AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523

BIBLIOGRAPHIC INPUT SHEET

FOR AID USE ONLY

1. SUBJECT CLASSIFICATION

A. PRIMARY: Food production and nutrition — AF60-1930-0000

B. SECONDARY: Plant physiology and biochemistry--Feed grasses

2. TITLE AND SUBTITLE

Effect of inoculation with Azospirillum and Azotobacter on turf-type bermuda genotypes

3. AUTHOR(S)

Baltensperger,A.A.; Schank,S.C.; Smith,R.L.; Littell,R.C.; Bouton,J.H.; Dudeck,A.E.

4. DOCUMENT DATE	5. NUMBER OF PAGES	6. ARC NUMBER
1978	4p.	ARC

7. REFERENCE ORGANIZATION NAME AND ADDRESS

Fla.

8. SUPPLEMENTARY NOTES (Sponsoring Organization, Publishers, Availability)

(In Crop Science, v.18, 1978, p.1043-1045)

9. ABSTRACT

10. CONTROL NUMBER

PN-AAG-430

11. PRICE OF DOCUMENT

12. DESCRIPTORS

Nitrogen fixation
Grasses
Yield

13. PROJECT NUMBER

931100400

14. CONTRACT NUMBER

AID/ta-C-1376 Res.

15. TYPE OF DOCUMENT

AID 590-1 (4-74)

Reprinted from CROP SCIENCE
Vol. 18, Nov.-Dec. 1978, p. 1043-1045

Effect of Inoculation with *Azospirillum* and *Azotobacter* on Turf-type Bermuda Genotypes[1]

A. A. Baltensperger, S. C. Schank, Rex L. Smith, R. C. Littell, J. H. Bouton, and A. E. Dudeck[2]

ABSTRACT

Eight selected bermudagrass (*Cynodon dactylon* L. Pers.) genotypes were inoculated with a mixed culture of the N_2-fixing bacteria, *Azospirillum* and *Azotobacter*, to determine plant growth response to inoculation under greenhouse conditions. Top, crown, and root growth and N content were measured. At low N fertility, top growth was increased 17% as a result of inoculation. Inoculation also resulted in increased total N accumulation in the top growth. Crown and root growth was not increased by inoculation and total dry weight of all plant parts was not significantly increased. The high fertility treatments gave significantly higher clipping yields, as expected, but no significant differences were observed between the inoculation treatments. No leaf color differences were observed as a result of inoculation. Bermudagrass genotypes were not significantly different in response to inoculation treatments.

Additional index words. N fixing bacteria, N content, Dry matter yield, *Cynodon dactylon* L. Pers.

NITROGENASE activity measured by acetylene reduction has been reported for roots or in the rhizosphere of numerous grasses in association with *Azospirillum brasilense* Krieg (8) (formerly *Spirillum lipoferum*), *Azotobacter paspali*, and other N-fixing bacteria (1, 3, 4, 5, 6, 9, 14, 15). Significant increases in plant growth as a result of inoculation with *Azospirillum* or *Azotobacter* have been reported (2, 10, 11, 12, 13). The exact mechanism causing these growth increases has not been established, and both N fixation and growth-regulating substances have been postulated (2, 15).

The objective of this study was to determine whether inoculation with *Azospirillum* and *Azotobacter* would increase growth and N content of bermudagrass under greenhouse conditions and whether selected turf bermudagrass (*Cynodon dactylon* L. Pers.) genotypes would respond differently to inoculation.

MATERIALS AND METHODS

Eight turf type bermudagrass (*Cynodon dactylon* L. Pers.) genotypes were established in 12.5 cm pots in the greenhouse. The soil mixture consisted of 1 part peat and 1 part Arredondo fine sand (Grossarenic Paleudult, loamy siliceous hyperthermic) obtained from a *Panicum maximum* Jacq. nursery. An attempt was made to maintain the natural microorganisms present in the field soil from the nursery. This soil mixture contained 6.15% organic matter and 1,328 ppm total N. Several bermudagrass genotypes were selected because in previous experiments they had remained green with less than optimum N fertilization. The identity of each of the vegetatively propagated Florida genotypes was as follows: #1 FB 119, #2 FB 61, #3 FB 19, #4 FB 133, #5 FB 157, #6 FB 141, and #7 FB 28. Genotype #8 was common bermudagrass obtained from New Mexico State Univ. and was the only entry established from seed.

Batch cultures of *A. brasilense* strains 7, 13t and JM 125A2 and *A. paspali* produced in a medium containing mineral salts, tryptocase, malate, succinate and yeast extract were obtained from Dr. M. E. Tyler, Dep. of Microbiology and Cell Science, Univ. of Florida, Gainesville. Inoculation consisted of 30 ml/pot of mixed culture containing equal parts of *A. brasilense* strains 7, 13t and JM 125A2 at 14 days after planting and 85 ml/pot of mixed culture containing equal parts of *A. brasilense* strains 7, 13t, JM 125A2 and *A. paspali* at 35 days after planting. Approximately 5.0×10^8 cells per ml were applied in the first treatment and approximately 1.0×10^9 cells/ml in the second treatment. High levels of mixed culture were used in an effort to maximize plant response. Control pots received the same amount of the above autoclaved cultures.

The experimental design was a split-split plot with fertilizer N levels as main plots, genotypes as subplots and two bacterial treatments as sub-subplots. Five replicates of main plots were made for a total of 160 pots in the experiment.

One week after transplanting, fertilizer was applied at the calibrated rate of 7.9 kg P, 39.1 kg K, and 5.5 kg of tritted trace elements (5g B, 5g Cu, 20g Fe, 12g Mn, 0.8g Mo, and 11g Zn)/ha. This fertilizer regime was used in an attempt to eliminate all nutrient deficiencies except combined N. One-half of the pots received no N fertilizer and the other half of the pots received an equivalent total of 100 kg N/ha as ammonium nitrate applied in three equal split applications 12, 42, and 62 days after planting.

Four harvests of top growth were made at 14, 28, 42, and 82 days following initial inoculation. Crowns and roots were also harvested after 82 days at the termination of the experiment. Top growth was clipped to a 3 cm stubble height at all harvests. Clippings and root weights were determined on an oven dry basis. Nitrogen in the top growth was determined by combining the top growth from all four cuttings, grinding through a 1 mm screen in a Wiley mill, digesting the samples using a block digester as previously described (7) and analyzing on a Technicon auto analyzer. No attempt was made to measure nitrogenase activity.

RESULTS AND DISCUSSION

Inoculation of bermudagrass with *A. brasilense* and *A. paspali* increased cumulative top growth 17% at the low N fertility level (Fig. 1). Significant top growth yield increases were measured from the inoculated treatments at the second and third harvests. The mean total accumulated top growth yield at the low N fertility level was 2.76 g/pot for the inoculated (live inoculum) and 2.35 g/pot for the control (autoclaved inoculum) treatment. The L.S.D. (0.05) for comparing these two means was 0.27 g with a C.V. of 23.6%. Inoculation had no effect on bermudagrass at the high N fertility level as measured by top growth.

Top growth means for the eight selected genotypes were significantly different at the low N fertility for only the first and second clipping and not for total

[1] Journal article 663, Agric. Exp. Stn., New Mexico State Univ., Las Cruces, NM 88003. The research was conducted in cooperation with the Univ. of Florida Institute of Food and Agric. Sci., Gainesville, FL 32611. The work was conducted while the senior author was on leave at the Univ. of Florida, and it was partially supported by USAID Contract ta-C-1576. Received 6 Mar. 1978.

[2] Professor of agronomy, New Mexico State Univ.; professor of agronomy, associate professor of agronomy, associate professor of statistics, former research associate in agronomy (now assistant professor of agronomy, Univ. of Georgia, Athens, GA 30602), and associate professor of ornamental horticulture, IFAS, Univ. of Florida, respectively.

top growth (Fig. 2) There was no significant genotype × inoculation interaction for any of the plant parts measured

The mean crown and root production and total cumulative dry matter production were not significantly different as a result of inoculation (Table 1). Crown and root growth and total dry matter production were different among genotypes as was expected.

Total N accumulated in the top growth was increased by 21% and total N accumulated in the total harvested material was increased by 13% as a result of inoculation (Table 2) Since inoculation did not affect percent nitrogen in the plant parts and inoculation increased top growth, it is concluded that the increased N in total harvested plant parts resulted primarily from increased top growth To the authors' knowledge, this is the first time that increased yield and accumulated total N have been reported as a result of applying N_2-fixing bacteria to bermudagrass

Leaf greenness was rated and no color differences were observed as a result of inoculation However, leaf greenness was greatly increased by N fertilization as expected Although a genotype × inoculation interaction was not found in this experiment, definite conclusions can not be drawn relative to the potential

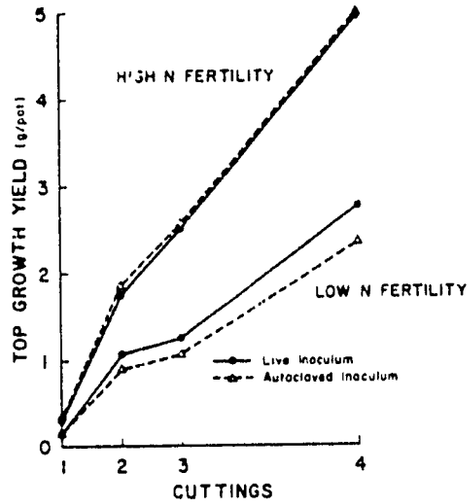

Fig. 1. Mean cumulative top growth dry matter yield of eight bermudagrass genotypes treated with live and autoclaved inoculum at low and high N fertility levels in a greenhouse experiment. Cuttings 1, 2, 3, and 4 were 14, 28, 42, and 82 days following initial inoculation

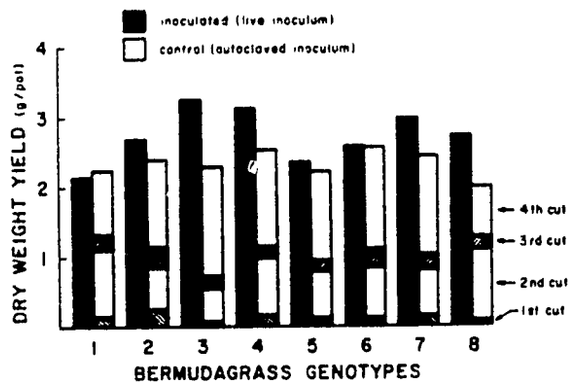

Fig. 2. Mean top growth dry weight yields of eight bermudagrass genotypes comparing inoculated grasses to controls for four harvests at low N fertility level; greenhouse experiment.

Table 1. Mean dry weight of crowns and roots and mean total plant dry weight of eight bermudagrass genotypes as influenced by inoculation (I) at low N fertility level, greenhouse experiment.

Genotype	Crowns and Roots			Total		
	I	Control	x̄	I	Control	x̄
	dry wt/pot, g					
1	1 64	1 46	1 55	3 82	3 72	3 77
2	2 84	3 88	3 36	5 54	6 28	5 91
3	4 26	5 16	4 71	7 54	7 46	7 50
4	3 84	3 66	3 75	7 00	6 20	6 60
5	2 82	3 30	3 06	5 20	5 56	5 38
6	3 54	3 48	3 51	6 14	6 06	6 10
7	2 46	2 84	2 65	5 42	5 30	5 36
8	1 60	0 64	1 12	4 38	2 66	3 52
x̄	2 88	3 05	2 96	5 63	5 40	5 52
L S D (0 05)						
Inoc (I)		NS			NS	
Geno (G)		0 66			0 35	
I × G		NS			NS	

Table 2. Mean N content of top growth, and crowns and roots of eight bermudagrass genotypes as influenced by inoculation (I) at low N fertility level greenhouse experiment

Genotype	N of total clippings			N of crowns and roots			Total N of tops			Total N crowns and roots			Total N of tops, crowns, and roots		
	I	Control	x̄	I	Control	x̄	I	Control	x̄	I	Control	x̄	I	Control	x̄
	%						mg								
1	2 07	1 93	2 00	0 53	0 55	0 54	45 0	44 3	44 7	9 2	8 6	8 9	54 2	52 9	53 6
2	1 66	1 78	1 72	0 57	0 53	0 55	44 8	42 3	43 6	15 8	20 7	18 3	60 6	62.9	61 7
3	1 78	1 59	1 69	0 63	0 64	0 64	57 5	35 3	46 4	27 0	33 5	30 2	84 5	68 8	76.7
4	1 79	1 69	1 74	0 56	0 50	0 53	58 0	42 8	50 4	22 0	18 6	20 4	80 2	61 4	70 8
5	1 74	1 85	1 79	0 55	0 60	0 58	51 0	34 0	42 0	15 8	20 5	18 1	56 9	63 6	60 2
6	1 83	1 74	1 79	0 51	0 57	0 54	47 2	45 4	46 3	18 5	20 4	19 4	65 6	65 7	65 7
7	1 79	1 60	1 69	0 59	0 56	0 57	54 7	39 3	47 0	14 6	16 3	15 5	69 3	55 6	62 4
8	1 57	1 58	1 58	0 65	0 63	0 64	44 0	32 0	38 0	11 5	4 0	7 7	55 4	35 9	45 6
x̄	1 78	1 72	1 75	0 57	0 57	0 57	49 0	40 5	44 8	16 8	17 8	17 3	65 8	58 4	62 1
L S D (0 05)															
Inoc (I)		NS			NS			5 8			NS			6 3	
Geno (G)		0 28			NS			NS			7 9			3 7	
I × G		NS			NS			NS			NS			NS	

for selecting bermudagrass plants more responsive to N-fixing bacteria. An evaluation of additional bermudagrass genotypes and additional bacterial strains would be helpful in determining if a genotype × inoculation interaction exists. Research toward explaining the mechanism(s) giving rise to increased top growth by inoculating bermudagrass is needed.

REFERENCES

1. 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.
2. Brown, M. E. 1976. Role of *Azotobacter paspali* in association with *Paspalum notatum*. J. Appl. Bacteriol. 40:341-348.
3. Day, J. M., M. D. P. Neves, and J. Dobereiner. 1975. Nitrogenase activity on the roots of tropical forage grasses. Soil Biol. Biochem. 7:107-112.
4. Dobereiner, 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.), Proc. 1 Int. Symp. Nitrogen Fixation, Washington State Univ., Pullman.
5. ———, ———, and J. F. W. Von Bulow. 1975. Associations of nitrogen-fixing bacteria with roots of forage grass and grain species. II. *In* V. Johnson (ed.) Proc. Int. Winter Wheat Conf., Zagreb, Yugoslavia (June).
6. Dommergues, Y., J. Balandreau, G. Rinaudo, and P. Weinhard. 1973. Non-symbiotic nitrogen fixation in the rhizospheres of rice, maize and different tropical grasses. Soil Biol. Biochem. 5:83-89.
7. 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.
8. Krieg, N. R. 1977. Taxonomic studies of *Spirillum lipoferum*. *In* Alexander Hollaender (ed.) Genetic engineering for nitrogen fixation. Plenum Press, N. Y.
9. Schank, S. C., J. M. Day, and E. Delgado de Lucas. 1977. Nitrogenase activity, nitrogen content, In vitro digestibility and yield of 30 tropical forage grasses in Brazil. Trop. Agric. 54:119-125.
10. ———, R. L. Smith, J. H. Bouton, and K. H. Quesenberry. 1977. Yield increases in tropical grain and forage grasses after inoculation with *Spirillum lipoferum*. p. 307-311. *In* A. Ayanaba and P. J. Dart (eds.) Biological nitrogen fixation in farming systems of the humid tropics. John Wiley and Sons, Chichester, England.
11. Smith, R. L., S. C. Schank, J. H. Bouton, and K. H. Quesenberry, M. E. Tyler, J. R. Milam, M. H. Gaskins, and R. C. Littell. 1976. Nitrogen fixation in grasses inoculated with *Spirillum lipoferum*. Science 193:1003-1005.
12. ———, ———, ———, and ——— 1976. Yield increases of tropical grasses after inoculation with *Spirillum lipoferum*. Proc. Int. Symp. Environmental Role of N_2 fixing Blue-green Algae and Symbiotic Bacteria. Uppsala, Sweden.
13. ———, ———, and K. H. Quesenberry. 1977. Biological nitrogen fixation. Opportunities for greater utilization of solar energy in food production. Agric. and Food Chem. Symp. on Renewable Resources. New Orleans.
14. Von Bulow, J. F. W., and J. Dobereiner. 1975. Potential for nitrogen fixation in maize genotypes in Brazil. Proc. Natl. Acad. Sci. USA 72:2389-2392.
15. Yoshida, T., and R. R. Ancajas. 1973. Nitrogen fixation in upland and flooded rice fields. Soil Sci. Soc. Am. Proc. 37:42-46.