# THE UNIVERSITY OF THE WEST INDIES
## DEPARTMENT OF SOIL SCIENCE
## CORNELL/UWI BIOLOGICAL NITROGEN FIXATION PROJECT
## PHASE I REPORT

3.0127

A. PROJECT TITLE: Maximizing the Grain Yield and Symbiotic Nitrogen Fixation by Grain Legumes in Trinidad

B. PRINCIPAL INVESTIGATORS: Spider K. Mughogho
Henry S. Lowendorf

C. REPORTING PERIOD: March, 1977 - March, 1979

D. INTRODUCTION:

Production and consumption of fertilizer nitrogen has increased by a factor of about 10 in the last twenty years. It has been predicted that twenty years from now, 200 x $10^6$ metric tons of fertilizer N will be required annually in absence of alternative techniques for producing fixed N. This has generated a greatly expanded interest in the Rhizobium - legume based symbiotic $N_2$-fixation. These conditions are essential for successful symbiotic $N_2$-fixation:

(a) The host plant and the Rhizobium must be matched; in other words, the fixation of nitrogen by legumes is governed by the effectiveness of the symbiotic assocation between a particular legume and a Rhizobium strain. Thus the Rhizobium strain has to be able to nodulate the legume and subsequently the nodule has to have the ability to fix nitrogen;

(b) The soil and environmental conditions must be suitable for the growth of the host plant. Adding required nutrients or eliminating toxic effects will increase the growth of legumes and thereby the need for N either from the soil or by symbiotic fixation;

(c) The soil and environmental conditions must be favourable for the survival of the Rhizobium strain. It is possible to select strains which are tolerant to stress conditions commonly found in the soils of the tropics, e.g. acidity, high temperatures, and long dry spells.

## E. *RESEARCH ACTIVITIES:*

After several discussions between Dr. Henry Lowendorf and me on one side and several people at the University of the West Indies working on grain legumes on the other side, we decided to set up some field experiments. We were advised to work on soybeans and/or cowpeas (blackeye peas).

### 1) Maximizing Grain Yield of Cowpeas (*Vigna unguiculata*) through Liming and Phosphorus Application on a Piarco Fine Sand

#### Introduction:

In soils, plant growth is likely to be affected more commonly by factors related to soil acidity such as aluminium and/or manganese toxicity, phosphorus, calcium and/or molybdenum deficiencies. It has been reported in literature that many tropical legumes are capable of growing in soils that are too acid for a good growth of many temperate legumes. This may be that tropical legumes can tolerate excessive Al and/or Mn, and that they are more efficient in extracting soil Ca. Excess Al and/or Mn may also depress the *Rhizobium* - host symbiosis. Work in Brazil has revealed that *Glycine* and *Phaseolus* developed nodules and fixed $N_2$ satisfactorily at surprisingly low pH levels, provided Mn toxicity is not the limiting factor (Dobereiner, 1966), or Al toxicity in case of *P. vulgaris* (Ruschel *et al* 1968).

In spite of exceptions, the general conclusion appears to have been reached that liming is unimportant for tropical legume production and that even on acid infertile soils sufficient Ca will likely be applied when triple superphosphate is used as a P source. It is commonly accepted that P additions are needed in most cases for optimum production of tropical legumes, and positive responses to P are almost universally found.

The present field study was undertaken to determine the effect of lime and phosphorus on the growth of cowpeas on an acid ultisol which has a great potential of having greater quantities of exchangeable Al in soil solution.

#### Materials and Methods:

The first experiment was conducted in 1977 on Piarco fine sand, a member of the fine sandy, clayey, kaolinitic, isohyperthermic family of aquoxic Tropudults at Wallerfield. The surface 0-15 cm had

a pH of about 4.6-5.0 while the 15-30 cm had a pH of 4.7-4.8 in KCl. The KCl-extractable Al saturation varied from 40-80% in the top soil and 63-84% in the subsoil (Tables I-1 and I-2).

The treatments consisted of factorial combinations of 0, and 4 tons/ha lime (88% $CaCO_3$, and 60 mesh), and triple superphosphate to supply 40 and 80 kg P/ha. Plots of 3 x 3.6 $m^2$ were arranged in a ran omplete block design with four replicates. The treatments were long with a basal application of 30 kg K/ha and 30 kg N/ha e of potash and urea respectively, and zinc was foliarly sprayed twice as zinc sulfate at 0.1% w/v solution.

They were seeded to cowpeas (Vigna unguiculata) at 4 seeds per stand at 15 cm apart in rows which were spaced at 50 cm. The seeds were inoculated with cowpea strain 13B originally isolated from Piarco fine sand. The seedlings were thinned to one per stand. Soil samples (0-15 cm at 15-30 cm) were taken at time of planting and at harvest and analysed for pH, Ca, Mg and P from selected plots.

The second experiment was conducted in 1978 on the same site as the first Lime x Phosphorus experiment. The purpose of this experiment was to investigate the residual effects of the lime and/or phosphorus to cowpeas. No further lime or phosphorus were applied to the plots. There were basal treatments of 30 kg K/ha, 30 kg N/ha, and 1 kg/ha Zn as muriate of potash, urea, and zinc sulphate respectively. The plots were seeded to cowpeas just as in 1977.

## Results and Discussion:

Examination of the 1977 treatment means of the grain yields suggested that phosphorus application greatly increased the grain yield of cowpeas, with the greatest response being at first increment of P (20 kg/ha P) on both the limed and unlimed treatments. The grain yield increased from 400 kg/ha in the control to 865 kg/ha at 20 kg/ha P (Table I-3 and Figure I-1). Sugsequent P increments had very little or no response to the first increment.

Lime had no effect on grain yield. It has been stated by several workers that ordinary superphosphate will adequately satisfy the Ca requirements of most tropical legumes, making liming unnecessary (Norris, 1958; Dobereine, 1966; Ruschel et al 1968; Spain, 1975). In the present study the failure to responses of lime applications and high P applications might have been due to nutrient imbalance, of which Zn and/or Mg deficiencies are suspected. This was reflected by the appearance of some deficiency symptons in the

field. There was a delayed maturity of over 40% of the grain, which is one of the effects caused by Zn deficiency.

The 1978 results were generally lower than those of 1977 (Table I-4). Several reasons might have contributed to this, among which was the incidence of Cercospora disease which might have greatly reduced the grain yield, although there were precautionary measures, e.g. spraying the crop with Diathane M-45 which proved to be very effective.

There was a linear relationship with P application and the grain yield in the unlimed plots. This would indicate that there was a residual effect to P application. It is interesting to note that the grain yields were higher in the limed than the unlimed treatments. This may indicate that lime is more beneficial if it is applied a few months before growing the legume crop. The failure for the response to further P applications over and above 40 kg/ha P may be due to the physiological problems associated with the balance of nutrients.

This experiment has shown that it is not advisable to plant blackeye peas following blackeye peas in a cropping sequence because this leads to incidence of diseases such as Cercospora and Altenaria. The P and lime residual effects have to be further investigated in a long term experiment.

Literature Cited:

1) Dobereiner, J. 1966. Manganese toxicity effects of nodulation and nitrogen fixation of beans (Phaseolus vulgaris L.) in acid soils. Plant and Soil 24: 153-166.

2) Norris, D.O. 1958. Lime in relation to the nodulation of tropical legumes. In Nutrition of the Legumes (ed. Hallsworth), Butterworths, London, pp. 164-182.

3) Ruschel, A.P., Alvahydo, R. and Sampio, I.B.M. 1968. Influencia de excesso de aluminio no feijao (Phaseolus vulgaris L.) cultivado en salucao nutritiva. Presg. Agropec. Bras. 3: 229-233.

4) Spain, J.M. 1975. Forage potential of Allic soils of the humid lowland tropics of Latin America, pp. 1-8. In Tropical Forages in Livestock Production Systems. Amer. Soc. Agron. Spec. Publ. No. 24, Madison, Wis.

Table I-1: Estimations of aluminium saturation in KCl-extracts of the Ultisol at Wallerfield

| Sample[1] | pH | Ca | Mg | Al | Σ Cations | % Al |
|---|---|---|---|---|---|---|
| | | me/100 g | | | | |
| 0-15 N | 5.0 | 1.60 | 0.61 | 1.50 | 3.71 | 40 |
| 15-30 N | 4.8 | 1.00 | 0.42 | 2.44 | 3.86 | 83 |
| 0-15 S | 4.6 | 0.40 | 0.19 | 2.37 | 2.96 | 80 |
| 15-30 S | 4.7 | 0.35 | 0.17 | 2.69 | 3.21 | 84 |

Table I-2: Some chemical analyses of the soil samples from Wallerfield [2]

| Sample[1] | pH | Ca | Mg | K | Zn | % O.M. | % O.M. |
|---|---|---|---|---|---|---|---|
| | | ppm | | | | | |
| 0-15 N | 2.0 | 275 | 70 | 30 | 1.5 | 3.4 | 0.22 |
| 15-30 N | 1.5 | 175 | 55 | 35 | 1.0 | 2.2 | 0.15 |
| 0-15 S | 1.0 | 75 | 30 | 15 | 1.5 | 2.2 | 0.14 |
| 15-30 S | 1.0 | 50 | 25 | 15 | 1.0 | 1.6 | 0.08 |

[1] 0-15 cm and 15-30 cm is depth in cm

N and S designates north and south respectively to show the gradient in nutrient levels as related to proximity to the pig pen which is N of the experimental site.

[2] $NH_4OAc$ - extractable cations

Table I-3: The effect of lime and phosphorus application on the grain yield of cowpeas (*Vigna unguiculata*)

| Treat/Rep. | I | II | III | IV | Mean |
|---|---|---|---|---|---|
| $L_0$ $P_0$ | 544 | 426 | 265 | 314 | 387 |
| $L_0$ $P_1$ | 454 | 926 | 1085 | 956 | 855 |
| $L_0$ $P_2$ | 1013 | 1149 | 880 | 1013 | 1014 |
| $L_0$ $P_4$ | 850 | 760 | 1000 | 698 | 827 |
| $L_4$ $P_0$ | 687 | 478 | 294 | 174 | 408 |
| $L_4$ $P_1$ | 914 | 767 | 1017 | 777 | 869 |
| $L_4$ $P_2$ | 702 | 1086 | 832 | 817 | 859 |
| $L_4$ $P_4$ | 450 | 1118 | 1022 | 893 | 871 |

A factorial analysis of variance indicated that there are no significant differences in yield resulting from lime treatment; but a significant difference was found for phosphate treatment ($P < 0.001$). No differences were established between $P_1$, $P_2$ and $P_4$.

Table 1-4: The effect of residual lime and phosphorus on the cowpea grain yield (*Vigna unguiculata*)

| Treat/Rep. | I | II | III | IV | Mean |
|---|---|---|---|---|---|
| $L_0$ $P_0$ | 384 | 415 | 485 | 357 | 410 |
| $L_0$ $P_1$ | 354 | 425 | 368 | 567 | 428 |
| $L_0$ $P_2$ | 594 | 307 | 433 | 583 | 479 |
| $L_0$ $P_4$ | 504 | 824 | 487 | 337 | 538 |
| $L_1$ $P_0$ | 981 | 306 | 372 | 409 | 517 |
| $L_1$ $P_1$ | 650 | 1390 | 588 | 588 | 804 |
| $L_1$ $P_2$ | 504 | 760 | 652 | 418 | 570 |
| $L_1$ $P_4$ | 766 | 592 | 640 | 416 | 604 |

## II) Yield and Response of Corn to Nitrogen Following Fallow, Corn and Cowpeas

### Introduction:

Leguminous plants are noted for their ability to fix atmospheric nitrogen. Legumes may be able to contribute nitrogen to the soil and/or to non-legumes in two ways. First, they may be associated with an intercropped non-legume, and second, the legumes may contribute to the succeeding non-legume through residual N remaining in the form of soil organic matter or plant residues. The attention is on the changes in organic N in the soil under three conditions when a non-legume succeeds legumes (a) green manures; (b) pastures/forages and (c) grain legumes. The biggest assumption one makes when dealing with residual N is that the organic N will mineralize in the legume residues (tops and roots) over time. The basic rationale is that the value of crop residues on succeeding crop is the product of decomposition factor and quantity of residue. A non-legume will benefit more following green manures, and these can be used as fallow. Less N is contributed from grain legumes since as much as 80% N is lost in the harvest (Hammond *et al* 1951).

The purpose of the present experiment was to determine the response of corn to residual and/or fertilizer N following fallow, corn or cowpeas. Both corn (*Zea mays*) and cowpeas (*Vigna unguiculata*) are important in human nutrition in Trinidad.

### Materials and Methods:

Plots of 36M x 4 m were divided into three sections of 12 m long. One section was left under fallow and the other two were planted to corn at 90 cm x 30 cm and cowpeas at 50 cm x 15 cm, replicated four times in the major season of 1977. Muriate of potash at 60 kg/ha K, and triple superphosphate at 100 kg/ha P were broadcast in all plots and rotovated under at 15 cm before sowing. Weed control was done regularly in all plots.

In the minor season (1977/78) all the plots were seeded to corn at 75 cm x 30 cm. The plots were then subdivided into subplots which were fertilized with urea at 0, 30, 60 and 120 kg/ha N. The statistical design was a randomized complete block design with a split plot arrangement of units, with the major season crops (fallow, corn, and cowpeas) forming the whole plots and N levels the subplots. A basal treatment of 30 kg/ha K as muriate of potash and 40 kg/ha P as triple superphosphate was banded with fertilizer N at time of planting.

The 120 kg/ha N treatment was split into 60 kg/ha N applied at time of planting and the rest as sidedress after four weeks of growth.

Grain yield and plant N uptake will be used as a measure of available N from fallow, corn and cowpeas. Yields of the former fallow plots will furnish a standard curve.

Results and Discussion:

The effect of N application is to increase the uptake of N in the plant tops (cobs, stalks, and grain) of corn in the minor season. At zero level of fertilizer N, the highest N uptake was with the corn which followed Vigna, but at higher fertilizer N levels, the fallow plots had higher N uptake (Table II-1). It can be argued that the corn in the fallow plots absorbed fertilizer N more efficiently than in plots which had corn or Vigna residues. This might have been due to the fact that the fertilizer N was immobilized by the residues. The N content in corn stalks was about 0.5% whereas that of Vigna was averaged at 1.10%. The ease with which the two types of residues mineralized would be different. In fact one would suspect an initial N immobilization by the corn residues.

There is an increase in the Harvest Index (H.I.) of N uptake in the corn following corn or fallow with increased fertilizer N. There is no trend in the corn following Vigna treatments (Table II-2):

$$\text{Harvest Index} = \text{H.I.} = \frac{\text{N uptake in grain}}{\text{total N plant uptake}}$$

In general, the H.I. (N uptake) is higher in Vigna plots than either the fallow or corn plots. It might be deduced that the corn in Vigna plots utilized some of the residual N from Vigna tops and roots mineralized during the growing period. This is despite the fact that about 75% of Vigna N is harvested in the grain while only 25% is left in the stalks. It is important to note that root and/or nodule N was not determined in the present investigation.

Corn yields in the major season were 51% higher than in the minor season despite the fact that fertilizer N was applied in the minor season. The lower yields in the minor season may have been caused by the low amounts of soil moisture since there was not much rain in the minor season.

Although the differences in corn yields following fallow, corn or Vigna are not very spectacular, it clearly shows that Vigna has a slight advantage over fallow and has even a greater effect over corn

following corn. The overall averages are in the order of 2514, 2810, and 2982 kg/ha grain yields of corn-corn; fallow-corn and Vigna-corn respectively.

The present exercise shows that Vigna is more efficient and economical in its utilization of soil N than corn by an amount equivalent to 81 kg/ha of fertilizer N or fallow by an amount equivalent to 45 kg/ha N. Shrader et al (1966) pointed out that a crop that followed a good legume crop rotation benefited to the extent of better than 110 kg/ha N once the legume residues are returned to the soil. Johnson (1970) pointed out that cowpeas enrich the soil sufficiently for subsequent plantings of maize to benefit from it for two years in succession. On the other hand, Bartholomew (1972) contends that the use of leguminous crops to supply N may not be justified in that the cost of N applied by leguminous crops in terms of labour and land use, is often too high to compete with fertilizer N except where fertilizer N is expensive and difficult to get. Besides this, leguminous crops cannot supply enough N to meet the needs of modern high yielding crops. This is supported by Lyon and Bizzell (1934).

The reasons why grain legumes such as Vigna don't contribute greater quantities of fixed N to non-legumes may be that (a) a major portion of the N accumulated by the plant is removed in the grain and (b) the mineralization of soil N under grain legumes is likely to be high and hence the potential decline in soil organic N is fairly high (Wetselaar, 1973).

One would conclude from the rather meager data set available that substitution of a grain legume in a cropping sequence is likely to have a minor effect on the N economy.

Literature Cited:

1) Bartholomew, W.V. 1972. Soil nitrogen and organic matter. In Soils of the Humid Tropics. New National Academcy of Sciences, Washington, D.C. pp. 63-81.

2) Hammond, L.C., Black, C.A. and Norman, A.G. 1951. Nutrient uptake by soybeans on two Iowa soils. Iowa Agric. Exp. Sta. Res. Bul. 384.

3) Lyon, T.L. and Bizzell, J.A. 1934. A comparison of several legumes with respect to nitrogen production. J. Amer. Soc. Agron. 26: 651-656.

4) Shrader, W.D., Fuller, W.A. and Cady, F.B. 1966. Estimation of a common nitrogen response function for corn (*Zea mays*) in different crop rotations. Agron. J. 58: 397-401.

5) Wetselaar, R., Jackson, J. and Chaplin, G.R. 1973. Nitrogen balance in crop systems in tropical Australia. Soil Biol. Biochem. 5: 35-40.

Table II-1: The effect of N application on nitrogen uptake in corn following fallow, corn and Vigna

| Treatment*/Rep. | I | II | III | IV | Mean |
|---|---|---|---|---|---|
| F $N_0$ | 4.52 | 7.40 | 7.03 | 7.35 | 6.58 |
| F $N_1$ | 7.26 | 6.26 | 7.66 | 10.83 | 8.00 |
| F $N_2$ | 7.18 | 12.66 | 9.95 | 10.24 | 10.01 |
| F $N_4$ | 6.80 | 14.27 | 9.25 | 9.31 | 9.91 |
| C $N_0$ | 3.59 | 4.78 | 4.48 | 5.37 | 4.56 |
| C $N_1$ | 4.35 | 5.78 | 5.80 | 7.93 | 5.97 |
| C $N_2$ | 6.65 | 7.00 | 8.34 | 8.82 | 7.70 |
| C $N_4$ | 6.95 | 7.92 | 7.46 | 11.12 | 8.36 |
| V $N_0$ | 7.57 | 5.87 | 7.02 | 6.53 | 6.75 |
| V $N_1$ | 8.09 | 8.46 | 6.82 | 7.78 | 7.79 |
| V $N_2$ | 9.90 | 8.32 | 8.29 | 8.42 | 8.73 |
| V $N_4$ | 9.74 | 9.57 | 10.56 | 8.99 | 9.72 |

*F = Fallow; C = Corn; V = Vigna

$N_0$ = 0; $N_1$ = 30; $N_2$ = 60; $N_4$ = 120 kg/ha N

Table II-2: Harvest Index for the N uptake as affected by fertilizer N application in the fallow, corn and Vigna crops

| Treatment*/Rep. | I | II | III | IV | Mean |
|---|---|---|---|---|---|
| F $N_0$ | 0.676 | 0.742 | 0.668 | 0.795 | 0.725 |
| F $N_1$ | 0.795 | 0.667 | 0.765 | 0.738 | 0.741 |
| F $N_2$ | 0.777 | 0.771 | 0.760 | 0.802 | 0.778 |
| F $N_4$ | 0.797 | 0.662 | 0.771 | 0.790 | 0.755 |
| C $N_0$ | 0.656 | 0.754 | 0.709 | 0.750 | 0.717 |
| C $N_1$ | 0.663 | 0.658 | 0.734 | 0.752 | 0.702 |
| C $N_2$ | 0.748 | 0.732 | 0.781 | 0.803 | 0.766 |
| C $N_4$ | 0.802 | 0.780 | 0.715 | 0.785 | 0.771 |
| V $N_0$ | 0.800 | 0.756 | 0.721 | 0.759 | 0.784 |
| V $N_1$ | 0.801 | 0.714 | 0.706 | 0.720 | 0.735 |
| V $N_2$ | 0.772 | 0.795 | 0.795 | 0.716 | 0.770 |
| V $N_4$ | 0.773 | 0.705 | 0.774 | 0.746 | 0.750 |

*F = Fallow; C = Corn; V = Vigna

$N_0$ = 0; $N_1$ = 30; $N_2$ = 60; $N_4$ = 120 kg/ha N

Table II-3: The effect of N application on corn yield following corn, fallow or Vigna, kg/ha

| Treatment | Replicates I | II | III | IV | Mean |
|---|---|---|---|---|---|
| F $N_0$ | 1602 | 2712 | 2385 | 3319 | 2506 |
| F $N_1$ | 2558 | 2830 | 2796 | 2264 | 2612 |
| F $N_2$ | 2683 | 2450 | 2741 | 3322 | 2799 |
| F $N_4$ | 3277 | 3745 | 3348 | 2925 | 3324 |
| C $N_0$ | 2051 | 2288 | 1420 | 2502 | 2065 |
| C $N_1$ | 2054 | 2638 | 1831 | 3278 | 2450 |
| C $N_2$ | 1938 | 2765 | 2192 | 3328 | 2655 |
| C $N_4$ | 2278 | 3626 | 2820 | 2976 | 2987 |
| V $N_0$ | 2688 | 2622 | 2535 | 2976 | 2705 |
| V $N_1$ | 2312 | 3404 | 2778 | 2885 | 2845 |
| V $N_2$ | 3138 | 2788 | 3030 | 3182 | 3034 |
| V $N_4$ | 3858 | 3520 | 2533 | 3468 | 3345 |

Significant effects of both major season treatment ($P = 0.05$) and nitrogen fertilization ($P = 0.01$) were found.

## III) The Effect of *Rhizobium* Inoculation and Fertilizer Nitrogen Application on Nitrogen Uptake and Cowpea (*Vigna unguiculata*) and Soybean (*Glycine max.*) Grain Yields on a Piarco Fine Sand

### Introduction:

Grain legumes such as cowpeas (*Vigna unguiculata*) and soybeans (*Glycine max.*) are important components in many crop production systems. They are expected to receive most of their N supply from symbiotic $N_2$ fixation. Optimal symbiotic $N_2$ fixation requires that the *Rhizobium* strain is compatible with the host plant and that edaphic and climatic factors are suitable for the survival and growth of the host plant and *Rhizobium*. Where these conditions are fulfilled, a *Rhizobium* - legume association can supply by fixation most of the N required for high yields. Thus, legumes generally conserve what soil N is available and add to it in direct proportion to the size of the "sink" created by the yield potential of the crop.

Thus, in planning a fertilizer experiment with grain legumes, it is necessary to secure a *Rhizobium* strain that is both infective and effective. There is also a need to investigate whether the selected strain will survive in the soil saprophytically in the absence of the host plant during the dry season or when the site is under fallow or under a different species. After survival in the soil, the particular strain should be both competitive (and thus infective) and effective.

An examination of grain legume production in various parts of the world illustrates that fertilizer N is recommended (Bazan, 1975) and in experimental plots, responses to fertilizer N are common. Thus, the best means of realizing some of the potentials of grain legumes is through increasing yields and provision of effective rhizobia. Currently, the yields of grain legumes in tropical regions are low even if they are important sources of dietary protein.

There is limited data on the effect of fertilizer N application and/or inoculation of soybeans or cowpeas on the symbiotic $N_2$ fixation or grain yields in Trinidad. It has been shown that on some soils in Trinidad, fertilizer N increases soybean yields (Brathwaite, 1974). In the present study, the purpose of the investigation is to observe the effect of fertilizer N and/or *Rhizobium* inoculants on symbiotic $N_2$ fixation and grain yields of soybeans and cowpeas on a Piarco fine sand.

Materials and Methods:

The experiment was conducted on Piarco fine sand (for detailed information - see Experiment I on lime-phosphorus) as a randomized complete block design. Treatments consisted of factorial combinations of fertilizer N as urea at the rates of 0, 60, and 120 kg/ha N banded, and *Rhizobium* strains included:

$Rh_1$ = no inoculum;

$Rh_2$ = 3I-Ib-138 and S7C for soybeans and cowpeas respectively. The soybean strain was imported from the USA and S7C was isolated from Princes Town clay.

$Rh_3$ = SOY 13B and 13B for soybean and cowpeas respectively. SOY 13B was imported from the USA and 13B was isolated from Piarco fine sand.

Basal treatments included lime at 3 tons/ha as $CaCO_3$, muriate of potash at 60 kg/ha K, triple superphosphate at 100 kg/ha P and magnesium sulphate at 50 kg/ha, which were broadcast and rotovated under at 15 cm before planting. During the growth period zinc sulphate was foliarly sprayed twice at 0.1% w/v solution.

The plots were seeded to soybeans, Jupiter variety (*Glycine max.*) on June 24, 1977 and cowpeas, California 5 (*Vigna unguiculata*) on June 23, 1977 at 4 seeds per stand and thinned to one seedling at 15 cm apart in rows spaced at 50 cm. The plots were 6 m x 3.6 m. The seeds were wetted with $Rh_2$ and $Rh_3$ treatments including a blank the night before seeding. During the week of sowing, torrential rains caused flooding despite the fact that camber beds were made, and as a result there was extremely poor germination. The crops (seedlings) were destroyed and ridges were made on camber beds at 50 cm apart to facilitate more surface drainage. The second seeding was on July 13 and 14, 1977 for cowpeas and soybeans respectively.

After one month of growth, destructive harvesting was done from one half of each plot (3 m x 3.6 m) at 3 plants per plot. This was repeated one more time for cowpeas after 2 weeks, whereas for soybeans, sampling was repeated 3 more times at 2 week intervals. The plants were harvested to determine nodule count, plant weight and subsequently N concentration and/or uptake. Grain yield was determined from the other half (3 m x 3.6 m) of the plots.

Results and Discussion:

Table III-1 presents the results of nodule counts as affected by different rhizobial strains and/or levels of N. There were significant differences between no inoculation and inoculation during the first two sampling periods on the soybean plants. The nodule count on the soybean roots showed a delayed nodule formation in the uninoculated plots ($Rh_1$) at all fertilizer N levels and that there was increased nodulation with time. There are several explanations to this. First, there might have been contaminants from inoculated plots ($Rh_2$ and $Rh_3$) to $Rh_1$ plots during weeding, thinning and destructive sampling, although great precautions were undertaken by sterilizing feet and hands of the workers and also of the tools used with ethyl alcohol. Second, there might have been "indigenous japonicum" rhizobia in the soil in low numbers and that with time these multiplied to cause effective nodulation. Generally, it is reported that R. japonicum is not always present where soybeans have never been grown previously. Inoculation with $Rh_2$ or $Rh_3$ greatly increased nodulation throughout the sampling period. Statistically, significant differences were found in period one and two for Rhizobium treatments ($P < 0.01$), but not three and four. No differences among nitrogen treatments were found.

On the other hand, nodule count in cowpeas was already high at 33 days and that there were no significant differences between treatments at this sampling (Table III-1b). At 42 days the nodule count was so low that the sampling was discontinued in the cowpea treatments since most of the nodules were already decaying. Cowpea rhizobia are always in abundance in this soil, and this is attributed to the fact that there were other wild legumes that had nodules and that they cross nodulate cowpeas. As opposed to soybeans, cowpeas are fast nodulators.

Table III-2 presents a summary of the data on N accumulation in soybeans at 29, 42, and 75 days and in cowpeas at 33 and 42 days after planting and also N harvested in the grain. At 29 days there was no significant difference among treatments in the soybean plots. With time the general trend was that fertilizer N greatly increased N uptake in soybeans, and this is in agreement with what Hanway and Weber (1971) found in their work with different soybean varieties.

Analysis of variance indicates that a highly significant difference ($P < 0.01$) is found among effects of nitrogen treatments in the soybeans at 42, 75 and at harvest. In all cases there was no significant difference caused by Rhizobium treatments.

The present work also shows that the rates of N accumulation were slow early in the season in the soybean plants whereas it was fairly rapid in the cowpea plants, and this is attributed to the fact that cowpea plants are fast growers and nodulators. On the other hand, the final harvest shows a low N harvest in the grain, and this is because the average percentage N in the cowpeas (3.71%) is almost half that of soybeans (6.92%). The cowpea grain yields were far much lower than those of soybeans.

The grain yields of both soybeans and cowpeas showed greatest responses to first increments of fertilizer N (60 kg/ha N) at all Rhizobium treatments (Tables III-3 and III-4). There was a slight decrease in grain yields at 120 kg/ha N over the first increment. It is important to note here that increased N application over and above 60 kg/ha reduces the N content of the plant material and the grain. This may be due to reduced efficiency of the rhizobia to fix atmospheric $N_2$ in the presence of high levels of combined N (Summerfield et al, 1977).

Although with cowpeas strain 13B ($Rh_3$) gave slightly higher grain yields than did S7C ($Rh_2$) or non-inoculated plants ($Rh_1$), the difference was not significant ($P = 0.084$). It may be that the high variability among replicates has obscured a real difference in inoculant treatments with cowpeas. $Rh_3$ was originally isolated from Piarco fine sand and is presumably more adapted to the adverse soil conditions prevailing. Perhaps the indigenous rhizobia in $Rh_1$ included ineffective strains. With soybeans, $Rh_1$ gave almost 50% more grain yield than either $Rh_2$ and $Rh_3$. The explanation to this may be that the strains originally present, though in low numbers initially, were more effective and efficient in the $N_2$ fixation than introduced strains. This is reflected in the N uptake in $Rh_1$ (Table III-2). This stresses the point that indigenous strains may be more adapted to the soil and environmental conditions, and this explains why $Rh_3$ is more effective than $Rh_2$in cowpeas since $Rh_2$ was isolated from Princes Town clay which has different soil properties altogether. The effect of several complex factors, isolated or interacting, and inherited properties of rhizobial strains determine their behaviour in the soil (Freire, 1976). Another school of thought is that late nodulation in $Rh_1$ in soybeans would provide enough N for grain yield production in soybeans.

The present study proved that the terms effective and ineffective are inadequate to describe nodulation responses. This is so because Rhizobium strains differ in effectiveness or ability to produce nodules and differ in ability to fix atmospheric $N_2$. A

correlation of nodulation and $N_2$ fixation characteristics with plant properties is essential. This would include a determination of the number, size, weight and colour of the nodules and also the N uptake of soil and atmospheric $N_2$.

1977/78 Minor Season Experiments at Wallerfield:

In the cowpea treatments in the major season Rhizobium - nitrogen experiment, sorghum was planted on the same ridges at the same spacing (50 cm x 15 cm). The purpose of this study was to observe the effect of residual N on sorghum grain yields. The major season soybean plots were divided into two equal sections of 3 m x 3.6 3.6 m. The first half was not inoculated while the second half was inoculated with soybean Rhizobium strain 13B ($Rh_3$) 31-1b-13B ($Rh_2$) for major season uninoculated plots and $Rh_2$ plots, and soy 13B ($Rh_3$) for major season $Rh_3$ plots.

The results in the minor season show that there was a great increase in soybean grain yields when compared with minor season crop. The main reasons are that management was greatly improved. Phosphorus, potassium and zinc were banded at 40 kg/ha, P 30 kg/ha, K and 5 kg/ha Zn as triple superphosphate, muriate of potash and zinc sulfate respectively. The introduction of zinc might have contributed to increased pod set, since zinc reduces flower dropping. Rain was just adequate for grain legume production since there was not any flooding. On the other hand it was difficult to determine nodule number because the ground was hard to dig. From the few plants that were dug, there was good nodulation in all plots irrespective of the treatment. On the average, inoculation with $Rh_3$ reduced soybean grain yield (Tables III-6 and 7).

Table III-8 presents sorghum grain yield data. There was no difference in grain yields with N treatments. On the other hand, $Rh_1$ plots had higher sorghum grain yields than the $Rh_2$ plots. To interpret these results, the N data of the soil and/or plant material is necessary, which is still to be analyzed.

1978 Cowpea and Soybean Re-inoculation Experiment:

Introduction:

Preliminary results at Wallerfield on a Piarco fine sand indicate that the uninoculated soybeans picked up an "indigenous" rhizobia strain which is believed to be a cowpea-type Rhizobium and

gave higher grain yields than soybeans inoculated with either of the two commercial strains imported from the USA. Greenhouse experiments carried out in Leonard jars at UWI have indicated that the Rhizobium isolated from soybeans nodules on Piarco fine sand (SN2) formed nodules on cowpeas, pigeon peas and soybeans which may indicate that the strain is a cowpea Rhizobium and not R. japonicum. Generally, it is reported that the R. japonicum is not always present where soybeans have never been grown previously.

In the cowpea plots, the Rhizobium strain 13B isolated from Piarco fine sand gave higher yields than the Rhizobium strain isolated from a Princes Town clay. The purpose of the present experiment is to test the effect of Rhizobium strain SN2 and 13B both isolated from Piarco fine sand on the N uptake and grain yields of both soybeans and cowpeas respectively.

Materials and Methods:

For the cowpeas, the plots were laid on the site that had previously been cultivated to cowpeas in the major season of 1977 and to sorghum in the 1977/78 minor season. In case of soybeans, the plots were laid on the site that was grown to soybeans in both the major and minor seasons

Treatments consisted of a factorial combination of fertilizer N as urea at the rates of 0, 30 and 60 kg/ha N banded and rhizobia strains 13B and SN2 for $Rh_2$ plots for cowpeas and soybeans respectively, and $Rh_1$ as the check plots. Basal treatments included triple superphosphate, muriate of potash, zinc sulphate, magnesium sulphate at the rates of 40 kg/ha P, 30 kg/ha K, 5 kg/ha Zn and 50 kg/ha $MgSO_4$ respectively.

The plots were seeded to cowpeas (California 5 variety) on June 20, 1978 at 4 seeds per stand, 15 cm apart within rows which were spaced at 50 cm apart within rows which were spaced at 50 cm apart. The seeds were inoculated with cowpea Rhizobium strain 13B or blank the night before planting. The seedlings were thinned to one per stand on July 11, 1978. Soybeans were planted the same way on August 10, 1978, but since germination was poor, the seedlings were uprooted and the plots reseeded on August 24. The seeds were inoculated with SN2 for $Rh_2$ and blank for $Rh_1$.

Destructive sampling of the cowpea seedlings was done on August 7 to determine the nodule number, plant weight and N content of the tops. It was difficult to uproot the seedlings in the soybean plots in September/October because the land was dry, and as such nodule count was not done.

Results and Discussion:

In the non-inoculated cowpea plants there was an increase in nodule count with increased N application, and that 30 kg/ha N had the highest nodule count (Table III-5). It may be argued that a starter application of fertilizer N is necessary to boost seedling growth before the plants nitrogen-fixing process begins (Agboola, 1978; Ezedinma, 1964). On the other hand, the highest nodule count in the uninoculated plots resulted at the 0 kg/ha N. The theory behind this may be that N application at the time of planting reduces infectiveness since the Rhizobium is near the fertilizer (Williams, 1970).

There was almost a linear increase in N uptake with fertilizer N application in the uninoculated cowpea plots (Table III-6). The N uptake in the uninoculated plots increased at the first increment of N and slightly decreased at the highest N rate, although it was still higher than the 0 kg/ha N.

Results in Table III-7 clearly show that fertilizer N increases grain yields when Rhizobium strain 13B is not used, and the trend is almost linear. Similar results were reported by Godfrey, 1973. He found that in nitrogen-depleted low pH (5.2) ferralitic soils of Sierra Leone where the cowpea roots had non-functional nodules, as much as 165 kg/ha N was required to obtain maximum grain yield in the uninoculated plants. The present results show that the grain yields in the inoculated plots are higher than in the uninoculated cowpea plots. In fact the grain yield at 0 kg/ha N in the inoculated plots is as high as the best yields in the uninoculated plots at 60 kg/ha N. This is similar to the results of 1977 whereby inoculation was just as good as fertilizer N.

From the present experiment, one may conclude that it is better to inoculate the seeds even where the Rhizobium strain was established. It may be that the Rhizobium that was used in the first season had lost its effectiveness after one year without the cowpea as the host plant. This raises the question as to whether the seeds should be inoculated every time a new crop is planted, although the general belief is that if the "appropriate" rhizobia were widespread, there is no need for re-inoculation. On the other hand, if there is a lack of an "appropriate" host crop over a period of time, the numbers of rhizobia may be reduced to low levels. Under such circumstances, response to inoculation may be obtained.

For the soybean crop, there was a short dry spell in September/October, 1978 and as a result the soil was so dry that destructive sampling by uprooting the plant was impractical. To date only the N content and the grain yields of the soybeans are reported. Results in

Table III-8 show that there were no significant differences in the percentage N in the plant tops between N and/or Rhizobium treatments. Generally, inoculated plants showed higher N contents than the uninoculated plants.

Fertilizer N almost increased the soybean grain yields linearly in both inoculated and uninoculated treatments. There was an indication that 60 kg/ha N rate was not adequate to sustain maximum grain yields in both rhizobial treatments (Table III-9). This is because soybeans have a high soil or fertilizer nitrogen requirement if the crop is not well nodulated or when the Rhizobium-host association is not efficient. In fact the present results show that Rhizobium inoculation did not have any beneficial effects over uninoculated plots.

Under suitable conditions, most or all of the nitrogen requirements of the grain legumes can be provided by the Rhizobium. In the absence of an "appropriate" rhizobial population, the legumes will fail to develop effective nodules. In the present study, these conditions were not met and the soybean crop depended on fertilizer N. On the other hand, fertilizer N gave no higher yields than a well-nodulated cowpea crop without it. Thus one may conclude that the contribution of fixed N to the growth and yield of grain legumes would depend on the existence of effective and efficient inoculant, given optimum edaphic and climatic factors. Future studies, then, would include a correlation of nodulation and $N_2$ fixation characteristics with plant properties. A determination of the number, size and distribution, weight and color of the nodules and also the N uptake of the soil N and atmospheric $N_2$ is essential.

Literature Cited:

Agboola, A.A. 1978. Influence of soil organic matter on cowpea's response to N fertilizer. Agron. J. 70: 25-28.

Bazan, R. 1975. Nitrogen fertilization and mangement of grain legumes in Central America. In E. Bornemisza and A. Alvarado (ed.), Soil Management in Tropical America. North Carolina State Univ., Raleigh, N.C.

Brathwaite, R.A.I., Hammerton, J.L. and Tai, E.A. 1974. Effects of fertilizer on yield, seed quality and plant characteristics of soybean (Glycine max. (L) Merrill). Twelfth Ann. Mtg. of the CFCS Jamaica, June 30-July 6, 1974.

Ezedinma, F.O.C. 1974. Effects of inoculation with local isolates of cowpea Rhizobium and application of nitrate-nitrogen on the development of cowpeas. Trop. Agric. (Trin.) 41: 243-249.

Freire, J.R.J. 1976. Inoculation of soybeans. In Exploiting the legume-Rhizobium symbiosis in Tropical Agriculture. Proceedings of Workshop, Kahului, Maui, Hawaii, August 23-28, 1976.

Godfrey-Sam-Aggrey, W. 1973. Effects of fertilizers on harvest time and yield of cowpeas (Vigna unguiculata) in Sierra Leone. Expl. Agric. 9: 315-320.

Hanway, J.J. and Weber, C.R. 1971. Accumulations of N, P, and K by soybean (Glycine max. (L) Merrill) plants. Agron. J. 63: 406-408.

Hardy, R.W.F. and Havelka, U.D. 1975. Nitrogen fixation research: A key to World food. In P.H. Abelson (ed.) Food: Politics, Economics, Nutrition and Research. Amer. Assoc. Adv. Sci. pp. 178-188.

Summerfield, R.J., Dart, P.J., Huxley, P.A., Eaglesham, A.R.J., Minchin, F.R. and Day, J.M. Nitrogen nutrition of cowpea (Vigna unguiculata). I. Effects of applied nitrogen and symbiotic nitrogen fixation on growth and seed yield. Expl. Agric. 13: 129-142.

Williams, C.H. 1970. Pasture nitrogen in Australia. J. Aust. Inst. Sci. 36: 199-205.

Table III-1a: The effect of *Rhizobium japonicum* inoculation and fertilizer N application on nodule count per plant of soybeans

| Sampling period | Treatments | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $N_1$ | | | $N_2$ | | | $N_3$ | | |
| | $Rh_1$ | $Rh_2$ | $Rh_3$ | $Rh_1$ | $Rh_2$ | $Rh_3$ | $Rh_1$ | $Rh_2$ | $Rh_3$ |
| First | 1 | 30 | 16 | 1 | 27 | 9 | 1 | 27 | 16 |
| Second | 9 | 24 | 19 | 4 | 23 | 15 | 4 | 19 | 15 |
| Third | 25 | 25 | 18 | 15 | 24 | 26 | 16 | 19 | 21 |
| Fourth | 22 | 28 | 23 | 19 | 30 | 20 | 25 | 20 | 20 |

Table III-1b: The effect of cowpea *Rhizobium* inoculation and fertilizer N application on nodule count per plant of cowpeas

| Sampling period | Treatments | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $N_1$ | | | $N_2$ | | | $N_3$ | | |
| | $Rh_1$ | $Rh_2$ | $Rh_3$ | $Rh_1$ | $Rh_2$ | $Rh_3$ | $Rh_1$ | $Rh_2$ | $Rh_3$ |
| First | 18 | 20 | 21 | 26 | 18 | 16 | 18 | 14 | 17 |
| Second | 3 | 9 | 6 | 7 | 11 | 2 | 1 | 9 | 1 |

The results in this table averages from 12 plants = 3 plants/plot and replicated four times.

Table II-2: Accumulation of N by soybean and cowpea plants at different sampling periods as influences by fertilizer nitrogen and inoculation (kg/ha)

| Treat/ period[1/] | soybeans | | | | cowpeas | | |
|---|---|---|---|---|---|---|---|
| | 29 | 42 | 75 | Harvest[2/] | 33 | 42 | Harvest[2/] |
| $Rh_1$ $N_1$ | 6.1 | 9.5 | 61.8 | 39.8 | 17.5 | 20.4 | 6.6 |
| $Rh_1$ $N_2$ | 9.2 | 28.5 | 76.7 | 79.4 | 19.8 | 25.0 | 16.1 |
| $Rh_1$ $N_3$ | 6.4 | 38.1 | 89.0 | 71.4 | 16.7 | 18.1 | 16.0 |
| $Rh_2$ $N_1$ | 7.2 | 17.6 | 55.4 | 27.1 | 14.0 | 15.8 | 6.1 |
| $Rh_2$ $N_2$ | 11.7 | 30.1 | 91.1 | 50.8 | 17.4 | 20.5 | 16.0 |
| $Rh_2$ $N_3$ | 10.3 | 29.4 | 102.0 | 43.9 | 12.7 | 13.9 | 12.5 |
| $Rh_3$ $N_1$ | 7.7 | 23.7 | 57.9 | 39.1 | 10.0 | 15.8 | 11.4 |
| $Rh_3$ $N_2$ | 8.9 | 26.3 | 71.1 | 46.2 | 15.8 | 30.6 | 20.4 |
| $Rh_3$ $N_3$ | 8.6 | 24.1 | 61.8 | 48.1 | 17.1 | 18.4 | 19.6 |

[1/] Days from planting

[2/] Harvest - N harvested in grain

Table III-3: The effect of *Rhizobium japonicum* inoculation and N application on the grain yield - kg/ha

| Treatment | Replicates (Blocks) | | | | |
|---|---|---|---|---|---|
| | I | II | III | IV | Mean |
| $Rh_1$ $N_1$ | 965 | 668 | 255 | 410 | 574 |
| $Rh_1$ $N_2$ | 1243 | 1318 | 1120 | 888 | 1142 |
| $Rh_1$ $N_3$ | 1027 | 1283 | 835 | 1073 | 1054 |
| $Rh_2$ $N_1$ | 1000 | 212 | 287 | 67 | 392 |
| $Rh_2$ $N_2$ | 1582 | 823 | 348 | 190 | 736 |
| $Rh_2$ $N_3$ | 285 | 1090 | 513 | 632 | 630 |
| $Rh_3$ $N_1$ | 620 | 660 | 343 | 603 | 556 |
| $Rh_3$ $N_2$ | 788 | 800 | 585 | 567 | 685 |
| $Rh_3$ $N_3$ | 1063 | 933 | 180 | 542 | 680 |

Factorial analysis of variance indicates highly significant difference among *Rhizobium* treatments ($P < 0.01$), and significant differences among nitrogen treatments ($P < 0.05$).

Table III-4: The effect of *Rhizobium* inoculation and fertilizer N application on cowpea grain yield - kg/ha

| Treatment | Replicates (Blocks) | | | | |
|---|---|---|---|---|---|
| | I | II | III | IV | Mean |
| $Rh_1$ $N_1$ | 244 | 200 | 140 | 178 | 191 |
| $Rh_1$ $N_2$ | 651 | 710 | 327 | 20 | 427 |
| $Rh_1$ $N_3$ | 767 | 476 | 236 | 148 | 407 |
| $Rh_2$ $N_1$ | 262 | 302 | 73 | 74 | 178 |
| $Rh_2$ $N_2$ | 792 | 677 | 75 | 201 | 436 |
| $Rh_2$ $N_3$ | 602 | 499 | 109 | 89 | 325 |
| $Rh_3$ $N_1$ | 453 | 536 | 243 | 38 | 318 |
| $Rh_3$ $N_2$ | 1025 | 546 | 227 | 354 | 538 |
| $Rh_3$ $N_3$ | 962 | 792 | 173 | 60 | 497 |

Factorial analysis of variance indicates a highly significant difference among nitrogen treatments ($P < 0.01$), and no significant difference between rhizobial treatments ($P = 0.084$).

Table III-5: Minor season soybean grain yields in uninoculated plots - kg/ha

| Treatment | Replicates (Blocks) | | | | |
|---|---|---|---|---|---|
| | I | II | III | IV | Mean |
| $Rh_1$ $N_1$ | 2917 | 1500 | 1676 | 718 | 1703 |
| $Rh_1$ $N_2$ | 2361 | 1273 | 1134 | 1574 | 1586 |
| $Rh_1$ $N_3$ | 3454 | 1454 | 1500 | 1134 | 1886 |
| $Rh_2$ $N_1$ | 977 | 1750 | 2088 | 1356 | 1543 |
| $Rh_2$ $N_2$ | 2602 | 2216 | 1292 | 958 | 1767 |
| $Rh_2$ $N_3$ | 1116 | 1630 | 819 | 1042 | 1152 |
| $Rh_3$ $N_1$ | 1829 | 2180 | 1792 | 1028 | 1707 |
| $Rh_3$ $N_2$ | 2081 | 1904 | 1486 | 1319 | 1698 |
| $Rh_3$ $N_3$ | 2810 | 1273 | 1648 | 1412 | 1786 |

Analysis of variance indicates no significant differences between inoculated and uninoculated plots, or any of the major season treatments.

Table III-6: Minor season soybean grain yields in the inoculated plots - kg/ha

| Treatment | Replicates (Blocks) | | | | |
|---|---|---|---|---|---|
| | I | II | III | IV | Mean |
| $Rh_1$ $N_1$ | 2810 | 1842 | 1180 | 458 | 1572 |
| $Rh_1$ $N_2$ | 1954 | 597 | 731 | 1227 | 1127 |
| $Rh_1$ $N_3$ | 727 | 2616 | 1088 | 1361 | 1448 |
| $Rh_2$ $N_1$ | 2620 | 1227 | 1282 | 1356 | 1621 |
| $Rh_2$ $N_2$ | 1481 | 1509 | 1180 | 1208 | 1344 |
| $Rh_2$ $N_3$ | 1537 | 2037 | 1319 | 1890 | 1696 |
| $Rh_3$ $N_1$ | 1546 | 1042 | 2000 | 940 | 1382 |
| $Rh_3$ $N_2$ | 2245 | 1394 | 940 | 1144 | 1431 |
| $Rh_3$ $N_3$ | 2412 | 1106 | 1602 | 1412 | 1633 |

Table III-7: The effect of major season treatments of cowpeas on the sorghum grain yield in the minor season - kg/ha

| Major Season Treatment | Replicates (Blocks) | | | |
|---|---|---|---|---|
| | I | II | III | Mean |
| $Rh_1$ $N_1$ | 3095 | 2964 | 1030 | 2363 |
| $Rh_1$ $N_2$ | 3490 | 1980 | 1325 | 2265 |
| $Rh_1$ $N_3$ | 2875 | 2495 | 1795 | 2388 |
| $Rh_2$ $N_1$ | 3032 | 2165 | 1260 | 2152 |
| $Rh_2$ $N_2$ | 3767 | 1417 | 1090 | 2091 |
| $Rh_2$ $N_3$ | 2230 | 1864 | 1680 | 1924 |
| $Rh_3$ $N_1$ | 3375 | 2100 | 1300 | 2257 |
| $Rh_3$ $N_2$ | 2866 | 2064 | 1037 | 1989 |
| $Rh_3$ $N_3$ | 1968 | 2544 | 1207 | 1906 |

A factorial analysis of variance determined that no significant differences occurred among yields as affected by the previous season's treatments - *Rhizobium*, nitrogen, nor cross effects of the two.

Table III-8: The effect of *Rhizobium* inoculation and fertilizer N application on the nodule count in cowpeas

| Treat/ Rep. | I | II | III | IV | V | VI | Mean |
|---|---|---|---|---|---|---|---|
| $Rh_1$ $N_1$ | 71 | 23 | 74 | 60 | 28 | 21 | 47.2 |
| $Rh_1$ $N_2$ | 41 | 270 | 91 | 48 | 22 | 39 | 85.2 |
| $Rh_1$ $N_3$ | 107 | 110 | 56 | 23 | 38 | 85 | 69.8 |
| $Rh_2$ $N_1$ | 15 | 88 | 72 | 133 | 304 | 126 | 123.0 |
| $Rh_2$ $N_2$ | 105 | 107 | 8 | 59 | 31 | 12 | 53.7 |
| $Rh_2$ $N_3$ | 98 | 104 | 78 | 3 | 37 | 71 | 65.2 |

Table III-9: The effect of *Rhizobium* inoculation and fertilizer N application on the uptake of N in cowpeas (g/3 plants) plants sampled at flowering

| Treat/ Rep. | I | II | III | IV | V | VI | Mean |
|---|---|---|---|---|---|---|---|
| $Rh_1$ $N_1$ | 0.85 | 1.09 | 1.30 | 0.94 | 0.56 | 0.33 | 0.85 |
| $Rh_1$ $N_2$ | 1.31 | 1.06 | 1.44 | 1.00 | 0.29 | 0.29 | 0.89 |
| $Rh_1$ $N_3$ | 1.34 | 1.34 | 1.83 | 0.48 | 0.48 | 0.20 | 0.94 |
| $Rh_2$ $N_1$ | 0.99 | 1.38 | 1.05 | 0.60 | 0.89 | 0.37 | 0.83 |
| $Rh_2$ $N_2$ | 1.54 | 1.17 | 1.51 | 1.35 | 0.32 | 0.33 | 1.04 |
| $Rh_2$ $N_3$ | 1.31 | 1.17 | 1.37 | 0.64 | 0.69 | 0.24 | 0.98 |

Table III-10: The effect of *Rhizobium* inoculation and fertilizer N application on the cowpea grain yield - kg/ha

| Treat/ Rep. | I | II | III | IV | V | VI | Mean |
|---|---|---|---|---|---|---|---|
| $Rh_1$ $N_1$ | 856 | 1643 | 985 | 904 | 662 | 554 | 934 |
| $Rh_1$ $N_2$ | 1858 | 1389 | 1237 | 933 | 434 | 229 | 1013 |
| $Rh_1$ $N_3$ | 1573 | 1910 | 1487 | 367 | 1074 | 318 | 1122 |
| $Rh_2$ $N_1$ | 1456 | 1482 | 1890 | 412 | 1097 | 613 | 1158 |
| $Rh_2$ $N_2$ | 1909 | 1267 | 1462 | 1179 | 387 | 418 | 1104 |
| $Rh_2$ $N_3$ | 1694 | 1872 | 1490 | 360 | 892 | 392 | 1117 |

Table III-11: The effect of *Rhizobium* inoculation and fertilizer N application on the N content of the soybean plant tops (% N)

| Treat/ Rep. | I | II | III | IV | V | VI | Mean |
|---|---|---|---|---|---|---|---|
| $Rh_1$ $N_1$ | 1.80 | 3.10 | 3.45 | 3.35 | 1.75 | 2.15 | 2.60 |
| $Rh_1$ $N_2$ | 2.15 | 2.10 | 2.95 | 2.25 | 1.95 | 2.10 | 2.25 |
| $Rh_1$ $N_3$ | 2.55 | 2.50 | 2.15 | 2.40 | 3.15 | 2.70 | 2.58 |
| $Rh_2$ $N_1$ | 2.50 | 2.85 | 3.45 | 2.40 | 3.60 | 1.80 | 2.76 |
| $Rh_2$ $N_2$ | 1.90 | 2.60 | 2.85 | 2.60 | 2.75 | 4.25 | 2.82 |
| $Rh_2$ $N_3$ | 2.50 | 2.75 | 2.60 | 1.80 | 2.45 | 2.40 | 2.42 |

Table III-12: The effect of *Rhizobium* inoculation and fertilizer N application on the soybean grain yields - kg/ha

| Treat/ Rep. | I | II | III | IV | V | VI | Mean |
|---|---|---|---|---|---|---|---|
| $Rh_1$ $N_1$ | 810 | 1288 | 1104 | 612 | 450 | 862 | 855 |
| $Rh_1$ $N_2$ | 1084 | 1814 | 1114 | 796 | 788 | 612 | 1065 |
| $Rh_1$ $N_3$ | 1866 | 1854 | 1278 | 890 | 1128 | 384 | 1266 |
| $Rh_2$ $N_1$ | 1840 | 908 | 888 | 798 | 474 | 482 | 899 |
| $Rh_2$ $N_2$ | 1798 | 1456 | 1296 | 630 | 786 | 388 | 1059 |
| $Rh_2$ $N_3$ | 1036 | 1652 | 2186 | 664 | 908 | 388 | 1166 |

## IV) The Effect of *Rhizobium japonicum* Inoculation and Fertilizer Nitrogen Application on Soybean Grain Yield and Nitrogen Uptake on a River Estate Loam

### Introduction:

In the absence of an appropriate rhizobial population, the grain legumes will fail to develop effective nodules. Their response to the additions of nutrient elements will be dependent on the level of nitrogen supply from the soil and/or biological nitrogen fixation. Inoculating the seeds has been reported to result in the nodulation of the grain legumes, but no mention was made of any attempt to see whether an effective nodule system has been developed. The inoculation of the seed with an effective strain, on its own, is no guarantee of the development of effective nodulation.

It has also been accepted that nitrogenous fertilizers depress nodulation, although small amounts are reported to have stimulating effect on nodulation. The third aspect is that additions of fertilizer N late in the growing season can enhance the N content of the legume crop.

The purpose of the present exercise was to investigate the effect of *Rhizobium* inoculation and fertilizer N application on the soybean grain yields and N content of the plant parts.

### Materials and Methods:

The experiment was conducted on a River Estate loam as a randomized complete block design. Treatments consisted of factorial combinations of fertilizer N as urea at the rates of 0 and 30 kg/ha N ($N_1$ and $N_2$ respectively) banded, and the *Rhizobium* strain SN2 isolated from Wallerfield on a Piarco fine sand was inoculated onto one set of seeds ($Rh_2$) and the other set was uninoculated ($Rh_1$). Basal treatments included muriate of potash at 30 kg/ha K, triple superphosphate at 45 kg/ha P, and 5 kg/ha Zn as zinc sulphate all banded at time of planting.

The plots were seeded to two soybean varieties, Jupiter ($S_1$) and Dapros ($S_2$) on October 3, 1978 at 2 seeds per 4 centimeter spacing within the rows which were 50 cm apart. The plots were 4 m x 2 m. The seeds were wetted with either the blank ($Rh_1$) or *Rhizobium* strain SN2 ($Rh_2$) the night before seeding. There was very poor germination of the Jupiter variety, and as such the seedlings were destroyed and

a new set of seeds was planted on October 16, 1978. The plots were thinned to one plant per stand on October 19 in case of Dapros variety and on October 26 for the Jupiter.

Destructive harvesting of the soybean plants was done at flowering and two weeks after flowering to determine the nodule number, plant weight and subsequently plant N content. Grain yield was determined at the time of final harvest.

Results and Discussion:

On the River Estate loam, Jupiter is a far much poorer nodulator than Dapros, and this was also observed in an experiment by Parasaram of CARDI (Personal Communication). There was practically no nodulation four weeks after planting in Jupiter treatments. Fertilizer N application greatly reduces nodule formation, and this is in agreement with what has been reported in literature. One of the reasons for the generally low nodule count in this soil is that there are relatively high levels of total N in the River Estate loam when compared with Piarco fine sand where the Rhizobium strain SN2 was isolated. Somehow, there were some nodules formed in the uninoculated plots despite the fact that in each Rhizobium treatment there was a one meter bare land to separate the plots (Table IV-1).

Fertilizer N application at time of planting greatly reduced the emergence of the soybeans, and hence poor growth during the growing season. Inoculation also reduced the growth by about 18.5% in the Jupiter variety and by only 4.4% in the Dapros (Table IV-2).

Whereas the nodule numbers decreased with N application, there was a reverse effect with regard to N content in the plant materials. Is it that the Rhizobium strain that was used was ineffective in fixing atmospheric $N_2$? The variety Dapros nodulates more on this soil than Jupiter, although the Rhizobium strain that was used (SN2) was isolated from the Jupiter variety.

The Dapros variety lodges during the later stages of growth, which made harvesting difficult. This variety also shatters, and harvesting had to be done during the early hours of the day. Results in Table IV-3 show that there was no significant difference in the grain yields in the Dapros variety. Jupiter, on the other hand, shows an increased grain yield with Rhizobium (SN2) inoculation. On the other hand there was no change in grain yield with fertilizer N application. It can be argued that the Rhizobium strain is efficient in fixing atmospheric $N_2$ at the appropriate time, e.g. at pod filling.

It can also be argued that Dapros, even if it is a better nodulator, failed to show an increase in grain yield in inoculated plots because the Rhizobium strain might have been specific to Jupiter variety.

It can be concluded from the present experiment results that there is a great need to compare different strains on different soybean varieties and on various soil types. Transferring a Rhizobium strain from one soil type to the other may not prove beneficial as was shown in Experiment III with both soybeans and cowpeas. It may also not be beneficial to use a strain isolated from one variety on a different variety. More work is needed to investigate the transferability of a Rhizobium strain.

Table IV-I: The effect of *Rhizobium* inoculation and fertilizer N application on nodule count on soybeans

| Treatment/ Rep. | FIRST HARVEST | | | | SECOND HARVEST | | | |
|---|---|---|---|---|---|---|---|---|
| | I | II | III | MEAN | I | II | III | MEAN |
| $S_1Rh_1N_1$ | 0 | 0 | 4 | 1.33 | 1 | 0 | 42 | 14.33 |
| $S_1Rh_1N_2$ | 0 | 0 | 0 | 0.00 | 0 | 1 | 18 | 6.33 |
| $S_1Rh_2N_1$ | 0 | 0 | 1 | 0.33 | 0 | 3 | 17 | 3.33 |
| $S_1Rh_2N_2$ | 0 | 0 | 1 | 0.33 | 0 | 0 | 0 | 0.00 |
| $S_2Rh_1N_1$ | 0 | 2 | 0 | 0.67 | 0 | 29 | 18 | 15.67 |
| $S_2Rh_1N_2$ | 0 | 0 | 0 | 0.00 | 0 | 2 | 5 | 2.33 |
| $S_2Rh_2N_1$ | 26 | 68 | 37 | 43.67 | 62 | 220 | 38 | 106.67 |
| $S_2Rh_2N_2$ | 0 | 18 | 13 | 10.33 | 24 | 90 | 8 | 43.67 |

Table IV-2: The effect of *Rhizobium* inoculation and fertilizer N application on soybean growth

| Treatment/ Rep. | FIRST HARVEST | | | | SECOND HARVEST | | | |
|---|---|---|---|---|---|---|---|---|
| | I | II | III | MEAN | I | II | III | MEAN |
| $S_1Rh_1N_1$ | - | - | - | - | 31.1 | 20.5 | 21.1 | 24.6 |
| $S_1Rh_1N_2$ | - | - | - | - | 12.6 | 29.6 | 22.9 | 21.7 |
| $S_1Rh_2N_1$ | - | - | - | - | 23.3 | 17.8 | 26.0 | 22.4 |
| $S_1Rh_2N_2$ | - | - | - | - | 14.8 | 12.1 | 22.2 | 16.4 |
| $S_2Rh_1N_1$ | 3.44 | 2.02 | 2.98 | 2.75 | 27.6 | 23.4 | 34.8 | 28.6 |
| $S_2Rh_1N_2$ | 3.11 | 2.97 | 3.20 | 3.09 | 19.6 | 39.0 | 29.1 | 29.2 |
| $S_2Rh_2N_1$ | 1.71 | 4.00 | 4.52 | 3.41 | 21.5 | 23.6 | 29.8 | 24.9 |
| $S_2Rh_2N_1$ | 1.75 | 3.26 | 4.08 | 3.03 | 20.7 | 25.6 | 25.4 | 23.9 |

$S_1$ = Jupiter
$S_2$ = Dapros
$Rh_1$ = No Inoculation
$Rh_2$ = Inoculated with *Rhizobium* strain SN2
$N_1$ = No fertilizer N
$N_2$ = 30 kg/ha N as urea

Table IV-3: The effect of *Rhizobium* inoculation and fertilizer N application on the nitrogen content (% N) of the top plant material

| Treatment/ Rep. | FIRST HARVEST | | | | SECOND HARVEST | | | |
|---|---|---|---|---|---|---|---|---|
| | I | II | III | Mean | I | II | III | Mean |
| $S_1Rh_1N_1$ | 4.00 | 4.25 | 4.40 | 4.22 | 3.75 | 3.15 | 2.95 | 3.28 |
| $S_1Rh_1N_2$ | 4.20 | 4.50 | 4.25 | 4.32 | 4.00 | 4.00 | 4.25 | 4.08 |
| $S_1Rh_2N_1$ | 4.50 | 4.30 | 4.25 | 4.35 | 4.25 | 4.25 | 3.15 | 3.88 |
| $S_1Rh_2N_2$ | 4.25 | 4.35 | 4.75 | 4.45 | 4.25 | 4.50 | 4.25 | 4.33 |
| $S_2Rh_1N_1$ | 5.00 | 4.75 | 5.25 | 5.00 | 4.50 | 2.20 | 2.10 | 2.95 |
| $S_2Rh_1N_2$ | 6.75 | 5.50 | 5.25 | 5.83 | 2.50 | 4.85 | 4.50 | 3.95 |
| $S_2Rh_2N_1$ | 4.25 | 5.00 | 4.75 | 4.67 | 3.15 | 1.80 | 4.80 | 3.25 |
| $S_2Rh_2N_2$ | 4.75 | 4.75 | 4.00 | 4.50 | 2.70 | 3.45 | 4.75 | 3.65 |

Table IV-4: The effect of Rhizobium and fertilizer N application on soybean grain yield kg/ha

| Treatment/ Rep. | I | II | III | Mean |
|---|---|---|---|---|
| $S_1Rh_1N_1$ | 2209 | 2320 | 2832 | 2454 |
| $S_1Rh_1N_2$ | 2328 | 2222 | 2684 | 2411 |
| $S_1Rh_2N_1$ | 2291 | 2939 | 2760 | 2663 |
| $S_1Rh_2N_2$ | 2291 | 2461 | 3265 | 2673 |
| $S_2Rh_1N_1$ | 2202 | 2956 | 2258 | 2472 |
| $S_2Rh_1N_2$ | 1931 | 3010 | 2354 | 2432 |
| $S_2Rh_2N_1$ | 2249 | 2195 | 2850 | 2431 |
| $S_2Rh_2N_2$ | 2394 | 2082 | 2765 | 2414 |

# THE UNIVERSITY OF THE WEST INDIES
## DEPARTMENT OF SOIL SCIENCE
## CORNELL/UWI BIOLOGICAL NITROGEN FIXATION PROJECT
## PHASE II PROPOSAL

A. *PROJECT TITLE:* Maximizing the Grain Yield and Symbiotic Nitrogen Fixation by Grain Legumes in Trinidad

B. *PRINCIPAL INVESTIGATORS:* Spider Kajera Mughogho

C. *PERIOD:* May 1979 - December 1981

D. *INTRODUCTION:*

For the legumes to utilize their ability to fix atmospheric nitrogen, it is necessary to ensure that there is an appropriate Rhizobium strain. It is also necessary to supply the legumes with a range of elements only required in traces. Thus, in planning for fertilizer experiments with legumes, it is consequently necessary to take into account three factors, as well as the levels of the major nutrient elements.

1) The first is that steps should be taken to secure a strain of rhizobia known to be effective on the legume concerned.

2) An effectively fixing nodule system should have a number of secondary and/or trace elements.

3) Methods of application of the secondary or trace elements to the crops need to be standardized between different locations.

A layman's observation of some of the deficiency symptoms revealed in the field of soybeans and winged beans has shown that there are several nutrient elements that are deficient at any one time and location in the highly weathered soils of Trinidad. Thus one needs to take this into consideration since applying just one nutrient element would cause nutrient imbalance. The author has observed that applying high levels of P causes "phosphorus toxicity" which may be P-induced zinc deficiency. Overliming also caused some deficiency symptoms to appear on soybeans which may be those of zinc or magnesium.

In view of these facts, several experiments are proposed to cover the "grant project" period.

## E. *RESEARCH ACTIVITIES:*

### I. The Effect of Lime and Magnesium Application on the Grain Yield and Symbiotic Nitrogen Fixation:

Introduction:

It is reported that when calcitic limestone is applied to soils, some of the exchangeable magnesium will be replaced by Ca. That is, plant deficiencies of Mg may result if the Ca/Mg is too high, and this may be true of Mg-deficient acid sandy soils such as Piarco fine sand. Some of the loss of green color beginning with bottom leaves, and premature drop of leaves observed on soybeans and winged beans may be attributed to Mg-deficiency.

The purpose of this experiment will be to study the effect of lime as $CaCO_3$ and Mg as magnesium sulphate on the growth, nutrient uptake and grain yield of cowpeas and symbiotic $N_2$ fixation on a highly weathered Ultisol.

Materials and Methods:

The experimental plots will be conducted at the UWI/Mc Gill Sugarcane Feeds Center (SFC) site at Longdenville. Lime will be applied as calcium carbonate (88% $CaCO_3$, 60 mesh) and Mg as magnesium sulphate as follows:

$L_0 Mg_0$ - zero lime; zero Mg
$L_0 Mg_1$ - zero lime; 12 Kg Mg/ha
$L_0 Mg_2$ - zero lime; 24 Kg Mg/ha

$L_1 Mg_0$ - one ton lime/ha; zero Mg
$L_1 Mg_1$ - one ton lime/ha; 12 Kg Mg/ha
$L_1 Mg_2$ - one ton lime/ha; 24 Kg Mg/ha

$L_4 Mg_0$ - 4 tons lime/ha; zero Mg
$L_4 Mg_1$ - 4 tons lime/ha; 12 Kg Mg/ha
$L_4 Mg_2$ - 4 tons lime/ha; 24 Kg Mg/ha

Note that dolomitic limestone is not available in the country, and as such, magnesium sulphate will have to be used. Basal treatments will include Zn at 5 Kg/ha as zinc sulphate; P at 40 Kg/ha as triple

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superphosphate; K at 40 Kg/ha as muriate of potash; Molybdenum will be foliarly applied at 20 grams per hectare; and N will be applied at 60 Kg/ha as urea. Cowpeas (California 5 variety) will be used in this test.

Soil samples will be taken at the beginning and end of the experiment to determine pH, Al, N, P, K, Ca, Mg, Zn and C. Four plants per plot will be sampled for total nutrient analysis: N, P, K, Ca, Mg and Zn.

Throughout the duration of the experiment, plants will be harvested destructively for nodule number, weight, nitrogenase activity, top and/or root weight, and possibly leaf area. In addition, nodules will be analyzed for the percentage recovery of the inoculant strain.

## II. The Effect of Phosphorus and Zinc Application on Symbiotic Nitrogen Fixation, Leaf Number, Pod Number and Grain Yield of Soybeans:

### Introduction:

The relationship between lime and zinc, or phosphorus and zinc is widely recognized. In most cases it has been observed that where incipient deficiencies are known to exist, overliming such soils may cause Zn deficiencies. Increased use of phosphatic fertilizers may cause "phosphorus toxicity". This is believed to be Zn adsorption in the soil caused by high phosphorus applications. Another school of thought contents that normal metabolism is dependent upon a physiological balance between P and Zn.

The proposed study will be undertaken to check if Zn applications would alleviate the poor growth that results from "phosphorus toxicity".

### Materials and Methods:

Since agricultural micronutrients are not available in the country, laboratory grade zinc sulphate ($ZnSO_4.7H_2O$) will be used in the present experiment as a source of Zn, and triple superphosphate will supply P as follows:

$P_0$ $Zn_0$ - zero P; zero Zn
$P_0$ $Zn_1$ - zero P; 5 Kg Zn/ha
$P_0$ $Zn_2$ - zero P; 10 Kg Zn/ha

$P_1 Zn_0$ - 40 Kg P/ha; zero Zn
$P_1 Zn_1$ - 40 Kg P/ha; 5 Kg Zn/ha
$P_1 Zn_2$ - 40 Kg P/ha; 10 Kg Zn/ha

$P_2 Zn_0$ - 80 Kg P/ha; zero Zn
$P_2 Zn_1$ - 80 Kg P/ha; 5 Kg Zn/ha
$P_2 Zn_2$ - 80 Kg P/ha; 10 Kg Zn/ha

Basal treatments will include 2 tons lime per hectare broadcast as $CaCO_3$ (88% $CaCO_3$; 60 mesh), muriate of potash at 40 Kg/ha $K_2O$; magnesium sulphate at 12 Kg Mg/ha; and urea at 30 Kg N/ha at planting and 60 Kg N/ha at flowering all banded. Molybdenum will be foliarly sprayed at 20 grams per hectare as sodium molybdate.

Seeds will be inoculated before sowing with a Rhizobium strain containing a selective marker (SN2, streptomycene resistant). Plant roots and tops and soil samples will be treated as in Experiment I.

## III. The Effect of Cowpea Rhizobium and Fertilizer Nitrogen Application on Cowpea Grain Yield and Symbiotic $N_2$ Fixation on an Ultisol:

### Introduction:

Preliminary results from the field experiments on a Piarco fine sand at Wallerfield indicate that a Rhizobium strain isolated from Piarco fine sand was quite an efficient inoculant with cowpeas (Mughogho, Phast I - Project Report 1977-78) or with pigeon peas (Quilt, Final ODM Project Report) on Piarco fine sand. In case of cowpeas, a rhizobia strain isolated from Princes Town clay proved to be very inefficient. It is important to note that whereas in 1977 both inoculated and uninoculated treatments responded to fertilizer N, this effect was observed only in the non-inoculated cowpea treatments in 1978.

This experiment is designed to determine the competitive and survival capacity of Rhizobium strain 13B when used to inoculate cowpeas on Piarco fine sand at Longdenville.

### Materials and Methods:

Streptomycene resistant Rhizobium strain 13B (marked strain) will be used in this experiment to determine the competitiveness of the marked strain.

| First Year | Second Year |
|---|---|
| $Rh_1$ $N_1$ | $Rh_1$ $N_1$ $Rh_2$ |
| $Rh_1$ $N_2$ | $Rh_1$ $N_2$ $Rh_2$ |
| $Rh_1$ $N_3$ | $Rh_1$ $N_3$ $Rh_2$ |
| $Rh_2$ $N_1$ | $Rh_1$ $N_1$ $Rh_2$ |
| $Rh_2$ $N_2$ | $Rh_1$ $N_2$ $Rh_2$ |
| $Rh_2$ $N_3$ | $Rh_1$ $N_3$ $Rh_2$ |
| Grass $N_1$ | $Rh_1$ $N_1$ $Rh_2$ |
| Grass $N_2$ | $Rh_1$ $N_2$ $Rh_2$ |
| Grass $N_3$ | $Rh_1$ $N_3$ $Rh_2$ |

One factor to take into consideration is that there is a high incidence of diseases when cowpeas are grown successively. Cercospora is one of the diseases which have been identified to cause a great damage to the crop. In view of this fact, grass will be grown between the first year and the second year crops to reduce the incidence of diseases.

$Rh_1$ = no inoculation

$Rh_2$ = inoculated with cowpea rhizobia strain 13B(S) isolated from Piarco fine sand

$N_1$ = no nitrogen

$N_2$ = 30 Kg N/ha

$N_3$ = 60 Kg N/ha

Basal treatments will include 2 tons of lime/ha; 40 Kg P/ha, 40 Kg K/ha; 5 Kg Zn/ha, and 12 Kg Mg/ha.

Soil samples will be taken at the beginning and end of the experiment for determining total N and C. After one month of growth and at flower setting, destructive harvesting will be done to determine nodule count, nitrogenase activity, shoot length and dry weight and subsequently N concentration. Grain yield will be calculated on a Kg/ha basis.

## IV. The Effect of *Rhizobium japonicum* and Fertilizer Nitrogen Application on Soybean Grain Yield and Symbiotic $N_2$ Fixation:

### Introduction:

Soybeans will fail to develop effective nodules in the absence of an appropriate rhizobial population. Over and above, their response to the additions of P, K and/or other secondary or trace elements in such cases will be dependent on the soil and/or fertilizer N instead of atmospheric $N_2$. Preliminary results at Wallerfield on a Piarco fine sand (PFS) indicate that uninoculated soybean plants were infected by an "indigenous" *Rhizobium* strain which resulted in higher grain yields than inoculating with two commercial strains imported from the United States of America. For both the uninoculated and inoculated soybean treatments, there was a response to fertilizer N.

This experiment is designed to compare the response to and survival of one of the commercial *Rhizobium japonicum* strains (3I-1b-138) with the response to and survival of the Piarco fine sand isolate on Piarco fine sand.

### Materials and Methods:

*R. japonicum* strain SN2 (s) was isolated from Piarco fine sand whereas 31-1b-138 is a commercial strain from the U.S.A. The treatments will be as follows:

| First Year | Second Year |
|---|---|
| $Rh_1$ $N_1$ | $Rh_1$ $N_1$<br>$Rh_2$ |
| $Rh_1$ $N_2$ | $Rh_1$ $N_2$<br>$Rh_2$ |

| | |
|---|---|
| $Rh_2$ $N_1$ | $Rh_1$ $N_1$<br>$Rh_2$ |
| $Rh_2$ $N_2$ | $Rh_1$ $N_2$<br>$Rh_2$ |
| $Rh_3$ $N_1$ | $Rh_1$ $N_1$<br>$Rh_2$ |
| $Rh_3$ $N_2$ | $Rh_1$ $N_2$<br>$Rh_2$ |
| Grass $N_1$ | $Rh_1$ $N_1$<br>$Rh_2$ |
| Grass $N_2$ | $Rh_1$ $N_2$<br>$Rh_2$ |

$Rh_1$ = no inoculation

$Rh_2$ = inoculated with imported R. japonicum strain 3I-1b-138

$Rh_3$ = inoculated with P.F.S. R. japonicum strain SN2

$N_1$ = no fertilizer N

$N_2$ = 60 Kg/ha N as urea banded at flowering.

Destructive harvesting will be done at time of flowering and three weeks later (i.e. at pod setting and filling) for shoot length and weight, nodule number and weight, leaf number, nitrogenase activity and N content of the tops. After harvesting the entire crop for grain yield, the plots will be in fallow. For the second year, the plots will be subdivided as outlined above. The same parameters will be studied. The Rhizobium strains will be marked for antibiotic resistance and percentage effectiveness by the applied strains will be determine from nodulated plants.

## V. The Effect of *Rhizobium japonicum* Inoculation and Rates and Times of Fertilizer Nitrogen Application on Soybean Grain Yield and Symbiotic $N_2$ Fixation on an Ultisol:

### Introduction:

It has been reported in literature that combined nitrogen depresses nodulation. A second aspect which has also been reported has been the stimulating effect of nitrogenous fertilizers on nodulation if the rates are low. The third aspect is the demonstration that fertilizer N applied late in the growing period increases the N content of the crop and the grain yields.

This experiment was designed to investigate the effects of nitrogen application at time of planting and at flowering on symbiotic $N_2$ fixation and soybean grain yield.

### Materials and Methods:

Sterilized soybean seeds will be inoculated with *Rhizobium japonicum* and sown to plots on a Piarco fine sand at Longdenville. Different plots will be treated to different levels of nitrogen fertilizer either at sowing or at the time of flower initiation (see table for variables).

| | | |
|---|---|---|
| 1 - | $Rh_1$ $N_1$ | $Rh_1$ = no inoculation |
| 2 - | $Rh_1$ $N_2$ | $Rh_2$ = inoculated with strain SN2 (S) |
| 3 - | $Rh_1$ $N_3$ | $N_1$ = no fertilizer N |
| 4 - | $Rh_1$ $N_4$ | $N_2$ = 30 Kg/ha N at time of planting |
| 5 - | $Rh_1$ $N_5$ | $N_3$ = 60 Kg/ha N at time of planting |
| 6 - | $Rh_2$ $N_1$ | $N_4$ = 60 Kg/ha N at time of flowering |
| 7 - | $Rh_2$ $N_2$ | $N_5$ = 30 Kg/ha N at time of planting and 60 Kg/ha N at time of flowering |
| 8 - | $Rh_2$ $N_3$ | |
| 9 - | $Rh_2$ $N_4$ | |
| 10 - | $Rh_2$ $N_5$ | |

Plants will be destructively harvested at time of flower initiation and 3 weeks thereafter for nodule count and weight, nitrogenase activity, shoot length and weight and leaf number and/or pod number, and N content of the plant tops. Final harvest will be for grain yield and Harvest Index (H.I.). Note that no attempt will be made to identify the infecting *Rhizobium*.