

Production and use of Rhizobial inoculants in Africa

Milestone reference number 3.4.1

Abdullahi Bala, Nancy Karanja, Mazvita Murwira, Lloyd Lwimbi, Robert Abaidoo and Ken Giller

Submission date: 31 January 2011

N2Africa

Putting nitrogen fixation to work for smallholder farmers in Africa



N2Africa is a project funded by The Bill & Melinda Gates Foundation by a grant to Plant Production Systems, Wageningen University who lead the project together with CIAT-TSBF, IITA and many partners in the Democratic Republic of Congo, Ghana, Kenya, Malawi, Mozambique, Nigeria, Rwanda and Zimbabwe.

Email: Internet:	n2africa.office@wur.nl www.N2Africa.org					
Authors of th	Authors of this report and contact details					
Name: Address: E-mail:	Abdullahi Bala IITA Kano, PMB 3112, Nigeria a.bala@cgiar.org	Partner acronym: IITA				
Name: Address: E-mail:	Nancy Karanja P.O.Box 30197 -00100, Nairobi, Kenya nancy.karanja@cgiar.org	Partner acronym: University of Nairobi				
Name: Address: E-mail:	Mazvita Murwira P. Bag 3757, 108 - 9th Crescent, Warren Park mazvita@gmail.com	Partner acronym: SPRL				
Name: Address: E-mail:	Lloyd Lwimbi	Partner acronym:				
Name: Address: E-mail:	Robert Abaidoo PMB 5320 OYO ROAD, Ibadan, Nigeria <u>r.abaidoo@cgiar.org</u>	Partner acronym: IITA				
Name: Address: Email:	Ken Giller Plant Production Systems, P.O. Box 430, 6700 ken.giller@wur.nl	Partner acronym: WU AK, Wageningen, The Netherlands				

If you want to cite a report that originally was meant for use within the project only, please make sure you are allowed to disseminate or cite this report. If so, please cite as follows:

Abdullahi Bala, Nancy Karanja, Mazvita Murwira, Lloyd Lwimbi, Robert Abaidoo and Ken Giller, 2011 Production and use of Rhizobial inoculants in Africa, www.N2Africa.org, 21 pp.

Disclaimer:

This publication has been funded by the Bill & Melinda Gates Foundation through a grant to Wageningen University entitled "Putting nitrogen fixation to work for smallholder farmers in Africa". Its content does not represent the official position of Bill & Melinda Gates Foundation, Wageningen University or any of the other partner organisations within the project and is entirely the responsibility of the authors.

This information in this document is provided as it is and no guarantee or warranty is given that the information is fit for any particular purpose. The user thereof uses the information at their own sole risk and liability.



Table of contents

Ir	Introduction5				
1	1 UNESCO MIRCEN				
2	2 Inoculant production and use in West Africa8				
3	Ino	culant production and use in East and Central Africa)		
	3.1	Kenya)		
	3.2	Tanzania10)		
	3.3	Uganda10)		
	3.4	Rwanda)		
4	Ino	culant production and use in southern Africa12	2		
4	Ino 4.1	culant production and use in southern Africa12 Zambia 12			
4			2		
4	4.1	Zambia12	2		
4	4.1 4.2	Zambia	3		
-	4.1 4.2 4.3 4.4	Zambia 12 Zimbabwe 13 South Africa 15	2 3 5 5		
C	4.1 4.2 4.3 4.4	Zambia 12 Zimbabwe 13 South Africa 15 Malawi 15	2 3 5 5 7		



Introduction

Inoculation studies have been conducted in Africa since the 1950s and the benefits of inoculation of legumes with suitable rhizobial strains have been amply demonstrated. Despite the clear evidence of response to inoculation by legume crops, there is relatively little use of inoculants by smallholder farmers in sub-Saharan Africa. Several programmes aimed at promoting the use of inoculants in smallholder farms have been supported by national governments, especially in East and southern Africa (e.g. Malawi, Zambia and Zimbabwe). Such programmes were often run with funding from international agencies, such as the Food and Agricultural Organisation (FAO), United Nations Educational, Scientific and Cultural Organisation (UNESCO) and the International Atomic Energy Agency (IAEA). While the projects lasted, some increase in inoculant use was often recorded. However, the scale of adoption declined once the projects came to an end. The various regions within sub-Saharan Africa have had varying degrees of success in promoting inoculant production and use. Table 1 shows some of the existing inoculant plants and their capacity. Some of the problems identified for the lack of sustainability of inoculant production in Africa include poorly developed marketing channels, inadequate quality assurance as well as inadequate capacity within the extension sector.



1 UNESCO MIRCEN

Efforts to strengthen research infrastructure in Africa led UNESCO to start a major regional initiative in the field of applied microbiology and biotechnology in the 1980s. The rationale was to develop the research potential and technological capacity already existing in African countries through the promotion of scientific co-operation between existing local institutions and regional professional societies within the framework of the regional microbiological resources centres (MIRCENs). Under this initiative, three MIRCEN centres were established for East and West Africa at the University of Nairobi and the Centre National de Recherches Agronomiques, Bambey, Senegal, and for the Arab States at the Ain Shams University, Cairo, Egypt. These MIRCENs function as the anchor of a framework that was aimed at promoting high value, low-cost technologies that improve rural agricultural practices, creating rural market economies and providing more technological avenues for employment, increased incomes and ultimate feeder industries to the urban sector.

Table 1. Production of rhizobial inoculants in sub-Saharan Africa during the 1990s (from Karanja et al., 2000).

Producer Country	Product name	Production yr ⁻¹ (kg)	Inoculant Carrier	Retail quantity (g)	Crops
Kenya, MIRCEN, University of Nairob	BIOFIX	1,500	Sterile filter mud	100	Bean, soybean, cowpea, groundnut, legume trees
Kenya, MEA LTD.	BIOFIX	2,000	Sterile filter mud	100,50,20,10	Bean, soybean, cowpea, groundnut, legume trees
South Africa, Pretoria	STIMULANT	9,000	Irradiated peat	250	Soybean, Groundnut, cowpea, bean, peas, fodder legumes
Tanzania, Sokoine University	NITROSUA	-	Sterile filter mud	100	Bean, soybean and lucerne
Uganda, Makerere University	Bio-N-Fix	2,000	Sterile peat	250	Soybean, bean ,lucerne and calliandra
Uganda, Madhvani industries	Bio-Fertilizer legume inoculant	8,000	Sterile peat	100	Soybean, groundnut and bean
Zambia, Mt.MakuluResearch Station	NITROZAM	16,000	Sterile peat	250	soybean, lucerne and bean
Zimbabwe, Marondera		3,000	Sterile bargasse	50	Soybean, bean, pea, fodder legumes



The Nairobi and Dakar MIRCENs had emphasis on research and training in the production of biofertilisers, whereas that in Cairo focused on biotechnological techniques in the recycling of biodegradable wastes. The MIRCENs for the East African and West African regions are engaged in activities in the field of nitrogen fixation by *Rhizobium*/legume systems. The main responsibilities of the centres include collection, identification, maintenance, testing and distribution of rhizobial cultures compatible with crops of the region. Other activities include the identification of problems pertinent to the deployment of local rhizobial inoculant technology and promotion of research. In line with these objectives, pilot rhizobial inoculant production facilities were installed at the centres, with the long-term objective of serving the role of technology incubation centres that lead to spin-off inoculant plants in the respective sub-regions. More than 3 decades on, the two centres in West and East Africa have had mixed fortunes.



2 Inoculant production and use in West Africa

Other than experiments that are limited to research farms, there is hardly any country in West Africa where rhizobial inoculants are regularly used by farmers. The Microbiological Resource Centre (MIRCEN) in Senegal conducts inoculation trials on grain legumes, especially cowpea (*Vigna unguiculata*), groundnut (*Arachis hypogaea*), soybean (*Glycine max*), and bean (*Phaseolus vulgaris*). The centre also works on inoculation responses by tree legumes, especially *Acacia* and *Sesbania* species. However, in spite of its more than 30-year existence, the Dakar MIRCEN still produces inoculant at the pilot scale level with little evidence of efforts to engage the private sector for commercial production. The French Institute of Scientific Research for Cooperative Development (ORSTOM) also owns a laboratory in Dakar that is involved in research activities focusing on legume-rhizobium symbioses as well as on actinorhizal microorganisms (*Frankia* spp) aimed at identifying the most effective symbiotic associations with *Casuarina* and *Allocasuarina* genotypes micropropagated *in vitro*.

The two major elements that appear to drive the widespread adoption and use of inoculants and inoculant technology are largely absent in West Africa. These are large-scale commercial production of soybean and an intensive livestock industry. Specific varieties of soybean are high-yielding and are widely cultivated in commercial farms. They, however, require rhizobial inoculants for high productivity. Similarly, high value meat or dairy production is often done under an intensive livestock production system that relies on breeding animals on improved pasture. The pasture legumes are often not indigenous to the soils on which the pastures are established and inoculation, therefore, becomes imperative. In West Africa, soybean is a relatively new crop, although the area of cultivation continues to expand rapidly. However, virtually all the soybean grown is of the promiscuous variety, which readily nodulates with indigenous rhizobia. The major grain legumes in the sub-region, mainly cowpea, groundnut and bambara groundnut (*Vigna* subterranean), are also highly permissive in their interaction with indigenous rhizobia and hardly respond to inoculation. Additionally, extensive system of livestock production is widespread in the area; hence the incentive for inoculant adoption is minimal.



3 Inoculant production and use in East and Central Africa

3.1 Kenya

The Nairobi MIRCEN project located at the University of Nairobi was founded in 1977 with funding from UNESCO. The project's mandate includes the promotion and transfer of biological nitrogen fixation (BNF) technology, including inoculant production, to agricultural stakeholders within the East African region. The Centre has a collection of more than 250 rhizobial strains from local and foreign sources. Majority of the strains are fast-growing strains. All cultures are stored at 4°C, with those sourced abroad were brought and stored in lyophilised form. Using some of the rhizobial strains in its collection, the centre has developed inoculants for various legumes including pulses, pasture legumes and trees. These include *Phaseolus vulgaris* (common bean), *Glycine max* (soybean), *Medicago sativa* (lucerne), *Arachis hypogaea* (groundnut), *Desmodium* spp. (desmodium), *Sesbania* spp. (sesbania), *Leucaena leucocephala* (leucaena), *Vigna unguiculata* (cowpea) and *Pisum sativum* (Garden pea).

Nairobi MIRCEN has since 1981 been producing an inoculant known as BIOFIX which was the main inoculant in the market. The product comes in 100 g packets, with one packet sufficient to adequately inoculate 10 kg of seeds needed per hectare of common beans and soybean. In response to the needs of peasant farmers who mostly own small parcels of land, the Centre also sold 20 g and 10 g mini packs, which are sufficient for about 2 kg and 1 kg, respectively, of beans or soybeans (Karanja et al., 1998). Between 1992 and 1993, the average sales of MIRCEN's inoculants were about 1,350 kg per year (Mugabe, 1994), the level at which production had stagnated throughout the 1990s (Karanja et al., 1998). Some factors responsible for the low demand for the inoculants included inadequate and inefficient marketing channels and outlets, as well as inadequate extension services covering inoculant use (Odame, 1997).

Since 2008, Biofix inoculants have been produced by a private company, MEA Fertilizer Company Ltd., based on a memorandum of understanding signed with the University of Nairobi. Quality control of the Biofix inoculants remains the responsibility of the university. MEA made a \$200,000 investment in an inoculant production plant that is located at Nakuru, 200 km east of Nairobi. The company started by producing 400 sachets of Biofix weekly at the cost of about \$2.5 per sachet. The company is targeting production at 1000 sachets per week in future. MEA's involvement production and marketing has led to a two fold increase in sales of Biofix.

Inoculant production at Nairobi MIRCEN has 4 steps as follows:

Carrier preparation: A large deposit of peat exists outside Nairobi but lies within a government conservation area. Therefore, the carrier used for inoculant production at Nairobi MIRCEN is filter mud, obtained from the Muhoroni sugarcane mill. The filter mud is sun-dried, ground using a hammer mill, and sieved with a 210 μ m mesh. The pH of the resulting powder is adjusted to 7.0 after which 50 g units are transferred into 150 gauge polyethylene bags and moistened with yeast extract mannitol broth at 15% moisture holding capacity. The bags are sealed, leaving a small vent to allow for gaseous exchange during autoclaving, which is done at 121 °C for 3 hours. The bags are then aseptically sealed and stored pending when they are to be inoculated with rhizobial broth. At this stage, random samples are taken and tested for sterility on yeast extract mannitol agar (YEMA).

Inoculum broth preparation: The desired inoculant strains are cultured in 1.5 L of yeast extract mannitol broth (Vincent, 1970) in a 2 L flask for 2-4 days to late log phase of growth. Each flask represents a batch and is aerated by gently bubbling sterile compressed air through the broth. At the end of the incubation, an aliquot of the broth is sampled for plating on YEMA to test for sterility and for cell counts.



Inoculation of carrier. The late log phase culture is aseptically diluted in 20% (v/v) solution of yeast extract mannitol. The pre-sterilised carrier material is aseptically injected with the diluted broth. A small area in a corner of the carrier bag where the injector needle is to be inserted is sterilised by swabbing with sterile cotton wool containing 70%. The puncture hole is immediately sealed with an adhesive label that bears the batch number and other relevant information about the inoculant. Inoculation of the carrier is done with YEM broth containing approximately 10^9 viable cells of *B. japonicum* strain USDA110 for soybean and *Rhizobium tropici* strain CIAT899 for bean.

Inoculants curing: The inoculated bags are incubated at 25-30 $^{\circ}$ C for 2 weeks. The rhizobia in the carrier are expected to reach maximum population during this period. The packets are then observed for contamination before being stored at 4 $^{\circ}$ C.

The quality assurance framework put in place provides for testing of samples at every stage of the production, with plant infection tests employed on every batch of inoculant before deployment to market. However, recent tests have raised concern about the quality of the product due to the much lower cell numbers observed in the laboratory.

3.2 Tanzania

In the 1990s, the Food and Agricultural Organisation (FAO) supported a project to select better strains of rhizobia in Tanzania (Mugabe, 1994). The project included the establishment of a fermenter for the production of inoculants at the University of Dar-es-Salaam. The University also had support from the University of Nijmegen, The Netherlands, for the training of microbiologists at postgraduate level and research on BNF.

The Sokoine University of Agriculture developed at a commercial level an inoculant called 'Nitrosua' for use in soybean production. In collaboration with the Ministry of Agriculture and some non-governmental organisations (NGOs), the university also established extension activities to disseminate the inoculants to local farmers. Nothing much is now known about the inoculant production activities at both the Dar-es-Salaam and Sokoine Universities. It would appear that the activities stopped when external funding dried up.

3.3 Uganda

Inoculants are produced in Uganda by at least two plants. The first is Madhavani Ltd, a sugar factory with inoculant research laboratory near Jinja, and the other is Biological Nitrogen Fixation Makerere University which was established with the aid of the United State Agency for International Development (USAID) in 1990. Average annual production in the 1990s at the Makerere plant was about 1,500 kg of unsterile inoculants in 250 g packets for use in Uganda. In 1994, however, this plant and the one near Jinja were both contracted by FAO to supply 14.2 tonnes of soybean inoculants between 1995 and 1997 for distribution in Rwanda. Inoculants at Makerere University are produced by impregnating sterile peat carrier with yeast extract mannitol (YEM) broth containing approximately 10^9 viable cells of B. *japonicum* strain USDA110 originally obtained from the University of Hawaii NifTAL Project (Rwakaikara-Silver, 1998). The peat carrier is mined from a local deposit in Uganda. The inoculants are packaged in 100 g units. Quality control measures required the inoculants to contain a minimum of 1.2×10^6 rhizobia and should have less than 1 contaminant organism per 100,000 rhizobia.

3.4 Rwanda

In Rwanda, the Food and Agricultural Organisation (FAO) helped to set up an inoculant production facility at the Institut des Sciences Agronomique du Rwanda (ISAR) in 1984 and had reached an annual production level of 2.4 tonnes by 1990 (Cassien and Woomer, 1998). Within 7 years, inoculum production increased 600-fold. Inoculants were packaged in units of 40 g which was enough to inoculate 200 m² of soybean field. The size of the packets was suited to the needs of the farmers who often cultivate small holdings and are not resource–endowed to require large supplies. However, activities were disrupted by the civil war in 1994.



The laboratory was destroyed and all of the rhizobial strains and their documentation were lost. The laboratory was rehabilitated later and new equipment installed. Between 1995 and 2005, the laboratory could produce between 6000 to 8000 packets of solid inoculant that were bought by a non-governmental organisation on yearly basis and distributed to soyabean farmers across several sites in the country. Since 2005, the laboratory has not been working at full capacity due to inadequate equipment and lack of qualified staff.



4 Inoculant production and use in southern Africa

4.1 Zambia

Early documented reports on inoculation activities in Zambia came from the work of Corby (1965), who reported that soybean variety, Hernon 147, effectively nodulated with indigenous rhizobia and did not respond to rhizobial inoculation with Bradyrhizobium japonicum at five of the six locations in Zambia and Zimbabwe where the trials were conducted. Subsequently, a programme on symbiotic nitrogen fixation in soybean and other grain and forage legumes was initiated in 1973, with field trials on response to inoculation conducted during the 1973/74 and 1974/75 seasons (Zayed, 1982). Results of these trials showed soybean increases of 93-102% due to inoculation on 3 varieties, Bossier, Geduld and Hale 3. These results led to the introduction in 1976 of a programme on commercial inoculant production for soybean at the Mount Makulu Research Station. Initial quantities produced were sufficient for about 1,500 ha, which increased to 2,500 ha in 1977. Inoculant production at that stage was carried out in the laboratory without recourse to sophisticated equipment. Inoculum broth was produced using a molasses medium containing 5% (w/v) molasses, 0.3% CaCo3, 0.1% yeast extract. The molasses served as a replacement to mannitol, the conventional energy source used in yeast extract mannitol (YEM) medium. Chemical analysis showed that the molasses contained 37% sugar, 0.81% calcium, 0.30% magnesium, 3.4% potassium, 0.55% sodium, 0.05% phosphorus and 0.015% zinc. The use of molasses reduced the cost of production medium by 85% compared to that of YEM. A by-product of sugarcane processing, the molasses was obtained from the Zambia State Sugar Company.

In 1983, the University of Hawaii NifTAL (Nitrogen Fixation in Tropical Agricultural Legumes) Project was subcontracted by the University of Illinois, under a USAID-funded programme, to provide a technical assistance in the establishment of a rhizobia inoculation production facility at the Mount Makulu Research Station. The objective was to improve the availability and performance of rhizobial inoculants for improved BNF in Zambia (Sloger et al., 1993). Production lasted up until 1990 when it was moved to the Balmoral Veterinary Research Institute (BVRI), Lusaka. This is basically a vaccine laboratory that uses spare capacity to make and sell rhizobium inoculants for commercial soyabean farmers. It allows the use of existing bio-reactors for vaccine production which would otherwise be too expensive for inoculant production. According to Sloger et al. (1993), the introduction of the NifTAL project led to the increase in the area planted with inoculated soybean from 6,550 ha in 1984 to 22,780 ha in 1992, with 48% of the yield attributed to the use of inoculants.

Inoculants have since 1990 been produced at the Balmoral Institute although it has remained at a pretty small scale (Table 2) and only inoculants for soybean are produced. Sterile peat is used as inoculant carrier and quality control tests are done by the Zambia Agricultural Research Institute (ZARI) at Mt Makulu Research Station. Current annual production ranges from 30,000 to 40,000 units of 250 g satchets, costing approximately US \$2.5 per satchet. The quantity of inoculant produced is based on pre-season orders by Zambia Farmers Cooperative Society and the Zambia Seeds Company. The bulk of the inoculants are sold to commercial farmers who account for 60-70% of soybean produced in Zambia (Sloger et al., 1993). Average soybean yield in the commercial farms is about1.8 t/ha compared to a yield average of 0.7 t/ha in smallholder farms. An initial major constraint to inoculant use in soybean among smallholder farmers was the unavailability of the inoculants in remote areas and lack of adequate storage facilities for the inoculum (Joshi and Javaheri, 1986). To overcome this problem, naturally nodulating (promiscuous) soybean varieties were developed (Javaheri and Nyembe, 1982; Joshi et al., 1985) and have been promoted among smallholder farms.



Year	Location	Volume	Unit size (g)	Unit price (Kwacha)	Unit price (US \$)
1984-85	Mt Makulu	8,000	400	3	1.50
1985-86	Mt Makulu	21,500	150	3	0.45
1986-87	Mt Makulu	16,000	250	5	0.35
1987-88	Mt Makulu	24,000	250	5	0.30
1988-89	Mt Makulu	26,000	250	5	0.25
1989-90	Mt Makulu	36,000	250	5	0.25
1990-91	BVRI	52,000	250	50	2.00
1991-92	BVRI	55,000	250	60	1.80
1992-93	BVRI	40,000	250	150	1.58

Table 2. Volume and sale	prices of inoculants	produced in Zambia	(From Sloger et al. 1	993)
		produced in Zambia	(i toin ologei et al., i	333)

4.2 Zimbabwe

In Zimbabwe, the inoculant production facility that is located at the Grasslands Research Institute, Marondera, is the only one of its kind in the country and one of the largest in sub-Saharan Africa. It is government owned and operated on a semi commercial basis by the Soil Productivity Research Laboratory. It is supported by an independent but publicly-owned microbiology laboratory that is responsible for quality control and the maintenance of the Grassland Rhizobium collection. The collection has over 500 rhizobial and bradyrhizobial strains of tropical and temperate origin, all of which are lyophilised.

Inoculant production at SPRL started in 1962, producing inoculants for pasture legumes, mainly lucerne and clover species. Production of soybean inoculants commenced in 1967 and currently accounts for more than 90% of the inoculants produced, although inoculants are also made for *Phaseolus vulgaris, Pisum sativum, Lens culinaris, Trifolium* spp., *Medicago sativa, Desmodium* spp., *Arachis hypogaea, Stylosanthes* spp., *Crotalaria juncea.* The soybean inoculants are made from pure cultures of *B. japonicum* (strains MAR1491 and 1495, which are USDA 110 and 122 respectively). The long history of inoculant production means that there is a solid body of expertise in inoculant production, including expert technical staff (Giller et al., 2010).

The inoculants are made up of bagasse-based carrier that is impregnated with nutrients. Bagasse is a waste product of sugarcane. It consists largely of parenchyma cells (pith) from sugarcane stalks (Marufu et al., 1995). The bagasse is supplied by a company, Triangle Ltd at the cost of \$700 USD per tonne. Inoculant production is by batch culture using single inoculants strains in a 4-step process as follows (Marufu et al., 1995):

Carrier processing: The bagasse is sieved using 710 um mesh to remove coarse lignocellulose residue. The sieved fraction is mixed with other materials in the following proportion: dry bagasse (1 kg), CaCO₃ (20 g), MgSO₄.7H₂O (1.33 g), NaCl (0.67 g), K₂HPO₄ (3.33 g), and tap water (4.6 L). The final product has a moisture content of about 40% and is weighed into a 13 x 16 cm 50 µm high density polyethylene bags at the rate of 37 g per bag. The bags are heat-sealed, leaving an opening of about 1.5 cm through which a drinking straw is inserted to allow for gaseous exchange during autoclaving. The bags are stacked in single layers on trays and left overnight at room temperatures to allow for the germination of any fungal or bacteria spores that may be contained in the carrier before autoclaving at 121 °C for 30 minutes. The openings in the sterile bags are sealed under aseptic conditions and stored in cardboard boxes until required.



Preparation of inoculum broth: Rhizobial strains are grown in a nutrient broth with the following composition (per litre of water): sucrose (7g), K_2HPO_4 (0.5 g), MgSO₄.7H₂O (0.2 g), NaCl (0.1 g), and yeast extract (0.5 g). This composition is similar to the conventional yeast extract mannitol medium (Vincent, 1970) often used in most laboratories, except for the substitution of 0.7% sucrose for the 1% mannitol that is contained in the Vincent media. This substitution substantially lowers production costs because mannitol is generally the best carbon source for rhizobia, but also the least available and most expensive. Broth batches of 1.5 L are produced in 2 L fermentor flasks and inoculated with 10 ml of broth culture containing the required rhizobial strain. The inoculated broth is incubated at 28 °C for 2-4 days during which the broth is aerated by passing sterilised compressed air.

Inoculation of carrier: The mature broth culture is aseptically diluted with sterile yeast extract sucrose (YES) solution at a ratio of 1:5 (broth:YES). The YES solution is made up of 22.86 g of sucrose and 1.3 g of Oxoid yeast extract in 1 L of water and is autoclaved to achieve sterility and cooled to room temperature before mixing with the broth. Fifteen millilitres of the diluted broth is injected into pre-packed carrier through the lining of the bag using a sterile 5 mL repeater syringe. Prior to the injector is to penetrate. The needle hole is immediately sealed with an adhesive label that carries information on the batch number, the rhizobial strain, the legume for which the inoculants is to be used, and the expiry date.

Curing of inoculants bags: The inoculated bags are kept at 28 °C for 14 days. This is to allow for multiplication of rhizobial numbers in the carrier before storage at 4 °C. At the end of this period, the bags are visually graded for fungal contamination and 2 sample packs are randomly selected from each batch for testing at the laboratory. Quality control measures require the inoculants to contain a minimum of 1.0×10^9 rhizobia.

Quality control is conducted on the mother culture and on all batches using plating for purity and counts, and plant infection tests for nodulation and nitrogen fixation. The inoculants have a shelf life of up to 6 months when refrigerated at 4°C and a shelf life of 4 months when stored at room temperature in clay pots.

The factory has an annual capacity of about 200,000 packs of 100g units. However, its current production level is about 60,000 units annually. The inoculant at Marondera was exclusively patronised by farmers in the commercial farming sector up until the late 1990s when a soybean promotion programme led to a widespread adoption and use of inoculants by smallholder farmers. Production of inoculants increased until the collapse of commercial agriculture in 2001 to a peak of 136,000 sachets (Figure 1). Since 2000 much of the inoculants produced have been used in smallholder agriculture, with production gradually gaining ground in response to demand. A sachet currently sells for \$6 per pack. Inoculant packs are marketed by agro-dealers that are approved by the Ministry of Agriculture. These are usually farmers' cooperatives and seed companies with branches throughout the country. Seed Co, the largest soybean seed house in Zimbabwe, sells an average of 30,000 units annually. The Agriculture Extension Department and Grains Marketing Board each has an annual sale of about 10,000 units. This arrangement ensures that seeds and inoculants are sold at the same market outlets. The bulk of the inoculant is sold within Zimbabwe; however, Seed Co exports some of the inoculant for sale in Zambia and there are also buyers from Mozambique who buy directly from SPRL.

Since the 2007/2008 season, production has been hampered by problems of intermittent electricity supply, and inadequate resources. The facilities are well-maintained under the circumstances, but in dire need of reinvestment as many of the autoclaves need repair (some date from the 1950's) and the glasshouses and buildings need renovation (Giller et al., 2010).



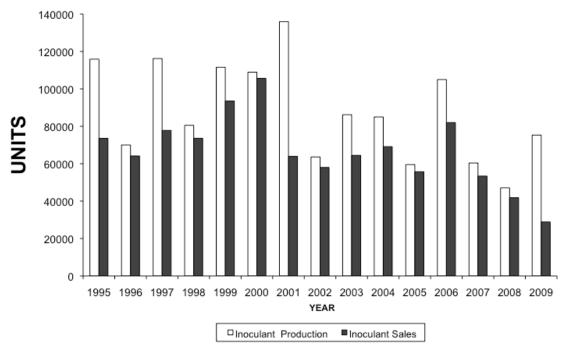


Figure 1. Production of soybean inoculants (indicated in number of 100 g sachets) at SPRL, Marondera, Zimbabwe has stagnated since 1995 (Giller et al., 2010).

4.3 South Africa

Commercially manufactured South African inoculants first appeared on the market in 1952 and rapidly expanded to warrant the introduction of an independent quality control system in the early 1970s (Strijdom, 1998). Beginning from 1966, the requirements for registration of inoculants with the South African Department of Agriculture included the use of a peat carrier and a proof that rhizobia could survive in specified minimum numbers in inoculants until the expiry date six months after their manufacture. Inoculants of each batch produced are submitted by the manufacturer for plate count and serological tests before marketing, or samples were taken at trade outlets by the regulatory agency for testing. Since 1976, all inoculants had to be manufactured with sterilised peat and must contain at least 5 x 108 rhizobial cells g⁻¹ of peat (Strijdom, 1998). A range of inoculants are produced for a number of crops including soybean, Medicago spp. and groundnut/cowpea (Deneyschen et al., 1998). However, there is little information available about production methods and quality control practices. A prominent inoculant producer in South Africa is Soygro Pty Ltd. This company is based in Potchefstroom, about an hour's drive from Johannesburg. It manufactures and produces microbiological formulations of various types, including rhizobial inoculants, for plants. According to the company's web site, it is the largest manufacturer in South Africa for microbiological inoculants for plants.

4.4 Malawi

In Malawi, inoculation activities probably started with soybean. Although this crop has been under cultivation in the country since about 1909 when it was recommended as green manure crop, the earliest records on inoculation activities were from 1951 when inoculation trials at Thyolo indicated the benefit of Rhizobium inoculant to soybean (Davis, 1979). Subsequent trials in the 1960s and 1970s at Chitedze demonstrated dramatic increases in soybean yields due to the application of locally produced inoculants. Commercially available inoculants produced by the Microbiology Section of Chitedze Agricultural Research Station, Lilongwe, were available starting from 1975. Going by the brand name of Fertilizer for



Legumes, inoculants for several pasture and grain legumes were produced and sold in 50 g packets (Khonje, 1989). These included cowpea, soybean, groundnut, common bean, lucerne (*Medicago sativa*), arch (*Macrotyloma axillare*), siratro (*Macroptilium atropurpureum*), *Centrosema pubescens, Aeschynomene americana, Desmodium* spp., *Stylosanthes guianensis, Neonotonia wightii, Lotononis bainesii, Leucaena leucocephala*, and guar (*Cyamopsis tetragonoloba*).

Sales rose from a paltry 450 packets in 1976 to about 1800 in 1987/88 (Table 3). The inoculants were usually purchased in bulk by the Agricultural Development Divisions and distributed to smallholder farmers. However, inoculant use by smallholder farmers had not been widely adopted largely because of inadequate extension but also due to poor quality products. Davis (1982) had earlier demonstrated that the inoculants produced in Malawi remained viable for up to 12 weeks at 26°C. Sadly, however, quality control in recent times has not been effectively implemented, with quality checks on the mother cultures being almost non-existent, and there is no quality control on the finished product. Nonetheless, the laboratory continues to produce inoculants; production figures since 2005 have been at about 20,000 units of 50 g per annum at the cost of about US 0.40 per unit (Lwimbi, pers. Comm.). Inoculants are currently produced for groundnut, common bean, soybean and pigeonpea using filter mud as carrier. Products are sold directly to farmers on the assurance that the inoculants contain 10^6 cells/g and a storage life of 6 weeks at room temperature. Given the parlous quality control practice at Chitedze, it is not unlikely that farmers are merely sold some other organisms in place of viable inoculant rhizobia.

Growing sea	son Number of	buyers Number of 50 g p	packets sold Total cost (Kwacha)*
1976/77	ND	448	224.00
1977/78	ND	616	308.00
1978/ <u>79</u>	14	1089	544.50
1979/80	20	872	436.00
1980/81	22	1179	520.00
1981/82	23	3481	1741.00
1982/83	11	1741	1384.25
1983/84	18	975	731.25
1984/85	20	1296	972.00
1985/86	16	1145	858.75
1986/87	38	1767	1325.25
1987/88	ND	1775	1221.25

Table 3. Total Rhizobium inoculant sales in Malawi, 1976-1988 (From Khonje, 1989).

ND = No data.

* = The cost of inoculant was 50 tambala per 50 g packet in 1976/77.

- In 1982/83 the price was raised to 75 tambala.



Conclusion

The greatest efforts at promoting inoculant production and use in sub-Saharan Africa were made by governments in East and Central as well as southern Africa. These efforts were often actualised through projects funded by international organisations with pilot inoculant plants being set up in universities or research stations. While these projects lasted, there was often an increase in inoculant production and sales in such countries. However, such spikes in availability and sales tended to wear off once the projects came to an end. It appears that a major impediment to the sustainability of such projects is the absence of private investors that can grow the venture beyond the pilot project stage. There is also the problem of inadequate resources and the absence of good quality control framework, which results in poor quality products and hence farmer apathy towards the products.



References

Cassien, B and Woomer, P.L. 1998. Recent history of the BNF activities at the Institut des Sciences Agronomique du Rwanda. In: F.D. Dakora (ed.) Biological Nitrogen Fixation in Africa: Linking Process to Progress, African Association for Biological Nitrogen Fixation, Cape Town, south Africa, pp. 33-34.

Corby, H.D.L., 1965. Report of national Rhizobium trial for soyabeans, 1963/1964. Mimeo, Rep. No. 236/59, Grasslands Research Station, Marandellas, Zimbabwe.

Davis, P.E. 1982. Legume microbiology research in Malawi. 1976-1981. Final report of the ODA Technical Cooperation Officer. February 1982. Lilongwe, Malawi.

Deneyschen, T., Strijdom, B.W. and Law, I.J. 1998. Comparison of South African legume inoculants manufactured in 1978-79 and in 1995-97. In: F.D. Dakora (ed.) Biological Nitrogen Fixation in Africa: Linking Process to Progress, African Association for Biological Nitrogen Fixation, Cape Town, south Africa, pp. 50-51.

Giller, K.E., Murwira, M.S., Dhliwayo, D.K.C., Mafongoya, P.L. and Mpepereki, S. 2010. Soyabeans and sustainable agriculture in southern Africa. Accepted for publication in International Journal of Sustainable Agriculture.

Javaheri, F. and Nyemba, R. 1982. Notes on soybean nodulation with the indigenous Rhizobium in Zambian soils. Soybean Genetics Newsletter 9, 173-174

Joshi, J.M., Javaheri, F. and Nkumbula, S. 1985. Development of promiscuous soybean varieties. Soybean Genetics Newsletter 12, 113-116.

Joshi, J.M. and Javaheri, F. 1986. Zambia. Genetics Newsletter 13, 209-212.

Khonje, D.J. 1989. Adoption of the rhizobium inoculation technology for pasture improvement in sub-Saharan Africa. In B.H. Dzowela, A.N. Said, Asrat Wendem-Agenehu and J.A. Kategile (Eds.), Utilization of research results on forage and agricultural by-product materials as animal feed resources in Africa. PANESA, Lilongwe, Malawi.

Karanja, N.K., Mwala, A.K., Kahindi, J.P. and Woomer, P.L. 1998. The East African Rhizobium MIRCEN: a review of the progress in research, training, and information dissemination. In: F.D. Dakora (ed.) Biological Nitrogen Fixation in Africa: Linking Process to Progress, African Association for Biological Nitrogen Fixation, Cape Town, south Africa, pp. 101-102

Marufu, L., Karanja, N. and Ryder, M. 1995. Legume inoculant production and use in East and southern Africa. Soil Biology and Biochemistry 27, 735-738.

Mugabe, J. 1994. Research on Biofertilizers: Kenya, Zimbabwe and Tanzania. Biotechnology and Development Monitor 18, 9-10.

Odame, H. 1997. Biofertilizer in Kenya: research, production and extension dilemmas. Biotechnology and Development Monitor 30, 20-23.

Rwakaikara-Silver, M.C., Luwata, C.K. and Katende, S.S. 1998. "Rhizobia for hope": legume inoculant production by Makerere University for emergency relief in Rwanda. In: F.D. Dakora (ed.) Biological Nitrogen Fixation in Africa: Linking Process to Progress, African Association for Biological Nitrogen Fixation, Cape Town, south Africa, 214 pp.

Sloger, C., Carr, T.H., Rourke, J.W. and Tembo, H. 1993. Review of legume inoculant use in Zambia: an assessment of the agronomic and economic impacts, 1984 to 1992. NifTAL, Hawaii, USA. 21 pp.

Strijdom, B.W. 1998. South African studies on biological nitrogen-fixing systems and the exploitation of the nodule bacterium-legume symbiosis. South African Journal of Science 94, 11-23.



Vincent, J. M. (1970). A Manual for the Practical Study of Root-Nodule Bacteria, Blackwell, Oxford.



List of project reports

- 1. N2Africa Steering Committee Terms of Reference
- 2. Policy on advanced training grants
- 3. Rhizobia Strain Isolation and Characterisation Protocol
- 4. Detailed country-by-country access plan for P and other agro-minerals
- 5. Workshop Report: Training of Master Trainers on Legume and Inoculant Technologies (Kisumu Hotel, Kisumu, Kenya-24-28 May 2010)
- 6. Plans for interaction with the Tropical Legumes II project (TLII) and for seed increase on a country-by-country basis
- 7. Implementation Plan for collaboration between N2Africa and the Soil Health and Market Access Programs of the Alliance for a Green Revolution in Africa (AGRA) plan
- 8. General approaches and country specific dissemination plans
- 9. Selected soybeans, common beans, cowpeas and groundnuts varieties with proven high BNF potential and sufficient seed availability in target impact zones of N2Africa Project
- 10. Project launch and workshop report
- 11. Advancing technical skills in rhizobiology: training report
- 12. Characterisation of the impact zones and mandate areas in the N2Africa project
- 13. Production and use of Rhizobial inoculants in Africa



Partners involved in the N2Africa project



Université Catholique de Bukavu

- Programme d'appui au developpement durable PAD (DRC) .
- Service d'Accompagnement et de Renforcement des capacities d'Auto promotion de la Femme en sigle – **SARCAF** (DRC)