EFFECT OF INCUBATION TIME ON ACETYLENE REDUCTION ACTIVITY OF COMMON COVER CROPS OF SRI LANKAN RUBBER PLANTATIONS

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ABSTRACT

A wide range of incubation times from a few minutes to severl hours have been used with different plant systems in investigations on acetylene reduction assays. However, the duration of incubation period is of utmost importance in obtaining nitrogen fixation estimates using this technology.

Time course experiment showed that acetylene reduction by nodules of *P. phaseoloides* and *D. ovalifolium* started without a lag but rate of the nitrogenase activity was reduced 4 hours after incubation while *M. invisa* showed a considerable activity even at the 8th hour of incubation. In the second experiment which was conducted to observe the relationship between acetylene reduction activity and incubation time upto one hour, there was a linear aelationship for both *P. phaseoloides* ($r = + 0.9832$) and *D. ovalifolium* ($r = + 0.9961$).

Key words:

Acetylene reduction activity, Incubation time, *Pueraria*, *Desmodium*, *Mimosa*

INTRODUCTION

The observation that acetylene is converted to ethylene by the nitrogen fixing enzymes and that acetylene is an inhibitor for nitrogen fixation (Dilworth, 1966; Schollhorn and Burris, 1967) provided the basis for the development of acetylene reduction (AR) assay as an indirect method for assaying biological nitrogen fixation.

Although certain drawbacks exist in this methodlogy (Hardy *et al.*, 1973; Knowles, 1981; Larue and patterson 1981; Herridge, 1982; Minchin *et al.*, 1983; Witty *et al*, 1984) many studies have been accomplished with the aid of this technique. Acetylene reduction activity in legume root nodule bacterial symbioses has served as the main or suplementary criterion in various types of comparison studies and the assessment of nitrogne fixing performance (Minchin and Pate, 1974; Ayanaba and Lawson 1977; Zary and Miller, 1980; Hardarson *et al.* 1981; Eaglesham *et al.*, 1982; Jessop *et al.*, 1984; Jakobsen, 1985; DuTean *et al.*, 1986; Miller *et al.*, 1986).

During this assay, the system under study is enclosed in a gas tight container exposed to an atmosphere containing acetylene. A sample is taken after a suitable incubation period and analysed for ethylene using a gas liquid chromatograph fitted with a flame ionization datector.

A wide range of incubation times from a few minutes to several hours have been used with different plant systems in investigations on AR assays. For instance 30 minutes incubation period has been used for *Glycine max* (Mague and Burris, 1972; Patterson and La Rue, 1983), 30 - 60 minutes for *Lupinus* spp. (Trinick *et al.*, 1976) aud three hours for *Vigna radiata* (Talekar and Kuo, 1979). The principal factors requiring attention in determining the (ncubation period for the experimental systems are; non-linear ethylene production in long incubation due to O_2 depletion (Hardy *et al.*, 1968), energy depletion, depression of nitrogenase and production of inhibitory products (Masterson and Murphy, 1980).

Therefore, the incubation period should be determined after considering the nature of the test plant. This is of utmost importance if these assays are to be used to obtain nitrogen fixation estimates.

For example, the AR assay and N^{15} technique were compared for measuring symbiotic nitrogen fixation by *Trifolium repens* in established pasture Goh *et al.*, 1978). These authors reported that a three hour iucubation time in the AR assay gave the best estimate of symbiotic nitrogen fixation relative

to the N^{15} technique. One hour incubat.on over estimated and six hour incubation under estimated the rate of symbiotic nitrogen fixation.

In this paper we report results of time course studies with three common cover crop legumes viz. *Pueraria phaseoloides* (Roxb) Benth., *Desmodium ovalifolium* (Prain) wall ex Ridley and *Mimosa invisa*. (Mart ex.) in rubber plantations of Sri Lanka in order to determine the most suitable time of incubation for AR activity assays.

MATERIALS AND METHODS

Test plants

P. phaseoloides, *D. ovalifolium* and *M. invisa* were used as test plants.

Preparation of samples

Plants were removed from the soil and the whole root system with nodules was incubated after gently shaking off the sand.

Incubation

Root systems with intact nodules were placed in 1000 ml assay containers and the reaction was initiated by injecting 10% commercial grade acetylene. Gas samples were withdrawn and assayed at 15 minute and one hour intervals in short term and long term experiments respectively. All the containers were maintained at a constant temperature around 26°C throughout the incubation period. Four replicates were used in all the experiments where observations were made at hourly intervals and three replicates were used for other experiments.

Gas chromatography

Separation of acetylene and ethylene was achieved on a porapak N glass column (80 - 100 mesh) run at 100°C in a Packard Model 642 chromatograph with a hydrogen flame ionization detector. The temperature of the injector and detector were 130°C, and 190°C respectively. One millilitre samples were used for analysis.

RESULTS

The results of the experiment are given in graphical form in figures 1, 2 and 3.

DISCUSSION

The rate of acetylene reduction aetivity (/u moles ethylene produced per gram nodule dry weight) was reduced four hours after incubation in both *P. phaseolides and D. ovalifolium* (Fig. 1 a and 2 a). *M. invisa* showed considerable acetylene reduction activity even at the 8th hour of incubation, but the rate was reduced thereafter (Fig. 3). This stabilization in the activity after a certain period of incubation may be due to oxygen depletion as showed by Hardy *et al.*, (1968) for nodulated soybean root systems.

When acetylene reduction activity was correlated to incubation time the correlation coefficients for *M. invisa* was +0. 9675 up to the 8th hour of incubation, But correlation coefficients for *P. phaseoloides* and *D. ovalifolium* were +0. 9333 and +0. 9442 respectively up to two hours incubation period. No further increase in the rate of the acetylene reduction activity was detected for all three crops beyond 8 hours incubation periods.

In the short term incubation up to one hour, there was a linear relationship for both *P. phaseoloides* and *D. ovalifolium* (Fig. 1 b and 2b). The correlation coefficient for *P. phaselides* was +0. 9832 and +0. 9961 for *D. ovalifolium.*

These results are generally in agreement with the findings for other crops such as *Glycine max* (Hardy *et al.*, 1968; Mague and Burris, 1972), *Vigna radiata* (Talekar and kuo; 1979) *Lupinus* spp. (Trinick *et al.*, 1976). For instance, *Lupinus* spp. samples on days of uninterrupted sunshine showed a linear reduction of acetylene with time for periods greater than 2 hours (Trinick *et al.*, 1976). Hardy *et al.*, (1968) demonstrated that the rate of acetylene reduction by nodulated roots or root soil cores of *Glycine max* was constant up to 60 minutes. Further, it was reported that for *Glycine max* production of ethylene was linear for abouut 80 minutes and continued at a decreasing rate for at least 7 hours (Mague end Burris, 1972).

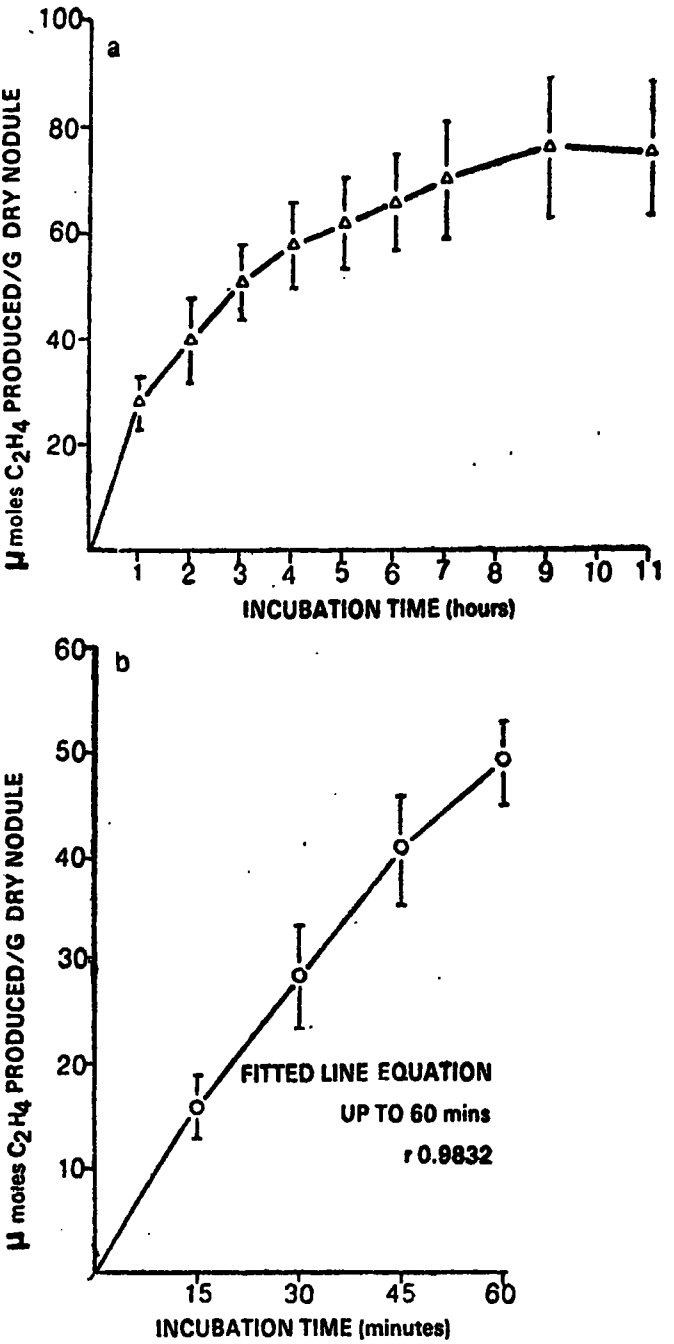

Figure 1. Time — course of acetylene reduction by P. phaseoloides measured at hourly (a) and 15 minute (b) intervals.

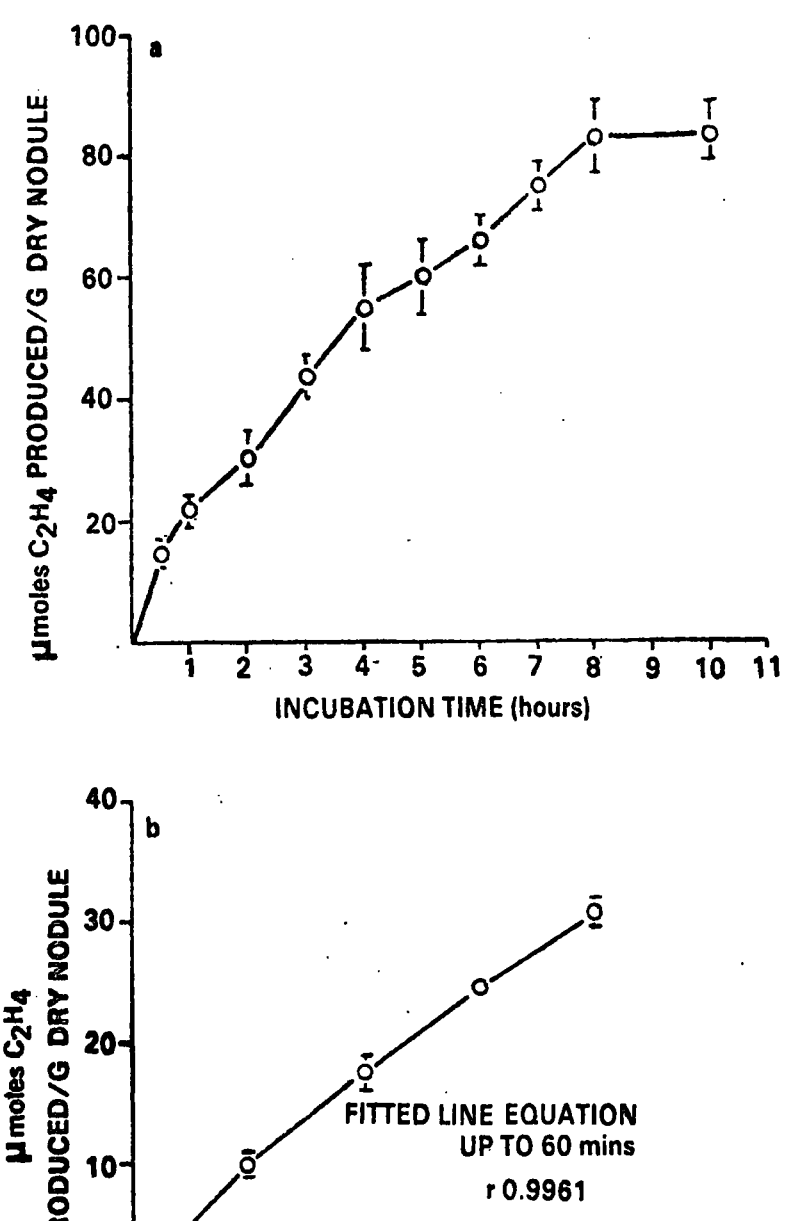

Figure 2. Time — course of acetylene reduction by D. ovalifolium measured at hourly (a) and 15 minute (b) intervals.

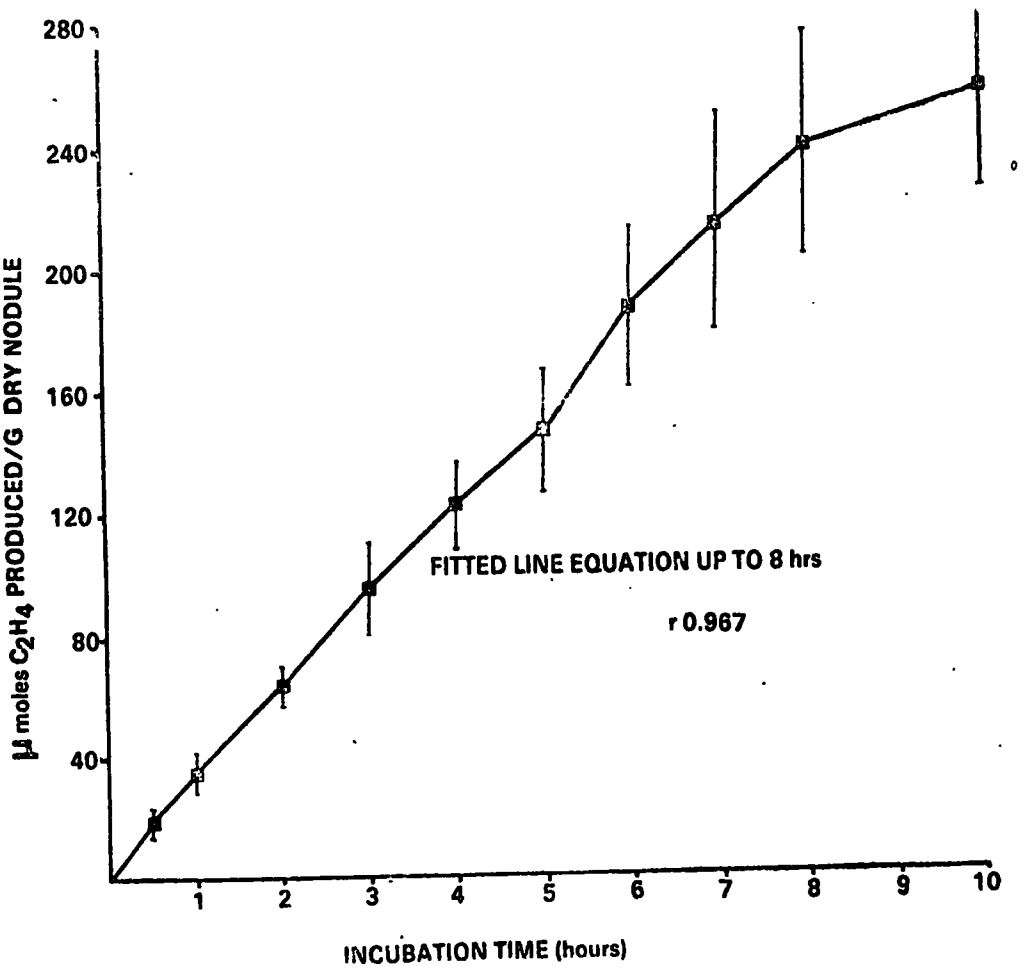

Figure 3 Time — course of acetylene reduction by M invisa measured at hourly intervals

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