PD-AAQ-223 31629

ANNUAL REPORT

JUNE 1977 - June 1978

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

NATIONAL ACADEMY OF SCIENCES
United States of America

Washington, D.C., December 1979

CONTENTS

I. INTRODUCTION AND BACKGROUND

II. HIGHLIGHTS OF THE PROGRAM, JUNE 1977 - JUNE 1978

III. SUMMARY OF RESEARCH, JUNE 1977 - JUNE 1978

IV. PERSONNEL RECEIVING SUPPORT FROM THE PROJECT

V. LIST OF PUBLICATIONS IN 1977/78

VI. FINANCIAL SUMMARY

I. Introduction and Background

The International Program of Cooperation in Training and Research in n Fixation in the Tropics was conceived at a meeting in November 1974 between a group of Brazilian scientists, headed by Dra. Johanna Dobereiner, and 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) at km 47 on the old highway from Rio de Janeiro to Sao Paulo (now known as EMBRAPA, 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.

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 the rapidly rising costs of nitrogenous fertilizer, as a result of higher 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 Cooperation.

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 2 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 1977 - June 1978

1. An important international symposium on nitrogen fixation was organized in Brasilia July 18-22, 1977. Nearly 200 participants, mainly from Brazil and other Latin American countries, but including a majority of the foremost authorities in the field from North America and Europe, spent five days reviewing progress in nitrogen fixation research principally in legumes and grasses. The symposium was organized by the University of Brasilia with the support of the CNPq, EMBRAPA, the UFRRJ, and a variety of U.S. and U.N. sources. The proceedings of the Symposium were published late in 1978. (See list of publications.)

2. In association with the Brasilia Symposium, a meeting of the International Advisory Committee was held at km 47. This meeting was reported in the Annual Report for 1976-1977.

3. Research highlights.

In legume nitrogen fixation research, work continued on the interaction of soil acidity factors. Phaseolus vulgaris, the common bean and a major Brazilian staple, was shown to have difficulty in extracting molybdenum from acid soils. When suitably treated with an ammonium molybdate solution, the seed, under good growing conditions, has been able to support yields of up to 3 t/ha with no additional nitrogen fertilizer. Investigations of the interaction between nitrate reductase activity and nitrogen fixation showed possibilities for increasing yields markedly through application of nitrogen fertilizer during the grain filling stage rather than early in the growth cycle.

Soybean varieties have been selected for improved nodulation and yield on cerrado soils which are capable of achieving moderate to high yields without nitrogenous fertilizer, thus permitting the extension of soybean production over large areas of these hitherto low-producing areas. Similar work is proceeding with various forage legumes.

In grass-bacterial nitrogen-fixing associations, efforts to identify the factors which affect the growth of the bacteria, now reclassified as Azospirilla, continued. Study of the contribution to the nitrogen nutrition of the plants through their fixation activities also proceeded. Evidence has been gained regarding the specificity of the bacteria for their plant host, which may explain earlier lack of success with inocula.

4. Training. A one-month intensive training course was organized with 20 participants from Brazil, Argentina, Colombia, and Mexico. Professor Bohlool from the University of Hawaii participated through the program and gave both research advice and training in immunofluorescent labeling techniques.

III. Summary of Research June 1977 - June 1978

The following summary is a synopsis prepared by Dra. Döbereiner:

Although research breakthroughs indicating possibilities for extending. biological nitrogen fixation to grasses and cereals were the key event to call attention to the Brazilian research group, legume nitrogen fixation has always been given at least equal consideration. The major research objective of devising modern productive agricultural systems where nitrogen input is based largely on biological fixation certainly must consider the use of grain, forage and green manure legumes as number one priority.

In contrast to concepts of most other groups of "Rhizobiologists," however, which have centered most of their attention on Rhizobium strain selection and inoculation practices, the research objectives at km 47 considered various aspects of microbiology, plant nutrition, plant breeding, and plant physiology which affect legume nitrogen fixation. There are great possibilities of substantially increasing nitrogen inputs through increased N_2 fixation by developing practices which optimize environmental conditions for N_2 fixation as well as inoculation of selected Rhizobium strains.

Earlier work on the interaction of soil acidity factors (mainly Mn and Al toxicities) has been intensified. Large differences between plant species and cultivars were recognized. Beans (Phaseolus), the major basic food of Brazilian people, were found to have difficulties in extracting sufficient Mo from acid soils even if Mo was applied. Mo is the key element in N_2 fixation and Mo deficiency may be a limiting factor for this process in beans. On the other hand, it was shown that when problems of soils, constant humidity, and plant genotype are overcome, beans can obtain all

the nitrogen they need from biological fixation for grain yields of 3t/ha. Studies of the interactions of soil nitrate metabolism (nitrate reductase activity) with nitrogen fixation showed possibilities of complementing nitrogen fixation with low mineral nitrogen doses during grain filling rather than early in the growth cycle.

Soybeans, on the other hand, have quite different requirements, and plant genotype effects seem the most serious problem. Most crosses bred for low latitudes used cultivars such as L2006 (H IAC-2) or Hardee for more indeterminate growth habits. From 11 such crosses, 10 presented the inherent nodulation problems of these cultivars. Special strains were selected which seem more competitive and are able to nodulate cultivars with nodulation problems. These results became specially important for the rapidly increasing soybean areas of the Central Brazilian savannas (cerrados) where Al toxicity, P fixation in the soil, and many still unknown problems aggravate nodulation difficulties. Already in the Cerrado Center of EMBRAPA, agricultural techniques (specific inoculants, four times the usual inoculant rates, proper P, Mo and lime applications) are now available to open cerrado with soybeans which produce 2 to 3t of grain without N fertilizer. 200 x 10^6 ha of such savanna land are available. Inoculants and technology to date in Brazil have reached a point where high yields of soybeans can be obtained all over the country without any nitrogen fertilizer, and millions of tons of fertilizers can be saved with this crop. Various forage legumes have also been studied for establishment in melassis grass hill pastures. While Stylosanthes can be successfully established with P fertilization (super or rockphosphate in plant holes) Centrosema and siratro need Mo and perhaps other minor elements in the

typical RYP or RYL pastures. In pots, N yield increases of 100 percent were obtained with Mo alone, but significant yield increases were also observed with Mn, Fe Zn and B. A biological Mo deficiency screening test was elaborated which showed highly significant correlations between plant response and chemical analyses. By this test almost 50 percent of the soils of Rio de Janeiro State, Sao Paulo, and South of Mato Grosso are Mo deficient. Intrinsic host plant specificity problems of Stylosanthes spp. remain unsolved so far, and in most cases nodulation by soil Rhizobium is more efficient than by inoculated strains. Establishment of this plant depends on early and rapid nodulation which is further enhanced by large cotyledons that play a role in supplying photosynthates for N_2 fixation.

Summarizing, we think that we can say now that under tropical conditions, soil and nutritional factors, constant humidity and plant genotype (but not photosynthesis) are the main limiting factors of nitrogen fixation in the legume symbiosis. Nitrogen fixation can supply all the nitrogen needed for high yields if these limiting factors are eliminated, and only in exceptional cases are legume yield increases obtained with addition of nitrogen fertilizer.

However, the extension of N_2 fixation to the major cereals and forage grasses represents a great challenge, even if only a smaller part of their nitrogen needs can be covered by associations with N_2 fixing facteria. Nitrogen fixation of this kind has now been found in a variety of forage grasses and in the major cereals. Besides large amounts of data obtained in experiments using the C_2H_2 reduction method in soil plant cores and with excised roots, there are now also data available which show $^{15}N_2$ incorporation into plants. The bacterium responsible for several associations was identified

as Azospirillum (syn. Spirillum lipoferum) which infects the roots and can proliferate in inter and intracellular spaces. In tropical regions these organisms are extremely numerous, specially when the land is first brought into cultivation. Numbers in the range of 10^7 or 10^8/g dry roots can be found during the whole growth cycle of maize. Surface sterilized roots contain many fewer bacteria early in the season, but these organisms increase greatly during tasseling when nitrogenase activity of maize, sorghum, wheat, and rice is maximal. It seems, therefore, that nitrogen fixation occurs mainly inside the roots.

Once the existence of associations of grasses and cereals with Azospirillum was established, research efforts were concentrated on the ecology and physiology of this most interesting organism. Reclassification with the creation of two new species became necessary (A. lipoferum and A. brasilense). Both organisms can dissimilate nitrate to nitrite, but there is a group of strains of A. brasilense which does not reduce nitrite further (nir^-). Most A. lipoferum strains also denitrify, that is, reduce nitrite to N_2 which is lost to the atmosphere. Nitrate dissimilation (or as it is often called, "NO_3 respiration") can replace O_2 to support nitrogen fixation. Various kinds of nitrate and nitrite reductase negative mutants were obtained which can fix N_2 in the presence of high NO_3 levels even when O_2 is used for respiration. Another possibility of protecting the nitrogenase from excessive O_2 is a very active uptake hydrogenase which recycles H_2 generated during nitrogen fixation and makes the process at the same time more efficient.

Understanding of all these characteristics in culture should eventually lead to better understanding of the physiology of the plant bacteria

associations. Recently the first experimental evidence of host plant specificity was obtained. Although the highly specific Paspalum notatum, Azotobacter paspali association has been known for years, initial attempts to inoculate grasses with Azospirillum in the U.S. neglected completely the possibility of specifity. Now it has been shown that from uniformly inoculated soil, maize selected for A. lipoferum while wheat and rice were infected almost exclusively by A. brasilense nir^- strains. When such strains were marked with streptomycin resistance, 100 percent of the isolates obtained from field grown wheat were identified as the inoculated strain. Once the kind of strains which infect the various plans were known, possibilities of using selected or manipulated strains for field inoculation became more promising.

Still, as in legumes, optimizing conditions for nitrogen fixation in grasses, e.g., plant genotype, soil fertility, etc., offers much better chances for substantial yield increases than inoculation. Identification of limiting factors and plant breeding practices are dependent, however, on better understanding of the mechanisms of N_2 fixation in grasses.

The simple knowledge of the existence of N_2 fixation in grasses and cereals should stimulate reassessments of economic levels of nitrogenous fertilizers and may already be responsible for considerable economy of this most expensive of the fertilizers.

Both legume and grass N_2 fixation research yielded a large number of publications which are listed below.

IV. Personnel Receiving Support from the Project

Dr. Avilio A. Franco, member of the Brazilian research group, attended the Rhizobium conference at the University of Florida, Gainesville, August 27 - September 3, 1977. His local expenses were covered by grant funds.

The second meeting of the International Advisory Committee was held at km 47 immediately following the International Symposium on Nitrogen Fixation, which the Brazilian Committee organized at the University of Brasilia. The following NAS Committee members and staff traveled to Brazil on grant funds (local expenses were covered by the CNPq):

Dr. R. H. Burris, University of Wisconsin, Madison

Dr. H. J. Evans, Oregon State University, Corvallis

Dr. S. C. Schank, University of Florida, Gainesville

Dr. S. H. West, University of Florida, Gainesville

Dr. M. G. C. McDonald Dow, BOSTID, National Academy of Sciences (staff coordinator), Washington, D.C.

Dr. B. Ben Bohlool of the University of Hawaii visited EMBRAPA for one month beginning June 27, 1978 to teach the immunofluorescence and ecology sections of the Latin American "Intensive Course on Nitrogen Fixation" held at the UFRRJ July 3 - 28, 1978. He also carried out preliminary research on serological characterization of Azospirillum isolates from different parts of the world.

V. List of Publications in 1977/78

BALDANI, J.I., PEREIRA, P.A.A., NEYRA, C.A. and DÖBEREINER, J., 1977. The initiation of acetylene reduction in isolated roots of maize: effect of carbon, oxygen and mineral nitrogen sources. In: Döbereiner, J. et al. (eds.). Limitations and potentials for biological nitrogen fixation in the tropics. Plenum Press, New York. p.356-357.

DAY, J.M., BERKUM, P. van and WITTY, J.F., 1978. Associative symbioses: their contributions through the nitrogen cycle and a critical analysis of the methodology employed in their study. Simposio Internacional sobre Limitações e Potenciais da Fixação de Nitrogênio nos Trópicos, Brasília, p. 38.

DE-POLLI, H., CARVALHO, S.R. de, LEMOS, P.F. and FRANCO, A.A., 1977. Effect of micronutrients on establishment of tropical legumes and persistence on a red yellow podzolic soil hill pasture. In: DÖBEREINER, J. et al. (eds). Limitations and potentials for biological nitrogen fixation in the tropics. Plenum Press, New York. p. 339-340.

DÖBEREINER, J., 1976. Physiological aspects of N_2 fixation in grass-bacteria associations. In: Recent Developments in Nitrogen Fixation (W. Newton, J.R. Postgate and C. Rodrigues-Barrueco eds.) Academic Press, London, p. 513-522.

DÖBEREINER, J., 1976. Environmental effects on the occurrence of Spirillum lipoferum in soils and roots. In: U. Granhall (ed.) Environmental Role of Nitrogen Fixing Blue-Green Algae and Asymbiotic Bacteria. Ecological Bulletin (NFR) 26: p. 343-352.

DÖBEREINER, J., 1977. Plant genotype effects on nitrogen fixation in grasses. In: A. Muhamed, R. Aksel and R.C. von Borstel (eds.). Genetic Diversity in Plants. Plenum Press, New York. p. 325-334.

DÖBEREINER, J., 1977. Fixação de nitrogênio em gramíneas. Revista Brasileira de Ciêcia de Solo: 1: 1-9.

DÖBEREINER, J., 1977. Nitrogen fixation in grass-bacteria associations in the tropics, Advisory Committee Meeting, IAES, Vienna.

DÖBEREINER, J., 1978. Role of Azospirillum in grass associations. Proceedings of the Steenbock-Kettering International Symposium on Nitrogen Fixation, p. 26.

DÖBEREINER, J., 1978. Potential for nitrogen fixation in tropical legumes and grasses. IN: Döbereiner et al. (eds.) Limitations and potentials for biological nitrogen fixation in the tropics. Plenum Press, New York. p. 13-24.

DÖBEREINER, J., 1978. N_2 fixation associated with nonleguminous plants. In. Hollaender, A. et al. (eds.). Genetic engineering for nitrogen fixation. Plenum Press, New York. p. 451-461.

DÖBEREINER, J., BURRIS, R.H., HOLLAENDER, A., FRANCO, A.A., NEYRA, C.A., SCOTT, D.B. (eds.), 1978. Limitations and potentials for biological nitrogen fixation in the tropics. Plenum Press, New York. 398pp.

FRANCO, A.A., 1978. Micronutrient requirements of legume-Phizobium symbiosis in the tropics. In: Döbereiner et al. (eds.). Limitations and potentials for biological nitrogen fixation in the tropics. Plenum Press, New York. p. 161-172.

FRANCO, A.A., FONSECA, O.O.M. da and MARRIEL, I.E., 1978. Effect of mineral nitrogen on the activity of nitrogenase and nitrato-reductase during the soybean cycle in the field. Revista Brasileira de Ciêcia de Solo (in press).

FRANCO, A.A., PERES, J.R.R. and NERY, M., 1978. Determination of molybdenum deficiency in soil due to nitrogenase activity of Azotobacter paspali in culture medium. Plant & Soil (in press).

FRANCO, A.A., 1978. Contribution of the legume-Rhizobium symbiosis to the ecosystem and food production. In: Döbereiner, J. et al. (eds.). Limitations and potentials for biological nitrogen fixation in the tropics. Plenum Press, New York. p. 65-79.

FRANCO, A.A., PEREIRA, J.C. and NEYRA, C.A., 1978. Nitrogen utilization in Phaseolus vulgaris L. In: Döbereiner, J. Nitrogen fixation in the tropics. Plenum Press, New York. p. 335.

FRANCO, A.A. and SOUTO, S.M., 1978. Importance of cotyledonary photosynthesis on symbiosis and early growth of Stylosanthes guyanensis. In: Döbereiner, J. et al (eds.). Limitations and potentials for biological nitrogen fixation in the tropics. Plenum Press, New York. p. 360-361.

FRANCO, A.A., PEREIRA, J.C. and NEYRA, C.A., 1978. Seasonal patterns of nitrate reductase and nitrogenase activities in Phaseolus vulgaris (L). Plant physiology (in press).

FREITAS, J.L.M. de and DÖBEREINER, J., 1978. Effects of oxygen on nitrogenase activity in a sterilized intact grass system inoculated with Azospirillum spp. In: Döbereiner, J. et al. (eds.). Limitations and potentials for biological nitrogen fixation in the tropics. Plenum Press, New York. p. 361-362.

LAERA, N.T., DAY, J.M. and FRANCO, A.A., 1978. Effect of calcium and pH on the response of N_2-fixation to Mo fertilizer in Phaseolus vulgaris. In: Döbereiner, J. et al. (eds.). Limitations and potentials for biological nitrogen fixation in the tropics. Plenum Press, New York. p. 364-365.

MAGALHAES, F.M.M., PATRIQUIN, D. and DÖBEREINER, J., 1979. Infection of field grown maize with Azospirillum spp. Proc. Bras. Acad. Sci. (submitted).

MAGALHAES, L.M.S., NEYRA, C.A. and DÖBEREINER, J., 1978. Nitrate reductase negative mutante of Azospirillum spp. Krieg. In: Döbereiner, J. et al. (eds.). Limitations and potentials for biological nitrogen fixation in the tropics. Plenum Press. New York. p. 351.

MARRIEL, I.E. and DÖBEREINER, J., 1978. Potential of fixation of N_2 and incorporation of mineral-N in soybeans. In: Döbereiner, J. et al. (eds.) Limitations and potentials for biological nitrogen fixation in the tropics. Plenum Press, New York. p.365-366.

NERY, M., ABRANTES, G.T.V., SANTOS, O. dos and DÖBEREINER, J., 1978. Nitrogen fixation (C_2H_2) in wheat. In: Döbereiner, J. et al. (eds.). Limitations and potentials for biological nitrogen fixation in the tropics. Plenum Press, New York. p.348.

NEVES, M.C.P., NERY, M. and DAY, J.M., 1976. Effect of temperature on nitrogen fixation by Spirillum isolates from Digitaria and millet. Anales de XV Congresso Brasileiro de Ciêcia de Solo, p.143-150.

NEYRA, C. A., 1978. Interactions of plant photosynthesis with dinitrogen fixation and nitrate assimilation. In: Döbereiner, J. et al. (eds.). Limitations and potentials for biological nitrogen fixation in the tropics. Plenum Press, New York. P.111-120.

PATRIQUIN, D.G. and DÖBEREINER, J., 1978. Light microscopy observations of tetrazolium-reducing bacteria in the endorhizosphere of maize and other grasses in Brazil. Can. J. Microbial; 24. p.734-742.

PATROQUIN, D.G. and DÖBEREINER, J., 1978. Bacteria in the endorhizosphere of maize in Brazil. In: Döbereiner, J. et al. (eds.). Limitations and potentials for biological nitrogen fixation in the tropics. Plenum Press, New York. p.349.

PEREIRA, P.A.A., BÜLOW, J.F.W. von and NEYRA, C.A., 1978. Nitrogenase and nitrato-reductase activity and accumulation of nitrogen in Zea mays L. (cv Piranão) at two levels of applied nitrogen. Revista Brasileira de Ciêcia de Solo: 2. p.28-33.

PEREIRA, P.A.A., NEYRA, C.A. and DÖBEREINER, J., 1978. Nitrogena activity, assimilation of NO_3 and dentrification in Brachiaria cores. In: Döbereiner, et al. (eds.). Limitations and potentials for biological nitrogen fixation in the tropics. Plenum Press, New York. p.367-368.

PERES, J.R.R., NERY, N. and FRANCO, A.A., 1976. Molybdenum deficiencies in various soils in Rio de Janeiro State according to microbiological tests. Anales de XV Congresso Brasileiro de Ciêcia de Solo. p.163-169.

SA, J.C. de M., DE-POLLI, H. and ALMEIDA, D.L. de, 1978. Determination of micronutrients in commercial calcium fertilizers in Rio de Janeiro State. (In preparation)

SAMPAIO, M.J.A., VASCONCELLOS, L. de and DÖBEREINER, J., 1978. Characterization of three groups within Spirillum lipoferum Beijerinck. IN: U. Granhall. (ed.). Environmental role of bacteria. Ecol. Bull. 26. p.364-365.

SCOTT, D.B. and NEYRA, C.A., 1977. Glutamine synthetase and assimilation of nitrate in sorghum leaves (Sorghum vulgare L.). Canadian Journal of Plant Physiology. (In press).

SCOTT, D.B., SCOTT, C.A., 1978. Nitrate dependent nitrogenase activity in Azospirillum spp. under low oxygen tensions. In: Döbereiner, J. et al. (eds.). Limitations and potentials for biological nitrogen fixation in the tropics. Plenum Press, New York. p.350-351.

SCOTT, D.B., 1978. Ammonia assimilation in N_2-fixing systems. In: Döbereiner, J. et al. (eds.). Limitations and potentials for biological nitrogen fixation in the tropics. Plenum Press, New York. p.223-235.

SCOTT, C.A., MAGALHAES, F.M.M., DIVAN, V.L. dos S. and SCOTT, D.B., 1978. Numbers of Azospirillum spp. associated with the roots of field-grown maize. In: Döbereiner, J. et al. (eds.). Limitations and potentials for biological nitrogen fixation in the tropics. Plenum Press, New York. p.371-372.

SCOTT, D.B., SCOTT, C.A. and DÖBEREINER, J., 1979. Nitrogenase activity and nitrate dissimilation in Azospirillum spp. under anaerobic conditions. Arch. Microbial (in press).

SERPA, A. and DE-POLLI- H., 1976. Genetic variability in the Centrosema-Rhizobium symbiosis. Pesquisas Agropecuarias Brasileiras Serie Zootecnicas 11. p.29-32.

SILVA, M.F.S. da and DÖBEREINER, J., 1978. Occurrence of Azospirillum spp. in soils and roots. In: Döbereiner, J. et al. (eds.). Limitations and potentials for biological nitrogen fixation in the tropics. Plenum Press, New York. p.372.

TARRAND, J.J., KRIEG, N.R. and DÖBEREINER, J., 1978. A taxonomic study of the Spirillum lipoferum group, with descriptions of a new genus Azospirillum gen. nov. and two species, Azospirillum lipoferum (Beijerinck) com. nov. and Azospirillum brasilense sp. nov. Can. J. Microbial: 24. p. 967-980.

VI. 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

Program Administration	Expenditures 7/1/77 - 6/30/78
1. Salaries	$ 2,837
2. Fringe Benefits	1,076
3. Travel-international	5,697
4. Communications & Shipping	124
5. Materials & Services	242
6. Indirect Costs	4,091
Subtotal	14,067
To and On Behalf of the Fellows	
7. Travel Grants	3,993
8. Equipment	---
9. Insurance	---
10. Indirect Costs	200
Subtotal	4,193
GRAND TOTAL	$ 18,260