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ANNUAL REPORT TO AID FOR 1981

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BIOLOGICAL NITROGEN FIXATION IN TROPICAL AGRICULTURE

Department of Agronomy

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Ithaca, NY 14853

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I. Research Activities

Cooperative studies with the University of the West Indies involved greenhouse and field tests. Twenty-nine Rhizobium strains, including local isolates and strains from international collections, were assessed for nodulating and nitrogen-fixing ability in symbiosis with Vigna unguiculata in Leonard jars. The Rhizobium strains were found to differ significantly in both nodulating capacity and nitrogen-fixing ability. Several local strains performed well and appear to have potential for improving nodulation and nitrogen fixation in the field. However, the recommended strain for cowpeas (CB 756) and other crops of the same cross-inoculation group was not among the best strains.

Six Rhizobium strains were selected for a study of the response to inoculation of cowpeas grown in soil in pots. The soils were Cunupia clay (pH 4.9) and St. Augustine loam (pH 5.8). Inoculation failed to increase nodule weight or total N significantly in St. Augustine loam. In Cunupia clay, one strain increased nodule weight significantly. In both soils, the addition of 50 ppm N gave total N values in excess of either inoculated or uninoculated treatments.

To ensure adequate seed material for host studies, cowpea seed material was procured, and the germplasm was multiplied in the field at St. Augustine. Observations were made during growth on time to flowering and maturity, as well as on pest and disease resistance. Seed stocks in excess of 2 kg now exist for 22 cowpea cultivars. Sixty-four accessions were obtained from the Plant Introduction Center, Georgia, and 40 of these are currently being multiplied and evaluated. Ten of the cultivars exhibited considerable variation in both total N fixed and nodulating capacity. California 5, the most commonly grown variety in Trinidad, performed well in both assessments. To determine whether the observed host differences are genetic and therefore constant, two cultivars, Laura B. and Ife Brown, are being screened against 5 Rhizobium strains. Five more varieties (ER7, Vita 1, Vita 4, Vita 5 and Los Banos Bush Sitao No. 1) are simultaneously being evaluated for nodulating capacity and nitrogen-fixing ability.

In field studies at the University of West Indies, the response of cowpeas (California 5) to inoculation was studied on a St. Augustine loam soil (pH 5.8). Inoculation with two Rhizobium strains had no significant effect on seed yield and seed weight or on nodule weight and total plant N. The application of 50 kg/ha N improved seed yield significantly without appearing to depress nodulation significantly.

Four Rhizobium strains, an uninoculated control and a control were used as treatments in another experiment with cowpeas on St. Augustine loam. Two of the rhizobia are isolates from the locality of cultivation. Two cultivars

(California 5 and Crimson) were used. Evaluation will be at 35 days for nodule weight and total plant N, and at harvest for seed yield and yield components.

A study was conducted of the effect of liming on nodulation of Phaseolus vulgaris grown in pots of St. Augustine loam. No nodules were found on any plants in any of the treatments, whether inoculated or not. However, liming appeared to improve plant growth. Plants supplied with N were significantly different from those in the uninoculated and inoculated treatments.

An experiment was initiated to see whether nodulation could be stimulated by either liming, the use of acid-tolerant strains of R. phaseoli or both. The acid tolerant strains were derived from studies conducted in Ithaca. Beans were grown in pots of St. Augustine loam. However, no nodules were found on any plants in any treatment. P. vulgaris has been known not to nodulate at temperatures greater than 30°C, and ambient temperatures in the greenhouse may go as high as 37°C. Twelve temperature-tolerant and acid-tolerant cultivars have been obtained from CIAT to continue these studies.

Bagasse, dried cowpea shoots and a compost (mixture of soil and decomposing plant material) were compared with irradiated and autoclaved peat for their ability to support Rhizobium growth. Preliminary results indicate that none of the potential carriers supported growth for very long, even when amended with a carbon source (glucose) or a vitamin source (yeast extract).

In studies conducted in Ithaca, differences among species of Rhizobium in ability to survive in acid soils were found. The tolerance to acid soils was greatest with cowpea rhizobia, less with R. phaseoli, and R. meliloti was the most sensitive. Within species differences have also been noted. For R. phaseoli, tolerance to acid soils correlated well with tolerance to high acidity and Al concentration in culture. Tolerant strains of this species, however, did not survive well in all acid soils tested. There seems to be no correlation between survival and soil pH, KCl-extractable Al, or Mn, all of which inhibit plant growth. For the tolerant strains, Ca in culture did not counteract Al inhibition, nor did H enhance it. How Al, Ca, and H interact in soil to affect rhizobial survival is now under investigation. In addition, how tolerant and intolerant strains nodulate Phaseolus vulgaris and fix nitrogen in acid soils is also being examined.

Differences among R. meliloti strains in ability to grow in acidified culture medium were found. However, strains could survive well in culture at pH values (4.7) well below the minimum pH allowing growth (5.3-5.9). In sterile soils, survival was good, and rhizobial growth was observed in a soil of pH 4.8. In nonsterile soils, on the other hand, survival was reduced in all soils below pH 6.0. Simple tests have not yet been found that predict the ability of R. meliloti to survive in acid soils, nor have strains been found which survive as well as R. phaseoli or cowpea strains.

One Al-sensitive and two Al-tolerant cowpea Rhizobium strains were tested for their ability to survive and to nodulate cowpeas in a soil with Al concentrations from < 0.06 to 0.95 meq/100 g. Stable antibiotic-resistant mutants were obtained to allow enumeration of the rhizobia in soil by plate counts on antibiotic-containing medium. Only minor differences in survival were evident

among the 3 strains after 7 weeks, regardless of the Al concentration or whether a plant was present. In soil with the highest Al concentration, plants inoculated with the Al-sensitive strain had significantly fewer nodules compared to plants inoculated with Al-tolerant strains. However, the total nodule weights of the plants inoculated with the Al-sensitive strain were the same, regardless of the Al concentration. Plant weights were not statistically different. These results show that soil Al is of minor importance to the survival of cowpea rhizobia but suggest that soil Al is important in nodulation.

The extent of survival of Rhizobium as the soils underwent drying was generally related to soil pH, clay content, organic matter and occasionally to the levels of the total available Al or P. Certain strains persist in large numbers during the drying of some mineral soils, suggesting that such soils might be good carriers for inocula of root-nodule bacteria. Therefore, soybean, alfalfa and kidney bean seeds were coated with both soil-base and peat-base inocula and allowed to dry at room temperature. After 6 weeks, the number of surviving rhizobia on seeds coated with soil-base inoculum was as high or higher than on seeds coated with peat-base inoculum.

Spontaneous mutants of R. japonicum resistant to benomyl or carboxin were obtained by stepwise exposure of parent cultures to higher concentrations of these fungicides. Both these mutants were also made resistant to streptomycin and erythromycin. The resistance markers were found to be retained on prolonged growth. The R. japonicum populations were then estimated in the rhizosphere of soybeans grown from seeds treated and not treated with benomyl. Populations were 1-12 times higher on 3- to 5-day seedlings from seeds treated with benomyl. There were no substantial population differences between the two treatments for the next 60 days. Total bacterial population, however, was 12-13 times higher for the first 3 days on seedlings from seeds not treated with benomyl, after which the populations were similar for both treatments. Protozoa in the rhizosphere were 14-180 times more abundant for the first 1 to 3 days on the seedlings from the seeds not treated with benomyl, but their numbers were similar in subsequent samplings for both treatments.

Experiments were conducted in the greenhouse to determine the effects of inoculation of benomyl- or carboxin-treated seed with benomyl or carboxin-resistant mutants. Seed treatment with benomyl or carboxin and rhizobium resulted in statistically greater plant and nodule weights and higher total nitrogen contents than all other treatments. A statistically significant response to inoculation was observed for only the benomyl treatment.

II. Cooperative Activities

Cooperation is continuing with University of West Indies. The bases for this cooperation is research, but training activities have also been involved; the trainees include Dr. Richard Graham, and a graduate student and undergraduate student at the College of Agriculture of the University of West Indies. Cooperation with faculty members in soil and crop sciences in that college has been excellent, and the AID-supported program has been the basis for developing additional cooperative ventures. Cornell faculty members (T. W. Scott, D. J. Lathwell, and M. Alexander) have visited the University of West Indies several times to discuss future research programs and other cooperative activities.

The Cornell staff member who is assigned to the University of West Indies (Dr. Richard Graham) has been integrated fully in research activities of that University.

Joint research projects are also being conducted with the International Institute of Tropical Agriculture in Nigeria, the International Rice Research Institute in the Philippines and the Boyce Thompson Institute located at Cornell University. The cooperative work with the International Institute of Tropical Agriculture has focussed on cowpeas and soybeans, and the cooperation with the International Rice Research Institute has been directed to devise means to control grazing animals feeding on nitrogen-fixing algae important in rice production. Some of these activities overlap with joint endeavors with the Boyce Thompson Institute and in addition, cooperation is underway with that Institute on other aspects of BNF. The program at the Ithaca campus involves not only collaborative research but joint seminars and frequent discussions on technical problems.

In addition, graduate students from developing countries in Latin America and Asia are pursuing programs at Cornell University. Graduate students from Mexico and Nigeria completed their doctoral research this year and returned home. One from Bengladesh is currently engaged in a Ph.D. dissertation dealing with BNF and soybeans.

M. Alexander continues to serve on the U. N. Microbiology Panel that is concerned with, among other things, the development and integration of international activities dealing with BNF. In addition, he has been involved with planning a training course in Venezuela dealing with root-nodule bacteria.

III. Workshop on BNF in the Caribbean

A workshop was held in January that was jointly sponsored by Cornell University and University of West Indies. Individuals from a number of countries came to these meetings, and the program was quite fruitful and considered a variety of aspects of BNF as applied to the Caribbean region. Visits were also made to various locations in Trinidad. Most of the papers presented at the meeting were brought together and published in a special number of Tropical Agriculture.

IV. Consultation

T. R. Scott consulted with soil and crop scientists at the University of West Indies and reviewed field, greenhouse and laboratory studies there. He also met with personnel in Barbados and Trinidad in regard to extending the BNF program.

D. J. Lathwell attended the workshops in University of West Indies and CIAT, presented papers at those meetings and participated in discussions on BNF.

M. Alexander participated in the workshop at the University of West Indies and met with faculty members of that University in regard to the joint research. In addition, he has been consulting with U. N. programs in regard

to the Microbiological Resource Centers concerned with BNF (in Brazil and Kenya). He also met with personnel of FAO in regard to planning activities in BNF.

Richard Graham visited Guyana to review and discuss methods of inoculant production, seed production and field trials dealing with legumes and BNF. He also participated in joint planning with Dr. Scott during visits to Barbados. Dr. Graham came to Cornell University to discuss the joint research program and to evaluate experimental findings. He also consulted with personnel at Boyce Thompson Institute.

Graduate students and postdoctorals concerned with the program attended the Rhizobium conference in Canada in August.

V. States-of-the-art Documents

All of the SOTA documents are completed. The choice of means of final publication is currently being considered. The SOTA on cross-inoculation groups has been released in tabular form. A computer-designed analysis of affinities among the cross-inoculation groups has also been completed and will be appended to the tabular material in connection with final publication.

VI. Personnel

Richard Graham, postdoctoral, assigned in Trinidad.

Henry Lowendorf, postdoctoral, working in Ithaca.

S. Singh, graduate student, working in Trinidad.

W.-L. Chao, graduate student, in Ithaca.

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VII. Publications

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Chao, W.-L., and M. Alexander. 1981. Interaction between protozoa and Rhizobium in chemically amended soil. Soil Sci. Soc. Am. J. 45: 48-50.