Soil Microbiology

Objectives

The objectives of the Soil Microbiology section were specified in 1980 as: a) to select promising legumes which do not need inoculating, b) to select Rhizobium strains for those which do need inoculating, and c) to evaluate the effect of inoculation techniques and pasture management practices on nodulation and nitrogen fixation in the field.

Need to inoculate tests in cores of undisturbed soil

A series of experiments was carried out to detect responses to inoculation and nitrogen fertilizer of twenty legumes in Categories III, and IV and V in cores of undisturbed Carimagua soil. It was found that all the legumes tested produced more nitrogen in the tops when fertilized with nitrogen, and none produced more when inoculated than when not inoculated (Figures 1, 2 and 3). The strains used for inoculation were: CIAT 79 for Pueraria phaseoloides and Aeschynomene histrix; CIAT 71 + 1238 for Stylosanthes capitata; CIAT 71 for S. macrocephala, S. leiocarpa, S. guianensis and Zornia brasiliensis; and CIAT 299 for Desmodium ovalifolium and D. canum.

The response to inoculation was sometimes negative (Z. brasiliensis, S. macrocephala), although this was not statistically significant.

Some legumes nodulated more in the uninoculated treatments than others. For example, C. brasilianum ecotypes nodulated more with native strains than C. pubescens and C. macrocarpum, and produced correspondingly more nitrogen in the tops (Figure 3). Nitrogen fertilization inhibited nodulation in some legumes whereas in others it did not.

Comparison of disturbed and undisturbed soil, and mineralization studies

Four legumes were tested for their response to inoculation and nitrogen fertilization in cores of undisturbed soil and in pots of the same soil which had been dried and broken into small pieces. Figure 4 shows that the difference between the treatments with and without nitrogen was much greater in the undisturbed than in the disturbed soil. This is partly due to greater production of N in the tops in the disturbed soil than in the undisturbed soil without nitrogen fertilizer. This indicates that disturbing the soil stimulates mineralization of organic matter to produce NO_3^--N which is taken up by the plant.

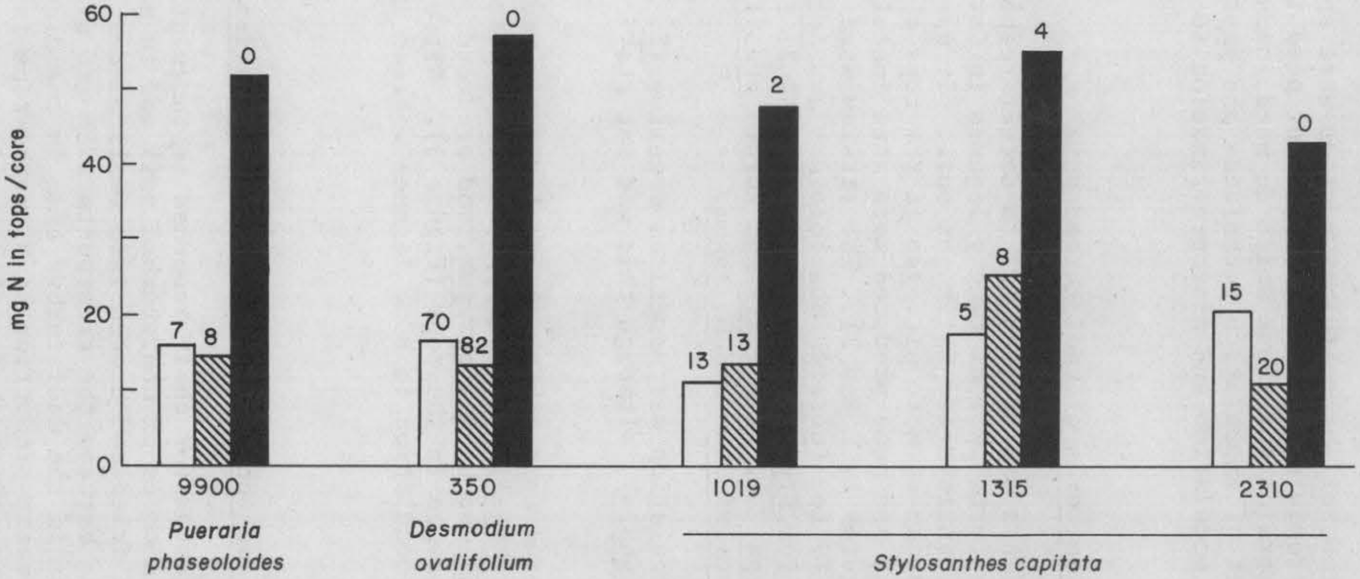

Figure 1. Response of forage legumes to inoculation (▨) and 150 kg N/ha (■) in cores of undisturbed Carimagua soil, and number of nodules per core.

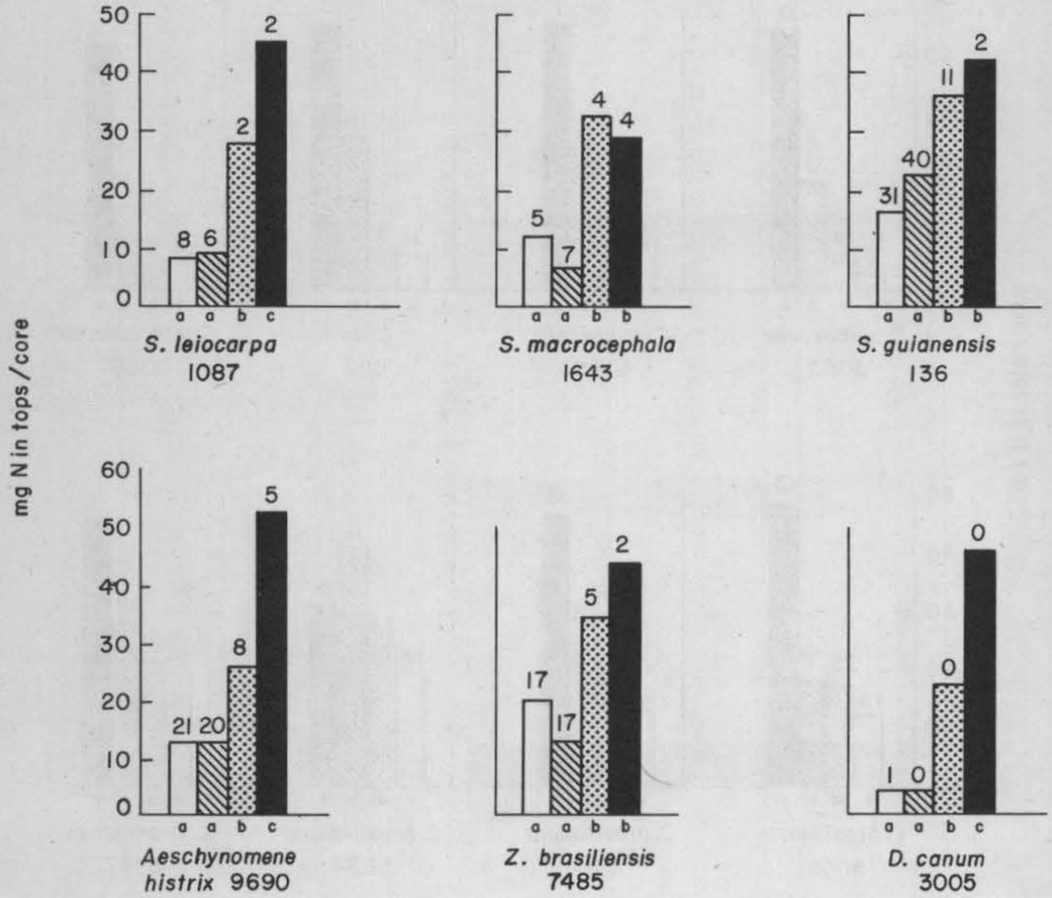

Figure 2. Response of forage legumes to inoculation ( ) and 75 ( ) or 150 ( ) kg N/ha in cores of undisturbed Carimagua soil, and number of nodules per core. Different letters represent significant differences in nitrogen in the tops.

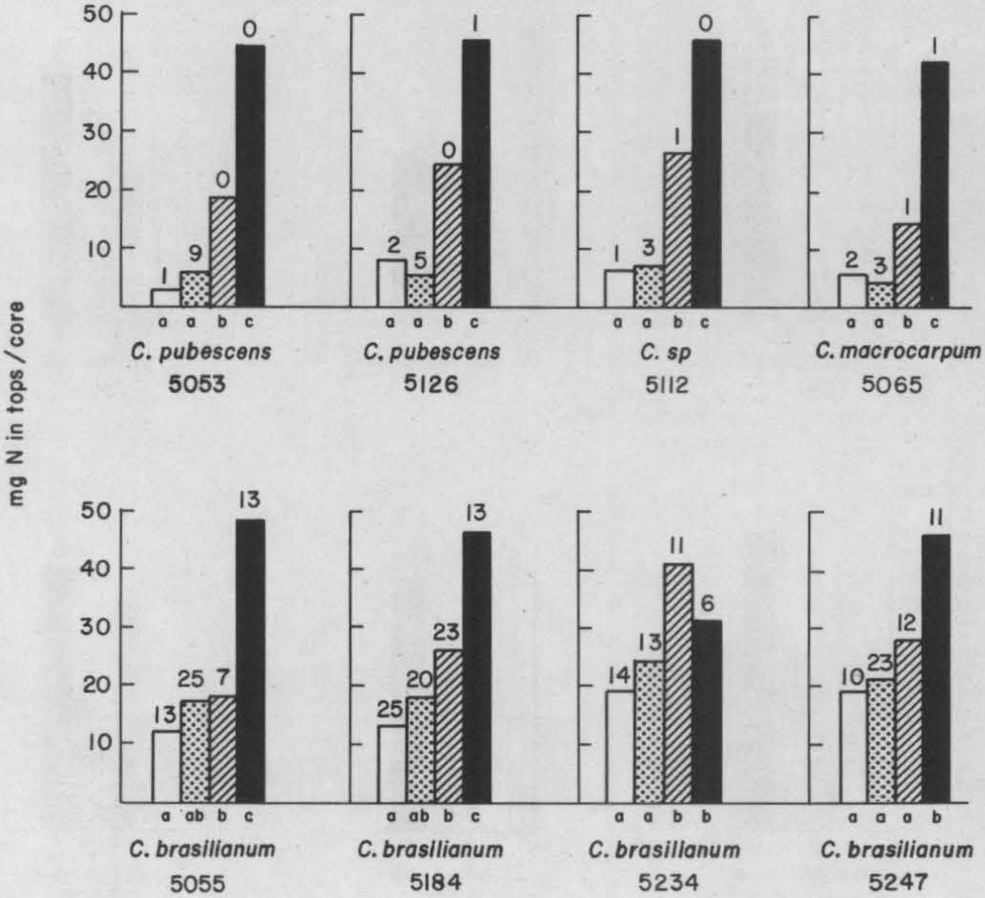

Figure 3. Response of eight Centrosema accessions to inoculation with CIAT strain 590 ( ) and 75 ( ) or 150 ( ) kg N/ha in cores of undisturbed Carimagua soil, and number of nodules per core. Different letters represent significant differences in nitrogen in the tops.

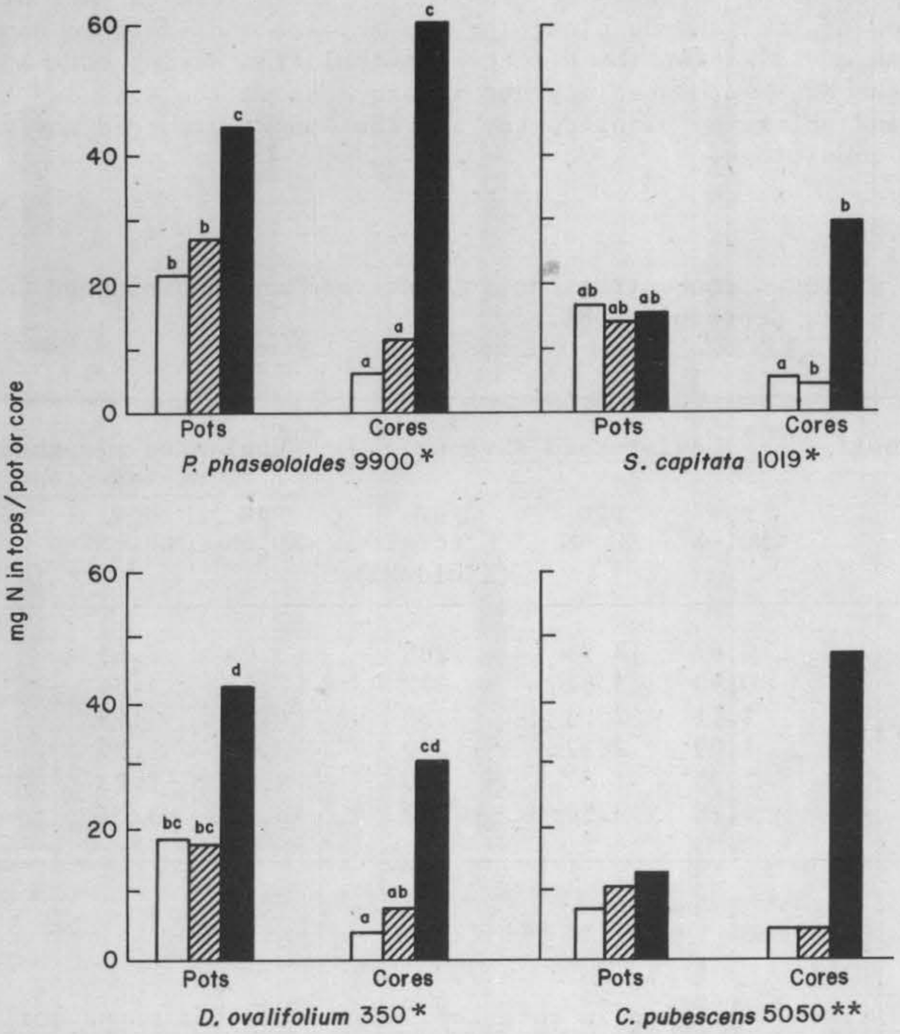

Figure 4. Response to inoculation (▨) and 210* or 120** kg N/ha (■) of four forage legumes in disturbed (pots) or undisturbed (cores) of unsterilized Carimagua soil after 12 weeks growth. Different letters represent significant differences in nitrogen in tops.

Table 1 shows that the soil under undisturbed savanna contains very low concentrations of NO_3^--N. However in soil which had been ploughed but not planted the levels were higher, and at 1 m depth there were 18 ppm NO_3^--N. The NH_4^+-N levels in the surface soil were correspondingly lower. In soil from different sites at Carimagua which had been ploughed and planted with S. capitata, the NO_3^--N levels varied between 1 and 3 ppm depending on the site (Table 2). These results indicate that preparation of the land by ploughing can release considerable amounts of NO_3^--N which may diminish the effect of inoculation during establishment. However, the NO_3^--N produced may not remain long in the soil due to leaching and uptake by plant roots, and the amount produced may vary with soil conditions.

Table 1. Nitrogen concentrations in disturbed and undisturbed Carimagua soil, September 1981.

Depth in soil (cm)	Undisturbed savanna			Unplanted ploughed and disked savanna		
	ppm NO_3^--N	ppm NH_4^+-N	ppm N total (Kjeldahl)	ppm NO_3^--N	ppm NH_4^+-N	ppm N total (Kjeldahl)
0 - 5	0.97	18.75	1288	3.94	7.53	1064
20 - 25	0.90	3.82	672	7.77	3.34	672
40 - 45	1.11	2.83	336	6.51	2.49	392
60 - 65	1.09	2.17	280	3.30	1.96	336
89 - 85	1.38	1.82	280	9.60	2.23	336
95 -100	1.18	2.50	280	18.23	3.01	280

Rhizobium strain selection in cores of undisturbed Carimagua soil

Over 1000 new strains of Rhizobium isolated from legumes for which previously few strains were available have been added to the collection this year. The strains are tested for their ability to nodulate "Siratro" if their cultural characteristics are not those typical of Rhizobium. Some of the strains are fast-growing or produce acid in neutral yeast mannitol agar or both, which is unusual for Rhizobium isolated from tropical pasture legumes. A small proportion of the strains grows better on acid than on neutral medium.

Preliminary experiments have been carried out to test the response of different legume hosts to inoculation with a range of Rhizobium strains (usually isolated from the same species) in cores of undisturbed Carimagua soil in order to determine which strains are most effective.

Table 2. Nitrogen concentrations in ploughed and disked soil planted with *Stylosanthes capitata* at Carimagua in June-August and sampled in September 1981.

Depth in soil (cm)	Hato 1 Yopare			Hato 6 Yopare			Hato 8 Alegría		
	ppm NO_3^--N	ppm NH_4^+-N	ppm N total (Kjeldahl)	ppm NO_3^--N	ppm NH_4^+-N	ppm N total (Kjeldahl)	ppm NO_3^--N	ppm NH_4^+-N	ppm N total (Kjeldahl)
0 - 5	2.17	14.78	1176	1.07	11.40	784	1.05	11.73	616
20 - 25	2.55	5.06	560	1.53	4.43	448	1.50	3.08	280
40 - 45	3.00	3.98	392	1.56	3.19	280	1.07	1.71	168
60 - 65	2.57	3.15	392	1.30	2.57	224	1.06	2.11	168
80 - 85	2.34	3.16	280	1.32	2.52	168	1.73	1.97	112
95 -100	2.12	4.75	280	1.60	3.87	168	1.27	1.74	112

Figure 5 shows the results obtained with Desmodium ovalifolium. The best strain increased N in tops of the uninoculated control by 18% (1.81 fold). However N in tops of the N fertilized control was much greater than any of the inoculated treatments, and the best strain was not significantly better than a group of 10 other strains, although it was significantly better than the uninoculated control. Possibly this was due to the limitation of nitrogen fixation by the low nutrient levels in the cores.

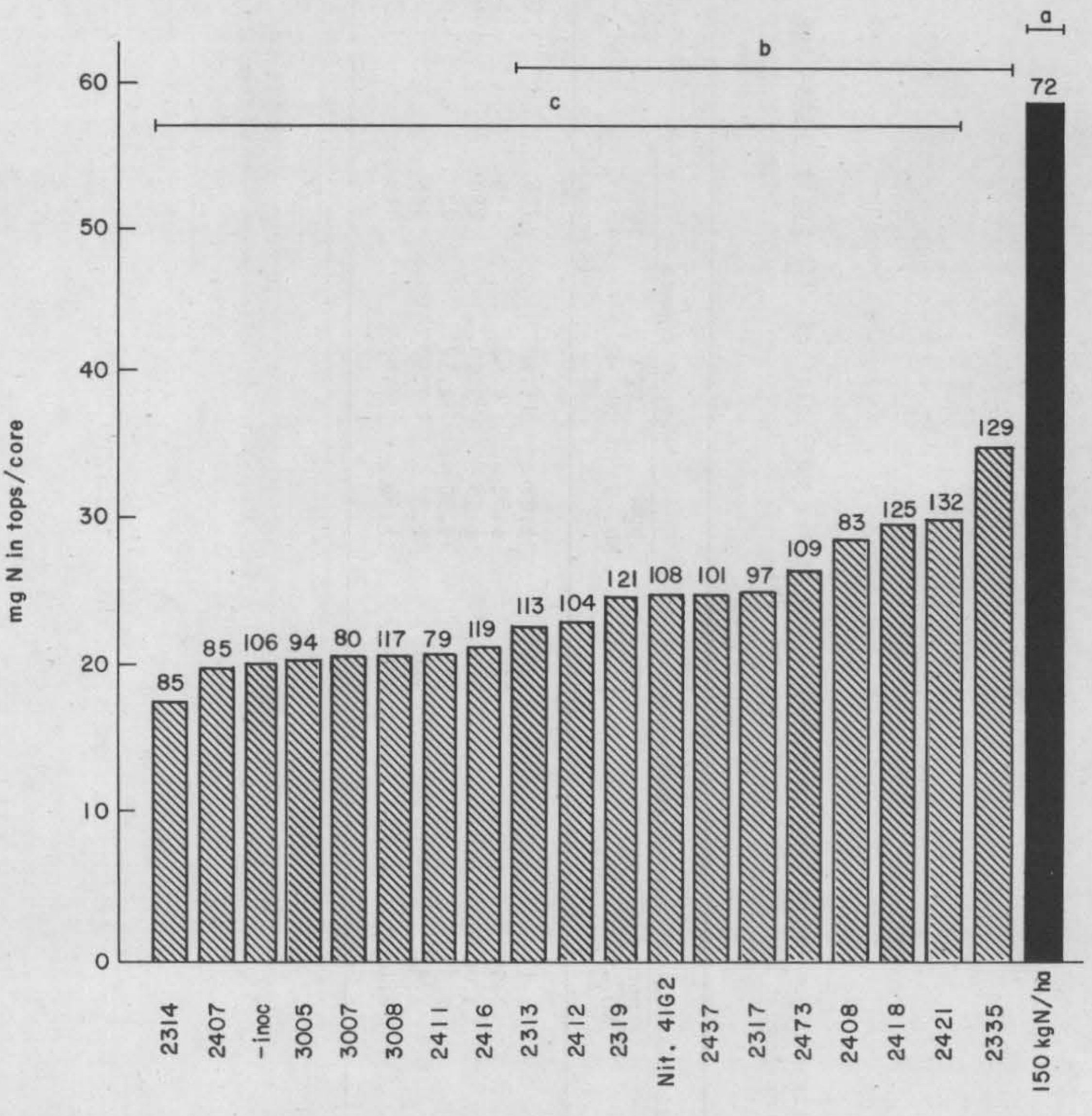

Figure 5. Response of Desmodium ovalifolium 350 to inoculation with different Rhizobium strains ( ) and nitrogen ( ) in cores of undisturbed Carimagua soil, and number of nodules per core.

Figure 6 shows that S. capitata responded to nitrogen, but the control without nitrogen or inoculation contained high nitrogen levels even though it had few nodules. The best strain increased N in tops of the uninoculated control by only 39% (1.39 fold) although the total N in these plants was much higher than in the equivalent D. ovalifolium plants.

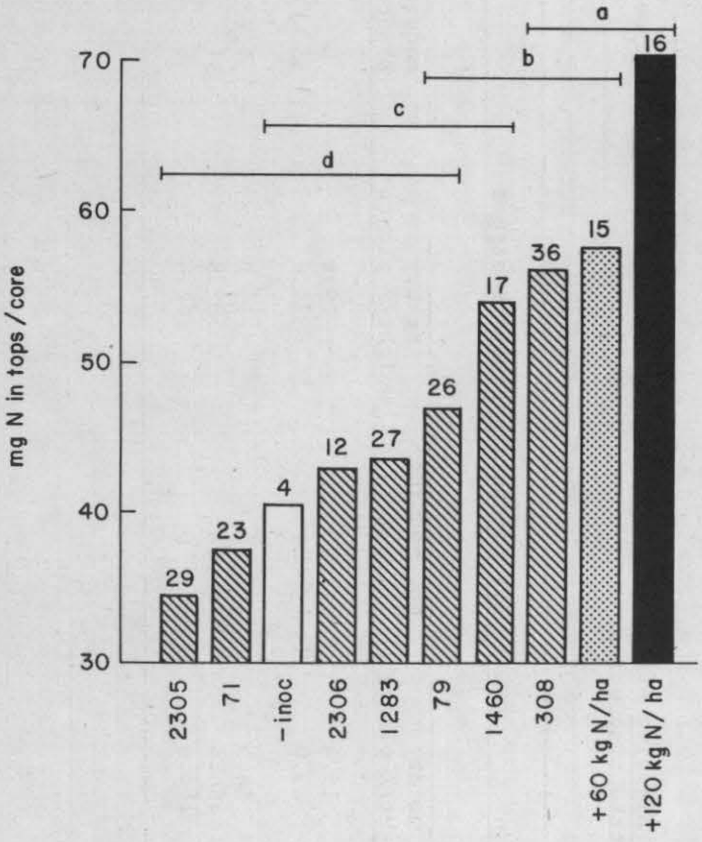

Figure 6. Response of Stylosanthes capitata 1019 to inoculation with different Rhizobium strains ( ) and two levels of N ( ) in cores of undisturbed Carimagua soil, and number of nodules per core.

This indicates that nodulation by S. capitata is very sensitive to low levels of soil nitrogen, which are used very efficiently by the plant. In sand and acid (pH 4.5) nutrient solution the difference between the controls with and without nitrogen was much greater, and CIAT strain 1460 was found to be very effective on three ecotypes of S. capitata (Table 3). Strain 308, which was effective in the cores of soil, was not effective in the sand and acid nutrient solution.

Table 3. Effect of inoculation of Rhizobium strains on nodulation and growth of three ecotypes of S. capitata in sand and acid (pH 4.5) nutrient solution.

	Stylosanthes capitata ecotypes					
	1019		1315		2310	
Treatment	No. nodules/ 2 plants	mg dry weight/2 plants	No. nodules/ 2 plants	mg dry weight/2 plants	No. nodules/ 2 plants	mg dry weight/2 plants
Not inoculated	0	15.4	0	19.4	0	17.0
Strain 308	11	23.0	31	36.6	35	28.8
Strains 71 + 1238	0.2	14.5	0.6	15.2	1.6	16.4
Strain 1460	28	456.4	24	502.8	31	606.6
10 ppm N	0	158.4	0	366.4	0	227.4

Centrosema macrocarpum 5065 and *Centrosema* sp. 5112 were inoculated with the same 11 strains of *Rhizobium*. For both legumes strain 1780 was effective increasing N in tops by 260% (3.6 fold) and 150% (2.6 fold), respectively (Figures 7 and 8). However, the three most effective strains of *Centrosema* sp. 5112 were among the four least effective for *C. macrocarpum*. Strain 2389 was completely ineffective for both legumes, even though it increased the number of nodules per plant. The two figures show that *Centrosema* sp. 5112 nodulated more effectively with a wider range of strains than *C. macrocarpum*.

In other similar experiments with *Pueraria phaseoloides* and *Desmodium canum*, strains 2434 and 1502 respectively, produced most nitrogen in the tops.

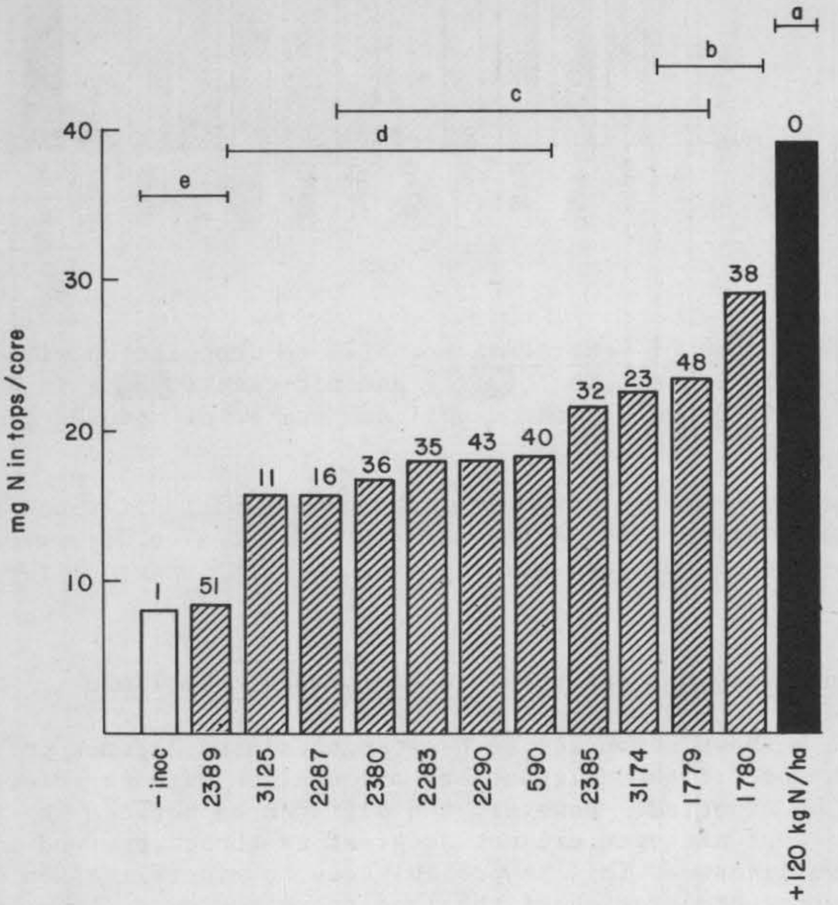

Figure 7. Response of *Centrosema macrocarpum* 5065 to inoculation with different *Rhizobium* strains (▬) and nitrogen (▨) in cores of undisturbed Carimagua soil, and number of nodules per core.

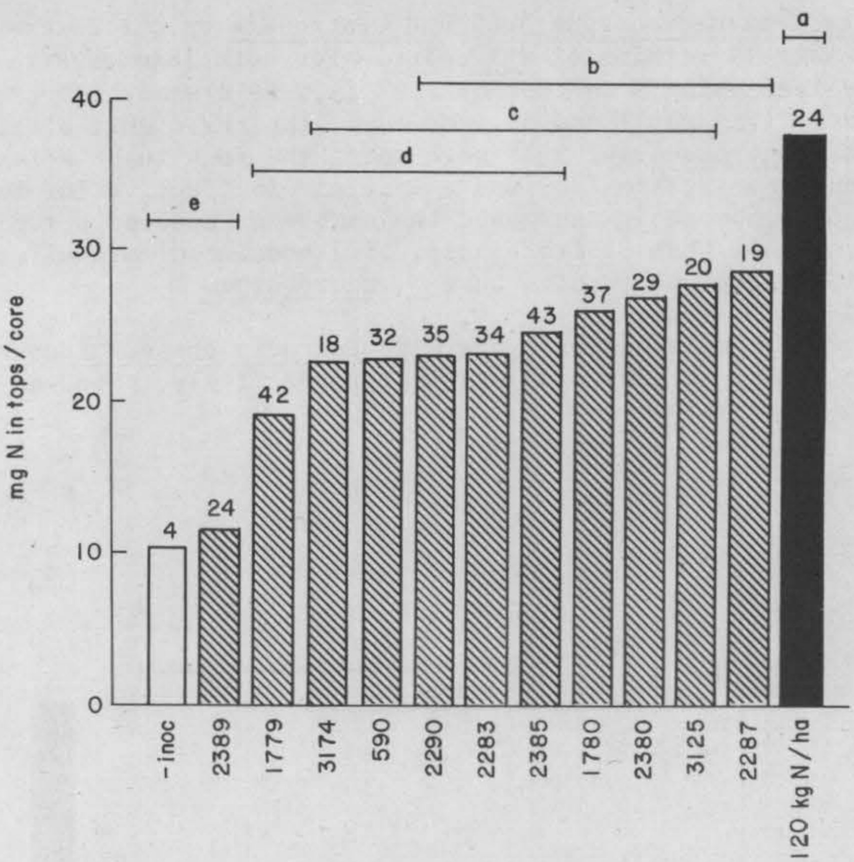

Figure 8. Response of Centrosema sp. 5112 to inoculation with different Rhizobium strains ( ) and nitrogen ( ) in cores of undisturbed Carimagua soil and number of nodules per core.

Further experiments are being carried out with different fertility levels and different soil in the undisturbed cores to determine whether the performance of the good strains is consistent under different conditions.

Response to nitrogen of uninoculated legumes in the field

Figure 9 shows responses to N of uninoculated legumes in the field. Thus, a response of these legumes to inoculation with an effective strain can be expected. However, the differences between the treatments with and without nitrogen are not as great as those observed in the disturbed soil cores. This is probably due to mineralization of soil nitrogen during preparation of the land for planting. These legumes were planted in pure stands. It is possible that if they were planted with a grass, nitrogen uptake by the grass would increase nitrogen deficiency in the uninoculated legumes. Further studies to detect responses of legumes to inoculation in the field will be carried out in soils expected to be deficient in nitrogen such as sandy soils, humid soils, soils prepared with minimum tillage, and in mixed swards with grasses.

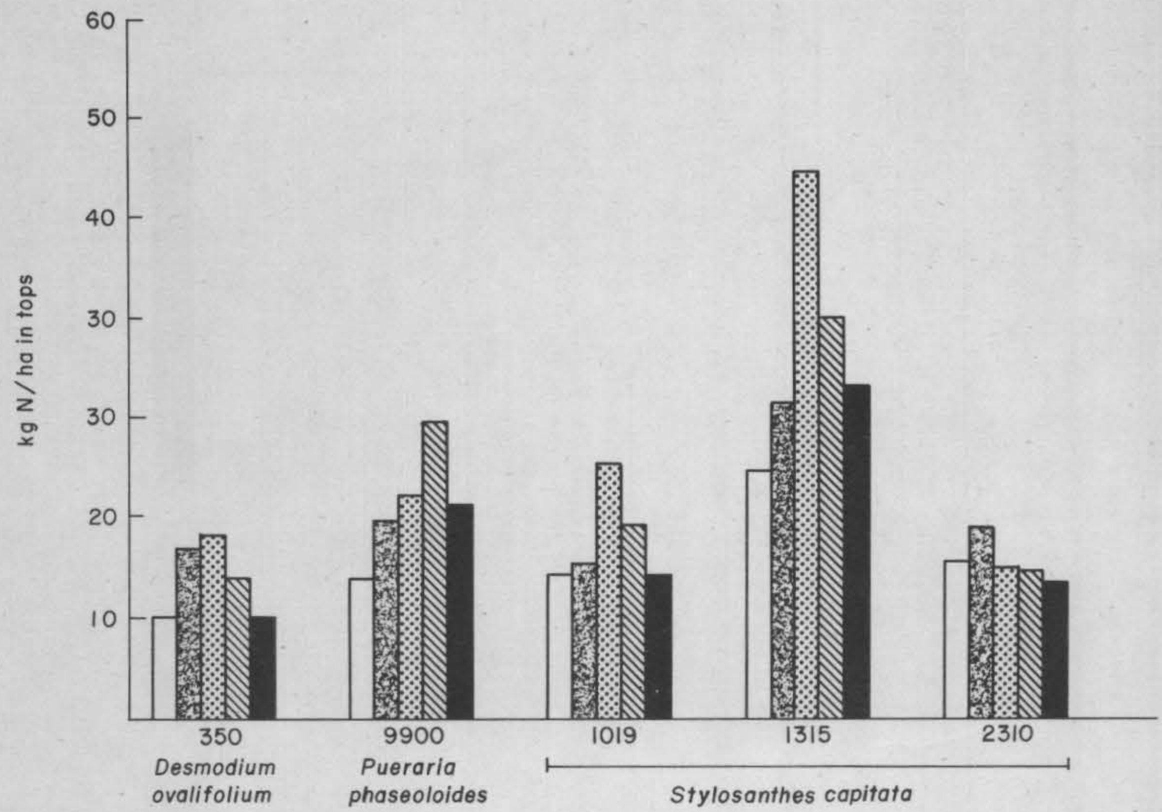

Figure 9. Effect of N (urea) 0 ( ) 25 ( ) 50 ( ) 100 ( ) and 200 ( ) kg N/ha on production of nitrogen in the tops of forage legumes at Carimagua, three months after planting.