THE EVALUATION OF ASSOCIATIVE N_2-FIXATION IN BAHIAGRASS AND CORN

BY

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DEDICATED TO

KAY AND MY PARENTS

FOR THEIR LOVE AND KINDNESS

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Abstract of Dissertation Presented to the Graduate Council
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THE EVALUATION OF ASSOCIATIVE N_2-FIXATION
IN BAHIAGRASS AND CORN

By

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Inoculation experiments were conducted on 21 _Paspalum notatum_ Flugge genotypes and 15 _Zea mays_ L. genotypes grown under field, greenhouse, and axenic conditions, using several N_2-fixing bacteria. This study sought to (1) compare acetylene reduction activity (ARA), yield, N balance, and ^{15}N-isotope dilution methods for estimating N_2-fixation; (2) develop quick methods for screening genotypes with superior ability to support N_2-fixation; and (3) evaluate the potential of bahiagrass and corn genotypes to support N_2-fixation.

Methods for estimating N_2-fixation gave different values of fixed N. Percentage of total N in bahiagrass tops from N_2-fixation due to inoculation was estimated to be 8% from yield differences and 2% by the ^{15}N-isotope dilution method. Estimates of N_2-fixation from ARA measurements were highly variable and inconsistent with those of other methods because control plants had higher nitrogenase activity than inoculated plants. Total amount of fixed N in inoculated and control

plant-soil systems was calculated by the N balance method. An overall mean of 0.094 g N in excess of all N inputs was observed which indicated 46% of the N in these systems was fixed. Similar calculations determined by ARA measurements and the ^{15}N-isotope dilution method were 12% and 6%, respectively. Nitrogen balance determinations indicated that 81% of fixed N was located in root-zone soil.

Corn genotypes did not respond consistently under field, greenhouse, and axenic conditions as determined by ARA. However, two genotypes, Asgrow RX-112 and Funk G-4864 had high ARA under most growing conditions. An overall significant response to inoculation for top growth and top N was observed in bahiagrass. However, no exceptional genotype could be identified. Tetraploid bahiagrass genotypes exhibited greater nitrogenase activity but were not as efficient as diploids for taking up fixed N and preventing its loss. Overall, bahiagrass genotypes assimilated 40% of ^{15}N-labeled fertilizer while 43% of it was lost from plant-soil system.

CHAPTER I

INTRODUCTION

Plant requirements for N exceed those of any other essential element for growth. Nitrogen is abundant in the atmosphere but in order to be available for plant growth it must go through a reduction process (fixation). The current input of fixed N in the world is about 116 x 10^6 MT of which 31% is fixed by industrial processes (Hardy and Havelka, 1975). Biological fixation and the depletion of soil N account for the remainder. Petroleum resources supply the large quantity of energy needed for industrial fixation. Energy supplies are limited and expensive and their availability is unreliable. This concern coupled with the fact that the world's food production capability has failed to keep pace with the world's population has prompted additional interest in biological N_2-fixation.

Legumes play a major role in the world's food production. The legume-*Rhizobium* symbiosis, supplies essentially all the N that is required for production of these crops. However, on a worldwide basis, cereal and forage crops are grown on 90% more acreage than legumes (F.A.O., 1977). If biological N_2-fixation in grasses could supply agronomically important amounts of N, i.e., 15-30 kg N/(ha·growing season), its impact on world food production would be of great significance.

Interest in N_2-fixation in grasses was revised with the discovery that the diazotroph, *Spirillum lipoferum* (now described as *Azospirillum*

brazilense and Azospirillum lipoferum), was associated with the roots of the tropical grass Digitaria decumbens (Döbereiner and Day, 1976; Day et al., 1975b). Prior to these studies nitrogenase activity was observed in the rhizosphere of Paspalum notatum Flugge (Döbereiner et al., 1973; Döbereiner et al., 1972). Since then many studies have described associations between N_2-fixing bacteria and numerous grass species including many of the world's most important grasses such as corn, rice, and wheat.

Yield responses to the inoculation of grasses with these N_2-fixing bacteria have been successful but erratic. In addition to fixing N, these diazotrophs produce plant growth hormones which could be involved in yield responses (Tien et al., 1979; Gaskins et al., 1977). Methods of estimating N_2-fixation other than yield differences between inoculated and control plants are needed to give a more direct estimate of N_2-fixation.

Almost all the literature of N_2-fixation deals with the measurement of acetylene reduction activity (ARA). Nitrogenase is able to reduce C_2H_2 to C_2H_4 so measurement of this reduction was proposed as an indirect method to assay for N_2-fixation (Hardy et al., 1973). The preincubated washed root modification of this method, introduced and used by Döbereiner et al. (1972) has been criticized because of nonlinear, over-estimated activity and long lag periods (van Berkum and Bohlool, 1980). The major problems with the ARA method are high variability and measurements which are short term and indirect. Also the theoretical assumption that for every three moles of C_2H_4 produced one mole of N_2 will be fixed has to be verified by $^{15}N_2$ incorporation. Estimates of N_2-fixation by ARA often fail to support yield increases or correlate with inoculation treatments. However, this may not be a methodology problem but may indicate something about N_2-fixation and yield responses.

Other methods of estimating N_2-fixation include N balance and ^{15}N-isotope dilution studies. These methods give more direct estimates of N_2-fixation than those based on ARA but they are not without problems. Large amounts of initial soil N in N balance studies create difficulty in estimating fixed N because the small values of N fixed by the associative system are within the limits of experimental error of N measurements. Growing plants in soil or medium low in N may avoid the above problem. Studies using the ^{15}N-isotope dilution method, i.e., methods using N fertilizer enriched with ^{15}N, require an accurate estimate of soil N mineralization either by analytical methods or by using nonfixing control plants having similar N uptake patterns. Unfortunately, the establishment and maintenance of control plants in the nonfixing state for very long is difficult. The mineralization control used in analytical methods must be maintained at conditions similar to the root zone and must give slow uniform changes in ^{14}N : ^{15}N ratios of available N over the experimental period. These results are difficult to accomplish. Studies utilizing N fertilizer that is enriched with ^{15}N provide estimates of N loss from the plant-soil system. These losses are from denitrificatin, volatilization, and leaching and must be considered in N balance studies. Also, estimates of percent recovery of fertilizer N by the plant can be calculated. The most direct and definitive method of estimating N_2-fixation is by the incorporation of ^{15}N from $^{15}N_2$. This method is laborious, expensive, and difficult to employ in field studies. However, this method must be used to verify measurements from other methods.

Each of the previously described methods for estimating N_2-fixation has its own merit and limitations. Concurrent utilization

of several methods is advisable if not necessary. However, the final desired result of N_2-fixation should be yield increases in the field or reduction of N fertilizer consumption.

Field experimentation is expensive, time consuming, and progress can be slow. Quick screening methods to reduce the number of genotypes tested in the field would be a great asset to a breeding program. Therefore, quick screening methods under greenhouse or growth chamber conditions should be developed to select genotypes for field evaluation of N_2-fixation potential. Very little research has been conducted in this area. Development of successful quick screening methods will depend on three factors. First, an accurate and consistent method of measuring N_2-fixation must be available. Secondly, genetic variation within a species for the ability to support N_2-fixation must exist for selection to be possible. Differences among genotypes, primarily determined by ARA, have been described within several species, including bahiagrass and corn. Lastely, genotype responses in the screening system must correlate with field responses.

The objectives of this study were

(1) to compare ARA, yield, N balance, and ^{15}N-isotope dilution methods for estimating N_2-fixation;

(2) to develop quick methods to screen genotypes for the ability to support N_2-fixation; and

(3) to evaluate the potential of bahiagrass and corn genotypes to support N_2-fixation.

CHAPTER II

EVALUATION OF N_2-FIXATION IN BAHIAGRASS BY ^{15}N-ISOTOPE DILUTION AND OTHER TECHNIQUES

Introduction

In most environments, nitrogen is the plant-growth-limiting resource. Energy to commercially fix N is expensive and limited. This concern, coupled with world food demand, has prompted recent interest in the associative symbiosis between N_2-fixing bacteria and the roots of grasses. It has been observed that these systems fix low but agronomically important amounts of N (Blue, 1974; Neyra and Döbereiner, 1977; Day et al., 1975a). Recent reviews on N_2-fixation were written by Neyra and Döbereiner (1977) and van Berkum and Bohlool (1980).

Significant increases in plant growth as a result of inoculation with N_2-fixing bacteria were reported with *Pennisetum americanum* (L.) K. Shum. (Bouton et al., 1979), *Cynodon dactylon* L. Pers. (Baltensperger et al., 1978), *Pancium maximum* Jacq. (Smith et al., 1978), and *Zea mays* L. (Kapulnik et al., 1981; Nur et al., 1980). However, a null response was reported with *Pennisetum glaucum* (L.) R. Br. (Barber et al., 1979) and *Z. mays* (Albrecht et al., 1981). Schank et al. (1979) reported a negative response with *P. americanum*. At best, yield responses to inoculation have been erratic and unpredictable.

Studies have shown genetic variation for N_2-fixation in *P. americanum* (Bouton, 1977), *Z. mays* (von Bülow and Döbereiner, 1975), and *P.*

notatum (Benzion and Quesenberry, 1978). Tetraploid ecotypes of P. notatum have been observed to exhibit greater diazotroph colonization, acetylene reduction activity, and greater amounts of photosynthate released from its roots than diploid ecotypes (Döbereiner and Campelo, 1971; Döbereiner et al., 1972; Vietor, 1982). Other studies with Z. mays (Albrecht et al., 1981) and C. dactylon (Baltensperger et al., 1978) have indicated no significant differences among genotypes for the ability to support N_2-fixation.

Acetylene reduction activity (ARA) estimates of N_2-fixation are often variable and fail to correlate with inoculum treatments (Albrecht et al., 1981); Weiser, 1980; Schank et al., 1979; Brown, 1976; Taylor, 1979; Smith and Schank, 1981). N_2-fixing bacteria produce plant growth hormones (Tien et al., 1979; Barea and Brown, 1974) which may promote root development (Tien et al., 1979; Schank et al., 1979; Umali-Garcia et al., 1978; Schank et al., 1981) and improve growth of young plants when measurable increases of ARA are not produced (Barea and Brown, 1974; Brown, 1976). Plant growth substances and N_2-fixation may both be responsible for reported stimulation of growth. More definitive techniques such as ^{15}N incorporation and N balance studies are needed for proper understanding of plant-bacterial association.

Incorporation of ^{15}N into plant tissue from $^{15}N_2$ is considered the most direct and definitive evidence for N_2-fixation. Yoshida and Yoneyama (1980) estimated N_2-fixation rates of 1.4 mg N/(plant·13 d) with Oryza sativa L. Japonica var. 'Koshihikari'. They calculated 20% of the fixed N was assimilated by the plant. Other grasses that have been reported to assimilate $^{15}N_2$ include P. notatum and Digitaria

decumbens Stent. (De-Polli et al., 1977), O. sativa (Ito et al., 1980) and Saccharum officinarum L. (Ruschel et al., 1975). However, Matsui et al. (1981) did not detect enrichment of ^{15}N in field-grown S. officinarum.

Estimations of N_2-fixation using ^{15}N-isotope dilution and 'A' value calculations require a healthy nonfixing control plant or an accurate estimate of soil N mineralization by analytical methods. Rennie (1980) found no significant difference between the yield difference and ^{15}N-isotope dilution method for calculating percentage fixed N in Z. mays. His calculations were achievable because plants were grown axenically in vermiculite, i.e. (1) a plant-growth medium system of which total plant-available N inputs were very low and defined so determinations of fixed N were not confounded, and (2) control plants did not exhibit ARA. Previous greenhouse and field studies in our lab indicate (1) control plants exhibit ARA and (2) N assimilated from soil sources greatly exceeds assimilated fixed N (Bouton, 1977). Thus, the amount of fixed N which is relatively small is within the limits of experimental error of N measurements.

The objectives of this study were to (1) screen a 21-genotype collection of bahiagrass for the ability to support N_2-fixation; (2) examine ploidy effects upon N_2-fixation potential in bahiagrass; (3) compare ARA, yield, N balance, and ^{15}N-isotopic dilution methods for estimating N_2-fixation; and (4) estimate percent N fertilizer loss from the plant-soil system and percent N fertilizer recovery of the plant.

Materials and Methods

Twenty-one bahiagrass clones, which included 12 tetraploid and nine diploid genotypes, were obtained, courtesy of G. W. Burton,[1] 21 Dec. 1979 (Table 1). These clones were maintained under a high fertility regime to promote rapid growth and provide adequate propagation material for the greenhouse study on 15 Mar. 1981. Ten uniform sprigs of each clone were planted, one each in a 15.2 cm plastic pot containing approximately 2,700 g pasteurized (24 h at 107°C and at a pressure of 6.9×10^4 Pa) growing medium "soil" consisting of 95% coarse builders sand and 5% bentonite clay (V/V), pH = 8.4, and 17 ppm total N.

A tygon tube (15.24 cm long, 1.27 cm diam) was inserted into each pot to facilitate subsurface irrigation and fertilization. The tube had emitting holes along its side and its bottom end was plugged with a stopper. It was believed that a dry medium surface would reduce diazotrophic contamination by providing a nonsuitable environment for bacterial growth.

In order to minimize light exposure of soil and prevent the growth of blue-green algae, a 1.9 cm layer of gravel was placed on the soil surface and the top of each pot was covered with aluminum foil, leaving only the plant exposed.

Pot drainage holes were closed with duct tape and small holes (0.5 mm) were punched to permit drainage. Plants were watered as needed but not enough to cause drainage. However, pots were flushed on a monthly basis to reduce salt accumulation.

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[1]Research geneticist, AR, SEA, USDA, and the University of Georgia, College of Agricultural Experiments Stations, Coastal Plain Station, Agronomy Department, Tifton, GA 31793.

Table 1. Twenty-one bahiagrass genotypes surveyed for N_2-fixation potential.

Genotype no.	Tifton genotype ID			Ploidy
	Material No.	Row no.	Plant No.	
1	43	27	66	4n
2	26	17	64	4n
3	33	29	63	4n
4	11	30	59	4n
5	14	10	59	4n
6	14	10	58	4n
7	38	4	59	4n
8	10	18	53	4n
9	10	18	54	4n
10	15	22	48	4n
11	15	22	47	4n
12	49	13	46	4n
	Tifton hybrid no.			
13	78 - 17			2n
14	78 - 24			2n
15	78 - 16			2n
16	78 - 23			2n
17	78 - 17			2n
18	15 - 21			2n
19	17 - 29			2n
20	78 - 13			2n
21	Pensacola†			2n

†Acquired from established sod, Plant Science Lab, University of Florida, Gainesville, FL.

Plants of each clone were graded according to size and sorted into five matched pairs; one of each pair was used for inoculation and the other was used for a control. The pairs were arranged in blocks and randomized according to a 21 x 5 type IV balanced incomplete block design (Cochran and Cox, 1957).

One-liter cultures for each of seven diazotrophs (Table 2) were prepared in aerated liquid succinate N free media (SNF) described by Tyler et al. (1979) with trypticase (Baltimore Biological Laboratories) and $(NH_4)_2SO_4$ were included at 1.0 and 0.5 g/L, respectively. Cultures were grown for 18 h at 35°C to approximately 10^8 cells/ml. Cells were washed twice, centrifuged at 2,500 rpm for 10 min and resuspended in distilled water. Cultures were obtained from J. R. Milam, Department of Microbiology and Cell Science, University of Florida, Gainesville, Florida.

Inoculated plants received 33 ml of a mixture containing equal culture volumes of each diazotroph. Control plants received the same amount of the above mixture after autoclaving. Plants were inoculated as above 6, 23, and 95 d after planting.

Plants were fertilized biweekly with a complete N free Hoagland's solution (Hoagland, 1950). Total amount applied was equivalent to 26 and 70 kg/ha, P and K, respectively. Nitrogen was applied monthly in the form of KNO_3 (2.23 atom % ^{15}N). A total of 0.064 g N/pot (a rate of 35 kg N/ha) was applied in four applications. Fertilization was omitted one month prior to harvest.

Table 2. Diazotrophs included in inoculation mixture.

Diazotroph	Plant of bacterial isolation	Bacteria source
Azotobacter paspali	*P. notatum*	ATTCC 23833
Azospirillum brasilense strain 13t	*D. decumbens*	J. Döbereiner, Rio de Janeiro, Brazil
W8 (*Erwina* spp.)	*Triticum aestivum* L. emend. Thell	R. V. Klucas, Univ. of Nebraska, Lincoln, Nebraska
S28-10A (*Klebsiella* spp.)	*Z. Mays*	R. L. Smith, Univ. of Florida, Gainesville, Florida
S24-5 (*Klebsiella* spp.)	*Z. Mays*	R. L. Smith, Univ. of Florida Gainesville, Florida
S85-9 *Enterobacter agglomerans*	*Z. Mays*	R. L. Smith, Univ. of Florida Gainesville, Florida
S143 (*Azospirillum* spp.)	*Paspalum urvillei* Steud.	R. L. Smith, Univ. of Florida, Gainesville, Florida

Acetylene Reduction

Plants were assayed for nitrogenase activity by acetylene reduction activity (ARA) 122 to 142 d after planting. Pots were placed in 10 L plexiglass cylinders (51 cm deep, 16.5 cm diam). A top with a fitted rubber gasket and a sampling port (1.3 cm diam) for insertion of a septum was bolted to the cylinder. Prior to fastening, vasoline was applied sparingly to the gasket to create an air-tight seal. Cylinders were flushed with argon for 1 min through the sampling port and sealed with a septum. Oxygen concentration in the cylinders after flushing was approximately 6%. Acetylene was added through the septum to an approximate conc of 10% in cylinder gas phase. Cylinders were incubated 16 to 21 h in a growth chamber maintained at 30°C. Ethylene evolution was measured by gas chromatography.

Yield, Total N, Exchangeable N, and ^{15}N-determinations

Plant tops (leaves and stolons) were harvested 156 d after planting, dried at 60°C, and weighed. Soil from each pot was collected and the five replications from each genotype-inoculation treatment was pooled. Remaining plant parts were transplanted in the field for further evaluation.

Plant tissue was ground through a 1 mm screen in a Wiley mill. In a procedure described by Weiser (1980), 0.2 g samples were block digested (Gallaher et al., 1975) in a mixture containing 1.5 g K_2SO_4, 1.5 ml $HgSO_4$ solution (12 H_2SO_4:88 H_2O:10 HgO V/V/W), and 6 ml conc H_2SO_4. Ammonium of the digestate was recovered by alkaline distillation, collected in boric acid-indicator solution, and titrated with standard acid as described by Bremmer and Edwards (1965), procedure (A).

Ethanol was distilled between sample distillations to remove traces of NH_4^+ from the distillation apparatus. The distillates were acidified with 2 ml 0.083 N H_2SO_4, condensed to approximately 3 ml, and analyzed for atom % ^{15}N with a VG Micromass 602 E mass spectrometer.

Total N and atom % ^{15}N of soil were determined by the above procedure except 4.0 g of screened soil was block digested in a mixture containing 3.2 g K_2SO_4:$CuSO_4$ (9 : 1 W/W) catalyst, 6 ml conc H_2SO_4 and 2 ml 30% H_2O_2 (Gallaher et al., 1976).

Exchangeable N from soil was extracted in KCl, steam distilled and titrated. Screened, 30 g soil samples were shaken for 1 h in 100 ml 1 N KCl and allowed to settle 24 h.

Determination of NH_4^+ and NO_3^- followed a steam-distillation-titration procedure using MgO and Devarda alloy described by Bremner (1965), except 75 ml aliquots were distilled.

Nitrogen Balance and Accumulation

Nitrogen accumulating in the plant-soil system (PSS) in excess of all N inputs was calculated as follows: [final plant nitrogen (Plant N) + final soil nitrogen (Soil N)] - [(initial sprig nitrogen) + (initial soil nitrogen) + (fertilizer nitrogen)]. Fertilizer and initial soil contributed 0.064 and 0.046 g N/pot, respectively. Total N was determined for representative sprigs of each genotype. Overall mean N content was 0.004 g/sprig.

Plant dry weights were estimated to compute plant N with the ratio 1 top dry weight : 1.64 plant dry weight. This ratio was determined from top, root, and plant dry weights of a similar study (Benzion, 1978).

^{15}N-Labeled Fertilizer Recovery

The amount of ^{15}N-labeled fertilizer remaining in the PSS was calculated with equations of Hauck and Bremner (1976):

(1) grams ^{15}N-labeled fertilizer remaining in the soil =

$$\frac{(\text{soil N})(\text{atom \% }^{15}\text{N soil} - 0.3663)}{(\text{atom \% }^{15}\text{N fert.} - 0.3663}\text{ ; and}$$

(2) grams ^{15}N-labeled fertilizer taken up by plant =

$$\frac{(\text{plant N})(\text{atom \% }^{15}\text{N plant} - 0.3663)}{(\text{atom \% }^{15}\text{N fert.} - 0.3663)}.$$

The above calculations relate to the currently accepted value of 0.3663 atom % ^{15}N for naturally occurring N.

The percent fertilizer N recovered by the plant and PSS can be calculated with the following equations:

(3) percent fertilizer N recovered by plant =

$$\frac{\text{equation (2)}}{0.064\text{ g N }(2.23\text{ atom \% }^{15}\text{N})} \times (100)\text{; and}$$

(4) percent fertilizer N recovered by PSS =

$$\frac{\text{equations (1) + (2)}}{0.064\text{ g N }(2.23\text{ atom \% }^{15}\text{N})} \times (100).$$

Results and Discussion

ARA, Top ^{15}N, and Top Yield

An overall significant response to inoculation was observed for most measured variables (Table 3). Overall, genotypes responded similarly to inoculation, indicated by the absence of significant genotype x inoculation interaction (G x I). A highly significant genotypic effect was observed for all measured variables, except top ^{15}N.

Acetylene reduction activity (ARA) was highly variable which was demonstrated by a C.V. = 70.0 and an activity range of 30 to 4420 n mole C_2H_4/(cylinder·h). This N_2-fixation estimate was inconsistent with inoculum treatments and negatively correlated with top dry weight (Table 4). Initial sprigs were soil grown and could not be sterilized so they were the probable source of unknown diazotrophs. Because of this and other contamination sources, control plants exhibited higher ARA rates than inoculated plants (Table 5). Other possible sources of contamination include transfer from inoculated plants, airborne organisms, and incompletely pasteurized soil. Possibly, bacterial populations of control plants grew from an initial low population to one comparable to inoculated plants at the time of ARA assays.

Tops of inoculated plants had a significantly ($P = 0.09$) greater ^{15}N-isotope dilution than control plants indicating greater fixation in the former (Table 5). If inoculated plants are considered fixing systems (fs) and control plants nonfixing systems (nfs), then the principle of ^{15}N-isotope dilution may be used to calculate the amount of N that was fixed due to inoculation (Rennie, 1979):

Table 3. Analysis of variance for acetylene reduction activity, top ^{15}N, top dry weight, top N, and top % N in bahiagrass.

Source	df	ARA		Top ^{15}N		Top dry weight		Top N		Top % N	
		MS	F	MS	F	MS	F	MS	F	MS	F
Block	20	751,814		0.01		1.99		0.00004		0.01	
Genotype	20	1,014,054	4.06**	0.02	1.52	17.46	13.89**	0.00021	8.87**	0.05	6.29**
Inoculation	1	1,394,459	5.58*	0.03	2.93	5.59	4.45*	0.00016	7.08**	0.01	1.09
Genotype x Inoc.	20	375,429	1.50	0.01	1.09	1.04	0.82	0.00002	0.74	0.01	0.80

*, **Significant at the $P = 0.05$ and 0.01 levels, respectively.

Table 4. Correlation coefficients of several N_2-fixation parameters.

	Top DW	Top N	Top ^{15}N	Soil N	Accumulated N	Percent ^{15}N-labeled fertilizer recovery	
						PSS	Plant
Acetylene reduction activity	-0.20**				-0.22**		
Top dry weight		0.82**		0.39**	0.61**	0.77**	0.79**
Top N				0.24**	0.52**	0.87**	0.93**
Top ^{15}N						0.23**	0.27**
Soil N					0.95**	0.39**	0.24**
Accumulated N						0.61**	0.51**
Percent ^{15}N-labeled fertilizer recovery of plant-soil system							0.93**
Percent ^{15}N-labeled fertilizer recovery of plant							

**Significant at the $P = 0.01$ level.

Table 5. Overall mean estimates of acetylene reduction activity, top ^{15}N, top dry weight, top N, and top % N in bahiagrass.

Measured variable	Adjusted inoculation means† Inoc (+)	Control (-)
	— n mole C_2H_4/(cylinder· h) —	
ARA	623	784
	— atom % ^{15}N —	
Top ^{15}N‡	1.6159	1.6430
	— grams —	
Top dry weight	5.73	5.40
Top N	0.024	0.022
	— % N —	
Top %N	0.447	0.432

†Adjusted means are required for comparisons between treatments in balanced incomplete block designs.

‡Each plant received 0.064 g N in the form of KNO_3 (2.23 atom % ^{15}N).

$$\text{percentage } N_2 \text{ fixed} = 1 - \frac{\text{atom \% } ^{15}\text{N excess (fs)}}{\text{atom \% } ^{15}\text{N excess (nfs)}} \times 100.$$

Calculations indicated 2% of top N was from fixation due to inoculation.

Top dry weight (top DW) and top N responses to inoculation were relatively more pronounced than responses of top ^{15}N-isotope dilution (Table 5). Genotype adjusted means of top DW and top N ranged from 3.27 to 9.25 g and 0.015 to 0.035 g, respectively. Inoculated plants had 8% more top N than control plants.

Estimates of percent top N from fixation due to inoculation differ when calculations are based on top N and top ^{15}N-dilution measurements. The former estimates, based on yield measurements and N determinations are subject to greater experimental error than ^{15}N-isotope dilution measurements, which only require a representative sample of the test crop for determination (Rennie and Rennie, 1973; Rennie et al., 1978). Thus an estimate of 2% top N from N_2-fixation due to inoculation, as determined by ^{15}N-isotope dilution may be more precise than an estimate of 8% based on differences in yield.

Top percent N was unaffected by inoculation (Tables 3 and 5). Significant differences of top percent N among genotypes were probably related to differences in yield. This is indicated by a significant negative correlation ($r = -0.41$; $P = 0.0001$) between top percent N and top DW.

Inoculation effect (IE) of each pair was computed (IE = inoculated plant minus control plant) and analyzed to identify genotypes with a

superior response to inoculation. All overall genotypic effect on IE was not significant for any measured variable ($P = 0.80$). This indicated that genotypes responded uniformly to inoculation for all measured variables.

Genotype comparisons were made on adjusted IE means by L.S.D. procedures (Table 6). A significance level of 0.01 was chosen because a nonsignificant G x I was observed earlier in Table 3 and a significant difference among genotypes for IE represents G x I. Therefore, conservative comparisons between genotypes are justified and preferred.

No genotype had a unique IE for any measured variable. This observation further demonstrates that basically, genotypes responded to inoculation uniformly. Differences in the sign of IE suggest G x I but this was not significant as shown in Table 3.

An overall response to inoculation was significant but minimal and genotypes responded similarly to inoculation. Thus, no exceptional genotype could be identified by its inoculation response. Consequently, identification of superior genotypes for N_2-fixation potential by inoculation response may be difficult. Caution must be used when N_2-fixation estimates are based on ARA, top growth, or both. Verification of suprior genotypes and quantification of N_2-fixation rates should include ^{15}N-data.

Nitrogen Balance and Accumulation

Because only the top growth was harvested, a top dry weight to plant dry weight ratio of 1 to 1.64 was used to estimate plant DW. This ratio was determined from top, root, and plant dry weights of

Table 6. Genotype comparisons of inoculation effect (IE) adjusted means.

Genotype no.[‡]	IE (inoculated - control) adjusted means for four variables[†] ARA	Top ^{15}N	Top dry weight	Top N
	— n mole C_2H_4/(cylinder·h) —	atom % ^{15}N —	— grams —	
1	159	-0.0325	-0.50	-0.001
2	-62	-0.0442	0.31	0.002
3	-116	0.0855	1.64	0.004
4	-351	0.0145	0.19	0.001
5	135	-0.0830	-0.55	-0.002
6	78	0.0646	0.51	0.002
7	-36	0.0535	-0.35	0.000
8	-461	-0.0316	0.32	0.002
9	226	-0.0166	-0.26	0.000
10	-246	0.0098	0.49	0.001
11	-218	0.0443	-0.40	0.000
12	1185	-0.0056	1.68	0.003
13	-296	-0.0483	-0.71	-0.003
14	807	0.0566	0.07	0.000
15	130	-0.0680	0.07	0.001
16	143	0.0213	-0.46	-0.001
17	-351	0.0704	-0.54	-0.004
18	-238	0.0261	0.69	0.003
19	116	-0.0573	0.46	0.001
20	189	0.0697	-0.48	-0.002
21	32	-0.1540	1.32	0.004

[†]Adjusted means are required for comparisons between treatments in balanced incomplete block designs.

[‡]Means within each column are not different at the $P = 0.01$ level as determined by pair wise t test.

a similar study (Benzion, 1978). This ratio is reasonable because of the similarity of Benzion's study with this one, i.e. (1) it was a 130 d greenhouse study surveying three tetraploid and three diploid bahiagrass genotypes for N_2-fixation potential, (2) plants were grown in 15.3 cm clay pots containing a loamy sand which was amended with N at a calibrated rate of 60 kg N/ha, and (3) plants were inoculated with _A. brasilense_ strain JM 125 A2 or _A. paspali_. Further, it is reasonable to calculate plant N with top percent N rather than plant percent N because Benzion was unable to detect a difference between top and root percent N. He reported mean values of 0.46 and 0.50% N, respectively. These values are comparable to the mean top N value of 0.43% determined in this study. Some error in the estimation of plant N is not serious because root N was estimated to only contribute about 7% of plant-soil system N (Table 7).

An analysis of variance was made on plant N but its purpose was to produce adjusted means of this parameter for N balance equations. Analysis of variance for soil percent N and soil N were not appropriate because soil samples were pooled; however, analysis of variance was made to produce adjusted means. Mean soil N was 63 ppm and ranged from 47 to 91 ppm N. Genotype adjusted means of soil N ranged from 0.128 to 0.246 g/pot. Differences among genotypes for soil N may be related to differences in root system development and mineral uptake capability which could (1) influence the amount of N loss from the plant-soil system and (2) influence the quantity of organic matter present in the plant-soil system. The first will be discussed in conjunction with plant recovery of ^{15}N-labeled fertilizer and the second is supported by several observations: (1) fine root matter was observed in soil samples

Table 7. Analysis of variance and inoculation means of N balance measurements.

Analysis of variance

Source	df	Plant N MS	Plant N F	Accumulated N/PSS MS	Accumulated N/PSS F
Block	20	0.0001		0.0001	
Genotype	20	0.0006	8.87**	0.0085	136.45**
Inoculation	1	0.0004	7.08**	0.0011	17.53**
Genotype x Inoculation	20	0.0001	0.74	0.0016	25.72**

Adjusted inoculation means†

	Inoc. (+)	Control (-)
	—— grams N ——	
Plant N	0.040	0.037
Soil N	0.170	0.169
Accumulated N/plant-soil system	0.096	0.092

*,**Significant at the P = 0.05 and 0.01 levels, respectively.

†Adjusted means are required for comparison between treatments in balanced incomplete block designs.

following screening; (2) there was a significant correlation between soil N and top DW or top N (Table 4); and (3) soil N was 63 ppm of which 15 ppm was extractable NH_4^+ N (only a trace amount of NO_3^- N was detected). Therefore 76% of soil N was organic and in either plant or microbial tissue.

Soil N accounted for 81% of the accumulated N (N accumulated in the plant-soil system in excess of all N inputs). The above was indicated by a correlation coefficient of 0.95 between soil N and accumulated N (Table 5). Yoshida and Yoneyama (1980) observed that 75 to 81% of total fixed N in rice plant-soil systems was located in root zone soil. A significant response to genotype and inoculation was observed for accumulated N (Table 7). Genotype adjusted means ranged from 0.054 to 0.192 g/PSS. A significant G x I indicated genotypes did not respond uniformly to inoculation. However, inoculated PSS tended to accumulate an average of 4% more N than controls.

The accumulated N is probably from N_2-fixation but a genotype's ability to utilize N and reduce its loss may relate to several factors such as (1) plant size, i.e., root system development, and (2) plant mineral uptake capability. Thus a genotype that has more fixed N in its PSS may be one that reduces N loss because of certain characteristics in its root system. The significant genotypic effect on accumulated N may relate to the above and will be discussed with ^{15}N-labeled fertilizer recovery.

An adjusted grand mean of 0.094 g accumulated N/PSS during the 156 d duration of the experiment or 0.60 mg N accumulated/(PSS·d) may be accurate and indicates considerable amounts of fixed N in inoculated and control PSS. An adjusted grand mean for ARA was 703 n mole C_2H_4/

(cylinder·h). This activity can be converted to fixed N/(PSS·d) by assuming that 3 moles of C_2H_4 are produced per 1 mole N_2 fixed. This calculation indicates 0.16 mg fixed N/(PSS·d). Estimates of amounts of fixed N by ARA and accumulated N may be extrapolated to (g N/(ha·d) by multiplying them by 553,191, a factor which equates the area of a pot to a hectare. Acetylene reduction activity and accumulated N measurements extrapolate to 90 and 330 g fixed N/(ha·d). It is difficult to explain why these estimates differ so much but they are independent methods of measurement and ARA depends on short term measurements unlike those of N balance.

Measurements of N in the entire PSS are important because they account for N which is unaccounted for with measurements of plant yield. Approximately 81% of accumulated N was located in root-zone soil. Therefore N_2-fixation may have its largest impact on root-zone soil so it is important to include this part of the PSS in N_2-fixation studies. Soil N which was 76% organic should eventually become available for plant growth.

^{15}N-labeled Fertilizer Recovery

Assumptions for use of ^{15}N are discussed by Hauck and Bremner (1976), two of which are important for the measurements of this study: (1) complex elements (those containing two or more isotopes) in the natural state have a constant isotope composition (0.3663 atom % ^{15}N); and (2) the distribution of applied N among different plant parts or within a plant part is uniform.

Analysis of variance was made on ^{15}N-parameters so that adjusted means could be obtained. Overall adjusted means for plant and soil

recovery of ^{15}N-labeled fertilizer were 0.026 and 0.010 g, respectively (Table 8). Genotype adjusted means for the above ranged from 0.017 to 0.040 g recovered by the plant and 0.006 to 0.017 g recovered in the soil. Adjusted means for percent ^{15}N-labeled fertilizer recovered by the PSS and plant were 56.9 and 40.4%, respectively. Genotype adjusted means for the above ranged from 39.9 to 79.4% for the PSS and 26.8 to 62.4% for the plant.

Approximately 72% of the total recovered ^{15}N-labeled fertilizer was located in the plant. Plant recovery rates of ^{15}N-labeled fertilizer were probably influenced by genotype. Plant factors that promote root system development and mineral uptake would enhance fertilizer recovery rates and reduce fertilizer N losses. It follows that a genotype with enhanced fertilizer uptake would have enhanced uptake of N from other sources such as biologically fixed N. Genotypes with high ^{15}N-labeled fertilizer recovery would be genotypes with reduced losses of fixed N and therefore have higher N accumulation values (accumulated N in excess of all N inputs). The above is demonstrated by significant correlations between accumulated N and percent ^{15}N-labeled fertilizer recovered by plant and PSS (Table 4).

A genotype with superior N efficiency may be a genotype that supports N_2-fixation by leaking photosynthate from its roots (Vietor, 1982), but it may also be a genotype that has enhanced N uptake abilities that allow it to take up fixed and soil N that otherwise may be lost.

The above supposition can be supported by comparing tetraploid and diploid means of several measured variables (Table 9). Tetraploid genotypes exhibited greater nitrogenase activity, i.e., support N_2-fixation

Table 8. Plant-soil recovery of ^{15}N-labeled fertilizer.

N-source	Adjusted means†		
	Inoculated	Control	Overall
	— grams ^{15}N-labeled fertilizer recovered‡ —		
Plant	0.026	0.025	0.026
Soil	0.010	0.011	0.010
Plant-soil system	0.036	0.036	0.036
	— percent recovery of ^{15}N-labeled fertilizer —		
Plant	41.5	39.3	40.4
Soil	15.7	17.2	16.4
Plant-soil system	57.0	56.7	56.9
	— atom % ^{15}N excess —		
Plant	1.2499	1.2771	1.2635
Soil	0.1148	0.1233	0.1195

†Adjusted means are required for comparison between treatments in balanced incomplete block designs.

‡Each plant-soil system received 0.064 g ^{15}N-labeled fertilizer.

Table 9. Diploid/tetraploid means and contrast procedure for various N_2-fixation parameters.

Measured variable	Means		Contrast 'Diploid minus tetraploid'	
	Diploids	Tetraploids	F	Probability > F
	n mole C_2H_4/(cylinder·h)			
ARA	484 ± 41	888 ± 68	31.87	0.0001
	atom % ^{15}N excess			
Top	1.2724 ± 0.0139	1.2553 ± 0.139	0.16	0.6861
Soil	0.0969 ± 0.0034	0.1307 ± 0.0029	-	-
	grams			
Top N	0.025 ± 0.0005	0.022 ± 0.0008	23.30	0.0001
Soil N	0.189 ± 0.003	0.154 ± 0.002	-	-
Accumulated N	0.117 ± 0.004	0.076 ± 0.002	1086	0.0001
Plant recovery of ^{15}N-labeled fertilizer	0.028 ± 0.0009	0.024 ± 0.0006	19.53	0.0001
Soil recovery of ^{15}N-labeled fertilizer	0.010 ± 0.0004	0.011 ± 0.0003	-	-
Loss of ^{15}N-labeled fertilizer from plant-soil system	0.0260 ± 0.0011	0.0284 ± 0.0006	-	-

better than diploids as observed by ARA. Vietor (1982) observed that the percentage of the ^{14}C-labeled photosynthate appearing in the root wash solution was significantly greater for tetraploids than diploids. Reports of greater diazotroph colonization and greater ARA in association with tetraploids compared with diploid roots (Döbereiner and Campelo, 1971; Döbereiner et al., 1972) are consistent with Vietor's data and the data of this study. Measurements of ^{15}N-isotope dilution of top growth indicate equal amounts of fixed N in diploid than tetraploid plants. However, measurements of ^{15}N-isotope dilution of soil indicated there is 26% more fixed N in the soil of diploids and tetraploids. Diploid PSS had 54% more fixed N. Diploid genotypes recovered 17% more ^{15}N-labeled fertilizer. Plant-soil systems of tetraploid plants exhibited 9% more ^{15}N-labeled fertilizer loss.

The above and previous data indicate that tetraploid genotypes exhibit greater nitrogenase activity, but are not as efficient as diploids for taking up fixed N and therefore experience greater losses of fixed N from the plant-soil system. Diploids retain greater amounts of N in their root-zone soil.

Two characteristics may be important in the selection for N efficiency: (1) the ability to support diazotrophic colonization; and (2) the ability to take up fixed N and not lose it. The interrelationship between these characteristics may become clearer with $^{15}N_2$-incorporation studies in conjunction with ARA, N uptake, and N accumulation measurements. Medium that is nearly N free should be used so as not to confound N accumulation measurements.

Inoculation did not affect fertilizer recovery rates (Table 8). However, soil of inoculated PSS exhibited a greater ^{15}N-isotope dilution.

If inoculated PSS are considered a fixing system and control PSS nonfixing, then calculations of ^{15}N-isotope dilution can be made to estimate the percentage soil N from fixation due to inoculation. Seven percent of soil N was from fixation due to inoculation. Earlier calculations estimated 2% of top N was from fixation due to inoculation. The amount of fixed N due to inoculation in the PSS can be estimated by adding 2% of the plant N to 7% of the soil N. Calculations indicate 0.013 g N are fixed due to inoculation. The above calculation does not estimate total amounts of fixed N in the PSS because these calculations considered control PSS as nonfixing while in reality they exhibited N_2-fixation rates nearly equal to inoculated rates.

An average of 43% of ^{15}N-labeled fertilizer was lost. Rennie (1979) reported losses of ^{15}N-labeled fertilizer of 22%. Possible sources of loss include denitrification, volatilization, or leaching. If the assumption that no significant isotope discrimination occurs during these loss processes and thus the $^{15}N/^{14}N$ ratio remains constant, then the isotope dilution procedure will remain unaffected. However, measurements based on total N are underestimated due to losses.

In summary, N_2-fixation contributed considerable amounts of N to the bahiagrass PSS (46% of PSS N was fixed). However, inoculation of these systems with N_2-fixing bacteria accounted for only 8 and 2% of top N, as estimated by differences between inoculated and control plants in yield and ^{15}N-isotope dilution measurements, respectively. Thus, estimates of N_2-fixation which were based on differences between inoculated and control plants in yield and ^{15}N-isotope dilution measurements account for only a portion of the total fixed N within the bahiagrass PSS.

CHAPTER III

POTENTIAL FOR N_2-FIXATION IN *ZEA MAYS* GENOTYPES GROWN IN FLORIDA

Introduction

The purpose of this introduction is to supplement the literature that has been previously cited with more information concerning N_2-fixation in *Zea mays* L. The reader is referred to the other introductions in this dissertation for a more complete review of associative N_2-fixation.

Reports of N_2-fixing bacteria living in association with the roots of *Z. mays* have been published (O'Hara et al., 1981; von Bülow and Döbereiner, 1975). Nitrogen fixing bacteria may be attracted to root exudates of several grasses including *Z. mays* (Alvares-Morales and Lemos-Pastrana, 1980). Following inoculation they have been observed adsorbing to root surfaces and found in the middle lamellae of root cells within one week (Umali-Garcia et al., 1978). The potential of these associations involving important cereal crops and forage grasses is significant because economically important amounts of nitrogen could be fixed biologically. The actual contribution of N_2-fixing bacteria to the N economy of *Z. mays* and other grasses remains elusive. Efforts to further understand this association are currently under investigation.

Significant yield increases in *Z. mays* due to inoculation with N_2-fixing bacteria have been reported (Kapulnik et al., 1981; Nur et al., 1980; O'Hara et al., 1981; Cohen et al., 1980; Rennie, 1980). However,

nonsignificant responses in yield of _Z. mays_ inoculated with N_2-fixing bacteria have been reported (Albrecht et al., 1981; Albrecht et al., 1977).

Inoculation has been observed to increase the diazotrophic population in the rhizosphere without concurrent increases in nitrogenase activity as estimated by acetylene reduction activity (O'Hara et al., 1981). Other reports indicate increased acetylene reduction activity (ARA) in inoculated roots but without apparent N benefit to the plants (Okon et al., 1977; Barber et al., 1976). This suggests that other factors such as bacterial-produced plant growth hormones are at least partially responsible for yield responses because diazotrophs have been reported to produce plant growth hormones (Gaskins et al., 1977; Tien et al., 1979).

Measurement of ARA is the most common method for estimating N_2-fixation. This technique is easily employed in field, greenhouse, and growth chamber studies. Unfortunately it is an indirect method of short duration which is extremely variable. Several factors influence ARA which include soil type or field location (von Bülow and Döbereiner, 1975; Cohen et al., 1980) climatic conditions such as air temperature and light (Cohen et al., 1980; Albrecht et al., 1977; Weier, 1980), soil moisture levels (Weier, 1980; Smith and Schank, 1981; Smith et al., 1982), reduced N levels in the growth medium or soil (Barber et al., 1979; Day et al., 1975b; Cohen et al., 1980; Smith et al., 1977), O_2 and CO_2 levels of assay vials or root-soil cores (Döbereiner et al., 1972; Smith et al., 1982) plant maturity (Albrecht et al., 1981; von Bülow and Döbereiner, 1975) and seasonal variations (Smith et al., 1982; Weier et al., 1981).

In addition, ARA is highly variable even when the above factors are held constant. Corroboration of ARA with other methods of estimating N_2-fixation such as N balance and ^{15}N-isotope dilution should be considered.

Tests for the ability to support N_2-fixation which are conducted under field conditions are closest to natural conditions, but are time-consuming and expensive. Development of quick methods, adapted to screening seedlings or young plants under greenhouse or growth chamber conditions would be helpful by predicting field results and permitting the simultaneous evaluation of large numbers of plant genotype-bacterial strain combinations for associative N_2-fixation. Superior genotypes selected by these methods would be evaluated further under field conditions.

Inoculation studies have been conducted using several methods for growing plants in growth chambers under initially axenic conditions. They include growing plants in vials containing vermiculite (Rennie, 1980), in bottles containing sand (Albrecht et al., 1981), in test tubes containing Fahraeus nitrogen and carbon free medium (Schank and Smith, 1980), in test tubes or "Whirl Pac" plastic bags containing autoclaved soil (Schank et al., 1979), and in Leonard's bottle-jar assemblies containing washed sand, soil, or loess (Cohen et al., 1980) or an acid washed sand-vermiculite mixture (Rennie and Larson, 1979). In addition to these systems, several have been developed for inoculation studies under greenhouse conditions. They include growing plants in large ceramic containers containing soil (Bouton et al., 1979), in plastic or mason jar assemblies containing soil (R. L. Smith and S. C. Schank, unpublished data, 1981), and sand-vermiculite (O'Hara et al., 1981) or sand-soil pot cultures (Albrecht et al., 1981).

Studies under greenhouse or growth chamber conditions usually have been unsuccessful in repeating field responses. Bouton (1977) was unable to repeat yield responses observed in the field with Pennisetum americanum L. 'Gahi-3' inoculated with Spirillium lipoferum in nonaxenic tests. Schank et al. (1979) grew genotypes of P. americanum that had been selected because of their high and low response to field inoculation in test tubes and plastic bags containing autoclaved soil. Yield responses were negatively correlated with inocualtion and it was concluded that the system did not provide a suitable environment for screening bacterial-plant associations and predicting their response in the field. Schank and Smith (1980) grew corn and millet plants axenically in test tubes containing nitrogen and carbon-free Fahraeus media and inoculated with Azospirillum. Acetylene reduction activity was high and bacteria colonized root surfaces but no consistency was observed with previous field inoculation responses. However, latter axenic test-tube and field experiments indicated that several corn and millet genotypes with high ARA in test tubes also had high ARA under field conditions (Schank and Smith, unpublished data, 1981). They also found that ARA under test-tube conditions was influenced by several factors including agar concentration, mineral salt composition, and inoculum strain. They felt that the latter may represent bacterial strain-plant cultivar complementation.

There is a need for further development and refinement of quick screening methods. In order for these methods to be successful, an accurate method for measuring N_2-fixation must be available and genetic variation within a species for the ability to support N_2-fixation must

exist. Variations among _Z. mays_ genotypes for ARA have been described by von Bülow and Döbereiner (1975).

The objectives of this study were to (1) develop quick methods to screen corn genotypes for the ability to support associative N_2-fixation; (2) compare these methods to core acetylene reduction assays of field-grown plants; and (3) evaluate the potential of corn genotypes to support N_2-fixation.

Materials and Methods

A collection of 26 _Zea mays_ genotypes (Table 10) were grown under field, greenhouse, and growth chamber conditions. Plants were grown in 2.2 L plastic jar assemblies in a greenhouse and axenically in test tubes in a growth chamber.

Field Experiments

Two experiments were conducted in the field at the University of Florida, Institute of Food and Agric. Sci., Beef Research Unit (B.R.U.), Gainesville, Florida. The first experiment tested the response of 15 corn genotypes to inoculated and indigenous N_2-fixing bacteria. The second experiment tested the response of these same genotypes to indigenous N_2-fixing bacteria. Previous studies at this location indicated abundant populations of undefined N_2-fixing bacteria. Soil at this location is primarily a Wauchula fine sand, a poorly drained hyperthermic siliceous Ultic Haplaquod, which was analyzed at the termination of the first experiment and found to be pH = 7.22, 0.70 ppm NH_4^+ N, 0.10 ppm NO_3^- N, and 1.16% organic matter. In both experiments, fertilizer was applied

Table 10. Corn genotypes surveyed for N_2-fixation potential.

Field experiments 1 and 2 Test-tube experiments 1-4		Greenhouse experiment 1		Greenhouse experiment 2	
Hybrid		Cargill description[†]		Hybrid	
Funk	G-4864	Caribbean Flint/Dent	single cross II	Funk	G-4864
Pioneer	3160	Alpine Flint	8 line synthetic	Pioneer	3160
Coker	22	Tuxpeno	single cross I	Coker	22
Northrup King	PX-95	North temperate zone Cuzco	composite	Northrup King	PX-95
Funk	G-4507A	North temperate zone Coroico	composite	Pioneer	3030
Pioneer	3030	Argentine Flint Race Cateto	single cross II	Dekalb	XL-80
Dekalb	XL-80	Argentine Flint Race Cateto	single cross I	Funk	G-4810
Asgrow	RX-112	Tuxpeno	single cross II	Pioneer	3320
Funk	G-4810	Caribbean Flint/Dent	single cross I	Jacques	247
Pioneer	3320	Hybrid		Dekalb	XL-82
Jacques	247	Funk	G-4507A	Coker	77B
Dekalb	XL-82	Coker	16	McCurdy	8150
Coker	16	Asgrow	RX-112	Tuxpeno	Single Cross I
Coker	77B	McNair	508		
McCurdy	8150	Univ. of Florida	Florida Stay Sweet		
McNair	508				
Univ. of Florida	Florida Stay Sweet				

[†]Obtained from Everett Gerrish, Technical Maize Research, Cargill, Inc., Grinnell, Iowa, 50112.

prior to planting at a rate of 20 kg P and 70 kg K/ha, and 4.48 kg/ha of fritted trace elements (FTE 503; 5 B, 5 Cu, 29 Fe, 12 Mn, 0.3 Mo, 11 Zn in g/100 kg). Nitrogen was applied as NH_4NO_3 after seedling emergence at a rate of 25 kg/ha.

Planting. In the first field experiment 15 genotypes were planted in a strip-plot design, replicated six times. Each block consisted of 15 rows (one row for each genotype) that were 91 cm apart. Each row (main plot) was divided into two 6.1 m subplots for inoculation treatments. Subplots within each row were separated by 3 m to minimize cross inoculation. Each subplot had 20 hills in each of which three seeds were planted.

The second field experiment had 16 genotypes planted in a randomized complete block design, replicated four times. Each block consisted of 16 rows (one row for each genotype) 91 cm apart and 6.1 m long. Twenty hills were planted in each row as before.

Field experiments were weeded and irrigated when needed. Lanate was applied several times for insect control.

Inoculation. Liquid cultures of S125 and S145 (two *Azospirillum* spp., isolated by R. L. Smith, Univ. of Florida; S125 was isolated from the B.R.U.) were grown in 10 L batches in a fermenter to an approximate conc of 10^8 cells/ml. Both diazotrophs were grown in aerated succinate N free medium with trypticase and $(NH_4)_2SO_4$ added, as described in Chapter II.

The appropriate subplots of the first experiment were inoculated two and ten days after planting with a mixture containing equal volumes

of each diazotroph at an approximate rate of 19 ml/m at the first inoculation and one half that for the second. Sprinkling cans were used to apply the inoculum. Control subplots received autoclaved inoculum at the same rate as specified above. Sprinkler irrigation was applied immediately after application.

Acetylene reduction activity, root dry weight, soil moisture and mineral analysis. Sampling for ARA involved taking 18 cm soil-root cores in metal tubes either 7.5 or 10.2 cm diam and 36 cm long. One end was sealed with a rubber cap clamped in place and the other end with a rubber septum. Both experiments were sampled twice, the first experiment 59 to 70 d and 83 to 93 d after planting and the second 77 to 84 d and 100 to 107 d after planting. After taking a core containing a corn plant cut off at ground level, each tube was capped, then flushed with argon for 1 min, followed by the addition of acetylene to approximately 10% of the gas phase volume. Tubes were incubated at 30°C in a growth chamber for 18 to 24 h and assayed for ethylene evolution by gas chromatography. Following ARA measurements root-soil masses were removed from each metal tube. Soil samples were taken for analyses and oven-dried to determine moisture content. Soil analyses for P, K, Ca, Al, Cu, Fe, Mg, Mn, Zn, NH_4^+ N, NO_3^- N, pH, and percent organic matter were conducted on four replications by the IFAS Soil Science Laboratories. Each root mass was washed over a 16-mesh metal screen, dried at 60°C, and weighed.

Greenhouse Experiments

Two greenhouse experiments were conducted to (1) reevaluate *Z. mays* genotypes grown in the field under conditions intermediate to field and axenic studies and (2) test a second group of genotypes in an effort to find greater genetic variation for the potential to support N_2-fixation (Table 10). In both experiments, plants were grown in 2.2 L plastic jar assemblies (Inmark Inc., Atlanta, Georgia) with drip irrigation and gravity drainage. Each jar was filled with soil from the B.R.U. that had been screened through a 16-mesh wire screen. A 3.8 cm hole through which plants could emerge was punched into each jar lid. A second hole, 1.3 cm diam, was drilled in the bottom of each jar for drainage. In order to minimize light exposure of soil and prevent the growth of blue-green algae each jug was sprayed with silver reflective paint and a 2 cm layer of waxed sand was placed on the soil surface.

Planting. In the first experiment, 14 genotypes were planted (three seeds per jar) and then thinned by selecting one uniform seedling. Jars were arranged in a complete block design with five replications. In the second experiment, 13 genotypes were planted and arranged in the above manner with four replications. In both experiments, control jars consisted of a complete jar assembly without a plant. General maintenance of irrigation and insect pest control with Lanate were used as needed. Plants of the first experiment showed signs of mineral deficiency (not N), approximately 43 d after planting so they were fertilized with a complete N free Hoagland's solution at a rate of 1.2 kg P and 12.6 kg K per hectare.

Acetylene reduction activity and yield measurement. Acetylene reduction activity procedure involved placing each jar assembly into 10 L plexiglass cylinders as described in Chapter II. Cylinders were flushed with argon for 1 min, followed by the addition of acetylene to an approximate conc of 10% in cylinder gas phase. Cylinders were incubated for 18 to 24 h and assayed for ethylene evoluation by gas chromatography. Both experiments were analyzed for ARA twice, the first experiment 45 to 52 d and 67 to 74 d after planting and the second experiment 30 to 35 d and 60 to 65 d after planting.

Following ARA measurements, tops were harvested, dried at 60°C and weighed. Root-soil mass was removed from each jar and root dry weight and soil moisture measurements were made as described on page 38.

Test-Tube Experiments

Sixteen *Z. mays* genotypes previously grown under field and greenhouse conditions were tested in four growth chamber experiments (Table 10). A description of each experiment can be seen in Table 11. Seedlings were grown axenically, according to a procedure described by Schank and Smith (1980), in 15 x 200 mm test tubes containing 60 ml of nitrogen and carbon free, semi-solid (6 g agar/L) Fahraeus medium (Fahraeus, 1957) with 4 ml/L of 0.5% bromothymol blue solution (dissolved in 10% ethanol as a pH indicator. The medium was adjusted with NaOH to pH = 7. In experiments 3 and 4 succinate (1.965 g/L) was included for a carbon source to stimulate bacterial growth because in experiments 1 and 2 low ARA was encountered with diazotrophs grown on medium without an added carbon source. Active bacterial populations should not be the limiting factor in this screening method and

Table 11. Genotypes and experiment parameters of test-tube experiments.

Experiment 1		Experiment 2		Experiment 3		Experiment 4	
Genotype							
Funk	G-4864	Pioneer	3030	Funk	G-4864	Funk	G-4810
Pioneer	3160	Asgrow	RX-112	Pioneer	3160	Pioneer	3320
Coker	22	Funk	G-4810	Coker	22	Jacques	247
Northrup King	PX-95	Pioneer	3320	Northrup King	PX-95	Dekalb	XL-82
Funk	G-4507A	Jacques	247	Funk	G-4507A	Coker	16
Dekalb	XL-80	Dekalb	XL-82	Pioneer	3030	Coker	77B
Coker	16	Coker	77B	Dekalb	XL-80	McCurdy	8150
Control		McCurdy	8150	Asgrow	RX-112	McNair	508
		Control		Control		Control	
Design							
6 replications in a Complete Randomized Design (C.R.D.)		6 replications in a C.R.D.		4 replications in a C.R.D.		3 replications in a C.R. D.	
Inoculation							
0.2 ml *A. brasilense* strain JM 125 A2		0.2 ml *A. brasilense* strain JM 125 A2		1.0 ml mixture *A. brasilense* strain JM 125 A2 *A. brasilense* strain CD		1.0 ml mixture *A. brasilense* strain JM 125 A2 *A. brasilense* strain CD	
Addition of succinate							
No		No		Yes		Yes	

differences in ARA should reflect plant contributions (root exudates released into the medium) to the plant-bacteria association.

Seeds were surface sterilized by consecutive dips in 95% ethanol for 30 s, two dips in 50% Clorox for 5 min each, and 3% hydrogen peroxide for 2 min. After thorough washing in sterile water, seeds were germinated on either agar plates (0.70% agar with 0.50% sucrose added to test for bacterial contamination) or sterile paper towels. Poor and uneven germination with the former method was experienced so paper towels were used. The towel method resolved the germination problem but seeds were not tested for bacterial contaimination. However, seedlings that were germinated on paper towels and then transferred to test tubes appeared to have a low contamination rate. Any contaiminated tubes were discarded.

Two days after seeds were sterilized test tubes were inoculated with a suspension of washed cells (approximately 10^8 to 10^9 cell/ml) of Azospirillum brasilense strain JM 125 A2 or a mixture containing equal volumes of the above diazotroph and Azospirillum brasilense strain CD (ATTCC 29729). Each test tube received either 0.2 or 1.0 ml of inoculum mixed in the warm (40 to 45°C) melted growth medium which then was allowed to solidify to its semi-solid state. Cultures of diazotrophs for inoculation were grown and cells washed as described in Chapter II.

Seedlings approximately 2 cm in length were transferred to test tubes 3 to 4 d after seeds were sterilized if germinated on paper towels and 3 to 8 d if germinated on agar plates. Seedlings were placed on stainless steel screens inserted into each test tube and suspended 0.5 cm above the medium in order to prevent leaching nutrients of the seed

from influencing bacterial growth and ARA. Control tubes without seedlings were included in the experiment and the analysis of variance to determine the effect of a plant on ARA. Plants were grown in a growth chamber maintained at 30°C with a 14 h day. Light intensity was 149 $\mu E/(m^2 \cdot s)$ at plant level. Acetylene reduction activity was measured 15 to 20 d after seedlings were transferred by adding 10% acetylene to each tube and incubating for 20 to 24 h in the growth chamber maintained as above. Ethylene evolution was measured by gas chromatography.

Results and Discussion

Field Experiments

Field Experiment 1. On the first coring date neither inoculation or genotype significantly affected ARA, root dry weight (Root DW), or soil moisture (Table 12). Analysis of variance from the second coring date indicated the same as above, except a genotype x inoculation interaction was significant (Table 13). Overall means of these measurements can be seen in Table 14. Acetylene reduction activity, Root DW and soil moisture measurements were highly variable as indicated by high coefficients of variation.

Combined analyses of both coring dates indicated ARA was not significantly different on each coring date but soil moisture was significantly higher and root dry weight significantly lower on the second coring date (Tables 14 and 15). The latter observation may relate to the fact that plants were beginning to show signs of senescence on the second coring date. Root dry weights were significantly different among genotypes which indicated differences in growth characteristics.

Table 12. Analysis of variance of first coring date, field experiment 1.

Source	df	ARA MS	ARA F	Root DW MS	Root DW F	Soil Moisture MS	Soil Moisture F
Rep	5	199282		756		106.70	
Genotype	14	17550	0.99	313	1.74	0.77	1.37
Rep x genotype	70	17714		180		0.56	
Inoculation	1	16207	0.86	236	1.81	0.01	0.02
Rep x inoculation	5	18841		131		0.18	
Genotype x inoculation	14	19309	1.07	163	1.62	0.29	1.20

*,**Significant at the P = 0.05 and 0.01 levels, respectively.

Table 13. Analysis of variance of second coring date, field experiment 1.

Source	df	ARA MS	ARA F	Root DW MS	Root DW F	Soil Moisture MS	Soil Moisture F
Rep	4	409,767		610		288.61	
Genotype	14	74,163	1.09	185	1.69	0.97	0.98
Rep x genotype	56	68,141		109		0.99	
Inoculation	1	17,539	0.23	248	2.63	0.01	0.00
Rep x inoculation	4	75,789		94		2.11	
Genotype x inoculation	14	117,957	1.21	34	0.86	0.74	2.21*

*,**Significant at the P = 0.05 and 0.01 levels, respectively.

Table 14. Overall means by coring date of acetylene reduction activity, root dry weight, and soil moisture, field experiment 1.

Parameter	Means: Grand	Inoc (+)	Control (-)	Range	C.V.
	n mole C_2H_4/(core·h)				
ARA 1	240 ± 11	249 ± 10	230 ± 12	22 - 1041	63
ARA 2	224 ± 25	213 ± 15	235 ± 32	24 - 3135	146
	Grams				
Root DW 1	24.27 ± 0.98	23.13 ± 0.77	24.42 ± 1.15	5.53 - 80.35	54
Root DW 2	20.64 ± 0.80	19.36 ± 0.75	21.94 ± 0.84	3.47 - 57.80	48
	Percent				
Soil Moisture 1	6.61 ± 0.14	6.61 ± 0.14	6.60 ± 0.13	2.35 - 12.36	23
Soil Moisture 2	8.35 ± 0.24	8.35 ± 0.23	8.34 ± 0.24	3.06 - 15.35	35

Table 15. Analysis of variance for the combined data of field experiment 1.

Source	df	ARA		Root DW		Soil Moisture	
		MS	F	MS	F	MS	F
Rep	5	139,618		705		84.47	
Genotype	14	54,540	1.49	371	2.30*	0.79	1.01
Rep x genotype	70	36,630		161		0.78	
Inoculation	1	22	0.00	483	3.10	0.01	0.01
Rep x inoculation	5	53,503		156		1.08	
Genotype x inoculation	14	74,597	1.27	119	1.65	0.49	1.62
Rep x genotype x inoculation	70	58,879		72		0.30	
Date	1	19,280	0.32	1075	8.83**	246.50	22.00**
Date x genotype	14	37,174		128		0.96	
Date x inoculation	1	33,723		2		0.01	
Date x genotype x inoculation	14	62,669		79		0.55	

*,** Significant at the P = 0.05 and 0.01 levels, respectively.

Correlations of combined data indicated ARA increased with increasing levels of soil moisture ($r = 0.29$, $P = 0.0001$) but was uncorrelated with inoculation or Root DW. Weier et al. (1981) and Smith et al. (1982) reported similar correlations between ARA and soil moisture. Root dry weight decreased with increasing levels of soil moisture ($r = -0.20$, $P = 0.0003$), but other factors, such as maturity may have caused this phenomenon. More coring dates during the experiment are needed to further investigate correlations between ARA, Root DW, and soil moisture.

Mineral analyses taken from soil samples of the first coring date indicated inoculated plants were not significantly different from control plants for all tested elements (Tables 16 - 18). Mineral analyses taken from the second coring date indicated the same as above except inoculated plants had significantly higher percent organic matter (Tables 19-21). Genotypes were significantly different for soil percent organic matter on the first coring date (Table 18). Soil samples from inoculated plants had higher overall mean soil mineral concentrations than controls in 79% of the comparisons in Tables 22 and 23. However, when data from each coring date were combined, only NH_4^+ N was significantly correlated with inoculation ($r = 0.20$, $P = 0.002$). Soil mineral concentrations of several elements were significantly correlated with soil moisture, pH, and Root DW. Soil mineral concentrations for all tested elements pH and organic matter were uncorrelated with ARA. Smith et al. (1982) found that soil Zn content was positively correlated and calcium soil content negatively correlated with ARA.

Table 16. Analysis of variance for soil mineral content from coring date 1, field experiment 1 (part 1).

		Al		K		Cu		Fe		P	
Source	df	MS	F	MS	F	MS	F	MS	F	MS	F
Rep	3	662		114.0		0.069		0.70		48.71	
Genotype	14	77	0.52	6.6	0.82	0.005	0.87	0.38	0.84	3.65	0.50
Rep x genotype	42	148		8.1		0.006		0.45		7.21	
Inoculation	1	145	0.70	1.2	0.77	0.015	0.96	0.32	3.96	20.61	1.96
Rep x inoculation	3	208		1.5		0.016		0.08		10.53	
Genotype x inoculation	14	89	1.46	3.2	1.33	0.002	0.80	0.14	0.94	5.06	1.27

*,**Significant at the P = 0.05 and 0.01 levels, respectively

Table 17. Analysis of variance for soil mineral content from coring date 1, field experiment 1 (part 2).

		Ca		Mg		Mn		Zn	
Source	df	MS	F	MS	F	MS	F	MS	F
Rep	3	186,199		7,491		0.75		7.66	
Genotype	14	5,132	0.60	681	0.64	0.13	1.24	1.66	0.80
Rep x genotype	42	8,576		1,065		0.11		2.08	
Inoculation	1	34,003	0.99	2,394	2.77	0.01	0.02	0.50	0.40
Rep x inoculation	3	34,440		864		0.23		1.24	
Genotype x inoculation	14	7,987	1.80	800	1.23	0.18	1.71	2.28	0.93

*,**Significant at the $P = 0.05$ and 0.01 levels, respectively.

Table 18. Analysis of variance for soil mineral content from coring date 1, field experiment 1 (part 3).

Source	df	NH_4^+ N		NO_3^- N		pH		% Organic Matter	
		MS	F	MS	F	MS	F	MS	F
Rep	3	0.84		0.089		0.50		0.835	
Genotype	14	0.17	1.68	0.005	0.93	0.03	0.97	0.103	2.28*
Rep x genotype	42	0.10		0.006		0.03		0.045	
Inoculation	1	0.04	2.07	0.011	1.03	0.07	2.51	0.008	0.10
Rep x inoculation	3	0.02		0.010		0.03		0.087	
Genotype x inoculation	14	0.01	0.33	0.002	1.03	0.02	1.10	0.053	1.06

*,**Significant at the $P = 0.05$ and 0.01 levels, respectively.

Table 19. Analysis of variance for soil mineral content from coring date 2, field experiment 1 (part 1).

		Al		K		Cu		Fe		P	
Source	df	MS	F	MS	F	MS	F	MS	F	MS	F
Rep	3	644		11.0		0.002		2.71		10.76	
Genotype	14	64	0.79	3.6	0.52	0.001	0.42	0.07	0.71	8.09	1.19
Rep x genotype	42	81		7.0		0.002		0.10		6.83	
Inoculation	1	374	2.15	6.5	0.82	0.003	1.30	0.20	0.62	5.89	0.86
Rep x inoculation	3	174		7.6		0.002		0.33		6.84	
Genotype x inoculation	14	45	1.62	2.2	0.61	0.001	0.48	0.07	0.59	3.63	0.65

*,**Significant at the P = 0.05 and 0.01 levels, respectively.

Table 20. Analysis of variance for soil mineral content from coring date 2, field experiment 1 (part 2).

Source	df	Ca		Mg		Mn		Zn	
		MS	F	MS	F	MS	F	MS	F
Rep	3	299,969		10,358		0.87		2.29	
Genotype	14	6,174	0.97	243	0.49	0.13	1.52	0.31	0.53
Rep x genotype	42	6,337		498		0.09		0.59	
Inoculation	1	74,401	3.04	1,555	2.44	0.08	2.39	0.04	0.31
Rep x inoculation	3	24,495		636		0.03		0.14	
Genotype x inoculation	14	2,881	0.66	184	0.61	0.04	0.74	0.25	0.62

*,**Significant at the P = 0.05 and 0.01 levels, respectively.

Table 21. Analysis of variance for soil mineral content from coring date 2, field experiment 1 (part 3).

Source	df	NH_4^+ N		NO_3^- N		pH		% Organic Matter	
		MS	F	MS	F	MS	F	MS	F
Rep	3	1.05		0.045		0.62		0.44	
Genotype	14	0.05	1.34	0.004	0.56	0.01	0.54	0.07	0.70
Rep x genotype	42	0.04		0.007		0.02		0.09	
Inoculation	1	0.04	3.39	0.004	0.12	0.06	1.68	0.25	10.43*
Rep x inoculation	3	0.01		0.004		0.03		0.02	
Genotype x inoculation	14	0.03	0.84	0.001	0.46	0.02	1.29	0.02	0.57

*,**Significant at the P = 0.05 ad 0.01 levels, respectively.

Table 22. Overall mean soil mineral content of field experiment 1 (part 1).

Al	K	Cu	Fe	P	Ca	Mg
	Soil ppm					
	inoculated, coring date 1					
27.80 ± 0.96	6.23 ± 0.29	0.19 ± 0.01	2.04 ± 0.04	4.85 ± 0.27	583.30 ± 11.92	117.63 ± 9.95
	control, coring date 1					
27.03 ± 0.97	5.93 ± 0.23	0.16 ± 0.00	2.15 ± 0.05	4.44 ± 0.18	568.20 ± 7.50	111.77 ± 1.96
	inoculated, coring date 2					
27.53 ± 0.84	6.40 ± 0.20	0.14 ± 0.00	2.00 ± 0.03	4.82 ± 0.27	582.33 ± 9.95	113.73 ± 3.93
	control, coring date 2					
24.43 ± 0.47	6.17 ± 0.22	0.14 ± 0.00	2.02 ± 0.04	4.55 ± 0.18	552.00 ± 7.47	110.27 ± 1.82

Table 23. Overall mean soil mineral content of field experiment 1 (part 2).

Mn	Zn	NH_4^+ N	NO_3^- N	pH	Organic Matter
	Soil ppm				
		inoculated, coring date 1			
1.81 ± 0.04	2.48 ± 0.16	0.65 ± 0.02	0.09 ± 0.01	7.15 ± 0.02	1.16 ± 0.02
		control, coring date 1			
1.87 ± 0.03	2.47 ± 0.11	0.69 ± 0.03	0.09 ± 0.00	7.12 ± 0.02	1.16 ± 0.02
		inoculated, coring date 2			
1.77 ± 0.03	1.94 ± 0.08	0.76 ± 0.03	0.10 ± 0.01	7.32 ± 0.02	1.21 ± 0.03
		control, coring date 2			
1.74 ± 0.03	1.93 ± 0.05	0.71 ± 0.02	0.10 ± 0.01	7.30 ± 0.02	1.15 ± 0.02

Field Experiment 2. A significant genotypic effect was not observed for ARA, Root DW or soil moisture for either coring date (Table 24). Data from both coring dates were combined and analyzed and indicated ARA and soil moisture were significantly higher on the second coring date (Tables 25 and 26). As observed in the first field experiment, ARA was positively correlated with soil moisture ($r = 0.60$, $P = 0.0001$). Soil moisture content was significantly different among genotypes when the data were combined.

In summary, corn genotypes grown under field conditions and inoculated with N_2-fixing bacteria or grown in the presence of indigenous N_2-fixing bacteria, were not significantly different for ARA. These measurements were highly variable but significantly correlated to soil moisture. High variation for ARA in field experiments exists but the failure of these experiments to identify genotypes for high ARA may not relate entirely to methodology. Insufficient genetic variation may have been present in the corn hybrids.

Greenhouse Experiments

Significant differences among genotypes were not observed for ARA in either greenhouse experiment (Table 27). Activities were not different for the first and second acetylene reduction assay within both experiments (Table 28). These measurements were highly variable as indicated by a high C.V. Top dry weights were significantly different among genotypes in experiment 1 and root dry weights were significantly different among genotypes in experiments 1 and 2. In experiment 1 Top DW exceeded Root DW and in experiment 2 the opposite was observed. Differences among genotypes for Top DW and Root DW indicated

Table 24. Analysis of variance of coring dates 1 and 2, field experiment 2.

Source	df	ARA		Root DW		Soil Moisture	
		MS	F	MS	F	MS	F
		coring date 1					
Rep	3	262,580		362		54.00	
Genotype	15	9,109	0.93	112	0.73	0.47	0.85
		coring date 2					
Rep	3	263,514		354		6.87	
Genotype	15	25,480	1.63	70	0.89	1.11	0.95

*,**Significant at the P = 0.05 and 0.01 levels, respectively.

Table 25. Analysis of variance for the combined data of field experiment 2.

Source	df	ARA		Root DW		Soil Moisture	
		MS	F	MS	F	MS	F
Rep	3	246,800		399		37.00	
Genotype	15	16,634	1.18	124	1.45	0.93	1.13*
Rep x genotype	45	14,068		85		0.82	
Date	1	701,446	25.00**	22	0.17	244.00	116.89**
Date x genotype	15	17,954		57		0.42	

*,**Significant at the P = 0.05 and 0.01 levels, respectively.

Table 26. Overall means by coring date of acetylene reduction activity, root dry weight, and soil moisture, field experiment 2.

Parameter	Mean	Range	C.V.
	n mole C_2H_4/(core·h)		
ARA 1	129 ± 18	3.0 - 613.0	114
ARA 2	277 ± 22	50.0 - 819.0	62
	grams		
Root DW 1	15.05 ± 2.09	2.66 - 55.78	79
Root DW 2	14.01 ± 1.18	2.64 - 65.11	67
	percent		
Soil moisture 1	8.07 ± 0.22	5.36 - 11.70	22
Soil moisture 2	10.84 ± 0.14	6.91 - 13.16	10

Table 27. Analysis of variance for greenhouse experiments 1 and 2.

Source	df	ARA 1 MS	ARA 1 F	ARA 2 MS	ARA 2 F	Top DW MS	Top DW F	Root DW MS	Root DW F	Soil Moisture MS	Soil Moisture F
Greenhouse experiment 1											
Rep	4	196,720		180,925		0.97		1.39		40	
Genotype	13	114,715	1.58	29,938	0.66	2.15	3.75**	0.58	6.85**	10	1.35
Greenhouse experiment 2											
Rep	3	8,415		88,058		0.40		0.43		10.66	
Genotype	12	34,946	0.94	16,472	1.11	0.07	0.41	1.43	2.84**	12.20	2.03*

*,**Significant at the $P = 0.05$ and 0.01 levels, respectively.

Table 28. Overall means of acetylene reduction activity by sample date, top dry weight, root dry weight, and soil moisture from greenhouse experiments 1 and 2.

Parameter	Mean	Range[†]	C.V.
Greenhouse experiment 1			
	n mole C_2H_4/(cylinder·h)		
ARA 1	387 ± 36	- 74 to 1,188	76
ARA 2	320 ± 28	-125 to 974	71
	grams		
Top DW	4.92 ± 0.11	3.53 to 7.06	19
Root DW	1.70 ± 0.06	0.64 to 3.11	30
	percent		
Soil Moisture	16.94 ± 0.39	3.96 to 20.04	19
Greenhouse experiment 2			
	n mole C_2H_4/(cylinder·h)		
ARA 1	270 ± 26	- 6 to 857	69
ARA 2	244 ± 20	- 19 to 553	58
	grams		
Top DW	1.34 ± 0.06	0.70 to 3.38	29
Root DW	3.02 ± 0.12	0.93 to 4.88	28
	percent		
Soil Moisture	18.07 ± 3.39	4.78 to 21.66	15

[†]Negative ARA values were not measured but obtained when ARA of control jars exceeded sample jars. Control jars did not contain a plant.

they had different growth characteristics. Soil moisture was significantly different among genotypes in experiment 2, but was uncorrelated with ARA of the second sample date. However, soil moisture was correlated ($r = 0.28$, $P = 0.02$) with ARA of the second sample date in experiment 1. In summary, corn genotypes grown under greenhouse conditions were not significantly different for ARA. These measurements were highly variable but only in a few cases did controls (no plant) exceed plant systems.

Test-Tube Experiments

Acetylene reduction activity was significantly different among genotypes in experiments 1 to 3 but not in experiment 4 (Table 29). In these analyses, controls (no plant) were considered a "genotype." In experiments 2 to 4, controls exhibited as high or higher ARA than plant systems. This indicates that the addition of a plant to the system does not contribute to ARA activity and some genotypes significantly decreased it. One explanation for this is that seedlings compete with bacterial populations for growth producing substances, i.e., mineral salts and possibly carbon sources. Asgrow RX-112 exhibited high ARA in experiments 2 and 3, while ARA of other genotypes was inconsistent. The addition of succinate as an energy source and use of greater amounts of inoculum enhanced ARA up to 2,729%.

Comparisons of Screening Methods

Genotype ARA from greenhouse and test-tube experiments was compared to field activity to determine if field performance could be predicted by using greenhouse and/or test-tube results (Table 30).

Table 29. Analysis of variance and genotype comparisons of test-tube experiments.

	Analysis of Variance															
		Experiment 1				Experiment 2				Experiment 3				Experiment 4		
Source	df	MS	F	P>F	df	MS	F	P>F	df	MS	F	P>F	df	MS	F	P>F
Genotype	7	14,433	2.19	0.06	8	5,617	2.54	0.02	7	177,399	2.53	0.05	8	38,078	2	0.11

Acetylene reduction means in n mole C_2H_4/(test tube·h)[†]

Experiment 1		Experiment 2		Experiment 3		Experiment 4	
Coker 16	153.3 A	Control	83.8 A	Asgrow RX-112	1,509.7 A	Control	1,073.2 A
Funk G-4507	70.8 AB	Asgrow RX-112	62.7 AB	Pioneer 3030	1,374.8 A	McCurdy 8150	1,004.5 AB
Funk G-4864	52.0 B	Funk G-4810	38.6 AB	Control	1,331.7 A	Coker 77B	955.5 AB
Pioneer 3160	51.0 B	Coker 77B	23.9 B	Coker 22	1,279.2 A	McNair 508	929.3 AB
Coker 22	46.5 B	Dekalb XL-82	6.7 B	Northrup King PX-95	1,248.5 A	Funk G-4810	909.7 AB
Control	17.7 B	Pioneer 3320	1.6 B	Funk G-4507A	1,149.0 AB	Jacques 247	850.8 AB
Dekalb XL-80	2.7 B	McCurdy 8150	1.0 B	Pioneer 3160	1,060.9 AB	Coker 16	769.1 B
Northrup King PX-95	0.61B	Pioneer 3030	0.8 B	Dekalb XL-80	743 B	Pioneer 3320	762.6 B
		Jacques 247	0.0 B			Dekalb XL-82	762.5 B

[†]Means within each column followed by the same letter are not different at the P = 0.05 level as determined by Duncan's Multiple Range Test. However, the probability level in experiments 1 and 4 was greater than P = 0.05. For the purpose of genotype comparisons between experiments they were included.

Table 30. Genotype means of acetylene reduction activity in n mole C_2H_4 evolved/(core·h) for field and greenhouse experiments.

Field experiment 1		Field experiment 2		Greenhouse experiment 1		Greenhouse experiment 2	
Genotype	Mean[†]	Genotype	Mean	Genotype	Mean	Genotype	Mean
Funk G-4864	372	Coker 22	269	Asgrow RX-112	510	Funk G-4810	364
Asgrow RX-112	288	Funk G-4864	258	Caribbean II	455	Dekalb XL-82	354
Coker 16	252	McNair 508	257	Caribbean I	423	Dekalb XL-80	350
Pioneer 3030	247	Coker 16	249	Tuxpeno II	419	Northrup King PX-95	296
Pioneer 3160	245	Jacques 247	244	Funk G-4507A	368	Pioneer 3160	280
Coker 77B	240	Funk G-4507A	241	McNair 508	356	Pioneer 3320	279
Funk G-4810	233	McCurdy 8150	207	Coker 16	353	Coker 22	257
Dekalb XL-82	230	Coker 77B	193	Argentine I	338	Pioneer 3030	231
Dekalb XL-80	225	Dekalb XL-82	193	Cuzco composite	334	Jacques 247	187
Pioneer 3320	222	Pioneer 3030	192	Argentine II	331	McCurdy 8150	184
Funk G-4507A	207	Northrup King PX-95	192	Florida Stay Sweet	223	Funk G-4864	159
McCurdy 8150	199	Dekalb XL-80	172	Tuxpeno I	311	Coker 77B	138
Coker 22	181	Pioneer 3320	158	Alpine I	211		
Northrup King PX-95	180	Funk G-4810	152	Coroico composite	211		
Jacques 247	170	Florida Stay Sweet	134				
		Asgrow RX-112	133				
		Pioneer 3160	-				

[†]Means are the average ARA of both coring dates in field experiments or both sample dates in greenhouse experiments.

These genotype comparisons are not statistically valid because in the field and greenhouse experiments ARA among genotypes was not significantly different ($P \geq 0.10$). However, more liberal observations may be warranted and useful in screening studies.

In general, genotypes responded erratically to all growing conditions. Asgrow RX-112 responded well in field experiment 1, test-tube experiments, and greenhouse experiment 1, but poorly in field experiment 2. Funk G-4864 responded well in both field experiments but poorly in greenhouse experiment 2 and on an average in test-tube experiment 1.

From the limited data in this study a correlation probably does not exist between a genotype's ARA under axenic, greenhouse, and field conditions. However, a few genotypes may show promise for consistently having high ARA under axenic, greenhouse, and field conditions. Asgrow RX-112 and Funk G-4864 are two genotypes that should be further tested.

CHAPTER IV

CONCLUSIONS

Methods of estimating N_2-fixation gave different values of fixed N in a bahiagrass study under greenhouse growing conditions. Percentage of total N in bahiagrass tops due to N_2-fixation from inoculation was 8% when estimated by yield differences between inoculated and control plants and 2% when estimated by the ^{15}N-isotope dilution method. Acetylene reduction activity (ARA) was highly variable and negatively correlated with the above methods of estimating N_2-fixation.

Estimates of N_2-fixation due to inoculation did not measure total fixation because control plants exhibited fixation rates nearly equal to inoculated plants. However, measurements of accumulated N in excess of all N inputs were measurements of total fixation, i.e., measurements from the N balance method. These measurements in inoculated and control plant-soil systems indicated 46% of the N in the system was fixed. Thus N_2-fixation estimates which were based on differences between inoculated and control plants underestimated and accounted for only a portion of total N_2-fixation. Estimates of total N_2-fixation were calculated from ARA and ^{15}N-isotope dilution measurements and indicated that 12% and 6% of the N in the plant-soil system was fixed. These total N_2-fixation estimates were lower than estimates from the N balance method. However, the three estimates are from independent measurements because (1) ARA depends on short-term measurements, and (2) ^{15}N-isotope dilution estimates considered

control plant-soil systems as nonfixing. Estimates of N_2-fixation from N balance calculations may be closest to actual fixation rates because of the above limitations of the other two methods. These calculations indicated that agronomically important amounts of N were fixed. It is important to remember that even when inoculation responses are low, significant N_2-fixation could be occurring.

The N-balance method of estimating N_2-fixation indicated that 81% of the N in excess of all N inputs was located in the root-zone soil. Therefore, N_2-fixation may have its largest impact on root-zone soil so it is important to include this part of the plant-soil system in N_2-fixation studies. Seventy-six percent of the soil N was in the organic form and was not available to plants. This N should be mineralized over time and should become available for plant use.

The utilization of ^{15}N-enriched fertilizer expanded the scope of the N balance study by permitting plant fertilizer N uptake and loss estimates from the plant-soil system. These measurements indicated that 40% of the ^{15}N-labeled fertilizer N was located in plant tissue while 16% remained in the soil. Forty-three percent of the ^{15}N-labeled-fertilizer N was lost. Since losses were not considered in the N_2-fixation calculations, estimates of N_2-fixation by the N balance method could be conservative.

Development of quick screening procedures for the ability to support N_2-fixation were difficult to evaluate because corn genotypes were not significantly different for ARA in field and greenhouse experiments. Significant differences among genotypes were observed within test-tube experiments but genotype ARA between experiments was

generally inconsistent. Acetylene reduction activity had large amounts of extraneous variation but the difficulty in obtaining significant genotypic responses for ARA may partially relate to insufficient genetic variation in ability to support associative N_2-fixation. However, more liberal observations indicate two genotypes, Asgrow RX-112 and Funk G-4864 show promise and should be tested further.

A significant overall response to inoculation was observed in the bahiagrass study. However, genotypes responded similarly to inoculation and no exceptional genotype could be identified because the inoculation response measured was small and within the limits of experimental error. Tetraploid bahiagrass genotypes exhibited greater nitrogenase activity but were not as efficient as diploids in taking up fixed N. Therefore, two characteristics may be important in the selection of N efficiency: (1) the ability to support diazotropic colonization and (2) ability to take up fixed N and prevent its loss. This second characteristic may be related to several plant factors such as root development and mineral uptake efficiency.

LITERATURE CITED

Albrecht, A. L., Y. Okon, and R. H. Burris. 1977. Effects of light and temperature on the association between Zea mays and Spirillum lipoferum. Plant Physiol. 60:528-531.

Albrecht, S. L., Y. Okon, J. Lonnquist, and R. H. Burris. 1981. Nitrogen fixation by corn-Azospirillum associations in a temperate climate. Crop Sci. 21:301-306.

Alvarez-Morales, R. A., and A. Lemos-Pastrana. 1980. Chemotaxis of Azospirillum-lipoferum and Azospirillum-brasilense towards root exudates of graminae. Rev. Latinoam. Microbiol. 22:131-136.

Baltensperger, A. A., S. C. Schank, R. L. Smith, R. C. Littell, J. H. Bouton, and A. E. Dudeck. 1978. Effect of inoculation with Azospirillum and Azotobacter on turf bermudagrass genotypes. Crop Sci. 18:1043-1045.

Barber, L. E., S. A. Russell, and H. J. Evans. 1979. Inoculation of millet with Azospirillum. Plant and Soil 52:49-57.

Barber, L. E., J. D. Tjepkema, S. A. Russell, and H. J. Evans. 1976. Acetylene reduction (nitrogen fixation) associated with corn inoculated with Spirillum. Appl. Environ. Microbiol. 32:108-113.

Barea, J. M., and M. E. Brown. 1974. Effects on plant growth produced by Azotobacter paspali related to synthesis of plant growth regulating substances. J. Appl. Bacteriol. 40:583-593.

Benzion, G. 1978. Interaction of Paspalum notatum genotypes with associative N_2-fixing bacteria. M. S. Thesis. Univ. Florida.

Benzion, G., and K. H. Quesenberry. 1978. Interaction of Paspalum notatum genotypes with associative N_2-fixing bacteria. Agron. Abstr. 70:70.

Blue, W. G. 1974. Efficiency of five nitrogen sources of Pensacola bahiagrass on Leon Fine Sand as affected by lime treatments. Soil and Crop Sci. Soc. Fla. Proc. 33:176-180.

Bouton, J. H. 1977. Response of pearl millet inbreds and hybrids to inoculation with Spirillum lipoferum. Ph.D. Dissertation. Univ. Florida. Univ. Microfilms. Ann Arbor, Mich. Diss. Abstr. Int. 38:5125-B. Order No. 7806676.

Bouton, J. H., R. L. Smith, S. C. Schank, G. W. Burton, M. E. Tyler, R. C. Littell, R. N. Gallaher, and K. H. Quesenberry. 1979. Response of pearl millet inbreds and hybrids to inoculation with Azospirillum-brasilense. Crop Sci. 19:12-16.

Bremner, J. M. 1965. Methods of soil analysis: Part 2. Chemical and microbiological properties. Agronomy 9:1195-1201.

Bremner, J. M., and A. P. Edwards. 1965. Determination and isotope-ratio analysis of different forms of nitrogen in soils: I. Apparatus and procedure for distillation and determination of ammonium. Soil Sci. Soc. Am. Proc. 29:341-348.

Brown, M. E., 1976. Role of Azotobacter paspali in association with Paspalum notatum. J. Appl. Bact. 40:341-348.

Cochran, W. G., and G. M. Cox. 1957. Experimental Designs. W. A. Shewhart and S. S. Wilks (ed.) John Wiley & Sons, Inc., New York, New York.

Cohen, E., Y. Okon, J. Kigel, I. Nur, and Y. Henis. 1980. Increase in dry weight and total nitrogen content in Zea mays and Setaria italica associated with nitrogen-fixing Azospirillum spp. Plant Physiol. 66:746-749.

Day, J. M., D. Harris, P. J. Dart, and P. van Berkum. 1975a. The broadbalk experiment. An investigation of nitrogen gains from nonsymbiotic fixation. p. 71-84. Nitrogen fixation by free-living microorganisms. International Biological Programme Series, Vol. 6. Cambridge University Press, Cambridge, England.

Day, J. M., M. C. P. Neves, and J. Döbereiner. 1975b. Nitrogenase activity on the roots of tropical forage grasses. Soil Biol. Biochem. 7:107-112.

De-Polli, H., E. Matsui, and J. Döbereiner. 1977. Confirmation of nitrogen fixation in two tropical grasses by $^{15}N_2$ incorporation. Soil Biol. Biochem. 9:119-123.

Döbereiner, J., and A. B. Campelo. 1971. Nonsymbiotic nitrogen fixing bacteria in tropical soils. Plant and Soil Spec. Vol.:457-470.

Döbereiner, J., J. M. Dart, and P. J. Dart. 1972. Nitrogenase activity and oxygen sensitivity of the Paspalum notatum-Azotobacter paspali association. J. Gen. Microbio. 71:103-116.

Döbereiner, J., and J. M. Day. 1976. Associative symbioses 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 First International Symposium on Nitrogen Fixation. Vol. 2. Washington State Univ. Press, Pullman, Washington.

Döbereiner, J., J. M. Day, and P. J. Dart. 1973. Rhizosphere associations between grasses and nitrogen-fixing bacteria: Effect of O_2 on nitrogenase activity in the rhizosphere of _Paspalum notatum_. Soil Biol. Biochem. 5:157-159.

Fahraeus, G. 1957. The infection of clover root hairs by nodule bacteria studied by a simple glass slide technique. J. Gen. Microbiol. 16:374-381.

Food and Agricultural Organization of the United Nations. 1977. Annual fertilizer review for 1977. Food and Agriculture Organization of the United Nations, Rome.

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.

Gallaher, R. N., C. O. Weldon, and J. G. Futral. 1975. An aluminum block digester for plant and soil analysis. Soil Sci. Soc. Am. Proc. 39:803-806.

Gaskins, M. H., M. Garcia, T. M. Tien, and D. H. Hubbell. 1977. N_2-Fixation and growth substance production by _Spirillum lipoferum_ and their effects on plant growth. Plant Physiol. 59:128.

Hardy, R. W. F., R. C. Burns, and R. D. Holsten. 1973. Applications of the acetylene-ethylene assay for measurement of nitrogen fixation. Soil Biol. Biochem. 5:47-81.

Hardy, R. W. F., and U. D. Havelka. 1975. Nitrogen fixation research: A key to world food? Science. 188:633-643.

Hauck, R. D., and J. M. Bremner. 1976. Use of tracers for soil and fertilizer nitrogen research. Adv. Agron. 28:219-265.

Hoagland, D. R., and D. I. Arnon. 1950. The water-culture method of growing plants without soil. Calif. Agric. Exp. Stan. Circular 347. Berkeley, California.

Ito, O., D. Cabrera, and I Watanabe. 1980. Fixation of dinitrogen-15 associated with rice plants. Appl. Environ. Microbio. 39:554-558.

Kapulnik, Y., S. Sarig, I. Nur, Y. Okon, J. Kigel, and Y. Henis. 1981. Yield increases in summer cereal crops in Israeli fields inoculated with _Azospirillum_. Expl. Agric. 17:179-187.

Matsui, E., P. B. Vose, N. S. Rodrigues, and A. P. Ruschel. 1981. Use of ^{15}N enriched gas to determine N_2-fixation by undisturbed sugarcane plants in the field. p. 153-161. In P. B. Vose and A. P. Ruschel (ed.) Associative N_2-Fixation. CRC Press, Boca Raton, Florida.

Neyra, C. A., and J. Döbereiner, 1977. Nitrogen fixation in grasses. Adv. Agron. 29:1-38.

Nur, I., Y. Okon, and Y. Henis. 1980. An increase in nitrogen content of Setaria italica and Zea mays inoculated with Azospirillum. Can. J. Microbiol. 26:482-485.

O'Hara, G. W., M. R. Davey, and J. A. Lucas. 1981. Effect of inoculation of Zea mays with Azospirillum brasilense strains under temperate conditions. Can. J. Microbiol. 27:871-877.

Okon, Y., S. L. Albrecht, and R. H. Burris. 1977. Methods for growing Spirillum lipoferum and for counting it in pure culture and in association with plants. Appl. Environ. Microbiol. 33:85-88.

Rennie, R. J. 1979. Comparison of ^{15}N-aided methods for determining symbiotic dinitrogen fixation. Rev. Ecol. Biol. Sol. 16:455-463.

Rennie, R. J. 1980. ^{15}N-isotope dilution as a measure of dinitrogen fixation by Azospirillum brasilense associated with maize. Can. J. Bot. 58:21-24.

Rennie, R. J., and R. I. Larson. 1979. Dinitrogen fixation associated with disomic chromosome substitution lines of spring wheat. An. J. Bot. 57:2771-2775.

Rennie, R. J., and D. A. Rennie. 1973. Standard isotope versus nitrogen balance criteria for assessing the efficiencies of nitrogen sources for barley. Can. J. Soil Sci. 53:73-77.

Rennie, R. J., D. A. Rennie, and M. Fried. 1978. Concepts of ^{15}N usage in dinitrogen fixation studies. p. 107-133. In Isotopes in biological dinitrogen fixation. International Atomic Energy Agency, Vienna.

Ruschel, A. P., Y. Henis, and E. Salati. 1975. Nitrogen-15 tracing of N-fixation with soil-grown sugar cane seedlings. Soil Biol. Biochem. 7:181-182.

Schank, S. C., and R. L. Smith. 1980. Growth responses and acetylene reduction of several grass/bacteria associations grown in vitro. Can. J. Genet. Cytol. 21:677.

Schank, S. C., R. L. Smith, and G. C. Weiser. 1979. Responses of two pearl millets grown in vitro after inoculation with Azospirillum brasilense. Soil Crop Sci. Soc. Fla. Proc. 39:112-115.

Schank, S. C., K. L. Weier, and I. C. MacRae. 1981. Plant yield and nitrogen content of a digitgrass in response to Azospirillum inoculation. Appl. Environ. Microbio. 41:342-345.

Smith, R. L., J. H. Bouton, S. C. Schank, and K. H. Quesenberry. 1977. Yield increases of tropical grain and forage grasses after inoculation with Spirillum lipoferum in Florida. p. 307-311. In A. Ayanaba and P. J. Dart (ed.) Biological N_2-fixation in farming systems of the tropics. John Wiley & Sons, Inc., New York, New York.

Smith, R. L., and S. C. Schank. 1981. Factors affecting nitrogenase activity of grass field plots. p. 495. In Current perspectives in nitrogen fixation. Australian Acad. Sci. Canberra.

Smith, R. L., S. C. Schank, J. H. Bouton, and K. H. Quesenberry. 1978. Yield increases of tropical grasses after inoculation with Spirillum lipoferum. Ecol. Bull. (Stockholm). 26:380-385.

Smith, R. L., S. C. Schank, J. R. Milam, and R. C. Littell. 1982. Statewide search for highly active associative N_2-fixation systems. (In press) Soil Crop Sci. Soc. Fla. Proc. 42.

Taylor, R. W. 1979. Response of two grasses to inoculation with Azospirillum spp. in Bahamian soil. Trop. Agric. 56:361-366.

Tien, T. M., M. H. Gaskins, and D. H. Hubbell. 1979. Plant growth substances produced by Azospirillum brasilense and their effect on the growth of pearl millet (Pennisteum americanum L.) Appl. Environ. Microbio. 37: 1016-1024.

Tyler, M. E., J. R. Milam, R. L. Smith, S. C. Schank, and D. A. Zuberer. 1979. Isolation of Azospirillum from diverse geographic regions. Can. J. Microbiol. 25:693-697.

Umali-Garcia, M., D. H. Hubbell, and M. H. Gaskins. 1978. Process of infection of Panicum maximum by Spirillum lipoferum. Ecol. Bull. (Stockholm). 26:373-379.

Van Berkum, P., and B. B. Bohlool. 1980. Evaluation of nitrogen fixation by bacteria in association with roots of tropical grasses. Microbio. Rev. 44:491-517.

Vietor, D. M. 1982. Variation of ^{14}C-labeled photosynthate recovery from roots and rooting media of warm season grasses. Crop Sci. 22:326-366.

von Bülow, J. F. W., and J. Döbereiner. 1975. Potential for nitrogen fixation in maize genotypes in Brazil. Proc. Natl. Acad. Sci. U.S.A. 72:2389-2393.

Weier, K. L. 1980.. Nitrogenase activity associated with three tropical grasses growing in undisturbed soil cores. Soil Biol. Biochem. 12:131-136.

Weier, K. L., I. C. Macrae, and J. Whittle. 1981. Seasonal variation in the nitrogenase activity of a _Panicum maximum_ var. Trichoglume pasture and identification of associated bacteria. Plant and Soil. 63:189-197.

Weiser, G. C. 1980. Evaluation of _Panicum virgatum_ L. for nitrogen related agronomic characters. Ph.D. Dissertation. Univ. Florida. Univ. Microflims. Ann Arbor, Mich. Diss. Abstr. Int. 41:1603-B. Order No. 8025401.

Yoshida, T., and T. Yoneyama. 1980. Atmospheric dinitrogen fixation in the flooded rice rhizosphere as determined by the N-15 isotope technique. Soil Sci. Plant Nutr. 26:551-559.

BIOGRAPHICAL SKETCH

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Rex L. Smith, Chairman
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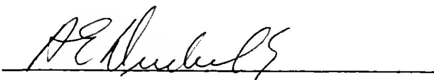

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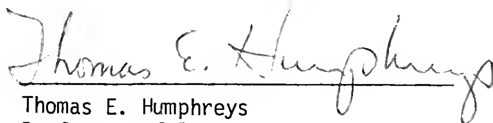

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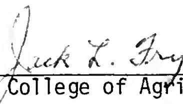
December 1982



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