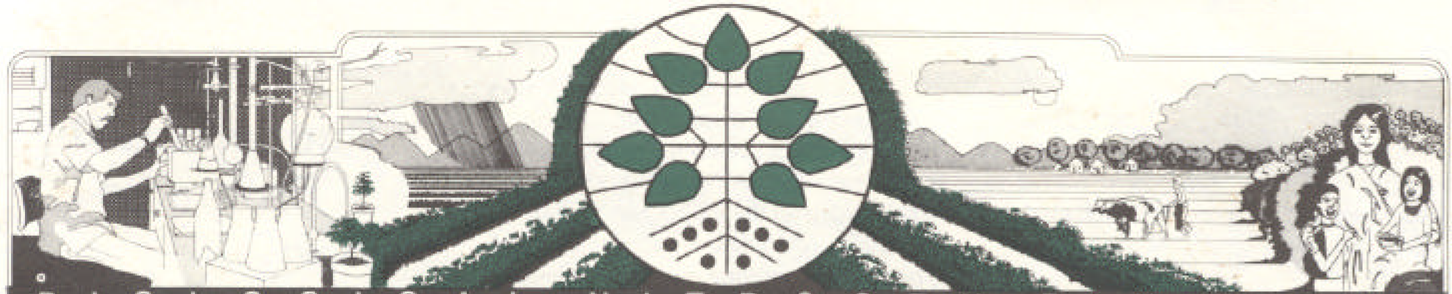

BIOLOGICAL NITROGEN FIXATION

BNF BULLETIN

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BNF Means Million$ for Zambia: Who Benefits?

The economic contribution of legume BNF to global agriculture is enormous—an estimated $85 billion per year. However, government policy-makers rarely have access to the information they need to evaluate investments in BNF programs. In Zambia, the fairly recent introduction of legume seed inoculation technologies provides a unique opportunity to monitor their impact.

In May 1993, a US Agency for International Development team reviewed the agronomic and economic impact of BNF technologies on soybean production in Zambia. **Charles Sloger**, USAID Bureau for Research and Development, was team leader. The group found that commercial farmers have been the first to benefit from inoculation technologies—they have earned millions of dollars in direct and indirect profits. However, adoption of inoculants by smallholder, subsistence farmers has lagged.

The BNF program for Zambia was supported by the Zambia Agricultural Research and Extension Project (ZAMARE), a USAID-funded consortium led by the University of Illinois and the Zambian Ministry of Agriculture and Water Development. ZAMARE was launched in 1981 to increase national food production and improve the welfare of smallholders through appropriate agricultural technologies. The project targeted the improvement of BNF for soybeans through two approaches: 1) a breeding program to develop "promiscuous" soybean varieties that nodulate with indigenous soil bradyrhizobia, eliminating the need for inoculation; and 2) a program to increase the availability of seed inoculants for commercial varieties.

The promiscuous "free-nodulating" soybean varieties were selected through extensive field trials. For the second approach, ZAMARE contracted NifTAL to establish an inoculant-production program at the Mt. Makulu Research Station in Chilanga. By 1984, NifTAL and Zimbabwean staff were producing soybean inoculant at Mt. Makulu. The carrier was sterilized peat, mined locally. NifTAL supervised production for three growing seasons. Production continued at Mt. Makulu until the 1989-1990 season, when it was moved to the Balmoral Veterinary Research Institute. Figure 1 shows the dramatic increase in area of inoculated crops over time.

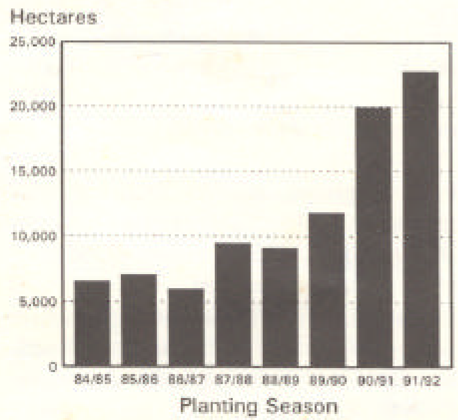


Figure 1. Soybean inoculant use in Zambia. Data from GRZ/MAFF, Statistical Bulletin, Mt. Makulu Research Station, and Balmoral Veterinary Research Institute.

The review team found that inoculants are popular among commercial the farmers who grow about 78% of Zambia's soybean crop. They can purchase inoculants through the ZAMSEED Company or the Zambia Farmers Cooperative Society. In the five years from 1988 to 1993, ZAMSEED sold approximately 100,000 units of inoculant, representing 53% of the retail market.

> In eight years, Zambia gained more than US$23 million using legume inoculants.

Increased inoculant use reflects the expansion of land under soybean production, increasing at a rate of 27% per year. Previously uncultivated areas lack the *Bradyrhizobium japonicum* specific to commercial soybean varieties. As a result, yield improvements after inoculation are high. Field data from **Ronnie Nyemba** and **Howard Tembo** of the Mt. Makulu Research Station indicate that average seed yields nearly doubled in three areas of Zambia due to inoculation. Their trials showed that inoculants costing US$3.60 per hectare matched yields achieved with about 300 kg of urea, costing about US$86.

Higher yields combined with fertilizer savings mean that Zambia received a net eco-

Continued on page 2

Continued from page 1
nomic benefit of more than US$23 million during the first eight years of inoculant use. Profits from inoculation are expected to increase even further with expanded legume production.

Investment in inoculants has been a boon to commercial farmers, but what about the estimated 380,000 subsistence farmers in Zambia? Nine years after the introduction of inoculant technology, many smallholders still do not have ready access to inoculant products. Perhaps as a result, commercial farmers enjoy average soybean yields of 1.8 metric tons per hectare, while smallholder yields average only 0.7 metric tons—less than half.

According to review team member **Thomas Carr**, NifTAL Private Enterprise Development Specialist, "The irony of this situation is double edged. Inoculants are very affordable, at only $3.60 per hectare, but only large-scale commercial farmers have access to them. Government policy has promoted the use of promiscuous 'free-nodulating' soybean varieties among smallholders, rather than encouraging inoculant use among this group."

"The AID review reveals a huge potential for BNF and a further challenge to widen the spectrum of inoculant users in Zambia."

Smallholders have widely adopted the "free nodulators", a mark of success for the project. But indigenous rhizobia are clearly not meeting the yield potentials of these promiscuous varieties. Experimental data indicate that inoculation of these varieties with *B. japonicum* can potentially double their yields. So why don't the subsistence farmers use inoculants?

Is there a perception that smallholders are not sufficiently "educated" to use inoculants effectively? Or that inoculants are not appropriate for smallholders because the rhizobia are sensitive to stresses encountered during distribution in rural areas? Is it perhaps a failure of the inoculant distribution network to reach small villages?

"These are all legitimate concerns," says Carr, "but any of these constraints can be overcome." The review team recommends a vigorous extension effort to educate smallholders in the proper handling of inoculants. This should broaden the base of producers benefitting from BNF technology. The team also encourages a concerted effort by government and donor agencies to facilitate the participation of the private sector in inoculant production. "The private sector can best exploit the untapped market for inoculants among smallholders and can ensure sustained production for established commercial users," says Carr.

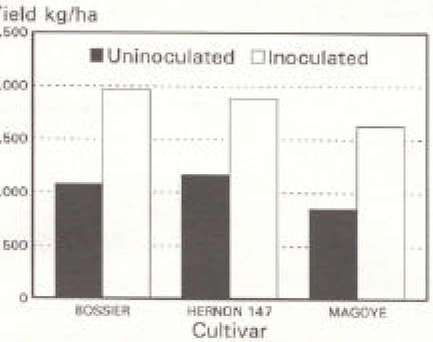


Figure 2. Response of specific (Bossier) and promiscuous (Hernon 147 and Magoye) soybean cultivars to inoculation at Mufulira 84/85 season. From S. Sonogho, Mt. Makulu Research Station.

So is it only the large commercial farmers who are benefitting from inoculants? Not according to review team member **Joe Rourke**, NifTAL Inoculant Production Specialist, who worked at Mt. Makulu during the 1984 and 1985 cropping seasons. On returning to Zambia in 1993, Rourke was encouraged to see that the government has continued inoculant production, but he was especially gratified to see that ancillary industries—such as production of high-protein, soy-based infant formulas—have emerged as a result of increased soybean production. According to Rourke, "If BNF helps to improve the nutrition of children, then everybody benefits."

Paul Singleton, NifTAL Director comments, "Millions of dollars of profits from BNF help the Zambian economy and society as a whole, not just the commercial farmers. The BNF project has been highly successful, and the Zambian government and participating donors and technical assistants should be proud of their efforts. The USAID review reveals a huge potential for BNF and a further challenge to widen the spectrum of inoculant users in Zambia."

FAO/IAEA Supports BNF Programs

by Dr. Gudni Hardarson

For more than two decades the Joint United Nations Food and Agriculture Organization (FAO) and International Atomic Energy Agency (IAEA) Division have coordinated international programs on biological nitrogen fixation in developing countries. The Agriculture Laboratory in Seibersdorf, Austria, also participates in the project. The main objectives of these programs are to develop and optimize the ^{15}N isotope dilution method for quantifying nitrogen fixation by legumes and to enhance BNF in various cropping systems.

The Division has supported BNF work through Coordinated Research Programs (CRP) and Technical Cooperation Projects. Previous programs focused on grain and forage legumes, *Azolla*, and the development of ^{15}N methodology. Current programs emphasize grain legumes in Asia, tree legumes, and microbial ecology. The programs have an average duration of five years. Scientists from 10 to 15 institutes participate in each CRP, either as funded contractors from developing countries or as cost-free agreement holders from developed countries or international institutes. Participants from approximately 30 countries throughout the world are now involved in FAO/IAEA BNF programs.

Many of the CRP experiments are conducted simultaneously in a number of countries under a wide range of environmental conditions. Cost-free experts help transfer technology to developing countries through research-coordination meetings, workshops, and fellowships. The Soil Science Unit at the Seibersdorf Laboratory supports implementation of the FAO/IAEA program by providing research, training, and isotope analyses of field samples.

Funding opportunities through the Coordinated Research Programs are advertised in the *Soils Newsletter*, a free publication of IAEA. For additional information write to Dr. Christian Hera, Head, Soil Fertility, Irrigation, and Crop Production Section, Joint FAO/IAEA Division, P.O. Box 100, Wagramerstr. 5, A-1400 Vienna, Austria.

BJSM: A Selective Medium for Soybean Bradyrhizobia

A major obstacle in monitoring rhizobia in the environment has been the inability to isolate the bacteria directly from matrices with diverse microbial populations. Enumerating rhizobia in soils or non-sterile inoculant carriers has required the use of complex immunological procedures or indirect methods such as plant-infection Most-Probable-Number (MPN) tests.

Zhaokun Tong and **Michael Sadowsky** of the University of Minnesota recently presented their research on a novel medium that may help to overcome this obstacle. Called BJSM, their medium selectively allows the growth of soybean symbionts *Bradyrhizobium japonicum* and *B. elkanii*. BJSM is based on the inherent resistance of *B. japonicum* and *B. elkanii* to high levels of zinc and cobalt. By combining these metals in the medium, the researchers were able to isolate and quantify soybean bradyrhizobia from soil and inoculants.

Selective media are not new. Researchers have previously combined antibiotics with various dyes and fungicides to isolate rhizobia from non-sterile matrices. The usefulness of this approach is limited, however, because of the specific intrinsic resistances of individual strains to various antibiotics. BJSM is unique because it does not contain any antibiotics. The reliance on heavy metals for selection of whole genera of bradyrhizobia evolved from research by Kinkle and colleagues (1987) who evaluated the tolerance of soybean bradyrhizobia to heavy metal concentrations in soils treated with sewage sludge.

In addition to zinc and cobalt, BJSM also contains brilliant green and pentachloronitrobenzene (PCNB), components of other selective media (Pattison and Skinner, 1974; Barber, 1979). This combination proved highly successful in inhibiting the growth of fungi and other bacteria. When a suspension of North American soil was plated on BJSM, 98% of all colonies nodulated soybean.

The development of BJSM was supported by a grant from NifTAL. NifTAL Research Director, **Harold Keyser**, is encouraged by the medium's potential for the inoculant production industry. Producers using non-sterile carriers have had to rely on MPN tests—which can take weeks—to enumerate rhizobia in their products. "We're excited because this medium can revolutionize inoculant quality control procedures for soybean," says Keyser. "We've compared the medium with MPN tests and found that we can accurately enumerate soybean strains by direct plating on the BJSM."

If you would like to test BJSM, please contact **Padma Somasegaran** at NifTAL.

References

Barber, L.E. 1979. Use of selective agent for recovery of *Rhizobium meliloti* from soil. Soil Sci Soc Am J 43:1145-1147.

Kinkle, M.T., J.S. Angle, and H.H. Keyser. 1987. Long-term effects of metal-rich sewage sludge application on soil population of *Bradyrhizobium japonicum*. Appl Environ Microbiol 53:315-319.

Pattison, A.C. and F.A. Skinner. 1974. The effect of antimicrobial substances on *Rhizobium spp.* and their use in selective media. J Appl Bacteriol 37:230-250.

Tong, Z. and M.J. Sadowsky. In press. A selective medium for the isolation and quantification of *Bradyrhizobium japonicum* and *Bradyrhizobium elkanii* strains from soils and inoculants. Appl Environ Microbiol.

NifTAL has trained 23 scientists and technicians from India's Biofertilizer Development Centers through a United Nations Food and Agriculture Organization program, "Development and Demonstration of Biofertilizers." NifTAL and the BNF Resource Center (BNFRC) in Thailand conducted two comprehensive five-week training courses covering the assessment of nitrogen fixation, microbiology of rhizobia, inoculant production, and quality control. The latest course was hosted by the Thai Department of Agriculture from 26 July to 27 August 1993, at the BNFRC in Bangkok. Instructors included Course Director Padma Somasegaran, FAO Consultant J. Thompson, BNFRC Director N. Boonkerd, and NifTAL Training Coordinator H. Hoben.

Examining legume roots for nodulation

Inoculating a glass fermentor

Conference on *Azospirillum* in Uruguay

Montevideo, Uruguay, was the venue for a workshop on "Agronomic Applications of *Azospirillum*," held in August 1993. Organizers **Yaacov Okon** of the Hebrew University of Jerusalem and **Carlos A. Labandera-González** of the Uruguay Ministry of Agriculture report that 30 participants came together to discuss their experiences with this associative nitrogen fixer. They represented research groups and inoculant producers in Uruguay and Argentina. The organizers are now preparing a review paper "Agronomic applications of *Azospirillum*: An evaluation of 20 years of world-wide field inoculation studies." For more information, contact Carlos Labandera, Laboratorio de Microbiologia, Burgues 3208, Montevideo, Uruguay, fax (598-2) 238152 or (598-2) 235992.

Micronutrients for Cowpeas in Sri Lanka

Dr. V. Arulnandhy from Eastern University, Sri Lanka, reports that micronutrient applications may enhance the growth and nodulation of cowpeas in Sri Lanka's sandy regosols. In a pot test, separate foliar applications of molybdenum (25 ppm), cobalt (1 ppm), and boron (0.5 ppm) increased nodule mass by 129, 39, and 81% respectively. Six weeks after planting, plant dry weight increased by 31, 18, and 20% respectively, with these inputs.

In Sri Lanka, traditional farmers grow cowpea over an area of about 50,000 hectares. They use few inputs. While yields under optimal management are potentially as high as 1.5 metric tons per hectare, the national average ranges from only 0.7 to 0.8 tons. Because cowpea nodulates with indigenous rhizobia, it is generally not inoculated, but appropriate management could increase the contribution of BNF significantly. Dr. Arulnandhy will take his investigations to the field to identify management inputs that could improve yields of this important crop.

For more information, contact Dr. Arulnandhy, Department of Agronomy, Eastern University, Vantharumoolai, Chenkalady, Sri Lanka.

BNF Awareness Grows in Uganda

Francis Oching reports that there is a growing awareness of the benefits of legume inoculants and BNF for sustainable agriculture in Uganda, thanks to the BNF/Legume Management/On-Farm Productivity Enhancement Program (BNF/LM/OFPEP), a consortium led by Agricultural Cooperative Development International (ACDI). ACDI collaborated with the BNF/Legume Management Pilot Program, Save the Children Foundation, the Cooperative Agriculture and Agribusiness Support (CAAS) Project, and NifTAL to hold a series of four skill-building workshops on applied BNF. The 108 participants came from 21 of the 33 districts of Uganda. They represented community groups, government agencies, and private voluntary organizations. Oching, BNF Country Coordinator for the USAID/ACDI CAAS program, conducted the most recent workshop in June 1993. He was assisted by local resource persons from Makerere University and the Ministry of Agriculture who had received training in the earlier workshops.

In 1990, NifTAL provided technical assistance to establish an inoculant-production facility at Makerere University research station. A recent CAAS project evaluation of Uganda's BNF program concluded that inoculant production should be privatized to insure a steady supply. Makerere University should play a vital role in providing quality control and technical assistance to the private sector. According to **Thomas Carr**, NifTAL's Private Enterprise Development Specialist, "Privatization is a natural progression to keep up with inoculant production in Uganda as the BNF workshops continue to increase farmer demand for inoculants."

Nitrogen Sources for Rhizobia Tested in Colombia

J. Ernesto Luque T. of the University of Nariño, Colombia, reports that he has tested 11 *Rhizobium leguminosarum* strains for their ability to grow on media containing various nitrogen sources. He found that certain nitrogen sources, such as glutamic acid, aspartic acid, and lysine, supported the growth of all strains, while none of the strains were able to grow on media with tryptophan, thiamin, or riboflavin as the sole nitrogen source. The strains differed in their abilities to grow on media containing urea, ammonium nitrate, ammonium chloride, potassium nitrate, alanine, valine, methionine, tyrosine, cysteine, and niacin. Luque proposes that this variation in the tolerance of strains to specific nitrogen sources may be exploited as a means of strain identification. For further information, contact Dr. Luque at Calle 4 Oeste 31-39, Pasto, Nariño, Colombia.

^{15}N Techniques in Egypt

Dr. Yehia Galal M. Galal, of the Atomic Energy Authority's Soil and Water Department in Cairo, reports that a successful training course on "Use of Radiation and Radioisotopes in Agriculture and Life Sciences" was held in July 1993. Topics included the use of stable isotopes in BNF research.

Dr. Galal and **Dr. Ismail G.L. Ghandour** are using ^{15}N isotope dilution techniques to quantify nitrogen fixation by biofertilizers. They report that inoculation with associative-fixers *Azotobacter* and *Azospirillum* increased the nitrogen-uptake efficiencies of wheat and maize. For further information, contact Dr. Galal at Soil and Water Department, P.O. Box 13759, Cairo, Egypt.

Attention All Inoculant Producers

NifTAL is compiling an inventory of global resources for legume inoculant production. The Information Section has assembled a draft directory of government and private inoculant producers throughout the world and has sent questionnaires to these agencies and companies to assess their needs and capabilities. NifTAL thanks all of the inoculant producers who have returned their questionnaires: They will receive a revised directory in 1994. Please contact NifTAL if you produce inoculants (even on a small scale) and have not yet received a questionnaire. NifTAL staff hope this inventory will eventually lead to the formation of an international organization of inoculant producers.

Soil Inoculum: A Low-Technology Alternative

by Lex Diatloff

Peat-based inoculants are not available in many areas where the need for inoculation is urgent. Soil-transfer inoculation is a low-technology alternative that uses readily available natural resources. The merits of soil-transfer inoculation may be debated, but at the village level this historic practice comfortably links the past with hope for the future.

The success of soil-transfer inoculation depends on the vigor of the original legume stand in the source-soil. I suggest establishing a 1-m^2, well-tended, nodulated-legume nursery. A friable, moist, high-organic soil is ideal for building a population of rhizobia through rhizosphere colonization and release from nodules. Soil from this plot will be sufficient to inoculate 5 to 10 hectares. The starter rhizobia may be collected from nodules of healthy crops or from soil transfer itself. Check the internal color of the nodules to insure that the plants in the plot are nodulated effectively.

It is very important to inspect the plants for root pathogens such as wilts, nematode galls, stem rots, borers, or weevils. In their discussion of the soil-transfer method, Fred and colleagues (1932) prudently pointed out the dangers of transferring diseases and pests.

If the plants are well nodulated and free of diseases and pests, the soil can be collected to a depth of 150 mm, then sieved, bagged in plastic, and stored in a cool place for later use. I have recorded rhizobial counts as high as 10^6 per gram of soil under perennial siratro pastures. Rhizobia survive well in soils with high clay contents.

There are several ways to use soil inoculum: 1) Coat large seeds (cowpea, *Arachis pintoi, Dolichos*) with a creamy, montmorillic clay slurry; 2) For medium-sized seeds (*Vigna* sp., *Pueraria*), roll dry soil pellets the size of the seed, mix them with seed at a rate of four to six pellets per seed, and plant pellets and seed together; 3) Plant three or four small seeds (*Lotononis, Wynn cassia, Stylosanthes* spp.) in a ball of soil inoculum; 4) For browse shrubs (*Leucaena, Gliricidia*), plant seeds in pots of soil inoculum molded with binding straw. Check for nodulation before transplanting.

For additional information, contact Lex Diatloff, Division of Plant Protection, Department of Primary Industries, 80 Meiers Road, Indooroopilly, Queensland 4068, Australia.

Reference

Fred, E.B., I.L. Baldwin and E. McCoy. 1932. Root nodule bacteria and leguminous plants. University of Wisconsin, Madison.

Editor's note: Modern inoculants typically contain 10^9 rhizobia per gram and are free of pathogens. It would take more than 1000 kg of soil inoculum to provide the same number of rhizobia as 1 kg of good-quality peat inoculant.

MEETINGS

World Soybean Research Conference V, 21 to 27 February 1994, Chiang Mai, Thailand. The conference theme is "Soybean: A universal crop for better global health." Contact the Secretariat, Conference Organizing Committee, Department of Agricultural Extension, P.O. Box 1081, Kasetsart, Bangkok 10903, Thailand, phone (66-2) 579-3936, or fax (66-2) 579-6635.

Gatlinburg Symposium on the Commercialization of Plant Science, 25 to 28 May 1994, University of Tennessee, Knoxville, TN, USA. For more information contact Gary Stacey, Director, Center for Legume Research, M409 Walters Life Sciences Building, University of Tennessee, Knoxville, TN 37996-0845, USA, phone (1-615) 974-4041, fax (1-615) 974-4007.

International Symposium on Environmental Agriculture, 5 to 8 June 1994, Travelodge Resort Hotel, Gold Coast, Queensland, Australia. For more information contact Paul Saffigna (Symposium Convenor), Head, Graduate School of Environmental Sciences and Engineering, Griffith University, Nathan Campus, Queensland, 4111 Australia, phone (61-7) 875-5332, fax (61-7) 875-5282.

International Expert Workshop on Nitrogen Fixing Trees for Acid Soils, 3 to 8 July 1994, Centro Agronómico de Investigación y Enseñanza (CATIE), Turrialba, Costa Rica. This workshop is co-organized by the Nitrogen Fixing Tree Association (NFTA). It will be conducted in English and Spanish. Contact Mark Powell, NFTA, 1010 Holomua Road, Paia, HI 96779, USA, phone (1-808) 579-9568, fax (1-808) 579-8516.

2nd World Cowpea Research Conference, 4 to 10 September 1994, at International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Contact Dr. F.M. Quin c/o L.W. Lambourn & Co., Carolyn House, 26 Dingwall Rd., Croydon CR9 3EE, England, fax (44-81) 681-8583.

6th Conference of the African Association for Biological Nitrogen Fixation (AABNF), 12 to 17 September 1994, Harare, Zimbabwe. Contact The Secretary, AABNF 6th Conference, Department of Soil Science, University of Zimbabwe, Box MP 167, Mt. Pleasant, Harare, Zimbabwe, fax (263-4) 732828.

FAO/IAEA International Symposium on Nuclear and Related Techniques in Soil/Plant Studies with Special Emphasis on Environmental Preservation and Sustainable Agriculture, 10 to 16 July 1994, Acapulco, Mexico. Send inquiries to Dr. Christian Hera, Head, Soil Fertility, Irrigation and Crop Production Section, Joint FAO/IAEA Division, P.O. Box 100, Wagramerstr. 5, A-1400, Vienna, Austria.

FAO/IAEA International Symposium on Nuclear and Related Techniques in Soil/Plant Studies on Sustainable Agriculture and Environmental Preservation, 17 to 21 October 1994, Vienna, Austria. The symposium will cover analytical methods, fertilizer and water management, soil conservation, and BNF in sustainable cropping systems. Send inquiries to Dr. Christian Hera, at the address above.

Rhizobia as Biocontrol Organisms?

by Abdul Ghaffar

Besides their capacity for nitrogen fixation, some strains of rhizobia have been found to inhibit the growth of root-infecting fungal pathogens. Rhizobia may protect the roots of legumes and nonlegumes by producing a toxic metabolite, rhizobitoxine (Chakraborty and Purkayashta, 1984), by producing antibiotics (Malajczuk, 1983), or by parasitizing the hyphae of pathogenic fungi directly (Tu, 1978) and lysing them (Malajckuk et al., 1984).

We identified four rhizobial strains that were successful antagonists, to varying extent, against the fungal pathogens *Macrophomina phaseolina*, *Rhizoctonia solani*, and *Fusarium* spp. (see table). Treating seed or soil with these strains frequently reduced pathogenic infection in both nonlegumes (sunflower and okra) and legumes (soybean and mungbean). There was also evidence of synergism between strains of rhizobia and other biocontrol agents and fungicides.

Pathogen	Treatment	Sunflower	Okra	Soybean	Mungbean
		(% infection)			
M. phaseolina	Control (no treatment)	91	83	91	91
	R. meliloti KUMH 139	50	75	58	81
	R. meliloti KUMH 555	67	83	75	83
	B. japonicum KUMH 569	83	33	67	75
	R. leguminosarum KUMH 55191	91	75	50	38
R. solani	Control	91	75	100	88
	R. meliloti KUMH 139	41	41	83	58
	R. meliloti KUMH 555	50	47	83	67
	B. japonicum KUMH 569	41	8	67	41
	R. leguminosarum KUMH 551	67	25	100	41
Fusarium spp.	Control	58	75	58	100
	R. meliloti KUMH 139	8	41	8	58
	R. meliloti KUMH 555	8	41	16	58
	B. japonicum KUMH 569	8	33	16	33
	R. leguminosarum KUMH 55158	8	58	25	38

The effect of seed treatment with strains of *Rhizobium meliloti*, *Bradyrhizobium japonicum*, and *R. leguminosarum* on infection of four plant species by fungal pathogens *Macrophomina phaseolina*, *Rhizoctonia solani*, and *Fusarium* spp. Plants are 60 days old. $LSD_{(.05)} = 6.2$.

For more information, contact Dr. Ghaffar at the Department of Botany, University of Karachi, University Road, Karachi 75270, Pakistan.

References

Chakraborty, U. and R.P. Purkayashta. 1984. Role of rhizobitoxine in protecting soybean roots from *Macrophomina phaseolina* infection. Can J Plant Pathol 8:140-146.

Malajczuk, N. 1983. Microbial antagonism to *Phytophtora*. p 197-218 *In* D.C. Erwin et al. (eds). *Phytophtora*: its biology, taxonomy, ecology, and pathology. St. Paul, MN, American Phytopathological Society.

Malajckuk, N. et al. 1984. Interactions between *Phytophtora cinnamoni* and *Rhizobium* isolates. Trans Br Mycol Soc 82:491-500.

Tu, J.C. 1978. Protection of soybean from severe *Phytophtora* root rot by *Rhizobium*. Physiol Plant Pathol 12:233-340.

Nitrogen Fixation Conference in Australia

The 10th Australian Nitrogen Fixation Conference took place in Brisbane from 7 to 10 September 1993. Organizer **H.V.A. Bushby** of CSIRO, Queensland, reports that the conference theme "Genetics, microbial ecology, and nitrogen fixation—Is there a sustainable symbiosis?" was designed to integrate the multifaceted aspects of BNF research. The conference reflected an awareness that BNF research is ultimately defined by its value to the end user, with research presented in terms of its potential for practical applications.

Ninety-five presentations from Australian and international scientists addressed seven major research areas. Bushby noted a strong interest in inoculants and field measurements of nitrogen fixation, with a particular focus on the use of the ureide and ^{15}N techniques. The genetics papers highlighted the practical significance of results, with reduced emphasis on methodology and sequencing details.

The conference proceedings will be published as a special issue of *Soil Biology and Biochemistry*. For more information, contact Dr. H.V.A. Bushby, CSIRO Australia, Division of Tropical Crops and Pastures, 306 Carmody Road, St. Lucia, Brisbane, Queensland 4067, Australia.

NEW PUBLICATIONS

A Practical Rhizobium-Legume Technology Manual, by D.P. Beck, L.A. Materon, and F. Afandi, has been published by the International Center for Agricultural Research in the Dry Areas (ICARDA). The manual covers rhizobial microbiology, inoculant production, and field and greenhouse experimentation to evaluate nitrogen fixation. The manual is dedicated to Professor James Vincent and Dr. Joe Burton, two respected pioneers in the BNF field. To obtain the manual, send US$35 to Publications Distribution Unit, ICARDA, P.O. Box 5466, Aleppo, Syria.

Where's NifTAL's Manual? The long awaited 2nd edition of NifTAL's 1985 *Methods in Legume-Rhizobium Technology* by P. Somasegaran and H. Hoben is in press at the Springer-Verlag publishing company in New York. Look for announcements of its release in future *BNF Bulletins*.

The Production, Management, and Use of Nitrogen Fixing Trees by NFTA and Heifer Project International is a complete training package including text, support materials, and slide set. The full package costs US$85; components may be ordered separately at lower prices. Contact NFTA, 1010 Holomua Road, Paia, HI 96779 USA.

Biofertilizers in Agriculture and Forestry (3rd edition), by N.S. Subba Rao, reviews the variety of microbial inoculants used as biofertilizers. The appendix contains information on media formulations and strains. The book costs US$55 plus shipping. Ordering information is available from International Science Publisher, 52 LaBombard Rd. North, Lebanon, NH 03766, USA.

Books available through B. Ben Bohlool Memorial Fund. Dr. J. K. Ladha has generously donated his royalties for the book ***BNF for Sustainable Agriculture*** edited by J.K. Ladha, T. George, and B.B. Bohlool, to the B. Ben Bohlool Memorial Fund. The Memorial Fund has been providing this book free to developing-country students with an interest in BNF. Ladha's donation extends the offer to both scientists and students in developing countries. Write to Dr. Ladha, Soil Microbiologist, IRRI, P.O. Box 933, 1099 Manila, Philippines, to request the book through the BBB Memorial Fund.

Legume Green Manures: Principles for Management Based on Recent Research, by D.J. Lathwell, and ***Dry Season Survival and the Effect on Succeeding Maize Crops***, by M.L. Burle and colleagues, are available from the USAID Soil Management Collaborative Research Program (TropSoils CRSP). Request these free publications (Bulletins 90-01 and 92-04) from T. McBride, TropSoils, Box 7113, North Carolina State University, Raleigh, NC 27695-7113, USA.

The Directory of BNF Resource Persons lists non-US scientists, inoculant producers, and extension persons with an interest in BNF for international development. NifTAL thanks all of its colleagues who have agreed to be listed in the Directory. Copies may be obtained from the NifTAL Communications Section, 1000 Holomua Road, Paia, Maui, Hawaii, 96779.

An Abridged Catalogue of Rhizobia, by P. Somasegaran, NifTAL germplasm curator, lists rhizobial germplasm available from the NifTAL Microbial Resource Center. Request catalogue from the NifTAL Communications Section at address above.

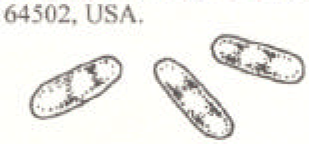
COMMERCIAL CORNER

Commercial Production in Bangladesh

Md. Zahurul Haque, Executive Director of the Bangladesh company BioLINK, reports that his firm has entered a joint venture agreement with Bangladesh Agricultural University (BAU) for commercial production of legume inoculants. **Dr. Shamsul Hoque** of BAU is the technical advisor to the BAU/BioLINK venture. BioLINK's annual production is now approximately 7,000 kg. Demand, now estimated at 20,000 kg, is expected to grow. BioLink plans to expand its product line to include *Azotobacter* inoculants, *Azolla*, and plant-based biopesticides. For more information, contact Z. Haque at BioLINK, 12 New Eskaton Rd., Moghbazar, Dhaka-1000, Bangladesh.

Frozen Rhizobia

Tom Wacek of Urbana Laboratories, Missouri, reports on a unique product—Urbana's Frozen *Rhizobium* Concentrate. The concentrate was successfully field tested for two years by **Bill Lindemann** of New Mexico State University, who applied the inoculant to bean crops via overhead irrigation. According to Wacek, "This product is not for everyone, but for certain situations, it's definitely a convenient method of inoculation." For further information, contact Tom Wacek at Urbana Laboratories, P.O. Box 1393, St. Joseph, MO 64502, USA.



Liquid Inoculants

Liquid inoculants are gaining popularity in North America among commercial farmers who need to inoculate large batches of seeds. They are sprayed in furrows or applied to seed in a hopper box or grain auger. Products include Urbana Laboratories' Liqui-Prep XT, containing a special sticker, and LiphaTech's CellTech™S, a sterile-liquid-base inoculant. **Stew Smith** of LiphaTech reports that rhizobia grow in the liquid to populations of $6x10^9$ rhizobia per ml, and maintain high populations for up to 25 months. For more information, contact T. Wacek at Urbana Laboratories (P.O. Box 1393, St. Joseph, MO 64502, USA) or S. Smith at LiphaTech, Inc., 3101 West Custer Ave., Milwaukee, WI 53209 USA.

Potential Strategies for Sustaining BNF in Drylands

by R. Venkateswarlu

Water-deficit stress can inhibit N_2-fixation in legumes and lead to reduced nitrogen accumulation, growth, and yields. At the Central Research Institute for Dryland Agriculture in Hyderabad, India, we are evaluating potential strategies for sustaining BNF in groundnut and cowpea through periods of water stress.

We consistently observed that the inhibitory effects of water stress on the plant and its symbiotic functioning are more critical than the effects on free-living *Rhizobium*. In alfisols, vertisols, and aridisols, native rhizobial populations declined to levels as low as 10 per gram of soil during the dry season but recovered to levels of 10^3 to 10^5 per gram of soil at the onset of the rainy season. These reduced rhizobial populations provided adequate nodulation in more than 90% of our sites.

These results led us to focus on genetic variation of the host plant. In cowpea, we found significant genotypic differences in the rate of new nodule formation following nodule shedding during water-deficit stress. Genotypes with a longer interval between flowering and physiological maturity were better able to regain nodule mass upon rewatering. Cowpea varieties also differed in the relative distribution of nodules on the tap and lateral roots. Since lateral-root nodules are shed with greater frequency than tap-root nodules, the distribution patterns may affect the stability of BNF under drought.

There were also significant differences in BNF among groundnut genotypes under dryland conditions. Genotypes that formed more nodules on the hypocotyl region suffered greater loss of specific nitrogenase activity during drought. More importantly, temporal differences in nodulation among varieties helped determine drought effects on BNF. Most of the groundnut crop in India is grown during the rainy season, and drought conditions at the end of the growing period commonly restrict the N-accumulation process. Genotypes that attained peak nodulation 50 to 60 days after planting accumulated more N and produced higher seed yields than genotypes that were initially slow to nodulate but continued to increase nodule biomass well into the maturity period. Early-nodulating genotypes remobilized and partitioned more N into the reproductive parts compared to late-nodulating genotypes. These results indicate that, for our conditions, N_2-fixation at a particular growth stage is more important than total fixation throughout the plant life cycle. These findings are now influencing our intra- and intervarietal selection programs.

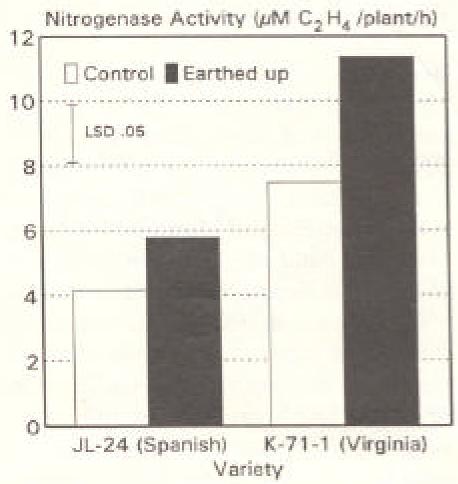


Figure 1. Influence of earthing-up on nitrogenase activity in two groundnut varieties 75 days after planting. The sample was taken after 7 rainless days when soil moisture had declined to below 50% of the available limit.

Agronomic practices such as mulching can help sustain BNF during soil dehydration. "Earthing-up" of groundnut crops is one effective practice. A plough is run between crop rows about 25 days after planting to form a 5- to 10-cm ridge covering the hypocotyl, collar, and basal portion of the stem. This simple practice maintains higher moisture around the roots and hypocotyl during dry periods and minimizes temperature effects on nodules.

Figure 1 shows the effect of earthing-up on nitrogenase activity of two varieties of groundnut following a seven-day dry period. Earthing-up resulted in higher activity in both genotypes, but the effect was more pronounced for K-71-1, a Virginia type that has a significant proportion of the nodules on the hypocotyl. Our results indicate that simple agronomic practices combined with exploiting specific nodulation traits of the host can help to stabilize BNF functions during water-deficit conditions.

The author has published a series of articles on this subject. Request references for this article from NifTAL, or contact Dr. Venkateswarlu at Central Research Institute for Dryland Agriculture, Santoshnagar, Hyderabad 500 659, India.

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