EFFECT OF DIFFERENT CENTRAL AMAZONIAN SOILS ON GROWTH, NODULATION, AND OCCURRENCE OF N_2 –FIXING *Azospirillum* spp. IN ROOTS OF SOME CROP PLANTS[1] /

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Resumo

Foram plantados feijão de asa, soja, feijão caupi e milho em diferentes solos da Amazônia Central, numa área protejida de chuva. As taxas de crescimiento das plantas, nodulação e ocorrência de Azospirillum *em latosolo amarelo argiloso e solo podsódico arenoso não queimados foram muito baixas. Em latosolo argiloso queimado o crescimento das plantas e a ocorrência de* Azospirillum *foram maiores, mas ocorreu pouca nodulação. Soja var. Jupiter nodulou espontaneamente em solo aluvial ("várzea"). Com inoculação de* Rhizobium *a nodulação de soja aumentou em solos da várzea e terra preta dos índios. A nodulação de feijão de asa e feijão caupi não aumentou com inoculação sem adubação em latosolo ou solo podsólico, mas em solo de várzea o crescimento e a nodulação de feijão de asa inoculado aumentaram. O crescimento e nodulação de feijão de asa inoculado e adubado aumentaram, especialmente em solo podsólico arenoso, enquanto o crescimento de feijão caupi aumentou em todos os solos, e nodulação somente em solo podsólico queimado e solo da várzea. Os dados indicaram que a ausência de nodulação em latosolo argiloso era parcialmente devida à presença de nitrogênio no solo.*

Introduction

Yellow latosol occurs over a large proportion of the Central Amazonia (4, 5, 8, 10). Much of this soil is very heavy, acidic (pH 4.0 – 5.0), has a high aluminium saturation level (over 50% of total C. E. C.) and low available phosphorus 1-2 ppm). Areas of a more sandy soil (red-yellow podsol) occur on the valley sides. This sandy soil is grey in colour at the surface but appears similar to yellow latosol when analysed by routine chemical tests. These two soils will be distinguished here by use of the terms latosol and podsol. Sandy podsol is often used in preference to clayey latosol for subsistence agriculture by the local inhabitants.

The natural vegetation on these two soils is forest which showed a living biomass of 473 t ha^{-1} (dry weight), containing 2.983 kg N ha^{-1} (6). However, when three parameters were used to estimate nitrogen-fixing activity (nodulation, acetylene-reducing activity and occurrence of N_2 –fixing *Azospirillum* spp.), little activity was found in association with roots of trees growing in areas of undisturbed forest on clayey latosol (12). Plants growing in sandy podsol and more fertile soils showed higher levels of activity.

Areas of forest in this region are being cleared for agricultural development, the trees being burned after felling which releases some plant nutrients into the soil and atmosphere. Growth of *Pennisetum purpureum* planted in recently cleared latosol near

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1 Received for publication August 13, 1980.
The authors thank the soil laboratories at CEPEC, Itabuna, Bahia; EMBRAPA Km 47, RJ and IAC, Campinas, SP for carrying out plant and soil analyses, and Valmique Silva de Souza for valuable technical assistance. A Portuguese version of this paper will be published in Acta Amazonica.

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Manaus was enhanced tenfold by fertilization with phosphorus but nitrogen gave little response. Maize and cowpea also showed little response to nitrogen in the first year of cultivation, but in succeeding years the response increased (1). This implies that nitrogen was not the limiting nutrient immediately after deforestation. However, continuous cultivation of the soil, especially if fertilized with phosphorus alone would rapidly cause depletion of the soil nitrogen and other nutrients. It is thus important that nitrogen-fixing plants should be incorporated into the agricultural systems being introduced in order to avoid the use of costly inorganic nitrogen fertilizers.

The objective of the investigations described here was to determine whether crop plants growing in representative Central Amazonian soils fix nitrogen and whether this activity can be enhanced by fertilization and inoculation of the appropriate bacteria.

Materials and Methods

Experiment I: Soil samples were collected from five sites considered to represent Central Amazonian soil types as follows: "alluvial" soil from the north bank of the Rio Solimões about 1 km above its confluence with the Rio Negro; "sandy podsol" at an experimental site at km 14, AM-010; "clayey latosol" at the Instituto Nacional de Pesquisas da Amazonia (INPA) Reserve (Estação Experimental de Silvicultura Tropical, EEST) at km 45 of the Manaus-Caracaraí road BV-8; "burned clayey latosol" in an area at EMBRAPA-UEPAE-Manaus (km 30, AM-010) which had recently been deforested and burned, and which therefore contained ash; "black earth" from the Estrada do Aleixo, Manaus, on the north bank of the Rio Negro opposite its confluence with the Rio Solimoes. Soil analyses and descriptions of alluvium and black earth are shown in Table 1. Samples were collected at five points at each site from 0-20 cm depth and mixed. The soil was distributed in plastic bags of 1.6 kg capacity covered with aluminium foil to prevent excessive temperatures. Soybean cv. Jupiter, winged bean (*Psophocarpus tetragonolobus* cv. IRI 30-91 from the Legume Research Institute, Singapore) and maize seeds were surface-sterilized in 0.1% acidified $HgCl_2$ and planted in four replicate bags of each soil. The plants were irrigated with distilled water. Plant dry weight, dry weight of nodules on the legumes, *Azospirillum* enrichment root culture activity for the maize, acetylene-reducing activity of the intact system and total foliar nitrogen were determined after 42 days growth in a rain-proof area. *Azospirillum* enrichment root culture activity was determined as previously described (12). Acetylene reduction was determined by enclosing the entire plant and soil system in a polyethylene bag and incubating with acetylene for three hours, after which gas samples were analysed for ethylene by gas chromatography (2).

Experiment II: Samples of the same soils as those used in Experiment I were taken. Eight bags of each soil type were planted with surface-sterilized soybean cv. Jupiter seeds, four with rhizobium inoculum and four without. The rhizobium culture used for the inoculum had been isolated from nodules on soybeans grown in Experiment I in alluvial soil. Dry weight of nodules and plants were determined after 45 days growth.

Experiment III: Clayey and medium latosol and sandy podsol were colllected from three sites close to the INPA EEST reserve at km 45 BV-8. Samples at each site were collected from primary forest and

Table 1. Analyses of soils used in Experiments I and II.*

Soil Type	P ppm	K ppm	Ca + Mg (meq./100 g)	Al (meq./100 g)	pH	Organic matter (%)	(Total) N %	C/N
Alluvium (A)	>30	144	14.3	0.8	5.0	0.4	0.12	2
Podsol (P)	15	28	0.7	0.5	4.7	0.8	0.07	7
Clayey latosol (CL)	2	66	0.5	2.5	3.6	5.0	0.32	9
Burned clayey Latosol (BCL)	1	56	1.7	0.5	4.6	2.2	0.18	7
Black earth (BE)	3	44	6.3	0.1	5.2	3.1	0.17	11

* Analyses carried out at EMBRAPA-Km 47-RJ. Black earth ("terra preta dos indíos") is a fertile soil containing many pieces of broken ceramics presumed to be left by the indians who once inhabited these sites (9). Alluvial or "várzea" soil is the fertile alluvial soil deposited in annual floods on the banks of sediment-loaded rivers such as the Rio Amazon.

from adjacent areas which had recently been deforested and burned. These as well as alluvial soil from the banks of the Rio Solimões were obtained using the same methods as those described for Experiment I. Surface-sterilized and germinated winged bean cv. IRI-30-91 and cowpea [*Vigna unguiculata* cv. VITA 3 (TVU 1190)] seeds were planted in bags of each soil with three treatments: untreated, inoculated and inoculated and fertilized, with five replicates of each treatment. The rhizobium inoculants used were prepared from cultures isolated from nodules on the respective plants growing locally and pretested for efficient nitrogen fixation on plants in sterile sand and nutrient solution. Fertilization rates of triple super phosphate, KCl and lime were calculated from the soil analyses (Table 2) following the recommendations of Souza (11). Nodule and plant dry weight were determined after 75 days growth.

Results

Table 3 shows that in Experiment I plant dry weight, nodule weight, *Azospirillum* enrichment culture activity and total plant nitrogen were all relatively low in unburned clayey latosol and sandy podsol.

In the other three more fertile soils (burned clayey latosol, black earth and alluvial soil), soybeans and winged beans grew equally well, whereas maize grew better in burned latosol than in alluvial soil or black earth.

The pattern of occurrence of nodulation and *Azospirillum* enrichment culture activity in the three more fertile soils was different for each plant. Soybeans showed some nodulation in alluvial soil, and negligible amounts in other soils. Winged beans showed good nodulation in both alluvium and black earth. They did not nodulate well in burned clayey latosol even though plant weight was relatively high in this soil. On the other hand activity of *Azospirillum* enrichment cultures from maize roots was higher in burned latosol and alluvium than black earth.

Total plant nitrogen was particularly high in burned clayey latosol for all three plants. The acetylene reduction assays carried out on the intact system at the end of the experiment showed acetylene reduction only by winged beans in alluvial soil and black earth.

Experiment II was set up in order to test further the nodulation of uninoculated soybeans in alluvial soil observed in Experiment I. Table 4 shows that inoculation of soybeans with *Rhizobium* isolated from the nodules formed on soybeans in alluvial soil in Experiment I caused better nodulation in alluvial soil and black earth. However, plant weight was only slightly higher in the inoculated treatment in alluvial soil.

The results of Experiment III are shown in Table 5. Although growth of the winged beans and cowpeas was better in burned than unburned latosol and podsol in no case was it as good as when the soil was fertilized. Even in alluvial soil growth of cowpeas was better with fertilization. Cowpeas in unburned unfertilized latosol and podsol either failed to germinate or died shortly after germination, despite various replantings.

Neither winged beans nor cowpeas nodulated better in burned than unburned latosol and podsol

Table 2. Analyses of soils* and fertilization rates used in Experiment III.

Soil Type**	P ppm	K ppm	Mg (meq./100 g)	Ca (meq./100 g)	Al (meq./100 g)	pH	Organic matter %	Fertilizer applied: Lime t ha⁻¹	Fertilizer applied: TSP** kg ha⁻¹	Fertilizer applied: KCl kg ha⁻¹
C	2	24	0.1	0.2	0.7	3.9	3.4	4.10	336	101
BC	4	102	0.6	1.3	0.1	5.0	5.0	0.65	285	60
M	2	28	0.2	0.2	0.7	4.0	3.4	3.98	336	101
BM	3	28	0.1	0.0	0.5	4.0	2.2	3.32	336	101
P	2	17	0.1	0.0	0.9	3.8	3.6	4.79	336	101
BP	2	21	0.1	0.0	1.0	3.6	4.2	4.95	336	101
A	52	106	2.0	9.9	0.1	5.0	1.9	0.00	143	101

* Carried out at IAC, CAMPINAS, SP.

** C = clayey latosol; BC = burned clayey latosol; M = medium latosol; BM = burned medium latosol; P = podsol; BP = burned podsol; A = alluvium; TSP = triple super phosphate.

Table 3. Levels of four plant parameters in different Central Amazonian soils (Experiment I). Values of the same parameter anotated with different letters are significantly different at the 50% level (Tukey's test).

Plant parameter	Soil type*	Soybean	Winged bean	Maize
Dry weight (g) plant $^{-1}$	A	1.39 [a]	1.20 [ab]	0.96 [b]
	P	0.24 [b]	0.75 [b]	0.20 [b]
	CL	0.24 [b]	0.52 [b]	0.56 [b]
	BCL	1.29 [a]	1.69 [a]	2.30 [a]
	BE	1.21 [a]	1.01 [ab]	0.90 [b]
Nodule weight (mg) plant $^{-1}$ (legumes) and root enrichment assay nmol C_2H_4 culture $^{-1}$ h^{-1} (maize)	A	20.70	62.70 [a]	205.08 [a]
	P	0.00	1.67 [b]	12.78 [b]
	CL	0.00	0.85 [b]	24.99 [b]
	BCL	0.00	0.47 [b]	198.29 [a]
	BE	0.10	41.37 [ab]	20.80 [ab]
Total N (mg) plant $^{-1}$	A	19.00 [b]	51.28 [b]	10.63 [b]
	P	6.88 [c]	26.38 [e]	3.64 [b]
	CL	8.51 [c]	22.66 [e]	9.48 [b]
	BCL	38.10 [a]	84.22 [a]	24.15 [a]
	BE	24.87 [b]	43.40 [cd]	9.93 [b]

* As in Table 1.

Table 4. Means and coefficients of variation (in brackets) of dry weights of nodules and whole soybean cv Jupiter plants in different inoculated and uninoculated Central Amazonian Soils (Experiment II).

Soil Type	mg nodules plant $^{-1}$		dry weight plant $^{-1}$ (g)	
	Uninoculated	Inoculated	Uninoculated	Inoculated
Alluvium	30.75 (187)	224.85 (33)	2.49 (30)	2.73 (10)
Podsol	0.00	22.75 (31)	0.81 (33)	0.65 (3)
Clayey latosol	0.00	0.50 (141)	0.53 (34)	0.41 (24)
Burned clayey latosol	0.00	63.85 (80)	2.27 (32)	1.67 (47)
Black earth	0.00	229.30 (27)	2.33 (13)	1.90 (44)

without fertilizer, even in the presence of inoculated *Rhizobium* spp. In alluvial soil inoculation alone improved both nodulation and growth of winged beans, but not of cowpeas.

In the fertilized and inoculated treatments winged beans nodulated well, particularly in the sandy podsol. Cowpeas on the other hand only showed good nodulation in fertilized and inoculated burned podsol and alluvial soil.

Discussion

The growth of winged beans, soybeans and maize in Experiment I varied markedly between the different soil types. The good growth observed in burned clayey latosol was presumably due to the nutrients contained in the ash. The legumes did not however nodulate in this soil, which indicates that it contained sufficient nitrogen to meet the plants' needs. Indeed, the total nitrogen data show that the plants grown in burned clayey latosol contained relatively high nitrogen levels.

Azospirillum enrichment culture activity was high in burned clayey latosol, but this does not necessarily imply that nitrogen was being fixed in the roots. Acetylene reduction assays of the intact maize plants in this soil were negative, although this might have been due to poor diffusion of the gases in and out of the soil. The higher enrichment culture activity observed may have been due to an increase in total numbers of bacteria including *Azospirillum* in the roots stimulated by more abundant energy-rich root exudates. The low *Azospirillum* enrichment culture activity observed in black earth was unexpected: previous observations showed abundant N_2–fixing *Azospirillum* in black earth samples (12). The difference may be due to the black earth having been collected from an uncultivated site, whereas previous investigations used samples from inhabited sites planted with fruit trees, cassava and other crops.

Soybeans are usually considered to require inoculation with *Rhizobium japonicum* for the establishment of effective nitrogen-fixing nodules, but rhizobia of the cowpea miscellany can form nodules on soybeans, although they are frequently ineffective (7, 13). Effective nodulation of low-yeilding soybeans of Asian origin by naturally-occurring cowpea rhizobia has been observed in African soils (3). In Experiment I and II surface-sterilized seed and soil from areas not previously planted with soybeans were used. The occurrence of nodules in uninoculated alluvial soil thus implies that this soil naturally contains rhizobia able to promote nodulation in Jupiter.

In Experiment II inoculation greatly increased nodulation of Jupiter in both alluvial soil and black earth, and to a certain extent in sandy podsol and burned clayey latosol. The lack of nodulation in the uninoculated black earth samples and its presence in the alluvial soil indicates that the rhizobia causing nodule formation were indeed of soil rather than seed origin because if they were of seed origin nodulation would have occurred in uninoculated black earth. However plant growth was no greater in inoculated than uninoculated treatments which implies that the strain of rhizobium used was inefficient on this variety of soybean. Further investigations would be needed to determine the specificity and efficiency of these naturally-occurring rhizobia.

The better growth of plants in fertilized than burned latosol and podsol observed in Experiment III indicates that the ash did not contain sufficient nutrients to support good growth. The quantity of nutrients contained in burned soil depends on the efficiency of the burning, the degree of leaching and run-off, and other factors. Results obtained with burned soil thus vary between experiments. It is possible that if crops were planted after a good burn before significant leaching had occurred they would not require additional fertilization.

In Experiment III inoculation alone did not overcome the lack of nodulation of winged beans or

Table 5. Means and coefficients of variation (in brackets) of dry weights of nodules and plants of winged bean and cowpea in different Central Amazonian soils (Experiment III).

Plant parameter	Soil type*	Winged Bean			Cowpea		
		Untreated	Inoculated	Inoculated and Fertilized	Untreated	Inoculated	Inoculated and Fertilized
Dry weight (g) plant^{-1}	C	0.42 (62)	0.62 (27)	4.88 (29)	–	0.30 (–)	3.95 (18)
	BC	1.87 (24)	1.84 (28)	5.15 (11)	0.69 (45)	0.66 (26)	4.38 (25)
	M	0.29 (21)	0.31 (–)	3.55 (18)	–	–	3.66 (43)
	BM	2.13 (37)	1.69 (31)	3.38 (23)	1.77 (18)	1.70 (43)	3.18 (24)
	P	0.46 (37)	0.22 (55)	5.29 (14)	–	–	3.00 (24)
	BP	2.28 (15)	2.56 (36)	4.05 (17)	1.05 (38)	1.09 (34)	4.38 (33)
	A	2.78 (29)	4.04 (25)	3.26 (32)	2.72 (16)	2.22 (28)	3.24 (4)
Dry nodule weight (mg) plant^{-1}	C	0.0	0.0	54.0 (103)	–	0.0	0.5 (200)
	BC	2.5 (200)	0.0	33.0 (86)	0.0	0.0	0.0
	M	0.0	0.0	46.0 (101)	–	–	1.3 (100)
	BM	0.0	0.0	64.0 (80)	0.0	0.0	1.4 (273)
	P	0.0	0.0	129.0 (72)	–	–	0.0
	BP	0.0	1.0	146.0 (23)	0.4 (225)	0.8 (225)	70.0 (137)
	A	59.0 (21)	183.0 (112)	121.0 (72)	13.2 (133)	12.2 (147)	45.0 (75)

* As in Table 2.

– Plants failed to germinate or died shortly after germination.

cowpeas growing in latosol or podsol even when it was burned. Once fertilized, however, the winged beans did nodulate. The more abundant nodulation of fertilized winged beans in sandy podsol than medium or clayey latosol, despite equally good growth in all three soil types indicates that there was more nitrogen available in medium and clayey latosol, thus partially inhibiting nodulation. A similar difference between clayey and sandy soil was observed when examining roots of vegetation (12) and is presumably due to more rapid leaching of nitrogen from sand than clay.

Cowpeas only nodulated in two of the inoculated and fertilized treatments: burned sandy podsol and alluvial soil. Plant growth was however more or less equal in all the treatments. This implies that cowpeas nodulate less readily than winged beans when nitrogen is present in the soil. Further experiments are needed to show the efficiency of nitrogen fixation by winged beans and cowpeas in different soils and whether it can be improved by inoculation of *Rhizobium*.

The failure of cowpeas to grow altogether or their death shortly after germination in unfertilized unburned latosol and sandy podsol was overcome both by burning and by fertilization. This may have been due to the greater vigour of the plants in more fertile soils enabling them to resist disease.

The data indicate that neither the winged bean nor the cowpea varieties used in these experiments would grow well in latosol or podsol without fertilization. Even the nutrients available after recent burning were not sufficient to support good growth and nodulation. It is also implied that the soils tested contained levels of nitrogen which were sufficient to inhibit nodulation in some cases. However, selection of the varieties used in the experiments described here was carried out without the determination of characteristics that would enable them to grow in soils of low natural fertility. The differences in nutrient uptake between varieties observed in these experiments imply that a wider range of varieties should be evaluated for ability to grow at low levels of nitrogen, phosphorus and lime, association with mycorrhizae and high rates of nitrogen fixation. It might then be possible to distribute these varieties to Amazonian farmers, thus avoiding as far as possible the application of expensive inorganic fertilizers.

Summary

Winged beans, soybeans, cowpeas and maize were planted in pots containing different Central Amazonian soils in a rainproof area. Plant growth, nodulation, and occurrence of *Azospirillum* in unburned yellow clayey latosol and sandy podsol were very low. In burned clayey latosol plant growth and incidence of *Azospirillum* increased but nodulation was still low. Soybeans cv. Jupiter nodulated spontaneously in alluvial soil, and inoculation with *Rhizobium* isolated from these nodules increased nodulation but not growth in alluvial soil and black earth. Inoculation alone did not increase nodulation of winged bean or cowpea in latosol or podsol, but in alluvial soil growth and nodulation of inoculated winged beans increased. Inoculated and fertilized winged beans showed an increase in both plant growth and nodulation, especially in podsol, whereas cowpeas showed an increase in plant growth in all the soils, and an increase in nodulation in burned podsol and alluvial soil. The data indicated that the lack of nodulation in clayey latosol was at least partially due to the presence of nitrogen in the soil.

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ARCHIVOS LATINOAMERICANOS DE NUTRICION

ORGANO OFICIAL DE LA
SOCIEDAD LATINOAMERICANA DE NUTRICION

VOL. XXXII JUNIO, 1982 No. 2

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