ANNUAL REPORT

JUNE 1978 - JULY 1979

U.S. PARTICIPATION IN AN INTERNATIONAL PROGRAM OF COOPERATION IN TRAINING AND RESEARCH IN NITROGEN FIXATION IN THE TROPICS

Report of activities undertaken by the Board on Science and Technology for International Development, National Academy of Sciences, in cooperation with the Brazilian National Research Council and Empresa Brasileira de Pesquisa Agropecuaria, with grant funds provided by the Office of Agriculture, Technical Assistance Bureau, Agency for International Development, Grant No. AID/ta-G-1329

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CONTENTS

I. Introduction and Background

The International Program of Cooperation in Training and Research in Nitrogen Fixation in the Tropics was conceived at a meeting in November 1974 between a group of Brazilian scientists, headed by Dra. Johanna Dobereiner, and its counterparts from the USA, UK, Canada, and Australia. The purpose of the meeting was to review and discuss the research and training program at the Soils Section, Instituto de Pesquisas Agropecuarias do Centro-Sul (IPEACS) - now known as Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA) - at km 47 on the old highway from Rio de Janeiro to Sao Paulo, 23460 Seropedica, Rio de Janeiro; and the Federal Rural University of Rio de Janeiro (UFRRJ) at the same location. The Brazilian National Research Council (CNPq) and the U.S. National Academy of Sciences (NAS) were also represented. These two organizations have a long-standing program of cooperation, and there also exists the possibility of further cooperation in this area of scientific endeavor linked to Brazilian agricultural development.

The meeting discussed recent advances in knowledge of nitrogen-fixing symbiotic associations in Brazil, including particularly the recent discovery by Dra. Dobereiner's group of bacteria (Spirillum lipoferum) which fix atmospheric nitrogen in the root cells of nonleguminous plants--tropical grasses and cereal crops grown on tropical soils in Brazil.

The meeting concluded that this area of research and training was especially significant because the shortage of nitrogen in tropical soils coupled with rapidly increasing costs of nitrogenous fertilizer, as a result of rising energy costs, places an increasingly important constraint

on the worldwide improvement of agricultural production from tropical soils. The prospect of supplying even a small part of this requirement from symbiotic fixation of atmospheric nitrogen led the group to recommend that the research and training at km 47 be strengthened, and to agree to support it through an International Program of Ccoperation.

The International Program provides a framework through which research on nitrogen fixation in both legumes and tropical cereal and forage crops can be supported at km 47 (1) by periodic exchanges of scientists, including post-doctoral research fellows from the United States and elsewhere to work at km 47 for periods of up to two years; (2) through assistance in purchasing essential items of equipment not readily available in Brazil; and (3) through periodic meetings of the International Advisory Committee to discuss progress in the research and training.

In 1976 the Office of Agriculture of AID's Technical Assistance Bureau provided a grant to the Academy for support of U.S. participation in the program.

II. Highlights of the Program, June 1978 - July 1979

1. An International Workshop on Associative Nitrogen Fixation was held at the Centro de Energia Nuclear na Agriculture (CENA), Piracicaba, Sao Paulo, July 2-6, 1979. A group of U.S. scientists (Drs. Payne, Hubbell, and Klucas) participated under the auspices of the Program. This, the second international symposium to be organized in Brazil, was well attended both by prominent researchers in the field from around the world and by large numbers of Brazilian students. The workshop report is expected later in 1980. A list of contributed papers is appended to this report.

2. Following the workshop, an informal meeting was held at Dra. Dobereiner's laboratory at km 47 to discuss continued international collaboration. Scientists from Europe, India, and the Caribbean participated in addition to those from the United States.

3. An evaluation of the grant to the NAS for support of U.S. participation in the Program was held in late 1978. Following the evaluation, the period of the grant was extended to September 30, 1980. Because of the difficulty in identifying suitably qualified candidates for long-term fellowships, sufficient funds remain to cover the costs of short-term visits by U.S. scientists to Brazil and Brazilian scientists to the United States for the remainder of the program.

4. The research on nitrogen fixation at km 47 continued to be divided roughly equally between symbiotic nitrogen fixation in legumes and associative nitrogen fixation in grasses and other nonleguminous plants.

Current staff and student levels (July 1979) total 26, with three senior program coordinators supported by five permanent staff, five Fellows supported by the CNPq, plus five graduate students and seven undergraduates. In addition, several other staff members of other institutions in Brazil (Dr. Neves from the Department of Plant Physiology at the UFRRJ, Dr. Pedrosa from the Department of Biochemistry at the University of Parana, and Dr. Keuk-Ki Lee, microbiologist at the EMBRAPA Rice and Bean Research Institute) work periodically at km 47 with Dra. Dobereiner. The staff is still somewhat limited in senior (postdoctoral) research personnel, and the periodic visits by NAS-supported short-term collaborators are welcomed for this reason.

The symbiotic nitrogen fixation studies involve numerous crops - soybeans, beans, cowpeas, peanuts and stylosanthes, covering such aspects as rhizobial strain selection, plant genotype, micronutrient requirements, and aluminum toxicity (in cerrado soils) as well as more basic research into nitrogen and carbon metabolism in root nodules.

The asscciative nitrogen fixation principally concerns Azospirillum species, including the physiology of the microorganism and its interaction with plants. Development continued during 1979 of the immunofluorescent and immunodiffusion research on types of Azospirillum in connection with host plant specificity in both C_3 and C_4 plants, and antibiotic resistance was observed in some strains found in grass roots. Preparations were concluded at km 47 for the ^{15}N isotope dilution experiment to measure net nitrogen fixation in grasses due to Azospirillum, the concrete sewer pipes were sunk, and their soils mixed with ^{15}N labeled ammonium sulfate, which is equilibrating. The experiment itself is scheduled to be undertaken over the next growing season. Some work was also undertaken on Derxia, and

there was an interesting minor project on nitrogen fixation and phosphate solubilization in organisms growing in "vinhaça," the stillage residue from sugar cane fermentation which contains C_5 sugars. Papers published, or submitted for publication in these areas, are listed under Section V.

III. Summary of Research, June 1978 - July 1979

The following summary is based on a presentation by Dr. R. Rennie summing up the CENA Workshop:

Some changes in terminology have been adopted. The term "associative symbiosis" has been replaced by "biocoenosis" in recognition of the redundancy implicit in all symbiosis being associative. Biocoenosis in the rhizosphere is therefore "rhizocoenosis," and in the leaf, "phyllocoenosis."

The nature of associations is being recognized as a complex ecological problem, rather than a simple agricultural phenomenon. It is known to involve a wide range of organisms, including Azotobacter, Enterobacter, Baijerinckiae, Azospirilla, and Bacilli, in which the only common factor is the ability to fix atmospheric nitrogen. The nature of the plants is also a factor; C_4 plants were believed to be more active than C_3 plants in both tropical and temperate regions. The key is apparently in the intimacy of the association. We now know that

i) the bacteria are in the roots - through electron microscopy;

ii) they are active in the roots;

iii) they reduce acetylene, reduce nitrogen; and

iv) the reduced nitrogen is transferred to the plant.

What is unknown is the nature of the initial attraction of the plant for the bacterium, and the route of the invasion - whether the bacteria invade via the root tip and spread within the root, or elsewhere. The mode by which the process is protected from oxygen is unclear, as is

the mechanism of the transfer of the fixed nitrogen, and the energetics of the process.

Economic exploitation of the association will depend on the following factors:

i) genetic
ii) reducing power
iii) energy
iv) cofactors (coenzymes I and II)
v) MoFe and Fe protein in equal concentration
vi) anaerobiosis

Recent information has highlighted the specificity of the association. Rennie has shown the absolute specificity of A. brasilense and Bacillus C - 11 - 25 in spring wheat. Other association specificities are well established - Azotobacter paspali : Paspalum notatum, for example; it appears that sugar cane may have associations with many bacteria, and A. lipoferum is specific for maize.

The associations are evidently ubiquitous - the same plants show the same associations on different continents. The magnitude of the fixation is quite impressive. While compared with soybean fixation levels the activity in grasses is of the order of four percent, in agricultural crops (rice) five percent, low in comparison with soya and particularly lucerne, which is about 136-170 percent of soybean levels. In nitrogen deficient soils in the tropics, of which there are extensive hectarages, even small levels of fixation can contribute huge amounts of fixed nitrogen to the nutrition of associated plants. The range of efficiencies currently indicates legumes at 55-300kg N/ha and bacteria 0.3kg N/ha; however, the relative amounts of hectarage indicate important fixation of nitrogen:

	Rate/ha.	Amount
legumes	140	35
rice	30	4
other cereals	5	5
grasses	15	45
forest	10	40

For the future, a reliable technique for the identification and quantification of nitrogen fixation is required. The acetylene reduction technique shows high variability; it is very sensitive to short-term kinetics, but extrapolation to net amounts over areas is impossible. Reduction of $^{15}N_2$ is the only absolute proof. At the meeting, only two papers out of 30 considered this method; other than proving the amount of fixation, it has very few advantages and very low sensitivity. ^{15}N isotope dilution has the advantage of being direct and allows integration of values over time; it is not kinetic, and a nonfixing control is not required. Analytical techniques must be improved.

Other areas of research which should be pursued include

enzymatic interference - hydrogenase activity
- manipulation of enzymes to increase efficiency
- nitrate/nitrite reductase activity

Among the material presented, the following were identified as highlights:

- the number of concrete examples of inoculation of bacteria giving increases in dry matter yield of crops in the field; this was not definitely demonstrated two years ago;
- the unique nature of the plant/bacteria association, which it is possible to control by manipulating the plant genotype;
- there is now excellent data on the invasion of the plant by bacteria, and it is possible to begin to localize the area, see the access, etc.
- ^{15}N is being increasingly used to quantify the fixation;

- the bacteria responsible for nitrogen fixation are being identified with increasingly sophisticated technology applied to their taxonomy. The use of serology to identify varieties and characterize their specificity, either by fluorescent labeling techniques or other, is an important area that needs to be well supported.
- streptomycin resistance is a significant phenomenon, although we do not yet know how many strains are resistant to streptomycin or what it means; this is another important area for future work.

In concluding, Dr. Rennie identified the following needs to support the research over the next several years:

- the rapid exchange of information on the biochemistry and genetics of nitrogen fixation;
- an approach to the rhizobium newsletter to include nonrhizobial fixation information; and
- the creation of a culture collection for nonrhizobial nitrogen fixers.

IV. Personnel Receiving Support from the Project

1. Helvecio De-Polli visited the University of Hawaii from October 17 to November 28, 1978 to collaborate with Dr. Ben Bohlool in applying the immunofluorescence technique to labeling nitrogen fixing bacteria. Mr. De-Polli's expenses while in the United States were covered by the program.
2. Dra. Johanna Dobereiner participated in the International Nitrogen Fixation Symposium, held at the University of Wisconsin, Madison, Wisconsin, in September 1978. Her expenses while in the United States were covered by the program.
3. Dr. W. Jackson Payne, Dean of the Franklin College of Arts and Sciences and Professor of Microbiology at the University of Georgia, Athens, Georgia; Dr. Robert Klucas, Associate Professor of Biochemistry, University of Nebraska, Lincoln, Nebraska; and Dr. David H. Hubbell, Professor of Plant Pathology at the New York State College of Agriculture and Life Sciences, Cornell University, Ithaca, New York, participated in the International Workshop on Associative Nitrogen Fixation, held at the Centro de Energia Nuclear na Agricultura, Piracicaba, Sao Paulo, Brazil, 2-6 July 1979. They were accompanied by Dr. M. G. C. McDonald Dow, Staff Coordinator of the Program at the NAS. International travel for the four U.S. participants was provided by the program; local expenses in Brazil were provided by the CNPq. Prior to the CENA workshop, Drs. Klucas and Hubbell visited Dra. Dobereiner's laboratory at km 47 to consult on the research; following the workshop, Drs. Payne and Dow visited km 47. Dr. Payne spent one week consulting with the research group; Dr. Dow spent three days.

4. Dr. Carlos Neyra, Assistant Professor of Plant Physiology at Rutgers University, Princeton, New Jersey, visited Dra. Dobereiner's laboratory from August 16 to September 18, 1979 to collaborate on research in nitrate reduction and nitrogenase activity in Brachiaria and corn species.

V. LIST OF PUBLICATIONS IN 1978-1979

PAPERS SUBMITTED OR PUBLISHED IN 1979 BY THE PROGRAMA FIXAÇÃO BIOLÓGICA DE NITROGÊNIO

1. BALDANI, J.I., BLASA, R.A.C. e DOBEREINER, J. 1979. Interaction of the enzyme activities nitrogenase and nitrate reductase in various maize genotypes. Intern. Workshop on Associative N_2-Fixation, Piracicaba.

2. BALDANI, J.I., BLASA, R.A.C. e DOBEREINER, J. 1979. Efeito do genótipo do milho na atividade da nitrogenase e da nitrato redutase. Pesq. Agropec. Bras. 14:165-173.

3. BALDANI, J.I., PEREIRA, P.A.A. e DOBEREINER, J. 1979. Host plant specificity of C_3 plants by Azospirillum spp. Inter. Workshop on Associative N_2-Fixation, Piracicaba.

4. BALDANI, J.I., PEREIRA, P.A.A. e NEYRA, C.A. 1979. Contribution to the methodology of excised root assays for evaluation of nitrogenase activities in grass roots. Intern. Workshop on Associative N_2-Fixation, Piracicaba.

5. BALDANI, V.L.D. e DOBEREINER, J. 1979. Host plant specificity in the infection of maize, wheat and rice with Azospirillum spp. Intern. Workshop on Associative N_2-Fixation, Piracicaba.

6. BALDANI, V.L.D. e DOBEREINER, J. 1979. Host plant specificity in the infection of cereals with Azospirillum spp. Soil Biol. Biochem. (in press).

7. BÔAS, F.C.S.V. e DOBEREINER, J. 1979. Efeito de diferentes níveis de nitrogênio mineral, na atividade da nitrogenase e nitrato-redutase em arroz inoculado com 2 estirpes de Azospirillum. Intern. Workshop on Associative N_2-Fixation, Piracicaba.

8. DE-POLLI, H., BOHLOOL, B.B. e DOBEREINER, J. 1979. Immunofluorescence differentiation of Azospirillum species belonging to different host-plant specificity groups. Arch. Microbiol. (in press).

9. DE-POLLI, H., BOHLOOL, B.B. e DOBEREINER, J. 1979. Grouping of Azospirillum spp. by immunofluorescence and immunodiffusion techniques. Intern. Workshop on Associative N_2-Fixation, Piracicaba.

10. DE-POLLI, H., CARVALHO, S.R., LEMOS, P.F. e FRANCO, A.A. 1979. Efeito de micrinutrientes no estabelecimento e persistência de leguminosas em pastagens de morro em solo P.V.A. Rev. Bras. Ciê. Solo (in press).

11. DIDONET, A.D. e DUQUE, F.F. 1979. Eficiência da simbiose de R. japonicum em variedades e linhagens de soja (G. max (L) Merril). XVII° Congr. Bras. Ciê. Solo, Manaus-Am.

12. DIDONET, A.D. e DUQUE, F.F. 1979. Interação entre Al trocável com a fixação biológica de N_2, em soja (G. max (L) Merril). XVII° Congr. Bras. Ciê. Solo, Manaus-Am.

13. DOBEREINER, J. 1979. Prospects for inoculation of grasses with Azospirillum. Intern. Workshop on Associative N_2-Fixation, Piracicaba.

14. DOBEREINER, J. 1979. Fixação de nitrogênio em gramíneas tropicais. Interciência. 4:200-205.

15. DOBEREINER, J., BALDANI, V.L.D., PEREIRA, P.A.A. e BALDANI, J.J. 1979. Increase of antibiotic resistant bacterian in grass roots. Intern. Workshop on Associative N_2-Fixation, Piracicaba.

16. DOBEREINER, J. e DE-POLLI, H. 1979. Diazotrophic Rhizocoenoses. Symposium on Nitrogen Fixation, Univ of Sussex, 17th-19th Sept.

17. DUQUE, F.F., MELO, J.C., SOUZA, R.L.P. e GOMIDE, R.L. 1979. Abertura do cerrado e sistematização do solo. In: V Simpósio sobre o cerrado Uso e Manejo, Brasília, DF.

18. FONSECA, O.O.M. da, DE-POLLI, H. e FRANCO, A.A. 1979. Adubação de micronutrientes para siratro e Stylosanthes em cinco solos do Estado do Rio de Janeiro. XVII° Congr. Bras. Ciê. Solo, Manaus-Am.

19. FRANCO, A.A. and DAY, J.M. 1980. Response of Phaseolus vulgaris L. to lime and Mo on acid soils. Turrialba (submitted).

20. FREITAS, J.L.M., PEREIRA, P.A.A. e DOBEREINER, J. 1979. Effect of organic matter and Azospirillum strain on N metabolism in sorghum vulgare. Intern. Workshop on Associative N_2-Fixation, Piracicaba.

21. NEYRA, C.A. e DOBEREINER, J. 1979. Current trends on nitrogen fixation by tropical rhizocoenosis. Workshop on Associative Symbiosis Held at the University of Florida, Gainesville, Florida, USA (November, 1979).

22. PEDROSA, F.O., STEPHAN, M.P. e DOBEREINER, J. 1979. Interaction of nitrogenase activity and uptake and hydrogenase in Azospirillum brasilense. Intern. Workshop on Associative N_2-Fixation, Piracicaba.

23. PEDROSA, F.O., DOBEREINER, J. e YATES, M.G. 1980. H_2-dependent growth and autotrophic CO_2 fixation by Derxia. J. Gen. Microbiol. (submitted).

24. PEREIRA, P.A.A., BALDANI, J.I., BLAÑA, R.A.G. e NEYRA, C.A. 1979. Efeito dos níveis de NO_3 na atividade de enzimas assimiladoras de nitrogênio e no crescimento reprodutivo de milho. XVII° Congr. Bras. Ciê. Solo, Manaus-Am.

25. PEREIRA, P.A.A., BALDANI, J.I., BLAÑA, R.A.G. e NEYRA, C.A. 1979. Assimilação e translocação de nitrogênio em relação a produção de grãos e proteínas em milho (Zea mays L.). Rev. Bras. Ciê. Solo,

26. PEREIRA, P.A.A., BALDANI, J.I., DOBEREINER, J. e NEYRA, C.A. 1979. Nitrate reduction and nitrogenase activity by excised corn roots. Plant Physiol. (submitted).

27. PEREIRA, P.A.A. e DOBEREINER, J. 1979. Nitrogenase activity and denitrification in intact soil plant core with Brachiaria genotypes. Intern. Workshop on Associative N_2-Fixation, Piracicaba.

28. PEREIRA, P.A.A., NEYRA, C.A. e DOBEREINER, J. 1979. Atividades da nitrogenase e nitrato redutase e níveis de NO_3 em cinco genótipos de Brachiaria. XVII° Congr. Bras. Ciê. Solo, Manaus-Am.

29. ROCHA, R.E.M., BALDANI, J.I., PEREIRA, P.A.A. e DOBEREINER, J. 1979. Host plant specificity in the infection of C_4 plants by Azospirillum spp. Intern. Workshop on Associative N_2-Fixation, Piracicaba.

30. SÁ, J.C.M., DE-POLLI, H. e ALMEIDA, D.L. 1979. Determinação de micronutrientes em diversos materiais calcários comercializados no Estado do Rió de Janeiro. XVII° Congr. Bras. Ciê. Solo, Manaus-Am.

31. STEPHAN, M.P., PEDROSA, F.O. e DOBEREINER, J. 1979. Physiology studies of Azospirillum spp. Intern. Workshop on Associative N_2-Fixation, Piracicaba.

32. VOLPON, A.G.T., DE-POLLI, H. e DOBEREINER, J. 1980. Growth physiology of Azospirillum lipoferum. J. Bacteriol. (submitted).

PAPERS LISTED IN THE 1978 REPORTED AS "IN PRESS" OR "SUBMITTED" AND NOW OUT FOR DISTRIBUTION:

1. FRANCO, A.A., PEREIRA, J.C. and NEYRA, C.A. 1979. Seasonal pattern on nitrate reductase and nitrogenase activities in Phaseolus vulgaris L. Plant Physiol. 63:421-424.

2. FRANCO, A.A., PERES, J.R.R. and NERY, M. 1978. The use of Azotobacter paspali N_2-ase (C_2H_2-reduction activity) to measure molybdenum deficiency in soils. Pl. Soil 50:1-11.

3. MAGALHÃES, F.M.M., PATRIQUIN, D. and DOBEREINER, J. 1979. Infection of field grown maize with Azospirillum spp. Rev. Brasil. Biol. 39:587-596.

4. SCOTT, D.B. and NEYRA, C.A. 1979. Glutamine synthetase and nitrate assimilation in sorghum (Sorghum vulgare) leaves. Can. J. Bot. 57:754-58.

5. SCOTT, D.B., SCOTT, C.A. and DOBEREINER, J. 1979. Nitrogenase activity and nitrate respiration in Azospirillum spp. Arch. Microbiol. 121:141-145.

6. VOLPON, A.G.T. 1979. Fisiologia do crescimento de Azospirillum lipoferum. Tese apresentada ao Decanato de Pesquisa e Pós-Graduação da U.F.R.R.J. para o grau de "Magister Scientiae".

VI. List of Papers Presented at the International Workshop on Associative Nitrogen-Fixation 2-6 July 1979 at CENA, Piracicaba, S.P., Brazil

Physiological interaction in the Azospirillum grass root association.
Hubbell, D.H. - Department of Soil Science, University of Florida, Gainesville; Tien, T.M., Gaskins, M.H.; and Lee, J.K. - Department of Agronomy, University of Florida, Gainesville.

Physiological studies with Azospirillum spp.
Stephan, Marilia Penteado; and Dobereiner, Johanna - Programa Fixacao Biologica de Nitrogenio, EMBRAPA/SNLCS-CNPq, KM 47, 23460 Seropedica, Rio de Janeiro, Brazil; Pedrosa, Fabio de O., Universidade Federal do Parana, Curitiba, Brazil.

Interaction of nitrogenase and uptake hydrogenase activities in Azospirillum brasilense.
Pedrosa, Fabio O. - Departamento de Bioquimica, Universidade Federal do Parana-CNPq-PFBN, Caixa Postal 939 - 80.000 Curitiba - Parana, Brazil; Stephan, Marilia S.P.; and Dobereiner, Johanna - Programa Fixacao Biologica de Nitrogenio, EMBRAPA/SNLCS-CNPq, Km 47, 23460 Seropedica, Rio de Janeiro, Brazil.

Some interactions between Azospirillum spp. and grass seedlings.
Glatzle, A.; and Martin, P. - Institut fur Pflanzenernahrung, Universitat Hohenheim, 7000 Stuttgart 70, W.Germany.

Nitrification-denitrification associated with plan roots.
Payne, W.J.; Sherr, B.F.; and Chalmers, A. - Department of Microbiology, University of Georgia, Athens, Georgia 30602.

Adsorption and mode of entry of Azospirillum brasilense to grass roots.
Umali-Garcia, Mercedes, College of Forestry, University of the Philippines at Los Banos - College, Laguna 3720; Hubbell, David H. and Gaskins, Murray H.- University of Florida, Gainesville; and Dazzo, Frank B. - Michigan State University, East Lansing.

Possible role of plasmid deoxyribonucleic acid in nitrogen fixation in Azospirillum brasilense.
Franco Lemos, Manuel Victor; Santos, Diogenes S.; Trabulsi, Luiz Rachid; and Azevedo, Joao L.

Increased bacterial colonization of the rhizosphere by controlling the soil protozoan population in the Rhizobium-legume system.
Ramirez, Carlos - Escuela de Fitotecnia, Universidad de Costa Rica; and Alexander, Martin - Laboratory of Soil Microbiology, Cornell University, Ithaca, N.Y.

Potential asymbiotic nitrogen fixation by Rhizobium phaseoli L.
Assis, Vera Lucia G. de - Assistant Professor, Universidade Federal de Pelotas, RS, Brazil; and Ruschel, Alaides P. - Centro de Energia Nuclear na Agricultura (CNEN/USP), Piracicaba, SP, Brazil.

Agronomic aspects of biological dinitrogen fixation by Azospirillum spp. in temperate region

Vlassak, K.; and Reynders, L. - K.U. Leuven, Faculty of Agricultural Sciences, Laboratory of Soil Fertility and Soil Biology, Leuven, Belgium.

Factors affecting $(C_2H_2)N_2$ase activity in the root of Pennisetum purpureum.

Samejima, Muneaki

Changes in efficiency of nitrogen fixation in various growth stages of batch cultures of Azospirillum lipoferum.

Volpon, Antonia G.T. - graduate student, Universidade Federal Rural do Rio de Janeiro; De-Polli, Helvecia; and Dobereiner, Johanna - Programa Fixacao Biologica de Nitrogenio, EMBRAPA/SNLCS - CNPq, Km 47, 23460 Seropedica, Rio de Janeiro, Brazil.

Immunofluorescence differentiation of Azospirillum species belonging to different host-plant specificity groups.

De-Polli, Helvecio; and Dobereiner, Johanna - Programa Fixacao Biologica de Nitrogenio, EMBRAPA/SNLCS - CNPq, Km 47, 23460 Seropedica, Rio de Janeiro, Brazil; Bohlool, Behzad Ben - Department of Microbiology, University of Hawaii, Snyder Hall 207, 2538 The Mall, Honolulu, HI 96822.

Nitrogen fixation associated with winter wheat, sorghum, and Kentucky bluegrass.

Klucas, R.V.; Pedersen, W.; and Wood, L.V. - Laboratory of Agricultural Biochemistry; and Shearman, R.C. - Department of Horticulture; all of University of Nebraska, Lincoln 68583.

Host plant specificity in the infection of maize, wheat and rice with Azospirillum spp.

Baldani, Vera Lucia D. - graduate student of Universidade Federal Rural do Rio de Janeiro; Dobereiner, Johanna - Program Fixacao Biologica de Nitrogenio, SNLCS/EMBRAPA - CNPq, KM 47, 23460 Seropedica, Rio de Janeiro.

Response of crops to Azospirillum inoculation in India.

Rao, N.S. Subba - Microbiology Division, Indian Agricultural Research Institute, New Delhi - 110 012, India.

Dinitrogen fixation associated with disomic chromosone substitution lines of spring wheat in the phytotron and in the field.

Rennie, R.J.; and Larson, R.I. - Research Station, Agriculture Canada, Lethbridge, Alberta, Canada T1J 4B1.

Effects of organic matter and inoculant with Azospirillum spp. on nitrogen metabolism of Sorghum vulgarae.

Freitas, Jose Luiz Morgado de; Pereira, Pedro Antonio Arraes; and Dobereiner, Johanna - Programa Fixacao Biologica de Nitrogenio, EMBRAPA/SNLCS - CNPq, Km 47, 23460 Seropedica, Rio de Janeiro, Brazil.

Interactions of nitrate reductase and nitrogenase activities in maize genotypes.

Baldanai, Jose Ivo; Blana, Roberto A.G.; and Dobereiner, Johanna - EMBRAPA, as above.

Nitrogen fixation associated with sorghum and millet.
Dart, P.J.; and Rao, R.V. Subba - ICRISAT, Hyderbad, India.

Plant characteristics and Nase-activity in five inbred lines of maize.
Silva, W.J. da; and Arruda, P. - Departamento de Genetica e Evoluçao - I.B., UNICAMP, Campinas, Sao Paulo, Brazil; Freitas, J.R. de; and Ruschel, A.P. - Centro de Energia Nuclear na Agricultura (CENA) - Piracicaba, Sao Paulo, Brazil.

N_2-fixing populations in seeds and plant parts of teosoide and mazoide plants from Zea mays x Zea mexicana (teosinte) crosses
Ruschel, Alaides P. - Centro de Energia Nuclear na Agricultura (CNEN/USP), Piracicaba, SP, Brazil; and Silva, William Jose - Instituto de Biologia, Departamento de Genetica e Evolucao, UNICAMP, Campinas, Sp, Brazil.

Influence of combined mineral nitrogen on rhizosphere fixation of nitrogen by maize
Pidello, A. - U.N.R., Facultad de Cs. Veterinarias, Laboratorio de Quimica Biologica, Bv. O. Lagos y Ruta 33, 2170 Casilda, Argentina.

La importancia de la fijacion asociativa de N_2 en regiones semiaridas de la Argentina
Merzari, Anibal H.; and Carpio, Dolly - Centro de Radiobiologia, Facultad de Agronomia, Universidad de Buenos Aires.

Infection of maize roots by Azospirillum spp.
Magalhaes, F.M.M.; Patriquin, D.; and Dobereiner, J. - EMBRAPA/SNLCS - CNPq, as above.

Host plant specificity in the infection of C_4 plants with Azospirillum spp.
Rocha, R.E.M.; Baldani, J.I.; and Dobereiner, J. - EMBRAPA/SNLCS - CNPq, as above.

Contribution to the excised root assay methodology for the evaluation of nitrogenase activity in grasses
Baldani, J.I.; Pereira, P.A.A.; Neyra, C.A.; and Dobereiner, J. - EMBRAPA/SNLCS - CNPq, as above.

Prospects for inoculation of grasses with Azospirillum spp.
Dobereiner, J.; and Baldani, Vera Lucia D. (graduate student of Universidade Federal Rural do Rio de Janeiro) - both of EMBRAPA/SNLCS - CNPq, as above.

Physiology of the associative symbiosis in salt marsh cord grass, Spartina alterniflora loisel
Patriquin, D.G.; Boyle, C.D.; Livingstone, D.C.; and McClung, C.R. - Biology Department, Dalhousie University, Halifax, N.S., Canada B3H 4J1.

Increase of antibiotic resistant bacteria in grass roots

Dobereiner, Johanna; and Baldani, Vera Lucia D. (graduate student of Universidade Federal Rural do Rio de Janeiro) - both of EMBRAPA/SNLCS - CNPq, as above.

Nitrogen-fixing bacteria isolated from diverse soils and grass roots in Amazonia

Magalhaes, Fatima Maria Moreira - Division of Agronomy, Instituto National de Pesquisas da Amazonia, Caixa Postal 478, 69000, Manaus, AM, Brazil.

Carbon metabolism of Spartina alterniflora roots and of root and rhizosphere soil diazotrophs

Boyle, C.D.; and Patriquin, D.G. - Biology Department, Dalhousie University, Halifax, Nova Scotia, B3H 4J1, Canada.

Acetylene reducing activity (ARA) by endorhizosphere diazotrophs

Boyle, C.D.; and Patriquin, D.G. - EMBRAPA/SNLCS - CNPq, as above.

Nitrogenase and nitrate reductase activities and denitrification in five genotypes of Brachiaria spp.

Pereira, Pedro A.A.; and Dobereiner, Johanna - EMBRAPA/SNLCS - CNPq, as above.

Nitrogenase and nitrate reductase activities in rice plants inoculated with various Azospirillum strains

Boas, Fernando Cesar S. Villas; and Dobereiner, Johanna - EMBRAPA/SNLCS - CNPq, as above.

$^{15}N_2$ incorporation in rice soils and by Azospirillum lipoferum from rice root association

Rao, V.R.; Charyulu, P.B.B.N.; Nayak, D.N.; and Ramakrishna, C. - Laboratory of Soil Microbiology, Central Rice Research Institute, Cuttack 753006, India.

Associative N_2-fixation by sugar cane

Ruschel, Alaides P. - Centro de Energia Nuclear na Agricultura (CNEN/USP, Piracicaba, SP, Brazil.

Microorganisms in the phyllosphere and rhizosphere of sugar cane

Graciolli, Luiz Antonio - Escola Superior de Agricultura de Paraguacu Paulista (ESAPP); postgraduate student at ESALQ, Piracicaba, SP, Brazil; Ruschel, Alaides Puppin - Centro de Energia Nuclear na Agricultura (CNEN/USP), Piracicaba, SP, Brazil.

Role of Azotobacter in sugar-cane culture and the effect of toxicants on its population in soil

Singh, Kishan; Sinha, A.P.; and Agnihotri, V.P. - Indian Institute of Sugarcane Research, Lucknow-2, India.

Seasonal variation in the microbial population of sugar-cane stalks
Costa, J.M. Ferro - ESALQ postgraduate student, CNPq fellowship, Piracicaba, Sao Paulo, Brazil; Ruschel, Alaides P. - Centro de Energia Nuclear na Agricultura (CNEN/USP), Piracicaba, Sao Paulo, Brazil.

Potential N_2-fixation by sugar cane in solution culture. II. Effect of inoculation; and dinitrogen fixation as directly measured by $^{15}N_2$.
Ruschel, A.P.; Matsui, E.; Vose, P.B. (UNDP, IAEA, Project at CENA); Salati, E. - all of Centro de Energia Nuclear na Agricultura, Piracicaba, Sao Paulo, Brazil.

Inheritance of N_2 fixing ability in sugar cane
Ruschel, Renato - IAA/Planalsucar, Piracicaba, SP, Brazil; Ruschel, Alaides Puppin - Centro de Energia Nuclear na Agricultura (CNEN/USP), Piracicaba, SP, Brazil.

Radiorespirometry studies as an indication of soil microbiological activity in relation to the root system in sugar cane, and comparison with other species
Freitas, Jose Renato de - graduate student in Agronomy, FAPESP fellowship, Piracicaba, SP, Brazil; Ruschel, Alaides P. - Centro de Energia Nuclear na Agricultura (CNEN/USP), Piracicaba, SP, Brazil; and Vose, Peter B. - UNDP, IAEA, Project at CENA.

Determination of the activity of N_2-fixing bacteria in sugar-cane roots and bean nodules using tritiated acetylene reduction technique and electron microautoradiography
Silva, D.M.; Ruschel, Alaides P.; Matsui, E.; Nogueira, Neusa de L.; and Vose, P.B. - Centro de Energia Nuclear na Agricultura, C.P. 96, 13400 Piracicaba, Sao Paulo, Brazil.

Use of ^{15}N enriched gas to determine N_2 fixation by undisturbed sugar-cane plant in the field
Matsui, E.; Vose, P.B. (UNDP/IAEA); Rodrigues, N.S.; and Ruschel, A.P. - Centro de Energia Nuclear na Agricultura, Piracicaba, SP, Brazil.

Relationship between Azospirillum spp. isolates
Saito, Siu M.T. - Centro de Energia Nuclear na Agricultura (CNEN/USP), Piracicaba, SP- Brazil; Graciolli, Luiz A. - Assistant Professor of FAC, Agronomy of Paraguacu Paulista (ESAPP), Sao Paulo, Brazil.

Inoculacion con Rhizobium japonicum en soya (Glycine max (L) Merril), intercalada a cana de azucar (Saccharum oficinarum (L) en el Valle del Cauca, Colombia
Belalcazar Gutierrez, D.J. - Universidad Nacional de Colombia, Facultad de Ciencias Agropecuarias-Palmira, Apartado aereo No. 237, Palmira, Colombia.

Nitrogen-fixing population and activity associated with wetland rice
Watanabe, Iwao; Ito, Osamu; and Barraquio, Wilfredo - Soil Microbiology Department, International Rice Research Institute, Los Banos, Laguna, Philippines

Use of *Azolla* and blue-green algae in rice cultivation in India
Singh, P.K. - Laboratory of blue-green Algae, Central Rice Research Institute, Cuttack-753 006, Orissa, India.

Mineral nutrition and N_2 fixation in *Azolla*
Malavolta, E.; Nakayama, L.I.; and Eimori, I. - ESALQ-UPS, Piracicaba, SP, Brazil; Ruschel, A.P.; and Krug, F.J. - CENA, Piracicaba, SP, Brazil.

Nitrogen fixation by *Azolla-Anabaena* in culture solution
Fiore, Marli de F. - graduate student in biology, FAPESP fellowship, Piracicaba, Sao Paulo, Brazil; and Ruschel, Alaides P - CNEN/USP, Piracicaba, Sao Paulo, Brazil.

Seasonal variations in nitrogenase activity of various rice varieties measured with an *in situ* acetylene reduction technique in the field
Boddey, R.M.; and Ahmad, N. - Department of Soil Science, The University of the West Indies, St. Augustine, Trinidad.

VII. Financial Summary
U.S. Participation in an International Program of Cooperation in Training and Research in Nitrogen Fixation in the Tropics
Grant No. AID/ta-G-1329

	Expenditures 7/1/78-6/30/79
Program Administration	
1. Salaries	$ 461
2. Fringe Benefits	88
3. Travel-international	1,535
4. Communications & Shipping	514
5. Materials & Services	3,221
6. Indirect Costs	719
Subtotal	6,538
To and On Behalf of the Fellows	
7. Travel Grants	3,210
8. Equipment	1,963
9. Insurance	--
10. Indirect Costs	273
Subtotal	5,446
GRANT TOTAL	$11,984