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Plant Yield and Nitrogen Content of a Digitgrass in Response to *Azospirillum* Inoculation†

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Two Australian soils, a vertisol (pH 6.8, 0.299% N) and a sandy yellow podzol (pH 6.2, 0.042% N), were used with digitgrass; *Digitaria* sp. X46-2 (PI 421785), in a growth room experiment. Comparisons were made between plants inoculated with live and autoclaved bacterial suspensions of Australian and Brazilian isolates of *Azospirillum brasilense*. Seedlings were inoculated on days 10 and 35. Acetylene-reducing activity was measured five times during the experiment. Dry matter yields of the digitgrass on the podzol (low N) inoculated with live bacteria were 23% higher than those of the controls. On the vertisol (high N), yield increases from inoculation with live bacteria were 8.5%. The higher-yielding plants had significantly lower percent nitrogen, but when total nitrogen of the tops was calculated, the inoculated plants had a higher total N than did the controls ($P = 0.04$). Acetylene-reducing activity was variable in the experiment, ranging from 0.5 to 11.9 μmol of C_2H_4 core^{-1} day^{-1}. Live bacterial treatment induced a proliferation of roots, possible earlier maturity, higher percent dry matter, and a higher total N in the tops.

The beneficial effect of the symbiotic N_2-fixing relationship between rhizobia and legumes is well known. Recent efforts to improve yields of tropical grasses by inoculating with N_2-fixing bacteria have been partially successful. Studies have shown that guineagrass (*Panicum maximum* Jacq.) and hybrid pearl millet (*Pennisetum americanum* (L.) K. Schum.) frequently produce higher dry matter yields in inoculated field plots (11–13). The increase in dry matter production has in some tests been 25%. However, since inoculation has not produced comparable increases in ethylene production as measured by acetylene reduction, there is some question about the mechanism responsible for the increased plant growth.

Bouton et al. (2) reported average yield increases on field-inoculated Gahi-3 and Bil 3B pearl millet of 19.2 and 14.0%, respectively. Baltensperger et al. (1) reported a 17% yield increase with eight genotypes of turf Bermuda grass (*Cynodon dactylon* L. Pers.) in a greenhouse study at low fertility. Benzion and Quesenberry (G. Benzion and K. H. Quesenberry, Agron. Abstr. 70:70, 1978) concluded that dry weight increases in *Paspalum* showed a definite bacterium genotype-plant genotype interaction. Umali-Garcia et al. (18) reported that more lateral roots and root hairs developed on inoculated roots than on sterile roots. Recent research in India (N. S. Subba Rao, K. V. B. R. Tilak, M. Lakshmi Kumari, and C. S. Singh, Proc. Steenbock-Kettering Int. Symp. Nitrogen Fixation, abstr. no. A40, 1978) and in the Bahamas (14) found significant yield increases after inoculation with *Azospirillum brasilense* on field crops and grasses, respectively. In Canada, inoculation significantly increased the plant yield of corn and also total N yield (8). The Canadian experiments used a controlled environment and ^{15}N analyses which suggested that significant amounts of atmospheric N_2 had been incorporated into the plants.

Surveys to detect grass nitrogen fixation in Texas (S. F. Wright, R. W. Weaver, M. W. Varanka, E. C. Holt, and O. E. Smith, Agron. Abstr. 71:166, 1979), Oregon (5, 7), Wisconsin (16), and Australia (K. L. Weier, Aust. Soc. Soil Sci. N. S. W. Branch, Soils Conf., p. 26–31, 1978) have shown variable rates of acetylene reduction which can be extrapolated up to 350 g of N ha^{-1} day^{-1}, much lower than rates reported by Von Bülow and Döbereiner (19). *Azospirillum* has been isolated from diverse geographic regions in Florida, Ecuador, Venezuela, and South Africa (17).

Gilmour et al. (C. M. Gilmour, S. Thome, S. M. Beck, and J. T. Gilmour, Agron. Abstr. 71: 157–158, 1979) showed that wheat seed inoculation with a mixture of five N_2-fixing bacteria

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increased acetylene-reducing activity of roots and also increased grain yield. These studies were done in growth chambers and in field experiments over 2 years.

A detailed analysis of root growth, acetylene-reducing activity, and dry matter yield under simulated tropical conditions could provide new information on grass response to *Azospirillum* inoculation. This study was therefore carried out in Australia in 1978. The general approach was to use the growth room to control environmental parameters which may confound field inoculation studies. The objectives were: (i) to compare yields of digitgrass on two Australian soil types after inoculation with isolates of *A. brasilense* from a different location in Australia and from Brazil; and (ii) to determine acetylene-reducing activity and N content of roots, stubble, and tops.

MATERIALS AND METHODS

The experiment involved one grass genotype, two soil types, and two inoculation treatments with five replications in a completely randomized arrangement.

Intact soil cores with no growing plants were obtained from Narayen (25°41′ S, 150°52′ E) vertisol (pH 6.8, 0.299% N) and from Beerwah (26°52′ S, 152°58′ E) sandy yellow podzol (pH 6.2, 0.042% N) (6). Cores were 12 cm in diameter by 16.5 cm in length and were taken with 14-gauge steel tubes which had been driven into the ground, removed, and sealed in plastic bags for transportation back to the laboratory. The top 3 cm of soil from the 10 cores of each soil type was removed, composited, screened twice through a 10-mm mesh screen, and replaced. The remainder of the core was kept intact. Soil moisture in all cores was brought to field capacity by soaking in water for 24 h, followed by draining for 24 h. The core weight, after draining, was recorded, and all cores were watered daily to the weight recorded.

Hybrid digitgrass seeds (*Digitaria* sp.) from X46-2 were planted in a concentric ring in the core, and three glass microscope slides were inserted into the center of each core, including unplanted cores. All cores were given an application of nitrogen-free nutrient solution (Weier, Aust. Soc. Soil Sci. N. S. W. Branch, Soils Conf., p. 26–31, 1978) at the beginning and again after 25 days of plant growth. Weak plants were removed from each core, and the 12 most vigorous plants were allowed to develop. Control cores with no growing plants were also set up. Inoculation was done 10 days after emergence, using a mixed culture (by volume) containing live *A. brasilense* strain 7 from Brazil and live *A. brasilense* isolated from an Australian *Bothriochloa insculpta* plant from Rockhampton. Cultures used for the inoculation step were grown in the peptone-succinate broth of Hylemon et al. (4) at 32°C for 24 h. The control cores received the same quantity and quality of each strain, but the control had been autoclaved just before inoculation. Bacterial dry weights of the inoculum were 28.8 and 21.3 mg/50 ml from the Brazilian and the Australian isolates, respectively. The medium supernatants contained 3.2 g of C per liter. Plants were reinoculated as above 25 days after the original inoculation to be sure inoculation had taken effect

Cores were placed in a constant-environment chamber with 33 to 30°C and 45 to 70% relative humidity during a 14-h photoperiod. Photosynthetic photon flux density for the plants at the center of the cores was 520 μEinsteins m^{-2} s^{-1}. To suppress development of algae on the soil surface, 50 g of black polyurethane beads were added to the soil surface of all cores.

One day after the first inoculation, when the plants were 11 days old, the first acetylene reduction assay was run. The intact cores were incubated for 24 h under 10% acetylene in sealed clear plastic chambers. Ten percent of the air was replaced by C_2H_2, and C_2H_4 production was measured by gas chromatography on a 0.5-ml sample removed from each chamber (Weier, Aust. Soc. Soil Sci. N. S. W. Branch, Soils Conf., p. 26–31, 1978). C_2H_4 production on time course studies did not deviate from a linear trend (21).

All 20 cores were rerandomized each week during the experiment. Plant heights, the number of tillers, and growth measurements were recorded during the experiment. Contact slides for fluorescent-antibody (10) studies were removed at 4, 6, and 8 weeks, immediately after the corresponding gas chromatographic analyses were completed on these dates.

The final harvest was taken 8 weeks after planting Green weights of tops were recorded immediately after harvest. Stubble and roots were allowed to soak overnight in water. Roots were screened out on the next day, dried, and weighed. Tops, stubble, and roots were dried at 60°C in forced-air ovens for 48 to 72 h. The samples were ground through a 1-mm screen, and total N was determined by the Kjeldahl method after pretreatment with salicylic acid and sodium thiosulfate (3).

RESULTS AND DISCUSSION

Analysis of variance procedures on combined data from sandy and clay soils showed significant ($P = 0.02$) green weight and dry matter yield increases from inoculated plants (Fig. 1). Inoculation increased the dry matter forage yields 23% in the sandy soil and 8.5% in the clay soil. Dry matter yield was multiplied by the percent nitrogen to give the total plant nitrogen in the tops. Again, combined data for inoculated plants were significantly higher in nitrogen ($P = 0.04$) than were noninoculated controls. The inoculated plants had a 20% greater root weight on both sandy and clay soils. We also observed, when washing the roots, that many more fine, filamentous roots were present in the inoculated plants on both soil types. Similar findings were also independently reported by Umali-Garcia et al. (18).

The Waller-Duncan K ratio test (20) was also performed on all variables and treatments. Data are summarized in Table 1. Plants were divided

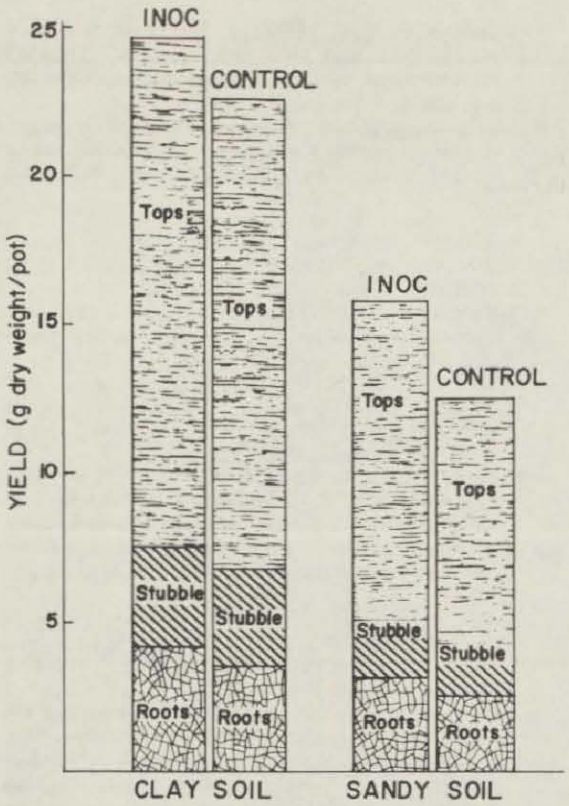

FIG. 1. *Yield increases of a hybrid digitgrass after inoculation with Azospirillum on two Australian soils.*

into tops, stubble, and roots at harvest time, and each component of the plant was analyzed for weight and percent N. The higher-yielding treatments had lower percent nitrogen in the tops and in the stubble. Root N content was not greatly affected by inoculation treatment. A similar observation was made by Baltensperger et al. (1) in Bermuda grass roots.

Percent N is often an indicator of maturity differences in forages. Since percent N of tops and stubble (Table 1) was inversely related to dry weight of the tops and stubble, other possible indications of maturity differences in the forage were examined. A trend in percent dry matter was inversely related to percent N in tops and stubble. In vitro digestibility (9) also indirectly gives a possible index of physiological maturity. Based on these observations, general plant appearance, and the significantly greater root mass (Table 1) of inoculated plants, we speculate that the inoculated plants not only grew faster and larger, but matured earlier as well. This resulted in a lower percent nitrogen content of the inoculated plants, but a higher dry matter yield.

Acetylene reduction data are also presented in Table 1 for five different assay dates during

TABLE 1. *Mean yield, in vitro digestibility, nitrogen, and acetylene reduction data from a digitgrass grown in the growth room on two Australian soils after inoculation with a mixture of N_2-fixing bacteria*[a]

Soil	Tops						Stubble			Roots			Tops, stubble, and roots		Acetylene reduction on day:				
	Green wt (g)	Dry wt (g)	DM (%)	IVD (%)	% N	Amt of N (g)	Dry wt (g)	% N	Amt of N (g)	Dry wt (g)	% N	Amt of N (g)	Dry wt (g)	Total N (g)	10/5	10/20	10/26	11/2	11/16
Clay																			
Inoculated	95.7a	17.3a	18.0a	57.0b	1.36c	0.232a	3.2a	1.05c	0.034b	4.3a	1.22ab	0.052a	24.8a	0.319a	3.7a	4.5a	3.7ab	2.7a	3.2b
Control	90.0a	15.9a	17.8a	57.9b	1.40c	0.221a	3.2a	1.19c	0.038ab	3.6b	1.38a	0.050a	22.7a	0.309a	5.9a	3.2a	3.4b	1.9a	3.4b
Sand																			
Inoculated	66.2b	10.6b	16.0ab	71.0a	1.85b	0.196b	1.9b	1.96b	0.038ab	3.1bc	1.16b	0.036b	15.6b	0.270b	7.1a	5.6a	5.4ab	3.3a	3.8b
Control	54.8c	8.4c	15.2b	69.0a	2.26a	0.186b	1.7b	2.61a	0.043a	2.6b	1.22ab	0.032b	12.7c	0.261b	4.9a	7.3a	5.8a	4.1a	6.0a

[a] Each value is the mean of five observations. Means followed by the same letter are not significantly different at the 0.05 significance level, using the Waller-Duncan *K* ratio test. Acetylene-reduction data is expressed as micromoles of C_2H_4 per core per day. DM, Dry matter; IVD, in vitro-digestibility.

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the experiment. All acetylene reduction data were somewhat lower than data for other forage grasses tested from field cores under similar conditions in Australia (21; Weier, Aust. Soc. Soil Sci. N. S. W. Branch, Soils Conf., p. 26–31, 1978). Results of our acetylene-reduction assays are not extrapolated to grams of N ha^{-1} day^{-1}. Our acetylene-reduction results indicate that only small amounts of ethylene were evolved after 24-h incubations in 10% acetylene. These mean rates ranged from 1.9 to 7.3 μmol of C_2H_4 $core^{-1}$ day^{-1}, commensurate with other recent results (5, 8; Wright et al., Agron. Abstr., **71:**166, 1979). Since all cores contained native or natural N_2 fixers as well as those added in the inoculum, a background of acetylene reduction in all cores was observed.

Use of the fluorescent-antibody technique (10) and reisolations at the end of the experiment established that *Azospirillum* was present and therefore could have contributed to increased yields. However, acetylene reduction assays performed on five occasions during the experiment showed no evidence that inoculation enhanced N_2 fixation. The yield increases, possible earlier maturity, and a higher total N in the tops may have been stimulated by bacterium-produced phytohormones which Tien et al. (15) suggested improve plant growth by their direct effects on metabolic processes. We conclude that *A. brasilense* inoculation significantly stimulated plant growth of digitgrass, although N_2 fixation was not enhanced appreciably.

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