BIOLOGICAL NITROGEN FIXATION

BNF BULLETIN

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Removing the Guesswork:
Predicting Response of Legume Crops to Inoculation

The use of appropriate, high-quality rhizobial inoculants does not guarantee increases in legume crop yields. Are there methods to predict when, where, and to what extent crops will respond to inoculation?

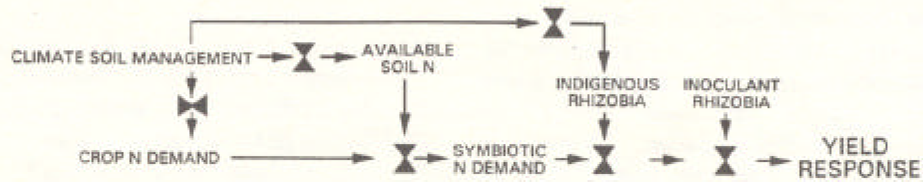

Figure 1. Conceptual model of response to inoculation

The exorbitant costs of petroleum-based nitrogen fertilizers during the oil crisis of the 1970s catalyzed a search for economical alternatives. Rhizobial inoculants for legumes were promoted as a panacea to meet demands for low input, high-protein food production in developing countries. Since then, inoculation technology has not lived up to those grand expectations. *Sometimes* inoculants cause huge increases in legume crop yields, and *often* they cause significant improvement in the quality of seed protein. "Sometimes" and "often" are not "always," and inoculation technology is now viewed with some skepticism. Were those grand expectations realistic?

To assess the effectiveness of inoculation technology in the tropics, NifTAL initiated the International Network of Legume Inoculation Trials (INLIT) in 1979. Over the next several years, collaborators conducted 228 standardized inoculation trials at two fertility levels on 19 legume species in more than 28 countries. In approximately 52% of the cases, inoculation resulted in significant yield increases. Obviously yield responses to inoculation were site specific, depending on location, species, fertility, or time. What are the underlying principles that determined these responses?

A conceptual model shown in Figure 1 identifies the major determinants of the response to inoculation. Legumes have two sources of nitrogen—mineral and biologically fixed. Legumes "prefer" mineral sources of nitrogen, and will assimilate fixed nitrogen only when mineral sources (*available soil N*) are insufficient to meet plant demand. The *crop demand for N* depends on the interaction between a complement of environmental and genetic factors that regulate plant growth. Thus *management* inputs that enhance plant growth also increase nitrogen demand, and consequently, the potential for positive inoculation response. For example, Figure 2 shows increased inoculation response with improved phosphorus fertility.

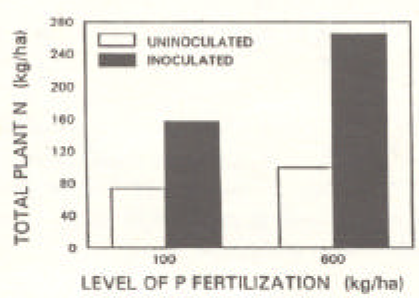

Figure 2. Total nitrogen accumulation of inoculated and uninoculated soybeans at two levels of phosphorus fertility. Management inputs that enhance plant growth potential also increase the response to inoculation.

The *demand for symbiotic N* can only be met if there are sufficient quality and quantity of rhizobia in the soil. If the *indigenous soil rhizobia* can meet the demand for fixed nitrogen, then introducing rhizobia through *inoculants* will not result in crop yield increases.

To quantify the complex relationship among the factors controlling the response to inoculation, NifTAL again used a network approach. With support from the National Science Foundation and USAID, NifTAL's Worldwide Rhizobial Ecology Network (WREN) evolved from the INLIT experience. The WREN approach combined standardized inoculation trials with extensive environmental data collection at diverse sites throughout the tropics and on NifTAL's home island of Maui. The underlying assumption of this research was that measurable ecological factors can be used to predict the effect of introduced rhizobia on legume crops at any given site.

As part of her doctoral research at NifTAL, **Janice Thies** conducted WREN standardized field trials at five ecologically distinct sites on Maui. The inoculation response of nine legumes in 29 legume X site combinations were measured. Crop management assured maximum potential yields at any given site. As expected, the response to inoculation varied by both site and legume species.

Thies developed mathematical models to describe these results. Most of the variation (59%) in observed inoculation response could be explained by the numbers of indigenous rhizobia estimated by Most Probable Number (MPN) assays of soils at each site. In general, crops did not respond to inoculation when there were more than 10 host-compatible indigenous rhizobia per gram of soil. When soil nitrogen data (as determined by laboratory mineralization tests) were incorporated into the models, the correlation between observed and estimated inoculation responses increased (r=0.83, Figure 3). This high correlation indicated that models using data from relatively simple soil tests (the MPN and nitrogen mineralization values) could be reliable predictors of the likelihood and magnitude of inoculation response at a given site.

These models have been incorporated into a first generation interactive computer program called *RESPONSE* that reduces the need for costly, site-specific field inoculation trials to estimate the effects of rhizobial inoculants in a particular area. Site soils throughout large regions can be sampled, and data from MPN and nitrogen-mineralization analyses can be used with *RESPONSE* to target areas where farmers are likely to benefit from inoculation. This predictive ability can help regional planners determine inoculation requirements of legumes introduced into new areas, and the requirements for inoculant production facilities.

Other researchers are continuing the WREN approach of using estimates of indigenous rhizobial populations as predictors of potential inoculation response. At the Fifth African Association for BNF Conference, **Nancy Karanja**, **Paul Woomer**, and **Sabastian Wangaruru** reported on the recent formation of the *Rhizobium* Ecology Network of East and Southern Africa (RENEASA), involving 11 institutes in eight countries.

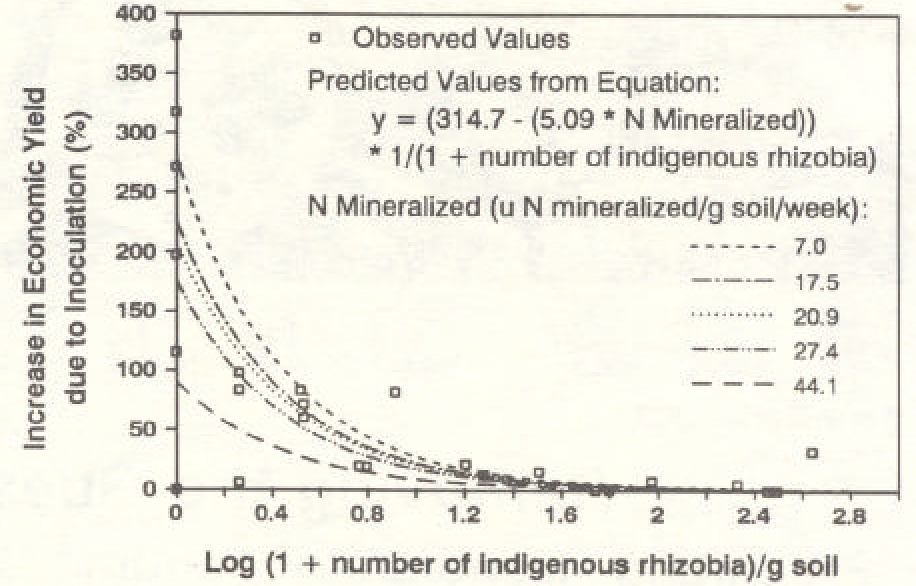

Figure 3. Comparison between observed inoculation responses and those estimated by a hyperbolic response model incorporating N mineralization values as an estimate of soil N availability

With support from USAID and the Thai government, Thai scientists **Nantakorn Boonkerd** and **Precha Wadisirisuk** of the BNF Resource Center and Department of Agriculture, and **Juckrit Homchan**, **Banyong Toomsan**, and **Ponsiri Patcharapreecha** of Khonkaen University are also using the WREN approach. These researchers are conducting extensive soil surveys and standardized inoculation trials in diverse regions of Thailand to develop response models that incorporate cropping system and management effects on rhizobial populations.

But wait a minute! Two soil assays and a mathematical equation—can it be that simple to predict the response to inoculation? So far, field data from international WREN collaborators generally fit the *RESPONSE* model, providing their MPN results meet standard criteria for reliability. But what about all of the other factors that can influence host growth and performance of the symbiosis?

The BNF BULLETIN invites your comments and questions. If you have an application for RESPONSE, we would like to hear from you. We hope to create a forum for some informative discussion.

References and Related Reading

Those marked with an asterisk can be requested directly from NifTAL.

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*Woomer, P. *et al.* 1988. Ecological indicators of native rhizobia in tropical soils. Appl Environ Microbiol 54(5):1112-16.

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Arachis pintoi The Tropical White Clover?

A little-known legume, *Arachis pintoi*, was introduced to CIAT in 1979 and is proving to be a most useful multi-purpose plant for tropical agroecosystems. *Arachis pintoi* was first collected near Belmonte, Brazil in 1954 by G.C. Pinto. It shares many characteristics with temperate white clover, being perennial with a stoloniferous growth habit. In tropical conditions, it flowers almost continuously, sets seeds throughout the wet season, and produces high seed yields.

Record animal live weight gains of more than 600 g/day have been measured in the savannas of Colombia when *A. pintoi* was grown in association with the grass *Brachiaria humidicola*. The legume has persisted under heavy grazing for more than six years. When grown on clay and sandy loam soils under high and low fertilizer treatments, the percent N derived from fixation remained high (81-89%).

This species has proved to be a successful ground cover for coffee plantations in Colombia. It outperforms other legumes by covering the soil rapidly and preventing soil erosion on steep hillsides. In Australia, it is used as a ground cover in orchards and plantations on the Queensland coast. The most successful inoculant strain to date is *Bradyrhizobium* CIAT 3101 (QA 1091), which was isolated from *Centrosema plumieri* in the north of Colombia. CIAT is currently studying the role of this legume in agropastoral systems in the acid-soil savannas of Latin America.

For more information, contact R.J. Thomas, CIAT, Apdo. Aereo 6713, Cali, Colombia, fax 57-23-647243.

Makerere University – Uganda

Charles Nkwiine, of Makerere University, reports that production and sales of legume inoculants are increasing significantly in Uganda. A total of 1477 250-g packets were sold in 1991-92 as compared to 340 in 1990-91. According to Nkwiine, "Increase in use of inoculants is a result of the rise in the awareness regarding the availability and use of inoculants for more economical farming."

IRRI-NifTAL Collaboration Moves to the Uplands

Following the successful completion of collaborative research on nitrogen cycling in the lowlands, IRRI and NifTAL are now initiating a new research project in the Philippine uplands. **Dr. Thomas George**, NifTAL scientist based at IRRI, is working with IRRI staff on this cooperative research effort on the phosphorous, nitrogen, and carbon dynamics in upland agroecosystems of Asia.

This research will address the influence of P management on the P cycle and, in turn, on the N and C cycles through effects on biological nitrogen fixation and soil organic matter. Diagnostic tools, such as the Phosphorous Decision Support System and phosphorous simulation models, will be tested and validated. The results will contribute to nutrient management recommendatations for the long-term sustainability of tropical upland soils.

Plant Molecular Genetics Knoxville, Tennessee

Dr. Peter M. Gresshoff reports that research at Plant Molecular Genetics, The University of Tennessee, Knoxville, continues to focus on the molecular analysis of nodulation-related genes, through positional cloning and DNA profiling using single primer amplification (DAF). The supernodulation (nts) gene has been tightly mapped to an RFLP marker on linkage group H; soybean YAC clones have been developed. Parallel attention is given to telomerea, non-nodulation loci as well as the loci controlling plant-mediated host range control in soybean.

NOTE TO OUR READERS

This bulletin belongs to everyone interested in BNF. Please send newsworthy items for future issues—human interest stories and photos, editorials and opinions, and notices about books and events. Extensive research data will not be published but short technical summaries are welcome.

NEW PUBLICATIONS

Symbiosis of Plants and Microbes, written by D. Werner, Department of Biology, Phillips University, Lahnberge, Germany. This new publication describes a variety of symbiotic relationships, their cell and molecular biology, and their agricultural and environmental applications. Of interest to the readers of the *BNF BULLETIN* will be the sections on the *Rhizobium/Bradyrhizobium*-Fabales and the *Bradyrhizobium-Parasponia* symbiotic associations. This book costs 60 pounds plus shipping charges, and discounts are available for orders of 10 copies or more. For more information, write Chapman & Hall Ltd., Cheritron House, North Way, Andover Hants, SP10 5BE, England, phone 071 522 9966.

Symbiosis in Nitrogen-fixing Trees, edited by N.S. Subba Rao and I.C. Rodriguez-Barrueco. The contents include sections on nitrogen fixation by legume trees, soil constraints, aerial and root nodules, mycorrhizal associations and functions in NFTs, utilization of VAM in establishment of NFTs, and contributions of molecular biology techniques and interactions of nodulated tree species with other microorganisms and plants. The cost is $74 US. For information on ordering, contact International Science Publisher, 2840 Broadway, New York, NY 10025, USA.

Other recent publications of interest:

Legumes in Farming Systems, A Joint ICARDA/IFPRI Report by Peter Oram and Abderrezak Belaid. For information, write to ICARDA, P.O. Box 5466, Aleppo, Syria.

Peanut Improvement: A Case Study in Indonesia, ACIAR Proceedings No. 40 of an ACIAR/AARD/QDPI collaborative review meeting; C.G. Wright and K.J. Middleton editors. For inquiries, please write to ACIAR, G.P.O. 1571, Canberra, A.C.T. 2601, Australia.

Legume Green Manures, Dry-season Survival and the Effect on Succeeding Maize Crops, Soil Management CRSP Bulletin Number 92-04. Available from TropSoils CRSP, Box 7113, North Carolina State University, Raleigh, NC 27695-7113, USA.

Breeding for Stress Tolerance in Cool-Season Food Legumes, edited by K.B. Singh & M.C. Saxena. Order from Sayce Publishing, 57 Marlborough Road, St Leonards, Exeter, EX2 4LN, England.

Nodulation-Promoting Rhizobacteria

By Russell K. Hynes

Reports of significant promotion of plant growth following seed inoculation with rhizobacteria has increased interest in these microorganisms. Plant-growth-promoting rhizobacteria (PGPR) are a specialized group of rhizobacteria, isolated from the rhizosphere, that colonize and persist in and on the roots of plants. They have been reported to improve growth of cereals, oil seed crops, vegetables, and annual and perennial legumes (Kloepper *et al.* 1988). The term "nodulation-promoting rhizobacteria" (NPR) was suggested following studies showing an increase in nodule number and mass for soybeans co-inoculated with PGPR and bradyrhizobia.

Further effects of NPR-rhizobia co-inoculation include improved root and shoot weight, plant vigor, N_2 fixation (C_2H_2 reduction), and yield. These have been observed on legumes such as bean (Grimes and Mount 1983), soybean (Halverson and Handelsman 1991, Singh and Subba Rao 1979, Polonenko *et al.* 1987, Li and Alexander 1988), alfalfa, clover (Handelsman *et al.* 1987), lentil, and pea (Chanway *et al.* 1990).

Growth promotion of legumes following colonization of the rhizosphere by NPR has been attributed to both direct and indirect mechanisms. Direct effects on plant growth result from NPR-mediated production of phytohormones or compounds such as siderophores that facilitate uptake of nutrients. The mechanisms by which the NPR maintain a competitive advantage for survival in the rhizosphere may indirectly promote plant growth. For example, a reduction in the population of deleterious rhizosphere microorganisms, particularly the fungal plant pathogens, may result from the excretion of antibiotics by NPR (Weller 1988). The end result is that NPR provides a root environment that is more conducive to the symbiotic association of rhizobia and its host plant.

The nature of the interactions between plant tissues and the NPR is not well understood. Increased root and shoot growth may be linked to the production of phytohormones by NPR that have been detected *in vitro*; their production in the rhizosphere has not been examined.

The involvement of NPR with the plant defense system is being investigated. Pathogenic microorganisms elicit a hypersensitive reaction (HR) in incompatable tissue and induce the accumulation of fungitoxic compounds called phytoalexins (Dixon 1986, Templeton and Lamb 1988). Symbiotic *Rhizobium* spp. have been reported to induce low levels of phytoalexin accumulation in root nodules of soybeans and faba beans (Wolff and Werner 1990). Recent evidence indicates that rhizosphere bacteria, or their products, have the capacity to activate plant defense genes.

Treatment with NPR induced production of HR and phytoalexin in white bean cotyledons and hypocotyls. The population size of NPR required to induce phytoalexin production was similar to that detected on white bean in natural systems. This ability of rhizobacteria to colonize the cotyledon in sufficient numbers to induce phytoalexin production has potential implications for disease control involving plant defense mechanisms.

Rhizobacteria are no longer regarded as passive bystanders in the rhizosphere, but rather as an integral component of this complex ecosystem. The ability of these microorganisms to interact with the legume plant and rhizobia, enhance nodulation, and improve yield has beneficial implications for sustainable agriculture.

Several challenges remain. Consistent yield increases must be obtained under a variety of environmental conditions, and the activity and viability of the microorganisms must be maintained in suitable carrier materials or seed coatings.

Complete references are listed on page 8.
For more information, contact Russell Hynes, Esso Ag Biochemicals, 402-15 Innovation Blvd., Saskatoon, SK, Canada S7N 2X8, phone 306 975 3840, fax 306 975 37550.

The Search for Inoculant Carriers in Egypt

"Egypt saves an estimated $80,000,000 per year by using rhizobial inoculants," claims **Dr. Samir Abdel-Wahab**, Head of Research, Soils and Water Research Institute (SWRI) in Giza. Approximately 95% of all soybeans planted in Egypt are inoculated. Each year, SWRI Biofertilizer Production Unit produces 300,000 units of 200-g bags of rhizobial inoculants called *OKADIN*, Arabic for "nodule," but production is dependent on the availability of peat carriers imported from U.S. and Canadian sources. To increase the potential for inoculant production, researchers in Egypt are continuing a search for suitable alternative carrier materials. During his recent visit to NifTAL, Dr. Abdel-Wahab described the carriers used in the past.

Inoculant production in Egypt started in 1950, using a carrier comprised of Nile silt, gelatin, charcoal, sugar, and phosphate. It supported approximately 10^6 rhizobia per gram, but was easily saturated, resulting in anaerobic conditions. This mixture was used until 1978.


Plastic-bag method of inoculation

From 1979 to 1982, "Jiffy 7" expandable peat-pot disks were inoculated with 50 ml broth culture and distributed in sterilized plastic cans. This novel method supported high numbers of rhizobia (10^9), but autoclaving was expensive and often did not prevent contamination, affected the water-holding capacity, and produced toxins.

Since 1982, imported fine-ground peat has been used. Peat is recognized as a superior carrier, but importation costs constrain inoculant production. Since Egypt has no natural peat deposits, a variety of alternatives have been tested, including bagasse and berseem straw. Thus far, "stretching" the imported peat by adding equivalent weights of indigenous vermiculitic clay produces the most suitable carrier.

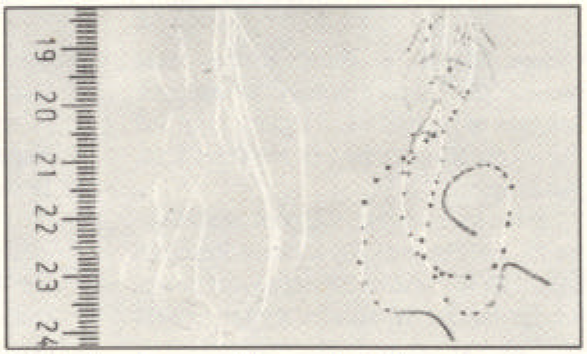
"Finding an alternative inoculant carrier is a priority for BNF research in Egypt," says Dr. Abdel-Wahab. He is intrigued by the potential for using liquid inoculants, but extensive testing is necessary to determine if the liquids are suitable for the often harsh environmental conditions in Egypt.

For more information, contact Dr. Abdel-Wahab at the Soils and Water Research Institute, Department of Soil Microbiology, Agricultural Research Center, 9 Gamma St., Giza, Egypt.

Nitrogen-Fixing Nodules on Cereals Near?

By Ivan R. Kennedy

The possibility that cereals such as wheat and rice might also be successfully "nodulated" for achieving nitrogen fixation has been considered merely a dream. Optimism has been renewed recently, due to several new experimental approaches, each of which has had limited success in laboratory trials. However, none of these approaches suggests that we will soon be inoculating wheat or rice with peat cultures on farms.



2,4-D induced *para*-nodules on wheat. The root system on the right side was treated with 0.5 ppm 2,4-D and inoculated with *Azospirillum brasilense* Sp. 7. The nodular structures have been stained with iodonitrotetrazolium salt.

As early as the 1940s and 1950s, pseudo-nodules were observed on legumes exposed to certain herbicides with auxin activity such as 2,4-D. In the early 1980s, Nie Yanfu at Shandong University in China imaginatively applied this observation to inducing nodular structures on the roots of wheat, inoculating at the same time with cultures of rhizobia or other diazotrophs that would sometimes colonize these structures. Attempts to assay nitrogen-fixing activity as acetylene reduction in these chemically induced structures (termed *para*-nodules) did not yield impressive results (Tchan and Kennedy 1989; Nie *et al.* 1992). In these nodular structures, recognized to be modified lateral roots, the bacteria usually grow between the plant cells. Where they occur intracelluarly as in legume nodules, the plant cells lack recognizable organelles such as nuclei or mitochondria and are probably dead.

In other work, such as that in Cocking's laboratory using cellulolytic pectolytic enzymes and polyethylene glycol (Al-Mallah *et al.* 1989, 1990) or genetically modified diazotrophs, similar nodular structures containing diazotrophs have been observed (reviewed in Kennedy and Tchan 1992; also see the proceedings of the 9th International Congress on Nitrogen Fixation in Mexico). None of these approaches has yet led to measurement of significant rates of nitrogen fixation using the acetylene reduction assay.

At the University of Sydney, the approach using 2,4-D has been refined, using *Azospirillum brasilense* as the inoculant (Tchan *et al.* 1992). In this system, significant rates of nitrogen fixation can be achieved after a period of induction (Sriskandarajah *et al.* 1993) but only by reducing oxygen levels to below 0.08 atmospheres. Maximum expression of nitrogenase requires an oxygen pressure of 0.01-0.02 atmospheres. These results have been confirmed in another laboratory (Christiansen-Weniger 1992) using an ammonium-excreting strain of *Azospirillum*. The ethylene formation has been verified as nitrogenase activity by its sensitivity to ammonium and nitrite ions and the use of $^{15}N_2$ (Yu *et al.* 1992).

Do these findings suggest that nodulated N_2-fixing cereals are near? Obviously, this is far from the case. Many problems remain including the oxygen sensitivity of the nitrogenase of diazotrophs, how to actually achieve any of these "nodulations" in the field, and the lack of a clear demonstration of any benefit of these nodular structures. It would be a mistake to consider that these artificial structures are in any way similar to legume nodules or that they will be developed to a point where they are beneficial to cereals. However, we consider that the results so far are promising and the prospects of eventual success are enhanced by the vast knowledge that exists about the factors controlling effective N_2 fixation with legume nodules.

For further information, contact Dr. Ivan R. Kennedy, The University of Sydney, Ross St. Building A03, Sydney, NSW 2006, Australia, phone 61 2 692 2439, fax 61 2 692 2945.

References

Al-Mallah, M.K., M.R. Davey and E.C. Cocking. 1989. A new approach to the nodulation of non-legumes by rhizobia and the transformation of cereals by agrobacteria using enzymatic treatment of root hairs. Int J Gen Manip Plants 5:1–7.

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Continued on page 8

An Effective Associative Nitrogen Fixation System: *Alcaligenes faecalis* and Wetland Rice

By Chongbiao You

The nitrogen-fixing diazotroph *Alcaligenes faecalis* was isolated from rice roots in 1977. There has been significant research since then to characterize and improve this associative nitrogen-fixing system to benefit China's rice production.

1. Researchers found *A. faecalis* synthesized nitrogenase even in high ammonium culture medium (30 mM), and the two component proteins were isolated and purified to homogeneity. Nitrogenase activity was absent, but when the ammonium in the culture medium was exhausted, nitrogenase activity was derepressed.

2. Non-nodular endorhizospheric nitrogen fixation was found. *Alcaligenes faecalis* not only accumulated on the rice root surface, but also entered the root cells. By using a variety of methods including immunofluorescence and scanning electron microscopy, *A. faecalis* was observed growing within rice root cells and living symbiotically with callus induced from the cells (Can J Microbiol, 1989, 35:403-408). *Alcaligenes faecalis* also grew and fixed nitrogen in inter and intracellular spaces in the rice plant. Recently, *A. faecalis* was incorporated into rice protoplasts. The bacteria were found to multiply in the leaves and roots of plants regenerated from the protoplasts, and ^{15}N analysis showed the bacteria were fixing nitrogen in these regenerated plants.

3. Transconjugants containing multiple copies of *nif*A or *ntr*C-*nif*A genes were constructed. These strains carried pCK3 and pCK5 plasmids containing *nif*A fragments, and pCA1841 containing *ntr*C-*nif*A genes. With aid of transposon Tn5, the *nif*A (or *ntr*C-*nif*A) was randomly inserted and integrated in the chromosomal genome of *A. faecalis*. These mutants can fix nitrogen in high-ammonium media and increase rice yields by 6% to 9%. The transconjugants were released for field use in China in 1989. During the past two years, more than 200,000 ha of wetland rice were inoculated with the transconjugants.

For more information, contact Dr. Chongbiao You, Professor, Laboratory of Nitrogen Fixation, Department of Biotechnology, IAAE, Chinese Academy of Agricultural Sciences, P.O. Box 5109, Beijing 100094, China.

READERS' CHOICE

Legume N_2 Fixation: An Efficient Source of Nitrogen for Cereal Production in Rain-Fed Systems of South Asia and Australia

By David F. Herridge

After water, nitrogen is the most important factor limiting yields of cereals. With wheat, the most commonly grown cereal in rain-fed agriculture, the soil must supply 30–40 kg N/ha in a plant-available form (usually as nitrate) for each ton of grain produced. In some of the developed countries and energy-rich developing countries, N is supplied in sufficient amounts as fertilizers. In the majority of countries, however, this is not possible principally because of the high cost.

One means of improving the supply of N to cereals is to use annual pulse and oilseed legumes as rotation crops. Research has shown that the soil following an annual legume crop often contains 30–60 kg N/ha more then after a cereal crop. This increased plant-available N is converted into increased cereal grain yield. The N benefits are due to the capacity of legumes to fix atmospheric N_2. Amounts fixed by annual crop legumes range from zero to more than 400 kg/ha. There is, however, scope to manage legume N_2 fixation for maximum benefit, through practices that optimize crop growth and minimize the suppressive effects of soil nitrate. One possible aid to management may come through the development and use of simple mathematical models that allow farmers to estimate amounts of N_2 fixed by a legume crop and the potential net N benefit resulting from that crop.

From its inception, the Australian Centre for International Agricultural Research (ACIAR) recognized the very great potential for legume N_2 fixation in agricultural systems and has maintained an unbroken portfolio of research projects dealing with various aspects of the process.

The collaboration began in 1992 with scientists from four Pakistani institutes—National Agricultural Research Centre, Islamabad; Barani Agricultural College, Rawalpindi; NWFP University, Peshawar; and Agricultural Research Institute, Tarnab. Nepal was represented by the National Agricultural Research Institute, Kathmandu. Australian scientists came from New South Wales Agriculture, Tamworth and CSIRO, Canberra.

The goal is to gain a better understanding of the factors that determine how much N is fixed by legumes in farmers' fields and how the fixed N can be exploited for improved production of subsequent cereal crops. Scientists agreed that the objectives of the joint project should be to:

1. Quantify, for the first time, amounts of N fixed by legumes in Pakistan and Nepal through surveys of farmers' crops.
2. Develop farming practices that maximize the inputs of N_2 fixed by the legumes.
3. Develop legume-cereal cropping sequences that maximize production of the cereals in the sequences as well as economic productivity.
4. Assess effects of cropping sequences on the organic fertility and long-term sustainability of the soil.
5. Synthesize the data on soil water, nitrate, yields of both legumes and cereals, and legume N_2 fixation to develop quantitative relationships between these factors for use as management tools.

The project is due to start in January 1994 following a six-month establishment period. In all, 12 scientists from the three countries will be involved. Discussions have commenced to link the project with similar programs at ICARDA and ICRISAT. For further information, contact Dr. D.F. Herridge, NSW Agriculture, Agric. Research Centre, RMB 944, Tamworth, NSW 2340, Australia.

MEETINGS

Rosewood (*Dalbergia* spp.) Multipurpose & High Value Timber Nitrogen Fixing Tree international workshop will be held at Tribhuvan University in Hetauda, Nepal, 31 May–4 June 1993. Information on all *Dalbergia* species is desirable. For more information, contact James Roshetko, NFTA, 1010 Holomua Road, Paia, Hawaii 96779, USA or Tom Hammett, Institute of Forestry Project, Tribhuvan University, P.O. Box 43, Pokhara, Nepal.

10th Australian Nitrogen Fixation Conference is planned for Brisbane, Australia, 7-10 September 1993. For additional information, contact H.V.A. Bushby, 306 Carmody Road, St. Lucia, Qld 4067, Australia, phone (07) 377-0209, fax (07) 371-3946.

Plant Genome Analysis Symposium takes place at the Center for Legume Research in Knoxville, Tennessee, USA, 9–12 June 1993. Contact Dr. Peter Gresshoff, Plant Molecular Genetics, 267 Ellington Plant Sci. Bldg., University of Tennessee, Knoxville, TN 37901-1071, USA, fax 615-974-2765. Plans are now being made for the 1994 symposium which will probably focus on plant cell signal transduction.

Conference on Sustainable Ecological Systems will be held in Flagstaff, Arizona, USA, 12–15 July 1993. Contact c/o duBois Conference Center, P.O. Box 15003, Flagstaff, AZ 86011-5003, USA, phone 602-404-0234.

Fourteenth North American Symbiotic Nitrogen Fixation Conference is coming up this summer in Minneapolis, Minnesota, USA, 25–30 July 1993. Contact Nancy Harvey, Program Coordinator, Educational Development System, 405 Coffey Hall, 1420 Eckles Ave., University of Minnesota, St. Paul, MN 55108-6068, USA, phone 612-625-8215, 800-367-5363 (USA), fax 612-625-2207, e-mail address INTERNET :nh@espmes. umn.edu.

Tenth International Symposium on Nitrogen Fixation with Non-Legumes will be held in Egypt, 6–10 September 1993. Contact Dr. Nabil Hegazi, Faculty of Agriculture, 12613 Cairo University, Giza, Egypt, phone 02-724-368, fax 02-349-761

International Symposium on Pulses Research is being organized for 4–8 December 1993 in Kanpur, India. Contact Dr. A.N. Asthana, ISPRD, Directorate of Pulses Research, Kanpur 208 024, India.

Sixth Conference African Association for Biological Nitrogen Fixation (AABNF) will take place at the University of Zimbabwe, Harare, Zimbabwe, 12–17 September 1994. Contact the Secretary, AABNF, Department of Soil Science, University of Zimbabwe, Box MP167, Mount Pleasant, Harare, Zimbabwe, phone 263-4-303211 ext 1412, fax 263-4-732828.

COMMERCIAL CORNER

New US Enterprise Markets NifTAL-Developed Fermentors

NifTAL-Developed Fermentors are being marketed by Hawaii-based Innovative Technology Associates, through its newly formed subsidiary, BNF (Biotechnologies for New Frontiers) Industries, Inc.

NifTAL developed the system for the mass culture of microorganisms through the USAID sponsored "Improved BNF Through Biotechnology" project. The system is economical and easy to operate — features that make the fermentors attractive to legume inoculant producers.

NifTAL transferred the systems to Innovative Technology Associates President, Mr. Thomas Reed, in September 1992. BNF Industries recently filled its first orders for five 50- and 100-liter systems in India and Myanmar. For more information, contact Thomas Reed at BNF Industries, Maui Research and Development Park, 590 Lipoa Parkway, Kihei, Hawaii 96753, USA, phone 808-875-2520, fax 808-875-2330; or contact the Private Enterprise Development Office at NifTAL.

ITA president Tom Reed and the 50-L fermentor

Micro-Production Unit

Recently, NifTAL's research activities have focused on appropriate technology for small-scale production of rhizobial inoculants. The **Micro Production Unit** approach to inoculant production relies on two key concepts 1) the use of sterile peat inoculant carrier and 2) the dilution of broth cultures of rhizobia. By integrating these concepts, decentralized small-scale commercial inoculant production is possible.

Advantages of producing rhizobial inoculants with these techniques include

* Significantly lower initial investment costs
* Simplified design
* Smaller fermentor vessels
* Flexibility in production scheduling
* Reduced time for filling orders
* High-quality product with increased inoculant shelf life
* Reduction of storage requirements
* Easier quality control procedures

For more information, contact the Private Enterprise Development Office at NifTAL.

FOR YOUR INFORMATION

Soil Biology Bibliographic Resource

EMBRAPA-CNPBS in Brazil, maintains a comprehensive database of about 20,000 references in the area of soil biology with the strongest emphasis in tropical BNF. Although keywords are in Portuguese, they are taken from a fixed list of legal keywords that are easily translatable; there are 350 keywords not counting scientific names. The database is on Micro-ISIS of UNESCO, occupies about 15 megabytes, and is in DOS for PC compatibility. For more details, contact the database specialist at EMBRAPA-CNPBS, Km 47, Seropedica, 23851-970, Itaguai, Rio de Janeiro, Brazil, phone 021 682-1500, fax 021 682-1230.

NifTAL 1993-1994 Course & Training Schedule

Note: This is a tentative schedule. Program dates and locations are subject to change. For more information, contact the NifTAL Training Coodinator.

* ***Legume Inoculant Production as a Business Enterprise*** — Location and 1993 dates to be determined
* ***Modern Methods for the Study of Rhizobia*** — 10–28 January 1994; Rabat, Morocco
* ***Methods in Legume BNF Technology*** — 4–22 April 1994; Aleppo, Syria
* ***Legume BNF Technology for Sustainable Agriculture*** — 1–26 August 1994; Chapingo, Mexico
* ***Legume BNF Technology for Extension Specialists*** — 7–18 November 1994; Nairobi, Kenya

Center for Legume Research

The Center for Legume Research, University of Tennessee-Knoxville, is dedicated to research, teaching, and technology transfer with regard to all leguminous crops. For information, contact Dr. Gary Stacey, Director, Center for Legume Research, M409 Walters Life Science Building, The University of Tennessee, Knoxville, TN 37995-0845, USA, phone 615-974-4041, fax 615-974-4007, e-mail GSTACEY@UTKVX.UTK.edu.

B. Ben Bohlool Memorial Fund Provides Books for Students

The B. Ben Bohlool Memorial Fund was established through contributions made by Ben's friends and colleagues to support students interested in BNF. This fund has been entrusted to NifTAL by his family.

A portion of the fund will provide the book *Biological Nitrogen Fixation for Sustainable Agriculture* by J. Ladha, T. George, and B. Bohlool (editors) on request to students from developing countries. To request a copy of this book, students should write to NifTAL Director indicating their interest in BNF.

Germplasm Catalogue and Directory of Inoculant Producers

Copies of the following may be requested from the NifTAL Communications Department:

Germplasm Catalogue: Current strain recommendations for a variety of tropical grain, forage, and tree legumes selected from NifTAL's MIRCEN collection of approximately 2000 strains

Directory of Inoculant Producers: Names and addresses of inoculant producers in 40 countries

N-FIXING TREE NOTES

Conference Produces Field Manual on Production and Use of *Erythrina*

The Nitrogen Fixing Tree Association (**NFTA**) and the Tropical Agronomical Center for Research and Training (**CATIE**) co-hosted an international conference on the genus *Erythrina* 19–23 October 1992 in Turrialba, Costa Rica. The conference marked the tenth year of continuous measurements in an experiment at CATIE to determine the value of *Erythrina* for alley farming. The conference was an opportunity to recognize **Dr. Gerardo Budowski** for his pioneering work with *Erythrina* and his contributions to agroforestry.

Scientists from the Americas, Africa, Asia, and Oceania shared their research and experiences with *Erythrina* species.

During the conference, working groups shared experiences and techniques that will be incorporated into a field manual on *Erythrina* production and use. Topics include *Erythrina* botany and ecology, establishment, fodder production, use as shade for perennial crops, use in annual cropping systems, other uses, pests, and diseases. Field manuals are currently available for the production and use of *Sesbania* and *Gliricidia*.

The conference proceedings and the field manual will be available from NFTA in mid-1993. For more information contact Mark Powell, NFTA, 1010 Holomua Road, Paia, HI 96779-9744, USA.

WHO'S WHO

The Nitrogen Fixing Tree Association (NFTA) is pleased to announce that **Sidney B. Westley** has recently joined them as the new Director of Communications for NFTA and NifTAL. She has just moved to Maui from Nairobi, Kenya where she was the Program Coordinator for Communications at ICRAF.

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SERVICES FROM NifTAL

RESEARCH

Rhizobial germplasm
Research quality inoculants
Custom antisera
Detailed field trial design for inoculation response studies
Methods for rapid, low-cost screening of rhizobia for soil stress tolerance

TRAINING

Basic six-week courses
Specialized extension and inoculant-production courses
Graduate degree support
Short courses on genetic technologies, serology, and commercial inoculant production
Training materials (manuals, slides sets)
Visiting scientists program

OUTREACH

Research and information networking
National research program design assistance
Technical backstopping to support developing-country entrepreneurs
Documents and information on BNF and tropical legumes
Technical assistance on inoculant-production systems
Advisory services on inoculant manufacture, distribution, and quality control

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For information on the NifTAL Center and to request services, contact NifTAL Center Director, NifTAL Center, 1000 Holomua Road, Paia, Hawaii 96779-9744, USA. Submissions to the *BNF BULLETIN* may be sent to the NifTAL Communications Section.

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