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## I. Research Activities

Several field studies were conducted in Trinidad to determine the effects of such factors as zinc, phosphorus, nitrogen, and Rhizobium japonicum on the nodulation and grain yield of soybeans. Field studies were also condducted to determine the effects of $CaCO_3$, magnesium, Rhizobium and nitrogen fertilizer timing and rates on cowpea grain yields.

In the field tests of soybeans, grain yields were significantly increased up to 40 kg/ha P. Zinc applications had a positive effect on yield up to 5 kg/ha on an Ultisol. In a comparison of commercial Rhizobium inoculants for soybean, Rhizobium japonicum isolated in Trinidad and two fertilizer N rates on soybean yield, there was an increase in pod number and grain yield with fertilizer N applications. Both strains of Rhizobium resulted in yields inferior to those from fertilizer N. In an additional study on the Ultisol to determine the best time of fertilizer application, a split application of 30 kg/ha at planting plus 30 kg/ha at flowering resulted in the highest yields. In this study, nodulation on all plots was poor.

In the field trials of cowpeas in Trinidad, increasing Mg rates up to 12 kg/ha increased grain yield but reduced nodule numbers where no lime was applied to an Ultisol. On limed soils, there was an increase in acetylene reduction with increased Mg rates.

A study was conducted to determine the effect of inoculation and fertilizer N on nodule activity and cowpea grain yields both before and after a corn crop in a cowpea, corn, cowpea rotation. The experiment has not been completed, but the results thus far indicate an increase in nodule number and weight with inoculation at the first harvest. Fertilizer N increased the grain yield in both inoculated and uninoculated plots. The trend so far has been for higher yields in the inoculated plots with fertilizer N. Also, both inoculation and fertilizer N promoted earlier maturity and reduced the incidence of disease and insect damage.

In a comparison of two cowpea varieties at 3 N rates and with and without inoculation, the ER-7 variety from IITA outyielded the California 5 variety. This variety (ER-7) did not seem to be affected by insects or diseases.

Several strains of three species of Rhizobium were studied to determine their ability to survive in acid soils. Strains of R. meliloti, R. phaseoli, and a cowpea rhizobium were inoculated into sterilized acid and limed soils at densities of $10^7$ to $10^8$ cells per gram, and changes in numbers were moni-

tored. Little change was found in the counts of the three strains in the soil at pH 6.9. In the pH 4.4 soil, however, the numbers of R. meliloti and R. phaseoli fell rapidly by three to four orders of magnitude, whereas the cowpea strain numbers remained at the initially high value. The cowpea strain, when inoculated into nonsterile soils, behaved the same for each soil despite differences in soil pH.

Strains of R. meliloti and R. phaseoli were sought which might survive as well as the cowpea strain in acid soils. A preliminary screening for such strains was attempted on the basis of growth of a small inoculum in defined, liquid medium. Strains were classified by the lowest pH (critical pH, $pH_c$) which permitted growth. The range of $pH_c$ among strains of R. meliloti was 0.6 pH units, and strain 411SE1 (pH 5.9) and GH1-1 (pH 5.3) were chosen for comparison for survival in acid soils.

Following Rhizobium inoculation in soils of pH 6.3, 5.2, and 4.8, the population densities of 411SE1 and GH1-1 behaved similarly. After a rapid initial decline of one to two orders of magnitude, the numbers of both strains returned to the initial number. In a sterile soil of pH 4.6, however, counts of 411SE1 fell rapidly by 6 orders of magnitude, whereas counts of GH1-1 declined only 2 log units and then maintained that level. In nonsterile soils inoculated with the two strains, 10-fold more GH1-1 than 411SE1 survived in soils of pH 6.4 and 5.4. However, in a pH 4.8 soil, 411SE1 virtually disappeared, whereas counts of GH1-1 dropped and then leveled off.

The critical pH for 13 strains of R. phaseoli was found to range from pH 3.8 to 4.6, and some strains tolerated aluminum concentrations in liquid culture of up to 50 μeq/liter. Six of these strains were then tested in sterile acid soils. In a soil of pH 6.3, the numbers of all strains remained at the level added. In a soil of pH 4.6, the density of two strains, CAR 36 and C-09, dropped 0.5 log units, whereas counts for the four remaining strains fell by two orders of magnitude. In a soil of pH 4.3, counts of C-09 declined from the initial value by 1.3 log units, but counts of CAR 36 and three of the other strains fell by 5 to 7 orders of magnitude.

Strain differences among R. meliloti and R. phaseoli were found which may be exploited in seeking inoculants capable of surviving in acid soils. However, none of the strains tested of the above species was as tolerant of acid soils as a cowpea strain.

A study was made to assess the possibility of using mineral soil to replace peat as an inoculum carrier for legume seeds. The survival of five strains of R. meliloti and one strain of R. phaseoli in nonsterile Lima silt loam was measured as the soil underwent air drying. The results showed that less than 2% of the cells of some strains were viable after 9 days, but as many as 23% of the cells of another strain were still viable. Thus, a study of the effect of inoculum size on the survival of Rhizobium in air-dried soil indicated that survival is better with the larger inoculum size. Evaluations of the survival of Rhizobium strains when first grown in sterilized silt loam and then subjected to desiccation revealed a high percentage survival even after three months storage (more than 30% for three of the six strains tested).

The strain that showed the best survival in soil was then grown in sterilized silt loam, and bean seeds were coated with this soil inoculum with the help of gum arabic (50%). The treated seeds were stored at room temperature. Periodic monitoring of survival of cells of this rhizobium showed that the number fell from $3.4 \times 10^8$ to $4.6 \times 10^5$ per seed in 45 days. However, the soil inoculum on the seeds did not dry uniformly, and our previous studies demonstrated that the rate of water loss is important to the survival of rhizobia. Therefore, a study was conducted to assess the survival of Rhizobium when subjected to rapid and extensive drying (in a desiccator). Under these conditions, the percentage survival was improved. We are presently investigating further the influence of rate of drying of the inoculum on seeds.

To u[illegible] the effect of soil pH, organic matter, texture, water content and [illegible] concentration on survival of Rhizobium in soils undergoing desiccation, 25 soils were used. The results indicate that at fairly constant clay content (25-30%), the survival of Rhizobium is closely related to the soil pH ($r = 0.85$, significant at 0.001 level). On the other hand, at near neutral soil pH (6.8-7.2), the survival of Rhizobium in soil is closely related to the clay content ($r = -0.86$, significant at 0.001 level). Organic matter level had no effect on the survival of Rhizobium in these soils.

## II. Cooperative Activities

Cooperation is continuing with the University of West Indies. As indicated above, much of this cooperation is through research. The faculty members at the University of West Indies have been very cooperative and are looking forward to extending the joint program in the future. Cornell faculty members have visited several times, and discussions on future courses of research and cooperative activities have been conducted. Moreover, the cooperation has resulted in the direct participation of Dr. S. Mughogho in academic and professional activities of the University of West Indies' training program. This has involved four individuals: (a) Sanjint Singh, who is now becoming actively involved with the program; (b) Lasane Krominch, who has been doing work on BNF of wing beans and will be preparing a paper to be presented at the January workshop; (c) Jane Awai, who is actively involved in sample analysis; and (d) Lucretia Gabrail, who is presently interacting with Dr. Mughogho in preparation of the draft her thesis.

In addition, joint projects are underway with the International Rice Research Institute in the Philippines, the International Institute of Tropical Agriculture in Nigeria and the Boyce Thompson Institute located at Cornell University. Visits of Cornell researchers on BNF have been conducted and field trials have been initiated or evaluated in both the Philippines and Nigeria, and laboratory work on BNF involving Rhizobium in Nigeria and blue-green algae in the Philippines is in progress. The staff at the Boyce Thompson Institute includes four senior scientists as well as a number of postdoctoral fellows, graduate students and technicians, and the interactions between members of the Department of Agronomy and Boyce Thompson Institute involve occasional collaborative research, frequent discussions on technical problems and joint seminars.

III. Workshop on BNF in Caribbean

A joint workshop involving the University of West Indies and Cornell University on promoting BNF in the Caribbean as well as other areas of the tropics will be conducted the first week of January 1981. The general approach was established in discussions between Drs. Alexander, Lathwell, Scott and Mughogho representing Cornell University and a number of faculty members representing the University of West Indies. The full details of the workshop have been developed and finalized by Dr. Mughogho and Dr. A. Donawa, as well as other faculty members at the University of West Indies. It is expected that about 35 people will be in attendance, and they will come from a number of areas in the Caribbean as well as from other regions.

IV. Consultation

Drs. Alexander, Lathwell, Lowendorf and Scott have all visited the University of West Indies to review various aspects of the research and cooperative activities. These involved extensive discussions between D. J. Lathwell, M. Alexander and S. K. Mughogho on the soil fertility aspects of the BNF program during a June visit and meetings with other members of the University of West Indies faculty. H. Lowendorf met with S. Mughogho and other members of the Department of Soil Science, University of West Indies in March in order to help overcome some of the problems that developed in the research. Most recently, T. W. Scott met with staff members in Trinidad to review the BNF research project underway in the field, to review plans for the BNF workshop and to discuss possible avenues for future research and cooperation.

S. Mughogho visited Guyana with students of the UWI faculty of agriculture, during which time he participated in the various field trips and met with H. B. Persad to further develop the workshop. S. Mughogho made a second visit to Guyana to participate in the symposium on "Management of Clay Soils." In addition, Mr. Herman Adams (CARDI, but formerly with the Guyana Ministry of Agriculture) met several times with S. Mughogho and provided him with six varieties of soybeans to evaluate under Trinidad conditions.

M. Alexander continues his collaborative work with U. N. agencies. He continues to serve on the UNEP-UNESCO-ICRO Microbiology Panel and chairs the Nitrogen Fixation Advisory Committee of that panel. As an extension of that committee, he was asked to participate in and ultimately chair the FAO planning workshop held in Rome in June, at which time a specific series of recommendations were developed to allow FAO to initiate a more extensive program on BNF.

Meetings were also held by various of the investigators concerned with BNF. These included meetings of the executive committee of the BNF consortium, national meetings of agronomy and microbiology societies, and a meeting in Kansas City of principal investigators on SEA/CR-AID projects.

## V. States-of-the-art documents

The last two SOTA documents are being completed. These include one on BNF in lowland rice, and this SOTA should be in its final form by March 1981. The review of the literature for the SOTA on cross-inoculation groups is complete, and all of the material has been brought together in a large number of tables. An overview of cross-inoculation groups and problems in this area is presently being prepared and should be completed January 1981.

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State of the Art

1. D. R. Bouldin, S. Mughogho, D. J. Lathwell, T. W. Scott. 1979
   Nitrogen fixation by legumes in the tropics.
   Cornell Int. Agr. Mimeo 75 (40 p.)
2. H. S. Lowendorf. 1980
   Survival of Rhizobium in soil.
   Adv. in Microbiol. Ecology 4:87-124.
3. M. Alexander.
   Cross-inoculation groups of Rhizobium and legumes.(In preparation).
4. H. S. Lowendorf.
   Biological nitrogen fixation in flooded rice fields.
   (Being reviewed and revised).

Other Publications

Pena-Cabriales, J. J., and M. Alexander. 1979.
Survival of Rhizobium in soils undergoing drying.
Soil Sci. Soc. Am. J. 43:962-966.

Osa-Afiana, L. O. and M. Alexander. 1979.
Effect of moisture on the survival of Rhizobium in soil.
Soil Sci. Soc. Am. J. 43:925-930.

Chao, W. L. and M. Alexander. 1981.
Interaction between protozoa and rhizobium in chemically-amended soils.
Soil Sci. Soc. Am. J. (in press)

Lowendorf, H. S., A. M. Baya and M. Alexander
Rhizobium survival in acid soils (Manuscript in preparation)

Theses finished

J. J. Pena-Cabriales.
Survival of Rhizobium in soil during drying.
Master's Thesis, Cornell Univ. 1978.

L. O. Osa-Afiana.
Moisture relation in the persistance of Rhizobium in soil.
Master's Thesis, Cornell Univ., 1979.

W. L. Chao.
The effect of a fungicide on the interaction between protozoa and fungicide-resistant Rhizobium in soil and rhizosphere.
Master's Thesis, Cornell Univ., 1980.

Students

J. J. Pena-Cabriales--Mexico
W. L. Chao--Taiwan
F. Mendez-Castro--Venezuela
L. O. Osa-Afiana--Nigeria

Postdoctorals

*H. S. Lowendorf (USA)
*R. Graham (Trinidad) ex-S. Mughogho (Zimbabwe)
A. M. Baya (Chile)
S. Odunfa (Nigeria)

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