Nitrogen fixation in *Nasutitermes* in Central Amazonia

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SUMMARY

Twenty one species of termites were collected from their nests in areas of pasture, secondary forest and primary forest at a cattle ranch in Central Amazonia, Brazil. They were tested immediately for nitrogen-fixing (acetylene-reducing) activity. The highest activities (more than 100 nmol C_2H_4 g dry weight $^{-1}$ h $^{-1}$) all occurred in the genus Nasutitermes collected in the pasture. Nasutitermes is known to be the commonest genus in the area. Acetylene-reducing activity associated with Nasutitermes spp. collected from the same nests on different occasions was very variable, and activity decreased with time after removal of the termites from the nests. Decapitation of the termites stopped activity immediately. The possible role of nitrogen fixation associated with Nasutitermes in the nitrogen cycle in Central Amazonia is discussed.

INTRODUCTION

The regeneration of a tropical rain forest requires a considerable input of nitrogen, part of which may be supplied by biological nitrogen fixation (Nye and Greenland, 1960). Little information is available concerning nitrogen fixation in tropical rain forest. Only since the development of the acetylene reduction technique (Hardy et al., 1968) have such studies been feasible in the tropics. Sylvester Bradley et al. (in press) found very low rates of acetylene reduction associated with roots of primary forest trees growing on heavy yellow latosol (oxisol) near Manaus, Brazil, even though some of the trees belonged to the family Leguminosae whose members are known for their ability to form nitrogen-fixing root nodules. This lack of nitrogen-fixing activity associated with roots in the rain forest might be due to inhibition of nodulation by the soil, which is acid, low in phosphorus, and has a high aluminium saturation of the cation exchange capacity. An alternative site for nitrogen fixation in the forest might be in the hind guts of termites, which form a large proportion of the soil fauna in Central Amazonia (Fittkau and Klinge, 1973) and which have been shown to possess nitrogen-fixing ability in North America (Benemann, 1973; Breznak et al., 1973) and in Australia (French et al., 1976).

Twenty one species of termites were collected from their nests in areas of pasture, primary forest and secondary forest in Central Amazonia. They were tested for nitrogenase activity by the acetylene reduction method. The highest activities (more than 100 nmol C_2H_4 g dry weight $^{-1}h^{-1}$) all occurred in the genus Nasutitermes collected in the pasture (Sylvester - Bradley et al., 1978, Figure 1).

In the experiments described here termites were sampled on several occasions from the same nests in order to obtain more information about their acetylene reducing activity.

MATERIALS AND METHODS

Preliminary experiments were carried out at a farm (Fazenda NAF-6) on the Manaus-Caracaraí road (BV-8). For these experiments the nests were broken open and the termites transferred with a paint brush into 7 ml screw-capped bottles. 10% acetylene was added to the gas phase and after incubation periods of up to five hours gas samples were withdrawn and analysed for ethylene and acetylene on a gas chromatograph with a flame ionization detector as described by Hardy et al. (1968).

In further experiments, termites from two nests of Nasutitermes spp. on the campus of the Instituto Nacional de Pesquisas da Amazonia (INPA, Manaus) were sampled and tested for acetylene-reducing activity on several occasions by different methods. Large pieces of nest containing termites were placed in dessicatiors into which 10% acetylene was injected, individual termites were placed in the barrels of 1 ml disposable syringes, or approximately fifty termites were placed in 7 ml screw-capped bottles as above.

Acetylene peak heights were used to correct the ethylene peak heights if the containers leaked.

RESULTS

Acetylene-reducing activities of termites collected on two sampling dates from the same nests at Fazenda NAF-6 did not correlate. Termites from one nest showed high activity on the first sampling date and no activity the following week, whereas other nests showed the opposite response (no activity on the first sampling date and high activity on the second).

Three large pieces of a nest of Nasutitermes sp. containing termites from

INPA collected at the same time and incubated in dessicators showed very different activities, and the activity decreased with time (Figure 2).

When between thirty and a hundred mixed soldier and worker termites from the same nest of Nasutitermes sp. were incubated with acetylene in 7 ml. bottles there was no apparent relationship between the number of termites and the amount of acetylene reduced (Figure 3.).

In order to determine whether this lack of consistency in the results was due to differences between individual termites, single worker or soldier termites of N. macrocephalus from a nest in INPA were incubated for five hours with acetylene in the barrels of 1 ml disposable syringes. The soldier termites reduced acetylene whereas the workers did not (Table 1.). By the end of the experiment the soldiers were still alive whereas the workers were dead.

In a further experiment on mixed worker and soldier termites from the same nest of N. macrocephalus some of the termites were deliberately killed by decapitation and their acetylene-reducing activity compared with that of live termites. The live termites reduced acetylene whereas the dead ones did not (Figure 4.).

Figure 5 shows that if the acetylene was injected into bottles containing N. macrocephalus soldiers immediately after they were collected from their nest, the activity was higher than if acetylene was injected six hours after removal of the termites from the nest. The activity of termites which had been removed from their nest six hours earlier was slightly higher if the bottles containing the termites were kept open during the six hours than if they were closed.

D I S C U S S I O N

The results presented here suggest that dead termites cannot reduce acetylene, even if tested immediately after their death. This is surprising in that the gut microflora of a termite would not be expected to die immediately on the death of its host. However, it implies that the activity of the termite gut microflora depends very closely on the physiological state of its host and that damage or disturbance of the host may inhibit the microflora's activity. The inconsistent results obtained when sampling the same nest more than once may have been due to changes in the behaviour and consequently the physiology of the termites in response to disturbance of the nest.

It is possible that the acetylene reduction method underestimates or misrepresents the nitrogen-fixing activity associated with termites. For example Benemann (1973) and Breznak et al. (1973) reported higher activity associated with worker than soldier termites, whereas here we show the opposite result. This might be due to the worker termites tested by us being more fragile than the soldiers, which caused the inhibition of the acetylene-reducing activity associated with them.

Sylvester-Bradley et al. (1978) concluded that the low rates of nitrogen fixation associated with Nasutitermes spp. in degraded pasture in Central Amazonia could be limiting the rate of decomposition in this ecosystem, and that the total amount of nitrogen fixed would not be sufficient to supply the needs of a regenerating forest calculated by Nye and Greenland. (1960).

In order to draw such conclusions it was necessary to assume a ratio of C_2H_2 reduced to N_2 fixed of 3:1, which is not necessarily a valid assumption (Hardy et al., 1973). The results presented here indicate

that estimates of nitrogen fixation by termites made with the acetylene reduction technique should be interpreted with great care. The rates of acetylene reduction associated with Nasutitermes spp. reported by Sylvester-Bradley et al. (1978) indicate that nitrogen fixation occurs in this genus. However, it is not clear whether the rates were under or overestimated or whether the other genera tested are able to fix nitrogen or not.

In view of the important role played by termites in the decomposition process in tropical ecosystems, the limitation of this process by levels of available nitrogen (Aho et al., 1974), and the lack of an appropriate method for estimating the rate of nitrogen fixation associated with termites it seems too early to conclude that the contribution of the process to the nitrogen economy of the ecosystem is insignificant.

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REFERENCES

Aho, P. E. Seidler, R. J., Evans, H. J. and Raju, P. N. (1974) Distribution, enumeration, and identification of nitrogen-fixing bacteria associated with decay in living white fir trees. Phytopathology 64: 1413-1420.

Benemann, J. R. (1973) Nitrogen fixation in termites. Science, Wash., 181: 164-165.

Breznak, J. A., Brill W. J., Mertins, J. W. and Coppel, H. C. (1973) Nitrogen fixation in termites. Nature, Lond., 244: 577-580.

Fittkau, E. J. and Klinge, H. (1973) On biomass and tropic structure of the Central Amazonian rain forest ecosystem. Biotropica, 5 (1): 12-14.

French, J. R. J., Turner, G. L. and Bradbury, J. F. (1976) Nitrogen fixation by bacteria from the hind-gut of termites. J. gen. Microbiol., 95: 202-206.

Hardy, R. W. Burns, R. C. and Holsten, R. D. (1973) Applications of the acetylene-ethylene assay for measurement of nitrogen fixation. Soil Biology and Biochemistry, 5: 47-81.

Hardy, R. W., Holsten, R. D., Jackson, E. K. and Burns, R. C. (1968). The acetylene-ethylene assay for N_2 fixation: laboratory and field evaluation. Plant Physiol. 43: 1185-1207.

Nye, P. H. and Greenland, D. J. (1960). The soil under shifting cultivation. Commonwealth Agricultural Bureau Soil Tech. Communication, No. 51.

Sylvester-Bradley, R., Bàndeira, A. G. and de Oliveira, L. A. (1978). Fixaçao (reduçao de acetileno) em cupins (Insecta: Isoptera) da Amazonia Central. Acta Amazonica 8(4): 621-627.

Sylvester Bradley, R. de Oliveira, L. A., de Podestà Filho, J. A. and St. John, T. V. (in press). Nodulation of legumes, nitrogenase activity of roots and occurrence of nitrogen-fixing Azospirillum spp. in representative soils of Central Amazonia.
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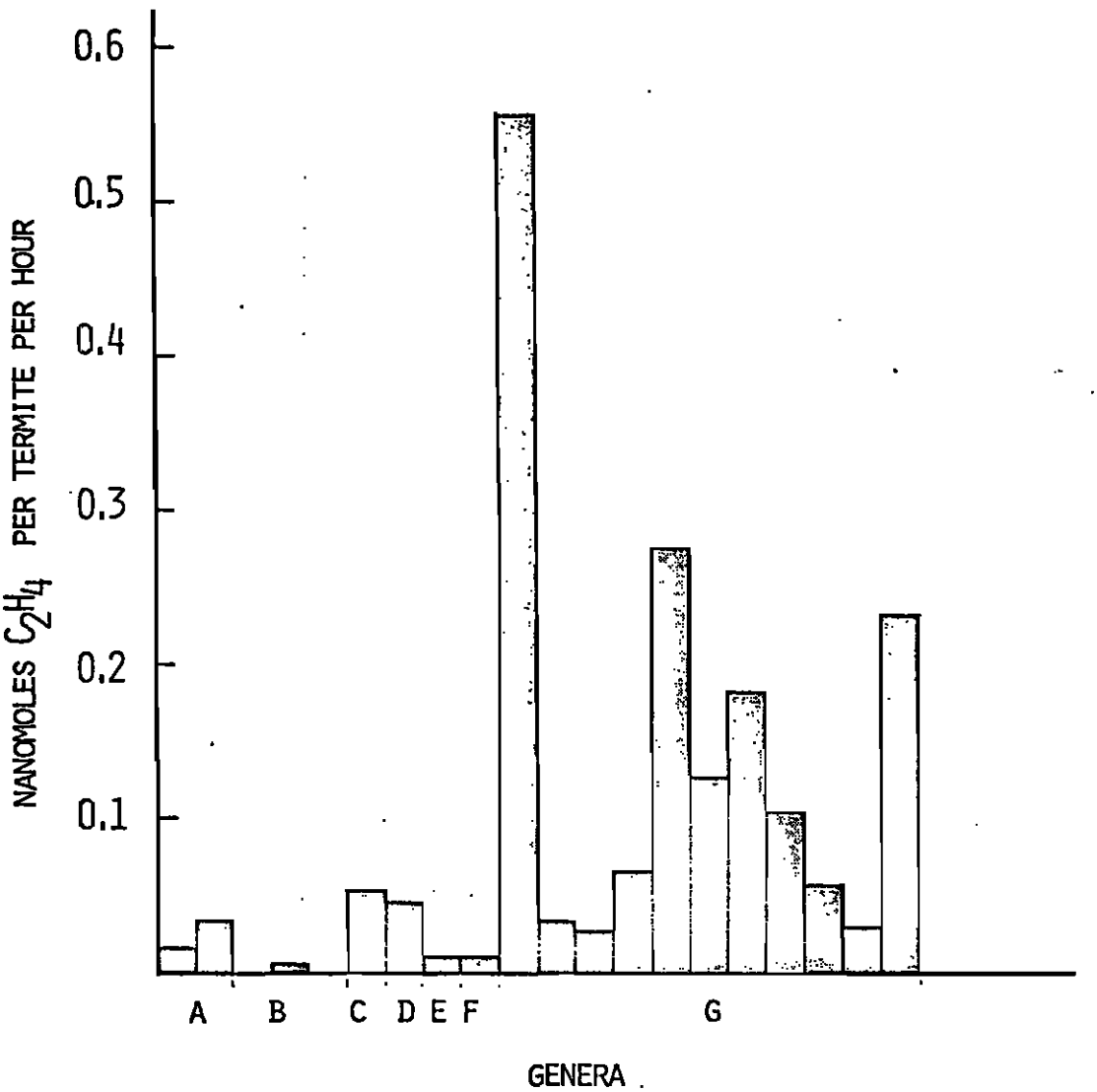
TABLE 1

Nanomoles C_2H_4 produced by individual N. macrocephalus termites incu - bated in 1 ml. syringes with C_2H_2.

Soldiers	workers
0.08	0.00
0.01	0.00
0.11	0.00
0.07	0.00
0.17	0.00

Fig. 1.:

Nanomoles C_2H_4 produced by different genera of termites collected in an area of degraded pasture in Central Amazonia.



A. Amitermes
B. Grigiotermes
C. Armitermes
D. Cornitermes
E. Heterotermes
F. Labiotermes
G. Nasutitermes

(Sylvester-Bradley et al., Acta Amazonica 8: 621-627, 1978)

Fig. 2.:

Micromoles C_2H_4 produced in dessicators containing Nasutitermes sp. within pieces of their nest.

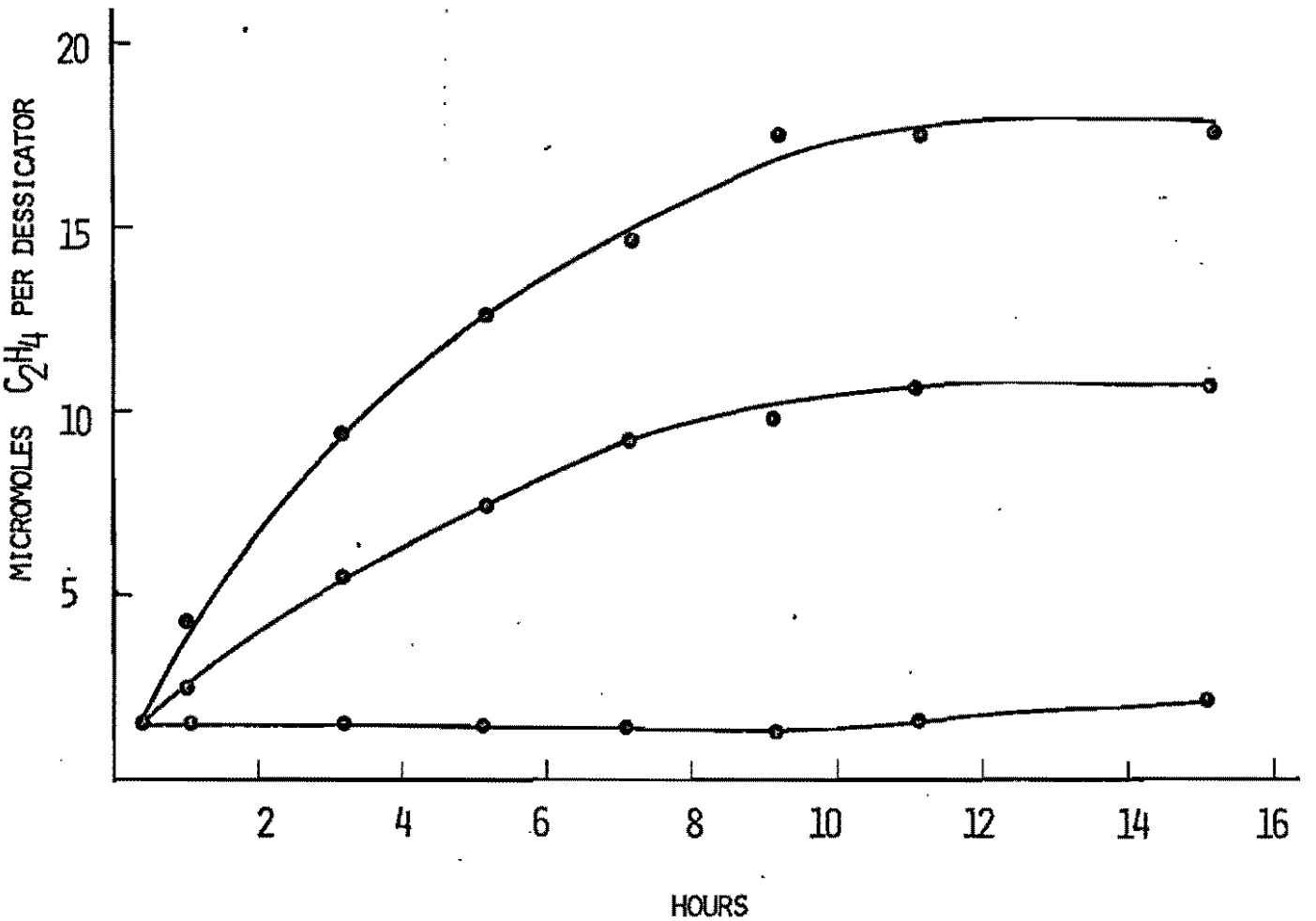


Figure 3.:

Nanomoles C_2H_4 produced in bottles containing between 30 and 100 mixed workers and soldiers of Nasutitermes sp.

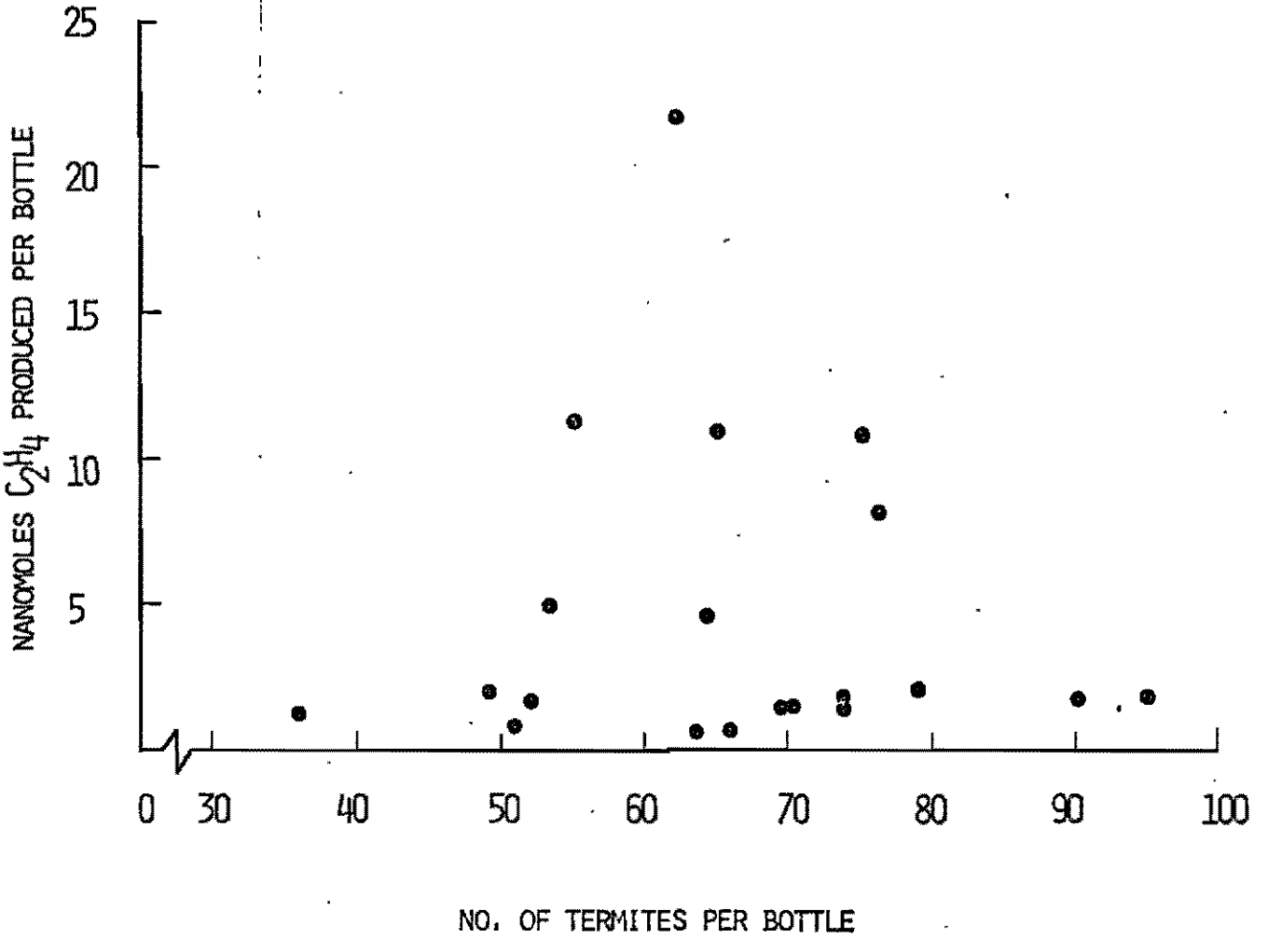


Fig. 4.:

Nanomoles C_2H_4 produced by live and dead (decapitated) workers and soldiers of Nasutitermes macrocephalus. (Mean of 3 replicates).

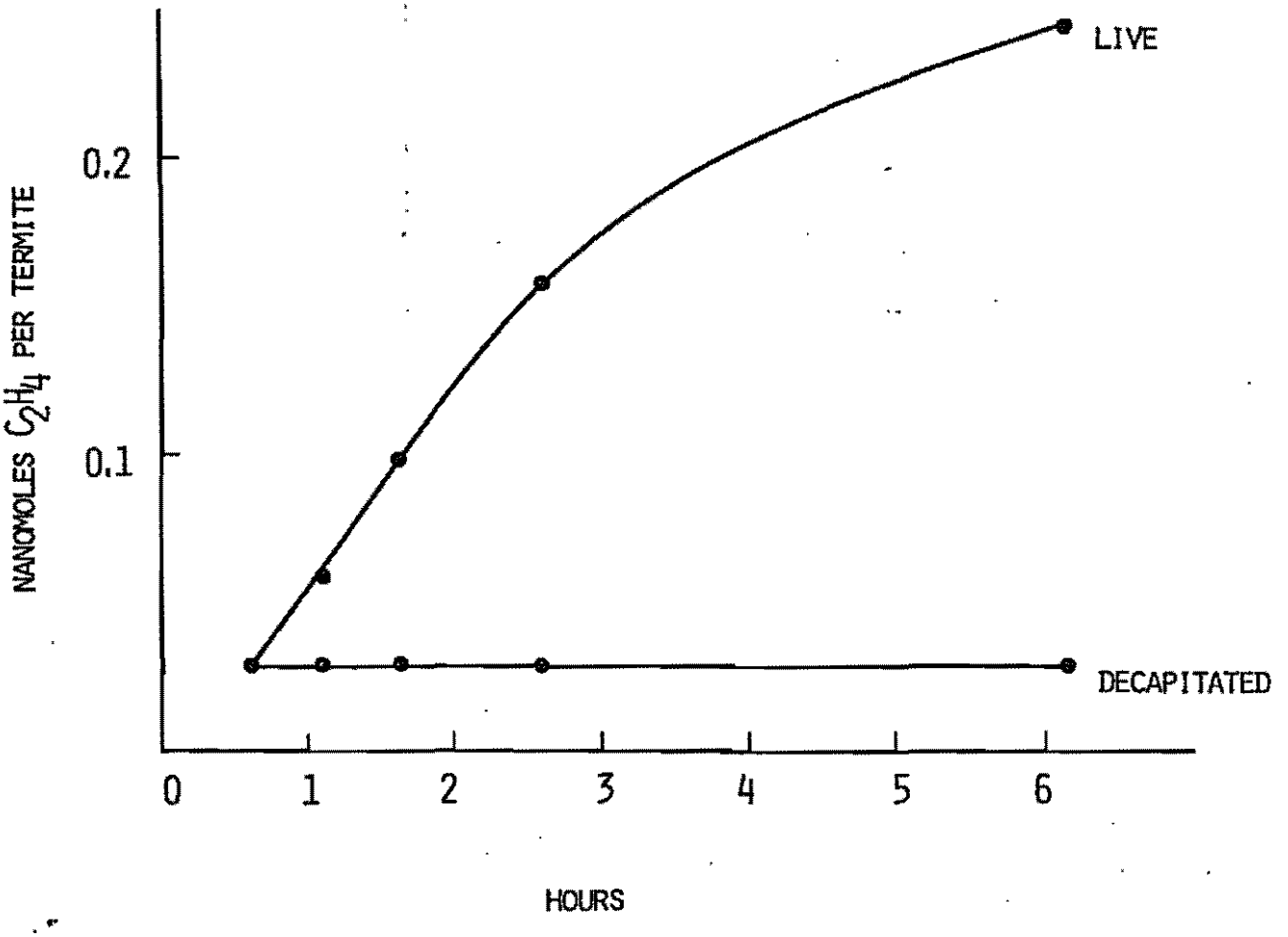
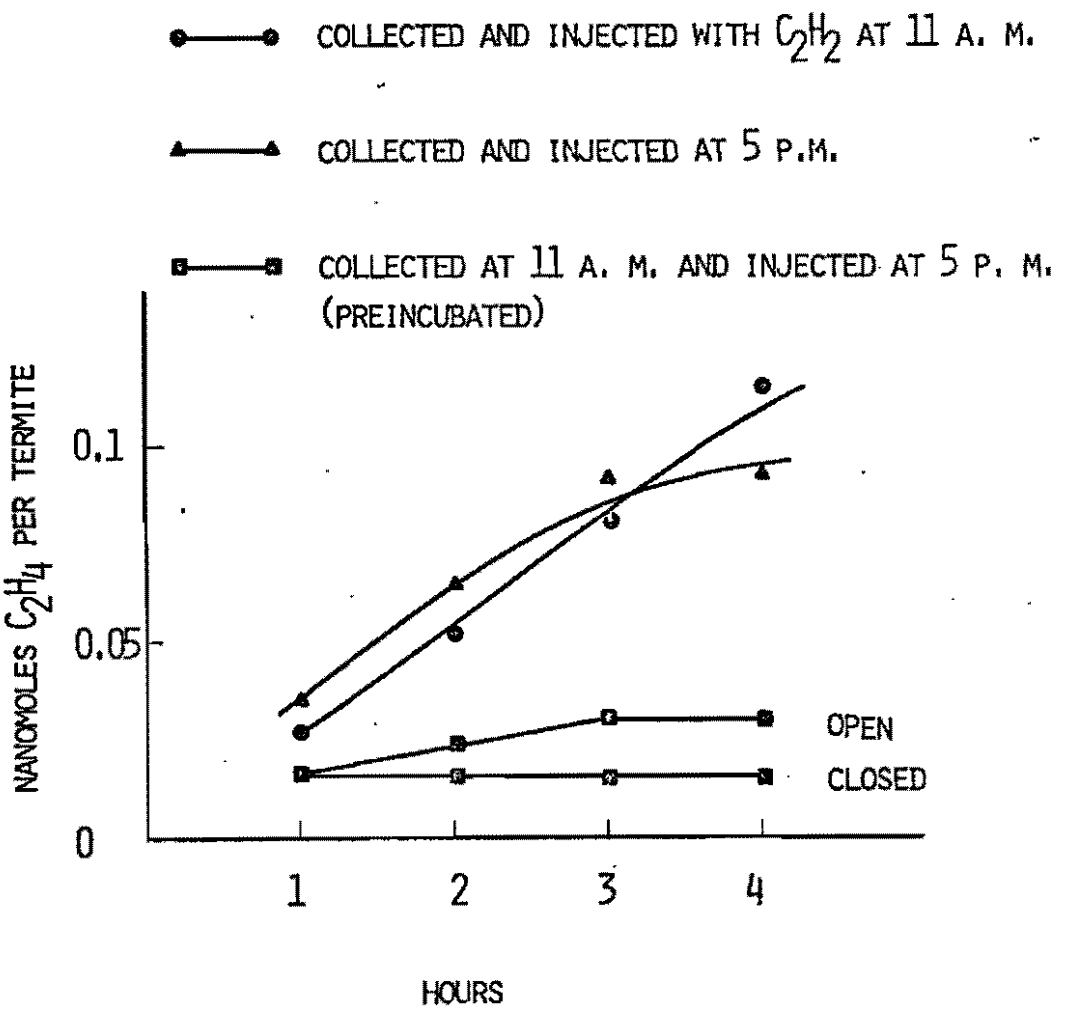


Fig. 5.:

Nanomoles C_2H_4 produced by soldier termites of N. macrocephalus collected at 11 a. m. or 5 p. m. and exposed to acetylene imme - diately, or collected at 11 a. m. and preincubated without acetylene until 5 p. m. Preincubation was carried out either in open or closed bottles .



Sylvester-Bradley, R., Bandeira, A. G. and de Oliveira, L. A. (1978) Fixaçao de nitrogênio (redução de acetileno) em cupins (Insecta: Isoptera) da Amazonia Central. Acta Amazonica 8: 621-627.

Sylvester-Bradley. R. (1979) Monitoring soils in agroforestry: Soil biology and biochemistry. In Mongi H. O. and Huxley, P. A. (eds.). Soils Research in Agroforestry. ICRAF, Nirobi. pp381-411.

Sylvester-Bradley, R., de Oliveira, L. A. de Podestà Filho, J. A., and St. John, T. V. (in press) Nodulation of legumes, nitrogenase activity of roots and occurrence of nitrogen-fixing Azospirillum spp. in representative soils of Central Amazonia. Agro-Ecosystems.

De Oliveira, L. A. and Sylvester-Bradley. R. (submitted to Turrialba) Effect of different Central Amazonian soils on growth, nodulation and occurrence of N_2 Azospirillum spp. in roots of some crop plants.

Sylvester Bradley, R., de Oliveira, L. A. and Bandeira, A. G. (1980) Nitrogen fixation in Nasutitermes in Central Amazonia. Paper to be presented at the International Symposium on Social Insects in the Tropics, 9-13 November, 1980, Cocoyoc, Mexico.

Sylvester-Bradley. R. (1980) Isolation and cultivation of Rhizobium strains for tropical forage legumes using acid media. X RELAR, Maracay, Venezuela. 15-19 September, 1980.

Sylvester Bradley, R. (1980) Comments for the Round Table Discussion "Endangered tropical ecosystems: identifying research priorities for microbial ecologists" at the Second International Symposium on Microbial Ecology at Warwick University. England, 7th-12th September, 1980.

Sylvester-Bradley, R., Bandeira, A. G. and de Oliveira, L. A. (1978) Fixaçao de nitrogênio (redução de acetileno) em cupins (Insecta: Isoptera) da Amazonia Central. Acta Amazonica 8: 621-627.

Sylvester-Bradley. R. (1979) Monitoring soils in agroforestry: Soil biology and biochemistry. In Mongi H. O. and Huxley, P. A. (eds.). Soils Research in Agroforestry. ICRAF, Nirobi. pp381-411.

Sylvester-Bradley, R., de Oliveira, L. A. de Podestà Filho, J. A., and St. John, T. V. (in press) Nodulation of legumes, nitrogenase activity of roots and occurrence of nitrogen-fixing Azospirillum spp. in representative soils of Central Amazonia. Agro-Ecosystems.

De Oliveira, L. A. and Sylvester-Bradley. R. (submitted to Turrialba) Effect of different Central Amazonian soils on growth, nodulation and occurrence of N_2 Azospirillum spp. in roots of some crop plants.

Sylvester Bradley, R., de Oliveira, L. A. and Bandeira, A. G. (1980) Nitrogen fixation in Nasutitermes in Central Amazonia. Paper to be presented at the International Symposium on Social Insects in the Tropics, 9-13 November, 1980, Cocoyoc, Mexico.

Sylvester-Bradley. R. (1980) Isolation and cultivation of Rhizobium strains for tropical forage legumes using acid media. X RELAR, Maracay, Venezuela. 15-19 September, 1980.

Sylvester Bradley, R. (1980) Comments for the Round Table Discussion "Endangered tropical ecosystems: identifying research priorities for microbial ecologists" at the Second International Symposium on Microbial Ecology at Warwick University. England, 7th-12th September, 1980.