Ho Chi Minh City
VIETNAM
Email: opi.vn@hcm.vnn.vn

Dr Nguyen Thanh Hien
Faculty of Biology
University of Natural Science, Hanoi
336 Nguyen Trai St., Hanoi
VIETNAM
Email: hnuniversity@vnn.vn

Dr Pham Van Toan
Vietnam Agricultural Science Institute
Thanh Tri District, Hanoi
VIETNAM
Email: pvtoan@hn.vnn.vn

Dr Cao Ngoc Diep
Biotechnology Research and Development Institute
Cantho University (CTU)
VIETNAM
Email: cndiep@ctu.edu.vn

Dr Nguyen Huu Hiep
Biotechnology Research and Development Institute
Cantho University, CTU
62/16A Tran Viet Chau, Cantho
VIETNAM
Email: nhhiep@ctu.edu.vn

Dr Nguyen Thi Lien Hoa
OPI, 171 —Ham nghi —
Ho Chi Minh City
VIETNAM
Email: opi.vn@hcm.vnn.vn

Ms Tran Yen Thao
OPI, 171 — Ham nghi —
Ho Chi Minh City
VIETNAM
Email: yenthao@hcm.ftp.vn

Mr Ha Huu Tien
IAS
Hung loc Agricultural Resource Center
Thong nhat, Dong nai
VIETNAM
Email: tienhanuyen@vol.vnn.vn

Mrs Tran Minh Hien
IAS (Institute of Agricultural Science)
121 Nguyen Binh Khiem
District 1, Ho Chi Minh City
VIETNAM
Email: saf@hcm.vnn.vn

Dr Do Nguyen Hai
Department of Soil Science
Faculty of Land Water Management
Hanoi Agricultural University
VIETNAM
Email: donguyenhai@yahoo.com

Dr Tony Fischer
ACIAR House
Traeger Court, Fern Hill Park
Bruce ACT 2617
Canberra ACT 2610
AUSTRALIA
Email: fisher@aciar.gov.au

Dr David F. Herridge
NSW Agriculture
Centre for Crop Improvement
RMB 944, Tamworth, NSW 7340
AUSTRALIA
Email: david.herridge@agric.nsw.gov.au

Dr Jo Slattery
Rutherglen Research Institute
Department of Natural Resources and Environment, Australia
RMB 1145 Rutherglen 3685, Victoria
AUSTRALIA
Email: jo.slattery@nre.vic.gov.au

Dr Graham O'Hara
The University of Western Australia
Nedlands, WA 6009
AUSTRALIA
Email: gohara@central.murdoch.edu.au

Dr Paul Singleton
NifTAL, 1000 Holumua Road
Paia, HI 96779
USA
Email: niftal@hawaii.edu

Dr Natakorn Boonkerd
School of Biotechnology
Institute of Agricultural Technology
Suranaree University of Technology
Nakhon Ratchasima
THAILAND
Email: nantakon@ccs.svt.ac.th