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Acetylene Reduction by Soil Cores of Maize and Sorghum in Brazil

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Nitrogenase activity was measured by the C_2H_2 reduction method in large soil cores (29 cm in diameter by 20 cm in depth) of maize (*Zea mays*) and sorghum (*Sorghum vulgare*). The activity was compared to that obtained by a method in which the roots were removed from the soil and assayed for nitrogenase activity after an overnight preincubation in 1% O_2. In a total of six experiments and 28 soil cores, the nitrogenase activity of the cores was an average of 14 times less than the activity of roots removed from the same cores and preincubated. Nitrogenase activity in the cores was very low and extrapolated to an average nitrogen fixation rate of 2.8 g of N/hectare per day. It was shown that inadequate gas exchange was not a reason for the lower activity in the soil cores, and the core method gave satisfactory results for nitrogenase activity of soybeans (*Glycine max*) and *Paspalum notatum*.

High rates of nitrogen fixation have been reported for maize growing in Brazil, using a method in which the roots were excised and preincubated overnight before measurement (8). It was suggested that the nitrogen fixation was due to *Spirillum* sp. associated with the maize roots. Researchers in the United States have inoculated maize with two strains of *Spirillum* sp. obtained from Brazil, and substantial nitrogenase activity was observed in response to the inoculation (3). But the activity was observed only in preincubated, excised roots and not in plants assayed in situ in large pots. This paper compares the nitrogenase activity of preincubated, excised roots and large soil cores of maize growing in Brazil.

MATERIALS AND METHODS

Nitrogenase activity of soil cores was measured by C_2H_2 reduction, using an adaptation of the method of Balandreau and Dommergues (2). Our method differed in that the plant top projected outside of the measuring cylinder (Fig. 1) and the cores were sometimes removed from the ground and assayed outdoors, with the bottoms sealed to a piece of sheet metal (Fig. 1). Also, the cores were driven 20 cm into the ground instead of 5 to 7 cm.

Cores were placed in the ground 2 to 4 days before measurements were begun. The soil was tamped down at the junction of the soil and cylinder and was watered with distilled water, both inside and outside the cores. C_2H_2 was generated from 8 to 9 g of CaC_2 placed in a dish inside the cylinders. The cores were shaded from the sun by covering with newspapers, to prevent overheating. The gas volume of the cores was calculated from the dimensions of the core device above the soil and from the assumption that one-fourth of the volume of soil within the cores was gas space. Measurement of C_2H_2 and C_2H_4 was by gas chromatography (8).

In experiment 5 (Table 1), cores were taken by digging out clumps of soil containing small sorghum plants. These were trimmed to approximately 22 cm in diameter and 20 cm in height. Newspapers were wrapped around the soil to shade it from the sun, and the cores were placed in Saran plastic bags. The cores were kept outdoors before and during assay, and the soil moisture was maintained with distilled water. To assay the cores, the plastic bags were sealed around the stems with aid of caulking compound and string. Commercial-grade C_2H_2 was injected into the bags, resulting in 0.1 to 0.2 atm of C_2H_2. There was very little leakage of C_2H_2 from the Saran bags in a 24-h period (4).

After the soil core assays were completed, the roots were removed from the soil and dipped immediately into distilled water, and all the roots from a given plant were placed in a bottle of 3.7-liter volume. In experiment 1 (Table 1), the roots were not from the plants assayed in the cores but from six similar plants. The bottles were filled with N_2 by water displacement, and 5% air was added (1% O_2). In the morning, after about 16 h of preincubation, 10% C_2H_2 and an additional 1% O_2 were added to the bottles. The preincubation and assay were at room temperature (27 to 31°C), and the length of the C_2H_2 assay was 4 h.

Sorghum vulgare, cultivar Redlan, and *Zea mays*, cultivar Piranao, were grown in plots without nitrogen fertilizer. Maize was sampled at the silking stage (experiments 2 and 7) or at the grain-filling stage (experiment 1). Sorghum was sampled at the

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flowering stage (experiment 6), or in a condition of flowering tillers and grain-filling main stems (experiments 3, 4, and 5).

RESULTS

In the experiment in Fig. 2, five soil cores of sorghum were measured both in situ (experiment 3, Table 1) and after removal from the soil (experiment 4, Table 1). The C_2H_4 present initially was a by-product of the generation of C_2H_2 from CaC_2. The results show that about 6 h was required for equilibration of C_2H_2 and C_2H_4 between the top and bottom of the soil cores. During the in situ measurements, gases could leak from the bottom of the cores, and this was probably the reason for the continued decrease in C_2H_2 in these cores. After the initial 6 h required for equilibration, the concentration of C_2H_4 in the cores increased slowly and approximately linearly.

Nitrogenase activities in soil cores and roots removed from the cores are compared in Table 1. The activity for the cores was measured for the period of about 6 to 27 h after the addition of C_2H_2. Experiments 1, 2, and 3 were done in situ, whereas the remainder was done with cores removed from the soil and their bottoms sealed to a piece of sheet metal. The rates of fixation for the cores measured in situ were calculated in two different ways. The smaller number in Table 1 is the value assuming no leakage of C_2H_4 from the cores. The second number is corrected for leakage of C_2H_4 from the cores, assuming that the rate of leakage is the same as for C_2H_2, using the calculation method of Balandreau and Dommergues (2). The plants showed no signs of wilting before or during the assays, although the lowermost green leaf often turned yellow or brown in the time between driving the cores into the ground

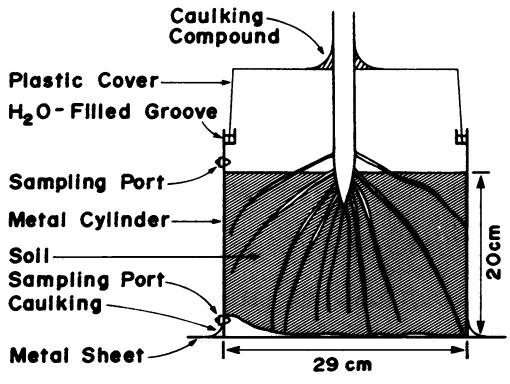

FIG. 1. *Soil core apparatus for measuring nitrogenase activity.*

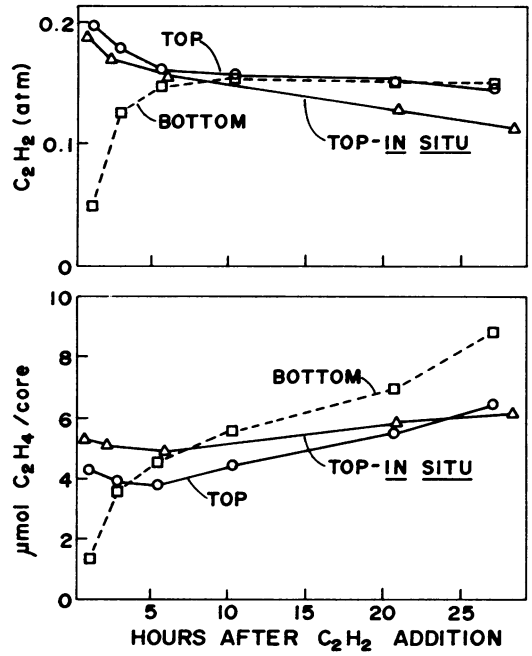

FIG. 2. *Time course for C_2H_2 and C_2H_4 in soil cores of sorghum, measured in situ and removed from the soil. For the latter, gas samples were taken at both the top and bottom of the cores.*

TABLE 1. *Comparison of nitrogenase activity in cores and isolated roots of maize and sorghum*

Expt no.	Date	Species	Location	No. of cores	Nitrogenase activity: μmol of C_2H_4/core per h		nmol of C_2H_4/h per g of dry roots
					Cores	Roots	
1	19 Feb.	Maize	Campo Novissimo	6	0.00–0.15	3.2	410
2	2 Mar.	Maize	Fitotecnia	6	0.60–0.79	1.7	103
3	8 Apr.	Sorghum	Fitotecnia	5	0.06–0.14		
4	13 Apr.	Sorghum	Fitotecnia	5	0.13	2.2	358
5	13 Apr.	Sorghum	Fitotecnia	3	0.29	3.9	1,840
6	20 Apr.	Sorghum	Campo Novissimo	3	0.044	0.64	147
7	24 Apr.	Maize	Campo Novissimo	5	0.077	1.1	91

and performing the nitrogenase assay. There was sunshine for at least half of the daylight period of each experiment, and the maximum soil temperature inside the cores at 5-cm depth varied from 30 to 35°C.

The results in Table 1 show that except for experiment 2, a much lower nitrogenase activity was observed for the intact cores than for the roots removed from them. The result of 2 March may have differed because the soil was nearly waterlogged. The rate of nitrogen fixation in the cores was very low. Assuming a ratio of 3 C_2H_2 to 1 N_2 and 50,000 cores (or plants) per hectare (ha), the average fixation rate was 2.8 g/ha per day. Even the fixation rate extrapolated from the activities of the preincubated roots was a maximum of only 43 g/ha per day (experiment 5). On the basis of activity per gram (dry weight) of roots, the activity of sorghum in experiment 5 approached some of the higher nitrogenase activities found previously for maize in Brazil (8), but since the root weight per plant was low, the activity per hectare was similar to that obtained in our other experiments. The reason for the high nitrogenase activity and low root weight in experiment 5 compared to those in experiment 4 is unknown, but the plants were from opposite ends of the experimental plot and the difference in nitrogenase activity was observed in other experiments. The varieties of maize previously reported to have very high nitrogenase activities (8) were not measured because no plants were available.

Since there was disagreement between the results obtained with the soil core and root assay methods, we checked our core method with other nitrogen-fixing systems. Figure 3 shows that moderate rates of C_2H_2 reduction were measured in situ for *Paspalum notatum* (six cores), with very little lag before linear rates were observed. The mean rate extrapolated to nitrogen fixation of 43 g of N/ha per day. Very similar results were obtained with 10-cm soil cores, using the method of Day et al. (5). We also obtained a linear time course for C_2H_2 reduction by soybeans, with a rate that extrapolated to nitrogen fixation of 1.7 kg of N/ha per day.

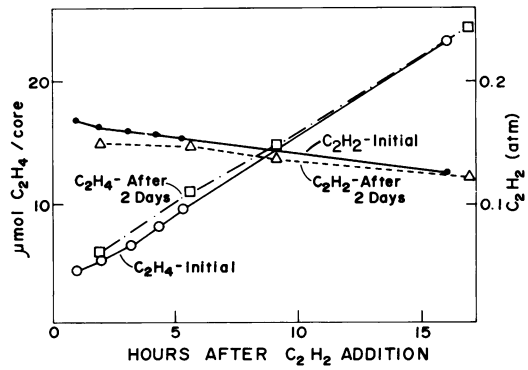

FIG. 3. *In situ measurements of C_2H_2 reduction by P. notatum. Measurements were made both immediately after the cores were driven in and 2 days later. The plastic cover was in place only during the 15 h of measurement.*

DISCUSSION

In Table 1, the nitrogenase activities of preincubated, excised roots were up to 20 times greater than the activities of the corresponding soil cores. Also there were individual soil cores that had no measurable nitrogenase activity, but had average activity in the excised root assays. Furthermore, the activities observed in the soil cores may not have been entirely associated with the roots. Part of the activity may have been due to C_2H_2 reduction by blue-green algae, or bacteria associated with decaying organic matter, or to endogenous C_2H_4 evolution by the roots or soil microorganisms. Thus, there was a large or possibly very large discrepancy in nitrogenase activity between soil cores and preincubated, excised roots of maize and sorghum under our experimental conditions. Similar results have been found by others for maize inoculated with *Spirillum* sp. (3; R. H. Burris, personal communication).

The reasons for the discrepancy between the two assay methods are not known. It has been found previously that isolated roots give lower activities than do soil cores for *P. notatum* (6) and *Digitaria decumbens* (1). We know of no reason why our core method might underestimate the nitrogenase activity. The method appears to be satisfactory for soybeans and *P. notatum*. The speed of gas diffusion is sufficient for the time period of the measurements, and the plants appear to remain healthy during the assay. After the initial period of about 6 h required for gas equilibration, the time course of C_2H_2 reduction was approximately linear, which is evidence that the system was not substantially changing during the course of the assay.

A possible explanation of the greater nitrogenase activity observed for the excised roots is that nitrogen-fixing bacteria, such as *Spirillum lipoferum*, may multiply during the preincubation. Immediately after collection of excised roots, the nitrogenase activity of the roots was negligible. After preincubation the activity increased to the high levels reported, but during such a preincubation Barber et al. (3) found

a 30-fold increase in nitrogen-fixing bacteria. Even larger increases in bacterial numbers during preincubation have been found by Okon et al. (7).

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