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Response of Pearl Millet Inbreds and Hybrids to Inoculation with *Azospirillum brasilense*[1]

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ABSTRACT

Plant yield and acetylene reduction were measured on six hybrids and 15 inbreds (including the hybrid parents) of pearl millet, *Pennisetum americanum* (L.) K. Shum., after field inoculation with *Azospirillum brasilense*, Sp 13t (reclassified from *Spirillum lipoferum* Beijerinck). During the first year of testing, inoculation responses were investigated among all plant genotypes by measuring plant dry weight, % N, total N, and acetylene reduction activity. A smaller population of genotypes were tested a second year for repeatability of inoculum response. The first year, one hybrid, Tift 23DA × Tift 186 ('Gahi 3'), gave significantly higher plant dry weight and total N in response to inoculation. Inoculated Gahi 3 produced 41.7% more dry weight and 37.4% more total plant N when compared to autoclaved inoculum controls. No inbred was found to respond. Acetylene reduction values were low among all genotypes (range 0.54 nmole/g dry root/½ hour) and did not support yield effects or confirm inoculum treatments. Inoculation responses were found to be repeatable for all genotypes tested during the second year (positive or negative) but not of a statistically significant magnitude. Of the genotypes tested both years, a combined analysis of the 2 year yields revealed significant dry weight increases after inoculation of 19.2 and 14.0% with Gahi 3 and the inbred, Bil 3B, respectively. Again, acetylene reduction values did not explain any data. A N balance study was conducted in greenhouse containers on inoculated Gahi 3 plants in an attempt to repeat the yield differences observed in the field and to monitor inputs of N into the soil plant system. No significant yield increase of N into the soil-plant system was found due to inoculation. Sampling error possibly negated an accurate measurement of all N.

Additional index words: Acetylene reduction, Grass N_2-fixation, *Pennisetum americanum* (L.) K. Shum., Plant yield, Plant N, and *Spirillum lipoferum*.

SIGNIFICANT acetylene reduction (nitrogenase activity) in 'Transvala' digitgrass, *Digitaria decumbens* Stent., led to the isolation of strains of the bacterium, *Azospirillum brasilense* (reclassified from *Spirillum lipoferum* Beijerinck [10]) from the roots of this forage grass (7). Strains of this bacterium were found to fix N in pure culture as evidenced by acetylene reduction, Kjeldahl total N, and $^{15}N_2$ incorporation (7, 12).

Differences in acetylene reduction were found among cultivars of *Paspalum* and *Digitaria* (7, 13). Von Bulow and Dobereiner (4) screened maize (*Zea mays* L.) for N_2-fixing ability by the acetylene reduction assay and reported variability between S_1 lines of the rust-resistant variety, UR 1. They estimated the best lines to fix up to 2 kg/ha/day and N_2 fixing *Azospirillum* strains were isolated from these active S_1 plants. Beneficial colonization of grass roots by bacteria could be a valuable plant breeding criterion and warrants further investigation.

Inoculation of legumes with *Rhizobium* bacteria facilitates their experimental and agricultural use. Likewise, constitution of grass-*Azospirillum* N_2 fixing associations through inoculation could be important. When used as inoculum in the field, *A. brasilense* (strain Sp 13t) produced significantly higher yields of dry matter than did uninoculated controls in field grown, lightly fertilized pearl millet, *Pennisetum americanum* (L.) K. Shum., and guineagrass, *Panicum maximum* Jacq. (11). Regression analyses projected up to 42 and 39 kg N/ha was replaced by Sp 13t inoculum in these two respective grasses. Work in Oregon with strain Sp 81 inoculum on maize showed less than 4 g N/ha/day fixed. Yield and N content were not increased, thus providing no evidence of appreciable N_2-fixation (1).

The purpose of this study was to measure differences in plant yield and acetylene reduction among pearl millet hybrids and inbreds (including the hybrid parents) after field inoculation with Sp 13t. Repeatability of inoculum response was also tested. A N balance study was conducted to monitor inputs of N into the soil-plant system of an inoculated hybrid.

MATERIALS AND METHODS

Inbreds and hybrids of pearl millet were screened for inoculation response. The hybrid, 23 DA × PMB004, was obtained by courtesy of Jimmy Barber, North American Plant Breeders, Hutchinson, Kan., and was the female of the three way cross shown to respond to bacterial inoculation in the field (14). These inbreds and hybrids are listed in Table 1. The A lines are cytoplasmic male sterile and B lines are maintainers for them. The D lines carry the dwarf gene. Varietal names of commercial hybrids are shown in parentheses.

Inoculum of strain Sp 13t (received from Dr. Johanna Dobereiner, Empresa Brazileira de Pesquisa Agropecuria, Rio de Janeiro, Brazil) was grown with aeration in 12 liter glass containers in N containing liquid medium of the following composition: 5 g/L malic acid, 0.1 g NaCl, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.02 g $CaCl_2$, 0.01 g $FeCl_3$, 6.0 g K_2HPO_4, 4 g KH_2PO_4, 0.002 g $NaMoO_4 \cdot 2H_2O$, 1.0 g NH_4Cl, 0.05 g yeast extract, 5.0 g Trypticase (16% N), 1 liter H_2O.

Throughout these studies, all fertilizer was applied at a calibrated per hectare rate of 60 kg N (as NH_4NO_3), 20 kg P, 70 kg K, and 9.0 kg of fritted trace elements (9 g B, 9 g Cu, 47 g Fe, 20 g Mn, 0.5 g Mo, and 18 g Zn). Fertilizer rates and Sp 13t were selected on the basis of previous success of enhancing growth in pearl millet with these two parameters as variables (14).

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Standardized Procedures

Increased plant dry weight, percent N, total N, and acetylene reduction were used as indicators of response to inoculation. Data were statistically analyzed by analysis of variance and comparisons between means were made by L.S.D.

Dry weight measurements were determined on soil and plant tissue dried at 60 C. for 48 hours in a forced air drier. Dried material was prepared for N analyses by screening soil four times through a 3 mm screen and grinding plant tissue through a 1 mm mesh screen in a Wiley mill or a Christy Norris disintegrator (Christy Norris LTD, Chelmsford, England). Material was stored in sealed, plastic bags in a freezer (−12 C) until ready for use.

Nitrogen determinations were conducted by digesting 2 g of the screened soil or 0.1 g of the ground plant tissue in 10 ml concentrated H_2SO_4, 2 ml concentrated H_2O_2 and 3.5 g catalyst salt mixture (90% K_2SO_4, 10% $CuSO_4$) with three boiling chips for 1.5 hours on an aluminum block heated to 400 C (8, 9). The digested liquid was then diluted to 75 ml with distilled water and analyzed by a Technicon® N autoanalyzer.

Acetylene reduction assays were conducted on root soil cores or preincubated excised washed roots. Preincubation denotes that time before acetylene is introduced into the chamber containing the roots. Acetylene reduction in freshly washed roots is characterized by a lag period in nitrogenase activity. Preincubation of washed roots has been used to overcome this lag (6), but is reported to overestimate nitrogenase activity (1). The core method allows a reasonable estimation of nitrogenase activity existing at sampling (15) and was used to overcome the problems created by preincubation.

Washed roots were sealed in plastic bags, then preincubated overnight in an atmosphere of 5% O_2 95% N_2 at 30 C. The plastic bags contained rubber septa taped on their sides to allow exchanging of gases. After preincubation, the atmosphere was replaced with a fresh O_2N_2 mixture and 10% acetylene added. After 3 hours incubation at 30 C, samples (11 cc) were withdrawn and placed in 8 cc evacuated tubes. The ethylene was subsequently measured by gas chromatography.

Core samples were prepared as follows: The tops of pearl millet plants were cut away at ground level. A soil core containing the roots was removed by inserting a metal cylinder (made by removing both ends of a soft drink can and sharpening the bottom rim) over the plant, forcing the cylinder into the soil, and then removing the soil core along with the cylinder. The intact soil core with the metal cylinder was sealed in a glass jar. Each metal lid of the jars contained a rubber septum for gas exchange. The atmosphere was replaced with gas containing partial pressures of 0.86 atm N_2, 0.05 atm O_2 and 0.09 atm acetylene. After a 4-hour incubation at 30 C, samples were taken and analyzed as described in the washed root procedure.

Field Screening Trials

In 1976, all inbreds and hybrids shown in Table 1 except 23DA × Bil 3B were tested in a field nursery for increased plant dry weight, nitrogen, and acetylene reduction due to Sp 13t inoculation. Soil was an Arredondo loamy fine sand, a member of the loamy, siliceous, hyperthermic family of Grossarenic Paleudults, pH 6.5. Fertilizer nutrients, except N, were applied before planting. Seeds of each genotype were planted in six row main plots, replicated three times. The 2.7-m rows were planted at the rate of 0.5 g seed/m of row. After seedling emergence, N was applied and subplots of Sp 13t inoculum or autoclaved inoculum drenched onto the second or fifth row with a watering can. The plots were then lightly irrigated to wash the bacteria into the soil. Inoculum was grown under standard conditions in N-containing medium and applied at the rate of 4 × 10^7 cells/cm of row. Inoculum treatments were reapplied 2 weeks later with a small push plow rigged with a CO_2 constant delivery sprayer. This device injected the liquid uniformly into the plant's root zone through a nozzle behind the furrow opener. This injection method avoided the need to irrigate as with the drench method.

General maintenance of hand weed control, irrigation, and insect pest control with Lanate were used as needed. Between anthesis and harvesting (70 to 85 days) plots were sampled four times for acetylene reduction by core and excised washed root assays. Full anthesis was reported by von Bulow and Dobereiner to be the best time to sample for highest acetylene reduction activity in maize (4). At harvesting four plants per treatment row were randomly taken for N determinations. The complete row was harvested with a flail harvester and total wet forage weighed. A subsample of the forage was dried for dry weight calculations.

Table 1. Pearl millet inbreds and hybrids used in screening for response to *Azospirillum brasilense*, Sp 13t, inoculum in field studies.

	Inbreds	
Tift 23DA	Tift 186	Tift 131
Tift 23DB	Tift 239B	Tift 123
Tift 23A	Tift 383	Tift 18DB
Tift 23B	Tiftlate	Tift 13
Tift 239A	Tift 23SB	Bil 3B
	Hybrids	
	Tift 23DA × Tiflate	
	Tift 23DA × Tift 18DB	
	Tift 23DA × Tift 186 (Gahi 3)	
	Tift 23DA × Tift 383 (Tifleaf)	
	Tift 23DA × PMB004	
	Tift 23DA × Bil 3B	
	Tift 23A × Bil 3B	

To test repeatability in 1977, the following genotypes were selected and field tested for response to inoculation with Sp 13t: Tift 23DA × Tift 186 (Gahi 3), Tift 23DA × Tift 186, and Bil 3B. The hybrid, Tift 23DA × Bil 3B was also used in 1977 to test the effects of common parentage. The nursery area was on land adjacent to the 1976 nursery. Planting, fertilization, inoculation, maintenance, and harvesting schedules were the same as in 1976. A similar experimental design was used except genotype main plots were replicated 10 times.

N Balance Study

Gahi 3, which responded to inoculation with increased yield in the field, was subjected to a N balance study to monitor inputs of nitrogen into the plant soil system.

Glazed, ceramic containers 23 cm in diam and [illegible] cm deep, with a drain for catching leachable liquid were used in this study. Nonautoclaved, bulk mixed soil from the field nursery area was weighed for each container and a sample taken for soil dry weight determination. Fertilizer was added to the soil and mixed in a clean cement mixer for 15 min per container. Two uniform seedlings of Gahi were transferred into each container and 100 ml of inoculum or autoclaved inoculum injected into each container with syringe and needle. Inoculum was grown under standard conditions to 10^8 cells/ml in N-containing medium. After fertilization and inoculation five soil subsamples were taken through the profile of each container to give the initial N determinations. The experiment thus consisted of two treatments, live inoculum and autoclaved inoculum, as pairs within each of 18 blocks. Throughout the study, each pair was rotated weekly within its block to overcome location effects in the greenhouse. At the end of the day, light was supplemented with incandescent and fluorescent sources to achieve a 13.5-hour photoperiod. Plants were hand watered daily to completely wet the soil profile. Any leachable liquid was collected and returned to its respective container.

After 42 days growth, five soil subsamples were taken as before. Plants were then removed from their container and washed free of remaining soil. Roots were excised from plant tops and each separately dried, weighed, and ground for N analysis.

RESULTS AND DISCUSSION

Field Screening Trials

Plant dry weight, percent N, and total N values for all hybrids tested in 1976 are presented in Table 2. The 23DA × 186 (Gahi 3) hybrid was the only hybrid to give a significant yield response. Dry weight in-

Table 2. Plant yield in dry weight, percent nitrogen, and total nitrogen of pearl millet hybrids after field inoculation with live and autoclaved *Azospirillum brasilense*, Sp 31t, during 1976.

Hybrid	Dry matter			N			Total N		
	Live inoc	Auto inoc	Difference (live inoc-auto inoc)	Live inoc	Auto inoc	Difference (live inoc-auto inoc)	Live inoc	Auto inoc	Difference (live inoc auto inoc)
	kg/ha			%			kg/ha		
Tift 23DA × Tift 18DB	11,234	10,900	+334	1.73	1 74	−0 01	194	191	+3
Tift 23DA × Tiflate	17,858	19,588	−1,730	1 58	1 43	+0 15	282	280	+1
Tift 23DA × Tift 383	10,712	11,523	−811	2.02	2 01	+0 01	215	231	−16
Tift 23DA × Tift 186	17,269	13,116	+4,153	1 57	1 50	+0 07	272	198	+74
Tift 23DA × PMB 004	11,241	11,791	−550	1 62	1 65	−0 03	180	195	−15
Tift 23A × Bil 3B	9,229	8,978	+251	1 60	1 68	−0 08	151	151	0
L.S D, p = 0 05			±2,372			±0 31			±54
C.V (%)			11 6			10 2			14 4

Table 3 Plant yield in dry weight, percent nitrogen, and total nitrogen of pearl millet inbreds after field inoculation with live and autoclaved *Azospirillum brasilense*, Sp 13t, during 1976.

Genotype	Dry matter			N			Total N		
	Live inoc	Auto inoc	Difference (live inoc-auto inoc)	Live inoc	Auto inoc	Difference (live inoc-auto inoc)	Live inoc	Auto inoc	Difference (live inoc auto inoc)
	kg/ha			%			kg/ha		
Tift 23DA	7,529	7,647	−118	2 10	1 89	+0 21	158	145	+13
Tift 23DB	6,168	7,018	−850	2 01	1 94	+0 07	124	136	−12
Tift 23A	7 927	8,269	−342	1 78	1 71	+0 07	141	141	0
Tift 23D	8,545	8,961	−416	1 65	1 59	+0 06	140	141	−1
Tift 239A	6,777	6 726	+51	1 90	1 91	−0 01	129	128	+1
Tift 239B	7,354	6 519	+835	1 83	1 75	+0 08	134	115	+19
Tift 186	10 368	11,363	−995	1 91	1 97	−0 06	198	224	−26
Tift 383	8,030	7,400	+630	1 96	2 05	−0 09	155	153	+2
Tiftiate	16,012	14,861	+1,151	1 55	1 48	+0 07	248	220	+28
Tift 23SB	9,527	10,113	−586	1 63	1 65	−0 02	155	168	−13
Bil 3B	8 023	6,831	+1,192	1 47	1 53	−0 06	118	105	+13
Tift 13	7,859	8,738	−879	1 52	1 54	−0 02	117	134	−17
Tift 131	9,271	8,749	+522	1 59	1 80	−0 21	148	155	−7
Tift 123	10,050	9 856	+194	1 89	1 91	−0 02	187	187	0
Tift 18DB	6,001	6,457	−456	2.24	2 07	+0 17	134	133	+1
L S D, p = 0 05			±1,917			±0 22			±36
C V (%)			13 5			7 8			14 6

creased significantly in Gahi 3 (p = 0 05) with Sp 13t inoculation when compared to its autoclaved control. In this hybrid, inoculation with live Sp 13t enhanced dry weight 31.7% and total N 37.4%. No inbred responded significantly to inoculation (Table 3).

Ov- ' dry weight yield was low the second year due in part to limited rainfall. Statistically significant increases in plant dry weight were not brought about by inoculation in any genotype (Table 4). The analysis of the combined 2-year yields in Table 5 revealed significant (p = 0 05) increases of inoculation over autoclaved controls of 19.2% in Gahi 3 and 14 0% in the Bil 3B inbred. Due to inherent variation in yield (hybrid vs. inbred) among genotypes tested for 2 years, L.S.D.'s were also calculated for each genotype. The significance of the combined yield is probably due in large part to the strength of the first year's data.

Acetylene reduction values among genotypes were low (range 0 to 54.3 nmoles C_2H_4/[g dry root × hour]) during the first year of testing and gave no significant support to yield differences or inoculum treatments The mean of inoculated plots was 10 7 nmoles C_2H_4/ (g dry root × four), while the mean of autoclaved control plots was 6.0 nmoles C_2H_4/ (g dry root × hour). Acetylene reduction was repeated the second year, and gave similar results. Due to reports in the literature (4) and the damage extensive assays would cause yield, sampling was conducted from anthesis to harvest or between 70 and 85 days after planting Also, it was felt that greater statistical strength would be gained by sampling the same row from which yield was taken. The possibility exists that acetylene reduction activity was not accurately measured due to the limited sampling period. For future studies, having paired rows of inoculum subplots would allow intensive sampling all during the growing season for both acetylene reduction and yield.

It was found that the same general responses (positive or negative) were repeated among this limited

Table 4. Plant dry weight yield of selected pearl millet genotypes after field inoculation with live and autoclaved *Azospirillum brasilense*, Sp 13t, during 1977.

Genotype	Live inoc	Auto inoc	Difference (live inoc-auto inoc)
	kg/ha		
Tift 23DA × Tift 186 (Gahi 3)	9,786	9,160	+626
Tift 186	6,235	6,318	−83
Tift 23DA	5,742	5,841	−99
Bil 3B	6,999	6,330	+669
Tift 23DA × Bil 3B	11,247	10,842	+405
	L.S.D., p = 0.05		±1,392
	C.V. (%)		19.3

population of genotypes. The probability of any genotype giving a similar positive or negative response to inoculation two consecutive years is 1/2. The probability of all four genotypes responding similarly is 1/2⁴ or 1/16, suggesting chance alone played a limited role. Though never of the significant magnitude of the positive responders, the repeatable negative values are noted.

With no significant increase in acetylene reduction activity, it was not possible to predict that N_2-fixation increased the growth of the responding genotypes. Also, the reasons for any negative responses are speculative. Whatever the mode of action of the inoculant, it is agronomically important. Pearl millet is a diploid tropical forage and cereal grass in which selfing and hybridization are easily achieved. Variability within this grass to associate with bacterial inoculum for increased yield is an important plant breeding consideration. However, more research is needed to clarify both the inoculant mode of action and the genetic role of the plant.

Nitrogen content (% N) was not increased by inoculation. Normally one expects N content to increase with better N nutrition of the plant, but with long harvesting schedules (90 days) % N has been reported not to increase in some tropical forage grasses until N fertilization was enhanced above 200 kg N/ha (16). Total plant N per unit of area might be a better predictor of added N to a grass especially in the range of N inputs of any associative N_2-fixation. However, total plant N cannot be the only predictor of N_2-fixation as bacterial inoculants other than *Rhizobium* were reported by Brown (2) to enhance overall plant yield by modes of action such as production of growth regulating substances, suppression of plant pathogens, or mineralization of soil phosphates. Care should be taken to monitor all sources of nitrogen to the plant before any predictions of gained nitrogen through fixation can be made. There are also reports that Sp 13t causes denitrification (11); therefore, under anaerobic conditions and in the presence of excess nitrate, it has the potential to decrease fixed soil N.

N Balance Study

The greenhouse N balance study was conducted to record increases or decreases of N in the inoculated Gahi 3 plant-soil system and to verify its field growth responses.

Table 5. Two-year dry weight yields of selected pearl millet genotypes after field inoculation with live and autoclaved *Azospirillum brasilense*, Sp 13t.

Genotype	2-year avg. yield: Live inoc	Auto inoc	Difference (live inoc-auto inoc)	L.S.D. p = 0.05	C.V.
	kg/ha				%
Tift 23DA × Tift 186 (Gahi 3)	11,513	10,073	+1,440	±771	8.3
Tift 186	7,188	7,482	−294	±878	13.9
Tift 23DA	6,169	6,258	−89	±260	4.9
Bil 3B	6,483	5,655	+828	±442	8.4
	L.S.D., p = 0.05		±598		
	C.V. (%)		9.8		

Table 6. Nitrogen balance comparisons of the 23DA × 186 (Gahi 3)—soil system after inoculation with live and autoclaved *Azospirillum brasilense*, Sp 13t.

Treatment	Total soil N: Initial	Terminal	Lost (Initial-Terminal)	Total Plant N	Balance (Plant N-Soil N lost)
	g/container			g	g N
Live Sp 13t	7.5693	7.4047	0.1646	0.3977	+0.2332
Autoclaved Sp 13t	7.5228	7.4339	0.0889	0.3814	+0.2925
$\bar{X}$ for combined treatments	7.5460	7.4194	0.1267	0.3895	+0.2628

A 3.2% increase (inoculum, 56.55 g dry wt/2 plants; autoclaved inoculum, 54.57 g dry wt/2 plants) in total yield (top + root) and a 4.3% increase (inoculum, 0.3977 g N/2 plants; autoclaved inoculum, 0.3814 g N/2 plants) in total plant N (tops + roots) were the responses observed on the various plant parameters due to inoculation. These responses were less than the increases observed in the field, but greenhouse conditions and root restrictions due to the pots gave an environment vastly different from the field. At termination of this N balance study, general plant yellowing was observed. This was not found in the field trials.

The N balance for the soil-plant system is presented in Table 6. With low plant responses, inoculation with Sp 13t did not increase the N content for the total system. An overall experimental N increase of 0.2628 g (combining all treatments) is seen. This amount of N represents 3.48% of the initial soil N and might be viewed as the sampling error within this N balance study. From previous work with pearl millet (14), it was predicted that Sp 13t inoculum would replace 42 kg N/ha if 60 kg N/ha was added as fertilizer. This soil was found to contain about 0.055% N, and if one assumes that one ha furrow slice of soil weighs 2,241,270 kg (3), then there would be 1,293 kg N/ha, after fertilizer addition. Therefore, 42 kg N/ha would be about 3.25% of this total. It appears that this amount of N is near the experimental sampling error and that total N determination may not be sensitive enough to show these and smaller levels of N_2-fixation as stated by Burris and Wilson (5). As there was no overall decrease in N, dentrification resulting in loss of N from the system was not observed. Sensitive

assays, such as $^{15}N_2$, should be used to evaluate N_2-fixation in the Gahi 3-Sp 13t system.

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REFERENCES

1. Barber, L E, J. D Tjepkema, S. A Russell, and H. J. Evans 1974 Acetylene reduction (nitrogen fixation) associated with corn inoculated with *Spirillum*. Appl. Environ. Microbiol 32 108 113
2. Brown, M E 1974 Seed and root bacterization. Ann. Rev. Phytopathol 12 181 197.
3. Buckman, H O, and N. C Brady 1969 The nature and properties of soils The Macmillan Co, London
4. Bulow, J F W. Von, and J Dobereiner 1975 Potential for nitrogen fixation in maize genotypes in Brazil. Proc. Natl Acad Sci USA 72 2389-2393
5. Burris, R H, and P W Wilson 1958 Methods for measurement of nitrogen fixation p 355-366 *In* S P. Colowick and N O Kaplan (ed) Methods in enzymology. Vol IV. Academic Press, Inc, New York.
6. Day, J M, M C P. Neves, and J Dobereiner. 1975. Nitrogenase activity on the roots of tropical grasses. Soil Biol. Biochem. 7:107 112
7. Dobereiner, J, and J. M. Day. 1974. Associative symbiosis in tropical grasses: Characterization of microorganisms and dinitrogen fixing sites p 518 538 *In* W E. Newton and C J Nyman (Ed) Proceedings of the International Symposium on Nitrogen Fixation: Interdisciplinary Discussions Vol II Washington State Univ Press
8. Gallaher, R N., C. O Weldon, and F C Boswell 1976 A semiautomated procedure for total nitrogen in plant and soil samples Soil Sci Soc Am. J 40 887 889
9. ———, ———, and J G Futral 1975 An aluminum block digester for plant and soil analysis Soil Sci. Am Proc. 39:803 806
10. Krieg, N R 1977 Taxonomic studies of *Spirillum lipoferum* *In* A Hollaendar (ed) Genetic engineering for nitrogen fixation Plenum Press, New York
11. Neyra, C A, J Dobereiner, F Lalande, and R Knowles 1977. Dinitrification by N_2 fixing *Spirillum lipoferum* Can J. Microbiol. 23 300-305.
12. Okon, Y, S L. Albrecht, and R H Burris 1976 Factors affecting growth and nitrogen fixation of *Spirillum lipoferum* J Bacteriol 127:1248 1254
13. Schank, S C, J. M Day, and E D deLucas 1977 Nitrogenase activity, nitrogen content, *in vitro* digestibility and yield of 30 tropical forage grasses in Brazil Trop Agric 54.119-135
14. Smith, R L, J H Bouton, S. C. Schank, K H Quesenberry, M E Tyler, J R Milam, M H Gaskins, and R C Littell 1976 Nitrogen fixation in grasses inoculated with *Spirillum lipoferum* Science 193 1003 1005
15. Tjepkema, J, and P Van Burkum 1977 Acetylene reduction by soil cores of maize and sorghum in Brazil Appl Environ Microbiol 33 626-629.
16. Vicente Chandler, J 1975 Intensive management of pastures and forages in Puerto Rico p 409-433 *In* F Bornemisza and A Alvarado (ed) Soil management in tropical America Soil Science Dep, North Carolina State Univ, Raleigh.