

NITROGEN FIXATION ASSOCIATED WITH SUGARCANE

By B. S. PURCHASE

South African Sugar Association Experiment Station, Mount Edgecombe *

Abstract

Nitrogen fixation associated with sugarcane was monitored using acetylene reduction tests and bacteriological techniques. Soil cores and trash from cane fields showed acetylene reducing activity. This activity was usually low in association with sandy soils. Where high activity was detected in soil cores it correlated with the wet weight of roots in each core.

Nitrogen-fixing bacteria resembling *Azospirillum* species were cultured from surface-sterilized roots of a number of cane varieties, the variety N7 being particularly well infected. Tenuous calculations based on acetylene reduction results and root weights suggest that sugarcane growing on Rydalvale series soil at the Experiment Station might derive about 25 kg of nitrogen per hectare per annum from nitrogen fixation.

Introduction

Nitrogen-fixing bacteria in the soils of the South African sugar industry were last studied in 1962 by Anderson¹. Since then new methods have revealed that some nitrogen-fixing bacteria form intimate associations with the roots of tropical grasses and that these bacteria have a greater potential for nitrogen fixation than the free-living bacteria studied by Anderson. Ruschel and Vose² have recently concluded that as much as 30% of the nitrogen in sugarcane growing in Brazil may be derived from bacteria. Such information, together with the recent dramatic increases in the price of fertilizer nitrogen, stimulated the present study. The aim was to determine whether appreciable nitrogen fixation takes place in association with cane grown in South Africa and to define optimum conditions for this fixation.

Methods

Acetylene reduction assays

Soil cores were collected in sharpened steel cylinders (150 x 70 mm) which fitted into 1-litre "Consol" jars. The lids of the jars were drilled and fitted with a rubber seal through which a hypodermic needle could be inserted for gas transfers. Acetylene was introduced by partially evacuating (75 mm mercury) the test jars and then allowing acetylene to enter the jars and restore atmospheric pressure. This gave an atmosphere containing approximately 15% acetylene.

Bacterial cultures were tested in bottles to which rubber seals could be fitted. A syringe was used to inject acetylene.

During exposure to acetylene the test systems were incubated at 32°C. Ethylene was analysed by gas chromatography using a 1 300 x 2 mm stainless steel column packed with "Poropak N" (80-100 mesh) and operated at 85°C with nitrogen carrier gas flowing at 30 ml per minute.

Bacteriology

The semi-solid medium of Dobereiner *et al*³, supplemented with trace elements, was used for detecting *Azospirillum* species. Freshly collected roots were washed in a jet of

water, then a 5 mm length was cut off and surface sterilized by immersion in 1% sodium hypochlorite for five minutes. After three rinses with sterile water the root pieces were transferred to 3 ml of medium in 7ml bottles. The bottles were incubated at 32°C and checked daily for the formation of white subsurface pellicles and blue colour in the medium.

Cultures showing these features were checked for acetylene reduction activity and those producing more than 5 η moles ethylene per hour were considered positive.

Results and Discussion

Acetylene reduction by soil cores

Preliminary investigations showed that acetylene reduction was initially relatively slow but it increased appreciably after 16 h of exposure to acetylene. Subsequent acetylene reduction rates were therefore measured over a 24 h period beginning 18 h after application of the acetylene.

Initially the soil core technique gave such variable results that it could not be used for the intended comparison of nitrogen fixation associated with different varieties and different soil types. In the absence of an alternative technique, effort had to be concentrated on determining the cause of variability. The relationship between acetylene reduction activity and root weight per core was investigated. In samples from a young ratoon crop on Rydalvale series soil there was a significant linear correlation between the fresh weight of roots per core and acetylene reduction (Figure 1). The dry weights of roots, however, did not correlate with acetylene reduction.

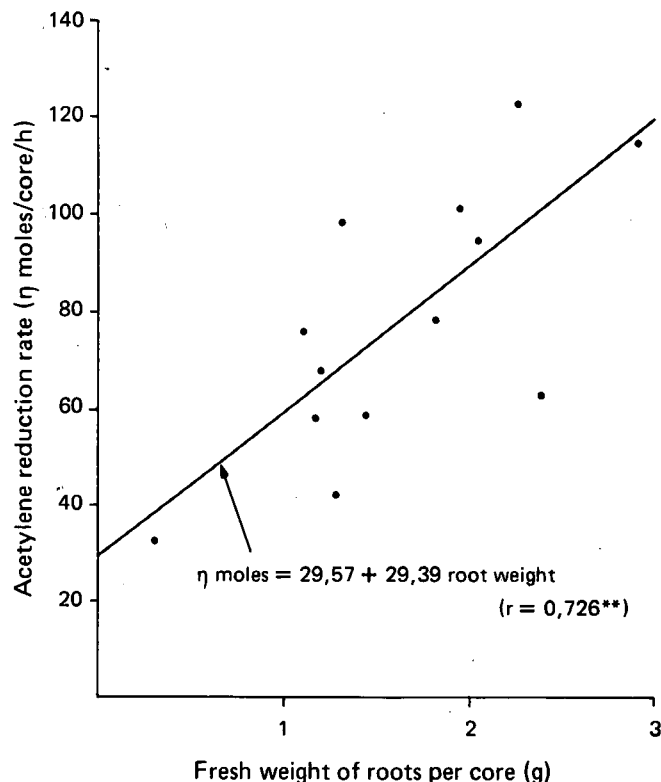


FIGURE 1 Relationship between acetylene reduction rate and root weight per core (young ratoon on wet Rydalvale series soil).

* Present address — Sugar Milling Research Institute, Durban.

The correlation between fresh weight of roots and acetylene reduction is significant because :

- (i) it indicates that nitrogen fixation in sugarcane is fairly intimately associated with the roots,
- (ii) an indication of the nitrogen fixation which is independent of roots is obtained by extrapolation to the point where root mass is zero,
- (iii) the fact that the correlation exists with wet mass of roots but not dry mass indicates that the bacteria are associated with young roots and are not merely using products of decomposition of old dry roots,
- (iv) it helps to explain the variability encountered with the soil core technique and suggests that this technique can be used for comparative studies if sufficient samples are taken to establish a regression equation relating acetylene reduction to root mass.

It can be misleading to calculate nitrogen fixation in terms of kg/ha per annum from acetylene reduction measurements obtained over a short period. Nevertheless, this calculation helps to give perspective to the acetylene reduction measurements. Assuming that there are 30 t (fresh weight) of roots per hectare (Wood⁴ measured up to 50 t/ha) and that each gram of root is capable of reducing 29,4 η moles of acetylene per hour (from regression equation) for 20 h per day and five months per year, and that the acetylene reduction: nitrogen fixation ratio is 3 : 1, then approximately 24 kg N/ha per annum is fixed. This amount is fixed in close association with the roots and an amount equivalent to 29,4 η moles acetylene per core per hour is fixed in the soil. On a surface area basis there are 2,6 x 10⁶ cores/ha so the soil-associated fixation is only about 2,6 kg/ha per annum in the top 12 cm of soil; assuming fixation for 24 h/day for five months per annum.

The most tenuous assumption in this calculation is that fixation is associated with all roots irrespective of their depth. There is no evidence to support or contradict this assumption. If fixation is confined to the layer of soil sampled (0-12 cm) then, on a surface area basis, the results indicate that only about 5 kg N/ha per annum is fixed.

When two different cane varieties were compared (Figure 2) most cores had an activity of about 50 η moles/h with no correlation between activity and root mass. Among the six cores with activity exceeding 70 η moles/h there was a significant correlation between activity and root mass. A possible explanation for this result is that the plants involved were from a mature ratoon containing numerous senescing stalks and some fresh shoots. Cores taken alongside senescing stalks are likely to have contained inactive roots. Comparison of the few active cores from the two varieties showed that roots from NCo 376 produced 22,3 η moles more ethylene/g.h than roots from NCo 334, but this difference was not statistically significant.

The same two varieties were again compared when growing as 13-month old plant crops on sandy soil (Clansthal series). The acetylene reduction activity was very low for both varieties and it was surmised that this could have been due to unsuitable soil. The influence of soil type was investigated by sampling from different soil types lying adjacent to one another at the root laboratory on the Experiment Station. The results (Figure 3) indicate that nitrogen fixation in sandy soils is likely to be much less than in heavier soils.

Bacteriological studies

The results of a survey to check for the presence of *Azospirillum* bacteria in roots in different sugarcane varieties are presented in Table 1.

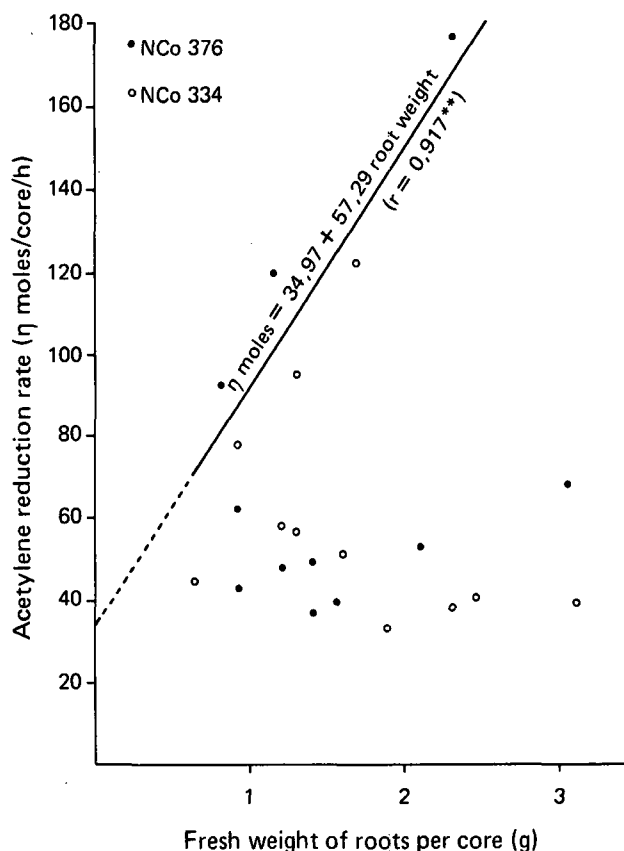


FIGURE 2 Relationship between acetylene reduction rate and root weight per core (mature ratoons on wet Rydalvale series soil).

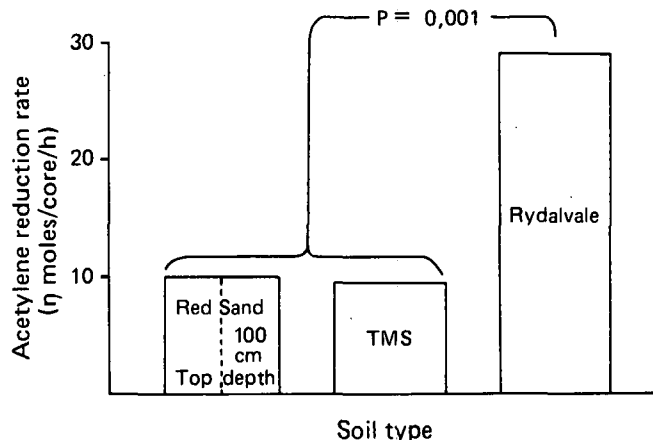


FIGURE 3 Rates of acetylene reduction by soil cores from different soil types at root laboratory (young ratoon crops).

TABLE 1
Influence of cane variety and locality on the percentage of root pieces infected with *Azospirillum* species

Variety	Locality	Expt. Stn. (heavy soil)	CFS (sandy soil)	Pongola (heavy soil)
N7	.	83	83	67
N8	.	50	66	43
NCo376	.	50	17	63
NCo310	.	50	17	63
NCo334	.	50	0	50
NCo382	.	33	0	43
NCo293	.	0	33	43
N55/805	.	17	33	—
N52/219	.	—	—	25

They indicate that *Azospirillum* species are intimately associated with some of the commercial varieties. The extent

of infection of the different varieties varied and was always highest for N7.

Experiments with trash

Nitrogen fixation associated with decomposing trash was monitored by acetylene reduction tests on trash contained in Consol jars. Three separate experiments indicated that the soil type can influence nitrogen fixation in the overlying trash (Table 2).

The trash from experiment 2 was incubated further in open jars at 32°C and periodically tested for acetylene reduction activity (Figure 4). The continuing difference between the

TABLE 2

Acetylene reduction (η moles/g.h) by wet trash from different soils

Experiment No.	Soil series		Significance
	Clansthal	Rydalvale	
2	0,57	7,07	**
3	1,04	3,63	*

two trash types, despite identical environmental conditions and absence of soil, suggested that the one trash type might be nutritionally better suited for nitrogen-fixing bacteria. Trash from sandy soil was then supplemented with various nutrients and incubated. After nine days of incubation there was no clear effect of added nutrients but after 16 days the trash whose nutrient supplementation included P and K reduced significantly more acetylene per hour than the trash without P and K (Figure 5).

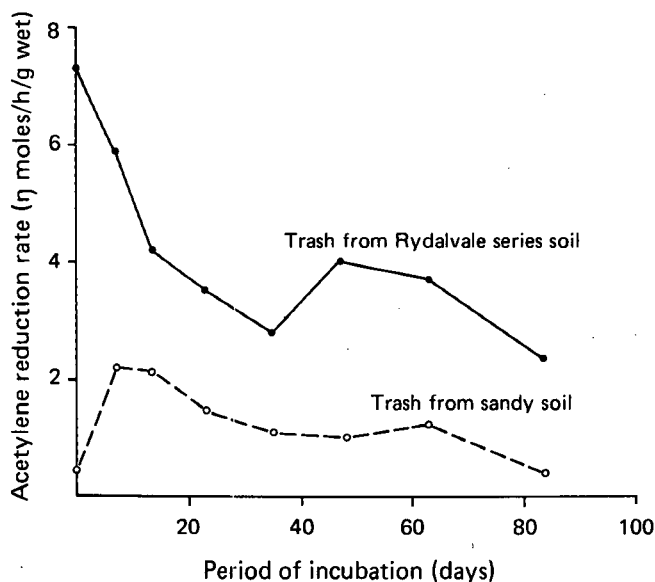


FIGURE 4 Acetylene reduction by trash during prolonged incubation (at every sampling date the difference in activity was statistically significant).

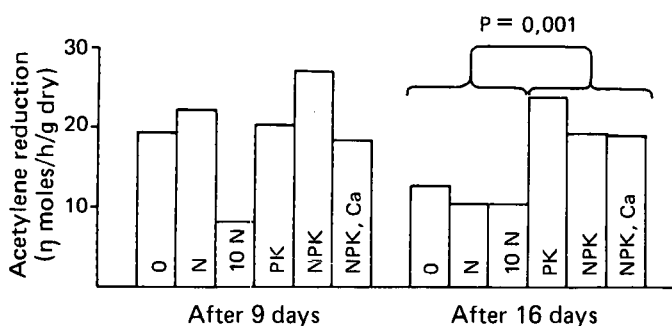


FIGURE 5 Acetylene reduction by trash supplemented with various nutrients.

Twelve trash samples were tested for presence of *Azospirillum* species and all gave positive results. *Azotobacter* and *Beijerinckia* species were also present.

General Discussion and Conclusions

Many findings reported here provide mere confirmation of findings in Brazil. Nitrogen fixation definitely takes place in association with sugarcane under some conditions in South Africa. The main activity is closely associated with the roots and there is some activity in trash. Preliminary results suggest that sandy soils are not conducive to nitrogen fixation and that P and/or K deficiency might sometimes limit fixation. The fact that many soil, root and trash samples have yielded organisms resembling *Azospirillum* species makes it

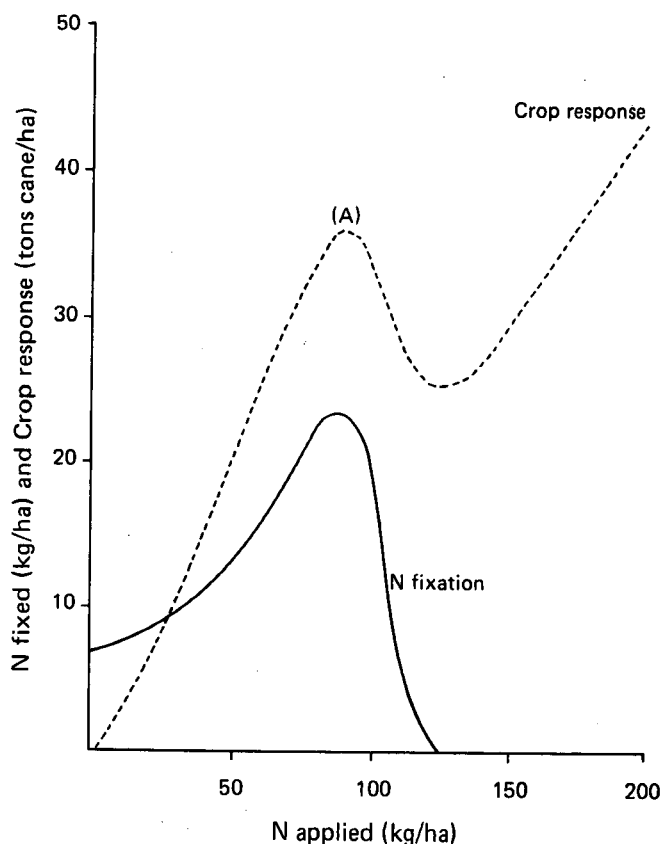


FIGURE 6 Hypothetical interactions between fertilizer N, nitrogen fixation and crop response.

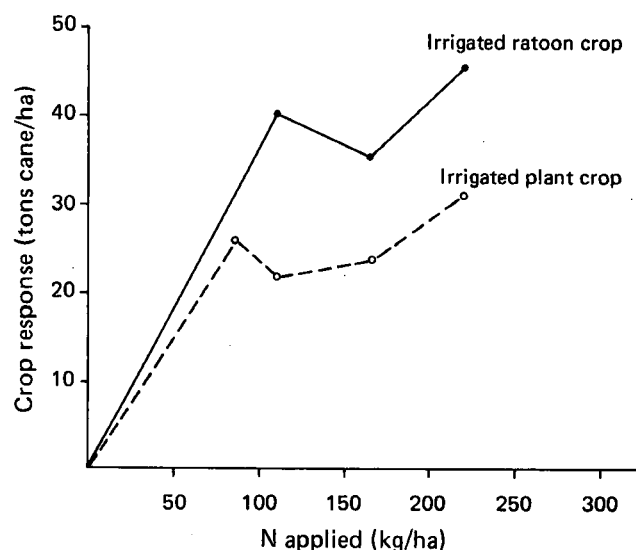


FIGURE 7 Crop responses to applied nitrogen (data from Wood, 1972).

difficult to accept the assertion of Ruschel and Vose² that this organism makes little contribution to nitrogen fixation in sugarcane.

The amount of nitrogen fixed is difficult to determine accurately. Estimates based on acetylene reduction results suggest that under suitable conditions it could be about 25 kg/ha per annum. Such fixation might explain why the curve relating response of irrigated cane to fertilizer nitrogen has an initial peak followed by a trough (Wood⁴). The peak might reflect the point at which nitrogen fixation is at its maximum (Figures 6 and 7).

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