Plant and Soil **141**: 1–11, 1992

PLSO SA01

Biological nitrogen fixation for sustainable agriculture: A perspective

B. B. BOHLOOL[1], J. K. LADHA[2], D. P. GARRITY[2] and T. GEORGE[1]
[1]*NifTAL Project, University of Hawaii, 1000 Holomua Avenue, Paia, HI 96779, USA and*
[2]*International Rice Research Institute (IRRI), P.O. Box 933, Manila, Philippines*

Key words: chemical fertilizer, crop production, developing countries, environment, inoculation, legume, pollution

Table of contents

Abstract

The economic and environmental costs of the heavy use of chemical N fertilizers in agriculture are a global concern. Sustainability considerations mandate that alternatives to N fertilizers must be urgently sought. Biological nitrogen fixation (BNF), a microbiological process which converts atmospheric nitrogen into a plant-usable form, offers this alternative. Nitrogen-fixing systems offer an economically attractive and ecologically sound means of reducing external inputs and improving internal resources. Symbiotic systems such as that of legumes and *Rhizobium* can be a major source of N in most cropping systems and that of Azolla and *Anabaena* can be of particular value to flooded rice crop. Nitrogen fixation by associative and free-living microorganisms can also be important. However, scientific and socio-cultural constraints limit the utilization of BNF systems in agriculture. While several environmental factors that affect BNF have been studied, uncertainties still remain on how organisms respond to a given situation. In the case of legumes, ecological models that predict the likelihood and the magnitude of response to rhizobial inoculation are now becoming available. Molecular biology has made it possible to introduce choice attributes into nitrogen-fixing organisms but limited knowledge on how they interact with the environment makes it difficult to tailor organisms to order. The difficulty in detecting introduced organisms in the field is still a major obstacle to assessing the success or failure of

inoculation. Production-level problems and socio-cultural factors also limit the integration of BNF systems into actual farming situations. Maximum benefit can be realized only through analysis and resolution of major constraints to BNF performance in the field and adoption and use of the technology by farmers.

Introduction

Chemical fertilizers have had a substantial impact on food production in the recent past, and are today an indispensable part of modern agricultural practices. The Green Revolution of the past half-century was fueled by technologies heavily dependent on synthetic fertilizers. Russell et al. (1989) estimate that in 1985, the use of 38.8 million Mt of N fertilizer on cereals globally resulted in increased world cereal production of 938 million Mt, more than half of the total cereal production in that year. They defined a relationship between world N fertilizer use on cereals (X) and mean world cereal yield (Y), between 1956 and 1985, which could be expressed by the equation $Y = 1202 + 13.3X$, with $R^2 = 0.983$ emphasizing the major driving force of N fertilizers in food production today.

There are, on the other hand, vast areas of the developing world where N fertilizers are neither available nor affordable. Furthermore, in most of these countries, removal of N fertilizer subsidies, due to balance of payment problems, have resulted in higher prices and lower supplies. Even in wealthier nations, economic and environmental considerations dictate that biological alternatives which can augment, and in some cases replace, N fertilizers must be sought. The process of biological nitrogen fixation (BNF) offers this alternative. In this paper, we outline sustainability issues that dictate increased use of BNF and the constraints to optimal use of BNF in agriculture.

The issue of sustainability in agriculture

Sustainability is defined as 'the successful management of resources to satisfy changing human needs while maintaining or enhancing the quality of the environment and conserving resources' (TAC, CGIAR, 1978). Economists measure sustainability as the ratio of output to input, taking into account stock depletion. Stocks in agriculture include soil, water, nonrenewable energy resources, and environmental quality.

Modern agriculture is based on maximum output in the short term, with inadequate concern for input efficiency or stock maintenance (Odum, 1989). Nitrogen fertilizer ranks first among the external inputs to maximize output in agriculture. Input efficiency of N fertilizer is one of the lowest among the plant nutrients and, in turn, contributes substantially to environmental pollution. The continued and unabated use of N fertilizers would further accelerate depletion of stocks of non-renewable energy resources used in fertilizer production. The removal of large quantities of crop produce from the land additionally depletes soil of its native N reserves. On the other hand, BNF, a microbiological process in the biosphere, converts atmospheric dinitrogen into a plant-usable form through the microbial enzyme nitrogenase. Nitrogen input through BNF can help maintain soil N reserves as well as substitute for N fertilizer to attain large crop yields (Peoples and Craswell, 1992).

Another issue which needs urgent attention is the decline in crop yields under continuous use of N fertilizers. The trend of the 1970s of increasing crop yields from fertilizer N addition has recently been slowed down both in the developed (Plucknett and Smith, 1986) and in the developing countries (Barker and Chapman, 1988; Byrlee, 1987). Odum (1989) indicated that a 4-fold increase in crop yield during the 1970s and 80s occurred at an 11-fold increase in fertilizer N use in Georgia, USA. An analysis of several years' data on rice yields from experimental stations in Philippines, Indonesia and Thailand by Pingali et al. (1990) shows trends of stagnation or decline.

Econc.nic considerations: The fossil-fuel outlook

The oil crisis of the 1970s and the current Middle

East problems are constant reminders of the vulnerability of our fossil fuel-dependent agricultural and food production systems. As the price of oil increases, so does that of agricultural inputs, notably of N fertilizers.

Industrial processes for the manufacturing of nitrogen fertilizer are heavily reliant on fossil fuel, utilizing a vast proportion of our energy supplies. Synthesis of compounds of nitrogen from N_2 gas (the Haber-Bosch process) requires large quantities of hydrogen, usually obtained from natural gas, in addition to substantial amounts of energy to establish and maintain the conditions of high temperature and pressure required for nitrogen to react with hydrogen to produce ammonia. In addition to the cost of production, a great deal of energy is also used for transportation, storage, and application of N fertilizer, all totalling to near 22,000 kilo calories of energy per kilogram of fertilizer-N processed, distributed, and applied (see Evans, 1975).

Capital costs for construction of a medium-size N fertilizer factory alone exceed $100 million. Considering that most of the hardware and know-how for construction of the factory comes from industrialized nations, it is difficult to promote expansion of the fertilizer manufacturing industry in developing countries strapped for hard currency.

Environmental quality considerations: Health and ecological impacts

The external costs of environmental degradation and human health far exceed economic concerns. Nitrate in groundwater is a major health concern in the corn belt of the U.S., and other intensively cultivated areas. Nitrogen in run-off and surface waters has led to extensive pollution and eutrophication of rivers and lakes. The gaseous oxides of nitrogen, derived from N fertilizers, are highly reactive and pose a threat to the stability of the ozone layer. Table 1, modified from Keeney (1982), summarizes the potential adverse impacts of excessive fertilizer N application.

Production sustainability: Management of internal resources

Long-term sustainability of agricultural systems must rely, as much as possible, on use and effective management of internal resources. Nitrogen-fixing plants offer an economically attractive and ecologically sound means of reducing external inputs and improving the quality and quantity of internal resources. Biological nitrogen fixation can be a major source of N in agriculture when symbiotic N_2-fixing systems are used; the amount of N input is reported to be as high as $360\,kg\,N\,ha^{-1}$ (Table 2). On the other hand, the nitrogen contributions from nonsymbiotic (associative and free-living) microorganisms are relatively minor, thus requiring fertilizer N supplementation.

Among symbiotic N_2-fixing systems, nodulated legumes have been used in cropping systems for centuries. They can serve a multitude of pur-

Table 1. Potential adverse environmental and health impacts of excessive N use (Keeney, 1982)

Impact	Causative agents
Human health	
Methemoglobinemia in infants	Excess NO_3 and NO_2 in waters and food
Cancer	Nitrosamine illness from NO_2, secondary amines
Respiratory illness	Peroxyacyl nitrates, alkyl nitrates, NO_3 aerosols, NO_2, HNO_3 vapor in urban atmospheres
Environmental health	
Environment	Excess NO_3 in feed and water
Eutrophication	Inorganic and organic N in surface waters
Materials and ecosystem damage	HNO_3 aerosols in rainfall
Plant toxicity	High levels of NO_2 in soils
Excessive plant growth	Excess available N
Stratospheric ozone depletion	Nitrous oxide from nitrification, denitrification, stack emissions

Table 2. Estimates of dinitrogen fixed by different N_2-fixing systems in agriculture

N_2-fixing system	N_2 fixed ($kg\,N\,ha^{-1}$)	References
Free-living/associative		
Rice-blue green algae	10– 80 $crop^{-1}$	Roger and Ladha (1992)
Rice-bacterial association	10– 30 $crop^{-1}$	Roger and Ladha (1992)
Sugarcane bacterial association	20–160 $crop^{-1}$	Urquiaga et al. (1989)
Symbiotic		
Rice-Azolla	20–100 $crop^{-1}$	Roger and Ladha (1992)
Legume-Rhizobium		
Leucaena leucocephala	100–300 yr^{-1}	Danso et al. (1992)
Glycine max	0–237 $crop^{-1}$	Keyser and Li (1992)
Trifolium repens	13–280 $crop^{-1}$	Ledgard and Steel (1992)
Sesbania rostrata	320–360 $crop^{-1}$	Ladha et al. (1990)
Non-legume-*Frankia Casuarina* sp.	40– 60 yr^{-1}	Gauthier et al. (1985)

poses in sustainable agriculture. They are used as primary sources of food, fuel, fiber and fertilizer, or, secondarily, to enrich the soil, preserve moisture and prevent soil erosion. They can also be used for windbreak, ground cover, trellis, hedgerow and shade, or as a source of resins, gums, dyes and oils. Some of the most ornamental flowering plants in the tropics are legumes (see NAS 1979 for more detail).

Some of the nodulated nonlegumes, notably species of *Casuarina*, are hardy nitrogen-fixing plants, which produce high-quality fuelwood on marginal lands, and have also been used for stabilizing sand dunes and eroding hillsides, as well as for reclaiming marshlands affected by fluctuating brackish/fresh water. The trees are useful for shade, windbreaks and hedges (for more detail see NRC, 1984).

Of particular practical value in rice production systems is the nitrogen-fixing symbiotic water fern Azolla. Azolla in symbiosis with cyanobacterium *Anabaena azollae* can fix 2–4 $kg\,N\,ha^{-1}\,day^{-1}$ (Lumpkin and Plucknett, 1982). Recently other benefits of Azolla have been recognized: a) weed suppressor, b) K scavenger from floodwater, c) animal feed, d) fish feed, e) P scavenger in sewage-treatment plants, and f) suppressor of ammonia volatilization. (Watanabe and Liu, 1992).

Associative and free-living microorganisms are believed to contribute to production sustainability of flooded rice production systems (Roger and Ladha, 1992). Approximately 50% of the N requirement of a flooded rice crop is met from the soil N pool (Bouldin, 1986) which is believed to be maintained through BNF by associative and free-living microorganisms (Koyama and App, 1979). Contributions from non-symbiotic N_2 fixation in upland agriculture is generally not substantial (Kennedy and Tohan, 1992), although N_2 fixation to the order of 160 $kg\,N\,ha^{-1}$ has been reported for sugarcane (Table 2).

Constraints to utilization of nitrogen-fixing systems

There are still many unknowns in the scientific understanding of N_2 fixation. Research into the basic mechanisms of the process is an important goal for improving N_2 fixation in the future (Ishizuka, 1992), but few, if any, of these unknowns are constraining implementation of the existing BNF technologies. So much of the existing knowledge is not being used, especially in developing countries, that it is essential to devote major efforts towards adoption of what is already known.

There are major technical, socio-economic and human-resource obstacles to fuller implementation of BNF technologies in cropping systems. The first can be addressed through comprehensive programs of basic and applied research. The latter two through education, training and private-enterprise development.

Full utilization of, and maximal benefit from, BNF systems can be realized only through analysis and resolution of major constraints to their optimal performance in the field, and their adoption and use by the farmers. These can be classified broadly into environmental, biological, methodological, production-level and socio-cultural constraints.

Environmental constraints

The revolution in modern molecular biology has made it possible to alter the genetic composition of living organisms and give them attributes desirable to man. However, our lack of understanding of how organisms interact with and within their environments has made it difficult to tailor organisms to order. The question is no longer 'can we genetically engineer beneficial organisms?', but 'what should we engineer them for?'. The challenge is to match the genetic potential of the living systems to the controlling parameters of the environment.

An essential consideration in establishment of nitrogen-fixing systems is how they would respond to the adversities of the soil environment. A thorough understanding of the ecology of the various nitrogen-fixing systems is crucial to the successful application and acceptance of BNF technologies for sustainable productivity.

The 'Law of the Minimum' states that plant productivity can be defined by a single limiting factor in the system. Amelioration of the limiting factor will increase productivity until another essential growth factor in the system becomes limiting. Therefore, no amount of biologically or chemically fixed nitrogen can increase production as long as some other factor is limiting growth. Several environmental factors that affect the performance of legume/rhizobia symbiosis (Alexander, 1985; Atkins, 1986) and actinorhizal (*Frankia*) symbiosis (Tjepkema et al., 1986; Torrey, 1978) have been studied. Gibson (1977), Munns and Francop (1982), Freire (1984) and Ladha et al. (1992) have reviewed the soil constraints to symbiotic performance. Major considerations affecting either the microbe, the host, or their symbiotic interaction include soil acidity (Munns, 1977), other acid-related factors including aluminum and manganese toxicity, and calcium deficiency (Singer and Munns, 1987), phosphorus (Almendras and Bottomley 1988; Cassman et al., 1981; Helyar and Munns, 1975, Keyser and Munns, 1979; Leung and Bottomley, 1987; Munns, 1979; Singleton et al., 1985), calcium (Beck and Munns, 1984, 1985), salinity (Singleton and Bohlool, 1983) and flooding (Ladha et al., 1992). Symbiotic activity within a plant community is also conditioned by the amount of N mineralized from organic sources (George et al., 1988).

Watanabe and Liu (1992) have discussed the diverse environmental constraints which limit the optimum performance of azolla. They include P deficiency, sensitivity to drought and high temperature.

One of the major obstacles to successful establishment and effective performance of introduced N_2-fixing systems is competition from native organisms. This has been extensively demonstrated for legume inoculants, and is likely to be a serious problem for other exotic organisms in new environments.

The complexities involved in competition for nodulation of legumes are numerous, as noted in the recent review by Dowling and Broughton (1986). Environmental influences such as soil temperature (Weber and Miller, 1972), or additions of P and K (Almendras and Bottomley, 1988) can alter competition patterns.

The population size of naturalized rhizobia affects the likelihood of inoculant establishment (Bohlool and Schmidt, 1973; Weaver and Frederick, 1974 a,b) and the magnitude of a legume crop's response to applied rhizobia (Singleton and Tavares, 1986). The yield and nodulation of a promiscuous host cowpea (*Vigna unguiculata* (L) Walp) can be increased with inoculation less frequently due in part to the population size and competitive ability of indigenous Bradyrhizobium sp. in tropical soils (Danso and Owiredu, 1988). However, the belief that tropical legumes do not respond to rhizobial inoculation is a misconception based on faulty assumptions and very little data (Singleton et al., 1991). Soybean, which has more specific rhizobial requirements, responds to inoculation with Bradyrhizobium rhizobia when first introduced into an area (Thies et al., 1991a). As the introduced rhizobia become naturalized, the yield response to inoculation is no longer observed in subsequent crops (Dunigan et al., 1984; Ellis et al., 1984). Thies et al. (1991b) have recently used indices of native rhizobial population size and the nitrogen status of the soil to develop ecological models for predicting the likelihood, and the magnitude, of legume response to rhizobial inoculation.

Biological constraints

What ultimately determines the success of a BNF practice is the genetic potential of the organisms and how that interacts with components of the environment. Figure 1 summarizes the data of Thies et al. (1991a) with legume inoculation. It compares the relative yield of uninoculated and inoculated legumes with that of N treatment (approximately 1000 kg of N per hectare, split 100 kg N per week), for a total of 29 combinations of 9 legume species at 5 diverse sites. The N treatment is taken as the 100% full yield potential for each species. The difference between uninoculated and inoculated plants represents opportunities for increasing yield of legumes with existing knowledge and inoculation technology. The gap between yields of inoculated and fertilized plants represents the window of opportunity for improving symbiosis by genetic manipulation, environmental management, or both.

A variety of biological factors may influence the expression of BNF in all nitrogen-fixing systems. In symbiotic associations, both partners are subject to biological constraints, such as disease and predation which can directly or indirectly affect the amount of N fixed, as well as the quantity made available to other components of the cropping system.

In general, and especially so in legumes, the amount of nitrogen fixed is directly related to the growth potential of the host in a particular system. When growth is limited, for example by disease, nitrogen fixation will be reduced accordingly.

Methodological constraints

The difficulty in identifying the N_2-fixing organisms introduced into the field is one of the major obstacles to assessing the success or failure of the technology. This is relatively easier with symbiotic legumes, since in most systems serological methods can be used to successfully identify and monitor the introduced rhizobia (for reviews, see

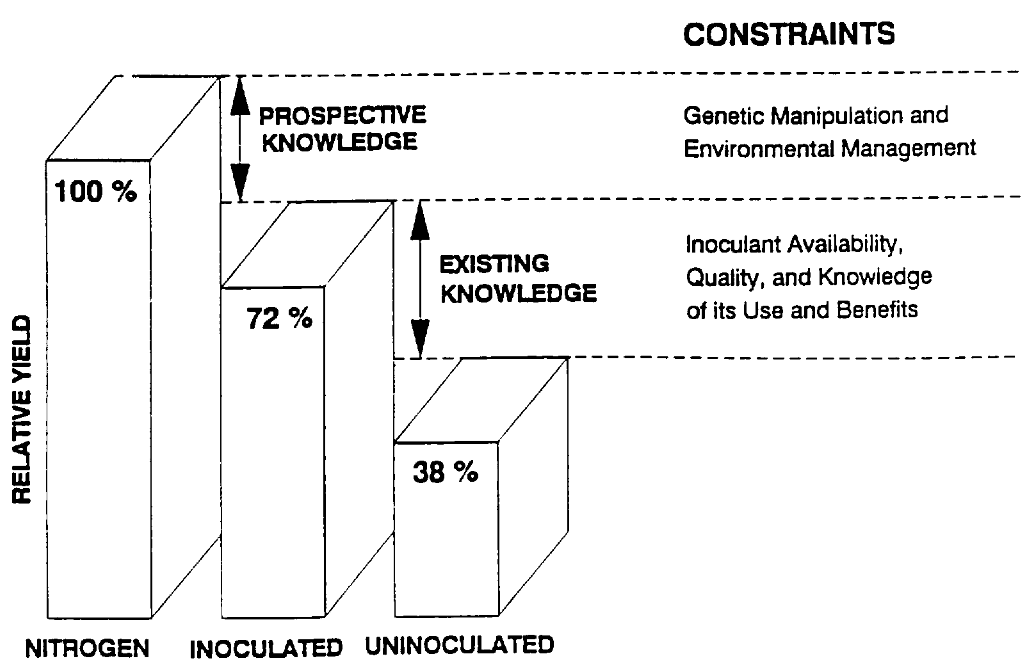

Fig. 1. Comparative benefits from rhizobial inoculation and N application. Results summarized from Thies et al. (1991a) with 9 legume species at five sites.

Bohlool and Schmidt, 1980; Bohlool, 1987). With the advent of DNA probes for genetic analysis at the strain level (Holben et al., 1988), it is likely that this barrier will be removed for most BNF systems in the near future.

Inoculant technology is well-developed for legume inoculants, but still in its infancy for actinorhizal and other BNF systems (Torrey, 1978). Although there have been some successes in growing *Frankia* in pure culture (Callaham et al., 1978; Diem et al., 1982), it still remains a problem to produce inoculum on a large scale. Even for legumes, the scale of production, the availability of suitable carrier material, preservation of germ plasm, and shelf-life of the finished product are serious constraints to use of inoculants, especially in developing countries.

A major methodological constraint to BNF use is the lack of reliable techniques for measuring nitrogen fixation in the field. This is particularly true for perennial pasture legumes and trees (Danso et al., 1992). When non-fixing genetic isolines are available, the N difference method can be a reliable estimator of the contribution of BNF. Unfortunately, however, only for soybeans, and to some extent for peas, have well characterized nod-nod isolines been reported. There are reports of nod-nod alfalfa, peanuts and chick peas, but these have not been studied as extensively. Several of the chapters in this volume cover the state of the art in methodologies for BNF measurement and discuss the strengths and limitations of various measurement techniques.

Production-level constraints

The development of an efficient BNF system carries no certainty that it will be successfully incorporated into prevailing farming systems. There are several field-level production constraints that limit the incorporation of BNF systems into farming systems.

Cereal crops dominate the agricultural landscape in both the temperate and tropical worlds. Fitting legumes into these systems is a task of challenging complexity, notwithstanding their significant BNF benefits. More niches for legumes must be found within the major cropping systems, and numerous agronomic limitations overcome, in order to exploit BNF's direct and indirect contributions.

Wide-scale production of leguminous crops has been particularly constrained in the humid tropics (Khanna-Chopra and Sinha, 1989). High precipitation and humidity during the rainy season induce insect and disease epidemics that have so far proven difficult to control, thus creating yield instability. Grain legume pods are also hygrophilic, and the seed deteriorates very rapidly in humid conditions (Shanmugasundaram, 1988). Special harvest precautions are needed compared to the cereals. Also, most legume cultivars have indeterminant-plant characteristics. They tend to have a low grain-to-biomass ratio when produced in rainy, cloudy weather patterns.

Due to these constraints, most legume production is undertaken in the wet-dry transition period at the end of the monsoon season, or during the dry-wet transition at the onset of the rains. These seasons are subject to great rainfall variability, resulting in alternating moisture stress or excess (George et al., 1992). Crop establishment is more difficult in the fluctuating moisture conditions, and grain yield and BNF contributions tend to be unreliable.

In the humid uplands, where Ultisols and Oxisols predominate on all three tropical continents, severe acidity and phosphorus deficiency are characteristic problems (Sanchez, 1980). Unless they can be alleviated through soil amelioration with external nutrient sources, production of conventional grain legumes is not favored. The contribution of BNF on these soils is also hampered by the fact that nitrogen is often not the primary limiting nutrient.

The approach of developing unconventional BNF systems for the domain of strongly acid soils is now receiving prominent attention. For sustaining intensive production, particular emphasis is directed to BNF in alley-cropping systems using leguminous trees or forages (Kang, 1990). For fallow-rotation systems, which occupy vast areas, managed fallows of leguminous cover crops are being developed. Until recently, little research attention had been devoted to these practices.

In the wetland rice production systems of the humid tropics, both leguminous green manures and grain legumes have traditionally played a critically important BNF role (King, 1911). The contribution of both declined in recent decades as fertilizer-N use became widespread. Research is developing new species to better exploit the available cropping-system niches. One striking example is the introduction of stem-nodulating green manure species (Ladha et al., 1992) that are better adapted to waterlogged conditions. However, the typical agronomic constraints common to all green manures remain as real barriers to adoption (Garrity, 1990). For most species, sustainable seed production is primary among them (Garrity et al., 1990), but successfully establishing the crop, and incorporating it into the soil during land preparation for rice, must be done with an absolutely minimal cost (Pradhan, 1988). Very little attention has been given to these critical agronomic issues.

Although Azolla is a potential BNF system for wetlands, the technology suffers from many farm-level constraints which limit any widespread use by farmers. Major constraints are: difficulty in maintaining and distributing inocula round the year and susceptibility to insects and diseases.

In the semi-arid tropics legumes occupy a more important role than in the humid tropics (Willey, 1979). They are commonly intercropped with cereals (Fujita et al., 1992): pigeon peas with sorghum in India, cowpeas with sorghum or maize in West Africa. The superiority of legume-cereal intercropping is well established for these long-duration annual crop associations. However, the long-term viability of these systems is still uncertain, unless they can be more effectively subjected to sustained improvements in agronomic efficiency.

In the temperate zone, legume oilseeds, particularly soybeans (Keyser and Li, 1992) and forages (Ledgard and Steele, 1992) are of major importance. Intensive agronomic research has assured their products as valued commodities. However, their indirect role as BNF suppliers to subsequent crops in rotations, although readily perceived by farmers, is discounted in practice. This occurs because of the lack of dependable quantitative measures of the actual N contribution, season by season, at the field level. Consequently, farmers usually ignore the contribution of the prior legume N when they calculate N fertilizer applications to the following cereal. Unless better tools to estimate legume N contributions are perfected, legumes may be found to unintentionally accelerate nitrate leaching into the groundwater, thus creating negative environmental consequences.

Socio-cultural constraints

It is important to emphasize that the constraints to fuller adoption of BNF technologies are not solely scientific but include cultural, educational, economic and political factors. A successful BNF-based program, therefore, must involve, in addition to scientific research, efforts in training, education, outreach and technical assistance. Evaluations of socio-economical constraints are needed to publicize the benefits of BNF technology and provide advance warnings about potential difficulties, facilitating their removal or circumvention.

Most farmers in developing countries do not know that legumes fix nitrogen in their root nodules. Yet traditional and modern farming systems of the tropics almost invariably include legumes. The legume cultivation results from recognition by farmers over many centuries that legumes are valuable components in farming systems rather than from intentional exploitation of biological nitrogen fixation per se.

The cost of inoculants is not usually a constraint to their use by farmers who outlay capital for seed. Inoculant cost will seldom exceed 1% of the seed cost. For subsistence farmers who do not ordinarily purchase seed off the farm, the capital outlay for inoculant, albeit small, may be a disincentive to the use of inoculants. Cost becomes a more important consideration with granular forms of inoculant in which the rate of application is much higher than with seed-applied inoculant.

BNF technology is a difficult technology to deliver by normal extension mechanisms. Thus a lack of illustrative and explanatory pamphlets and other aids, both for extension agents and farmers with whom they have contact, is also a constraint to implementation of BNF technology at the farm level.

Furthermore, few of the senior administrators and decision makers who determine agricultural policy in developing countries are fully aware of the opportunities for legume-based BNF technology in the agriculture sector of their countries. Most policy-makers are aware of some of the attributes of legumes, but relatively few appreciate the role played by biological nitrogen fixation in legumes. Among those few an even smaller proportion recognizes that it may be essential to employ specific technologies to ensure that nitrogen fixation occurs at all, let alone at a maximal rate. Thus, there is a need for educational material, especially developed for this clientele group, bringing to their attention the real need to adapt currently available technology to the particular circumstances in which it is to be employed in their country.

The task of training and educating persons to deliver BNF information and material has also been made difficult by the critical shortage of specialists with knowledge and interest in practical and applied aspects of the technology. The mass exodus of microbiologists and plant biologists from applied BNF to molecular biology and genetics has created a serious void in programs addressing BNF needs of sustainable agriculture systems in developing countries.

National and international subsidies for nitrogen fertilizers, in both developed and developing countries, have been a disincentive for farmers to use BNF products. However, as national debts rise and subsidies are phased out, BNF becomes more attractive and acceptable. Donor agencies should do more to educate and train in-country personnel to be able to respond to increasing demands for BNF, as sustainability and environmental issues demand biological alternatives to the use of industrial fertilizers.

Crop subsidies and support programs are, in some countries, limited to cereal crops. The United States is a major example. This situation distorts the entire agricultural system. It provides a de-facto incentive to abandon legume-cereal crop rotations in favor of cereal mono-cropping. In countries where virtually no differential crop support is practiced, for example in Australia, exploitation of cereal-legume systems is a dominant feature of agriculture.

Conclusion

Fertilizer nitrogen has become a major input in crop production around the world. Farmers have become increasingly dependent on off-farm supplies, which requires cash and may not always be available on time. The harmful effects on the environment of heavy use of N fertilizer are becoming more evident. Further, the fossil fuels which are used in the production of N fertilizer are becoming scarcer and more expensive. At the same time, the demand for food is going up as populations increase. Therefore, there is a great need to search for all possible avenues to improve biological nitrogen fixation and its use by farmers. There is a need of sustainable farming which maintains soil fertility by using renewable resources easily and cheaply available on the farm. Examples are: rotating cereal crops with legumes, recycling manure and other organic wastes, and using chemical fertilizers moderately, but efficiently.

A quantitative understanding of the ecological factors that control the fate and performance of BNF systems in the field is essential for promotion and successful adoption of these technologies. We can no longer afford the time and expense of trial-and-error experimentation, which has been the hallmark of experimental agricultural sciences in the past. Future research will have to be directed towards approaches that generate information that is transferable from one site to another. Scientists from biophysical and socio-economical fields should work together to identify and eliminate farmers' limitations in using BNF technology.

Recently, research efforts on basic aspects of biochemistry and genetics of biological nitrogen fixation have increased considerably. Yet, we are still hoping for breakthroughs in *nif* gene transfer from legumes to nonlegumes. Much more basic work will still be required. However, in the meantime, we should ask whether basic research is pursued at the expense of applied research. Relevant basic research must continue, but of equal or greater importance is applied, farm-level research.

References

Alexander M 1985 Ecological constraints on nitrogen fixation in agricultural ecosystems. Adv. Microb. Ecol. 8, 163–168.

Almendras A S and Bottomley P J 1988 Cation and phosphate influences on the nodulating characteristics of indigenous serogroups of *Rhizobium trifolii* on soil grown *Trifolium subteraneum* L. Soil Biol. Biochem. 20, 345–352.

Atkins C A 1986 The legume/Rhizobium symbiosis: Limitations to maximizing nitrogen fixation. Outl. Agric. 15, 128–134.

Barker R and Chapman D 1988 The Economics of Sustainable Agricultural Systems in developing Countries. Mimeo, Cornell University, Ithaca, NY.

Beck D P and Munns D N 1984 Phosphate nutrition of *Rhizobium* spp. Appl. Environ. Microbiol. 47, 278–282.

Beck D P and Munns D N 1985 Effect of calcium on the phosphorus nutrition of *Rhizobium meliloti*. Soil Sci. Soc. Am. J. 49, 334–337.

Bohlool B B 1987 Fluorescence methods for study of Rhizobium in culture and *in situ*. *In* Symbiotic Nitrogen Fixation Technology. Ed. G H Elkan. pp. 127–147. Marcel Dekker, Inc., New York.

Bohlool B B and Schmidt E L 1973 Persistence and competitive aspects of *Rhizobium japonicum* observed in soil by immunofluorescence microscopy. Soil Sci. Soc. Am. Proc. 37, 561–564.

Bohlool B B and Schmidt E L 1980 The immunofluorescence approach in microbial ecology. *In* Advances in Ecology. Ed. M Alexander. pp 203–235. Vol. 4. Plenum Press, New York, London.

Bouldin D R 1986 The chemistry and biology of flooded soils in relation to the nitrogen economy in rice fields. *In* Nitrogen Economy of Flooded Rice Soils. Eds. S K De Datta and W H Patrick, pp 1–14. Martinus Nijhoff Publishers, Dordrecht, The Netherlands.

Byrlee D 1987 Maintaining the momentum in post-green revolution agriculture: A micro-level perspective from Asia. MSU International Development Paper No. 10, Michigan State University, East Lansing, MI.

Callaham D, Del Tredici P and Torrey J G 1978 Isolation and cultivation *in vitro* of the actinomycete causing root nodulation in Comptonia. Science 199, 899–902.

Cassman K G, Munns D N and Beck D P 1981 Growth of Rhizobium strains at low concentrations of phosphate. Soil Sci. Soc. Am. J. 45, 520–523.

Danso S K A and Owiredu J D 1988 Competitiveness of introduced and indigenous cowpea Bradyrhizobium strains for nodule formation on cowpeas *Vigna unguiculata* (L.) (Walp.) in three soils. Soil Biol. Biochem. 20, 305–310.

Danso S K, Bowen G D and Sanginga N 1992 Biological nitrogen fixation in trees in agroecosystems. Plant and Soil 141, 177–196.

Diem H G, Gauthier D and Dommergues Y R 1982 Isolation of *Frankia* from nodules of *Casuarina equisetifolia*. Can. J. Microbiol. 28, 526–530.

Dowling D N and Broughton W J 1986 Competition for nodulation of legumes. Annu. Rev. Microbiol. 40, 131–157.

Dunigan E P, Bollick P K, Huchinson R L, Hicks P M, Azubrecher F C, School S G and Mowers R P 1984 Introduction and survival of an inoculant strain of *Rhizobium japonicum* in soil. Agron. J. 76, 463–466.

Ellis W R, Ham G E and Schmidt E L 1984 Persistence and recovery of *Rhizobium japonicum* in a field. Agron. J. 76, 573–576.

Evans H J 1975 Enhancing Biological Nitrogen Fixation. National Science Foundation, Dir. Biological and Medical Sciences, Washington, DC 20550.

Freire J R 1984 Important limiting factors in soil for the Rhizobium-legume symbiosis. *In* BNF Ecology, Technology and Physiology. Ed. M Alexander. pp 51–74. Plenum Press, New York, London.

Fujita K, Ofosu-Budu K G and Ogata S 1992 Biological nitrogen fixation in mixed legume-cereal cropping systems. Plant and Soil 141, 155–176.

Garrity D P 1990 Agronomic research on biofertilizers at IRRI: Overcoming systems-level constraints. *In* Proc. of the Nat. Symp. on Bio- and Organic Fertilizers. Univ. of the Philippines, Los Baños, Philippines. pp 309–320.

Garrity, D P, Roberto T B, Crecensia C B, Pye Tin and Riaz M 1990 *Indigofera tinctoria:* Farmer-proven green manure for rainfed ricelands. *In* Proc. of the Nat. Symp. on Bio- and Organic Fertilizers. Univ. of the Philippines, Los Baños, Philippines. pp 261–284.

Gauthier D, Diem H G, Dommergues Y R and Ganry F 1985 Assessment of N_2 fixation by *Casuarina equisetifolia* inoculated with *Frankia* ORS021001 using ^{15}N methods. Soil Biol. Biochem. 17, 375–379.

George T, Ladha J K, Buresh R J and Garrity D P 1992 Managing native and legume-fixed nitrogen in lowland rice-based cropping systems. Plant and Soil 141, 69–91.

George T, Singleton P W and Bohlool B B 1988 Yield, soil nitrogen uptake, and nitrogen fixation by soybean from four maturity groups grown at three elevations. Agron. J. 80, 63–567.

Gibson A H 1977 The influence of environmental and managerial practices in the legume-Rhizobium symbiosis. *In* A Treatise on Dinitrogen Fixation. Section IV: Agronomy and Ecology. Eds. R W F Hardy and A H Gibson. pp 393–450. Wiley, New York.

Helyar K R and Munns D N 1975 Phosphate fluxes in the soil-plant system. Hilgardia 43, 103–130.

Holben W E, Jansson J K, Chelm B K and Tiedje J M 1988 DNA probe method for the detection of specific microorganisms in the soil bacterial community. Appl. Environ. Microbiol. 54, 703–711.

Ishizuka J 1992 Trends in biological nitrogen fixation research and application. Plant and Soil 141, 197–209.

Kang B T 1990. Alley farming. Adv. Agron. 43, 315–359.

Keeney D 1982 Nitrogen management for maximum efficiency and minimum pollution. *In* Nitrogen in Agricultural Soils. Ed. F J Stevenson. Agronomy Monograph 22, pp 605–649. ASA, Madison, WI.

Keyser H H and Munns D N 1979 Effects of calcium, manganese, and aluminum on growth of rhizobia in acid media. Soil Sci. Soc. Am. J. 43, 500–503.

Keyser H H and Li F 1992 Potential for increasing biological nitrogen fixation in soybean. Plant and Soil 141, 119–135.

Khanna-Chopra R and Sinha R 1989 Impact of climate variation on production of pulses. *In* Climate and Food Security. pp 219–236. The Int. Rice Research Inst., Los Baños, Philippines.

King F H 1911 Farmers of Forty Centuries. Rodale Press, Inc., Emmaus, PA. 441 p.

Koyama T and App A 1979 Nitrogen balance in flooded rice soils. *In* Nitrogen and Rice. The Int. Rice Research Inst. pp 95–104. Los Baños, Philippines.

Ladha J K, Pareek R P and Becker M 1992 Stem-nodulating legume-Rhizobium-symbiosis and its agronomic use in lowland rice. Adv. Soil Sci. *In press*.

Ladha J K, Pareek R P, So R and Becker M 1990 Stem nodule symbiosis and its unusual properties. *In* Nitrogen Fixation: Achievements and Objectives. Eds. P M Gresshoff, L E Roth, G Stacey and W L Newton. pp 633–640. Chapman and Hall, New York, London.

Ledgard S F and Steele K W 1992 Biological nitrogen fixation in mixed legume/grass pastures. Plant and Soil 141, 137–153.

Leung K and Bottomley P J 1987 Influence of phosphate on the growth and nodulation characteristics of *Rhizobium trifolii*. Appl. Environ. Microbiol. 53, 2098–2105.

Lumpkin T A and Plucknett D L 1982 Azolla as a green manure: Use and management in crop production. Westview Tropical Agriculture Series No. 5, Westview Press, Boulder, CO. 225 p.

Munns D N 1977 Soil acidity and related matters. *In* Exploiting the Legume-Rhizobium Symbiosis in Tropical Agriculture. Eds. J M Vincent, A S Whitney and J Bose. pp 211–236. Univ. Hawaii Coll. Trop. Agr. Misc. Publ. 145.

Munns D N and Franco A A 1982 Soil constraints to legume production. *In* Biological Nitrogen Fixation Technology for Tropical Agriculture. Eds. P H Graham and S C Harris. pp 133–152. CIAT Workshop 1981, Calí, Columbia.

National Academy of Sciences 1979 Tropical Legumes: Resources for the Future. Washington, DC. 331 p.

National Research Council (US) 1984 Casuarinas: Nitrogen-fixing Trees for Adverse Sites. National Academy Press, Washington, DC. 118 p.

Odum E P 1989 Input management of production systems. Science 243, 177–182.

Peoples M B and Crasswell E T 1992 Biological nitrogen fixation: Investments, expectations and actual contributions to agriculture. Plant and Soil 141, 13–39.

Pingali P L, Moya P F and Velasco L E 1990 The post-green revolution blues in asian rice production – The diminished gap between experiment station and farmer yields. IRRI Social Science Division paper No. 90-01.

Plucknett D L and Smith N J H 1986 Sustaining agricultural yields. Bioscience 36, 40–45.

Pradhan P 1988 The establishment requirements of *Sesbania rostrata* as a pre-rice green manure. M.Sc. Thesis. Univ. of the Philippines, Los Baños, Philippines. 189 p.

Roger P A and Ladha J K 1992 Biological N_2 fixation in wetland rice fields: Estimation and contribution to nitrogen balance. Plant and Soil 141, 41–55.

Russell J P, Beech D F and Jone P N 1989 Grain legume productivity in subsistence agriculture. Food Policy 14, 129–142.

Sanchez P A 1980 Management considerations for acid soils with high phosphorus fixation capacity. *In* The Role of Phosphorus in Agriculture. pp 471–514. American Society of Agronomy, Madison, WI.

Shanmugasundaram S 1988 Seed production and management of mungbean and soybean. *In* Sustainable Agriculture: Green Manure in Rice Farming. pp 359–376. The Int. Rice Research Inst., Los Baños, Philippines.

Singer M J and Munns D N 1987 Soils, an Introduction. Macmillan, New York, 492 p.

Singleton P W, Abdel-Magid H M and Tavares J W 1985 The effect of phosphorus on the effectiveness of strains of *Rhizobium japonicum*. Soil Sci. Soc. Am. J. 49, 613–616.

Singleton P W and Bohlool B B 1983 Effect of salinity on the functional components of the soybean-*Rhizobium japonicum* symbiosis. Crop Sci. 23, 259–262.

Singleton P W, Bohlool B B and Nakao P 1992 Legume response to rhizobial inoculation in the tropics: Myths and realities. *In* Myths and Science of the Soils of the Tropics. Eds. R Lal and P Sanchez. Soil Sci. Soc. Am. Spec. Publ. No. 29. Madison, WI. *In press*.

Singleton P W and Tavares J W 1986 Inoculation response of legumes in relation to the number and effectiveness of indigenous Rhizobium populations. Appl. Environ. Microbiol. 51, 1013–1018.

TAC CGIAR 1988 Sustainable agricultural production: Implications for international agricultural research. CGIAR (Consultative Group on International Agricultural Research) Meeting, Berlin, Germany.

Thies J E, Singleton P W and Bohlool B B 1991a Influence of the size of indigenous rhizobial populations on establishment and symbiotic performance of introduced rhizobia on field-grown legumes. Appl. Environ. Microbiol. 57, 19–28.

Thies J E, Singleton P W and Bohlool B B 1991b Modeling symbiotic performance of introduced rhizobia in the field by use of indices of indigenous population size and nitrogen status of the soil. Appl. Environ. Microbiol. 57, 29–37.

Tjepkema J D, Schwintzer C R and Bensen D R 1986 Physiology of actinorhizal nodules. Annu. Rev. Plant Physiol. 36, 209–232.

Torrey J G 1978 Nitrogen fixation by actinomycete-nodulated angiosperms. Bioscience 28, 586–592.

Urquiaga S, Botteon P B L and Boddey R M 1989 Selection of sugarcane cultivars for associated biological nitrogen fixation using ^{15}N labelled soil. *In* Nitrogen Fixation with Non-Legumes. Eds. F A Skinner, R M Boddey and I Fendrik. pp 311–319. Kluwer Academic Publisher, Dordrecht, The Netherlands.

Watanabe I and Liu C C 1992 Improving nitrogen-fixing systems and integrating them into sustainable rice farming. Plant and Soil 141, 57–67.

Weaver R W and Frederick L R 1974a Effect of inoculum rate on competitive nodulation of *Glycine max* L. Merrill. I. Greenhouse studies. Agron. J. 66, 229–232.

Weaver R W and Frederick L R 1974b Effect of inoculum rate on competitive nodulation of *Glycine max* L. Merrill. II. Field studies. Agron. J. 66, 233–236.

Weber D F and Miller V L 1972 Effect of soil temperature on *Rhizobium japonicum* serogroup distribution in soybean nodules. Agron. J. 64, 796–798.

Willey R W 1979 Intercropping – its importance and research needs. Part 2: Agronomy and research approaches. Field Crops Abstr. 32, 73–85.