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Australian Centre for International Agricultural Research

Final report

project

Pasture development for community livestock production in the Eastern Cape province of South Africa

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Contents

1 Acknowledgments

This project, 'Pasture development for community livestock production in the Eastern Cape Province of South Africa' generally referred to as ECCAL (Eastern Cape Community Arable Lands) was envisioned at a time (in 2004) when Australian aid investments in South Africa for Agricultural Research were in their infancy. There was no firm road map to work with in this part of the world and as such we had to develop new relationships and new procedures.

This project would not have been possible without the unqualified enthusiasm and continued investment and belief in the science of legumes from the staff and management of the Dohne Research Station, Eastern Cape Department of Agriculture, RSA. The staff at Dohne were an inspiration and a pleasure to work with.

Further, the community leaders with whom we interacted for the duration of the project, and who provided their land and resources to us, deserve our thanks. We specifically thank Mr Mlumbi from Roxeni, Colonel Dudumashe from the Dudamashe community, and the Lushington, Nyandeni and Kubledana communities. We trust the legumes have improved your farming systems and will continue to do so.

ECCAL greatly benefitted from the continued energy and support of several scientists associated with Murdoch University, from the first legume experiments planted in September 2006 until the last in 2013. We would specifically thank Mr Neil Ballard, Mr Pieter Conradie, Mr Gideon Jordaan, Mr John Davis and Dr Ron Yates for giving their time and effort to this project.

At various times in the project we benefitted from interaction with academics in RSA, no more so than Prof Ben-Erik van Wyk from Johannesburg University, who is an expert in legume taxonomy.

ACIAR, and in particular, Dr Peter Horne and Catherine Hanley are thanked for their support. We also thank Bill Winter (ACIAR) and Amie Auchamp (NWGA, RSA) for early comments on the funding proposal.

2 Executive summary

The Eastern Cape agricultural lands in RSA are characterised by a rolling veld, dominated by several perennial C4 grass genera such as *Themeda, Eragrostis* and *Hyparrhenia*. The soils are red brown earths, moderately acidic, deficient in P, N and K, but with excellent agricultural potential because of their relatively high clay content and water holding capacity. Whilst predominantly grazing lands, this potential has seen them cultivated in recent history, for maize production. This practice, which includes repeated deep tillage, leads to erosion and a loss of soil structure, and ultimately abandonment of cropping – hence the term “abandoned arable lands”. Low value grasses such as *Cynodon,* however, can come to dominate after disturbance and the veld does not return to its former productive state.

This project, called ECCAL, was borne out of the need for the cropped arable lands to be stabilised back into permanent grazing. Associations such as the National Woolgrowers Association had identified that improving the feedbase was one route towards improving the productivity and profitability of the emerging farmers in the region. Cultivation had changed the ecology of the grass systems, and whilst they might eventually recover, there was both a need, and an opportunity, to increase the value of the forage during this recovery period. Legumes were considered as a means to achieve this, however the challenge became that of matching legume species, to the soil type and the relatively uncontrolled grazing pressures.

It was initially difficult to see an obvious fit for the commercially available grazing legumes to the climate and soils of the Eastern Cape (EC). Was the area in a temperate environment with appreciable summer rain, or perhaps a cool subtropical environment with significant winter rainfall? Local experience told us that the global “staple” legumes such as white clover, annual medics, lotus, lucerne and crown vetch from temperate regions, and siratro, stylosanthes, desmodium from the sub tropics were not suited to much of the EC in a climatic sense, and certainly did not tolerate the grazing management used. Our first experiments in 2006, therefore, were a series of legume “genebank” explorations at three research stations spread over a 500 km north-south range from Tsolo near Umthata to Mpofu, sown in both spring and autumn. The new legumes evaluated included the hard seeded annual species domesticated in WA during the 1990s, as well as the subtropical species that emerged from the parallel CSIRO program in Brisbane. The response to inoculation with appropriate rhizobia was also evaluated in these experiments.

It became evident that several hardy, acid tolerant species were well suited to the edaphic environment, but there was significant variation across the latitudes and altitudes. The perennial species Lespedeza, Lotononis and Desmodium were successful at some sites, whilst the annual species biserrula, serradella and arrowleaf clover were broadly successful, particularly in the southern sites which received winter rainfall.

The program moved to small plot trials on community lands in 2008, where grazing was imposed, and evolved to sites as large as 10 ha by 2010. There were some spectacular successes, with some legumes beginning to colonise and even dominate some sites, despite relatively uncontrolled grazing. *Lespedeza cuneata* was an outstanding success at Lushington. A mixture of arrowleaf clover, biserrula and common vetch produced in excess of six tonnes of biomass over winter at Roxeni, when the perennial grasses were inactive. This provided high value stock feed during the traditional time of animal starvation, and controlled experiments showed a doubling of live-weight gain for sheep grazing the legumes relative to those on unimproved veld. At Kubledana, the stock preferred to graze the legumes rather than the new shoots of regenerating grasses.

Seed increase activities for the successful (but non-commercially available) species were established as a small business opportunity by several communities in 2013 to supply seed on a commercial basis in the future.

In conclusion, grazing-tolerant legumes of different phenologies can be introduced to the veld in the EC to assist in rehabilitation of ploughed lands and to increase soil fertility, and that large improvements in sheep health and production can result. The ECCAL project showed the potential application of pasture legumes in this region to be massive. ECCAL funds leveraged large inputs from the ECDA, and working with local Departments of Agriculture is a model worthy of consideration for small project investment in Africa.

The research will also be of significant benefit to Australian farmers through the discovery, in the Western Cape region, of new grazing legumes such as *Lebeckia ambigua*. This plant has proven adapted to the deep and infertile sands of Western Australia, which receive low and variable rainfall. Agronomic experiments in the wheatbelt of WA are optimising the rhizobia for this legume (Howieson et al 2013; de Meyer et al 2013a,b,c) whilst simultaneously developing the first cultivars for commercialisation. Implementation of *L. ambigua* across WA and NSW could be worth in excess of $50 million pa in the long term, if the drying climate trends continue and farmers move towards permanent grazing enterprises on some soils.

3 Background

The majority of the Eastern Cape (EC) Province of South Africa (Figure 1) is occupied by 4 million African people living in pastoral communities that are characterised by a subsistence economy based primarily on livestock production (cattle, goats, sheep), underpinned by state welfare transfers and urban migrant remittances. However, agricultural potential is high as most of the EC receives 500-900mm annual rainfall and many areas have soils of good structure. The National Government places a high priority on the development of the EC economy, with a particular emphasis on the development of the livestock sector, given the high demand for beef, mutton and goat meats and the high populations (but poor off-take) of these livestock within the community groups.

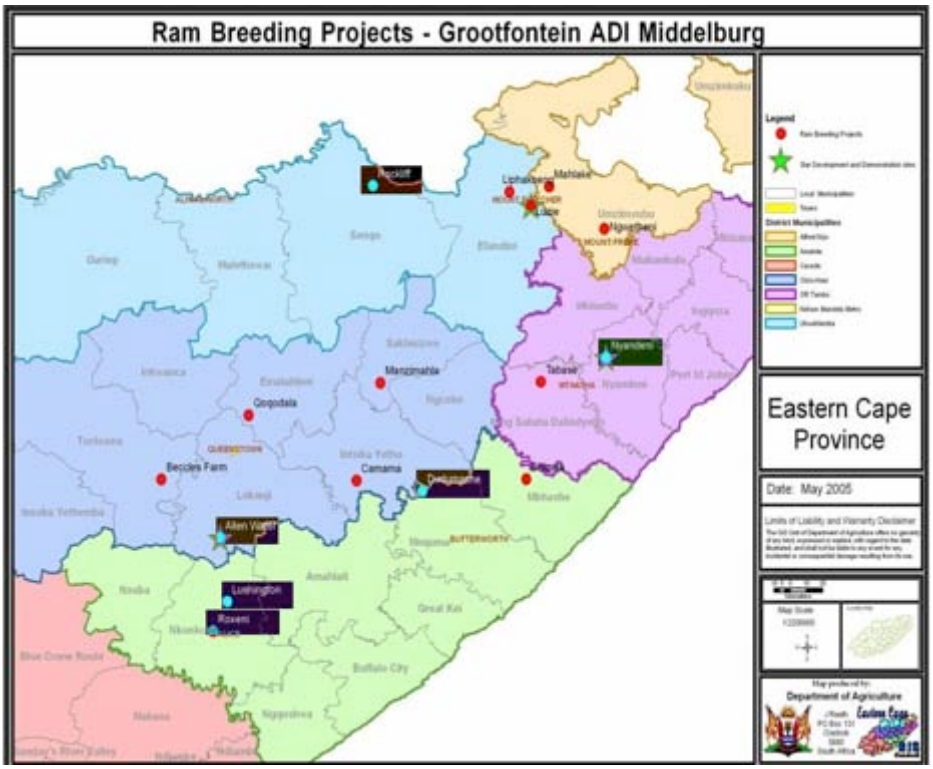


Figure 1. The EC region of RSA showing the location of ram breeding projects which identified the need for an improved pasture base.

The goal of ECCAL was to improve the livelihoods of the communal pastoral households of the EC by substantially and measurably increasing livestock production from improved pastures on abandoned arable lands, which were underutilised as grazing resources and at severe risk of erosion. These lands were cultivated and planted with maize and other crops by commercial farmers during the early 1900's onwards. From the early 1950's the Transkei and Ciskei of the Eastern Cape became the former homelands and were allocated as communal land to the communities of today. Since then the lands have been 'abandoned' in a sense that fencing systems broke down, animals continuously grazed these lands, cultivation skills were lost, no cultivation implements like tractors existed with these farmers, and eventually these old lands were overgrown with unpalatable pioneer grasses. Today these lands are again utilized as 'natural grazing' but could be restored with improved pastures to their original-increased potential.

Once the project interventions had improved and increased the fodder flow, it was expected that grazing demand would rapidly exceed the supply, especially during the winter. This would cause an influx of animals to move onto these unfenced lands. The project would thus aim (a) train farmers and demonstrate basic veld and grazing management systems and (b) increase off take of animals via marketing of 'surplus' animals (the culture of these farmers is to hold on to animal numbers as a proof of wealth). This becomes a significant target for the land management workshops to be run in the communities.

Description of communally managed lands:

The **veld** consists of natural vegetation, which is grazed continuously by goats, sheep and cattle at high stocking rates, is not rested, and has no applied fertilizer; sweet veld occurs in low rainfall areas but is of high quality, while sour veld occurs in higher rainfall areas but is of lower quality, particularly in winter. Virtually no supplementary feeding takes place, except during periods of severe drought. The area of veld within any single community varies from 50 to 90% of the available land area.

Abandoned arable land refers to lands that were previously cultivated for crops, but have been abandoned in the last 20-30 yrs. They generally comprise what were once the more fertile portions of the landscape. The area of abandoned land varies from 5 to 50% of the land area of any one community. The arable lands represent the largest opportunity for increases in forage production but are currently covered by poor quality pioneer grasses, e.g. *Cynodon dactylon* (couch) and *Eragrostis plana*. These lands are also at the highest risk of infestation by alien vegetation and erosion, particularly as very little stabilisation work was done at the time they were removed from cultivation.

Homestead gardens are individually fenced, cultivated areas used for household vegetable crops and maize production for human consumption. The area comprises 0.5 to 10% of the available area within any one community.

Across the whole of South Africa it is estimated that 28% of the total ruminant livestock are owned by people in the 'second economy' (the subsistence sector), but that their share of the commercial meat and fibre markets is less than 5%. Specifically, the rural communities of the EC principally produce livestock products, with the Province carrying 50% of the goats, 85% of the sheep and 40% of the cattle within the 'second economy' of South Africa, *vis* 3.2 million sheep, 2 million cattle and 1.8 million goats. The village communities in EC own and manage areas of communal land of between 200 ha to 2000 ha, depending on the nature and history of the settlement. About 4 million people are dependent on 5M ha of communally managed land, which comprises veld (for grazing), abandoned arable lands (used for grazing), cropping lands and homestead gardens and residential areas. The abandoned arable lands, which are the primary focus of ECCAL, occupy *ca* 750 000 ha of the communal lands of the Eastern Cape.

ECCAL aimed to address two of the major constraints to lifting livestock production - the quality and quantity of forages, and effective communal management of feed resources (from a social, economic and biological sense). The team worked with livestock farmers in the communal areas to improve pasture production –specifically by bringing abandoned arable lands back into production - through the introduction of legumes and rhizobia, P fertiliser and by developing innovative, participatory management strategies for the future utilisation of this pasturage. The project brought together forage and rhizobium experts who have successfully developed new legumes for similar soils in southern Australia, sociologists from both Australia and South Africa who are currently engaged with the

partner communities in the EC, pasture and animal scientists from South Africa, as well as the communities themselves. Many of the forage species evaluated were indigenous to South Africa but they could also be used in combating the development of secondary salinity in southern Australia.

This project built upon initiatives led by the Agricultural Research Council (ARC) and the National Woolgrowers' Association (NWGA) to increase wool production by communal African sheep-farmers in the EC. Training in wool sheep management and wool classing had resulted in a fourfold increase in the income from wool for the group of communal farmers involved: the major factor then limiting livestock production was the shortage of feed. The project was expected to work closely with the EC Dept of Agriculture (ECDA) "Integrated Livestock and Crop Farming Systems" development program that built on the earlier work. This program has established relationships with the farming communities and project outputs were being seamlessly integrated into the extension activities of that program.

A secondary objective of the work in the region was to continue exploration of South African legumes that might be domesticated to play a role in agriculture exposed to climate change in both South Africa and Australia. Our interest in the legumes of South Africa began with a series of surveys funded by the GRDC in 2000-2002, in which we noted the large potential for domesticating legumes from South Africa to suit southern Australia. None of those legumes had yet been fully explored, nor had their nodule bacteria been collected and studied. These activities are fundamental undertakings of the legume-rhizobium program at Murdoch University.

4 Objectives

The project aimed to increase household livelihoods from livestock production in the EC by developing forage species that increase production from the abandoned arable areas within the communal lands. The objectives were:

1. To assist community groups to develop procedures for the equitable and sustainable use of communal grazing land.

2. To evaluate a limited set of forage species on abandoned arable areas within communally managed lands for their impact on forage availability and animal production.

3. To evaluate a wider range of exotic and Eastern Cape legume forages, and their associated Rhizobia, for potential local and Australian use.

Project outputs were expected to include a suite of forage species suited to local management conditions; demonstration on a large scale of pasture and animal production; community structures and protocols that will enable effective and equitable use of the improved forage supply/quality; the identification of new forage species that may be suited to either the communal grazing systems and/or for use in salinity management in Australia; and enhanced capacity of individuals and institutions to implement genuinely participatory interactions with community groups.

The cultivated lands of the EC are evident in this photo (left panel), as is the erosion that results (foreground). The winters lack adequate feed which reduces animal productivity (below right), but which can be provided through winter grown legumes (below left).

Sinqumeni cultivation and erosion

Sinqumeni needs winter feed

Annual legumes grown through winter at Roxeni in 2009. Note the senesced veld in the background. Photos from P. Conradie and J. Howieson

5 Methodology

Where was the work undertaken?

To begin the research we accumulated more than 100 lines of different pasture legumes and their rhizobial inoculants from genebanks and repositories around Australia. We included sub-tropical, temperate and Mediterranean legumes in this initial scoping evaluation because we were not entirely sure of the variable climates, soils and seasons in the target zones, and how to match legume growth with these seasons. More details of methodology can be found in the reports and papers noted in section 10.2.

The first phase of the project was a broad evaluation of these legumes for their growth potential across several regions in the Eastern Cape. Information about potential legumes was sourced from scientists experienced in temperate and sub-tropical agriculture, and the germplasm of seed and rhizobia acquired. Because the project was asked to begin quite rapidly to fit the ACIAR funding cycle, in 2006 we chose three ECDA-managed research stations to undertake the initial experiments. This meant logistics were manageable for a rapid start up. Experiments of 200-300 plots were hand sown at Mpofu, Dohne, and Tsolo which represented a climatic gradient from south (with cool but wet winters) to the north. Plots were rated for germination, vigour and seed production, and the response to inoculation was recorded.

Small plots being established at Dohne in September 2006, and the result inspected by Neil Ballard and John Davis in March at Tsolo on the right. Photos J. Howieson and R. Yates.

The preparation of land for these experiments utilised rotary hoes and roundup for weed control, but we discontinued this practice after the first season as it was incompatible with community resources and expectations. It would also lead to over-cultivation and erosion.

During this period, the sociological work began, with communities within reach of the trial sites being approached for access to their land. This was facilitated initially by the ARC, and then with direct input from extension officers at the ECDA and Murdoch University.

In the second phase of the project, we identified nine communities and continued working with most of these for the following four years. Legumes that were phenologically adapted *viz*. able to grow prolifically and to set seed in the first series of experiments, were selected for evaluation in small plots of land held by each of these communities.

A small seeder capable of direct drilling into compacted soils was purchased and 2m x 30m plots of the promising legumes were sown with replication, and with split plots for rates of P application. The small seeds were established using minimum tillage directly into heavily grazed veld that had been sprayed once with glyphosate. We thus removed

any necessity for land cultivation, which we felt was essential to be removed because of the widespread evidence of erosion following deep tillage for maize cultivation in the regions.

We assessed the response to sowing time in March and October, as it was unclear which provided the most suitable seasonal conditions. In these trials grazing was “controlled” by communities, although it soon became evident that fences and gates were insufficient to manage the grazing at some sites. At other sites, somewhat counter intuitively, it was difficult to organise sufficient grazing pressure leading into the winter, as this was traditionally the season when animals would be penned and hand fed. Some of the communities were reticent to allow the animals access to the legumes in the fear they would be grazed too hard.

The ECCAL seeder was based at Dohne Research Station, near Stutterheim, and used to establish small plots across the range of latitudes. Some results are evident above in the right panel which is being inspected by Howieson and Yates. Photos by P. Conradie and G. Jordaan.

Nonetheless, the legumes that were able to tolerate the edaphic conditions and the grazing soon became evident, and the project began to monitor the longer-term performance of these legumes. Would the annual species be able to set seed, would the perennial species persist, would frost be an issue, would the seed harden sufficiently in the soft spring seasons to produce a hard seed bank? These questions were tackled by a combination of action research, monitoring and community participatory evaluation.

Plots at Lushington grazed hard during winter 2010 (left), then the Lespedeza and Lotononis regenerating at the same site the following summer (right panel). Photos by J. Howieson and G. Jordaan.

By 2011, the interest in the legumes was beginning to gather momentum. Commercial farmers were making enquiries for seed and expertise, and staff at Dohne established field sites of several hectares for experiments to rotate the winter growing legumes with summer grown maize, or to assess their performance with the companion grasses under development at Dohne. Additional communities approached the ECCAL staff to become involved with the project. Dohne appointed a specialist extension officer to work with ECCAL and the interested communities and three post graduate research projects were initiated with Dohne staff enrolled at local Universities.

A roll out of ECCAL also began in 2011 with an extension of funding provided by ACIAR. Plots in excess of 1 ha, and as large as 10 ha were established north and south of Dohne. It quickly became evident that demand for legume seed exceeded our financial ability to purchase and export from Australia, hence a program of seed multiplication and attendant skills were taught to locals by Neil Ballard.

A 10 ha roll out site at the Dudumashe community in 2011, with enthusiasm for the aerial seeding clovers shown by Colonel Dudumashe. Photo P. Conradie.

Who was involved with the work?

The sociologists from Murdoch University and the ECDA began the task of documenting the relationships and stakeholders in ECCAL.

1. Stakeholders whom the ECCAL project sought to influence or change
    a. Sheep owners
    b. Stock farmers
    c. Extension officers
    d. Land owners
    e. Community committee members
    f. Village community
    g. Youth

2. Stakeholders who were expected to assist the ECCAL project achieve its goals
    a. Chiefs and headmen
    b. Ward councillors
    c. Grootfontein
    d. Chief Dudumashe
    e. Control Agricultural development technicians
    f. Farmers Associations
    g. Researchers of legumes
    h. Social Science researchers
    i. Local Municipalities
    j. Mr Mlumbi
    k. Mr Mangqishi
    l. Extension officers

3. Stakeholders who needed to be informed of project performance
    a. Donors (Australia, ARC)
    b. Department of Land Affairs
    c. Eastern Cape Department of Agriculture (ECDA)
    d. Senior Management of ECDA
    e. District Municipalities
    f. School Science teachers
    g. Department of Environmental Affairs

4. End-users of ECCAL results
    a. Youths
    b. Sheep farmers
    c. Donors/ funders
    d. ECDA
    e. District Municipality
    f. Media
    g. Community committees
    h. Department of Land Affairs

Objective 3. Australian benefit.

Part of the incentive to become more closely involved with the aid work in this part of South Africa was our discovery at the beginning of the millennium of a wide range of under-utilised perennial browsing legumes in this region, as we were searching for new tools to combat climate change in Australian farming systems (Howieson *et al* 2008). To

tackle this objective, germplasm collections were undertaken in 2007 and 2010, with an emphasis on perennial legumes found in the Western Cape growing on deep acid sands. This part of the project was greatly assisted by Professor Ben-Erik van Wyk, from the University of Johannesburg.

6 Achievements against activities and outputs/milestones

Objective 1: To assist community groups to develop procedures for the equitable and sustainable use of community grazing land

no.	activity	outputs/ milestones	completion date	comments
1.1	Consult with communities and select experimental sites	Research protocols defined, partner communities identified	October 2007, but ongoing as more information produced. Communities identified by May 2007. Expanded in 2011.	Engaged a number of communities with expansion in 2011/12
1.2	Document socioeconomic livelihood data in respect of livestock production indices	Baseline information on community well being and livestock preferences	March 2008 but ongoing in respect of the latest communities to come on board	Communities are showing greater understanding of livestock management over winter in relation to land tenure and access
1.3	Document local knowledge and practice	Data collated outlining state of knowledge, current practices and expectations	The information was substantially collected by March 2008, but continued	DVDs containing short videos of legumes filmed in December 2008 were distributed to the participating communities in July 2009. Sociological studies to track the drivers for change conducted.

PC = partner country, A = Australia

Objective 2: To evaluate a limited set of forage species on abandoned arable areas within communally managed land

no.	activity	outputs/ milestones	completion date	comments
2.1	Select appropriate trial sites on 6 communities and 2 ECDA farms, prepare sites	Sites selected, fenced, soil sampled	March 2008 but extended with project expansion in 2010/11	New sites added to program with improved access and visibility – Kubledana, Fokotolo and community on main road adjacent to Lushington
2.2	Sow experiments, apply fertiliser and microbial inoculants, sow grasses	Assess growth, response of legumes to soil type and fertiliser, capacity for reproduction and regeneration	March 2008, but ongoing until March 2009	Studies conducted at post graduate level to trace nitrogen dynamics
2.3	For selected communities, establish larger trial areas	Measure forage persistence and animal performance	By March 2009 but expanded with project extension	10 ha sown into Kubledana 2011/12. Seed increase opportunities identified for species unavailable commercially.

PC = partner country, A = Australia

Objective 3: To evaluate a wider range of exotic and Eastern Cape species for potential local and Australian use

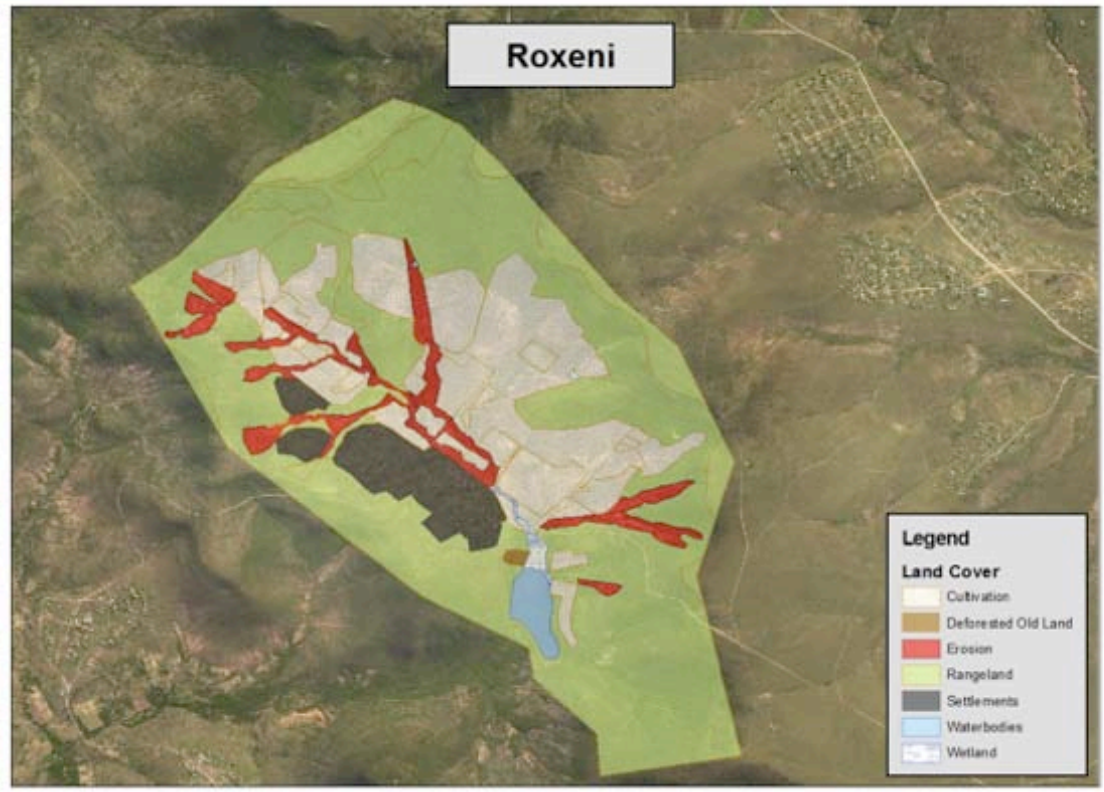
no.	activity	outputs/ milestones	completion date	comments
3.1	Collection of exotic and Eastern Cape (EC) grasses and legumes, and root-nodules	Collection of seeds, nodules, compilation of legume flora	November 2007	A major collection of species and root-nodule bacteria was undertaken in November 2007 and transitioned quarantine and Biosecurity Australia by 2009.
3.2	Seed increase of new species and isolation of rhizobia	Seed increased and matched with strains of rhizobia	ongoing	Key genera have been targetted for seed increase and rhizobial matching, including Desmodium and Lotononis from the EC and Lessertia from the WC
3.3	Evaluation of EC material and exotic species	Measure forage persistence and animal performance	By March 2009	Lebeckia ambigua grazed in 2011. Anticipated for commercialisation 2014/15. Significant progress made in developing commercial quality rhizobium inoculant also. IP clarified.

7 Key results and discussion

The grazed veld has a long and special place in the history of the Eastern Cape. It is a robust system when managed well, but disturbance by cropping or overgrazing can upset the ecological balance of the dominant perennial grasses (le Roux *et al* 1994). The ensuing decrease in productivity is well documented with the emerging dominance of inferior species such as *Cynodon dactylon* (couch) and *Eragrostis plana*. There have been historical attempts to develop legumes compatible with the veld grasses to overcome N deficiency (and "Dohne Desmodium" is an example of this), but these were hampered by a lack of available legume and rhizobium germplasm to evaluate, and often by the isolation of the legume scientist in a grass dominant ecology.

ECCAL was able to bring specialist legume and rhizobium scientists into this robust research environment. Through a combination of small plot trials on research stations to identify legumes adapted to the soils and climate, followed by larger plot experiments within the community lands exposed to grazing animals, and finally by sowing fields as large as 10 ha with minimum tillage, we were able to demonstrate the complementarities between legume and grass production and the impact on animal health and production.

The ECDA was able to support this research by provision of research station access, engaging scientific staff, and an introduction into the surrounding small- holder communities. GIS services were engaged through the ECDA staff and land capability mapping of the communities was undertaken, as seen in the example below for Roxeni community lands. The extent of cultivation of these lands is evident, as is the erosion it caused. This GIS capacity allowed us to understand that the extent of cultivation was greater than previous estimated (panel below prepared by T. Morgenthal, ECDA).



The key results from the research that was undertaken in ECCAL between 2006 and 2013 listed under the 3 Objectives were:

1. To assist community groups to develop procedures for the equitable and sustainable use of communal grazing land.

- A land capability assessment for each community was undertaken and published locally which enabled a more accurate assessment of the extent of land cultivation and erosion.
- The establishment and persistence of legumes in several communities was monitored by GIS and presented locally and internationally (see powerpoint Morgenthal *et al.* 2013, attachment 3)
- There was an absolute requirement for a strong sociological component in this research to increase adoption of recommendations within the communities.
- ECDA appointed a full time extension officer in recognition of this requirement
- ECDA also allocated staff to communities either north or south of the Kei river for mentoring.
- A broader understanding of the value of minimum tillage rather than deep ploughing in the eroded arable lands was developed.
- Several of the annual legumes were sown in rotation with maize on Dohne Research Station, and Biserrula in particular appeared suited to a winter legume: summer maize rotation potentiated by minimum tillage.
- Community leaders such as Colonel Dudumashe (Dudumashe) and Mr Mlumbi (Roxeni) assumed the responsibility for managing grazing on the “common lands”
- Practices, such as hard grazing of the veld prior to sowing the legumes, and rotationally grazing the legumes, were brought to the notice of the communities and largely adopted.
- Other sociological outcomes are listed below

2. To evaluate a limited set of forage species on abandoned arable areas within communally managed lands for their impact on forage availability and animal production.

- The acid soils of the EC were both N and P deficient, but had good water holding capacity, despite being moderately acidic (pH 5).
- In the southern EC, in most seasons there was approximately 200 mm of winter rain and this was enough to sustain winter growing annual legumes
- The mild winters and moist warm summers throughout the EC were suitable for the cultivation of perennial legumes
- All legumes responded to inoculation with rhizobia, and to application of P
- The traditional perennial legumes such as white clover and lucerne could not tolerate the combined stresses of severe grazing, acid soils and winter droughts sufficiently to be productive and long-lived. Thus while white clover is abundant in the gardens and parks of the region, it is not found in the grazed veld.
- Hard seeded annual legumes that were tolerant of grazing were able to produce abundant seed and formed seed banks that provided potential longevity in the system. *Biserrula pelecinus* and hard seeded *Ornithopus sativus* were the most successful, although *Trifolium vesiculosum* was also productive.
- These legumes had a phenology counter to the perennial grasses of the veld and seemed to be synergistic with them in their growth patterns and water usage.
- Several hardy perennial legumes were persistent and productive. The most adapted appeared to be *Lespedeza cuneata*, however *Desmodium subsericeae* and *Lotononis bainesii* were also impressive in some seasons.
- The availability of the annual legumes through winter allowed the sheep grazing them to increase their body weight and wool cut, whereas animals without access to the legumes in winter lost weight. 10 weeks grazing forage legumes in winter

saw sheep weight double, whereas the control group lost weight. An extra 1 kg of wool was cut from each sheep.

3. To evaluate a wider range of exotic and Eastern Cape legume forages, and their associated Rhizobia, for potential local and Australian use.

- Local legumes, such as “Dohne Desmodium”, and legumes known locally such as crown vetch and lespedeza were investigated for their agronomic value in RSA
- Three genera of legumes (Lotononis, Lessertia and Lebeckia) were collected from RSA and evaluated comprehensively in Australia (see publications).
- A detailed understanding of the nodule bacteria of perennial legumes indigenous to RSA was developed, including the full genome sequence determined for several (see publications).

Discussion

At the October 2013 meeting, which was arranged to begin the handover of ECCAL to the ECDA, team members present were asked to write down their perception of the project in terms of positives and challenges. Listed below are their comments exactly as they were provided:

Positives:

- Breaking new ground
- New research information available
- Find a solution for old/poor/unused lands
- Simple, robust grazing pasture system
- Engaging farmers and extension officers
- Consultation with community leaders, regular visits made them know the project.
- Legumes available during the most crucial time (winter) let to enthusiasm of farmers.
- Ability to work with community leaders and get the buy-in from communal leaders in areas like Roxeni, Tsolo and Dudumashe
- Managing to impact in communal farming systems by incorporating the legumes in the system
- Getting to be exposed as technical staff on the variety of leguminous pastures that we would not have got exposure to without the project
- Understanding how the minimum no-till system works (paradigm shift)
- Very good results, what works and where
- Adaptability of project, ability to change and adapt
- Multi-disciplinary approach
- Team approach w.r.t. research
- Existing and new technology tested and proved
- Overall goal to team
- Stimulated international collaboration
- Engagement with 6 communities
- Built technical expertise
- Options for overwintering with other spin offs
- External funding provides flexibility
- Improvement of communal lands so as to add value to their end product
- Opportunity for scientists to evaluate different legumes on various climatic gradients
- Place Döhne on the map with various farmers throughout the EC

Challenges

- Low/slow uptake of technology by Department and communities
- Limited success in some communities due to climatic limitations
- Need more preparation and input in site selection
- Single species small plot work in communities are problematic – mixtures should be considered
- Organising a team and delegating responsibilities will always be a problem but more effort should have been put there
- The mind blog some of the old timers had in the beginning
- Expectations on publications maybe too high
- Improper replications in the initial row plantings
- Pulling out of the training centres that we initially targeted as nursery sites
- Unavailability of seed and equipment
- Inaccessible land offered by farmers
- Capacity building of extension officers and farmers only done when they accepted the new technology
- Seed availability
- Seed cost
- Farmers need to understand that it is not a quick fix but will take time to establish
- Amount of time and effort going into planting only to have the lands destroyed by mismanagement e.g. fire and grazing duration
- Not having a trial site near the coast.

These positive comments combined with an enunciation of the challenges that ECCAL presented serve as an apt summary of the project in sociological terms.

Australian benefit.

Part of the motivation for developing ECCAL was to remain connected with RSA with a view to domesticating new pasture legumes that might be adapted to climate change.

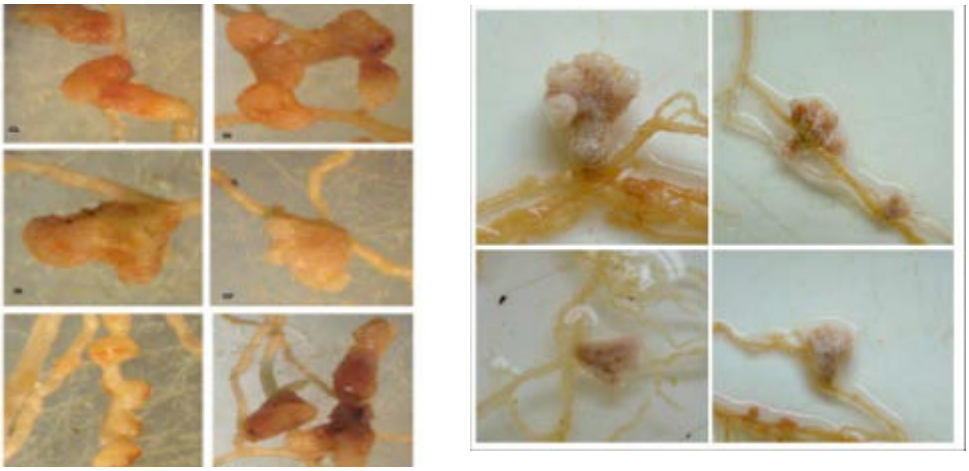
The agronomy and rhizobiology of several genera of under-researched perennial legumes from the Western Cape region was studied at Murdoch University, with a view to developing species adapted to acid infertile soils, in regions where rainfall is becoming uncertain.

Thus, from the inception of ECCAL, promising legumes and nodule bacteria were collected in targeted expeditions to the Western and Southern Capes, transitioned through quarantine at the Centre for Rhizobium Studies (CRS) Murdoch University, seed increased in small plots, the nodule bacteria characterised, and then agronomic evaluation began in Western Australia on acid and infertile sands, as shown in the plates below.

The first genus of interest was *Lotononis*, and we began an extensive evaluation program of *L. banesii* (already known as a pasture legume in Queensland) before delivering the outputs of this collection and breeding activity to the CRC Future Farm Industries based at UWA. However, it became clear over time that the very small seed size of that species would make it very difficult for the Australian seed industry to commercialise it. We thus began a hybridisation program between *L bainesii* and a related species we collected, *L angolensis*, however this failed to increase the seed size by more than 30 %. Some details can be seen in the research publications in section 10.2, later in this report. There would still be value in pursuing *Lotononis* as a source of new perennial legumes for Australia.

Evaluation of hybrids between *L. bainesii* and *L. angolensis* near Northam WA. Photo: Dr Ron Yates.

The second genus we evaluated thoroughly was *Lessertia*. It produced several years of exciting results in WA, however under field experimentation it became clear that this genus was nodulated competitively by sub clover rhizobia when sown into our soils. The negative aspect of this was that these nodules were unable to fix nitrogen. In RSA *Lessertia* is predominantly nodulated by mesorhizobia (see publications on *Lessertia* in section 9). We considered it very unlikely that we could solve this nodulation issue satisfactorily in WA, hence we changed our focus to a third genus. However, like *Lotononis, Lessertia* would still be a genus worthy of domestication, and some further experiments are underway with a colleague in Chile.



Nodules on Lessertia formed by background rhizobia strains in WA soils (left) or by inoculant mesorhizobia (right). Notice the pink colour and normal shape of those on the right. Photos. Dr M. Gerding.

The third genus that captured our attention during the collection missions was *Lebeckia*. This we found on the deep sands of the fynbos, and populations extended towards the fringes of the Kalahari desert. The taxonomy of the genus was poorly understood when we began this evaluation, but together with the team of legume taxonomists lead by Dr

van Wyk at Johannesburg University, we have come to focus upon the species *L. ambigua*.

Several accessions of *L. ambigua* are very promising for Australian conditions and have exhibited the potential to over-summer in the wheatbelt of WA, where there is often the complete absence of rain for 6 months. We are selecting accessions that persist, produce abundant seeds in pods which do not dehisce, and which are suitable for grazing by sheep. The site pictured below (left) is now 4 years old and the plants are still very robust, having withstood grazing by sheep, and are recruiting from a “hard“ seed bank.

Dr Steve Carr inspecting a three yr old stand of Lebeckia at Tincurrin, WA with Prof J. Howieson in 2012 (left panel) and a view of the field in 2013 (right). In these parts of rural WA summer rainfall varies from zero to 200 mm, and thus plants must be robust and have physiological traits adapting them to extreme heat and drought (Howieson *et al* 2008). Photos by N. Ballard.

An IP arrangement and royalty sharing agreement has been under development with RSA authorities, DAFWA and Murdoch University since 2012. The first of these perennial legumes should be commercialised in the near future, having shown remarkable adaptation to the hot and dry summers of Western Australia. The nodule bacteria of the *Lebeckia* are new to science and we have described and named three new species. In doing so we honoured two legume scientists of International repute (see publications in section 10).

8 Impacts

8.1 Scientific impacts – now and in 5 years

ECCAL provided evidence that annual hard-seeded legume species of temperate origin could grow in the winter period of the EC when the sub-tropical grasses are normally dormant, thereby closing a winter feed gap for sheep. This is an unique and exciting finding.

Knowledge of legume species and agronomy appropriate for the EC was vastly increased and opportunities for producing local varieties adapted to the region were identified. This provided incentive for ECDA staff to begin their own experiments with legumes, both in grazing and cropping systems.

The application of minimum tillage in association with a single spray of glyphosate to establish legumes in both winter and spring seasons was demonstrated at all communities and on Research Stations. Minimum tillage was given a greater profile through ECCAL.

The benefit of improved forage quality on animal production in the veld communities was demonstrated and quantified and this has provided impetus for local scientists to continue such evaluations.

Several perennial legume species and their nodule bacteria indigenous to RSA were shown to be adapted to the acidic, dry and infertile soils in Western Australia, and this should deliver significant Australian benefit in the future.

The essential role that nodule bacteria play in legume domestication was exemplified through the results of experiments in Western Australia. It is futile to attempt to domesticate new legumes without parallel focus on their nodule bacteria.

The nodule bacteria associated with indigenous legumes in South Africa were explored, several new species were named, and several had their genomes sequenced (see publications section).

In 5 years we will have commercialised well-adapted legumes for incorporation into farming systems both in the EC and in WA.

8.2 Capacity impacts – now and in 5 years

Training of local Agriculture Department Research staff in legume agronomy and N fixation was undertaken, with three postgraduate enrolments at local Universities.

A culture of adopting legumes rather than inorganic N fertiliser has been initiated. For example, experiments began on Dohne research Station to rotate biserrula (grown in winter) with maize (grown in summer).

Employment (by ECDA) of an extension officer expanded the skill capacity of the staff at the Research Station.

At the CRS, and Murdoch University generally, a core group of researchers and sociologists was developed that became comfortable working with the communities in South Africa.

In 5 years it is anticipated the ECDA staff will be actively engaged in developing and implementing minimum tillage farming systems based upon nitrogen fixation.

8.3 Community impacts – now and in 5 years

Development of a Xhosa word for “legume” in their language which will help in the development of an understanding of the value of legume N fixation in improving fertility of veld lands without the need to apply inorganic fertiliser.

Development of an understanding of improved pasture quality on animal productivity.

Development of small business opportunities in legume seed production and establishment of legume pastures in the eroded veld.

A greater understanding of the risks associated with cropping and deep tillage in the arable veld lands.

In 5 years we would expect several successful small community-based businesses developed around growing and sowing legumes into degraded veld, and in maize cropping systems.

8.3.1 Economic impacts

The economic benefits of this research to the communities, if these results are put into practice, are difficult to accurately quantify. The small data set on animal benefits suggested an extra kg of wool per hectare could be achieved. However the greater value might be seen in the winter legumes reducing lamb or ewe mortality by substantially closing the feed gap. The region in which this research is applicable covers up to 10% of the small holder lands in the EC – an area in total of approximately 750,000 ha, but for each community up to 200 ha. If these 200 ha in each community could be established to improved pastures for holding stock condition over winter, then the original estimates of direct value addition to the flock– of AUD$3-4 million pa – seem conservative and achievable.

A seed increase activity was initiated by communities at Lushington, Roxeni and Allen Water, RSA, with a view to developing a small business. An economic return calculated on seed produced sold into the local markets would be expected.

New legumes species adapted to climate change should become available to WA and NSW farmers as a result of ECCAL. In both states, *Lebeckia ambigua* should increase the value of deep infertile sands by $25 per ha. This would accrue a value of $50 million pa in the long term.

8.3.2 Social impacts

The legumes identified were resilient to uncontrolled grazing and thus can circumvent “the tragedy of the commons” as it refers to unmanaged grazing and its potential decimation of rangeland improvement activities.

The impact of the social program meant that by the conclusion of ECCAL we were able to:

- Identify the land tenure, social and economic factors which influenced the possibilities for integration of legume-based pastures into farming systems on communal arable lands.
- Characterise the ownership patterns for arable lands in the ECCAL project sites.
- Describe the land management systems currently in use for each site.
- Document the recent cultivation or grazing history of arable lands.
- Document socio-economic determinants of land use practices on the communal arable lands in the former Ciskei and Transkei
- Ensure that ownership of the legume sites by the rural areas was promoted through full engagement of the participants.

The ECDA employed a sociologist to work with communities engaged with ECCAL. To demonstrate the impacts, in March 2012 members of a neighbouring community walked

10 km to meet with us at Lushington, and asked our team to develop legumes on their lands.

8.3.3 Environmental impacts

An understanding that legume N can replace bag fertiliser N, with concomitant benefit to the environment, has been engendered in this region.

The knowledge and adoption of minimum tillage was, and still is, very weak in this region. There is the opportunity to strengthen recognition of the value of this soil management technique throughout the EC. This applies to the summer cropping systems, more so than the management of the veld.

The roll-out of legumes into the degraded community lands will decrease grazing pressure on these eroded lands, and the neighbouring fields, which should lead to decreased environmental damage in the weakened or degraded areas.

8.4 Communication and dissemination activities

Mr Gideon Joordan was given major responsibility for the Ciskei sites, whilst Ms Nobuntu Mapeyi and Ms Unathi Gulwa worked closely with the Transkei sites further north.

Training days for communities and research station staff were delivered at every opportunity in the project, including specialist courses in small seed production and handling, and in inoculation of legumes. Approximately 20 training days and workshops were delivered to some 500 farmers and scientists over the course of the project.

Meetings with community members took place upon every scientific visit.

Publications of the work were prepared for local and international grasslands conferences.

Bi-annual meetings were held formally with senior management at Dohne.

A key presentation was ‘Improving grassland quality in communal arable lands in the Eastern Cape Province, South Africa: ACIAR project ECCAL’ by Theunis L. Morgenthal, Pieter W. Conradie, Gideon Jordaan, Unathi Gulwa, Neil Ballard & John Howieson (attachment 3).

A formal “handover” of ECCAL to the ECDA will take place in the last week of July 2014.

9 Conclusions and recommendations

9.1 Conclusions

The ECCAL project has provided evidence that the disturbed perennial grass systems of the EC veld are amenable to improvement through the introduction of annual and perennial legumes that are adapted to heavy grazing and acid soil infertility. This may be achieved with minimum tillage on lands that have been formerly cropped, but subsequently abandoned, and left to evolve back to permanent grazing. The inputs required are a combination of legume selection for the particular regions and sociology to impart the knowledge for establishment and subsequent management of the legumes.

The availability of the annual legumes through winter allowed the sheep grazing them to increase their body weight and wool cut, whereas animals without access to the legumes in winter lost weight. Approximately 10 weeks of grazing forage legumes in winter 2012 saw sheep weight double, whereas the control group lost weight. An extra 1 kg of wool was cut from each group of sheep per hectare.

The exploration of the legume flora of RSA, which began in 2002 and which ECCAL supported, has revealed there are probably 100 under-researched legumes adapted to grazing by sheep that could be developed to provide Australian and RSA farmers with alternative species to grow, especially in regions which are becoming hotter and dryer. This would provide considerable long-term value to Australia from this project, potentially $50 million pa.

9.2 Recommendations

ECCAL will be “handed over” to the ECDA and communities in a closing ceremony in July 2014. All the stakeholders in the region, from the top tiers of Government to social workers who operate within the communities, have been primed to adopt the principles and findings of ECCAL. It is up to ACIAR to decide if they wish to be a partner in this next phase, and whether this would be an efficient investment of their funds in the African continent.

Recommendation 1. That the use of minimum tillage and wider use of legumes in the Eastern Cape be encouraged. Whether the driver is ACIAR or other organisations such as N2Africa[1], SIMLESA[2] or the ECDA will obviously be an ACIAR policy decision in association with local Government organisations.

Recommendation 2. That the integration of maize cropping with winter- grown, self regenerating annual legumes, adopting minimum tillage, N free fertilisers and glyphosate be the basis of any possible further research and development project.

Recommendation 3. That the fundamentals of the legume and rhizobia research conducted within this project be used as the basis of further ACIAR R & D cropping and grazing livestock research in ACIAR partner countries.

[1] http://www.n2africa.org. N2AFRICA is a large scale, science-based “research-in-development” project focused on putting nitrogen fixation to work for smallholder farmers growing legume crops in Africa and has funding from the Bill & Melinda Gates Foundation.

[2] http://simlesa.cimmyt.org SIMLESA - Sustainable Intensification of Maize-Legume cropping systems for food security in Eastern and Southern Africa program

Recommendation 4. That the research on the promising legumes and their associated rhizobia be promoted to Australian funding bodies (eg MLA, GRDC & AWI) and also be used as a basis for ACIAR promotional opportunities.

It is also appropriate to comment on how the ACIAR funds 'hit the ground' in ECCAL. Unlike greater sub-Saharan Africa, there is no Consultative Group (CG) system available for interaction within RSA, and the ARC underwent significant change during this project. Thus, the local Department of Agriculture became the institutional foci for ECCAL and this delivered several benefits. Through the ECDA the transaction costs were minimal and the funds invested were leveraged greatly into support of the project from research and technical staff from the ECDA offices. The ECDA even employed sociology staff out of their consolidated revenue to support ECCAL. We estimate we achieved a 3:1 leveraging of the ACIAR funds in this project. It is perhaps a model for aid investment in Africa worthy of future consideration.

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11 Appendix: Abbreviations

ARC	Agricultural Research Council, South Africa
C4 grasses	C4 plants are more adapted to warm or hot seasonal conditions under moist or dry environments.
CG	Consultative Group
CRS	Centre for Rhizobium Studies
EC	Eastern Cape
ECCAL	Eastern Cape Community Arable Lands
ECDA	Eastern Cape Department of Agriculture
NWGA	National Woolgrowers' Association
RSA	Republic of South Africa

12 Attachments

Attachment 1. Prospects for the future use of legumes

Attachment 2. Technical issues relating to agricultural microbial genetic resources (AMiGRs),

Attachment 3. Improving grassland quality in communal arable lands in the Eastern Cape Province, South Africa: ACIAR project ‘ECCAL’

CHAPTER 12

PROSPECTS FOR THE FUTURE USE OF LEGUMES

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1. INTRODUCTION

As rhizobiologists or soil scientists our reflex is to think of legumes in the primary role of providing fixed nitrogen to otherwise depauperate soils. However, other scientists see them as vital food or forage plants, as essential rotational species to improve cereal yields, or as a forestry commodity providing wood for fuel or shelter. Some scientists now see them as a source of pharmaceutical drugs for a range of maladies. This latter role is not unrealistic when we note that legumes have been components of traditional medicines for many centuries (Duke, 1981). No matter the end use, the symbiotic association between root nodule bacteria (hereafter rhizobia) and legumes plays a significant role in world agricultural productivity by reducing approximately 100 million MT (metric tonnes) of atmospheric dinitrogen into ammonia (Freiberg *et al.,* 1997; Herridge and Rose, 2000; Graham, Chapter 11) and saving $US 10 billion on fertilizer N each year. After photosynthesis, we might consider biological nitrogen fixation (BNF) by legumes as the most fundamentally important biological process on the planet. This is a critical issue, as many countries (both developing and advanced) have not fully embraced BNF and are substantially reliant upon fertiliser nitrogen to drive agricultural productivity. Lack of adoption is attributed to many factors: from a paucity of knowledge and expertise in manufacturing inoculants, to growing and inoculating legumes with rhizobia (Giller, 2001), to government subsidies in some advanced economies that mitigate against the use of biological N_2 fixation. Sadly, with the price of fossil fuels inevitably increasing, small economies will be faced with either food shortages or an inflated bill for fertiliser nitrogen. Many developing countries, such as those in SE Asia, rely upon buying urea for rice production (Thein and Hein, 1997). Their declining purchasing power in real terms will be deleterious for food production. This problem must be addressed, as current reviews forecast that food production will need to

M. J. Dilworth, E. K. James, J. I. Sprent, and W. E. Newton (eds), Leguminous Nitrogen-Fixing Symbioses, 000-000.

double by 2020 to feed our expanding population (Byerlee and White, 2000) and this cannot happen without inputs of N.

The Leguminosae is one of the largest families of flowering plants with more than 18,000 species classified into 650 genera (Sprent, 2001), just under one-twelfth of all known flowering plants. Not all legumes fix atmospheric N_2 and amongst the subfamilies of the Leguminosae, the species within the *Fabaceae* are recognised as those of primary agricultural importance. Herbaceous and woody legumes from the *Fabaceae* have been traditionally used for pastures, conserved animal feed, erosion control, agro-forestry and green-manuring. They also yield important substances like tannin, dyes, perfumes, insecticides, biofuels and resins (Tyler *et al.,* 1981). Some of our most valuable food crops - peas (*Pisum)*, beans (*Phaseolu*s), peanuts (*Arachis*) and soybeans (*Glycine*) - are legumes which produce high protein grains for human consumption. Of all the plants that man uses for food perhaps only the grasses (Graminiae) are more important than the legumes (Graham and Vance, 2003). While considerable resources have been directed towards developing grasses such as rice (whose full genome sequence is available), maize (whose genome is currently being sequenced), and wheat, only peanuts and soybeans within the legumes have been as thoroughly examined (Vietmeyer, 1986). Our increasing population, and the need to adequately feed people and prevent particular health problems, will necessitate a larger dietary contribution from legumes (Morris, 2003).

Apart from the direct benefits of N_2 fixation, legumes provide added value in weed, insect and pathogen control, improving soil stability and increasing soil organic matter when rotated with crops in farming systems (Robson, 1990; O'Hara *et al*., 2002). In the USA alone, alfalfa (*Medicago sativa*) is now estimated to be the third or fourth most valuable crop and is worth nearly $7 billion annually (Graham and Vance, 2003). However, pressures from biological, environmental, human health and economic sources dictate that the suite of legumes and how they are used in modern civilisation be not fixed, but dynamic. This chapter outlines how legumes and their rhizobia can be developed for future exploitation, including opportunities outside mainstream agriculture.

2. CURRENT AND PAST LEGUME USAGE PATTERNS

2.1 Legume use in antiquity, in undisturbed environments around the globe, and in today's agricultural systems

The evidence that legumes have been an integral component of human diets on all the continents for millennia is found in archaeological deposits containing seeds or sometimes DNA (Sprent, 2001), in religious scripts and mythologies from the earliest records, as well as in commentaries on daily life in such ancient sources as the Egyptian hieroglyphs, Indian Vedas and the Sumerian texts (Hancock, 2002). *Phaseolus* (dry beans) and *Lupinus* (lupin) in central and south America, *Cajanus* (pigeonpea) and *Vigna sinensis* (cowpea) in south-east Asia and central Africa, and *Lens, Lupinus, Cicer, Pisum* and *Vicia* in parts of west Asia, north Africa, the Mediterranean basin and the Asian sub-continent, have provided a major protein

source for humans as agriculture evolved over the last 10,000 years in these disparate civilisations. Simultaneously, as key animals were domesticated, forage legumes of the rangelands and forests provided high value grazing, as evidenced by the stomach contents of frozen animals in recently thawed Arctic ice (Hapgood, 1958). These forage legumes contributed nitrogen to the herbs and grasses growing alongside them in mixed swards. Recognition of the value of legumes in cereal rotations in early agriculture came well before modern science understood the role of micro-organisms in the N_2-fixing process. The Romans wrote of the re-invigoration by lupins of cropping lands for cereal production almost 2000 years before microbiologists actually isolated rhizobia from nodules (Gladstones, 1998).

Surprisingly little has altered in legume usage patterns in the Mediterranean basin since antiquity. A Roman farmer transported to the 21st century would see substantially the same suite of grain legumes (*i.e.*, pulses and oilseeds) in the fields, and his animals would graze the same wide diversity of forage legumes in the rangelands. Even *Lupinus angustifolius*, one of the few grain legumes to have been domesticated since biblical times (Hamblin, 1998), was widespread on mildly acid to neutral sandy soils of the western and northern Mediterranean basin in the Roman era. Without doubt the greatest global changes in grain legume usage have occurred in the tropics, sub-tropics and warm temperate zones of Africa, Asia and America. In these regions *Glycine max* (soybean) now dominates grain legume production, with nearly 70 million MT produced annually in the USA, and 34 and 53 million MT in Argentina and Brazil, respectively (USDA, 2005). Compared with the global trade in cool season grains of approximately 60 million MT (Kelley *et al.,* 2000), soybean is probably the world's single largest traded legume.

As for soybean in the Americas, large tracts of land have been cleared of their native vegetation in central Asia, temperate America and southern Australia and planted to cool season forage legumes from two main genera; *Trifolium* (clovers) and *Medicago* (medics). The perennial forage *M. sativa* has wide adaptation to soil and climate, notwithstanding intolerance of soil acidity (Cheng *et al.,* 2005), and has therefore spread from its centre of origin in the temperate zones of Persia to become a dominant forage on all continents except Antarctica (Lesins and Lesins, 1979). No perennial form of *Trifolium* has achieved such prominence. Possible reasons for this are covered later in this review. Annual clovers and medics were established across 25 million ha of arable land throughout southern Australia during the 150 years preceding the current millennium. The N fixed from these pastures produces more than 50% of the protein exported in cereal grains from the same agricultural region (when grown in rotation with the pastures; Herridge, Chapter 3).

However, despite examples of success in legume breeding and adoption, it is of concern that there are perhaps only 50 species of forage legumes and less than 15 species of grain legumes in wide global commercial trade (Kelley *et al.,* 2000). Is it prudent, from a gene conservation perspective, to cover the globe so completely with only 65 of a potential 18,000 species of legume?

2.2 Legumes in modern temperate and tropical agriculture

Current legume usage patterns reveal a substantial dichotomy in the global development of legumes species between warm and cool environments. Forage legume development for the cool environments has greatly outstripped that for warm environments, whilst it has been very much the reverse for the grain legumes. For example, soybean plantings have increased from 2 to 60 million ha in sub-tropical America during the last 30 years but grain legume sowings have actually decreased in the traditional, cool (mostly Mediterranean) environments over the last decade (Byerlee and White, 2000). Disease pressures are at least partly to blame for this latter pattern (Porta-Puglia *et al.,* 2000). In contrast, forage breeding of cool season species has been enormously successful in this same period. As an example, the development of a second generation of ley species for southern Australia has been an outstanding commercial success, with new cultivars, species and genera planted on more than 3 million ha since 1996 (Howieson *et al.,* 2000a; Loi *et al.,* 2005). Despite substantial investment, few new legume species, with the possible exception of *Stylosanthes scabra,* have been widely adopted in warm environments during this period (Miles, 2001). A recent analysis of outcomes in tropical forage legume breeding and adoption has listed some 30 species adopted world-wide since 1980, but only on 5 million ha (Shelton *et al.,* 2005).

We can speculate on the reasons for this disparity. In warm environments, C_4 grasses are adapted to tolerate heat and have an efficient water usage that provides them with a competitive advantage over legumes. Grasses in the tropics can produce large quantities of forage (100 tonnes per ha per yr) and grow as tall as 2 m. The legumes that have evolved to co-exist with these grasses climb to avoid being shaded by the tall grasses. These climbing legumes are not grazing tolerant, because their growing points (apical meristems) are exposed to the grazing animals, whereas the grasses conceal theirs at ground level or below. Therefore, when grazed, the tropical legumes do not persist, even when planted as pure swards. Furthermore, most temperate legumes introduced to tropical environments withstand neither the grass competition nor the extreme summer heat.

In contrast to the tropical grasses, temperate grasses (mostly C_3) are not as highly productive. For example, perennial ryegrass (*Lolium perenne*) is one of the most productive temperate species, but produces only 12 to 15 tonnes per ha dry matter annually, and only when supplied with N fertilizer. The height of C_3 grasses is also much less than the tropical grasses. The optimum height for grazing many temperate grasses is 20 cm (down to 5 cm after grazing) and this is compatible with legume phenology. Temperate legumes can co-exist and, in fact, be complementary to these grasses under normal grazing pressures (1-25 sheep per ha), sometimes developing into pure swards if they have reduced palatability. To assist their competition with grasses and herbs, temperate forage legumes have also evolved reproductive strategies such as high fecundity, hard seed, delayed imbibition and seeds whose shape and size can allow them to pass through the animal digestive tract undamaged (Loi *et al.,* 2005). Exploiting these attributes in breeding forage legumes for the cool environments has been highly successful over the last 50 years. We believe this is

because the strategies evolved by temperate legumes to make them competitive with C_3 grasses suit them for agricultural exploitation. Perhaps the recent development of new tropical cultivars of butterfly pea (*Clitoria ternatea*) and burgundy bean (*Macroptilium bracteatum*) can reverse this apparent imbalance between the warm and cool environments for commercially successful forage breeding (Conway *et al.,* 2001). For the grain legumes, there are opportunities in the future development of *Cicer, Lupinus, Vicia, Lens, Vigna* and others.

2.3 Constraints to breeding new legumes

Given that very few of the 18,000 species of legumes have been commercially exploited, what have been the major constraints to their broader development? The four key constraints appear to be difficulties in:

- breeding acceptable quality traits in legumes for human consumption,
- placing legumes into farming systems (which is particularly difficult for forages in warm climates),
- selecting legumes (and rhizobia) well adapted to both soil and climate,
- discovering and then acquiring suitable germplasm.

Breeding new grain legumes with high nutritional quality for monogastric or human consumption has to contend with the many anti-nutritional factors prevalent in wild legumes. These include non-protein amino acids, alkaloids, glycosides, tannins, saponins and protease inhibitors (Enneking and Wink, 2000). Whilst some societies have dealt with these anti-nutritional factors by processing (boiling, soaking, leaching, fermentation or dehulling; Uauy *et al.,* 1995), this is often not practical to undertake in today's large economies. Although some anti-nutritional traits are governed by single genes (Gladstones, 1998), it is not a trivial task to combine all the genes required for domestication into a species that will then displace (or augment) the suite of contemporary grain legumes in farming systems and in markets. The modern example of the domestication of *L. angustifolius* in the 1970s, followed by its adoption on acid, sandy, infertile soils in Western Australia (Nelson and Hawthorne, 2000), highlights some of the difficulties of market penetration by novel legumes. Despite *L. angustifolius* acquiring a very important niche in rotation with cereals on 750, 000 ha annually, the price paid for lupin seed constrains its wider adoption. Lupin is considered primarily as an animal feed in the marketplace, whereas traditional cool season grain legumes are grown for human consumption (*e.g.*, *Cicer, Vicia* and *Lens*) and fetch higher prices. Lupins remain popular in this particular farming system because they can fix more than 100 units of N per ha (Unkovich *et al.,* 1994), whilst providing the many additional rotational benefits associated with legume cultivation (Robson, 1990).

There are other technical and social barriers to legume adoption in the modern world. Farming systems that are distorted by price subsidisation often ignore the direct and associated benefits of cultivating legumes - common practice in the rural economies of North America and Europe (Carrouee *et al.,* 2000). Direct financial

support to farmers ensures that arable lands remain occupied, but removes the incentive to develop efficient farming systems based upon biologically fixed N. Recognition of environmental pollution in the manufacture and utilisation of fertiliser N is slowly increasing the pressure to embrace BNF in these regions. In other circumstances, the investment in legumes is often not realised for several growing seasons and the opportunity lost in growing legumes instead of crops that generate higher immediate cash flow is significant. Just as importantly, in communally owned lands, it is often difficult to manage the longer term custody of improved forage to retrieve the benefit to the investor.

There are also complex biological hurdles to legume adoption. These include unfavorable soil type or climate (Graham, 1992) that can affect any of the components of the legume symbiosis (Robson, 1969), and the presence of competitive yet ineffective rhizobia that compromise N_2 fixation (Brockwell *et al.,* 1995). Legumes are also often more difficult to grow, in an agronomic sense, than cereals. A final consideration is that legumes introduced to new environments often require parallel selection of appropriate rhizobial inoculants (Howieson and Ballard, 2004) and then the commercial manufacture of these inoculants. The expertise required to nurture a high quality inoculant manufacturing industry should not be underestimated (Deaker *et al.,* 2004). Some of the factors limiting legume exploitation have recently been reviewed in more detail by Sessitsch *et al.* (2002) and O'Hara *et al.* (2002).

It remains to acknowledge the key role of woody and herbaceous legumes, both annual and perennial, in communal rangelands in drier regions, and in forests (both tropical and sub-tropical) around the globe. Where forestry has not disturbed them, the majority of our legume and rhizobial diversity is found *in situ* in these non-arable lands. These repositories are now being recognised for their extremely high conservation value, particularly as *ex situ* germplasm centres become expensive to retain (Maxted, 1999; Sabanci, 1999). It is from these *in situ* repositories that many of the legumes and their root-nodule bacteria with unique future roles in agriculture, horticulture and medicine will be drawn.

3. NEW USES FOR LEGUMES

Global agriculture is facing unprecedented challenges in sustainability, whether those challenges arise from environmental, economic or biological constraints (Howieson *et al.*, 2000a). Since that review, the price of oil has doubled, and this will eventually inflate the price of nitrogenous fertiliser manufactured through the Haber-Bosch process. Legumes offer relief from reliance on fertiliser nitrogen in broad-acre cereal production and in more intensive primary production systems where N is limiting, such as in aquaculture. In animal feed lots which have traditionally used waste products such as offal to provide protein, this practice must now be abandoned because of bovine spongiform encephalopathy (mad cow disease). To realise the benefits of legume N, however, new cultivars or even new species must often be developed to precisely satisfy the demands of the production system, or the end-user. It is new uses for legumes that currently offer major

opportunities in legume discovery, selection and breeding outside of traditional agriculture.

3.1 Developing legumes for their pharmaceutical and health benefits

There is a strong consumer-driven trend for natural products in the USA and Europe. Of the active compounds that are in prescribed pharmaceuticals, 25% are derived from flowering plants and this is expected to increase to 30% over the next ten years. Moreover, of the antineoplastic drugs prescribed in Western countries and Japan, 54% are natural products or their analogs (Kinghorn *et al*., 2003). The global market for natural product pharmaceuticals has been estimated at US$30 billion and growing at 6% per annum (RIRDC, 2000). Many consumers want natural drugs, believing that the natural drugs are safer than synthetics. Herbs (including many legumes) possessing anti-cancer or penile potency properties are the focus of smuggling into markets into Europe, Japan and the USA (Hoareau and DaSilva, 1999). Advances in analytical chemical techniques such as high performance liquid chromatography (HPLC), mass spectroscopy (MS) and nuclear magnetic resonance (NMR) allow the rapid identification of novel compounds that are increasing the value of legumes, in particular, to the pharmaceutical industry. Dixon and Sumner (2003) propose that the legume species combine emerging genomic accessibility with biochemistry that is of acute relevance to human health.

Non-traditional benefits from legumes in human diets have been emphasized in recent years: alfalfa sprouts (*M. sativa*) and soybeans as sources of phytoestrogens to reduce menopause symptoms and to maintain bone health in women are good examples. We cover the phytoestrogens in greater detail later in this review. In Chinese medicine one of the oldest known beneficial plants is licorice (*Glycyrrhiza glabra*), a legume herb whose roots contain an anti-inflammatory and anti-ulcer agent. Legumes contain chemicals that may prove useful for their anti-oxidant, anti-viral, anti-microbial, anti-diabetic, anti-allergenic and anti-inflammatory activities (Tyler *et al*., 1981). The Plant Genetic Resource Conservation unit within the USDA is conserving 17 species of legumes that contain phytochemicals with potential human health impact. Some of these species include butterfly pea (*Clitoria ternatea* L.) for antifungal proteins, hyacinth bean (*Lablab purpureus* L.) for its anti-hypertensive properties, and Kudzu (*Pueraria montana* var. *lobata* Willd.) which contains the isoflavone daidzein for anti-inflammatory, antimicrobial and cancer preventive treatments (Morris, 1999). Five pyrano-isoflavones were isolated by Drewes *et al.* (2002) from the rootstock of *Eriosema kraussianum* (family Fabaceae). The most active of these compounds had an activity 75% of that of Viagra in increasing blood flow to rat penile tissue. *Trigonella foenum-graecum* L., widely known in Indian herbal medicine for increasing lactation (Duke, 1981), contains numerous chemical components of interest to the modern pharmaceutical industry, such as diosgenin and coumarin (Bhardwaj *et al.,* 1977; Liu *et al.,* 2002). A comprehensive cross-referenced compendium of compounds isolated from legumes, including a section on pharmacological applications, has recently been compiled by S. Hughes and A. Humphries (unpublished).

3.1.1 Phytoestrogens from legumes

Legumes contain phytoestrogens with broad biological activities and are now being applied to humans as treatments for menopause and osteoporosis. Phytoestrogens are plant-derived molecules so named because they possess both oestrogenic and anti-oestrogenic activity, although much less potent than the endogenously produced human oestrogens (Stephens, 1997). Isoflavonoids are one major class of phytoestrogens, including genistein, daidzein and equol, and are among the classes of phytoestrogens most extensively researched. Isoflavonoids are particularly prevalent in the *Fabaceae* subfamily of the Leguminosae and the most extensively studied are those from soybeans and red clover (*Trifolium pratense* L). Isoflavonoid extracts from red clover and soybean are now used as alternative compounds for hormone replacement therapy (HRT) for menopausal disorders (Beck *et al.,* 2003).

Soybeans are the main dietary source in humans of two isoflavonoids, genistein and daidzein, which are present in the form of their glycosides. Consumption of foods containing soy-based products results in high plasma, urine and prostate fluid concentrations of phytoestrogens. Epidemiological studies suggest that women in Asian countries with a typically high dietary intake of phytoestrogens have a decreased risk of breast cancer (Adlercreutz, 1998) and a lower incidence of menopausal symptoms (Albertazzi, 2003). As well, Asian men consuming a traditional diet high in soy products have a lower incidence of prostrate cancer compared to European and American men (Adlercreutz *et al.,* 1993). Although these examples may only provide correlative evidence, numerous *in vitro* studies support a role of genistein in inhibiting the growth of a number of cancers (Ren *et al.,* 2001). We have hypothesised that temperate legumes may also offer the same anti-cancer benefits as genistein extracted from soybean. *In vitro* studies in our laboratories have produced alcohol extracts from a wide range of legume leaf and stem tissues that inhibit the growth of MCF7 breast and LNCaP prostate cancer cells (P. Leedman, V. Russell, S. F. Wang, K. Foster and J. G. Howieson, Royal Perth Hospital, unpublished). The isoflavones from soybean may also have a role in maintaining healthy brain tissue and in treating age-associated cognitive declines such as Alzheimer's disease (Gleason *et al.,* 2004) or improving cognitive function (File *et al.,* 2001).

Many of these secondary plant compounds are frequently found in small quantities and tend to be synthesised in specialised plant cells or at specific growth stages. This makes their extraction and purification more challenging (Balandrin *et al.,* 1985) yet, with the equipment now available, we are likely to see a rapid expansion of the role for legumes or their extracted compounds in human medicines.

3.2 Developing legumes for specific antihelminthic benefits to ruminants

As for the emergence of resistance to herbicides in weed populations (*e.g.*, Burnet *et al*., 1994), developing and implementing chemical control (anthelminthic) programs for gastrointestinal parasites in grazing animals is a balance between seeking

efficacy and avoiding the creation of resistance. Sheep nematodes such as *Ostertagia (Teladorsagia) circumcincta, Haemonchus contortus* and *Trichostrongylus* species are major causes of livestock mortalities and reduced production, and widespread resistance to anthelmintics threatens effective control (Besier and Love, 2003). There is good evidence, however, that plant tannins which occur naturally in many forage legumes can reduce worm burdens in grazing animals, hence reducing the requirement for drenching and potentially providing a new weapon in the management of antihelminthic resistance.

The tannins of interest for their potential antihelminthic properties (condensed tannins or CTs) are described as proanthrocyanidins, phenolic compounds present in varying concentrations in a wide range of plants including leguminous forages. These CTs form part of the chemical defences of plants against bacterial and insect predation, and grazing by herbivores. CTs may also have a positive effect on ruminant nutrition by increasing the efficiency of protein utilisation. Through reversible binding to plant proteins, CTs are postulated to interfere with the activity of proteases produced by rumen microorganisms, thus reducing protein degradation in the rumen and allowing a greater proportion of protein to reach the small intestine (Aerts *et al.,* 1999; Min *et al.,* 2003). However, despite demonstrated benefits in terms of increased wool growth, milk production, reproductive rates and bloat control, high concentrations of tannins can also reduce voluntary feed intake, resulting in reduced animal performance (Min *et al.,* 2003). The effects of CTs evidently vary according to the nature, concentration and structure of different compounds, and potential anthelminthic benefits must be considered in this light.

3.2.1 Worm control with CTs

Positive effects of various CT forages in reducing sheep worm burdens have been noted in numerous studies. In field trials, significant reductions in worm burdens have occurred in sheep grazing tanniferous forages such as sulla (*Hedysarum coronarium*) (Niezen *et al.,* 1998a, 2002a), lotus (*Lotus pedunculatus*) (Niezen *et al.,* 1998b), birdsfoot trefoil (*L. corniculatus*) (Marley *et al.,*2003a) and chicory (*Cichorium intybus*) (Scales *et al.,* 1995; Marley *et al.,* 2003a; Tzamaloukas *et al.,* 2005). Pen studies with a tannin extract (Quebracho) also indicated a reduction in sheep worm egg counts and reduced worm burdens (Athanasiadou *et al.,* 2000; Max *et al.*, 2005). In goats, pen studies with Quebracho (Paolini *et al.,* 2003) and a tanniferous tree ration (Kahiya *et al.,* 2003) reduced numbers of *Haemonchus contortus*, and significant anti-parasitic effects were obtained with the forage *Sericia lespedeza* in goats in both pen (Min *et al.*, 2004) and grazing trials (Shaik *et al.*, 2006). In general, worm egg counts have been reduced within a week of introduction to CT pastures or rations, with most reductions in total worm numbers of the order of 30-50% in comparison to non-CT groups.

However, the role of CT forages as an alternative to chemical antihelminthics is far from clear, as the results and conclusions from various studies vary considerably. In contrast to earlier studies, little or no effect was seen in grazing studies with sulla (Tzamaloukas *et al.,* 2005) or *L. pedunculatus* (Niezen *et al.,* 1998a). Variation in

the effect on different worm species has also been shown: several authors report reductions in burdens of *Teladorsagia (Ostertagia) circumcincta* but not *Trichostrongylus* spp. (Niezen *et al.,* 1998b; Marley *et al.,* 2003a), whereas Athanasiadou *et al.* (2000) found effects on intestinal but not abomasal species. It is not clear whether these inconsistencies reflect varying concentrations of CTs, or the presence of different CT compounds.

There is also uncertainty regarding the mode of action of CTs on worm populations. In particular, it is not clear whether effects are due to the high nutritive value of proteins protected from rumen degradation, or to a direct antihelminthic action of CTs on various stages of the nematode life cycle. The effects of high protein diets on the immunological competence of livestock have been well-established (*e.g.*, Coop and Kyriazakis, 1999), although this does not necessarily explain all anti-parasitic effects seen in sheep grazing pastures high in protein. Direct effects on worms are reported in *in vitro* studies, including the inhibition of worm egg hatching and larval migration of *H. contortus*, *T. circumcincta* and *Tr. colubriformis* with sulla extracts (Molan *et al.,* 2000a), and similar effects with sulla, birdsfoot trefoil, lotus, sainfoin (*Onobrychus viciifolia*) and *Dorycnium* spp. on *Tr. colubriformis* of sheep (Molan *et al.*, 2000a) and nematodes of deer (Molan *et al.* 2000b). Similarly, larval development was reduced in faecal cultures from sheep fed chicory (Marley *et al.,* 2003b), *Dorycnium* spp. and *L. pedunculatus* (Niezen *et al.,* 2002b). However, the significance of these effects in the natural situation is not apparent, as *in vitro* egg hatching results have not always accorded with those from field trials (Waghorn *et al.*, 2006).

Further investigations, both *in vitro* and field-based, are essential to indicate whether CT-containing forages are likely to become a reliably effective addition to non-chemical worm control in livestock. Such studies should report CT concentrations and the proportions of different worm species involved, and note animal production effects. The mechanism of action requires elucidation to explain the variable results obtained in grazing trials. The identification of specific compounds associated with dose-dependent inhibitory effects against nematode developmental stages (Molan *et al.,* 2003) will provide an objective basis for relating the results of laboratory assays to those occurring in the field.

Authors of the reports cited have often noted that CT-containing forages are relatively more difficult and expensive to establish and maintain than traditional pastures. As we note later, unless the economic benefits of new legume species are clear - in terms of both antihelminthic effect and pasture management costs - and the sociological effects are considered, their uptake may be compromised.

3.3 Developing legumes to replace fishmeal in aquaculture feeds

Aquaculture has expanded so rapidly over the last decade that it now provides more than 30% of global fishery products (Allan, 2000). Whilst marine-based ingredients such as fishmeal and fish oil remain the preferred source of protein and energy to aquaculture, it is predicted that by 2006 50% of the global fish catch will be directed towards manufacturing aquaculture feeds (Tacon, 1996). Modern intensive

aquaculture is thus perceived as a net fish-user rather than producer (Naylor *et al.,* 2000), which is clearly undesirable. Soybean meal extracts have already been accepted as an alternative protein and energy source by the aquaculture industries; sweet lupin (*L. angustifolius*) and other grain extracts are currently being evaluated and appear to be adequate substitutes for soybean. Can other legumes, particularly those that can be produced inexpensively, satisfy this increasing demand for protein and energy in the aquaculture industries?

Fish do not require carbohydrates. Their presence in grain legumes can lead to reduced digestibility in fishmeal produced from legumes and concomitant reduced protein retention (Allan, 2000). Yet fish require protein (particularly S-rich amino acids), oils, fatty acids or lipids in their diets (Glencross, 2000). Although these may be provided by legumes in various ratios (Wang *et al.,* 2003), anti-nutritional factors similar to those previously listed for humans and monogastrics affect fish, notably protease inhibitors, saponins, oligosaccharides and a high cellulose/fibre content. These and other potential tainting molecules (*e.g.,* coumarins, Wang *et al.,* 1999) cannot be ignored in formulating fish diets and removing them from legumes requires either expensive processing or breeding programs.

An important role for fish in human health relates to the ratio of long chain (more than 18 carbon atoms in a straight chain) omega-3 to omega-6 oils in marine products. There are two issues of importance here in relation to the oils from plants. Firstly, legumes produce predominantly C18 oils, rather than the C20-C22 fish oils that are noted as beneficial to human health. Fresh water fish can synthesise C22 fats from C18 precursors, but marine fish, particularly those from cold waters, are much less able to. Secondly, as can be seen from Table 1, the ratio of omega-3 to omega-6 oils varies considerably between legumes and fishmeal (a difference of more than 100-fold). For increased human health a high ratio of omega-3 to omega-6 oils is desirable (Dry and Vincent, 1991). If the low ratio of omega-3 to omega-6 oils in some legumes is ultimately reflected in the fatty acid content of the aquaculture end-products, the value of legume-fed fish in human diets might need to be re-assessed.

Nonetheless, the substitution of fishmeal in aquaculture fish diets with high protein grains is attractive, particularly grains that contain omega-3 and omega-6 fats. Before embarking upon a program to breed pulse legumes specifically for fish feeds, we should ask whether any naturally available legume seeds contain the range of nutritional factors essential for aquaculture feeds. Table 1 indicates that, amongst the clover species, *T. glanduliferum* might be a candidate for future research. It combines an average level of total fats of 5.7% with a high proportion of these (40%) being present as omega-3 fats. This compares well with *L. angustifolius* (already advanced as a fishmeal substitute) which contains 6% fats but with only 5.3% present as omega-3 fats, and an omega-3/omega-6 ratio one tenth that of *T. glanduliferum*. Both species have acceptably high levels of protein. Amongst the legume species adapted to alkaline soils, *Trigonella balansa* contains a relatively high level of omega-3 and omega-6 fats, but a lower omega-3 /omega-6 ratio than *T. glanduliferum*. A broader search of the legume family may well uncover other agronomically adapted species that are nutritionally adequate for aquaculture diets. The aerial seeding clovers (such as *T. glanduliferum*) appeal as likely candidates for

Table 1. A comparison of the levels of fats and proteins in soybean meal and fishmeal with those in kernels of canola and a range of legumes that might be considered as alternatives to fishmeal in aquaculture diets.

	% total fats	% of fats as omega-3*	% of fats as omega-6*	Ratio of omega-3 to omega-6	% crude protein (N x 6.25)
Fishmeal	7.9	33.9	2.3	14.7	65
Soybean meal	19.6	7.5	56.6	0.13	48
canola	13.6	12.7	19.6	0.65	35
Lupinus angustifolius	6.0	5.3	37.5	0.14	38
Trigonella balansa	5.5	23	43	0.53	38
Trifolium strictum	3.8	29.3	40.9	0.71	32
Trifolium glanduliferum	5.7	40.1	25.3	1.6	32
Trifolium dichroanthum	7.1	18.6	41.5	0.45	40
Onobrychus aurantiaca	13.4	nd	nd		58

*these include C14 to C22 fats. nd – not determined
Data from van Barnefeld (1999); Petterson (2000); Glencross (2000), and S. F. Wang, unpublished).

fishmeal substitution because, under low input conditions, they can produce large quantities of seeds which are readily harvested by conventional machinery (Loi *et al.*, 2005). This attribute will be essential if new species are to be price competitive with soymeal. The agronomic potential of *Onobrychus aurantiaca* is unknown, but its protein and oil concentrations approach those of soybean meal. The crude protein estimate of 58% for *O. aurantiaca* (based on N analysis) is very high, raising the question of whether much of the N in that particular species is actually present as non-protein N.

3.4 New perennial legumes with deeper roots for increased access to water

A further opportunity for the future use of legumes is in providing hydrological stability to low input agricultural ecosystems. Undisturbed grassland and rangeland ecosystems often contain a mix of annual and perennial species that include herbs, shrubs, trees and grasses. This mix of bio-types in temperate climates has contributed to hydrological stability in the groundwater systems of much of the global land mass, with the deeper-rooting species translocating water from depth during the drier autumn and summer periods. In southern Australia, the natural mixture of perennial shrubs, trees, annual grasses and herbs was violently perturbed with the clear felling of 25 million ha for agriculture in the 19th and 20th centuries. Large areas of southern Australia have since become seriously affected by the

combination of salinity and waterlogging as a result of rising water tables consequent on decreased water utilisation. The current estimate of affected land exceeds 5 million ha (Rogers *et al.,* 2005). Pasture for use by livestock has been recognised as the large scale land use with the greatest potential for remediating this disaster (Ewing and Dolling, 2003). Farming systems in southern Australia are therefore likely to be redesigned in this century to mimic the water use patterns of native flora (Lefroy and Stirzaker, 1999), the key to this activity being discovery of plants with both economic and hydrological benefit.

Perennial legumes are projected to play a key role in this redesigned agriculture. Cocks (2003) estimates *M. sativa* is adapted to 96% of the soil types of south-eastern Australia where soils are fertile and alkaline. Many of the perennial legume species found in the rangelands surrounding the Mediterranean basin (Gintzburger and Le Houerou, 2003) might be evaluated against *M. sativa* in this setting if improvements are required. However, for the acid and coarse-textured soils of south-western Australia, which represent approximately 30% of the agricultural land in this region, a different suite of perennial legumes and rhizobia to those currently exploited in agriculture will need to be developed. None of the current commercial species is adapted to the combined edaphic stresses of aridity, infertility and acidity that typify this region (Howieson and Ballard, 2004), thus providing another major opportunity for developing legumes for future uses.

Remarkably little is known about the essential reproductive, agronomic, rhizobiological and physiological characteristics of perennial forage legumes other than perhaps for *M. sativa*, *T. repens*, *T. pratense* and *Lotus corniculatus,* which are used commercially in many parts of the world. This lack of knowledge is a serious constraint to the development of other perennial legumes for future agricultural usage. These constraints are further discussed in relation to the genus *Trifolium*, under the column headings in Table 2 - rhizobiology, seed and herbage production and seedling vigour.

3.4.1 The mode of reproduction of perennial legumes

Many perennial legumes must cross-pollinate to produce seed (*i.e.,* they are allogamous). Allogamy has two immediate impacts in breeding programs: it requires the presence of appropriate pollinating insects or vectors, and it also generates variability within seed stocks. Both strictures require the breeder to take elaborate precautions to ensure his seed stocks are replenished while remaining pure. In contrast, self-pollinating legumes (such as most annual species) are usually highly genetically stable and fecund, and therefore relatively simple to propagate and preserve. For a perennial legume to be extensively studied (and in the longer term to be economically attractive), adequate and inexpensive seed supplies must be available. However, perennial legume seed crops are perceived as high risk, and recent trends, particularly in New Zealand, show a decline in their production and, concomitantly, in research about them (Rolston, 2003). In association with a reduced fecundity, perennial species also tend to concentrate their resources into vegetative reproduction rather than seed production. This is counter to some of the baseline selection parameters developed for the ideotypic commercial legume (Howieson *et*

al., 2000a). To develop cultivars producing consistently high levels of seed is thus the major initial challenge when researching new perennial forage legumes.

The phenology of perenniality presents additional challenges for the plant breeder. Seedling establishment is comparatively slow in perennial plants as they prefer to secure a substantial rooting system prior to the development of aerial foliage. Annual plants, in contrast, are often very well adapted to rapid establishment because this attribute assists their primary reproductive strategy of seed production from aerial parts, as well as allowing them to be competitive with weeds. The slow establishment of perennial species is a second major constraint to the development of novel commercial species.

3.4.2 The rhizobiology of perennial legumes

Producing compatible rhizobial inoculants is a third impediment to the utilisation of perennial legumes. This is exemplified by our current understanding in the well researched genus *Trifolium.* Substantial specificity for root-nodule bacteria is seen between different clover species, between the same species growing in different geographic origins, and between annual and perennial species (Yates *et al.,* 2003). Many of the cross-reactions between clover and rhizobial strains may, in actuality, be parasitic (Friedericks *et al.,* 1990). Howieson *et al.* (2005) describe both 'geographic' and 'phenological' barriers to effective nodulation in this genus. These authors consider it very difficult to select inoculants with a sufficiently broad host range to fix N_2 in association with both annual and perennial clovers, particularly if their centres of origin are disparate. The annual clovers so far exploited in global commerce originate primarily from the Mediterranean basin. In Australia, the inoculant strain for clovers, WSM1325, although broadly effective with a wide range of annual clovers (Howieson *et al.,* 2000b*;* Loi *et al.,* 2005), is restricted almost entirely in terms of N_2-fixation to annual clovers from the Mediterranean basin (Howieson *et al.,* 2005).

Table 2 groups perennial clovers according to their rhizobial associations and then comments on their commercial adoption. It illustrates that the majority of currently commercial perennial clovers arise from the Euro-Mediterranean region (Zohary and Heller, 1984) and share common strains of effective rhizobia (primarily group 3). Most cross-inoculation reactions between these commercial perennial clovers therefore lead to compatible nodulation (effective for N_2 fixation). Very few perennial clovers have been successfully commercialised from either the American or African continents, where rhizobial specificities are marked (*i.e.,* the different clover species require different inoculants, groups 4-11). Within the Euro-Mediterranean region, a species such as *T. ochroleucum* might offer rhizobial compatibility with commercial perennial clovers but be deficient in other essential agronomic characteristics, such as seed production. Further, few inoculants for perennial clovers are effective on the annuals such as *T. subterraneum* (group 1, Table 2) and in fact may be competitive for nodulation, yet ineffective. Yates *et al.* (2003) emphasised that the release of new perennial clover cultivars with specific inoculants must be undertaken with care to ensure that this activity is not detrimental to annual clovers already established in the target farming system.

Table 2. Some perennial *Trifolium* species and their characteristics relative to the annual species T. subterraneum that have contributed to, or inhibited, their commercial exploitation.

Trifolium species	Centre of diversity	rnb grouping*	Seedling vigour	Herbage production**	Seed production characteristics***	Commercial adoption
T. subterraneum	Euro-Mediterranean	1	F	H	I	Yes
T. ambiguum	Euro-Mediterranean	2	I	I	I	Yes
T. fragiferum	Euro-Mediterranean	3	I	H	H	Yes
T. hybridum	Euro-Mediterranean	3	I	I	I	Yes
T. medium	Euro-Mediterranean	3	I	I	I	Yes
T. ochroleucum	Euro-Mediterranean	3	S	I	L	No
T. pratense	Euro-Mediterranean	3	F	H	H	Yes
T. repens	Euro-Mediterranean	3(1)	F	H	H	Yes
T. tumens	Euro-Mediterranean	3	S	L	L	No
T. uniflorum	Euro-Mediterranean	3	S	L	L	No
T. polymorphum	South America	4	S	L	L	No
T. longipes	North America	5	S	I	L	No
T. wigginsii	North America	6	S	I	L	No
T. wormskioldii	North America	7	S	L	L	No
T. africanum	Africa	8	S	I	L	No
T. burchellianum	Africa	9	S	I	L	No
T. cryptopodium	Africa	10	S	L	L	No
T. semipilosum	Africa	11	I	I	I	Yes

F – Fast, I – Intermediate, S – Slow, H – High, L – Low

* *those with the same numeral are cross compatible for nodulation and N_2 fixation (from Howieson et al., 2005)*

** *an assessment of leaf to stem ratio and woodiness*

*** *amount of seed production, ease of seed capture and cleaning after harvest*

Other attributes common to the successful perennial clover species listed in Table 2 appear to be intermediate (or better) seed and herbage production, combined with seedling vigour. Table 3 indicates that of the commercial perennial clovers in Australia, only *T. repens* can be considered as a successful species relative to the widely sown annual *T. subterraneum,* on the basis of certified seed production in the years 2000-2004. It is no coincidence that *T. repens* can nodulate and fix N_2 reasonably well with rhizobia from annual clover species originating in the European centre of origin of the genus (Howieson *et al*., 2005).

In summary, the few successful perennial clover species such as *T. repens* (Table 3) appear to be those that have been bred to include the key attributes that we actually find widespread in the annual forage legumes – high seed production, rapid

seedling development, intermediate or greater herbage production and broad compatibility with root-nodule bacteria. These attributes should become baseline selection parameters when identifying perennial forage legumes in other legume genera for future domestication.

3.4.3 The requirement to develop new perennial forage legumes for acid and infertile soils

The globe contains large pockets of acidic and acidifying soils in important climatic zones such as the Temperate, Mediterranean and elevated sub-tropical regions (Andrew, 1978). It comes as a surprise that there are few well-adapted and commercial perennial forage legumes for this edaphic niche, particularly where annual rainfall is low. As we have indicated, the premier perennial forage species for temperate zones is *M. sativa,* which is productive on fertile, well drained soils in the pH range 6-9. If *M. sativa* is not well suited to the farming systems on these soils, then species from the genera *Onobrychus, Hedysarum* or *Astragalus* offer many opportunities for commercialisation. Unfortunately, the suite of perennial legume herbs commercialised for acidic, infertile soils in temperate, sub-tropical and Mediterranean zones is very narrow, and non-existent if rainfall is below 500 mm.

Table 3. Certified seed production (MT) for five perennial and one annual *Trifolium* sp in Australia under the OECD, AOSCA and domestic seed certification schemes (2000 – 2004[#]).

Trifolium species	2000	2001	2002	2003	2004	Total
T. ambiguum	<1	<1	<1	<1	<1	?
T. fragiferum	75	67	7	4	57	210
T. pratense	5	<1	<1	13	80	98
T. repens	424	451	1732	657	1926	5190
T. semipilosum	<1	<1	<1	<1	<1	?
*T. subterraneum**	1484	461	1981	1568	2569	8063

* Annual *Trifolium*

[#] Compiled by Australian Seeds Authority Ltd from data provided by Seed Services Australia, AgriQuality Ltd, AGWEST, NSW Agriculture, QSEED Pty Ltd and Tasmanian Dept of Primary Industries, Water & Environment

It is essential in developing novel perennial legumes for commerce that species are matched for key soil characteristics such as clay content, pH, cation exchange capacity and inorganic fertility. Although there are exceptions, as these individual parameters decrease, abiotic stress on plants and rhizobia substantially increases. Sometimes, these abiotic stresses occur together, and where they do it represents a significant challenge to the establishment of symbiotic plants (Zahran, 1999; Howieson and Ballard, 2004). In this context, the exploration of world flora to develop novel perennial legumes for acid soils in arid regions should focus largely upon edaphic homologs. At present, the reflex for many plant collectors is to focus their activities on climatic homologs. These can be found by interrogation of simple

climate matching models, but are of little real value where the target environment for plant improvement harbours the abiotic stresses summarised above.

A surprisingly diverse suite of palatable and herbaceous perennial legumes has emerged from a recent botanical exploration of the Cape regions of South Africa. The perennial legumes there grow under rangeland conditions, with annual rainfall between 150 mm and 600 mm, and where coarse-textured, low pH and infertile soils are common (J. G. Howieson, R. J. Yates, D. Real, I. Law and B. E. Van Wyk, unpublished). The climate is dry-Mediterranean and thus these species might augment the narrow suite of perennial legumes available for this edaphic niche. Perhaps the first genus to explore for agriculture might be the *Lessertia,* predominantly from the Western Cape. *L. incana, L. diffusa, L. capitata,* and *L. excisa* exhibit many of the attributes we are seeking in new perennial legumes adapted to acid and infertile soils. Like many successful annual legumes, they are self-fertile and prolific seeders, with large seeds that germinate vigorously, and whose fruits adhere relatively strongly to the stems. The *Lessertia* are grazed in their natural habitat and become prostrate under high grazing pressure. Unusual characteristics found in other South African herbaceous legumes that might assist their adaptation to agricultural farming systems are their ability to root from stolons or rhizomes (as in several species of *Lotononis*), and to store carbohydrate in subterranean woody organs, as has been reported in *L. hirsuta* (van Wyk, 1991).

The rhizobiology of these herbaceous South African legumes is somewhat complicated, with recent reports of beta-bacteria from the genus *Burkholderia* (Moulin *et al.,* 2001) and both pigmented and non-pigmented, slow-growing *Methylobacterium* (Sy *et al.,* 2001; Jaftha *et al.,* 2002) among the unusual nodule occupants. Adding to this, we have isolated *Burkholderia* from the nodules of *Rhynchosia ferulifolia* and fast-growing, pink-pigmented *Methylobacterium* from species of *Lotononis* other than *L. bainesii* (Yates *et al.* 2007). The latter appear to be taxonomically distinct from the microsymbiont for *L. bainesii* previously reported by Norris (1958) and Jaftha *et al.* (2002). Parenthetically, pink-pigmented bacteria arise quite frequently in our isolations from non-traditional legumes and we can only assume that they have been overlooked in the past because of the routine use of Congo Red dye in rhizobial media (Vincent, 1970). Although the *Lessertia* are nodulated by *Mesorhizobium sp* (J.G. Howieson and R.J. Yates, unpublished), the majority of the nodule occupants from these South African Cape legumes await taxonomic identification.

In selecting novel legume species for the acid and infertile soils of the world, we should perhaps initially focus upon legume species that form a symbiotic association with rhizobia from the genera *Bradyrhizobium* and *Mesorhizobium* which have proven adaptation to stressful soils (Parker *et al.,* 1977; Howieson and Ballard, 2004). A corollary to this is that if we release new genera of rhizobia into agricultural soils, issues of inter-strain competition are probably greatly diminished.

4. MATCHING LEGUMES AND THE SYMBIOSIS TO EDAPHIC AND ECONOMIC PARAMETERS

4.1 The mechanics of developing new legumes and their root-nodule bacteria for production systems

We see five key steps in developing new legumes for agriculture.

1. The identification of the requirement for a new legume (*i.e.*, data on where and why current species fail).
2. An assessment of the economic and social issues relating to the introduction (or domestication) of a new species and its likely adoption.
3. The identification of appropriate germplasm with which to experiment.
4. Selection or breeding of legumes and their rhizobial genotypes adapted to each other, and to the target edaphic niche.
5. Assessment of the broader biological implications of introduction of both plant and microsymbiont, including "duty of care" issues such as understanding any biological threat posed by the new material.

We have discussed environmental issues such as salinity and rising water tables in this manuscript, acid soils, and the global requirement for higher protein diets, which are examples of the need to develop new species of legumes for agriculture (Step 1). A new legume must have a definable role in the farming system of the region and must be manageable in the social context of that farming system (Step 2). A flow diagram for sensible decision making in an economic and biological framework has been reported by Sessitsch *et al.* (2002) and this also covers the need to develop inoculant rhizobia. Herridge (Chapter 3) also reviews methodology for developing experimental evidence of the need to inoculate. Some constraints to legume use and adoption have been reviewed recently: economic, farming systems and social issues by Sessitsch *et al.* (2002), and marketing issues in the volume edited by Knight (2000).

A major consideration when domesticating new species is that matching plants to climate is more complex for perennial than for annual species. This relates to Step 3. Perennial legumes must survive for several years, whereas annual plants may need to survive for only a few months in every year – their dormant seed may carry them through to the next growing season. To match plants to climates for only half of the year is much more readily achieved than matching them for the whole year. Annual species in cold climates are often dormant in winter, but grow during the spring and summer on conserved moisture, or from melting ice or snow. These same species may be sown in subtropical or Mediterranean environments for winter production, because these winters are relatively mild. Thus the warm-season pulse *Cajanus* is now grown under cool-season conditions in northern Nigeria, and the cool-season pulse *Cicer* is grown in warmer environments such as southern India (Byerlee and White, 2000). Whereas perennial species from cold areas might grow during winter in other climatic zones, they rarely survive the warmer months and the drier summers, with low ambient humidity. In the development of novel perennial legumes, it is likely to be more important to closely match species to a similar climate than it has been in the past for annual plants (Step 3).

We have also covered some of the other decision-making processes that relate to the acquisition of appropriate germplasm for Step 3, from *ex situ* or *in situ* repositories, preferably in edaphic homologs. Part of the reason for the broad adaptation of legumes may be that, in their complex root systems, they seem to have adopted many characteristics from other plant families acknowledged as essential for survival in harsh soils. Apart from nodulation, these features include cluster roots, mycorrhizal associations (both ecto- and endo-; Sprent, 2001) and (as noted above) root tubers that store water and carbohydrate. It is important to identify the factors likely to limit legume cultivation before deciding upon where to source new germplasm and then focus upon regions where legumes may have evolved with adaptation to these factors. The Cape legumes illustrate this critical aspect of Step 3 in a search for concurrent adaptation to acidity and low rainfall.

Although legumes can exist under harsh conditions, many species are substantially more productive if given fertiliser. Very few legumes, however, can be cultivated economically (especially in broad-acre agriculture) when reliant upon large inputs of nutrients (N, P) and water, because the cost of these inputs exceeds the value of the end-products. Exceptions include legumes whose production is subsidised, legume sprouts grown horticulturally for salads, *T. pratense* sold into the nutriceutical market, *G. max, Phaseolus vulgaris* and forages such as *M. sativa*, whose seeds or hay fetch a high price. For most new legumes, it is mandatory that close to maximum growth and N_2 fixation is achieved under low input conditions, and this relates to Step 4 above.

For maximum N_2-fixation in the longer term (Step 4), the legume must be intimately matched to an appropriate strain of rhizobia, and the symbiosis then be robust in the target edaphic niche. The Centre for *Rhizobium* Studies at Murdoch University has developed a set of protocols that assess these attributes, including the saprophytic competence of strains to persist in soils over several seasons (Chatel and Parker, 1973). As an example, in the development of the second-generation ley species of annual clovers (Loi *et al.,* 2005) it quickly became apparent that the narrow host range of the long-term Australian inoculant strain for subterranean clover (WU95) would compromise agronomic evaluations of other annual *Trifolium* spp. (Howieson, 1999). The WSM strain collection was searched for strains with a broad host range through glasshouse studies on N_2-fixation (Howieson *et al.,* 2000b). Approximately 10 highly effective, broad host-range strains were then assessed for their adaptation to the target acid soils in Australia (Watkin *et al.,* 2000) and Uruguay (Real *et al.,* 2005; Yates *et al.,* 2005). After more than six years of experimentation, strains WSM409 and subsequently WSM1325 were released commercially, and have since proven to be very successful (Bullard *et al.,* 2005).

Other technical aspects of applying N_2 fixation in the field (Step 4) have been brought together in two recent journal special issues *viz.*, Graham and Vance (2000) and Herridge *et al.* (2005). Rather than attempting to summarise the information in those volumes, we focus in the remainder of this section on some newer concepts.

Legume exploitation today often involves their utilisation outside their geographic centres of origin, potentially exposing both legumes and rhizobia to conditions under which they have not evolved. Yet this may not necessarily hinder

their success if the process of selection is expert. After legumes and rhizobia have been introduced to a new environment (the final stage of Step 4), the challenge arises of maintaining the symbiosis in a state of maximum N_2-fixation. This is just one aspect of Step 5.

4.2 Maintaining an effective symbiosis

Brockwell and Bottomley (1995) and Giller (2001) give many examples of sub-optimal N_2-fixation in world agricultural systems. A major cause is considered to be competition for nodulation by ineffective soil-borne rhizobia. Such competition is rarely simple to manage, and at times it has been difficult even to determine the origin of competitive, yet ineffective, strains. Rhizobia are highly mobile as contaminants of dust and seed. For example, Stepkowski *et al*. (2005) have demonstrated that many of the contemporary lupin and serradella nodule occupants in farmland in South Africa and Western Australia derive from contaminant European strains imported to those continents unintentionally, probably in the preceding 200 years. In the Australian environment, high quality, commercial inocula for medics and clovers have been made available to farmers since the early 1900s (Bullard *et al.,* 2005), yet these same inoculant strains are rarely recovered from nodules (Brockwell *et al.,* 1995; McInnes, 2002) and nodule occupants are frequently ineffective (Ballard *et al.,* 2003). A similar scenario exists for soybeans in some parts of the USA (O'Hara *et al.,* 2002) and Brazil (Hungria and Vargas, 2000; Graham, Chapter 11).

Evolutionary theorists argue that host sanctions against ineffective nodule occupants should reduce the prevalence of mediocre symbioses (Kiers *et al.,* Chapter 2). As this has not yet become evident in the agricultural legumes examined, perhaps the time-frame for such evolution is much greater than the few hundred years in which agriculture has been exploiting legumes outside their centres of evolution. However, even in relatively undisturbed ecosystems, there is abundant evidence of the prevalence of ineffective rhizobial strains. Mesorhizobia that are poorly effective on the annual forage legume *Biserrula pelecinus* were isolated (5 of 33 strains) from nodules of this species growing in the grasslands of Morocco, Sardinia and Greece (Howieson, 1999). Perhaps more dramatically, only 1 of 8 strains isolated from the annual herb *Hymenocarpus circinnatus* growing in its natural environment of the Cyclades Greek Islands was capable of N_2 fixation with this host (Howieson, 2000). If the host is indeed placing sanctions upon ineffective nodule occupants, it would appear that there is still scope for the survival of these strains, in both agricultural and natural settings.

This serious issue of competition for nodulation by ineffective rhizobia might be averted, at least initially, by the introduction of novel species or even genera of rhizobia to agricultural environments as inoculants for alternative legumes. Because of genetic incompatibility, these novel genotypes would be incapable of nodulating the existing legumes. The grasslands of Uruguay, where production from grasses is

limited by nitrogen deficiency, provide an example of this approach. A native perennial clover (*T. polymorphum*), though well established in these grasslands, is not a highly productive species. It would therefore be beneficial to overall system productivity to supplement the growth of this clover with other legumes. A successful approach was the sourcing of alternative legumes which did not nodulate with the dominant rhizobial ecotypes and whose specific rhizobia could be introduced to that environment (Real *et al.,* 2005).

This approach may only offer transient relief from competition, however, as eventually we expect diversification in the rhizobia for the alternative legumes. How does this diversity of soil-inhabiting rhizobial strains arise and can it be managed?

4.3 Diversification of rhizobia in situ

Recent research by Sullivan and Ronson (1998) has revealed a potential mechanism for the evolution of diversity in rhizobia in agricultural settings. They have described the transfer of symbiotic DNA in discrete units termed 'symbiosis islands' from legume inoculants to soil bacteria. Nandasena *et al.* (2006) have subsequently described for the first time how the transfer of a symbiosis island from inoculant mesorhizobia to soil bacteria resulted in the rapid evolution of ineffective strains in the soil. Nodule isolates were recovered from *Biserrula pelecinus* six years after its introduction (with inoculant) to a new environment free of rhizobia capable of nodulating this legume. Of 88 nodule isolates, 81 very closely resembled the original inoculant strain of *Mesorhizobium spp.* (WSM1271), and produced equivalent amounts of N_2 fixation under glasshouse conditions. So, six years post-inoculation, and after two intervening cereal crops, nearly 90% of the nodule occupants remained identifiable progeny of the inoculant strain. However, several of the other seven isolates were very poor at N_2 fixation. These isolates (termed novel isolates) had mismatches with WSM1271 in the 16S rRNA gene of between three and 23 nucleotides, and clustered separately to WSM1271 in phylogenetic trees constructed using intragenic fragments of the *16S rRNA*, *dnaK* and *GSII* genes. The novel isolates also had distinct carbon source utilization patterns indicating they were different organisms, yet they contained identical sequences for the intragenic regions of *nifH, nodA* and *intS* to WSM1271. This provided strong evidence of an exchange of symbiotic DNA leading to the development of an ineffective suite of nodulating bacteria, within six years. This key finding explains how ineffective nodule occupants have arisen in agricultural systems where inoculation by elite strains has been strictly controlled.

The future management of this phenomenon, perhaps through understanding and manipulating the role of genes for excision and integration of the symbiosis island, is one avenue towards maximising long-term N_2 fixation in agriculture. In the future this may be a "duty of care" issue in Step 5 above. It is also possible that host-mediated sanctions against the mediocre symbioses will prevent the domination of the soil rhizobial populations by these "novel" strains. This sanctions theory (Denison, 2000) is more fully discussed by Kiers *et al.* (Chapter 2). However, the

emergence of the novel, ineffective but competitive strains described above, seems to present evidence against the global application of this theory.

5. UTILISING THE BASIC ADVANCES

How does our exploitation of legumes and nitrogen fixation benefit from three decades of molecular investigation? As many chapters in this volume testify, molecular technologies now provide unique tools and approaches that greatly expedite our exploration of the legume symbiosis. In the 1980s we saw the benefit of reporter gene fusions to indicate gene transcription. Insertional mutagenesis a decade later enabled phenotyping of knock-out mutations, whilst the modern 'omic' eras have provided information on enzyme, protein and gene structure and activity. In rhizobiology, these techniques have perhaps been applied most extensively to unravelling the complex signal pathways that govern the early stages of legume – bacterial recognition (Chapters 4, 5, and 9). Further, molecular marker techniques such as IVET (*in vivo* expression technology) allow us to now monitor gene expression directly in complex environments, such as in the rhizosphere (Allaway *et al.,* 2001) and thus providing the opportunity to understand success or failure of strain genotypes in these environments. Molecular markers have also greatly enhanced the efficiency of legume breeding, for example in rapidly developing cultivars resistant to anthracnose and phomopsis diseases of *L. angustifolius* (Yang *et al.,* 2004). But can molecular intervention be employed to create transgenic individuals of the nodule bacteria or the legume which have been modified in any way to enhance function in a given environment? Although there are few outcomes of engineering that have been adopted in current agricultural products, the exploitation of engineered herbicide resistance in *Glycine max* (soybean), insect resistance in cotton via the *Bt* toxin genes, and enhanced β-carotene production in rice, are evidence that applied outputs can be achieved by basic advances. In the latter example, seven foreign genes from two separate pathways were engineered into rice (Potrykus, 2001), although it is contested that the level of β-carotene produced in the transgenic plant will alleviate vitamin A deficiency.

Foreign gene insertion is an especially appealing approach to modify characters which respond to manipulation of a single gene. Unfortunately, many of the processes which are the subject of current research interest are highly complex – stress tolerance, control of recognition and infection, N_2-fixation, bacterial competition and saprophytic competence (Chapter 11) are all polygenic traits. Further, genes governing some of these traits are now known to be controlled by an associated set of regulatory gene products. The sigma factors are a good example of regulatory proteins, or protein subunits, whose task it is to ensure that RNA polymerase binds stably at a specific promoter site on DNA. Bacteria use alternative sigma factors to control sets of genes required for specific conditions. Thus, sigma factors may recognise, and then potentiate a rapid bacterial response to, extracellular signals (Gross, 1996). So, when molecular scientists planned and initiated the molecular manipulation of groups of genes in the late 1980s, they quickly discovered a further level of complexity that involved the regulation of these genes.

Is it thus folly to consider that we will be able to eventually manage the transfer of complex polygenic traits in either the prokaryotic or the eukaryotic components of N_2-fixation systems? Are there examples where we have achieved this?

There is the greatest likelihood of successful manipulation of a complex trait where the complete set of genes that govern and regulate that trait are found on discrete units of DNA, rather than distributed around the chromosome. Localisation of genes that govern a major trait into an operon may allow them to be transferred *in toto*. This appears to be the situation with the symbiosis islands that control nodulation and N_2-fixation in some microbial genera. The recent evidence of the *in situ* transfer of a symbiosis island from both *Lotus* and *Biserrula* mesorhizobia (Sullivan and Ronson, 1998; Nandasena *et al.,* 2006) to soil bacteria to create new nodulating organisms strongly suggests that we should be able to exploit this mechanism in the laboratory. As nature already has, we should be able to produce bacteria with an altered host range for nodulation. For other genera, such as *Sinorhizobium* and *Rhizobium,* where symbiotic genes appear to be located on discrete plasmids, it is feasible that we might also transfer the nodulation character between genera. Will this provide benefit to agriculture however? A scenario where this might be beneficial is in the acid-sensitive symbiosis between *M. sativa* and *S. meliloti.* This symbiosis is universally accepted as extremely acid-sensitive (Dilworth *et al.,* 2001). In contrast, the symbiosis between *P. vulgaris* and *R. tropici* is considered to be acid tolerant (Graham *et al*., 1994). The pH-sensitive component of both symbioses resides primarily (but not entirely) with the prokaryotic partner (Vargas and Graham, 1988; Cheng *et al*., 2005). The bacterial genes for nodulation and N_2 fixation in both symbioses are found on plasmids. The opportunity exists, therefore, to alter the host range of *R. tropici,* to enable it to nodulate *M. sativa.* This approach would potentially create an acid-tolerant microsymbiont for the most important perennial forage legume on the planet.

The current molecular era provides unprecedented opportunities in researching complex traits, because complete genome sequences of symbiotic organisms are becoming available. The phenotype(s) expressed following directed or focussed gene disruption provide unprecedented clarity of information on gene function. The availability of rapid sequencing has also been applied to phylogenetic and taxonomic research, and this has produced a revolution in the classification of the nodulating prokaryotyes (Chapter 1). Nodule bacteria now include several representatives of the *beta*-bacteria (Chen *et al*., 2003). Access to molecular methodology for bacterial classification has been especially useful to confront the uncertainty that arises when a legume is apparently nodulated by unusual microsymbionts. In such situations it has been possible to probe nodule isolates for symbiosis-essential genes, such as *nodA* and *nifH* and, if these are present, to then sequence the 16S rRNA gene of the organism to assist in its eventual identification.

There have also been enticing hints that intervention in the genetics of the micro-symbiont (Bosworth *et al.,* 1994) or the legume (Carroll *et al*., 1985) can increase N_2 fixation. Many years on from these reports, these advances have not yet seen large commercial adoption. This is, perhaps, because in situations where the symbiosis is mildly sub-optimal, the genetics of the symbiotic partners are less

limiting to N_2 fixation in the field than are other factors such as light, moisture and nutrient supply. Where the symbiosis is substantially sub-optimal, straightforward empirical screening, such as that reported by Brockwell *et al.* (1995) and Howieson *et al.* (2000b) can provide a cost-effective and immediate solution to mediocre N_2 fixation.

6. CONCLUSIONS

Despite their widespread biological benefits, most of our legumes have never been surveyed for their potential contribution to primary production systems, nor indeed for their biologically active constituents. This review has attempted to highlight some future uses to which our legumes may be put and pathways to achieve this development. However, our genetic biodiversity is constantly under threat through loss of habitat, desertification, overgrazing or illegal trade of medicinal plants. Of the 6,000 Latin American legume species, many are considered to be at risk of extinction in the next few decades (Rumbaugh, 1990). Currently, ten species of *Trifolium* endemic to the USA have been identified as threatened and sixteen Old World taxa are known or suspected to be endangered or vulnerable (Morris and Greene, 2001). For several centuries, medicinal plants have also been used by farmers and pastoralists as a primary source of prevention and control of livestock diseases (Hoareau and DaSilva, 1999). With the rapid loss of ethnic cultures and customs, some of these plants used in organized traditional medical systems will also no doubt disappear. It is becoming more important now than ever before that we explore and preserve these species before they are lost to science. While most of the agronomic research into legumes over the last century was directed at increasing yields in food and fibre plants (Abelson, 1994), considerably more emphasis in this century needs to be focused on research to identify plants with potential to supply valuable products for pharmaceutical and nutriceutical use, and for other alternative but valuable roles in modern society. This chapter has attempted to project forward and anticipate some of those roles that may be applicable to legumes and their rhizobia. To quote Akerele (1988); we need to “Save Plants that Save Lives”. A research focus on the continued exploitation of the enormous natural genetic variation available in both legumes and their microsymbionts will contribute to continued field application of biological N_2 fixation, which is undeniably one of the key biological processes on this planet.

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COMMISSION ON GENETIC RESOURCES FOR FOOD AND AGRICULTURE

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TECHNICAL ISSUES RELATING TO AGRICULTURAL MICROBIAL GENETIC RESOURCES (AMIGRS), INCLUDING THEIR CHARACTERISTICS, UTILIZATION, PRESERVATION AND DISTRIBUTION

A DRAFTINFORMATION PAPER PREPARED FOR THE GENETIC RESOURCES POLICY COMMITTEE (GRPC) OF THE CGIAR

This document has been prepared by, and is circulated at the request of, the CGIAR, in the language in which it was received.

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EXECUTIVE SUMMARY

PART 1

[ES.1] Plants and animals can not grow optimally without microbes, and 90 percent of flowering plants form some association with microbes to enhance their growth. Biological Nitrogen Fixation (BNF), for example, is one of the most important biological processes on the planet, turning inert nitrogen gas from the air into a form that plants and animals can use to make protein.

[ES.2] Agricultural Microbial Genetic Resources (AMiGRs) may be defined as microbes that assist the production of plants or animals, either directly or indirectly, in agricultural settings. AMiGRs can be differentiated from Microbial Genetic Resources (MGRs) utilized in food, medicine and industry, but for many this can only be upon the basis of their functionality (or end-use), as species overlap both categories. AMiGRs have been preserved in a series of *ex situ* repositories associated with institutions or individuals around the globe, in more modern times as freeze dried or frozen (-80°C) cultures.

[ES.3] After root nodule bacteria (RNB), the most preserved microbes appear to be pathogenic fungi and bacteria that are used as type specimens in breeding efforts. Germplasm repositories for bacteria, in particular, have embraced lyophilization as the preferred storage method.

[ES.4] There is evidence that germplasm collections are discarded as the key curator retires, particularly if the germplasm is not freeze dried. Only about half the germplasm repositories surveyed seem to have an accessible electronic database.

[ES.5] The development of a series of *in situ* plant repositories coordinated by ICARDA in West Asia provides an opportunity for associated preservation of AMiGRs for plants and insects, but perhaps not for animal microbes. The AMiGRs most likely to be successful are those that are endophytic (i.e. they invade host tissue) because organisms that only colonize the surface of the target are often non-competitive against microbes already well adapted to that environment

[ES.6] AMiGRs have been used since antiquity, but they have only been properly scientifically described since the late 19th and early 20th centuries, and this process is ongoing

[ES.7] Many currently exploited AMiGRs evolved in developing countries, but were transported to alien shores by accident through contamination of plants, animals or fodder, or in jet-streams. AMiGRs are considered in some quarters to be the ‘bio-prospecting’ entities of tomorrow, as plants are today. International coordination of genetic resources has yet to focus upon, or manage, AMiGRs. There is uncertainty whether AMiGRs utilized today can be reliably traced to their origins, even with the genetic techniques now available.

[ES.8] RNB seem to have been de-emphasized in the CGIAR system in the last decade, perhaps because many consider the work with them to have been completed. However, there appears to be continuing advances with RNB in other agricultural economies. It seems incongruous that many projects are built around microbial germplasm repositories that are uniformly poorly resourced. Some countries, such as China, India and the former Soviet Union, have a cultural history of utilizing AMiGRs, and this is becoming reflected in the nature of the AMiGRs held by some CGIAR centres.

PART 2

[ES.9] It is complex to assess the needs of, or potential benefits from, applying AMiGRs in agriculture because responses are often species and environment specific. This is the greatest challenge in embracing AMiGRs. AMiGR usage in developing countries is often limited by lack of manufacturing capacity and quality control. This needs to be addressed. Developing countries often subsidize imported N[itrogen] fertilizer to make this affordable for their farmers. An alternative is to develop RNB. A global benefit of this is that legume N fixation does not contribute to greenhouse gas emissions, whereas the manufacture of 1 tonne of urea burns 1 tonne of fossil fuels.

[ES.10] AMiGR adoption in developing countries would benefit from the availability of a 'core set' of AMiGRs with which to experiment. This would remove the initial time-consuming need to authenticate cultures and to establish their phenotype. A core set of AMiGRs could readily be developed by scientists who have collaborated in microbial germplasm exchange and evaluation programmes with the CGIAR centres. Countries that hold AMiGRs, for example in *in situ* repositories, may not yet be exploiting them, and thus the cost of conserving germplasm is borne by them for no immediate reward. Developed countries are prominent users of AMiGRs, mainly RNB, but they distribute a narrow range of organisms over vast acreages, and this has implications for loss of microbial biodiversity in these regions.

[ES.11] It is difficult to foresee where (geographically) the next range of exploitable microbes may arise. For example, Australia's microbes may become globally useful in bioremediation, and hence her current role as a net user of agricultural genetic resources (without contributing to the cost of their preservation) might well be reversed. Although the usage of AMiGRs in some agricultural systems might be routine (e.g. RNB), and the benefit of this application may be high (as estimated by the monetary cost of replacing N fixed by RNB with fertilizer N), the wholesale value of manufactured microbes is much lower, and thus any royalties levied on production are not likely to be of high value. The major obstacles to uptake of AMiGRs in developing countries are discovering, preserving and cataloguing the available AMiGR biodiversity, accurately ascertaining the beneficial properties of any AMiGR, and then manufacturing, distributing and utilizing high quality inoculants.

[ES.12] The USDA has moved to centralization of curatorial responsibility for MGRs in the USA. There is evidence that South American countries are utilizing this centralized facility. It could be possible to assign curatorial responsibility for one mainstream group of AMiGRs to each continent.

[ES.13] There has emerged an 'official' approach to acquisition and exchange of AMiGRs over the last decade, with Material Transfer Agreements (MTAs) and Memoranda of Understanding (MOUs) covering acquisition and exchange as well as future control of any commercial outcomes.

[ES.14] The taxonomy of microbes, particularly bacteria, in the 21st century, is unsettled. It is difficult to develop standards for the identification for many microbes, except for type strains whose genome is fully sequenced. AMiGRs may be separated from MGRs on the basis of functionality, but there is overlap, and for some microbes this distinction may have to be at the species level. AMiGRs are delivered live, whilst MGRs generally transform a process and are then eliminated—a major difference between AMiGRs and MGRs that can influence the possibility of obtaining and enforcing patents for MGRs used in food, medicine and industry. The situation for AMiGRs is clouded by their rapid rate of reproduction, and potential change during culture.

[ES.15] Quarantine and biosecurity concerns are reducing the extent of germplasm exchange, more so than issues of 'ownership'. Commercial entities in the 21st century are patenting AMiGR manufacturing and delivery technologies, rather than the microbes themselves. This reflects that a major challenge in utilization of AMiGRs is the development of appropriate manufacturing and delivery technologies.

PART 1

I. INTRODUCTION: HISTORICAL PERSPECTIVE

[1] Although the Romans wrote of the beneficial effects of cultivating nitrogen-fixing legumes such as lupins and pulses in rotation with cereals, the deliberate utilization of microbes in agriculture awaited the advances in manufacturing technologies that were developed towards the end of the 19th century. The invention of fine instrumentation for observing microorganisms, the subsequent development of specific growth media and then microbe purification enabled the microscopic world to be studied in detail. The root nodule bacteria (RNB) for legumes were almost certainly the first group of agricultural microbes to be studied at the microscopic level, and this was in the same decade that proof emerged (in 1883) that microbes such as *Vibrio* spp. were the causative agents of serious human and animal illnesses, such as cholera. RNB were, in fact, manufactured as agricultural amendments within a few years of Beyerinck isolating and growing the bacteria, and Wilfarth and Hellriegel identifying their role in legume nodulation and nitrogen fixation in 1887. This was only 6 years after Koch first cultured bacteria on gelatin. The early adoption of RNB inoculants was achieved by transferring soil from field to field, or soil to seed before planting, but this was quickly replaced by the supply to farmers of pure cultures on agar slants, then as broths. The first inoculant industries for RNB developed in the 1920s, with peat carriers available from the 1950s (Deaker et al. 2004). Global inoculation of legumes with RNB is valued at in excess of US$ 10 billion annually (calculated on the basis of the cost of replacing RNB-fixed N with manufactured N; Herridge 2005). This equation does not include the additional benefit that legume N fixation is a net user of greenhouse gases, whereas the manufacture of fertilizer N is energy demanding, and thus a net producer of greenhouse gases.

[2] Concomitant with the isolation of RNB from nodules, the understanding of the diversity of microbes interacting in symbioses with plants was expanded with the discovery of the relationship between certain fungal hyphae and plant nutrient acquisition. Frank described the fungus-root interaction with mycorrhizae in 1885, and it is now realized that about 95 percent of all vascular plants are involved in symbiotic associations with fungi. The most notable of these roles is with vesicular arbuscular mycorrhizae (VAM) and ectomycorrhizae in the acquisition of phosphate. It was not long into the 20th century before the role of the soil microflora in the development of plant disease and also in nutrient cycling in the soil ecosystem could be quantified. The concept of the ‘rhizosphere’ and its role in plant growth was described in the 1950s, and the capacity for rhizosphere organisms to affect plant growth by hormone production, diazotrophy (non-symbiotic N fixation) or nutrient acquisition reported soon after. Rumen microbiology had become a discrete science by the 1970s, and the molecular communication between microbes and plant roots (or animal cells), leading to regulation of gene cascades, was revealed in the 1980s. The latest phase in the discovery of microbes as plant symbionts is in their role as intercellular endophytes. Within (or between) plant cells, secondary metabolites from endophytic microbes elicit plant responses. The best described of these associations is with *Aceotobacter* sp. in Brazilian sugar-cane systems, which has the capacity to provide N in excess of 30 kg/ha. Unfortunately, difficulties with culturing the endophytic VAMs (for phosphate acquisition) has restricted their widespread adoption.

[3] It is now accepted that without these multiple aspects of microbial activity in the soil and rhizosphere, as well as in plant and animal tissues or cells, healthy plant and animal growth would not be possible. However, another facet of AMiGRs which is in a phase of development is in the use of microbes as indirect agents of plant growth (i.e. restricting a competitor or predator, rather than as plant symbionts or initiators per se). Thus, we now see a range of AMiGRs being considered as biocontrol agents for crop insects (e.g. *Metarhizium* spp.; nuclear polyhedrosis viruses) and fungal plant pathogens (e.g. *Bacillus subtilis*) to protect crop plants from disease. This field is termed ‘entomopathogenicity’ and there are several registered products currently on the market. One of these, *Metarhizium anisopliae,* has been used successfully to avert grasshopper plagues developing in outback Australia, prior to them moving towards farmers’ crops. This sort of application of AMiGRs, together with RNB, entomopathogens and VAM, has great potential in developing countries. It is

worth noting here the historical widespread use of two classes of AMiGRs in China, India and the former USSR: organisms that stimulate root growth through hormone production or through diazotrophic N production, commonly termed 'yield increasing bacteria' (YIB), have gained substantial acceptance in the rural communities of these nations.

[4] Thus, AMiGRs have been used, in one way or another, since antiquity, with the science of their interactions with plants, insects and animals only elucidated in the last 125 years. There is still much to learn about the microbes that enhance and protect animal growth in both natural and agricultural settings.

II. SCOPE OF THE REVIEW

[5] The scope of the review was defined by the Terms of Reference (see Appendix 4).

III. MICROBIAL GENETIC RESOURCES FOR FOOD AND AGRICULTURE AS A DISTINCT SUBSET OF MICROBIAL GENETIC RESOURCES

[6] Following the development of RNB as inoculants in the late 19th century, other microbes are now applied in agriculture, in a relatively wide variety of roles. These disparate roles can be summarized from a functional perspective, and then compared with microbes used in food, industrial processes and in medicine production. Differences between the two groups of microbes are considered, and there is a discussion of how effectively we have captured these roles to enhance agricultural production.

[7] The main functional roles of microbes in agriculture are considered to be as:

- plant microsymbionts;
- associative organisms (i.e. eliciting or enhancing a positive reaction or effect when in intimate proximity to a plant or animal);
- rumen organisms;
- biocontrol agents (pathogens of weeds, fungi, insects or nematodes);
- pathogens of plants or animals;
- agents for nutrient solubilization, bioremediation or biodegradation;
- agents for production of biofuels; or
- agents facilitating DNA or gene transfer.

Examples of some AMiGR within these functional roles are given below, with more details to be found in Appendix 1.

Plant microsymbionts (specifically RNB) — overwhelmingly the most successful AMiGR in agriculture

[8] RNB, like legumes, are found on all continents. The RNB nodulate the Leguminosae, which is one of the largest families of flowering plants, with more than 18 000 species classified into 650 genera (Sprent, 2001), just under one-twelfth of all known flowering plants. RNB tend to colonize the soils in association with their host legumes, although there is speculation (and indeed evidence) that some species of RNB 'invade' soils well in advance of their host. Not all the legumes fix atmospheric N, however, and amongst the subfamilies of the Leguminosae, the species within the Fabaceae are recognized as those of greatest agricultural importance. Some of our most valuable food crops, such as pea (*Pisum* spp.), beans (*Phaseolu*s spp.), ground-nut (*Arachis* spp.) and soybean (*Glycine* spp.) are Fabaceae, producing high-protein grains for human consumption. Of all the plants that man uses for

food, perhaps only the grasses (Graminiae) are more important than the legumes (Graham and Vance, 2003).

[9] The symbiotic association between RNB and legumes plays a significant role in world agricultural productivity by annually converting approximately 100 million tonnes of atmospheric nitrogen into ammonia (Herridge and Rose, 2000), and saving $US 10 billion in fertilizer N. This is a critical issue, as many countries (both developing and advanced) have not fully embraced biological nitrogen fixation and are substantially reliant upon fertilizer nitrogen. This lack of adoption of RNB is attributed to many factors: from a lack of knowledge and expertise in growing and inoculating legumes with rhizobia (Giller, 2001), to government subsidies in both developing and advanced economies that militate against the use of biological nitrogen fixation. Sadly, with the price of fossil fuels inevitably increasing, small economies will be faced with either food shortages or an inflated bill for fertilizer N. Many developing countries rely upon buying urea for rice production (Thein and Hein, 1997). Their declining purchasing power in real terms will be deleterious for food production; this must be addressed, as current reviews forecast that food production will need to double by 2020 to feed our expanding population (Byerlee and White, 2000).

Vesicular arbuscular mycorrhizae (VAM) and ectomycorrhizae

[10] Approximately 90 percent of all flowering plant species belong to families that form mycorrhizal associations. Mycorrhizae can be either endophytic (exist within cells) or grow between cells (ectophytic) of plant roots. Both patterns of development can be viewed as providing an extension of the plant root systems for the purpose of exploring a greater soil volume for nutrient uptake. Mycorrhizae and their interactions profoundly affect forest site productivity through capture and uptake of nutrients, protection against pathogens, maintenance of soil structure and buffering against moisture stress. The nutrients that are most often limiting plant growth are fixed nitrogen (N) and phosphorus (P), and it is for alleviating deficiencies of the latter that mycorrhizae have proven efficacious. Where soil P levels fall to 1 or 2 ppm, plant growth is usually constrained. Unfortunately, many heavily leached tropical soils are at or below this level and it is in these environments, as well as in severely eroded regions, that applications of mycorrhizae can be effective. Although the VAM are difficult to culture, they are the preferred type of inoculant, so we see cottage industries in tropical and subtropical countries where soils containing VAM are used to inoculate trees in nursery situations. When planted out into degraded lands, the VAM-inoculated seedling trees have a distinct advantage over uninoculated trees. VAM utilization has not spread to broad-acre crops for two main reasons. Firstly, it is difficult to inoculate crops with soil containing VAM over wide acreages, and, secondly, P fertilizers can effectively replace VAM. Despite this, VAM is a *bona fide* AMiGR in horticulture and forestry applications, and in rehabilitation exercises.

Microalgae, including Cyanobacteria

[11] Cyanobacteria (formerly termed blue-green algae) are photosynthetic prokaryotes, usually unicellular, some of which have the capacity to fix atmospheric nitrogen. The capacity of Cyanobacteria to fix N has long been utilized in paddy rice fields to provide additional N to the rice-growing system, reducing the need to supply all the crop N requirements from combined fertilizer. The Cyanobacteria utilize the water and phosphorus applied to the rice crop, and sunlight as an energy source. The species of Cyanobacteria most commonly utilized in paddy fields is the filamentous algae *Nostoc* spp., which forms a symbiotic association with the water fern Azolla in paddy fields. *Nostoc* spp. may also associate with *Gunnera* spp. and the terrestrial Cycads. *Nostoc* spp. has been transformed by the addition of *Bacillus thuringiensis* (*Bt*) genes to investigate the potential of this alga to control insects in rice production.

Associative organisms: Plant Growth Promoting Rhizosphere (PGPR) organisms or Yield Increasing Bacteria (YIB)

[12] China, India and the former Soviet Union have a long history of experimenting with, reporting and even manufacturing microbes that can be classified as PGPR or YIB. These microbes fit within functional group 2 (see Figure 1). The microbes are generally bacteria that form close associations with plant root systems, but may also be actinomycetes, fungi or endophytes. As a result of a plentiful supply of nutrients exuded from the roots in the rhizosphere, the PGPR have the capacity to grow and produce of enzymes such as ACC deaminase, whose action reduces the production of ethylene under stress conditions. Hormones, such as indole acetic acid (IAA), which affect root growth, branching and hair formation, are also commonly produced by PGPR, together with some N fixation (albeit in small amounts). There are many more mechanisms in which PGPR may benefit their hosts, from disease protection, nutrient solubilization to controlled exchange of mutually desirable proteins. With the cloning era, it has become possible to investigate more elaborately the relationship between PGPR and the host plant, and it is becoming obvious that many of the relationships are established by a complex pathway of low molecular weight (LMW) biochemical signals that control gene expression.

[13] Many of the commonly reported PGPR microorganisms are ubiquitous and it is possible to isolate them from garden, farm and forest soils. Because of the ease of isolation of the common PGPR, there is little exchange of this sort of germplasm per se. For those more difficult to culture, such as the actinomycetes and endophytes, there is substantial laboratory-to-laboratory exchange. Appendix 1 contains descriptions of some of the microbes commonly referred to as PGPR. The Pseudomonads have been used extensively in broad-acre agriculture for many years, but there is very little hard and convincing data that proves yield enhancement from their application. Similarly, *Penicillium* spp. have been developed as agents for solubilization of soil-bound phosphate, although modern studies have questioned this role and attributed their efficacy to direct impacts on plant growth.

Rumen organisms

[14] Some animals have a second stomach called the rumen, in which a suite of microbes assist in the breakdown of otherwise indigestible forages. The best researched rumen microbes are those that enable the digestion of forage containing high tannin levels, but other rumen microbes enhance fibre and cellulose digestion, and mitigate anti-nutritional factors. In cellulose degradation, a complex suite of microbial-mediated actions is initiated by anaerobic prokaryotes and protozoans, which liberate carbohydrates from cellulose. The carbohydrates are then fermented to gaseous end products. There is continuing research to select rumen microbes that minimize the release of methane (a greenhouse gas) to the atmosphere. The rumen microbes eventually overflow into adjacent stomach compartments, where their degradation by stomach acids yields amino acids and sugars that provide animal nutrition. Apart from minimizing methane production, other research interests include modifying the rumen microflora to metabolize toxic compounds found in some forages, such as the fluoroacetate found in many legumes. There is evidence that the rumen microflora can naturally evolve in response to the nutritional environment of their host, and that this response can be transferred from animal to animal.

Biocontrol agents, such as Metarhizium anisopliae (an insecticide), Bacillus subtilis (a fungicide) and B. thuringiensis (an insecticide)

[15] There are approximately 15 different biopesticides in current commerce, with *Bacillus thuringiensis* (*Bt*) accounting for approximately 45 percent of the market. A related species, *B. subtilis,* has been developed as a root-active fungicide, for protecting horticultural plants from pathogens. *B. subtilis* is sold as a fungicide for application to flower and ornamental seeds, and to agricultural seeds including cotton, vegetables, ground-nut and soybean. The bacterium colonizes the developing root system of the plant and competes with fungal disease organisms. The fungal genus *Metarhizium* is another AMiGR that has long shown promise as an insecticide. The successful mass culture of *M. anisopliae* and development of methods of mass-producing infective spores has led to the commercial uptake of this fungus as a microbial ‘insecticide’. *M. anisopliae* is grown on a large scale

in semi-solid fermentation and the spores are then formulated as a dust suspended in oil. This may be aerially applied to insect plagues. In Australian trials, application of *M. anisopliae* from aircraft in remote Queensland controlled a developing locust plague by killing 90 percent of the insects.

Pathogens of plants or animals

[16] Plant and animal pathogens need to be considered as AMiGRs because they are held in germplasm collections to facilitate breeding or selection programmes to find resistance to them. For plants, the pathogens are dominantly fungi, bacteria and viruses, most of which are ubiquitous at the genus level, but many of which have distinctive 'landraces' that are geographically separated. The transport of agricultural plants and animals to new geographical locations is now strictly regulated to control transfer of such pathogens, yet it appears the transfer of pathogenic microbes eventually follows the movement of their hosts. For example, the development of *Cicer arietinum* (chickpea) as an industry in Australia flourished in the early 1990s, but has since been seriously constrained by the development of Ascochyta blight disease, which was previously unrecorded in that country. There has been substantial success in managing the unwanted transfer of animal pathogens. For example, the Foot-and-Mouth virus, in the genus *Aphthovirus,* has been effectively excluded from many major meat producing regions by restrictive quarantine efforts.

AMiGRs as agents for nutrient solubilization, bioremediation or biodegradation

[17] This group of AMiGRs can be considered as separate from the associative organisms in Functional group 2 (Figure 1) principally because they interact with inanimate and inorganic targets (in contrast to the plants or animals that host the associative microbes). Targets for this group of AMiGRs include the (substantial) pool of inorganic phosphate held in the soil, toxic chemicals inadvertently accumulated or deposited in the soil, such as DDT, heavy metals and fossil fuels.

AMiGR for production of biofuels

[18] Biofuels, such as ethanol, have been considered an expedient alternative to fossil fuels since the petroleum fuel crisis of the 1970s. Essentially, carbohydrates derived from sugar-rich plants such as cassava, sugar beet or sugar-cane are fermented to ethanol by yeast in anaerobic respiration, but also occasionally by some bacteria. These microbes might be considered as AMiGRs because of their strong linkages to broad-acre agricultural enterprises.

AMiGRs facilitating DNA or gene transfer

[19] Although bacteria have been exchanging DNA since life formed on the planet, the cloning era began in earnest post-1985 with the deliberate laboratory transfer of whole genes, or parts of genes, between bacteria. Such transfer is now routine in many laboratories, between almost all higher lifeforms. There are universal vehicles for facilitating the transfer of DNA. The most common vectors in agricultural research are *Agrobacterium* spp. for plant-to-plant transfer and *Escherichia coli* for inter-bacterial transfer. Thus, these microbe vectors should be considered as AMiGRs because of their direct relevance to agricultural research.

[20] Fuller descriptions of some of the microbes that fill these functional roles can be found in Appendix 1. It is noteworthy that by far the most successful AMiGRs in broad-acre agriculture appear to be those that are endophytic, i.e. they invade the tissues of their host, for all or part of their life cycle, rather than residing on the surface of the target plant or animal. On the surface they may become exposed to competition from resident organisms that are, perhaps, better adapted to that particular environment.

Grouping the AMiGR into functional roles

[21] AMiGR may be broadly grouped as in Figure 1. Functional roles 6 and 7 in Figure 1 group together microbes that interact with nutrients, biomass or pollutants for bioremediation or fuel production. The beneficial symbiotic organisms (1, 3) can be grouped with those that also increase growth of plants or animals as associative microbes (2). The pathogens (4, 5) can be grouped, whether they are directly beneficial or not, because their modes of action are similar (i.e. they decrease growth of the target organism). These last-named two functional groupings (highlighted) contain those microbes that have seen major exploitation in agriculture.

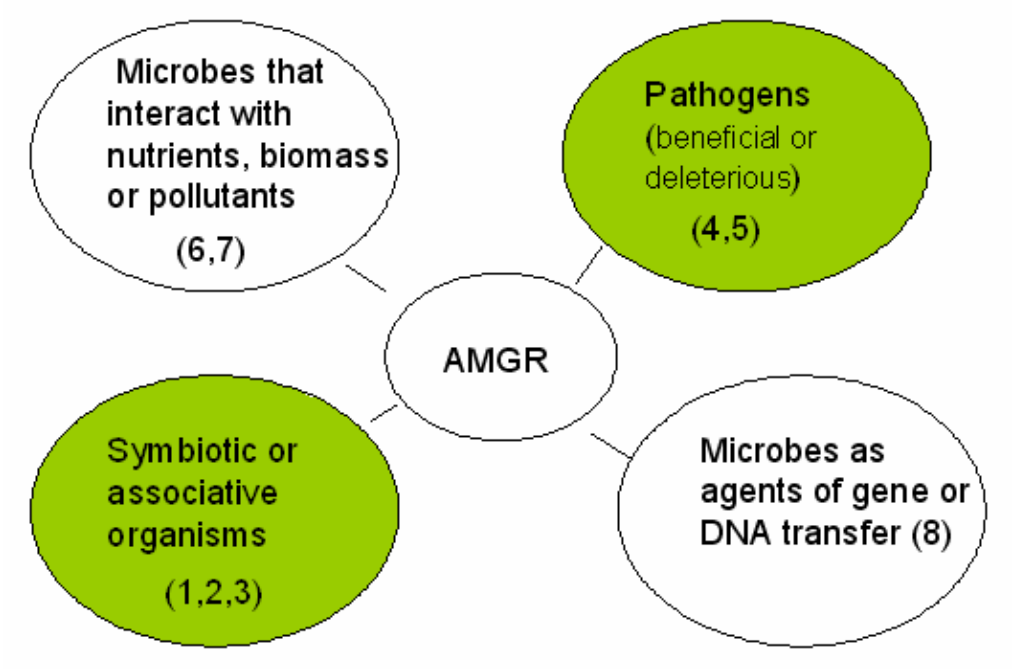

Figure 1. AMiGRs assigned to functional groups, with the highlighted groups being those most exploited in agriculture.

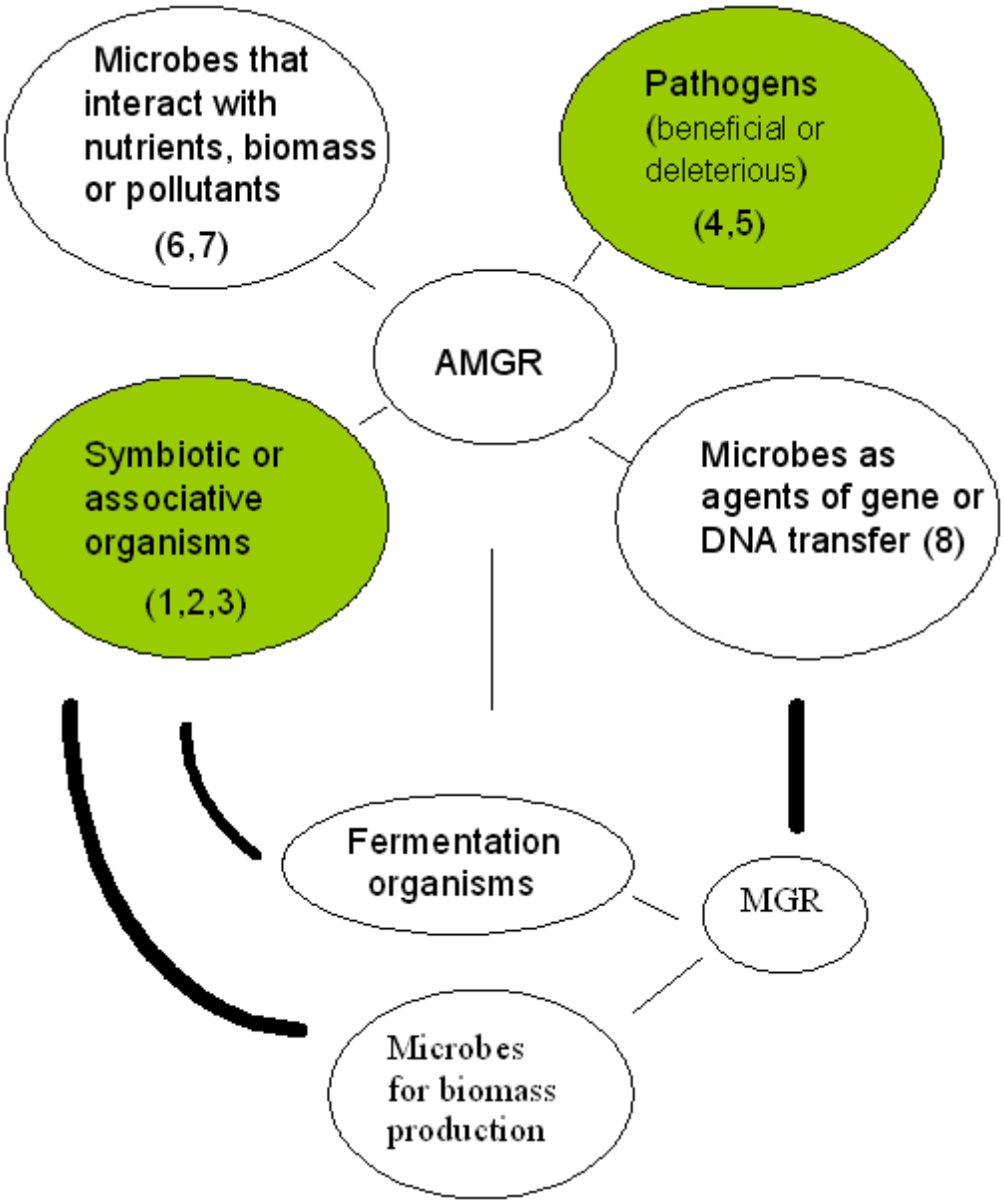

Figure 2. Functional groups of MGR added to those of AMiGR, and with an indication of areas of overlap with those used in Agriculture (thick bars).

Microbes in food, medicine or industry

[22] The main functional roles for microbes in food, medical science and industry (i.e. MGRs) are considered to be in:

- fermentation of foods and beverages;
- manufacture of medicines and pharmaceuticals;
- gene or DNA transfer; or
- mass culture as a source of pigments or antioxidants, or as a feed base for higher organisms.

[23] Fuller descriptions of these functional roles can be found in Appendix 1, but for comparison they are placed alongside those used in agriculture in Figure 2.

Areas of overlap. How may the agriculturally relevant groups be best separated from those utilized in food or medicine?

[24] There appear to be at least three functional roles that directly overlap between agriculture and either food, medicine or industry. The first is the use of microbes as agents for transfer of genes or DNA. Examples are *E. coli* and *Agrobacterium* spp., both of which are used extensively as carriers of plasmids holding DNA. *Agrobacterium* spp. is well described as a parasite of agricultural relevance. The second overlapping role is in microbes (usually microalgae) used in mass culture, that might also be associated with plants in agricultural settings. Specifically, microalgae can be utilized to produce pigments or antioxidants (e.g. beta-carotene, astaxanthin), fine chemicals (e.g. phycocyanin from *Spirulina* spp.) or to produce bulk feed in aquaculture industries (e.g. *Chlorella* spp.). However, the Cyanobacterium genus *Nostoc* is widely utilized to fix atmospheric nitrogen in association with rice production. Thirdly, there is significant overlap in the functions of fermentation for foods and associative organisms for plants. Microbe genera that overlap in these two groups include *Penicillium* spp. and *Acetobacter* spp., which are used in the production of fermented dairy products, as well as being important rhizosphere or endophytic microbes for plants. Yeasts, of course, are essential in fermentation of food and beverages, but are also a key microbe in the production of ethanol as a biofuel.

[25] However, it might be pragmatic to delineate AMiGRs from MGRs on the basis of their role in primary production. Thus AMiGRs might be considered (*vide* Figure 1) as:

> "microbes that are utilized, directly or indirectly, to assist the production of plants or animals in agricultural settings"

Adherence to this definition would separate those MGRs utilized for biomass production in aquaculture (e.g. microalgae) or for food fermentation from those microbes utilized *in situ* in agricultural settings. Microbes routinely used for gene or DNA transfer (such as *E. coli* and *Agrobacterium* spp.) and for fermentation would then overlap both sectors, as shown in Figure 2.However, can the groups realistically be separated in this way, or are the overlaps just too numerous? If we look further there are other areas of overlap in industry, where *E. coli* and *Clostridium* spp. are used in ethanol or butane production (which is a fermentation process), *Penicillium* spp., yeasts and *E. coli* are exploited in production of antibiotics, alkaloids, steroids, insulin and growth hormones (outside of agricultural settings), and *Aspergillus* spp. and *Bacillus* spp. produce enzymes utilized in food or health processes. All of these genera are, or may be, utilized as AMiGRs. The challenge in defining a distinct set of AMiGRs then becomes one of separating the functional groups at the species rather than the genus level. It is an outstanding question as to whether this enterprise is warranted.

IV. THE PHYSICAL NATURE OF COLLECTIONS AND HOW THEY DIFFER

[26] Microbe 'collections' can be considered as either *in situ* or *ex situ. In situ* collections may be of two types:

- the remaining undisturbed areas of the globe where microbes evolved and remain to this day relatively undisturbed as a component of the natural biodiversity; or
- in disturbed sites where, because of the general resilience of microbes, the perturbation to the environment has not eliminated them.

There is some evidence that in perturbed sites, such as long-term polluted sites, the microbe populations have been enriched in those organisms capable of remediating the pollutants.

[27] In both forms of *in situ* repository, the microbes are probably dependent upon some form of host interaction for their survival and multiplication, whether with plants, animals, insects or other microbes. Few microbes are competent saprophytes in isolation.

[28] *Ex situ* collections are of three major forms and the major difference from *in situ* repositories is that in these collections the microbe is usually cultured in pure form, in the absence of any host. The full metabolic requirements of the microbe must be met from artificial sources. *Ex situ* collections may be:

- collections amalgamated and fostered in the care of an individual;
- collections associated with institutions, and, more correctly, departments of institutions, which accept curatorial responsibility for them; or
- in association with commercial entities that exploit the microbes.

V. THE HISTORY AND ACTUAL GLOBAL PATTERNS OF DISTRIBUTION OF THESE ORGANISMS

General considerations concerning AMiGR distribution and exchange patterns

[29] Many AMiGRs are microscopic bacteria, or form spores, and it is difficult to contain such microbes geographically. As for pathogens, AMiGR will cross borders in aerosol form, in dirt or as unintentional contaminants. This re-distribution of microbes has been concomitant with exploration of the globe by man. The implications of this are that the geographical origin of many microbes is difficult to ascertain, and, further, that widespread application of an AMiGR will eventually lead to the widespread availability of that AMiGR. This is compounded by the fact that AMiGRs, unlike most microbes used in food reactions, are delivered to their target in a live state. Without a comprehensive and expensive border quarantine effort, it is unlikely that any unwanted re-distribution of an AMiGR could be prevented. For example, DNA is currently exchanged between laboratories through postal services by simply applying a small quantity of DNA to a sheet of paper and circling that spot on the letter. The recipient simply elutes the DNA from the paper and then amplifies it for use via the polymerase chain reaction (PCR) process.

[30] CGIAR, university and institutional scientists have historically and routinely exchanged AMiGRs or components of them (plasmids, DNA) for centuries. Culture repositories around the globe now hold thousands of cultures that have been accumulated in this fashion. By doing so, the science and exploitation of AMiGRs has rapidly advanced. The implications of this are that whilst there has been valuable preservation of genetic material *ex situ,* control of AMiGR at the species level has become clouded. Further, the value of any particular AMiGR is attached overwhelmingly to its manufactured form rather than to its germplasm form. As an example of this, culture collections sometimes contain over 1000 representatives (strains) of an organism. Individuals of this collection only become valuable after special attributes of them are identified, and the strain subsequently commercialized.

[31] In recognition of the above points, a policy of facilated exchange of AMiGR with a harmonized form of multilateral benefit sharing seems most practicable. Proof of geographical origin and of strain identity will, in many cases, be impossible to provide and thecosts of enforcing a rigid constrainment policy will also far exceed the value of the AMiGR, and will reduce the global exploitation of beneficial microbes. There may be some resistance to this approach by countries who perceive themselves as the countries of origin of AMiGRs. To counter this, the development of a core set of authenticated AMiGRs for facilitated distribution, with benefits flowing to developing countries in general, but not to particular suppliers, is suggested.

[32] A policy of facilitated exchange of AMiGR must be seen as separate to individual country policies on microbial biosecurity, as those policies might logically be applicable to any manufactured product, or to importation and distribution of pathogenic microbes.

GLOBAL CHANGES

Root Nodule Bacteria

[33] Without doubt, the greatest global changes in RNB distribution have come about with man's exploration of the world in the 18th century and then with their use as inoculants for legumes, particularly in the 20th century. Massive changes have occurred in the tropics, subtropics and warm temperate zones of Africa, Asia and America, where *Glycine max* (soybean) inoculated with *Bradyrhizobium japonicum* now dominates grain legume production. There is nearly 70 million tonne of inoculated soybean produced annually in the USA, in addition to 34 and 53 million tonne in Argentina and Brazil, respectively (USDA, 2005). This compares with the global trade in cool season grains of approximately 60 million tonne (Kelley et al., 2000), which suggests that soybean is probably the single largest traded legume commodity in the world. The RNB inoculants for this crop, which probably evolved in China, have thus been distributed over more than 150 million hectares of the Americas in the last 30 years.

[34] Similarly to soybean in the Americas, large tracts of land have been cleared of their native vegetation in central Asia, temperate America and southern Australia and planted to cool season forage legumes from two main genera, *Trifolium* (clovers) and *Medicago* (medics). Again, the majority of these legumes have been inoculated at some stage in their production with (the AMiGR) RNB. The perennial forage *M. sativa* (alfalfa; lucerne) has wide adaptation to soil and climate and because of this has spread from its centre of origin (believed to be in the temperate zones of Persia) to become the dominant forage on all continents in the last three millennia, carrying its RNB with it. No perennial form of a *Trifolium* species has achieved such prominence. Annual clovers and medics were established across 25 million hectares of arable land throughout southern Australia in the 19th and 20th centuries, with RNB inoculants available since 1896. As for the tropics, this represents a massive global change in distribution of RNB.

[35] At the same time, despite these examples of success in legume breeding and adoption, it is of concern that there are perhaps only 50 species of forage legumes and less than 15 species of grain legumes in wide global commercial trade (Kelley et al., 2000). Is it prudent, from a gene conservation perspective, to cover the globe so completely with only 65 of a potential 18 000 species of legume inoculated with only relatively few strains of RNB? We have evidence that these inoculants displace the original RNB. What is this doing to the *in situ* conservation of AMiGR biodiversity?

[36] The Australian usage of RNB AMiGR provides a good example for analysis of some of the issues relevant to this review. The value of RNB to Australian agriculture is estimated at AUD$ 3 billion annually, in terms of N fixed estimated by the replacement cost of N as urea fertilizer. All of this N fixation is by strains that were originally exotic to Australia, originating from the Mediterranean basin and western Asia for the temperate strains, and a range of tropical origins, including Africa and South America, for the tropical inoculants. Further, almost all of the strains that are commercially manufactured in Australia and that have been developed over the last 40 years have come from germplasm collected either *in situ* in focused collection missions, or *ex situ* from genebanks. This suggests a commercial exploitation of AMiGR by Australian agriculture from resources held by developing countries. However, the manufacturing industry that produces these inoculants has a wholesale value of less than AUD$6 million. Thus, the $3 billion benefit accrues from a $6 million industry, and it is the latter from which returns could be made to the country of origin of these inoculants. However, there is one pertinent example in this scenario that cannot be ignored. The lupin inoculant accounts for over 55 percent of RNB sales in Australia. The strain utilized, WU425, was originally isolated from naturalized serradella nodules found in Western Australia. It is believed that both the serradella and the rhizobial strain arrived by accident on Australian shores in the 19th century transport of animal fodder. This illustrates the difficulty of attempting to manage AMiGR movements around the globe, because microbes have moved accidentally with the development of global shipping. To reinforce this, recent genetic analysis of 50 lupin nodule isolates from Western

Australian fields examined by Thomas Strepkowski in Poland revealed that all of the isolates were from Europe, and that none had been deliberately introduced to Australia.

[37] So managing AMiGR exploitation by developed countries in such a way that the country(ies) of origin of the microorganism (often developing countries) may benefit faces dual difficulties, namely that:

- the value of AMiGR manufacture may be several orders of magnitude less than the value of their impact; and
- AMiGR (and pathogens) demonstrably transit country borders unaided

[38] This example of lupins in Australia is paralleled by that of soybean in the USA and South America, i.e. the current commercial inoculant strains evolved outside the geographical boundaries of these countries (actually in China), and were unintentionally transmitted to the New World, originally as contaminants on seed or in trash. All three soybean inoculants in America came from isolates made from naturalized soil populations. A similar scenario exists with alfalfa (lucerne). The movement of plant pathogens such as rusts (*Puccinia* spp.*)* and blights (e.g. *Phytopthera* spp.) from continent to continent is strong evidence for microbial transfer in aerosol form via the stratosphere.

[39] The scenario with newly developed legumes and their inoculants differs from the examples given above. In Australia there has arisen a 'second generation' of pasture legume species in the last decade (Howieson et al., 2000). Several species that form this second generation are new to agriculture and hence their inoculants have not always accidentally been carried around the world. For these legumes, the inoculants arose following targeted acquisition activities and their pedigree can be clearly traced. It is likely that some of these new species will ultimately be sown across tens of millions of hectares. However, the wholesale value of their inoculant manufacture will be measured in the tens of thousands of dollars per annum, and thus royalties from these, were they to be imposed, would be almost insignificant. Royalties are not currently paid on commercially manufactured rhizobial inoculants in Australia and the strains are distributed to manufacturers on the basis of a non-exclusive licence.

VAMs and ectomycorrhizae

[40]Uptake of VAMs and ectomycorrhizae has been significant, particularly in subtropical and tropical agriculture in Asia, where aid programmes have demonstrated the benefits of inoculation in the nursery phase. As with RNB, there is not always a response to inoculation with mycorrhizae, because many soils already contain naturally effective strains. The challenge in utilizing VAMs more widely is to develop regional knowledge of where positive responses are likely to occur, and to develop strains of VAM that are adapted to both the soils and crops of interest. This has happened, for example, in rattan plantations in southern China, where selection of locally effective VAM strains has resulted in increased production of rattan. There appears to be a gradual increase in VAM application around the globe and this may spread to developed countries as P fertilizers become more expensive.

Biocontrol agents, such as Metarhizium spp., B. subtilis and B. thuringiensis

[41] *Bacillus subtilis* is naturally widespread globally, and was actually one of the first bacteria to have its genome fully sequenced. The uptake of this AMiGR has been predominantly in horticulture or intensive agriculture in developed countries. Of greater impact has been the related species *B. thuringiensis,* used as an insecticide in many countries since the 1950s. *B. thuringiensis* produces a range of crystal proteins with varying degrees of toxicity to coleopteran and lepidopteran insects. Genes isolated from *B. thuringiensis* have been incorporated into commercial plant genomes for protection against insect pests, the most notable of which is the cotton boll weevil. The Pasteur Institute has a broad collection of both genes and strains of *B. thuringiensis* available for research purposes. Several genes have been patented since 1980. Although target organisms evolve resistance

to *Bt* toxins, the combined application of chemicals and biopesticides such as *B. thuringiensis* is a seen as a desirable development in integrated pest management.

[42] *Metarhizium* fungal spores can be produced in large-scale fermentors, but they can also be grown on sterilized rice in plastic bags for small-scale production. One limitation to widespread *Metarhizium* development is its sensitivity to temperature extremes; spore viability decreases as storage temperatures increase and virulence decreases at low temperatures. However, the broader application of *Metarhizium* to control cockroaches and white ants may increase its uptake. As for mycorrhizae, there has been a slow but steady uptake of AMiGRs as biopesticides since the 1950s, when the environmental implications of widespread chemical pesticides were first understood and publicized.

China and India, and the use of PGPRs or YIBs

[43] There has been historical acceptance of PGPRs in China, India and the former Soviet Union agriculture, with a research effort dating back some 50 years. The majority of these applications are of the diazotrophic microbes, in search of N accretion. It appears the use of PGPRs is static in these countries, neither declining nor becoming a mainstream activity. This influence is now spreading to South-East Asia, where co-inoculation of rice paddy fields with PGPR microbes (again predominantly diazotrophs) is gaining acceptance. There is certainly substantial research activity exploring the role of PGPR in rice growing in this region. Analysis of the published data on PGPR globally suggests that in more than 30 percent of reported applications of PGPR (generally associative N fixing *Azotobacter, Azospirillum* or *Clostridium*), a yield increase of 5 to 10 percent has been statistically demonstrated. It is difficult to gauge how much unreported experimentation with PGPRs is undertaken, and the range of the results of this work.

[44] In developing countries, the focus of PGPR application is on phosphate solubilization, stimulation of root length and early root growth, disease suppression, and nodulation enhancement. There is little doubt that inoculation of agricultural plants with PGPR can elicit a measurable response in the plant for all these factors. It is more problematic to transfer this plant response into an actual increase in grain yield.

VI. SURVEY TO ASSESS THE PHYSICAL NATURE OF CGIAR CENTRES HOLDINGS OF AMIGRS

Current status

[45] The Street (2000) review of AMiGR holdings in CGIAR Centres reported the breakdown of the microbial resources held at that time. A comparison is provided with the current situation in Table 1.

Table 1. Microbial resources held in CGIAR Centres in 2000 compared to 2005.

Microbe or functional group	Number in 2000†	Number in 2005
RNB	7780	6816
Animal pathogens	1326	na
Aquatic free-living N fixers	740	na
Plant pathogens	Undocumented	>1000
Entomopathogens	Undocumented	125
Mycorrhizae	Undocumented	>100
Rumen microorganisms	Undocumented	na
Non symbiotic beneficial microbes	Na	>600
Total (documented)	9846	8641

NOTES: † Data from 2000 derives from the review by Street (2000).na = data not available.

[46] From information received for the CGIAR survey (December 2005), the situation has altered somewhat since the Street (2000) review. ICRISAT, for example, in addition to 715 RNB, now holds significant numbers of plant pathogenic fungi (>1000), as well as a range of PGPR microbes (306). Interestingly, ICRISAT has also accumulated a number of entomopathogens (120) in the last few years. This evolution reflects the changing global patterns of AMiGR research quite well (although global usage of AMiGR is still dominated by RNB). The number of PGPR microbes held by ICRISAT is also consistent with the historical acceptance of these forms of AMiGR in Indian agriculture. During the mid-1980s and until 1995, ICARDA had as many as five scientists working with AMiGRs, predominantly with RNB. There are now no scientists active in this area at ICARDA, and no projects are being serviced from the collection. However, the RNB germplasm has been lyophilized and an electronic database is kept updated. There is, however, activity in integrated pest management using biopesticides, so this represents a further indication of trends in AMiGR usage in the CGIAR system. The downturn in active research with RNB at ICARDA has coincided with an increase in the usage of RNB in west Asia and North Africa, where farmers are inoculating pulses with cultures of rhizobium strains selected and manufactured locally.

[47] To provide a contrast to the response of the CGIAR Future Harvest Centres vis-à-vis other organizations, the questionnaire was also circulated within Australia. In Australia, holdings of RNB numbered approximately 7000, whilst there were collectively approximately 2500 plant pathogenic fungi, bacteria and viruses. The major institutions in Australia (e.g. CSIRO, State Departments of Agriculture, large universities) held collections of PGPR microbes and plant pathogens. The Grains Research and Development Corporation (GRDC) had implemented an AUD$ 10 million programme on Soil Biology (2003–2008), a large proportion of which is allocated to studying microbe-plant interactions.

[48] It seems incongruous that many projects (seven at the time of the 2005 survey) were built around microbial germplasm repositories that were uniformly poorly resourced. In Australia, a current research emphasis on the development of novel perennial legumes would be severely constrained without immediate access to RNB germplasm**.**

PART 2

VII. BASIC NEEDS AND CHALLENGES IN USING THESE AMIGRS IN THE GENERAL CONTEXT OF AGRICULTURAL DEVELOPMENT FOR THE COMING YEARS

[49] The primary needs and challenges can be distilled down to four:

- Discovering, preserving and cataloguing the available AMiGR biodiversity.
- Accurately ascertaining the beneficial properties of any AMiGR.
- Manufacturing, distributing and utilizing high quality AMiGR inoculants.
- Ensuring equitable access to AMiGR and sharing benefits associated with their use .

These challenges are discussed below. However, a substantial aid to the adoption of AMiGRs by developing countries would be the availability of a core set of AMiGRs (perhaps with representatives from each of the functional groups) that could satisfy the first two of the four requirements.

Preserving biodiversity

[50] CBD sets out principles of conservation and accesss and benefit sharing concerninggenetic resources. The application of the access and benefit sharing principles of the CBD is challenging in relation to AMiGR because microbes can easily transcend borders, as described earlier. CBD also suggests scientific experiments should be undertaken within the country of origin of the genetic resources, where possible. This is likely to be a difficult or impossible undertaking with AMiGRs because response to inoculation is likely to be species and environment specific.

[51] *In situ* repositories are, of course, relatively inexpensive to maintain, but there are substantial sociological, legislative and community consultation procedures to work through to ensure they succeed. With continued development of arable land, are we certain that maximum genetic diversity can be protected in these repositories? *Ex situ* collections are the converse: with relatively low diversity and expensive to maintain. The very positive outcomes of the current ICARDA project in biodiversity conservation with *in situ* repositories should provide a framework for further development of such collections. AMiGRs for plants are inevitably preserved wherever *in situ* repositories are proclaimed, but they must be large to preserve microbes associated with animals. There is some debate as to how many *in situ* repositories are required to capture a wide sample of AMiGRs. While many of the AMiGRs are ubiquitous at the genus level, stress-tolerant strains or species of AMiGR usually evolve in the presence of that stress, and these situations may be local.

[52] It is also pertinent here to discuss the loss of hosts for AMiGRs as an issue relative to loss of physical habitat of the AMiGRs. We can sometimes fall into the error of considering the AMiGRs in their habitat, but in isolation from their hosts. In reality, the loss of the host is more a threat to conservation of AMiGRs than the loss of diverse habitats, and this is more likely with animals than with plants. It is realistic to assume that whenever higher forms of life become extinct on this planet, then there is the strong likelihood that specific microbes associated with these lifeforms are also lost.

[53] The biosecurity aspects of exchange of AMiGRs cannot be ignored. The key issues here are, from the recipients' viewpoint, the potential loss of microbial biodiversity *in situ* following the application of an AMiGR to a new environment (i.e. competition for survival of microbes within that environment), the introduction of unwanted microbes, and the introduction of known pathogens. These are clearly matters of concern for sovereign governments, but are subjects of internal policy that should not be confused with the global exchange of AMiGRs.

Differentiating strains of AMiGRs

[54] While species of AMiGR may be nearly ubiquitous, strains vary considerably. For example, strains of RNB that belong to a single species and that nodulate a single species of legume can differ greatly in their N fixation and ecological properties. Molecular techniques, usually based upon some form of PCR (such as PCR-RFLP [polymerase chain reaction - restriction fragment length polymorphism]) can reliably differentiate microbial species at the strain level, yet not all microbes are amenable to PCR. Techniques for reliably differentiating strains within the broad suite of AMiGRs (fungi, bacteria, archae, viruses, algae, etc.) would need to be developed. These techniques are almost certainly likely to be based upon molecular methods.

Classifying microbes

[55] As with the discussion on differentiating microbes, despite the wealth of molecular tools available, microbial taxonomy is in a state of rapid change as we learn more about lateral transfer of genes on mobile genetic elements. There is little consensus amongst microbiologists on how to reliably classify many microbes below the genus level, particularly the bacteria. Nomenclatural changes have the potential to unwittingly confuse the origins of some AMiGRs in collections.

Handling AMiGRs

[56]Microbes replicate very quickly and the conditions under which they are cultured can lead to genetic change (drift), mainly through loss of plasmids or DNA units bearing non-essential genes. Bacteria and fungi can be readily freeze dried or lyophilized in glass ampoules, and this should be the preferred mode of preservation. If ampoules are kept below 15°C, the microbes commonly have a life span of over 50 years. However, not all microbes can be lyophilized or stored at -80°C. The microalgae are one such class of AMiGR that must be routinely subcultured, which is expensive and unreliable. So the optimal methods for handling some types of microbes for long-term storage needs further research.

Code of conduct

[57] With MOSAICS [Microorganisms Sustainable use and Access management Integrated Conveyance System – an EU initiative], a voluntary and guiding code of conduct already exists to assist suppliers and receivers of materials ensure that they are in compliance with the basic tenets of the CBD, namely that materials are accessed subject to prior informed consent (PIC) and on mutually agreed terms. This covers access to and circulation of MGRs, a pathway that tracks utilization and potential commercial benefits arising from exploitation of MGRs. This could be adopted for AMiGRs. MOSAICS is premised on the notion that suppliers and access seekers will negotiate new terms and conditions for each case. One possibly very useful value-added approach would be to develop a harmonized, pre-agreed set of terms and conditions that could be used for exchanges between a wide range of parties for specified purposes, such as research, conservation, etc. Such a harmonized approach would usefully complement the development of an internationally publicly available core set of AMiGRs, as discussed elsewhere.

Institutional continuity

[58] The world AMiGR collections appear to be associated with individuals rather than institutions, and thus when the individual relinquishes their position, the germplasm collection suffers. This seems to be the case for most CGIAR collections, which are ‘working collections’ rather than genebanks per se. The contrast here might be made with herbaria, seed banks or some microbial collections, such as those at USDA-ARS and Ghent (Laboratorium voor Microbiologie, Universiteit Ghent) where there is substantial funding for long-term curatorial purposes. Of significance is that a well-maintained culture collection is the product of many work-hours of collection, propagation, preservation, experimentation, authentication and documentation. It represents intellectual property that should not

be summarily dispensed with, and successional planning through the appointment of a curatorial position is the best way to achieve security.

Trends in amalgamating AMiGR collections

[59] In both the USA and Australia, the trend over the last two decades has been towards amalgamation of collections, particularly for RNB. With the withdrawal of CSIRO from rhizobiology, the Australian CSIRO collections (prefix CB (Brisbane), and CC (Canberra)) have been amalgamated into the WSM (curator Howieson, Perth), SARDI (curator Ballard, Adelaide) and US (curator Kennedy, Sydney) genebanks. However, only the lyophilized cultures were transferred (some 1000 cultures), with those held on agar slopes being destroyed. The Sydney US genebank is considered to be vulnerable, with the imminent retirement of Professor Ivan Kennedy. This situation reflects the generally poor long-term planning in relation to germplasm of AMiGR at the global level, even where the value of these microbes is acknowledged.

Recognizing and attaching value to AMiGRs

[60] In many traditional disciplines of biology, the value or role of microbes is not (transparently) recognized. For example, in the International Union of Forest Research Organizations (IUFRO) there is the IURFO Root Physiology and Symbiosis Unit. This unit has no public policy on the preservation of forest microbial genetic resources. It seems that most collections of forest microbes are privately owned and held in universities.

[61] However, in the USA, the USDA ARS has assumed responsibility for the majority of RNB collections held on the North American continent (curator Peter van Berkum). This raises the possibility of a model for AMiGR collections, with one repository per continent being nominated as the key core collection.

[62] A more detailed look at the USDA Agricultural Research Service (ARS) system in relation to AMiGR is provided below.

USDA ARS NATIONAL MICROBIAL GERMPLASM PROGRAM

[63] The goal of this programme is to ensure that the genetic diversity of agriculturally important microorganisms is maintained to enhance and increase agricultural efficiency and profitability. The programme will collect, authenticate and characterize potentially useful microbial germplasm; preserve microbial genetic diversity; and facilitate distribution and utilization of microbial germplasm for research and industry [Author's note: this is in the context of benefit to the USA as presented in the US Farm Bill outlined in Appendix 3].

[64] ARS in fact maintains several microbial germplasm collections, including:

- ARS Culture collection
- ARS Collection of Entomopathogenic Fungal Cultures (ARSEF)
- ARS National Rhizobium Genetic Resource Center
- ARS National Fungus Collections

The ARS National Rhizobium Genetic Resource Center has allocated funding of US$ 140 000 per annum in addition to the salary of its curator.

[65] Some aspects of the management and policies of these collections are relevant to this review:

➢ Identifying and acknowledging ARSEF strains in publications

> 'We ask that all publications using or referring to strains obtained from ARSEF acknowledge the ARSEF culture collection and state the ARSEF accession numbers of these strains. We would greatly appreciate receiving reprints of all past, current, and future publications involving ARSEF strains.'

Accession numbers of strains from commercial culture collections, such as the American Type Culture Collection (ATCC), Centralbureau voor Schimmelcultures (CBS), CAB International Mycological Institute (IMI), and the University of Alberta Microfungus Collection (UAMH), are listed in this catalogue only for the sake of providing complete information. Cultures received from ARSEF should be referred to by their ARSEF numbers only. Citation of cultures obtained from ARSEF by any corresponding ATCC, CBS, IMI or UAMH accession numbers they may also have is a violation of trademark laws; persons doing so are subject to prosecution.

➢ Updated, special, and electronic catalogues

Periodic updates of the general and special ARSEF catalogues and the update to the printed 1992 catalogue will be mounted on the Web page. Printed copies of the 1992 catalogue of ARSEF isolates (covering isolates up through 3736) are available without cost upon request to the curator. Complimentary copies of the ARSEF database and the customized application used to manage it can be obtained upon consultation with the curator of the ARSEF collection. It was anticipated that a fully interactive, searchable version of ARSEF culture accession data would be made available on the Web site in 2004.

➢ Depositing and exchanging cultures

The ARSEF culture collection encourages deposition of entomopathogenic fungal cultures—particularly strains used in published studies—as well as of voucher and reference specimens to its herbarium. Depositors may reserve the right to limit redistribution of any culture deposited with ARSEF for specified periods upon consultation with the curator. Depositors can receive subcultures of their own depositions at any time; these cultures do not affect any allowances for free cultures. Exchanges of cultures between ARSEF and other research or general collections of fungal cultures are encouraged and are not subject to numerical limits.

[66] Prior to shipping cultures from countries outside the United States, contact the Curator to obtain the appropriate needed importation permit from the U.S. Department of Agriculture, Animal and Plant Health Inspection Services, Plant Protection and Quarantine. When sending cultures and/or specimens to ARSEF, it is very important to include as much of the following information as possible:

- Scientific name (and taxonomic authority) of the fungus.
- Common and scientific name (with taxonomic authority) of the host.
- Order and family of the host. [This is essential information!]
- Date and site of collection.
- Name of collector.
- Date and name of isolator.
- Any collection, accession, or other identifier number(s) applied by the collector or sender.
- Medium on which a culture is sent.
- Any special requirements or conditions for growth (such as medium, temperature, pH).

➢ Diagnostic Services for Cultures and Specimens

Specimens and cultures of unidentified fungi from invertebrates can be submitted to ARSEF for diagnosis. This service is an important function of the ARS Collections of Entomopathogenic Fungi and is provided without charge. Identifications and information about the disposition of specimens will be mailed to the sender.

➢ Release of ARSEF Cultures from Containment or Quarantine

Neither the curator nor any employee of ARSEF or of the Plant Protection Research Unit is entitled to authorize the release of any culture it provides from laboratory containment or quarantine in the United States or elsewhere. Recipients of ARSEF cultures are responsible for obtaining all appropriate and necessary permissions from or for providing official notifications to State and Federal regulatory agencies.

The pragmatic value of a core set of authenticated AMiGRs

[67] This document hypothesises that a core set of 'authenticated' AMiGR might be developed by scientists and institutions who have historically collaborated in exchange of microbial germplasm. A core set would be different from a type set: the latter providing a taxonomic basis, the former providing a proven phenotype. The benefits of a core set would be two-fold. Firstly, it has been identified that the development of AMiGRs is hindered by the need for researchers to devote substantial time to procurement of microbes, followed by purification (if the organism is not from a reliable source), identification, laboratory or glasshouse evaluation, and finally *in situ* experimentation. The steps between procurement and *in situ* experimentation are considered as authentication. The second advantage is that developing countries (from which many AMiGR have been sourced) would perhaps be great beneficiaries of such a scheme, as the authentication steps can be difficult. As an example, if a research group were interested in developing a plant growth promoting organism based on the enzyme ACC deaminase, there may be several work-years required for isolation, purification, development of the bioassay for production of the enzyme, then selection of isolates for evaluation *in situ.* This same process has been undertaken in many laboratories over the last 20 years, and by now there should be available a set of strains, probably representing many species, that are well characterized for this enzyme. Selections from among these would represent a core set of ACC deaminase strains from which new projects might be developed. They could be thought of as 'control' strains for comparison with new isolates, or possible strains for commercial development in their own right. The concept of a core set parallels the 'type' strains available for serious diseases, or cancerous cell lines, which are widely distributed in medical research laboratories. It differs from current taxonomic 'Type Strains' in culture collections in the sense that the phenotypes of the core set of microbes would be substantially well researched. For example, the taxonomic Type Strain for *Sinorhizobium meliloti* is Sm1021. Although much is known about Sm1021 genetically, it is poor at nitrogen fixation when in association with many species of its host genus *Medicago.* Sm1021 would thus not be very useful as a core strain for evaluation in agricultural settings.

[68] So, how might this core set concept work in practice? The concept might initially be floated at the major international microbiology conferences. If there was general enthusiasm for the concept at the individual level, which was then supported at the institutional level, there would follow development of a working party to assess which AMiGR groups might be suitable for inclusion in the core set. Obvious candidates are the RNBs, PGPRs, pathogens, pathogen suppressors and probably others from the major functional groups 1–8 in Figure 1. An ensuing Web-based activitymight then be suitable for the process of deciding which AMiGR groups, and then which individual species and strains, might be accepted as the core set for each group. The strains finally accepted into the core group would be based upon agreed standard levels of authentication and, importantly, *in situ* performance from a number of valid tests.

[69] The major costs in developing and then servicing a core set of AMiGR are difficult to predict. The development phase might be potentiated by direct donations of strains from individuals or institutions. The costs of servicing the core set would be determined to a substantial extent by the demand. An estimate of the cost might be gained from enquiry through the USDA in relation to their RNB, or the Pasteur Institute for their *Bacillus thuringiensis* collection.

[70] Suitable partners in developing a core set of AMiGRs in the initial phase would be the CGIAR genebanks and public institutions such as the USDA, which have demonstrated a willingness to hold publicly available materials and supply them internationally. If the concept were favourably received (and there was appropriate recognition for acceptance of an organism into a core set, such as journal publication), it is possible that donations to the core set might rapidly gather momentum.

[71] A further consideration concerns how much of the usefulresources currently held by organizations could actually be globally, publicly distributed. This is a question that would require extensive review of each accession's legal status with reference international laws, national laws, intellectual property ownership, and the conditions under which those materials were supplied (and by whom) to the organizations concerned.

VIII. OBSTACLES FOUND IN USING AMIGRS, WITH EMPHASIS ON DEVELOPING COUNTRIES

Accurately ascertaining the beneficial properties of any AMiGR and demonstrating bona fide responses to inoculation of AMiGR

[72] The data for AMiGR response, apart from RNB and mycorrhiza, is seldom convincing. For RNB, the USDA NifTAL programme, and its follow up, the Worldwide Rhizobial Ecology Network, noted that where rhizobial populations of compatible strains were less than 10 per gram of soil, 93 percent of experiments produced yield increases in excess of 140 percent. However, where soil numbers were higher, 10 to 100 per gram of soil, the response dropped dramatically, to 68 percent frequency and 8 percent magnitude (Herridge, 2005). Determining the need to inoculate and then the response for other AMiGRs represents a substantial barrier to their scientific credibility and their adoption. As noted previously, for associative diazotrophes the frequency of response to inoculation drops to around 30 percent of published reports, but the magnitude to an alarmingly low 10 to 30 percent. These responses are difficult to accurately measure. In China, India and the former USSR, the relatively widespread use of AMiGRs seems to be more a cultural phenomenon than scientifically based. Perhaps there is merit in accepting that responses to AMiGRs will rarely, if ever, be comparable with those from RNB.

Decision-making in relation to the opportunities or benefits arising from application of AMiGRs

[73] The information explosion has delivered a multitude of reports relating to successes or failures with AMiGRs. Access to these reports is becoming more efficient, with on-line journals, although the information transfer to developing countries is certainly slower than for developed countries. Notwithstanding this, most reviewers acknowledge that responses to inoculation with AMiGRs are site and species specific. Thus, a major obstacle in developing countries to uptake of AMiGRs is in assessing whether there is likely to be local benefit from them. Although decision-making of this kind is not simple, Figures 3 to 5 illustrate a model developed for legumes and inoculation with RNB that could be adapted by regional scientists for application to a broader range of AMiGRs.

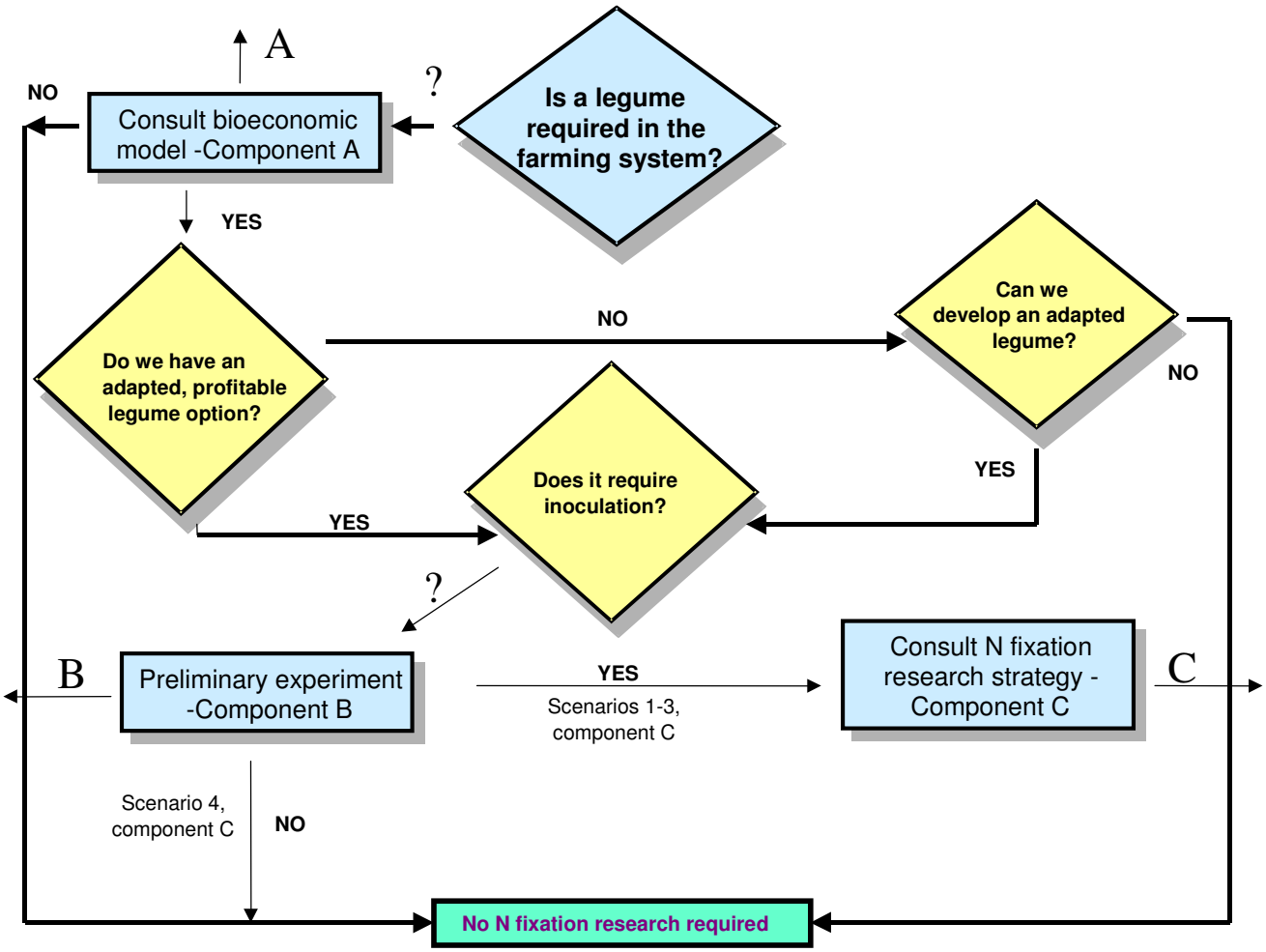

Figure 3. A flow chart illustrating the range of decisions required prior to initiating a legume or rhizobial selection programme.

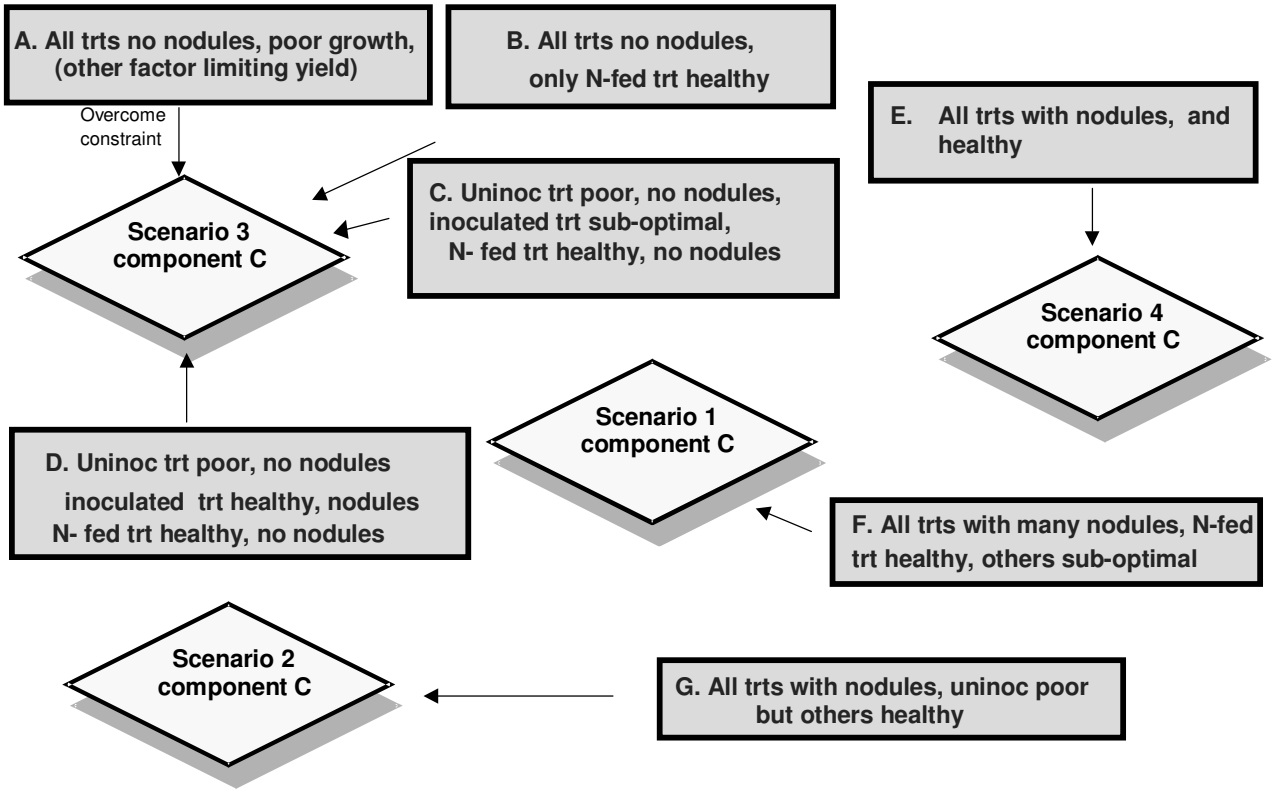

Figure 4. Component B. The possible outcomes of a preliminary inoculation experiment to determine if a legume requires inoculation in a particular soil. The experiment has three legume treatments- uninoculated, inoculated with a "best bet" strain and N-fertilized.The ensuing research requirements are represented in Component C (see Figure 5).

Notes: trt(s) = treatment(s). uninoc = uninoculated.

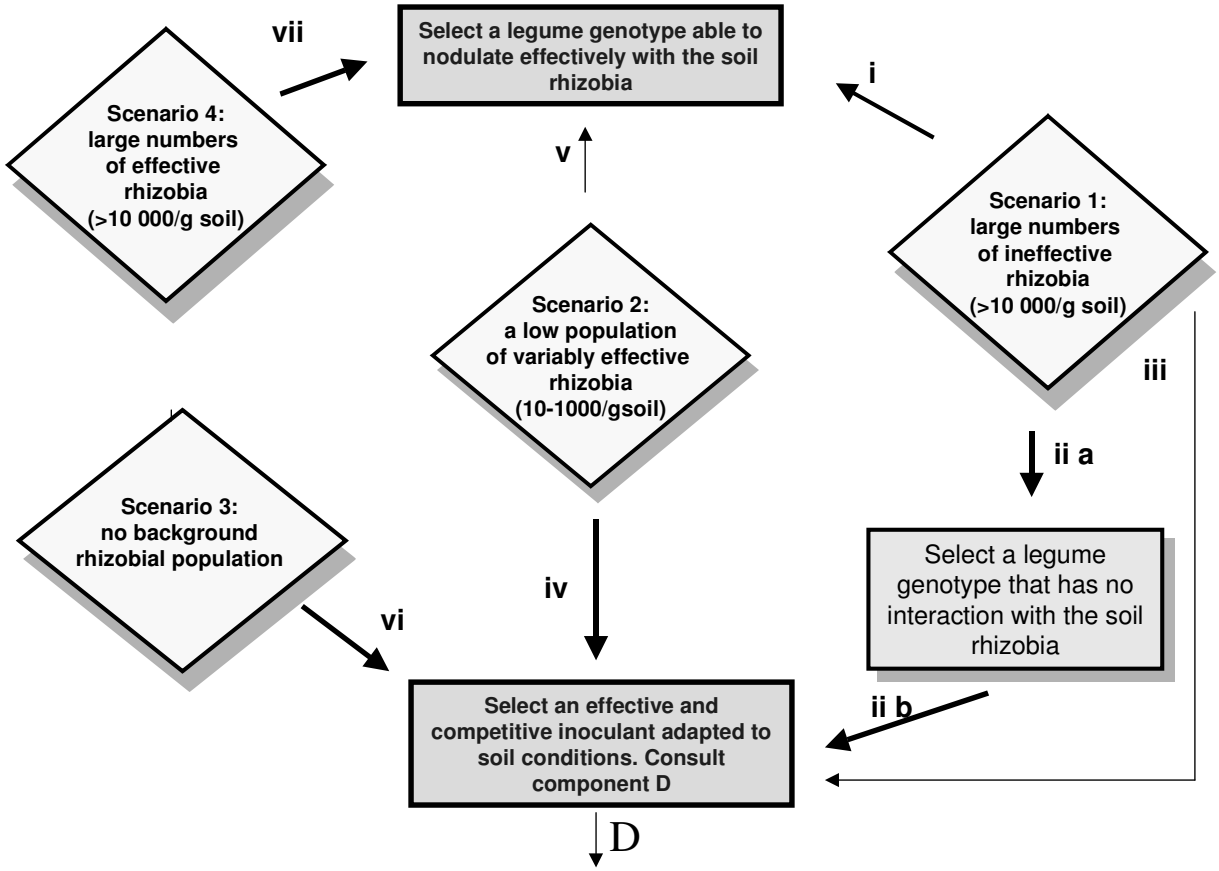

Figure 5. Component C. Research strategies for increasing N2 fixation (after Sessitsch et al., 2002).

[74] As previously mentioned, the availability of a core set of AMiGR with which to experiment within the boundaries of this decision-making model would perhaps greatly benefit the uptake of AMiGRs in developing countries. A core set of AMiGRs could readily be developed by scientists who have collaborated in microbial germplasm exchange and evaluation programmes with the CGIAR centres.

MANUFACTURING, DISTRIBUTING AND UTILIZING MICROBES

[75] After recognizing the value of AMiGRs and demonstrating responses to inoculation with them, the next step in utilizing AMiGRs is to manufacture them in sufficient quantity and with sufficient quality to ensure their adoption. In developed countries, the factors militating against adoption include the ease of applying alternatives to AMiGRs (chemicals, fertilizers). In developing countries, the problems are more microbiological.

Problems with manufacturing technologies in developing countries

[76] If the decision is made to manufacture AMiGR, then bacterial or fungal inoculants need to be produced in a fermentation process, usually under conditions of controlled sterility. The key challenges in manufacture of AMiGRs include:

- ensuring that the right organism is cultured during the fermentation step, i.e. that the inoculant is the desired organism and the growth phase is uncontaminated; and
- ensuring the fermentation is carried to completion (i.e. to achieve high numbers) and harvested without injury to the microbe.

[77] After fermentation, the microbes must be stored in a 'carrier' material until applied to seed, plants or soil. Carriers include:

- soils, such as ground peat, coal or lignite;
- plant material, such as charcoal, composted straw, bagasse, rice husks or coir dust;
- inert materials, such as vermiculite, perlite, bentonite, clay, phosphate rock, talc or alginate; or
- combinations of the above, such as a mixture of soil, clay and compost.

[78] The type of carrier developed usually depends on the availability of materials in reasonable proximity to the fermentation facilities. The key challenges in carrier selection are:

- ensuring the carrier protects the organism for a period sufficient to utilize the inoculant; and
- ensuring the carrier maintains high numbers of the inoculant capable of engaging with the target plant, insect or animal.

[79] Low rates of usage of AMiGR in many countries may reflect problems of supply or regional access to AMiGR, rather than reflect the actual intent of the farmer, who might be amenable to the purchase of a bona fide inoculant. Thus, lack of a reliable infrastructure for AMiGR production may restrict adoption, even if the organisms have proven efficacy.

Documentation and databases to aid transfer and to track acquisition and usage

[80] As with any scientific pursuit, it is essential that records be kept of experimental outcomes. However, because the nature of AMiGR research is long term, it is even more essential that good databases are developed to record the information generated with any series of experiments with AMiGR. This is essential where AMiGR repositories mature to become associated with institutions rather than individuals. In the case of the CGIAR system, the use of electronic databases to record and track the use of AMiGR acquisition and outcomes of experiments with them is strongly recommended.

IX. INFORMAL (NON-LEGALIZED) CUSTOMS DEVELOPED FOR THE ACQUISITION, DISTRIBUTION OR EXCHANGES OF AMIGRS

Record-keeping

[81] Many curators historically recorded the distribution of their cultures, more as a thorough record-keeping exercise than as a legal requirement. There was generally an understanding between scientists that the culture would be referred to with its initial accession number in any publication in an international forum and this provided some tracing of cultures. This has changed somewhat over the last 15 years. There currently exists relatively substantial record-keeping relating to acquisition and exchange of at least some AMiGRs. In the case of RNB, acquisition activities by institutions are now only undertaken with the full knowledge and cooperation of the country of origin. Material from collecting missions is then usually shared between collaborators at the point of collection, or after isolation and preservation has been achieved. The acquisition activity is usually preceded by written declarations of intent that describe the intended scope of the activities. This scenario differs significantly from that which existed pre-1994, where acquisition activities were frequently undertaken without the written consent of legally constituted authorities in the country of origin and following established access and benefit sharing laws, since relevant international standards and national laws generally did not exist.

X. TOWARDS CODIFICATION OF ACTIVITIES: DIRECTIONS AND ORGANIZATION TYPES GENERALLY INVOLVED

MTAs and MOUs

[82] There is now a general requirement for the preparation and signing of Memoranda that deal exclusively with the acquisition, exchange, research and future commercialization of any AMiGR. These documents are usually inter-institutional, rather than inter-governmental. In the case of the distribution of cultures from germplasm resource centres, requests for cultures may now often be met with Material Transfer Agreements (MTAs) that specify, amongst other things, that negotiation is required with the 'owners' of the material before commercial activities are to be undertaken. Usually, AMiGRs cannot be forwarded to a third party. An example of a current MTA is appended (Appendix 3). The exchange of AMiGRs has thus moved substantially towards an official activity, with record keeping and commitments by both parties.

Re-selection

[83] The issue of re-selection is significant. Microbes may divide and double their number within 30 minutes, and the offspring may be slightly different to the parents, depending upon how they are cultured. For example, at a mutation rate of 1 in 100 million, which is not high, any plate of bacteria is likely to have up to ten colonies that differ from the parents. It is, for example, a very basic step in microbiology to select for natural antibiotic resistance mutants. This rapid rate of change clearly has the potential to make claims for intellectual property ownership a significant challenge.

XI. POSSIBLE DIFFERENCES AMONG CODIFICATIONS APPLICABLE TO AMIGRS AND TO MGRS

AMiGRs differ from MGRs

[84] AMiGRs are generally delivered live to their target (soil, plant, insect, animal) whilst MGRs transform a process and then are eliminated. This is a major difference between AMiGRs and MGRs that affects their codification. For almost all AMiGRs, the organism itself is manufactured then utilized in a live state. Thus, to elicit the required response the microbe is distributed by inoculation of or placed in the vicinity of the target organism, using live cells, or fruiting bodies that should develop into live cultures. This contrasts with the utilization of MGRs, the vast majority of which act as microbial catalysts in a production sequence where the end product contains no live cells of the microbial agent. For example, yeasts ferment grape juice into wine or champagne and then die, with no live cells usually present in the final product. Similarly, whilst *Agrobacterium* might produce transformed cells, the bacteria itself is ultimately removed from the target organelle (although there are exceptions, such as the lactobacillus used in yoghurt manufacture). Because MGRs are generally utilized within a contained process, there has followed the "ownership and protection" of MGRs. Breweries have their favourite yeasts, which they closely guard, and laboratories store their unique transformation vehicles. The same protection is not available to AMiGRs, because once they are released into the environment, it is generally a simple matter to recover the organism.

XII. IMPACTS OF NATIONAL QUARANTINE LAWS

Labelling

[85] The primary requirements for import and export of agricultural microbes relate to labelling, in particular in relation to any potential hazardous substance. These must be disclosed and penalties for not doing so may be applied to both the exporting and importing agent. For animal pathogens, in particular those that are the subject of global quarantine efforts, access to microbial germplasm remains as strictly controlled as that of animal shipment. Aside from this, there is very little

monitoring of the exchange of agricultural microbes in most countries, and the unintentional trade of microbes across the globe continues to increase in association with shipments of grain, animals and fodder. However, those countries with strict quarantine laws are becoming increasingly stringent about the importation of microbes. Thus AQIS, the Australian Quarantine Inspection Service, prohibits the import of AMiGRs without special permits, and this is having a substantial impact upon the development of AMiGRs in that country.

XIII. TRENDS IN PATENTING OF UNMODIFIED AND MODIFIED MICROBIALS

AMiGRs and intellectual property

[86] In any handful of soil, from most places on the planet, there is likely to be in excess of 100 populations of different microbes, some of which will exceed 1 billion individuals in that handful. From this handful of soil there is the potential to develop one or more AMiGR inoculants. The widespread availability or natural distribution of microbes has several implications. The first is that it is almost impossible to demonstrate the origins of an AMiGR unless that AMiGR is highly specific. An example of a highly specific AMiGR might be the bacteria from which DNA polymerase for many PCR reactions originates, i.e. from thermal pools, which are a relatively restricted environment. Other than these rare examples, AMiGRs from common environments are ubiquitous. The intellectual property in their development, therefore, is associated with proving theirutility/industrial application rather than discovering the organism per se. This in turn means that it might be difficult to protect the intellectual property associated with many AMiGRs. For example, it is public knowledge that RNB fix nitrogen, RNB are ubiquitous, and therefore it is a relatively simple matter to isolate RNB from legumes to develop inoculants that simply can not be protected by intellectual property (IP) rights legislation. Whilst procaryotic AMiGRs can be patented, this is not a common practice.

[87] As noted earlier, microbes routinely double their number within 30 minutes when grown under favourable conditions. This provides the opportunity to generate inoculants within days, and the implications of this are that a competent manufacturer may develop a commercial-quality inoculant from a starter culture within a very short time-frame. This makes AMiGRs uniquely attractive as a small business opportunity in developing countries where local fermentation expertise is available. This is why many aid programmes, such as USAid (through NifTal), have focused upon AMiGRs. There is, however, a down side to rapid reproduction. The first consideration is that with the rapid rate of reproduction comes a potential for rapid mutation, or change. If the altered genotype is favoured in the production environment, the new genotype will soon dominate the population (this could be considered evolution). This, in turn, has implications: firstly, if the change is not beneficial then the inoculant may not be efficacious (and hence AMiGR production requires stringent quality control), and, secondly, if the original AMiGR was protected by patent, it is unlikely that the patent would apply to the evolved genotype. A similar scenario can occur for microbes delivered to soil. There is substantial acceptance and donation of DNA between even distantly related organisms, which leads to relatively rapid evolution or change. A major implication of rapid reproduction, then, is that it brings with it difficulties in intellectual property protection associated with the difficulties in proving identity.

Trends in patenting

[88] It seems commercial manufacture of AMiGRs is accompanied by patent applications, more so than through the activities of the genebank curators themselves. Executives of Becker Underwood in Australia were contacted on 10 August 2005. Becker Underwood are a major global manufacturer of AMiGRs. They had patented the use of microorganisms as biocontrol agents (although not RNB) in the USA and in Australia. These patented AMiGRs are not genetically modified and are occur naturally in the environment. The AMiGRs under patent have been selected in research programmes for specific purposes (e.g. *Metarizium* as an inseticide). This suggests that some patent laws now recognize and offer protection forinvestment in scientific researchof microbes that have been isolated from the environment and used in specific ways.

[89] A second manufacturer of AMiGRs in Australia, ALOSCA Pty Ltd were contacted on 14 August 2005. At that time, they had patented their delivery technology rather than specific microbes.

[90] New strains developed through scientific research and then made available for commerce are provided to Australian manufacturers free of charge under the conditions of a non-exclusive licensing arrangement, but only to those manufacturers who are participants in the Australian quality control programme (ALIRU).

Procaryotes protectable as intellectual property

[91] Although this document presents some pragmatic challenges associated with patenting of AMiGRs, the case of *Diamond v Chakrabarty* in the US Supreme Court has shown prokaryotes may be patent protectable under law in the USA, a decision that remains unchallenged today. The most current issue of Bergey's Manual (2005) has a paper by R.D. Meredith that examined the 1998 position on protecting IP in prokaryotes. In summary, at that time, prokaryotes were protectable if they were considered new inventions, of practical value and not simple variants of an entity already anticipated in the public domain. It was noted by Meredith that the law is evolving. The case was decided in favour of the applicant (5-4), which, in the opinion of the author, is indicative of a challengeable position. In the sense of AMiGR, it would be difficult in many cases to establish that a similar entity was not anticipated in the public domain. Procaryotes that are deliberately genetically altered to deliver a unique product (e.g. insulin) would be an obvious exception.

XIV. CONCLUSIONS

[92] Drawing on the preceding sections of this paper by Dr Howieson, the Genetic Resources Policy Committee of the CGIAR seeks to highlight a number of potentially important issues:

1. It is possible to develop a working definition of agricultural microbial genetic resources (AMiGR) on the basis of the function for which those resources are used, i.e. the fact that that they assist in the production of plants or animals, either directly or indirectly, in agricultural settings.
2. Because of a combination of factors concerning microbes used in agriculture—for example, their deployment in open environments; their extremely fast rates of reproduction and variation; their small size and portability; and historical patterns of use and distribution—it is difficult, and often impossible, to subject them to legal forms of control or appropriation. A large number of patents, however, have been granted in some countries over microbes as well as genes and proteins derived therefrom.
3. AMiGR are potentially extremely important for the sustainable improvement of productivity in developing countries, subject to biosafety considerations. However, they are as yet not widely exploited in a systematic manner in developing countries.
4. One possible way to increase the availability to, and use of AMiGRs by, developing countries would be to develop a 'virtual' core collection of screened materials currently held by public organizations around the world that wanted to participate. A critical aspect of this enterprise would be to agree upon harmonized terms and conditions for the distribution of those materials, in conformity with international law. The process for considering the establishment of such a base collection and the terms and conditions for its use would need to be highly participatory, with costs, legal status, partners, administrative responsibilities and other issues identified and exhaustively considered.

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APPENDIX 1

A BRIEF DESCRIPTION OF SOME COMMON AMIGRS WITHIN THEIR ASSIGNED FUNCTIONAL GROUPS

PLANT SYMBIONTS

[A1.1] Plant symbionts are microbes whose actions directly improve plant growth, usually by supply of otherwise limiting nutrients such as nitrogen or phosphorus. Root nodulating bacteria (RNB) are the best-researched example of microsymbionts for plant growth, reducing inert di-nitrogen gas in the atmosphere to a form that legumes can metabolize, usually amino acids. There are six main genera of RNB, including the phyllosphere microorganism *Azorhizobium* that forms stem nodules on Sesbania. The stem nodules and their microbial occupants may also be photosynthetic. Actinorhizae are fungi that form Frankia-type nodules on non-legumes, within which N fixation also takes place. The microbe genus *Frankia* can now be cultured on complex media and hence *Frankia* spp. are suitable as AMiGRs and can be applied to at least seven families of non-leguminous plants, the most utilized plants being in the genera *Casuarina* and *Alnus*.

[A1.2] The Cyanobacteria may also be listed under this heading, as they have the capacity to form symbiotic associations with eukaryotes and to fix atmospheric N. *Nostoc* is the most exploited genus of this group.

[A1.3] Mycorrhizae are root-fungus associations that effectively extend the rooting-zone of plants. There are six major types of mycorrhizae. The endomycorrhizae are of particular interest, although they can not be grown without the plant and therefore remain difficult in an AMiGR context.

RUMEN ORGANISMS

[A1.4] The rumen of methane-producing animals such as sheep and cattle contains a large and diverse microbial community of anaerobic fungi, such as *Neocallimastix,* prokaryotes, ciliates and protozoans. There may be as many as 1×10^{12} organisms per millilitre of rumen fluid. These microbes act together to break down the cellulosic plant components, mainly through the action of anaerobic prokaryotes and protozoans. Other bacteria then ferment carbohydrates to volatile fatty acids, carbon dioxide and methane, which the Archaea produce from acetate, carbon dioxide and hydrogen gas. Having performed their tasks, the rumen microorganisms are digested in the adjacent stomachs to yield amino acids and sugars for ruminant metabolism.

ASSOCIATIVE ORGANISMS

[A1.5] Associative organisms are organisms that elicit or potentiate a positive reaction or effect when in intimate proximity with a plant or animal. The best known are Plant Growth Promoting Rhizosphere (PGPR) organisms and Yield Increasing Bacteria (YIB)

[A1.6] The most common of these are the diazotrophs, including *Azotobacter, Azospirillum, Acetobacter, Azoarcus, Clostridium*, Enterobacteriaceae and *Herbaspirillum*, as well as the facultative nodule bacteria *Burkholderia, Rhizobium* and *Azorhizobium,* which have been shown to have additional associative effects in cereals. Most of these associative organisms may supply small amounts of N to crop plants, which may be useful in N-deficient systems, and this can be measured using the %ndfa natural abundance technique. *Azospirillum* has been shown to increase yield by 5 to 30 percent in about 70 percent of reported trials. However, they may also have a range of other functions related to hormone, siderophore or chelate production, or nutrient solubilization. Another class of microbes that is becoming well-researched in contemporary laboratories is the ACC group. This group deaminates 1-amino cyclopropane -1-carboxylate, which is a precursor to ethylene. Ethylene may be injurious to plants grown under stress. Avoidance of exposure to ethylene can

increase plant growth. The most studied ACC organism is *Pseudomonas putida*. There are accepted methodologies to assay for these functions.

BIO-CONTROL AGENTS (PATHOGENS OF WEEDS, FUNGI, INSECTS OR NEMATODES)

[A1.7] The use of microbes to control pests through parasitism, pathogenicity or competition is considered an environmentally sound use of AMiGRs, with significant potential in agriculture. A well documented bioinsecticide is *Bacillus thuringiensis,* which produces toxin crystals effective in controlling coleopteran and lepidopteran insects. *Bt* has been utilized for over 20 years in cotton crops to control the Boll Weevil, and *Bt* toxin genes have been transferred into both plants and bacteria for similar purposes. A large collection of *Bt* toxin genes are maintained by the Pasteur Institute in Paris. Other examples of the current application of AMiGRs as biocontrol agents include *Bacillus subtilis* as a pathogen of fungi; *Agrobacterium* cured of the *Ti* plasmid as a competitor against Crown gall-inducing *Agrobacterium*; *Pseudomonads* as weed control agents; *Metarhizium* as a control agent for locusts and grasshoppers; and the twist fungus as an inhibitor of nematode and *Corynebacterium* induced toxicity of annual ryegrass. The nuclear polyhedrosis viruses (NPVs) have proven effective against lucerne and celery loopers, and could be employed in genetic modification studies to control insect pests of agricultural plants.

FERMENTATION OF FOODS AND BEVERAGES

[A1.8] Yeasts are used in bread, beer and wine manufacture; *Streptococcus* and *Lactococcus* in dairy products such as cheese and yoghurt, as well as in nisin production, which may be used as an anti-spoilage treatment; *Penicillium camamberti* is used in the later stages of camembert production; *Acetobacter* in wine-vinegar production; *Lactobacillus* in production of fermented meats; *Aspergillus* and *Rhizopus* in soy fermentation. Many of these genera have a role elsewhere in Agriculture.

MASS CULTURE OF MICROALGAE AS A SOURCE OF PIGMENTS OR ANTIOXIDANTS, OR AS A FEED BASE FOR HIGHER ORGANISMS

[A1.9] Mass culture of microalgae is routinely undertaken in aquaculture facilities for production of feed-base to provide bulk for fish, cattle, pig or poultry feed. Examples include *Chlorella* spp., *Isochrysis* spp. and *Pavlova* spp. Microalgae may also be grown in high volume culture for fine chemical production, such as phycocyanin from the Cyanobacterium *Spirulina*; beta-carotene from *Dunaliella salina*; and astaxanthan from *Haematococcus pluvialis.* These are global aquacultural industrial processes. Cyanobacteria have a key role in rice production.

APPENDIX 2

AN EXTRACT FROM THE CURRENT US FARMBILL

US Farmbill

Appendix I
104 STAT.3744
Public Law 101-624-Nov. 28, 1990
Title XVI

Subtitle C--National Genetic Resources Program

7 USC 5841.

SEC. 1632. Establishment, Purpose, and Functions of the National Genetic Resources Program

(a) IN GENERAL.--The Secretary of Agriculture shall provide for a National Genetic Resources Program.

(b) PURPOSE.--The program is established for the purpose of maintaining and enhancing a program providing for the collection, preservation, and dissemination of genetic material of importance to American food and agriculture production.

(c) ADMINISTRATION.--The program shall be administered by the Secretary through the Agricultural Research Service.

(d) FUNCTIONS.--The Secretary, acting through the program, shall--

(1) provide for the collection, classification, preservation, and dissemination of genetic material of importance to the food and agriculture sectors of the United States;

(2) conduct research on the genetic materials collected and on methods for storage and preservation of those materials;

(3) coordinate the activities of the program with similar activities occurring domestically;

(4) unless otherwise prohibited by law, have the right to make available upon request, without charge and without regard to the country from which such request originates, the genetic material which the program assembles;

(5) expand the types of genetic resources included in the program to develop a comprehensive genetic resources program which includes plants (including silvicultural species), animal, aquatic, insect, microbiological, and other types of genetic resources of importance to food and agriculture, as resources permit; and

(6) engage in such other activities as the Secretary determines appropriate and as the resources of the program permit.

APPENDIX 3

AN EXAMPLE OF AN MATERIAL TRANSFER AGREEMENT (MTA) THAT RELATES TO MICROBES

The CHIEF EXECUTIVE OFFICER OF THE INSTITUTION, a body corporate under the *xxxxx Act 1988* (COUNTRY) having its offices at xxxxxxxxxxxxx and the Recipient requires the following Details set out in Schedules 1, 2 and 3 to be provided to allow for the exchange of Genetic Material (hereinafter called 'Material') under the Terms and Conditions of this Agreement.

	Item	SCHEDULE 1: Details		
MATERIAL	1	Description of Material to be transferred (If further details are attached please tick the box below and complete Schedule 2) □ Further Details Provided in Schedule 2		Common Name: Species: Identifying Codes: Other attributes:
	2	Quantity and form of Material		Quantity:
	3	Nominated Delivery Date		Date:
AGREEMENT DETAIL	4	Recipient's Details	Organization: Delivery Address: Contact Name	
	5	Purposes for which the Recipient can use the Material (Please place an "X" in only one of the boxes on right, and complete Schedule 3 if Purpose 5 is chosen)	Purpose 1 ☐ Purpose 2 ☐ Purpose 3 ☐ Purpose 4 ☐ Purpose 5 ☐	As parental material for crossing with genetic material only As reselection material only As testing and evaluation material only As genetic manipulation material only For any Purpose above where special conditions apply, a combination of Purposes listed above apply, or where Material is to be used for a purpose not covered above
	6	Commencement Date:		7 Expiry Date:
	8	INSTITUTE Authorised Signatory	Name: Position: Telephone: Email:	
	9	Recipient's Authorised Signatory	Name: Position: Telephone: Email:	

EXECUTION CLAUSE	By countersigning below, both parties agree to the Terms and Conditions of this Agreement and have provided the Details as required in Schedules 1, 2, and 3	
	Dated this day of 20__	Dated this day of 20__
	Signed for and on behalf of INSTITUTE ……..…….. Authorised Signatory (signature) …… Witness (signature) …… Witness Name and Title …… Manager Business Development (counter-signature)	Signed for and on behalf of the Recipient ……..…… Recipient Authorised Signatory (signature) …….... Recipient Witness (signature) …….... Recipient Witness Name and Title …….. Recipient Authorised counter-signatory, if applicable (signature)
Office use only GMTA ID……		GMTA prepared by:……

SCHEDULE 2: Further information describing the Material to be supplied

SCHEDULE 3: Further information describing the purposes for which the Material may be used and subsequent obligations of both parties
1. Purpose(s) for which the Material may be used:
2. Special conditions relating to the use of the Material:

SIGNING	**By countersigning below, both INSTITUTE and the Recipient's Authorised Signatory have checked the information set out in Schedule 2 and 3 and any associated attachments and agree that all information is true and correct and in accordance with the wishes of their organization.**	
	Dated this day of 20__	**Dated this day of 20__**
	..... INSTITUTE **Authorised Signatory (Signature)**	..... Recipient **Authorised Signatory (Signature)**

TERMS and CONDITIONS

All Item numbers referred to in the Terms and Conditions refer to Items within Schedule 1, 2 or 3 unless otherwise stated.

By providing the Details and countersigning Schedule 1 and if applicable, providing further information and countersigning Schedule 2 and 3, both INSTITUTE and the Recipient agree to the following:

1. GENERAL OBLIGATIONS

1.1) The Recipient acknowledges it accepts the Material at its own risk and that INSTITUTE is supplying the Material without any expressed or implied warranty as to the utility of the Material for the Purpose

1.2) INSTITUTE hereby grants the Recipient (Item 4 of Schedule 1) the right to use the Material (Item 1 of Schedule 1 or Schedule 2, as applicable) solely for the purposes defined in Item 5 of Schedule 1 or Item 1 of Schedule 3, as applicable.

1.3) The quantity and form of Material (Item 2 of Schedule 1) shall be delivered at INSTITUTE expense to the Delivery Address of the Recipient (Item 4 of Schedule 1) by the Delivery Date (Item 3 of Schedule 1) or as soon as practicable thereafter.

1.4) The Recipient shall take all necessary precautions to ensure the security of the Material, including but not restricted to adequate confidential identification as mutually agreed. The Recipient must detail such security measures in reports as required in Clause 1.8.

1.5) If the Recipient ceases to have a use for the Material, or if this Agreement expires or is terminated, or if INSTITUTE so requests, all Material shall be destroyed or returned to INSTITUTE (at INSTITUTE's election) and evidence to INSTITUTE's satisfaction of such destruction shall be immediately forwarded to INSTITUTE.

1.6) INSTITUTE shall have access to the Material and the relevant trialling, testing, modification or experimenting sites and all associated results, information and data at any point in the duration of the Agreement, subject to reasonable notification being given by INSTITUTE to the Recipient.

1.7) The transfer of any other material from the Recipient to INSTITUTE including, where applicable, crossbred breeding lines whose parent is the Material supplied by INSTITUTE will occur on the basis of like terms and conditions to those set out in this Agreement.

1.8) The Recipient shall deliver to INSTITUTE an identical copy of all summary reports produced by the Recipient on the performance and security of the Material at least every twelve (12) months following the Commencement Date, for the duration of the agreement.

2. DURATION OF THE AGREEMENT

2.1) This agreement shall commence on the Commencement Date (Item 6 of Schedule 1) and expire on the Expiry Date (Item 7 of Schedule 1). The Recipient shall complete all obligations under this Agreement by the Expiry Date (Item 7 of Schedule 1).

2.2) CONFIDENTIALITY

2.3) For the duration of the Agreement and for a period of three (3) years thereafter INSTITUTE and the Recipient shall keep confidential all information in relation to the supplied Material and all subsequent testing, modifications or experiments in relation to the Material. Either party may reveal information within the confidentiality period upon written approval from the other party.

2.4) Nothing in this Agreement prevents or inhibits INSTITUTE from providing information to the Minister of the Crown in right of the COUNTRY having responsibility for the INSTITUTE. Further, nothing in this Agreement prevents or inhibits that Minister of the Crown from providing to the Parliament of COUNTRY information concerning any conduct or operation of INSTITUTE in such a manner and to such an extent as the Minister thinks reasonable and appropriate.

3. OWNERSHIP OF MATERIAL AND INTELLECTUAL PROPERTY

4.1) Notwithstanding the Recipient's right to use the Material to the purposes defined in Item 5 and Item 1 of Schedule 3 as applicable, and unless Clauses 5.2 and/or 5.5 of this Agreement apply, the Recipient acknowledges and agrees that the Material and all associated industrial and intellectual property rights are owned in perpetuity by

INSTITUTE and the Material cannot be transferred by the Recipient to any third party, under any circumstances.

4. SPECIFIC OBLIGATIONS

4.2) The Recipient may only use the Material for the purposes set out in Item 5 of Schedule 1 or Item 1 of Schedule 3 as applicable, and accordingly agrees to the following specific obligations:

4.3) **As parental material**: If the Material is used in its supplied form as parental material for crossing with other genetic material being either breeding lines or commercial plant varieties (Purpose 1 of Item 5 of Schedule 1) the Recipient does not need any further approval for such activity. Provided that any new material is not considered to be essentially derived within the meaning of the Plant Breeder's Rights Act 1994 (Cth), any new material that results from such crossing with other genetic material will be solely owned by the Recipient provided that the Recipient agrees that progeny derived from material received by INSTITUTE from the Recipient shall be owned solely INSTITUTE. INSTITUTE's role in the parentage of the new material should be acknowledged in any subsequent trialling, modifying, Plant Breeders Rights registration or commercialization process.

4.4) **For reselection**: If the Material is used for reselection purposes (Purpose 2 of Item 5 of Schedule 1) any new material that results from such reselection will be solely owned by INSTITUTE. Such new material cannot be modified, improved, experimented on, or commercialised without both parties entering into a meaningful agreement allowing such activity.

4.5) **For testing and evaluation**: If the Material is used for testing and evaluation purposes (Purpose 3 of Item 5 of Schedule 1) the Recipient does not need any further approval for such activity. Any new material that results from such testing and evaluation shall be solely owned by INSTITUTE. Such new material cannot be modified, improved, experimented on, or commercialised without both parties entering into a meaningful agreement allowing such activity.

4.6) **For genetic manipulation**: If the Material is used in its current form as genetic manipulation material for the insertion of extraneous deoxyribonucleic acid or 'DNA' (Purpose 4 of Item 5 of Schedule 1) the Recipient does not need any further approval for such activity. Any new material that results from such genetic manipulation and DNA insertion, will be jointly owned by INSTITUTE and the Recipient. Such new material cannot be modified, improved, experimented on, or commercialised without both parties entering into a written agreement allowing such activity

4.7) **For some other purpose or combination of purposes**: If the Material is used for any other single purpose or combined purposes (Purpose 5 of Item 5 of Schedule 1) as specified in Schedule 3 the Recipient shall meet all of the obligations set out in Schedule 3.

APPENDIX 4

THE TERMS OF REFERENCE DEFINING THE SCOPE OF THE REVIEW

Terms of Reference for a Consultant to conduct study on technical issues related to developing harmonized management policies, guidelines and practices concerning acquisition, use and distribution of Agricultural Microbial Genetic Resources (AMiGR)

The consultant will:

1. Participate in a research initiation brain-storming session, either in person or over the phone, with members of the Genetic Resources Policy Committee (GRPC), Inter-Center Working Group – Genetic Resources (ICWG-GR), FAO, MOSAICC and other interested parties concerning the research activities he or she will undertake;

2. Address the question whether there is a distinct subset of microbial genetic resources that can be called microbial genetic resources for food and agriculture, or agricultural microbial genetic resource (AMiGR). In this context, the consultant will, among other things, consider data concerning the physical nature of AMiGR and their broad categories and uses, the history and actual patterns of the distribution of AMiGR around the globe, and other possible factors that may distinguish AMiGR from other forms of MGRs, for example, those that are used for pharmaceutical or industrial purposes. It is understood that it is probably not possible to exhaustively define the outer limits of this sub-class of resources, given the highly dynamic nature of their various sources, uses and distribution. Of course, it also the case that some MGRs are used for both agricultural purposes and other purposes. However, it is desirable to at least establish a 'moving' definition. We may also consider the use of the MGR as a discriminating factor (as for PGRFA).

3. Conduct a survey of management policies, guidelines and practices of organizations concerning the acquisition, use, collection and distribution of AMiGR materials. In this context, the consultant will focus on:

- Future Harvest Centres holding AMiGRs. Regarding the Future Harvest Centres, the consultant will use the System-wide Genetic Resources Program (SGRP) report entitled "A Discussion Paper on the Status of Microbial Genetic Resources Held by the CGIAR Centres," by Dr Kenneth Street as a starting point. The consultant will also develop a questionnaire and circulate it through the SGRP of the CGIAR to obtain new data and critical reflections from AMiGR managers within the Future Harvest Centres; and
- other organizations studying, holding or using similar AMiGRs, including the World Federation of Culture Collections, ICIPE, the United States' Department of Agriculture, the Belgian Coordinated Collections of Micro-organisms (which coordinated the development of the Micro-Organisms Sustainable use and Access regulation International Code of Conduct (MOSAICC)), Biological Resource Centres as developed by the OECD, organizations that have developed codes of responsible behaviour for forestry plantation management concerning uses of MGRs, the International Union of Forest Research Organization (IUFRO), including working group 2.01.13 "Root physiology and symbiosis", and so on. The consultant will identify a range of other similar AMiGR collection-holders and/or users whose management policies, guidelines or practices could usefully be reviewed in the context of this study.

4. Address the following questions:

- What are the basic needs and challenges in using these AMiGR in the general context of agricultural development for the next years?
- What obstacles are countries having in using these AMiGR, (with a special emphasis on developing countries)?

- Are there informal (non-legalized) customs developed for the acquisition, distribution, and or exchanges of these AMiGR? Are there movements towards codification of those activities? In what direction are they moving and what kinds of organizations are generally involved in developing those codes?
- Are the informal customs and movements towards codification with respect to these AMiGR different than those resources being used, e.g., biological control, bio fertilization, food industry, etc? If so, why?
- Is there evidence that national access laws are having an impact on the transfer, use of, and research concerning, these resources?
- What are the trends in patenting of unmodified and modified microbials?

Improving grassland quality in communal arable lands in the Eastern Cape Province, South Africa

ACIAR project ECCAL

Theunis L. Morgenthal, Pieter W. Conradie,
Gideon Jordaan, Unathi Gulwa, Neil Ballard & John Howieson

(Döhne ADI, Stutterheim, South Africa
Murdoch University, Perth, Australia

22nd International Grassland Congress
Sydney, Australia, 14 – 20 September 2013

Eastern Cape Department of Rural Development and Agrarian Reform
Döhne Agricultural Development Institute

The Eastern Cape Arable Lands Project (ECCAL)

- **ACIAR funded Project (ACIAR LPS/2004/022)**
- **Collaborate project between**
  - Eastern Cape Department of Rural Development and Agrarian Reform
  - SA Agricultural Research Council
  - Murdoch University, Perth
- **SA National Wool Growers Association**
  - Project was build on ongoing work by the NWGA
  - Community involvement based on existing partnerships

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The Eastern Cape Arable Lands Project (ECCAL)

- Project was initiated in 2006 and ended in 2013
- Project focussed on alleviating winter feed deficiencies
- Rehabilitation of “abandoned” cultivated lands to pastures
- Introduction of winter annual and perennial legumes
- Specifically using cultivars suited for marginal soils
- Project phases
  - Screening phase (row plantings)
  - Roll-out phase (mix plantings)

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Why the focus on cultivated lands?

- Betterment scheme: large areas of land cultivated prior to 1960
- Over the years many of these arable lands abandoned or only cultivated occasionally
- Government intervention greatly dictates usage
- Insufficient grazing land to sustain livestock
- Grazing land diminishing due to urbanisation
- Consequence of land tenure
- Arable land holds the key for improving livestock production in South African Communal Areas

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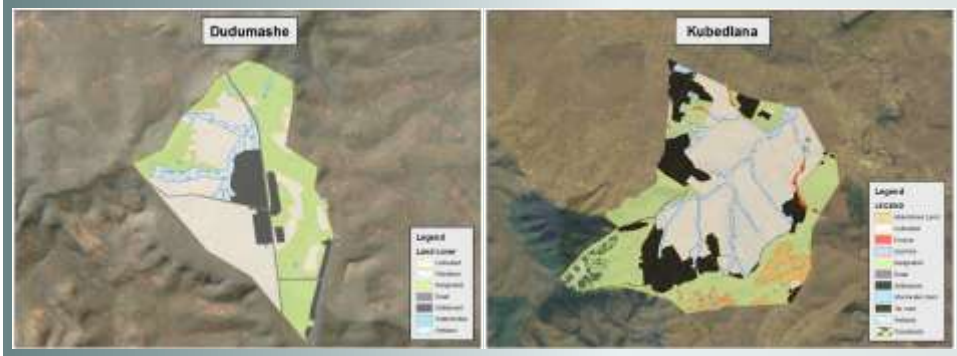
Why the focus on arable lands?



What is the solution to rehabilitate abandoned cultivated lands to improve pasture quality?

Create improved pastures by introducing a diversity of annual winter and perennial legumes

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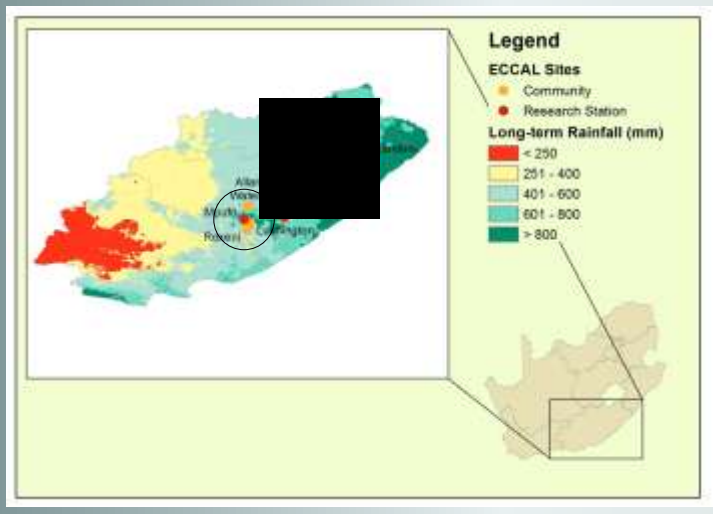
Testing the hypothesis of using legumes



Trial localities for the evaluation of suitable legume species

Testing the hypothesis of using legumes

- **Unsuccessful species**
  - *Desmodium intortum*
  - *Desmanthus virgatus*
  - *Lotus hispidus*
  - *Lotus subbiflorus*
  - *Ornithopus pinnatus*
  - Stylosanthes guianensis
  - Stylosanthes scabra
  - Trifolium hirtum
  - Trifolium pratense
  - Vicia sativa

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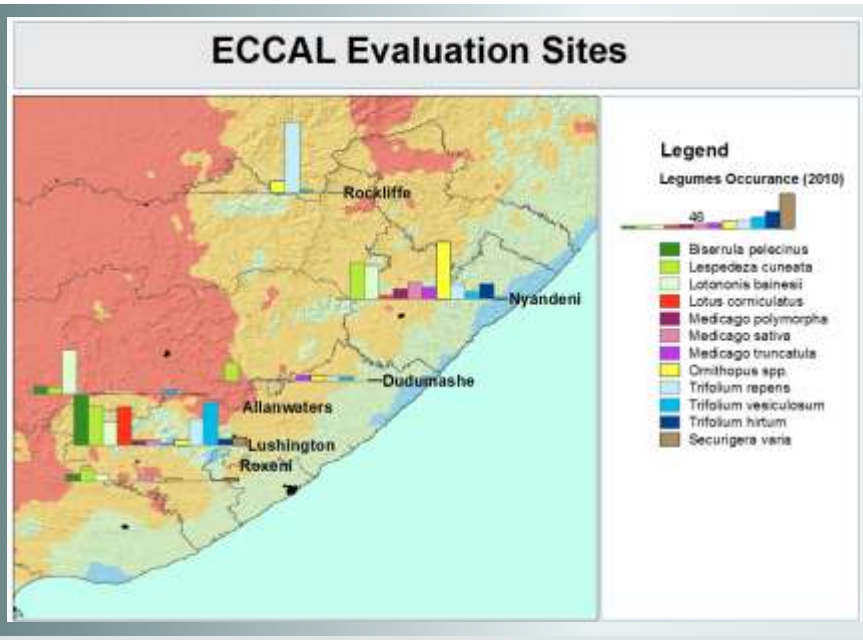
Testing the hypothesis of using legumes

- **Successful species**
  - *Biserrula pelecinus/Astralagus pelecinus* (Hard seeded cultivars)
  - *Ornithopus compressus* *(Hard seeded cultivars)*
  - *Ornithopus sativus*
  - *Medicago polymorpha* (Hard seeded cultivars)
  - *Trifolium repens*
  - *Trifolium vesiculosum*
  - *Trifolium hirtum*
  - *Lespedeza cuneata*
  - *Lotus corniculatus*
  - *Lotononis bainsii /Listia bainsii*



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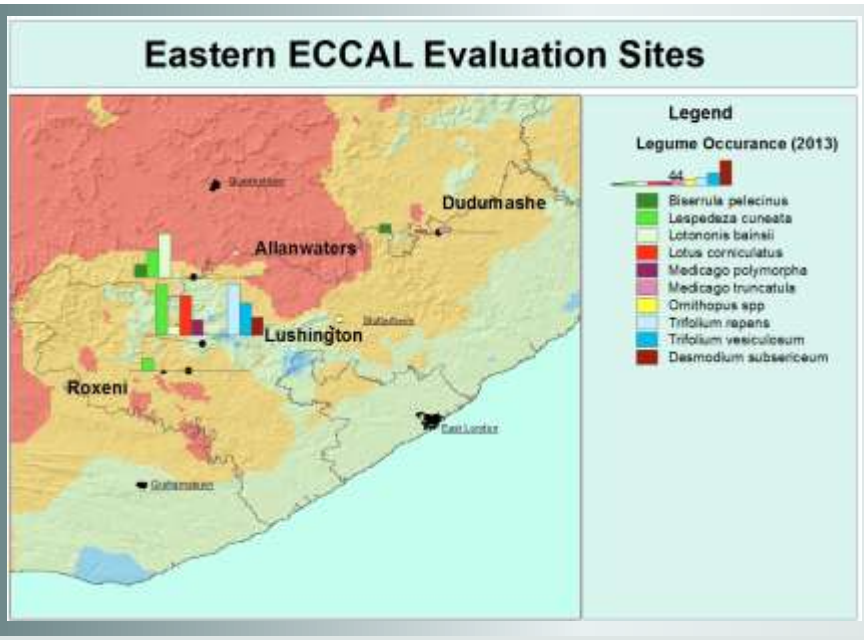

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Lushington – softer Ciskei, heavily grazed but species showing resilience

Heavy grazing, May 2011

Regrowth by early November 2011

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Initial establishment at Dudumashe very promising

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Nyandeni with a good stand of Arrow-leaf clover

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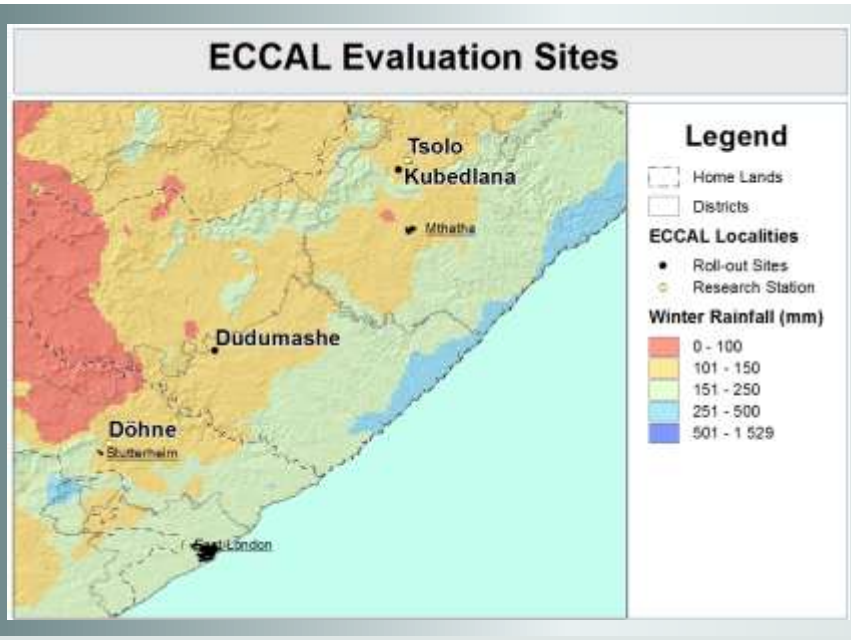

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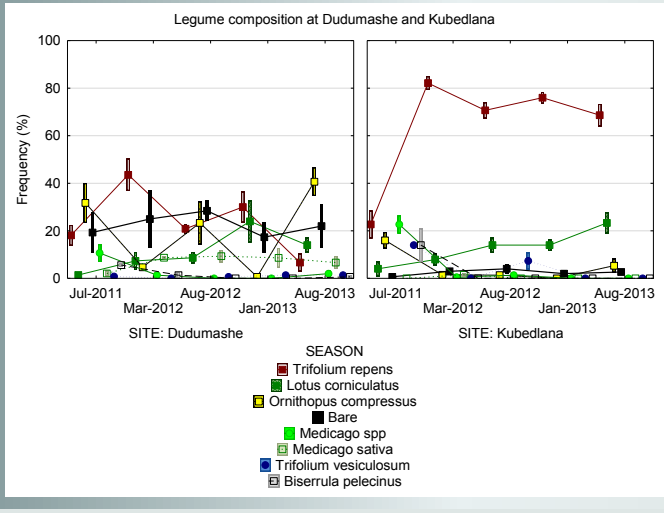
Change in legume composition



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Forage quality

Site		Season	Protein	Phosphorus Percentage	
			Grass Quality		
Dudumashe	Mixture of Legumes	Aug-2011	4.2(0.47)	0.3	(0.04)
Dudumashe		Mar-2012	6.0(0.55)	0.3	(0.05)
Kubedlana		Jul-2011	4.8(0.46)	0.2	(0.07)
Kubedlana		Mar-2012	5.5(0.87)	0.3	(0.16)
			Legume Biomass Quality		
Dudumashe	Mixture of Legumes	Aug-2011	16.4(2.37)	1.2	(0.27)
Dudumashe		Mar-2012	15.6(1.23)	1.5	(0.32)
Kubedlana		Jul-2011	12.7(1.17)	1.1	(0.12)
Kubedlana		Mar-2012	12.1(0.84)	1.2	(0.14)
			Grass Quality (Control Plots)		
Dudumashe	Control	Mar-2012	5.7(0.48)	0.3	(0.06)
Kubedlana		Mar-2012	4.0(0.5)	0.2	(0.05)

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Dudumashe – a 10 ha sowing in the Transkei 2009 grew very well in 2011

Inspecting the regrowth Nov 2011

Showing the formerly cultivated lands in the background

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Demonstrating the potential of legumes

Legume pasture provides green fodder during dry winter months at Kubedlana

Demonstrating the potential of legumes

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Conclusions and Recommendations

Redistribution of Serradella seed through small mammals

Conclusions and Recommendations

Reasons for success

- Well adapted legumes
  - Drought tolerant
  - Can tolerate acidic soils
  - Strong rhizobium mutualism
- High hard seediness and seed yield
- grazing tolerance especially stoloniferous types

A new planting of mixed pastures at Kubedlana - 2012

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Conclusions and Recommendations

Way Forward

- potential for 1000s of ha, therefore we need tonnes of seed
- continuation of minimum till
- Using seed mixtures ensures diversity and survival

Conclusions and Recommendations

A success story: a mixed pasture of grass and a diversity of legumes

Acknowledgements

ACIAR for funding the project
Murdoch University for their collaboration and
Regional Agricultural Extension

The communities involved in the project especially
Colonel Dudumashe and Mr Berand Mlunbi from Roxeni

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