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Department of Agronomy

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States-of-the-Art

Two of the SOTA documents have been completed and submitted to AID. The third, dealing with BNF associated with lowland rice, has been written and submitted for review to Cornell staff members and to others concerned with the BNF program. The fourth, dealing with cross-inoculation groups of *Rhizobium*-legumes, has essentially been completed, and the summary table is now being prepared together with the summary text. It will shortly be submitted for review to Cornell staff members and to other investigators concerned with BNF.

Advisory Activities and Collaboration

The Cornell program has been coordinated with the other three universities concerned with BNF on the AID program. This has been done by means of correspondence, executive committee meetings and visits to the University of Hawaii and Puerto Rico. In addition, the principal investigator has made trips to Nigeria, Australia, the University of Hawaii and France in connection with the BNF program. The BNF program at Cornell, which is funded by AID and UNDP, has also involved joint research, collaboration and other cooperative activities with the University of West Indies in Trinidad, International Rice Research Institute in the Philippines, International Institute of Tropical Agriculture in Nigeria and the Boyce Thompson Institute in Ithaca, New York. Each

of these centers has their own nitrogen-fixation program, and the Cornell activity has been carried out in coordination with these other programs as well.

The principal investigator is serving as chairman of the Nitrogen Fixation Advisory Committee of U. N. Environment Program/UNESCO/ICRO Panel on Microbiology, and at the present time he is endeavoring to coordinate the UNEP and UNESCO activities with the advisory committee which is probably to be established by the Food and Agricultural Organization of the United Nations. He has also participated in a conference on field testing of Rhizobium strains and helped to plan a series of nitrogen fixation training courses for UNESCO which will be held in a number of developing countries. The principal investigator has also served as the international organizer for BNF training courses held this year in Brazil and Kenya.

Professor T. W. Scott visited the University of West Indies for discussions on the BNF project and related BNF activities with staff members of the Faculty of Agriculture, University of West Indies. While in Trinidad, he also reviewed field experiments with Dr. S. Mughogho, the postdoctoral researcher of Cornell University, who is conducting the field work in Trinidad.

Drs. H. S. Lowendorf, S. Mughogho and T. W. Scott visited Dr. L. Frederick at AID to review the Cornell BNF work in Trinidad. In addition, Dr. Mughogho went to the Dominican Republic for a research meeting and presented a paper on some of the BNF field work done in collaboration with the University of West Indies. Furthermore, Dr. Mughogho presented a summary and status report of the Cornell BNF program at a grain legume workshop held at the University of West Indies in June.

Dr. Mughogho has received an adjunct appointment at the University of West Indies so his work is now integrated into their college of agriculture program.

Field Activities

Dr. Mughogho met with Drs. M. Alexander, T. W. Scott, D. R. Bouldin, R. F. Lucey, T. Grove and H. S. Lowendorf in Ithaca to discuss the field program. During that time, the general direction of the research activity was established. The plan of the program was also submitted to a number of other individuals for review and comment. Land was acquired at the University of West Indies/McGill University Sugarcane Feeds Center in Longdenville, Trinidad. Soils here are believed to be Las Romas, Piarco fine sand and Cunupia, which forms a catena. Soil samples were taken from that site for chemical analyses.

This has been an exceptionally wet year for field work. In June and July, it rained almost every day to such an extent that field work was almost impossible to set up. Two experiments, however, were established. These involved testing the fungicide-resistant Rhizobium strains inoculated on Phaseolus vulgaris as well as the use of a streptomycin-resistant strain of Rhizobium together with nitrogen application rates on soybeans. These experiments were unsuccessful because of the rain.

A report on the on-going field experiments has been prepared and submitted to AID. A phase two revised proposal is now being prepared and will be forwarded to AID once completed.

Laboratory Studies

Several different areas of investigation are being pursued. One program involves ascertaining whether a connection exists between the ability of Rhizobium phaseoli and R. meliloti to grow in acid media and their survival in acid soils. These two bacteria represent the slow and fast-growing rhizobia. In addition, studies are underway to isolate strains that tolerate acid soils and to determine what properties of acid soils are most important in limiting

survival of some strains and what features among the tolerant strains allow them to grow or survive under acid conditions. A total of 42 cowpea and soybean strains of Rhizobium are being tested for tolerance to acidity and aluminum. This screening may subsequently include drought resistance.

Forty strains of cowpea and soybean rhizobia which are spontaneously resistant to antibiotics have been obtained. These strains are now being used in studies of the effect of both temperature and moisture on the growth of the rhizobia in soil. The experiments involve inoculating the labelled rhizobia into soil that has been amended with a carbon source and determining the numbers of rhizobia. The enumeration is not difficult because of the marking of the strains. A parallel study will be conducted soon of the influence of temperature and moisture on the survival of the organisms in both unamended and in carbon-amended soil. Hence, the variables will be studied in terms of both growth and survival.

Work has also been initiated to assess further the differences among rhizobia in their responses to desiccation. The preliminary experiments on survival during drying stress are being conducted with Rhizobium strains for cowpeas, soybeans, beans, alfalfa, lupines and clover. These studies are being conducted with a view to establishing which strains are most tolerant and to determine the possible characteristics of the organisms, for example, extracellular polysaccharides, which are associated with drought tolerance.

Inoculation of R. phaseoli into soil in large numbers led to their decline, and their fall in numbers was correlated with an increase in the abundance of protozoa. However, when the bacteria, which were labelled with resistance traits to permit counting, were inoculated into a soil that was rendered free of protozoa, the population declined only slightly. These data suggest that protozoa are responsible for the decline in numbers of Rhizobium when added

in population densities equivalent to that associated with standard inoculation practices or to that released from the nodule.

When R. phaseoli was introduced into nonsterile soil amended with either thiram or cycloheximide, it survived better than when added to soil not amended with these antiprotozoan chemicals. The data suggest that the effect on survival is not one associated with total numbers of protozoa but with the specific composition of the protozoan community, some groups being more effective in eliminating the root-nodule bacteria from soil than others. The addition to soil of thiram-treated seeds together with the thiram-resistant R. phaseoli resulted in a far smaller decline in the numbers of the root-nodule bacteria in the root zone than on plants developing from seed not amended with the antiprotozoan chemical. These differences in numbers in the rhizosphere suggest that protozoa are causing a marked decline in the numbers of Rhizobium available for infection and subsequent nodulation of bean plants. The data suggest that by altering or reducing the protozoan population, the survival of a Rhizobium inoculum can be improved.

The experiments conducted to date have all been with chemicals, usually fungicides, applied directly to the seed. It would be advantageous to have a systemic fungicide which is translocated downward so that it can be applied to the foliage and have an effect on the microbial community, both rhizobia and protozoa, developing around the root system. To this end, studies have been conducted and are still underway to determine the effect of systemic fungicides on the nodulation of legumes and on the survival of Rhizobium in the legume rhizosphere. The systemic fungicides that have been used are Ridomil, Terricole, Bayleton and Previcur. However, the differences between numbers of Rhizobium in the rhizospheres of fungicide-treated and untreated plants were not statistically significant. Moreover, the number of nodules

were not different between fungicide-treated plants and the controls. The tests, however, are being continued.

Training Activities

At the present time, seven graduate students are involved in the BNF program in the Department of Agronomy. Some of these students are supported by the AID grant, but others are supported by other funds. These students come from Mexico, Nigeria, Venezuela, Taiwan as well as the United States. In addition, there are four postdoctorals involved in the BNF program, two being supported by the AID program. Of the two supported by the AID program, one is from Malawi and the other is an American.

Conclusions

Problems have been encountered in the first phase of the field program, but these have not seriously affected the development of the program.

Considerable progress has been made in laboratory studies of the growth, survival and potential manipulation of *Rhizobium* for improving legume yields.

The collaborative and advisory portions of the program are developing readily.

Recommendations

The program is developing largely in line with our original plan, and no change in emphasis or direction is recommended.

Plans

The future year will involve activities in the same program areas as delineated above and in our original project proposal. A plan for Phase II of the field work will be submitted shortly. The laboratory studies will proceed on the topics that have been explored during the first year of the project.

Advisory activities, collaboration and training activities will be of the same type as conducted in year one.