N2Africa Podcaster no. 23
November and December 2013

Introduction

The end of a year is always busy – and this year especially so for N2Africa. While we work on the final reporting on many milestones and on the consolidated report of Phase I of the N2Africa project, we are winding up for Phase II. Adverts for the staff positions are posted on the website – and Phase II officially starts on the 1st January 2014. Please help us to circulate these as widely as possible. The success of N2Africa depends on pulling together a winning team!

One of the major operations at present is to collate and archive all of the data collected by N2Africa over the past years. We have an agreement with the Bill & Melinda Gates Foundation that all data will be made openly available. We have good back-up systems in place to ensure that we can safeguard data for you so that it does not get lost. So we are relying on all staff and partners to assist in collating all of the data, reports and other information. The website continues to be updated – do have a look at the N2 Map and N2 Media pages which are quite new features you may not yet have seen.

In this issue we have reports from some of our students, Amaral Chibeba from Mozambique who is studying in Brazil, and two Dutch students Elise Bressers and Jori Langwerden who are in the Usambara Mountains in Tanzania. This is where I started my own work on nitrogen fixation in beans in Africa back in 1986!

We also have reports on a recent visit I paid to Bruce Knight of LegumeTechnology UK Ltd and a report from Pedro Lage, Lage y Cía. S.A. on inoculant development in Uruguay. One of the photos that Pedro sent in shows him with Prof Carlos Labandera and Dr Yakov Okon who the older ones amongst the nitrogen fixation community will remember well.

One positition is available via the Wageningen UR website:
• Farming systems researcher (Post Doc)
Closing date for this submission is 2 January 2014

We wish you all a very Merry Christmas and a Happy New Year – with the N2Africa Christmas Tree Greeting that Charlotte prepared – and look forward to working with you in 2014!

Ken Giller

N2Africa position announcements

Within N2Africa a number of job positions are open. Seven-position announcements can be found at the IITA website to which is linked below for the different functions:
• Senior Business Development Officer - N2Africa
• Monitoring and Evaluation Specialist - N2Africa
• Project Coordinator - N2Africa
• Country Coordinator - N2Africa - Ghana
• Country Coordinator - N2Africa - Nigeria

MSc students doing field research in the Usambara Mountains, northern Tanzania

Jori Langwerden and Elise Bressers, two MSc students from Amsterdam and Wageningen, are currently working in the Usambara Mountains, northern Tanzania. The Usambara Mountains are an important bean growing area because of its favourable climatic conditions and natural resources. However, many of the production areas are located on steep slopes which are intensively cultivated and highly degraded over the last couple of years. Jori and Elise have set up several experimental field trials within the area of Lushoto district to study the soil and plant nutrient deficiencies of common bean, and how the production can be improved with use of Rhizobium inoculation and fertilizers (especially P and K).

Over the last month they have selected and planted eleven trials on farmer’s fields. Even though the fields are within a small area, they found that there is a lot of diversity in soil types, rainfall patterns and land use. They especially...
enjoyed the collaboration with the local farmers during planting, and at one location the assistance of the local primary school students. Although the rainy season was a bit delayed this year, their beans are growing well and the first treatment differences are already becoming visible at some locations. The coming period they will be busy managing the field trials and carrying out lab analyses at NM-AIST in Arusha.

You may already have seen a first update on the activities of Jori and Elise on N2Africa Facebook and we hope that during their research they will share more of their experiences with us this way.

Jori Langwerden Elise Bressers and Charlotte Schilt

From humble beginnings: LegumeTechnology UK Ltd.

Following on from a visit by Dr Mahamadhi Dianda (IITA) to LegumeTechnology in June this year to learn about quality control methods, I met with Dr Bruce Knight at his factory near Nottingham in the UK to discuss his involvement in Phase II of N2Africa. LegumeTechnology manufactures high quality, zero contamination inoculants for markets throughout the world, and consistently achieves the N2Africa AA standard. Part of our discussions focused on the next generation of equipment to produce top quality inoculants in small packets.
LegumeTechnology UK have agreed to continue supplying N2Africa with inoculants on demand. Further, Bruce has agreed to supply bespoke orders of high quality inoculants of specified strains for experimental purposes, and to provide sterile packets so that N2Africa scientists can make these as required. This will facilitate the testing of elite strains against each other to ensure they are delivered onto the seed in the most effective way to ensure that strain tests are not compromised by using different inoculant formulations.

In addition to visiting the new factory that LegumeTechnology moved into last year, we stopped at the first premises where the company started out. Bruce started out with a strong vision in 2000, with very simple equipment such as a small pressure cooker for sterilization. His whole operation was at first housed in a small room situated in some semi-derelict farm buildings (see photo) from which he built up his company which now sells more than 120 tonnes of top-quality inoculant each year. Hopefully this can provide inspiration for investors to start up similar inoculant production companies in Africa.

Ken Giller

Some similarities between the development of the inoculant market in Uruguay and Africa

During March 2012, while surfing the web for information about inoculants in Africa, I read in N2Africa Podcaster #12 that “the N2Africa proposed strategy suggested was “to import high quality inoculants, and focus on ensuring an effective supply chain for inoculants in the areas where N2Africa is working until we know that there is sufficient demand to warrant local production”. I wrote an email to Dr. Ken Giller offering him samples for trials and since then we have been mailing from time to time. Recently, he asked me to write a small report on what we are doing in Africa. My idea is to set a comparison between the development of inoculants in my country and what N2Africa is doing.

During the decade of 1950, the Uruguayan government had designed a strategy to improve our natural pastures. By those days, New Zealand and Australia were already leaders in the seed industry so scientists from their National Research Institutions were invited to visit the country in order to make a diagnosis of the pasture situation. Their opinion was that Uruguay should incorporate more legumes in order to have better food for cattle, so farmers could increase milk and meat production.

National researcher Dr. Alberto Boerger was searching for someone who could help him to introduce specific
symbiotic *Rhizobium*, for the legumes he was studying, in order to allow them fix nitrogen from the air. During 1956, he asked Laboratorios Dispert if they could supply him specific inoculants for alfalfa, clovers and lotus. Dr. Carlos Batthyany, Dispert’s microbiologist, started analysing the project, and asked ATCC and Australian Labs to send them several recommended strains for the target legumes and simultaneously isolated native strains to be identified as specific or not. After selecting the most suitable strain for each legume, Dispert started the scaling, that ended in year 1963 in a 750 litres fermenter which allowed them to have good inoculant productivity.

During the 1960s, the Ministry of Agriculture created the “Plan Agropecuario Institute” (MA-PAI) which had in its structure the Soil Microbiology and Inoculant Control Laboratory (SMICL) in order to verify the quality of the inoculants delivered to the market. Dr. Carlos Batthyany accepted the invitation to be it’s first Director. He asked Dr. Richard Date to establish the first protocols for inoculant control and had the initiative to train local human resources, so he sent Agronomist Carlos Labandera to take his MSc degree in Soil Microbiology in Australia. Immediately after coming back, Prof. Labandera conducted Soil Microbiology courses at our University.

Photo 1: Left to right: Pedro Lage, Prof. Yaacov Okon, Microbiologist Cecilia Herrmann, Microbiologist Emilia Monteleone, Prof. Carlos Labandera-Gonzalez at Lage & Cía.’s facilities in Montevideo, Uruguay

The SMICL set the initial standards for inoculants in more than $1 \times 10^8$ viable cells per gram when produced and more than $1 \times 10^7$ viable cells per gram of inoculant at expiry date. During each legume planting season MA-PAI sent several agronomists to prepare meetings in order to train farmers in the new inoculation technology: how to keep the inoculants, dosage and preparation of the slurry for seed treatment, maximum time to plant the inoculated seeds, etc. Another important point to note is that our National Bank was encouraged by the Government to offer very low rate loans to enhance the purchase of legal seed with proper germination and free of weeds, fertilisers and inoculants. The money was not given to the farmer: the bank paid the distributor that sold him any of the products in order to ensure that the money would be spent in improving the pastures.

Due to this strategy, Uruguay multiplied its cattle and dairy productivity several-fold, in relation to what it was 60 years ago.

My father Carlos Lage, who was Laboratorios Dispert technical manager decided to leave the company. Part of the agreement was that Laboratorios Dispert would not produce legume inoculants and that he would continue producing them in his new company, Lage y Cía. S.A. that he founded in 1978. The first inoculants Lage y Cía. S.A. produced were formulated on a non-sterile peat carrier, which was the technology available in those times. A few years later Prof. Labandera attended a congress in which researchers showed the improvements when using carriers with low numbers of contaminants, so immediately after Labandera’s return, SMICL suggested that the peat should be sterilised in order to obtain better quality inoculants. Also concentration standards were raised: inoculants should contain more than $5 \times 10^8$ viable cells per gram when produced and more than $1 \times 10^8$ viable cells by expiry date. A year later, the standards were upgraded to more than $1 \times 10^9$ viable cells per grams when produced and more than $1 \times 10^8$ viable cells at expiry date. But as soon as the company increased sales, another sterilisation method was required. In 1985 two other companies were in the market and SMICL suggested the three companies to use gammaradiation treatment for the peat in order to be able to sterilise larger amounts of it and simultaneously increased again the standard that an inoculant should achieve to be allowed to be delivered to the market, setting a minimum of $2 \times 10^9$ viable cells per gram when produced and more than $1 \times 10^8$ viable cells by the expiry date, free of contaminants. These standards are still in force and to require more than $1 \times 10^9$ Rhizobium by expiry date, setting Uruguay as the country with highest concentration standard in the world at expiry date.

Soyabean area started increasing in 2002 and large farmers demanded a more practical inoculation system as peat based products were not easy to use. We decided to develop a liquid formulation for soyabean seeds that could simplify the inoculation process. SMICL gave instructions that if a company decided to launch a liquid formulation, it could not be produced in large scales and initially allowed each company to introduce inoculants for no more than 1000 ha. SMICL set the rate of 150 ml of inoculant to treat 50 kg of soyabean seeds. The idea was that SMICL could trace the liquid inoculants at the distributors warehouses in order to control their quality during the whole soyabean season.
Putting nitrogen fixation to work for smallholder farmers in Africa

If we compare the process between Uruguayan SMICL and N2AFRICA, we can find in Table 1 that the process has been almost identical, but with very important aspects to remark in the African process: N2Africa has attempted in 4 years almost the same activities (and surely in major quantities) that we succeeded in achieving over almost 5 decades.

Since 2002, we have delivered millions of inoculum packets to Argentina, Brazil, Paraguay, Bolivia, South Africa and Zambia. This year, small amounts for trial purposes have been sent to Ghana, Central and North America.

It has not been easy at all to export when you come from a country that most people have hardly heard about and that is only known thanks to our football players Suarez, Cavani and Forlán. But the job of SMICL giving guidance in what regards to kind of carrier, suitable sterilising techniques, setting standards for absence of contaminants and a concentration 10 times higher than our competitors, helped us improve our quality to match the global players’ products and helped us to open new markets for our products. Being an independent inoculant company and also having a little bit of good luck have been the reasons of finding suitable partners as Microbial Solutions in South Africa and Albida Agriculture in Zambia. To match with delighting people in both countries has been the principal factor to start business and another important issue is that they marketed other biological products before importing our inoculants, which I consider the basic characteristic a distributor should have to properly deliver the inoculants to the market. It is not easy to think “Bio” when you are a chemical company.

Having met the most suitable partners and their continuous effort to introduce LIKUIQ™ inoculants has been the reason for raising sales each season. We try to visit them at least once a year or receive them in Uruguay, in order to adjust order details and also give technical support.

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<thead>
<tr>
<th></th>
<th>Uruguay</th>
<th>N2Africa</th>
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<tbody>
<tr>
<td>Countries</td>
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<td>Legume market survey</td>
<td>Dr. Alberto Boerger</td>
<td>N2Africa</td>
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<tr>
<td>Soil Microbiology Laboratories for QC</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>SMICL initially directed by foreign researchers</td>
<td>Dr. Carlos Batthyany</td>
<td>Many scientists</td>
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<td>Training of specialised Human Resources</td>
<td>Prof. Carlos Labandera et al.</td>
<td>Several</td>
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<td>Selection of specific suitable strains</td>
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<td>Yes</td>
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<tr>
<td>SMICL setting concentration standards for inoculants</td>
<td>Approved / Rejected</td>
<td>Grades AA, A, B</td>
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<td>Extensionists to transfer inoculation technology</td>
<td>MA-PAI</td>
<td>Many – differing by country</td>
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<td>State loans with low rates to buy seeds, inoculants</td>
<td>BANCO DE LA REPUBLICA</td>
<td>Searching</td>
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<td>Support to local inoculant companies to assure availability</td>
<td>Yes</td>
<td>SLM Kalambo, MEA, Inoculant factory Marondera</td>
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<td>Years performing the project</td>
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<td>4</td>
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Ghana is a completely different story. I thought that attending an important agrochemical congress in Accra in 2012 would help us find at least one “Bio” distributor for many different countries. Now, it is obvious for me to say that I was in the wrong place as I could not find any distributor interested in inoculants. Incredibly, a few months ago, our Italian importer started doing business with TT Brothers, a company located in Tema, Ghana and has asked us to deliver them a small amount of LIKUIQ™ for soyabean and GRAMINOSOIL™, our Azospirillum based inoculant for maize.

Our expansion in Africa will be directly depend on our ability to find suitable new distributors. The degree of success of those inoculant companies in each country depends on several factors: commercial, regulatory, technical and technological. Nevertheless, the importance given by each government to the diffusion of the technology and to assure that farmers receive a high quality inoculant is the reason why there is such a difference in inoculated/uninoculated areas in most countries. It is important to emphasize that in those of them where the inoculation technology is successful, with a high level of adoption in commercial legume pastures or crops, there has been a permanent and intimate relation between researchers, universities, public entities, extensionists, farmers and the private inoculant factories. Cooperative efforts among all these sectors permit a dynamic development of the BNF research work, which allows enormous economic benefits in productive terms.

Today and the same as in Africa, soyabean are the legume crop in South America to which most rhizobiological work has been dedicated. In relation to other crops as groundnuts and beans, the inoculation practice does not have high levels of adoption in nearby countries, and in most situations the reason is not the lack of positive productive responses. Although we may consider there is great research work dedicated to it in relation to BNF in other crops, it has not been transferred to the productive sector (farmers) in the same manner as it occurred with soyabean.

It seems to me that the Uruguayan inoculant industry developed based in some solid pillars that have allowed us to grow locally and also exporting all around the world. N2Africa´s pillars are exactly the same, so I am sure that in a near future, new African inoculant companies will be in the market.

Pedro Lage, E-mail: lage@lageycia.com

Symbiotic effectiveness of indigenous Bradyrhizobia strains and strategies to maximize the contribution of Biological Nitrogen Fixation on soyabeans in Mozambique

In August 2012 Amaral Chibeba started his PhD research in Mozambique under the supervision of Dr. Mariangela Hungria (Embrapa), Dr. Maria de Fátima Guimarães (Universidade Estatal de Londrina) and Dr. Stephen Boahen (International Institute of Tropical Agriculture).

We enjoy sharing the summary of his study.

Bradyrhizobium spp. have been used in commercial inoculants for effective nodulation of soyabeans (Glycine max (L.) Merr) in the main producing countries where this crop is exotic. Currently used commercial strains are classified in three species of the genus Bradyrhizobium: B. japonicum, B. elkanii and B. diazoefficiens. To reduce the dependence of inoculation of soyabeans with Bradyrhizobium, researchers at the International Institute of Tropical Agriculture developed a line of genotypes for Africa known as TGx (Tropical Glycine cross), which nodulates with populations of Bradyrhizobium spp. indigenous to African soils. However, in some locations, TGx genotypes have shown symptoms of nitrogen deficiency, which suggests that, for these locations, genotypes require inoculation with selected Bradyrhizobium for obtaining high yields.

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The characterization of indigenous Bradyrhizobium populations is essential for developing strategies to improve soyabeans nodulation. TGx genotypes are among the most promotes soyabeans varieties in Mozambique. For the characterization of the indigenous Bradyrhizobium, soyabeans nodules of TGx genotypes were sampled at 15 locations in Mozambique. A total of 105 isolates were obtained, 7 from each location, and are being screened for symbiotic effi-

Figure 1: Amaral Chibeba during thinning on 11 December 2013
ciency in the greenhouse at Embrapa. The trial was sown on 03rd December and thinning was done on 11 December 2013 (Figure 1). From this trial, the best performing isolates will be tested in the field in two cropping seasons, 2014/15 and 2015/16. Simultaneously, trials with elite soyabean *Bradyrhizobium* strains will be conducted in both Brazil and Mozambique to evaluate their performance, and co-inoculation with *Azospirillum* will be studied both under greenhouse and field conditions. Based on the results of the field trials, the best strategies will be recommended for soyabean production in Mozambique.

**Announcement 16th conference of the AABNF, 2-6 November 2014**

The Moroccan Association of Microbiology (AMM) and the African association for Biological Nitrogen Fixation (AABNF) will organize the 16th congress of the AABNF and the 4th international congress on microbial Biotechnologies under the topic of the integrated soil fertilization management 2 (ISFM2), from 2 to 6 November 2014, at Ecole normale Supérieure, Mohamed V-Agdal University, Rabat, Morocco.

This Conference organized in cooperation with the “Ecole Normale Supérieure”, Mohamed V-Agdal University, will consolidate the first version of ISFM1 organized in Nairobi in 2012 by the AABNF and the AFnet.

For more information see their first circular.


Ce Congrès organisé en coopération avec l’Ecole Nationale Supérieure, Université Mohamed V-Agdal, consolidera la première version de l’ISFM1 organisé à Nairobi en 2012 par l’AABNF et l’AFnet.

Pour plus d’information veuillez consulter leur première circulaire.