BIOLOGICAL NITROGEN FIXATION

BNF BULLETIN

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Ben Bohlool, In Memoriam

The BNF community throughout the world was shocked and saddened by the recent death of Dr. B. Ben Bohlool, Director of the University of Hawaii's NifTAL Project. Dr. Bohlool died the morning of Thursday, May 16, 1991, as the result of a single car automobile accident that occurred on his way to work. Funeral services were held on the grounds of the NifTAL Project site in Paia, Hawaii, on May 21.

Dr. Bohlool was born in Tehran, Iran, in 1940. He earned a Bachelor's degree in zoology and chemistry from the University of Wisconsin, and a Master's degree from the University of Minnesota in St. Paul. In 1971, he graduated with a PhD in microbial ecology from the University of Minnesota at Minneapolis under Dr. E. L. Schmidt. His doctoral work focused on the use of immunofluorescence in the study of the ecology of *Rhizobium japonicum* in soils. Further post-doctoral research with Dr. Schmidt resulted in their formulation of the lectin hypothesis. This pioneering work suggested that lectins were a possible basis for specificity in the legume-rhizobia symbiosis.

Ben also conducted post-doctoral research at the University of Wisconsin under Dr. T. D. Brock studying the ecology of microorganisms inhabiting thermal and acidic conditions. In 1974, he was appointed Research Scientist at the Cawthron Institute in New Zealand to investigate the microbial aspects of water pollution and eutrophication. While in New Zealand he patented a process for making protein-binding microspheres.

In 1976, Ben joined the faculty of the University of Hawaii as an Assistant Professor of Microbiology and advisor to the NifTAL Project. He was promoted to Associate Professor of Microbiology in 1980 and to Professor in 1984. His research at the University of Hawaii encompassed several major areas including the ecology of rhizobia in tropical soils, assessing the potential of fast-growing soybean rhizobia from China, and studying the host regulation of the legume-rhizobia symbiosis. During his tenure at the University of Hawaii, Ben was the recipient of the Alexander von Humboldt Fellowship, and spent six months with the molecular genetics group at Max-Planck Institute in Cologne, Germany.

In 1985, Ben was appointed Director and Principal Investigator of the NifTAL Project. As NifTAL's Director, Ben led the Project in fostering international outreach and training activities to promote the use of biological nitrogen fixation in agricultural development. He was nominated for the World Food Prize for these efforts. Ben also maintained his interest in research and supported an international network of researchers in rhizobial ecology to study environmental effects on the occurrence, persistence, and performance of rhizobia in tropical soils.

Ben's research in microbial ecology was diverse, ranging from purely fundamental to applied. He published over 60 scientific papers and several book chapters in the area of soil and plant microbiology, biological nitrogen fixation, immunological techniques, thermophilic microbes, aquatic microbes, and pollution microbiology.

Ben is remembered by his friends and colleagues as an excellent and creative scientist. He was blessed with an insatiable curiosity, social gregariousness, and the ability to savor all aspects of life. Besides his own scientific contributions, Ben's legacy includes the continuing work of his former graduate students. All of us who were fortunate to know and work with Ben have been indelibly influenced by both his intellect and his passion for life.

Ben is survived by his wife, Carol, and their fourteen-year-old daughter, Kiaora, who will continue to reside on Maui. It is the wish of the family that memorial donations be applied toward a college fund for Kiaora. Donations made out to the "Kiaora Bohlool Scholarship Fund" may be sent to the office of the Maui Economic Development Board, 300 Ohukai Road, Kihei, HI 96753.

At the memorial service, Kiaora Bohlool shared the following poem which she found in her father's briefcase on the day of his death.

Though nothing can bring back
The hour of splendor in the grass
And the glory of the flower,
Still we shall grieve not;
Rather find strength in things
we leave behind.

Remembrances of Ben by Colleagues and Friends

I said goodbye to Ben on Tuesday, May 21, 1991. The flood of memories and remembrances of 26 years of companionship with Ben started welling a few days before with the tragic news, and will continue long after that goodbye.

I remember Ben as a young graduate student applicant interviewing in my office in the fall of 1965. It was my good fortune, the good fortune of microbial ecology, and the good fortune of hundreds whose lives have been touched indelibly by Ben in the pursuit of his profession, for that interview launched his career.

I remember Ben the scientist. The agile mind, the innovative insights, and the nimble hands: the consummate experimantalist. Ben was as relentless in detecting a flaw in his own experimental design as in the data of anyone else. The bright new thoughts or the incisive experiments that Ben laid out in the meeting halls, in the cocktail lounges, and in the publications will be sorely missed where microbiologists gather.

I remember Ben the person. Clearly charismatic, Ben related easily and meaningfully to everyone irrespective of age, background, or position. Despite his talents, Ben was completely unpretentious. Anyone, until circumstances or events proved otherwise, was to Ben a fellow being and a gateway to savor yet another aspect of living. I never knew Ben to be other than patient, humble, and considerate in all of the non-science aspects of his life. His own brightness made life brighter for so many.

I remember Ben the companion - the collaborator in science and the cherished friend in life. I remember conflicting views vigorously argued, and I remember euphoric moments of research success vigorously shared and vigorously celebrated. I remember the many things we did together in many settings with remarkable frequency considering the miles that consistently seperated us. But mostly, I remember a son that I was privileged to know and love for those 26 years.

***Dr. Edwin L. Schmidt** is Emeratus Professor of Microbiology at the University of Minnesota. Dr. Bohlool received his PhD with him, and they continued a long and productive relationship.*

When all is said and done, and I wipe away the last tear and get on with my life, all I know is that I will miss Ben dearly. Ben was my mentor, my teacher, my friend. He taught me to respect and, at the same time, to enjoy science. He taught me a way to ask questions and a way to find the answers. But most of all, he taught me how to put my science and my life in perspective. His means of interacting with students and friends is a model to which I strive for even today. He treated his graduate students as friends and colleagues, not as underlings, with the result being that we loved and learned from each other. I try to do this with my own students now. Ben also taught me the way to love life, which we all know, he did to the fullest. A day would not go by when we did not laugh together about some humorous situation. Ben was also serious when necessary, and I have fond memories of our lunchtime discussions concerning the ailings of the world. While in my heart I know there will never be another B. Ben Bohlool, I will do my best to keep his memory and teachings alive in myself and others. In that way, maybe I can give to my students and friends what he gave to me, love and friendship.

***Dr. Michael Sadowsky** is an Assistant Professor in the Departments of Soil Science and Microbiology and in the Microbial Ecology Program at the University of Minnesota. He was a PhD graduate student of Dr. Bohlool's at the University of Hawaii.*

To all of us who knew Ben, it was clear what an excellent and creative scientist he was. He was very stimulating to work with and always full of enthusiasm. In addition to all his intellect and accomplishments, what I enjoyed so much about Ben was his zest for life and full enjoyment of all it has to offer. I don't think he was cheated out of a day while he was here.

I knew Ben for some 13 years, beginning with all-night poker games at scientific meetings, through research collaboration, and finally working with him at NifTAL. He was just plain fun to be with, especially on road trips. I have fond memories of laughing with him through many countries and in many situations. It was a challenge to keep up with him. For instance at scientific meetings it sometimes took two or three of us to coordinate it - deciding, "OK, who's gonna stay up with Ben tonight, and who's gonna get some sleep?" Then, the next day at a scientific session his thinking and comments were sharp as ever, while we were half baked.

Ben was also a risk taker and a gambler. He had integrity, and always honored his bets and his losses. He enjoyed the political aspects of his job, and relished victories there as well. Recently, when he explained to me a rather bold move he had taken in this regard, my more conservative nature lead me to gasp and say "Ben, not everything is a poker game!" He leaned across his desk and with a big smile said, "You wanna bet?"

Ben was a breath of fresh air. To many scientists around the world he leaves some impressive contributions. To those of us lucky enough to have know him, he also leaves his thorough enjoyment of life, which can live on through us. His being so alive while here with us makes it even harder to grasp his absence. The love he shared with his family was full and wonderful. To Carol and Kiaora, and all of Ben's family, we offer our love.

*Remarks made by **Dr. Harold H. Keyser** at Dr. Bohlool's funeral. Dr. Keyser is a Plant/Soil Microbiologist on the scientific staff of NifTAL Project and is currently serving as Research Coordinator.*

If you are thinking a year ahead, sow seed.
If you are thinking ten years ahead, plant a tree.
If you are thinking one hundred years ahead, educate the people.
By sowing seed, you will harvest once.
By planting a tree, you will harvest tenfold.
By educating the people, you will harvest one hundredfold.

Anonymous Chinese poet, 400 B.C.

POINT OF VIEW

Dr. Paul Stangel, President and Chief Executive Officer of the International Fertilizer Development Center (IFDC) visited NifTAL Project in June. He shared a distinctive viewpoint of the world agricultural situation from the perspective of fertilizer use and the availability of agrominerals.

The "green revolution" of the sixties and seventies provided technologies to increase food production throughout the world. Many regions now have a food surplus. In the mid to late nineties food shortages are expected to reoccur with varying degrees of severity in different parts of the world. A 3% annual increase in food production is necessary if food supply is to meet demands of growing populations in developing countries. The rate of increase in food production lags behind the population increase in Africa. The most critical situation is in Sub-Saharan Africa where the population is growing at 3-4% per year (expected to double in 17-23 years) with a current annual food production increase of slightly over 1%.

There are several situations that have contributed to problems in meeting the world need for food. The focus has been on using greater amounts of fertilizer with a narrow focus on cereal crops, mainly rice and wheat. In addition, land use practices and population pressures led to degradation of soil resources.

Dr. Stangel noted that many countries in the past failed to maintain their capital stock in face of operating budget constraints and devoted too little to research and development for the future. Also, during this time agriculture was tied to national trade policies with a high degree of state-owned business, price controls, and subsidies. Future restructuring of agriculture industries will require further reforms. Economic support is dwindling, and funding sources are not expected to supply additional resources for research without a guarantee that technology will be transferred to the farmers.

As possible solutions, Dr. Stangel iscussed several interesting ideas. He sees the need for a shift from the production of cereal crops to diversified agricultural crops such as fruits, vegetables, high-protein crops, cooking oils, and ground nuts. With these commodities, market prices are generally higher, generating more income and a better standard of living. In conjunction with this trend he also sees marketing and production moving to the private sector.

He proposed a multiple approach to increasing agricultural production in areas, such as Sub-Sahara Africa, characterized by soil erosion, low soil fertility, and lack of fertilizer supplies. Because it is too costly to transport argrominerals to these regions, they need to develop their indigenous deposits of minerals, such as rock phosphate, including proper mining and grinding techniques. Combined with legume-BNF cropping practices and the development of agroforestry practices for erosion control, fuel wood, and mulch, the situation could be alleviated.

To accomplish these objectives, Dr. Stangel sees a collaboration of research, development, and training in the areas of legume-BNF and agromineral-fertilizers. Complete technology packages should include the development of irrigation, fertilizers, extension services, training, research, and improved seed stocks.

COMMERCIAL CORNER

According to a news release from the Bureau for Science and Technology, USAID, an inoculant production process developed by NifTAL is now being used by *Titre Inc.*, a commercial company located in Montana, indicating NifTAL's impact on U.S. agribusiness.

According to Dr. Eid M.A. Megeed, a consultant to *Titre, Inc.*, 128,000 kg of inoculant can be produced in six months when the plant is in full operation. The NifTAL production system is very simple and has the potential to be expanded to double capacity with only 60 percent of the original investment. The next move will be to incorporate the small, simple, cost-effective fermentors also developed by NifTAL. *Titre Inc.* uses a small crew: two microbiologists, two biologists, four administrators, and 12 assistants.

Titre inoculant is also marketed in Canada, England, Denmark, Brazil, Argentina, Uruguay, Australia, Hungary, and Italy, with test markets in Turkey, Guatemala, Saudi Arabia, Spain, France, and Yugoslavia.

NEW PUBLICATIONS

Perennial *Sesbania* Production and Use: A Manual of Practical Information for Extension Agents and Development Workers

Perennial *Sesbania* are found in diverse agroforestry systems in tropical and subtropical regions, and are used for fodder, firewood, wood products, soil improvement, and human food. Because research indicates considerable potential for enhancing farming systems, a workshop was organized by the Nitrogen Fixing Tree Association (NFTA) and the International Council for Research in Agroforestry (ICRAF) to explore various aspects of perennial *Sesbania*. It was held in Nairobi, Kenya, March 27-31, 1989. As a result of this conference, a manual was produced which summarizes the present knowledge of these plants and provides information on the symbiotic relationships, growth,utilization utiliutiliza utilization, and practical aspects for field workers. The manual and the proceedings of the workshop are available from NFTA, PO Box 680, Waimanalo, HI 96795, USA.

Agroforestry Extension Training Sourcebook

Louise E. Buck has prepared this guide for training agroforestry project staff to work more efficiently with farmers and other rural people. The ten modules are organized in a systematic learning process. Topics include beginning agroforestry extension training, introduction to agroforestry, project approach to agroforestry extension, agroforestry extension communication, land use diagnosis, agroforestry design, planning-monitoring-evaluation of agroforestry extension activities, seed supply, nursery management and tree planting, protection, and management. The Sourcebook is available for $27, plus postage, from CARE, Agricultural and Natural Resources Unit, 660 First Avenue, New York, NY 10016.

CIAT

Change of Emphasis at CIAT

By Richard J. Thomas

After many years as pioneers in the area of collecting and selecting efficient strains of *Bradyrhizobium* and *Rhizobium* for tropical forage legumes, the Soil Microbiology Section of CIAT's Tropical Pasture Program has undergone a change of emphasis. The new direction focuses on the role of the legume in nutrient cycling of sustainable pasture-based systems including crop-pasture alternatives for the acid soils of tropical savannas. Nitrogen fixation is a central component of this work along with studies on recycling of nitrogen in grazed pastures via plant litter and animal excreta.

A multidisciplinary group which includes an ecophysiologist, a plant nutritionist, a soil scientist, and an animal nutritionist is undertaking a large, long-term experiment on nutrient cycling which is open to participation from other parties.

The CIAT collection of *Bradyrhizobium* and *Rhizobium* bacteria includes some 4,000 strains with emphasis on strains for the most frequently requested forage legume species and *Phaseolus vulgaris*. The collection will be maintained and inoculants for research purposes will continue to be supplied free of charge.

For further information contact R.J. Thomas, Tropical Pasture Program, CIAT, Apartado Aereo 6713, Cali, Columbia.

NOTE TO BNF WORKERS

This Bulletin belongs to all people interested in various aspects of BNF. We welcome from our readers newsworthy items for inclusion in future issues. Human interest stories and photos, editorials and opinions, and notices of recent and upcoming books and events are especially welcome. It is editorial policy not to include extensive unpublished research data in the BNF BULLETIN.

NifTAL-IITA Collaborative Research

NifTAL and the International Institute of Tropical Agriculture (IITA) in Nigeria are collaborating on the study of the symbiosis between promiscuously nodulating soybean genotypes and their affiliated *Bradyrhizobium* spp. Under the supervision of soybean breeder Dr. Ken Dashiell, Mr. Robert Abaidoo will examine the indigenous bradyrhizobia which nodulate the IITA lines. Studies will include the nature of this group in relation to total indigenous bradyrhizobia, their presence in representative soils in Africa, and their symbiotic effectiveness across several IITA lines. Results from this collaborative work could have important management implications for these soon-to-be released varieties.

Mr. Abaidoo was previously sponsored by IAEA at the NifTAL Project campus as a Visiting Scientist in 1987, working with Drs. P. Singleton, C. van Kessel, and B. Bohlool on competition and N^{15} methodology with soybeans. Mr. Abaidoo is a native of Ghana, and is on leave from the University of Science and Technology in Kumasi. Complementary work on this topic will be conducted at NifTAL by Drs. P. Singleton and H. Keyser.

Upcoming NifTAL Course in Applied Legume BNF Technology for Extension Specialists

Two sesions will be offered in San Sai, Chang Mai, Thailand 16 - 27, 1992. The objective of this course is for participants to achieve a comprehensive and realistic understanding of the BNF process and the applied aspects of legume BNF technology. They will learn to assess BNF problems in the field, design appropriate tests and demonstrations, and provide farmers with practical solutions to problems. For more information contact the Training Coordinator, NifTAL Project, 1000 Holomua Ave, Paia, HI 96779-9744.

IRRI

Conservation and Use of Soil and Atmospheric Nitrogen Through Legumes in Lowland Rice-Based Cropping Systems

A NifTAL-IRRI Collaborative Research Program

By Thomas George

Rice, the world's major food staple is grown on 145 m ha, of which about 87% are in lowlands. Although fertilizer nitrogen is an essential input in modern lowland rice production, it is inefficient. Large amounts of soil nitrogen are lost from lowland rice soils during the transition between flooded and nonflooded conditions. If this loss of soil nitrogen is prevented, its use efficiency is increased.

At the end of a flooded rice crop, organic and ammonium forms of nitrogen dominate in the soil with negligible level of nitrate nitrogen. Subsequent soil drying favors aerobic nitrogen transformations; while mineralization of organic nitrogen produces ammonium which is rapidly nitrified. As a result, nitrate accumulates in the aerobic soil during the dry season. The soil undergoes transition from an aerobic to an anaerobic phase with the onset of rainfall and subsequent flooding for the wet season rice crop. Nitrate-nitrogen is subject to loss through denitrification or leaching during this transition period which is typified by intermittent, then continuous, soil flooding. Plants, including legumes, can capture this nitrogen through assimilation. In addition to preventing soil nitrate loss, legumes, through the process of BNF, have the potential to increase the quantity of nitrogen conserved.

Reducing fertilizer nitrogen input while maintaining and enhancing crop output is desirable from both an environmental and an economic perspective. This may be possible by minimizing the loss of nitrogen gained through legume BNF, and recycling nitrogen captured between crop seasons through plant residues. Managing native soil nitrogen and nitrogen derived

through BNF has great significance for the long-term sustainability of lowland rice-based cropping systems.

To effectively manage soil nitrogen and legume BNF, we need quantitative data on the effects of the flooded-nonflooded sequence on soil nitrogen dynamics and on how legume BNF fits into this complex. Our understanding is limited on soil nitrogen dynamics particularly during the nonflooded aerobic and the intermittently flooded transition phases, soil/crop management effects on soil nitrogen dynamics, legume BNF-soil nitrogen interactions, and the role of legume BNF in nitrogen nutrition of lowland rice. Lack of data is primarily due to lack of suitable methodologies to reliably quantify the dynamics of soil nitrogen and legume BNF in lowland rice-based cropping systems.

To fill this gap in knowledge, NifTAL and IRRI are collaborating to develop suitable methodologies, investigate the processes and magnitudes of soil nitrate accumulation and loss, and to define the roles of grain and green manure legumes in capturing soil and atmospheric nitrogen.

NifTAL and IRRI have been cooperating in this work since October 1989. NifTAL has assigned Dr. Thomas George to IRRI to carry out this research program in cooperation with IRRI scientists Drs. J.K. Ladha and D.P. Garriety and Dr. R.J. Buresh from IFDC. The agreement will support the research and development of BNF applications for rice-based cropping systems, the training of research personnel in BNF technology, and the maintenance of rhizobial germplasm.

News from the University of Hawaii Biotechnology Program

By Dulal Borthakur

Researchers in the UH Biotechnology Program have identified and cloned the symbiotic region of the *Rhizobium* strain TAL 1145 which nodulates tropical tree legumes such as leucaena. They have also identified at least two chromosomal genes involved in nitrogen fixation in TAL 1145. A 2.0 kb region of TAL 1145 DNA, which is required for both nodulation and exopolysaccharide synthesis by this strain, has been cloned and sequenced.

Legume Production and Demand for Inoculants Increases in Western Canada

By Chris van Kessel, Senior Research Scientist, University of Saskatchewan

Production of grain legumes, particularly pea and lentil, has increased dramatically in Western Canada. Prior to the seventies, production of these grains was virtually nonexistent; however, the total area planted in 1991 will be approximately 200,000 and 250,000 ha, respectively. The increase in acreage planted in legumes is due to several factors including

lower prices for cereals
higher demand for peas by European livestock producers
the desire to change crop rotation sequences for better disease control and improved soil structure.

Both lentil and pea are nodulated by *Rhizobium leguminosarum*. It has been estimated that 85-90% of the farmers use an inoculant, and farmers have been using the same inoculant for both crops. Pea, which is less drought tolerant than lentil, are usually grown in the wetter, more northerly regions of Western Canada, while lentil is more common in the southern part of the Prairies. Inoculation responses by lentil have been spectacular due to the low indigenous populations of *R. leguminosarum* in drier areas. Researchers at the University of Saskatchewan are looking at environmental parameters which might enhance the survival of introduced rhizobia in wetter areas and if these newly-introduced rhizobia can compete for nodulation with the indigenous rhizobia.

As legumes become part of the crop rotation, research is being conducted on the effect of residual legumes on subsequent crops. Avenues are being sought to replace part of the inorganic fertilizers with biologically-fixed N through the use of a green-manure cover crop during the summer fallow year. In these systems, up to 20% of the N recovered by spring wheat was derived from the incorporated green manure. The recovery of applied N fertilizer was between 23% and 34% depending on whether a crop residue was incorporated or not, and on the residue type of a previous crop. Total N losses have been consistently higher when fertilizer N was used as a source of N compared to green-manure N. However, N losses from green-manure crops did occur and were largely due to ammonia volatilization after cutting and to denitrification after periods of heavy rainfall.

The success of this agricultural trend is also evident in growth of Western Canadian agribusiness. In the seventies, *Nitragin* (located in Milwaukee, Wisconsin) was only major company that had inoculants available in Canada. Recently, various new companies (*Philom Bios*, *MicroBio-RhizoGen*) are producing and selling inoculants developed especially for growing conditions encountered in Western Canada. Even an oil company has entered the inoculant business with a new branch known as *ESSO-Ag. Biologicals*.

Training Course in Plant Nutrient Management for Sustainable Agriculture

NifTAL will participate in a training course in Plant Nutrient Management for Sustainable Agriculture, October 7-18, 1991, in Muscle Shoals, Alabama, sponsored by IFDC. NifTAL's section is titled Biological Nitrogen Fixation: Constraints, Optimization, and Impacts on Fertilizer Nitrogen Needs.

Recent Meeting in Canberra, Australia

The Ninth Australian Nitrogen Fixation Conference was held January 21-25, 1991, at the Australian National University in Canberra. Topics covered non-symbiotic nitrogen fixation, metabolic pathways in rhizobia and in symbiosis, oxygen regulation of nitrogen fixation, plant-microbe interactions--legumes, plant-microbe interactions--non-legumes, acid tolerance in rhizobia and symbiosis, nitrogen fixation involving trees and shrubs, nitrogen fixation in the field, and the ecology of rhizobia. Copies of the proceedings are available for $20 Australian from Dr. A.H. Gibson, CSIRO Division of Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia.

Studies on Rhizobial Population Structure and Dynamics at Oregon State University

Peter Bottomley at Oregon State University reports that research there is leading to a better understanding of the composition of soil rhizobial populations and the environmental factors that influence them. Several research sites are being evaluated in western Oregon which differ in regard to management style and amount of improvement. The locations also differ in the type and percentage of forage legumes that contribute to the plant community. Of particular interest are the resident populations of *Rhizobium leguminosarum* biovars *trifolii* and *viceae* which nodulate the clovers and vetch species of importance to hill farms in western Oregon. Researchers are defining the populations at each site, determining the diversity within it, and exploring the possibility of influencing their dynamics and nodulating success.

To date there has been little data generated about the structure of complex native rhizobial populations despite the fact that they occupy most of the nodules of field-grown legumes and are often sub-optimally effective on leguminous species of agricultural importance. Bottomley is concerned about the methods used to delineate the diversity and the application of findings from one location to another. Will the population structure determined at one location be confirmed at another? Is each situation unique? What role do population diversity and structure play in the ability of field-grown legumes to achieve their nitrogen-fixing potential?

One of the most pressing issues has been that of methodology. While traditional methods have the potential to discriminate between subpopulations; they, unfortunately, provide no information on genetic relationships. Currently, Bottomley's group is comparing data on population structure determined by serological methods to the data from techniques more amenable to genetic analysis such as multilocus allozyme electrophoresis and restriction fragment length polymorphisms.

One observation, which should be of immediate importance to rhizobial workers, is that serotype-clonal relationships can be found within soil rhizobial populations. However, other serotypes can exist within the same population in which clonal relationships are not followed. Bottomley believes that those who extensively use serological techniques should be aware of this possibility occurring within their own populations since it could cause misinterpretation of data.

One of the key questions Bottomley's group addresses is why certain subpopulations of soil rhizobia occupy the majority of nodules. Although much information exists about soybean rhizobia in this regard, studies about *Rhizobium* species are predominantly discontinuous, site-specific, and scattered across continents. Along with this complication, Bottomley states that advances in the molecular biology of competitiveness have resulted in the need to consider the possibility that plasmid-borne information is critical to nodulation success and might cross chromosomal subpopulation boundaries. Currently, their examination of population structure is giving equal emphasis to chromosomal and plasmid differences.

Despite the methodology issues, information about nodulation success in the field continues to be generated. At one field site, the serotype dominating nodules of subclover is also the most numerous in the soil. However, it does not dominate the root nodules of all clovers in that soil. For many vetch and perennial clover species, the researchers have found no evidence for any overwhelmingly competitive chromosomal types in the field site. This conclusion might be modified when other analytical methods are used to subdivide the population, hence, the work continues.

Looking at a Promising Biotechnology

By Doug Rice

Biotechnology offers opportunities for innovative research that will further improve BNF technology. In its continuing efforts to respond to the demand for more and better techniques and products, NifTAL is making full use of its molecular biology potential. One particularly promising research area is isolating cloned gene segments that can be used as specific DNA hybridization probes to identify elite tropical rhizobial strains.

DNA restriction fragment length polymorphism (RFLP) or "DNA fingerprinting" has the potential ability to detect genetic heterogeneity within serogroups. To realize this potentially more detailed genetic analysis requires careful hybridization probe selection. Also, it would be very desirable if a strain identification probe could identify other *Rhizobium* spp. and detect genetic rearrangements or mutations of a rhizobial strain. A class of genetic elements that are likely to fulfill these probe selection criteria are mobile genetic elements such as transposons and insertion sequences.

Wheatcroft and Watson (1), and more recently, Simon *et al* (2) have used insertion sequence (IS) elements from *Rhizobium meliloti* as hybridization probes to identify primarily *R. meliloti* strains by "IS fingerprinting" (a descriptive name that includes the type of probe used in the RFLP analysis). A given IS element isolated from one *R. meliloti* strain will also be found in 10% (1/10) to 100% (10/10) of other *R. meliloti* strains, producing a unique IS fingerprint for each strain containing the IS element (1, 2, 3).

IS elements are relatively small sequences of ``mobile DNA'' with the ability to copy themselves and insert themselves into another location of DNA with resulting copy numbers from 1 up to 40 (4). They are "jumping genes" like transposons which carry selectable antibiotic resistance genes, but differ from transposons because IS elements only carry the genes necessary for their mobility. Consequently, IS*Rm1* (from *R. meliloti* strain 1021), the first IS element isolated from rhizobia, was discovered as the causative agent of frequent spontaneous *nif* (insertion) mutations in strain 1021 (5).

Positive selection, broad-host-range bacterial vectors, such as pUCD800, are now IS elements (2,6). pUCD 800 entraps an IS element by insertional inactivation of

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N-FIXING TREE NOTES

Updates from NFTA

For information on any NFTA publications or activities, write to NFTA, PO Box 680, Waimanalo, HI 96795, USA.

Workshop on the Economics of Agroforestry

One objective of this workshop is to evaluate the impact of "trees on" versus "trees off" small farms. Another feature is a series of case studies in agroforestry economics from several countries including the Philippines, Costa Rica, Niger, and Burkino Faso. The keynote speaker Marcelino Avila, of the International Council for Research in Agroforestry (Kenya), addresses "Financial and economic analysis of agroforestry systems". The proceedings will be available later this year or early in 1992.

Poster of Native Hawaiian Nitrogen-Fixing Trees

A beautiful, colorful poster illustrating the many uses of the five nitrogen fixing trees native to Hawaii is available free to new associates joining NFTA or to anyone working in education in Hawaii. The poster was painted by Ipo Nihipali, a local artist noted for her depictions of native Hawaiian birds.

NFT Highlights

Since 1982, NFTA has been publishing two-page extension leaflets on various nitrogen-fixing trees species; there are now 36 in the series. The most recent ones feature *Alanus nepalensis*, *Acacia tortilis*, and *Enterolobium cyclocarpum*. A full set is available to anyone in developing countries.

Third Training on NFTs for Fodder

NFTA and the Heifer Project International (HPI) are continuing their training sessions on "Use of nitrogen fixing trees for animal production in the tropics". The latest will be held in Kampala, Uganda 7-11 October 1991.

The Plant-Infection Assay Applied to Trees

By Dan Turk

Recent work at NifTAL has demonstrated that it is possible to get reliable most-probable-number estimates of rhizobial populations for a variety of trees using the plant-infection assay. Good agreement between plate counts and MPN estimates of pure cultures (plate count:MPN ratio < 16) have been obtained for several trees using agar slants or growth pouches.

Because of slow early root growth in small-seeded trees, only 15 ml of agar was used to make the agar slants. This promoted contact between roots and rhizobia at the bottom of the 25 mm x 250 mm glass tubes. Evaluation of nodulation at weekly intervals suggested that tubes and pouches should be scored for nodulation after at least seven and five weeks respectively. MPN estimates using *Acacia* species in pouches tended to underestimate pure culture plate counts by 100 to over 1000 fold. The possibility of seriously underestimating rhizobial densities with some growth system-tree combinations emphasizes the need to determine the suitability of growth systems prior to enumerating rhizobial populations in soil.

Agar Slants	Growth Pouches
Acacia mangium	*Calliandra calothyrsus*
Acacia mearnsii	*Gliricidia sepium*
Leucaena diversifolia	*Leucaena diversifolia*
Paraserianthes falcataria	*Robinia pseudoacacia*
Robinia pseudoacacia	*Sesbania grandiflora*
	Sesbania sesban

Restoring Degraded Land in Hawaii with an Endemic Nitrogen Fixing Tree

Windswept and desolate, the uninhabited Kaho'olawe Island in the Hawaiian archipelago is the epitome of ecological disaster in the tropics. At one time, a unique dryland forest dominated by an endemic leguminous tree, *Erythrina sandwicensis* (wiliwili), grew on the island. Overuse of forest resources, accidental fires, and uncontrolled grazing by ferral goats contributed to the devegetation of the island and left the soil vulnerable to the scouring forces of the Pacific trade winds and tropical storms. Today, thousands of acres of the island's summit are characterized by barren hard clay pans dissected by eroded gorges. Only a few isolated pockets of mature wiliwili trees remain.

Supported in part by The Nature Conservancy of Hawaii and the U.S. Department of Navy, NifTAL Project's objectives are to identify soil microbial and nutritional constraints which limit the success of wiliwili in recolonizing eroded sites on Kaho'olawe. These eroded soils typically suffer from low fertility and a paucity of the beneficial soil micro-symbionts which contributed to the success of the original ecosystem. Pot and field experiments have demonstrated the benefit of introducing both appropriate rhizobia and vesicular arbuscular mycorrhizal fungi (VAM), and of raising the phosphorus fertility level of the soil.

Results from these studies will assist land stewards in determining suitable management practices for the reintroduction of wiliwili to Kaho'olawe. Current policies to rehabilitate degraded lands emphasize the introduction of alien, fast-growing, multipurpose nitrogen-fixing trees into agroforestry systems. The researchers hope this case will serve as a model for the reestablishment of native species in degraded lands and the preservation of biological diversity throughout the tropics. In addition to encouraging the regeneration of the original associated floral and faunal community, the use of native species can also strengthen the cultural ties of the indigenous peoples to their land.

Graduate Students

Surya Tewari

Surya is from the twin island Republic of Trinidad and Tabago in the southern Caribbean. She received her BSc in General Agriculture from The University of the West Indies, St. Augustine, Trinidad, with an emphasis in Agriculture and completed her MSc at Wye College, University of London. Her research is on the influence of soil nitrogen on early soybean growth and development with a view to improving crop modeling techniques based on the data collected. After a year of course work at Manoa during the next academic year, she will complete her research at NifTAL.

Bruce Lindquist

After receiving a BS from Texas A&M at College Station, Bruce went to University of California at Davis to continue his graduate work. He completed an MS degree in International Agricultural Development. Presently, he is persuing a PhD in Ecology. He is working with Ken Cassman, Paul Singleton, and Bill Rains on phosphous uptake in legumes as affected by root length, mycorrhizal fungi, and symbiosis with rhizobia.

Marcelo Bartolini

This Masters in Business Administration student at the University of Hawaii is working with Paul Singleton and Joe Rourke. His goal is to develop a computer model for inoculant production cost analysis. One of NifTAL's goals in to encourage entrepreneurs to get into the inoculant production business. Bartolini's model will give potential producers a method to determine the price that is necessary to charge for inoculants in order to have a profitable business. The model is expected to be applicable to any set of circumstances in any country. Bartolini and Rourke will be visiting inoculant producing facilities in Australia, Indonesia, Philippines, and Thailand, to obtain information of the real costs of producing inoculants.

Bartolini, who is from Argentina, received an agricultural engineering degree from Universidade Nacional de Cuyo in Mendoza, and an MSc in Food Science and Technology from the University of Georgia. He will receive his degree in 1992.

Visiting Scientists

Dr. Nesime Cebel

Dr. Cebel is from the Soil and Fertilizer Research Institute in Ankara, Turkey, and received her doctorate from Ankara University, Faculty of Agriculture. She will be at NifTAL for at least eight months with support from the Organization for Economic Cooperation and Development (OECD). Her research is on the development and characterization of strain/group specific DNA probes for the identification of chickpea nodulating rhizobia.

This spring, Dr. Wolff was at NifTAL working on plant defense mechanisms. He is particularly interested in correlation between the amount of tannin and the number of root nodules. When he returns to NifTAL, he will set up field experiments to explore the effects of elevation and stress conditions on inoculation. He is in the Department of Biology at the University of Marburg in Germany

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the *sacB* gene. The wild type *sacB* gene product, levansucrase, causes bacterial lysis or inhibition of growth when 5% sucrose is included in the growth medium.

Preliminary work done by NifTAL in this area will be reported at the 13th North American Symbiotic Nitrogen Fixation Conference in Banff, Canada, and in later issues of this newsletter.

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SERVICES FROM NIFTAL

OUTREACH

- 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 6 week courses
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- Graduate degree support
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- Research and Information Networking
- National research program design assistance
- Technical backstopping to suppport 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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