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SANTO DOMINGO, REPUBLICA DOMINICANA

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THE EFFECT OF *RHIZOBIUM* INOCULATION AND FERTILIZER NITROGEN APPLICATION ON NITROGEN UPTAKE AND COWPEA AND SOYBEAN GRAIN YIELDS ON AN ULTISOL IN TRINIDAD

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INTRODUCCION:

Production and consumption of fertilizer nitrogen has increased tenfold in the past twenty years, and the requirements for fixed are expected to increase even more over the next twenty-five years (Hardy and Havelka, 1975). Even where nitrogen fertilizers are available, their costs and difficulties in distribution render them impractical for farmers in many areas of the world. Lately after the oil crises of 1973, the situation has been accentuated by the high energy costs. In view of these facts, the *Rhizobium* legume based symbiosis offers an alternative for increasing nitrogen input to food production.

Grain legumes such as cowpeas (*Vigna unguiculata*) and soybeans (*Glycine*) 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 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. 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 N₂ fixation or grain yields in Trinidad. It has been shown that on some soils in Trinidad fertilizer N increase 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 N₂ fixation and grain yields of soybeans and cowpeas on an Ultisol.

1977 Cowpea and Soybean Inoculation Experiment:

A study was undertaken to determine the effect of introduced strains of *Rhizobium* on the cowpeas and soybeans. Strains 97C (Rh2) and 13B (Rh3) isolated from a Princes Town clay and Piarco fine sand respectively were inoculated onto cowpeas and strains 31-1b-138 (Rh2) and SOY 13B (Rh3) imported from U.S.A were inoculated onto soybean. The purpose of this preliminary experiment was to assess the need for and effectiveness of inoculation compared with fertilizer N at, 0, 60, and 120 kg/ha applied as urea. This experiment was conducted on a Piarco fine sand, a highly weathered and leached acid Ultisol (Tables 1 and 2). This is a member of the fine sand, clay, kaolinitic, isohyperthermic family of aquoxic Tropodults. The surface 0-15 cm had a pH of about 4.8-5.0 while the 15-30 cm had a pH of 4.7-4.8 in KCl. The KCl extractable aluminium saturation varied from 40-80% in the top soil and 63-84% in the subsoil.

Preliminary results show that the uninoculated soybeans picked up an "indigenous" cowpea-type *Rhizobium* strain. Generally, it is reported that *R. japonicum* is not always present where soybeans have never grown previously. Inoculation greatly increased nodulation throughout the growth period. The uninoculated plots had a slow start, and nodulation greatly increased with time (Figure 1). Nodule count in cowpeas was already high at 33 days (Figure 2) and that there was no significant differences between treatments. At 42 days a lot of nodules had decayed, and this is because cowpeas are fast nodulators and are also short season crops.

A summary of the N accumulation data is presented in Table 3. The rates of N accumulation were slow early in the season in soybean plants whereas it was fairly fast in the cowpea plants, and this again is attributed to the fact that cowpeas are fast growers and nodulators. Analysis of variance indicates that a highly significant (P < 0.01) difference is found among effects of nitrogen treatments in soybeans at 42 and 75 days and at harvest. In all cases there was no significant difference caused by *Rhizobium* treatments.

The grain yields of both the soybeans and cowpeas showed greatest responses to first increments of fertilizer N (60 kg/ha) at all rhizobial treatments (Figure 3), and N application over and above 60 kg/ha reduced grain yields. This may be due to reduced efficiency of the rhizobia to fix atmospheric N₂ in the presence of high levels of combined N (Summerfield et al, 1977). Cowpea strain Rh3 isolated from an Ultisol gave slightly higher yields than non-inoculated or Rh2 plots. On the other hand, uninoculated plots which picked up an "indigenous" *Rhizobium* strain gave almost 50% more grain yields than when inoculated with either of the strains imported from the USA

N/ha 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 plots. In fact the grain yields in the uninoculated plots at 60 kg N/ha. 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.

There was a short dry spell in September/October, 1978 and as a result the soil was so desiccated that destructive sampling was impractical. To date only the N content and the grain yields of the soybeans are reported. Results in Table 7 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 plants. There was an indication that 60 kg N/ha rate was not adequate to sustain maximum grain yields in both rhizobial treatments (Table 7). This is because soybeans have inoculation did not have any beneficial effects over uninoculated plots except when no N was applied. One may argue that re-inoculation of soybeans is not necessary when the *R. japonicum* is established (Freire, 1976).

Under suitable conditions most of 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 N₂ 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 N₂ is essential;

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

| Sample ¹ | pH | Ca | Mg | Al | Cations | o/oAl |
|----------------------|-----|------|------|------|---------|-------|
| ————— 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 | 63 |
| 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 2. Some chemical analyses of the soil samples from
Wallerfield^{2/}

| Sample ^{1/} | P | Ca | Mg | K | Zn | o/o O.M | o/o N |
|----------------------|-----|-----|----|----|-----|---------|-------|
| ————— 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 | 0.06 |

1/ 0-15 cm and 15-30 is depth in cm

H 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/ NH₄ OAc - extractable cations.

Table 3: Accumulation of N by Soybean and cowpea plants at different Sampling periods as influenced by fertilizer nitrogen and inoculation (Kg/ha)

| Treat/ Period 1/ | | Soybeans | | | Harvest 2/ | | Cowpeas | |
|---------------------|----------------|----------|------|-------|------------|------|-----------|------|
| | | 29 | 42 | 75 | 33 | 42 | Harvest2/ | |
| 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 | 16.1 |
| 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 | 102.6 | 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 | 7.1 | 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 4: 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 | 50 | 29 | 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 5: 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 | 1.08 | 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 | 0.49 | 0.48 | 0.20 | 0.94 |
| Rh ₂ N ₁ | 0.99 | 1.38 | 1.05 | 0.60 | 0.89 | 0.67 | 0.88 |
| 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 6: 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 7: 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 8: 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 | 868 | 798 | 474 | 482 | 899 |
| Rh ₂ N ₂ | 1798 | 1456 | 1296 | 630 | 687 | 388 | 1059 |
| Rh ₂ N ₃ | 1036 | 1652 | 2186 | 664 | 908 | 388 | 1166 |

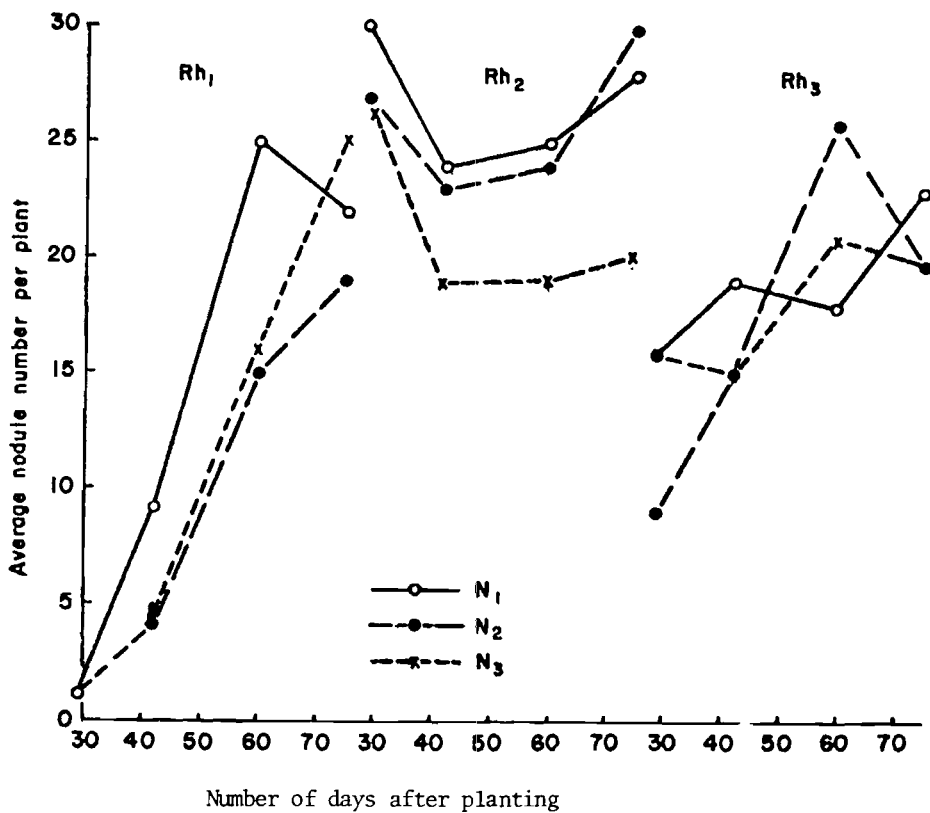


Figure 1. Change in nodule count in soybeans with time

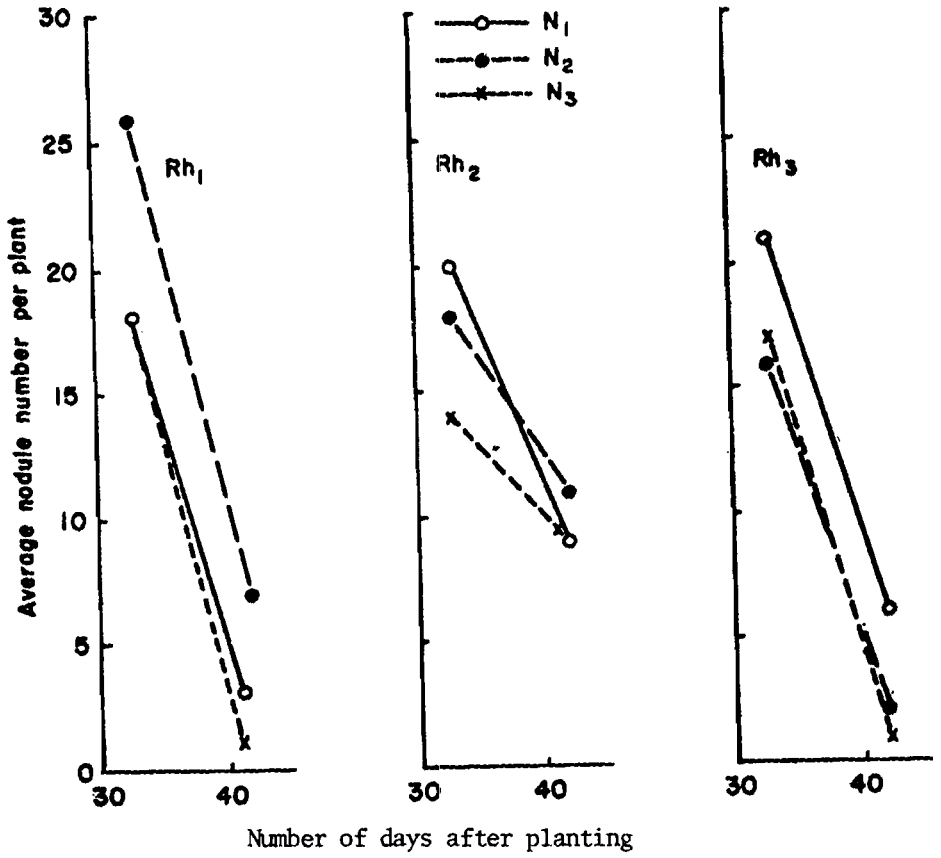


Figure 2. Change in nodule count on cowpeas with time

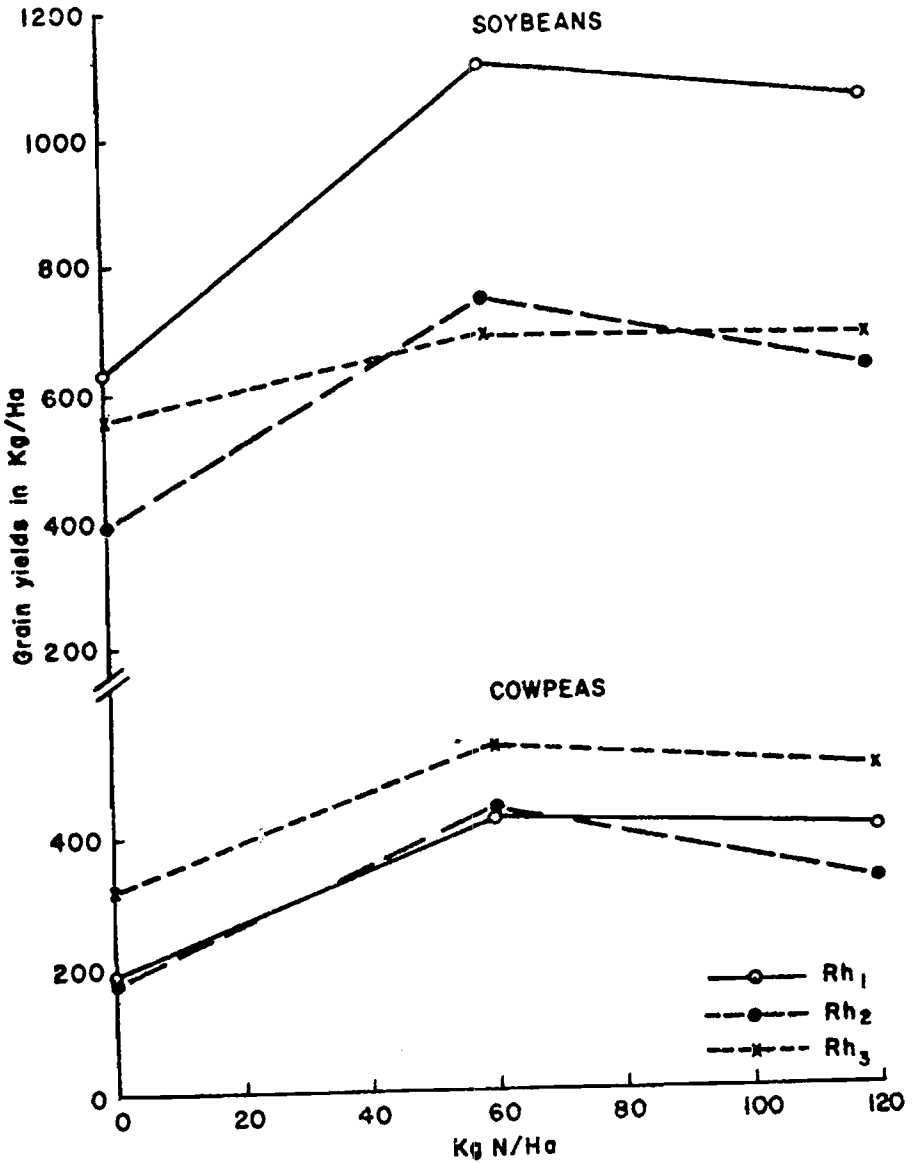


Figure 3. The effect of Nitrogen and Rhizobial inoculation on the grain yield of the soybeans (*Glycine max*) and the cowpeas (*Vigna unguiculata*).

(Rh2 and Rh3). This is attributed to the fact that *Rhizobium* from this Ultisol is presumably more adapted to the adverse soil conditions prevailing.

1978 Cowpea and Soybeans Re-inoculation Experiment:

Materials and Methods.:

For the cowpeas, the plots were laid on the site that had previously been cultivated to cowpeas in the major rainy 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 N/ha banded and rhizobia strains 13B and SN2 for Rh2 plots for cowpeas and soybeans respectively, and Rh1 as the check plots. Basal treatments included triple superphosphate, muriate of potash, zinc sulfate, magnesium sulfate at the rates of 40 kg P/ha, 5 kg Zn/ha, and 60 kg/ha Mg SO₄ respectively.

The plots were seeded to cowpeas, California 5 variety on June 20, 1978 at 4 seeds per stand 15 cm apart within the row 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 Rh2 and blank for the Rh1.

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 plants there was an increase in nodule count with increased N application, and that 30 kg N/ha had the highest nodule count (Table 4). It may be argued that a starter application of fertilizer N is necessary to best seedling growth before the plants nitrogen-fixing process begins (Ezedinma, 1964). On the other hand, the highest nodule count in the uninoculated plots resulted at the 0 kg N/ha. The theory behind this may be that N application at the time of planting reduces infectiveness since the *Rhizobium* is near the fertilizer

There was almost a linear increase in N uptake with fertilizer N application in the uninoculated plots (Table 5). The N uptake in the inoculated 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 N/ha.

Results in Table 5 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