DIURNAL VARIATION IN NITROGENASE ACTIVITY OF COMMON COVER CROPS IN RUBBER PLANTATIONS OF SRI LANKA

C. K. Jayasinghe. C. A. Parker & S. A. Kulasooriya

ABSTRACT

Diurnal fluctuations in nitrogenase activity (acetylene reducing activity) have been observed in several nitrogen fixing systems. All experimental plants viz. *P. phasides D. ovalifolium* and *M. invisa* demonstrated diurnal rhythm with light intensity. The lowest acetylene reduction activities were detected around midnight and they were actively fixing nitrogen by 8.00 am. *M. invsia* responded to high soil temperature in addition to light intensity, A marked drop in acetylene reduction activity was observed by about 4.00 pm when the soil temperature was around 38°C.

Key words:

Nitrogenase activity, Diurnal variations, *Pueraria, Desmodium, Mimosa*

INTRODUCTION

After the development of the acetylene reduction assay (Dilworth, 1966; Schollhorn and Burris, 1967), several investigators contributed information relating to the diurnal variation in nitrogenase activity of several nitrogen fixing legume systems (Hardy et al., 1968; Bergersen, 1970; Magne and Burris, 1972; Minchin and Pate, 1974; Sloger et al., 1975; Anon, 1978; Talekar and Kuo, 1979; Zary and Miller, 1980), In most of these studies attempts were made to relate the observed fluctuations in nitrogenase activity to the daily cycle of changes in the environment especially to the temperature, light intensity and duration of light (Minchin and Pate, 1974).

Except in a few investigations (Trinick et al.. 1976 and Talekar and Kuo, 1979), nitrogenase activities were higher in the day than night. This midday peak is attributed to the fact that newly translocated carbohydrate and ATP for fixation will be more readily available during time of photosynthesis (Wheeler, 1969; 1971; Minchin and Pate, 1974). Lawrie and Wheeler (1973) further suggested that carbohydrate in a specific compartment within the nodule is the critical factor since the overall carbohydrate status of the nodule did not correlate well with acetylene reduction activity.

However, it is worthy of note that other environmental factors in addition to light, such as temperature and humidity which affect transpiration, can also greatly influence the diurnal rhythm (Minchin and Pate 1974; Lawrie and Wheeler, 1973). For instance, it was reported that there was no diurnal variation in acetylene reduction activity in *Trifolium repens* when incubated at a temperature of 21°C but responded well in ambient temperatures (Masterson and Murphy, 1976). Contrary to these findings, a marked diurnal effect has been shown for *Glycine max* grown in a glass house using an incubation temperature of 25°C (Bergersen, 1970).

Today, use of leguminous cover crops as *pueraria phaseoloides* (Roxb) Benth, *Desmodium ovalifolium* (Prain) Wall ex Ridley and *Mimosa invisa* (Mart ex.) is a standard practice in Sri Lankan rubber plantations to conserve the soil and maintain the soil fertility. The following investigations have been carried out to examine the diurnal variation in nitrogenase activity of above cover crops.

MATERIALS & METHODS

1 Plant Culture

Test plants, *P. phaseoloides*, *D. ovalifolium* and *M. invisa* were grown in fertile soil (total N 0.236% and Org. C 1.867%) in 5 kg pots. Basal nutrients (Brockwell, 1980) were added to the soil to make sure that there were no limiting nutrients.

Acid treated seeds (Waidyanatha and Ariyaratna, 1976) of test plants were planted in each pot at about 2 cm depth. Pots were maintained in the glass house (28 ± 4 and 70 - 85% RH) for 3 1/2 months; their position being changed once a week to minimize the variations in the green house environment. There were 32 pots for each test plant.

II Assay technique

The effect of the normal day/night light cycle and of temperature on nitrogenase activity were examined at four-hour intervals viz. 0800 h, 1200 h, 1600 h, 2000 h, 2400 h and 0400 h over an entire day. Acetylene reduction activity was measured in root systems with intact nodules using one hour incubation periods. Root systems were incubated in 1000 ml assay containers and they were kept at room temperature (28±2) under normal dark and light regime. 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 analytical purposes. Four replicates were used for each treatment.

Soil moisture was maintained at an appropriate level throughout the assaying period, and atmospheric and soil temperatures were recorded. Light intensity was measured using a steady state porometer model LI–1600.

RESULTS AND DISCUSSION

There was a significant ($P < 0.05$) fall in acetylene reduction (/u moles ethylene produced/g nodule dry weight/hour) in *Pueraria* between 4 pm and 8 pm and continued at a low level until the following morning (*Fig. 1a*). In *Desmodium* a significant ($P < 0.05$) fall was observed only after 8 pm and acttvity continued at a markedly reduced rate until the following morning (*Fig. 2a*). With *Mimosa* acetylene reduction activity was significantly ($P < 0.05$) affected even at 4.00 pm and low rates continued until midnight. There was an increase in activity around 4.00 am (*Fig. 3a*).

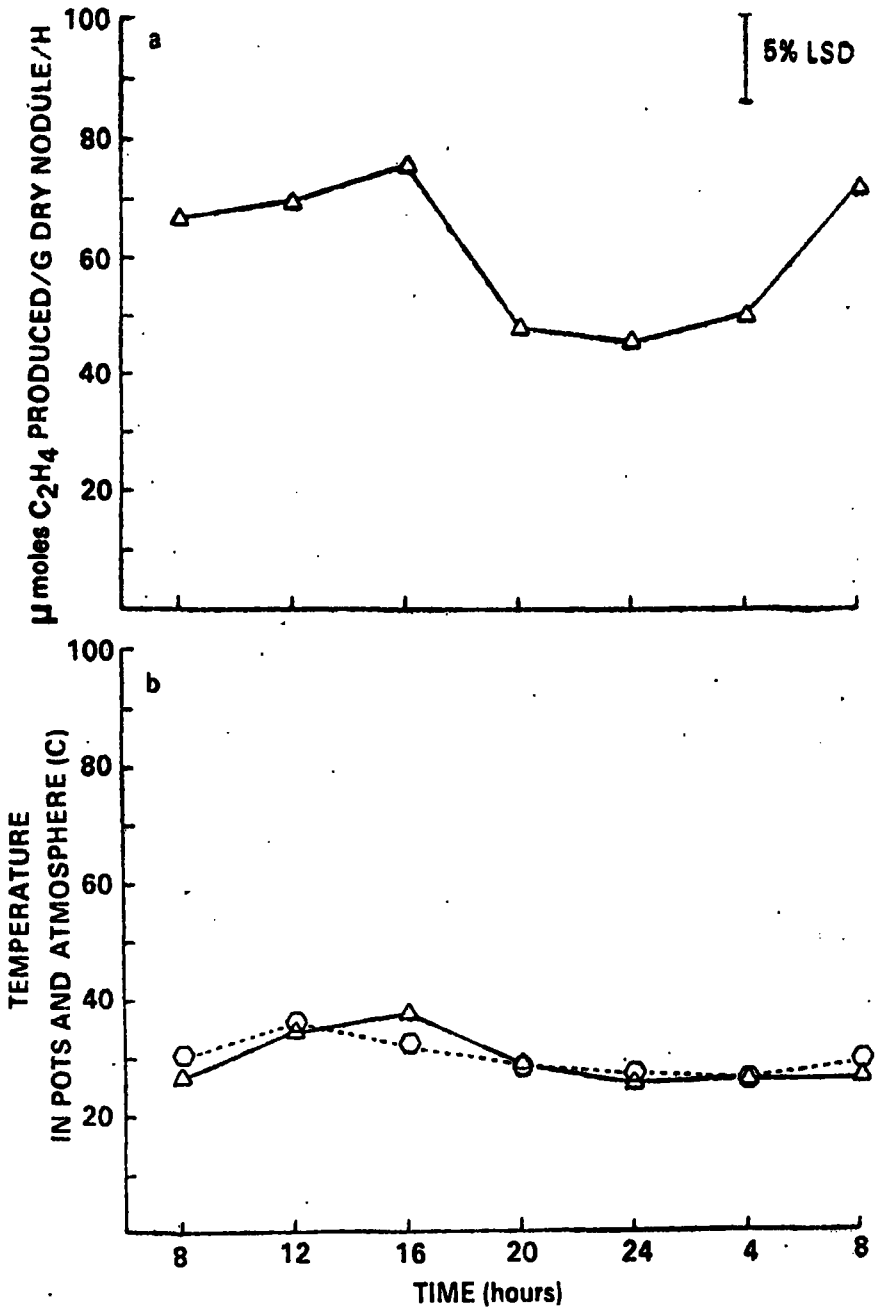

Figure 1 Acetylene reduction by P. Phaseoloides (a) temperature of pots (B△) and atmosphere (b○) in relation to time of day.

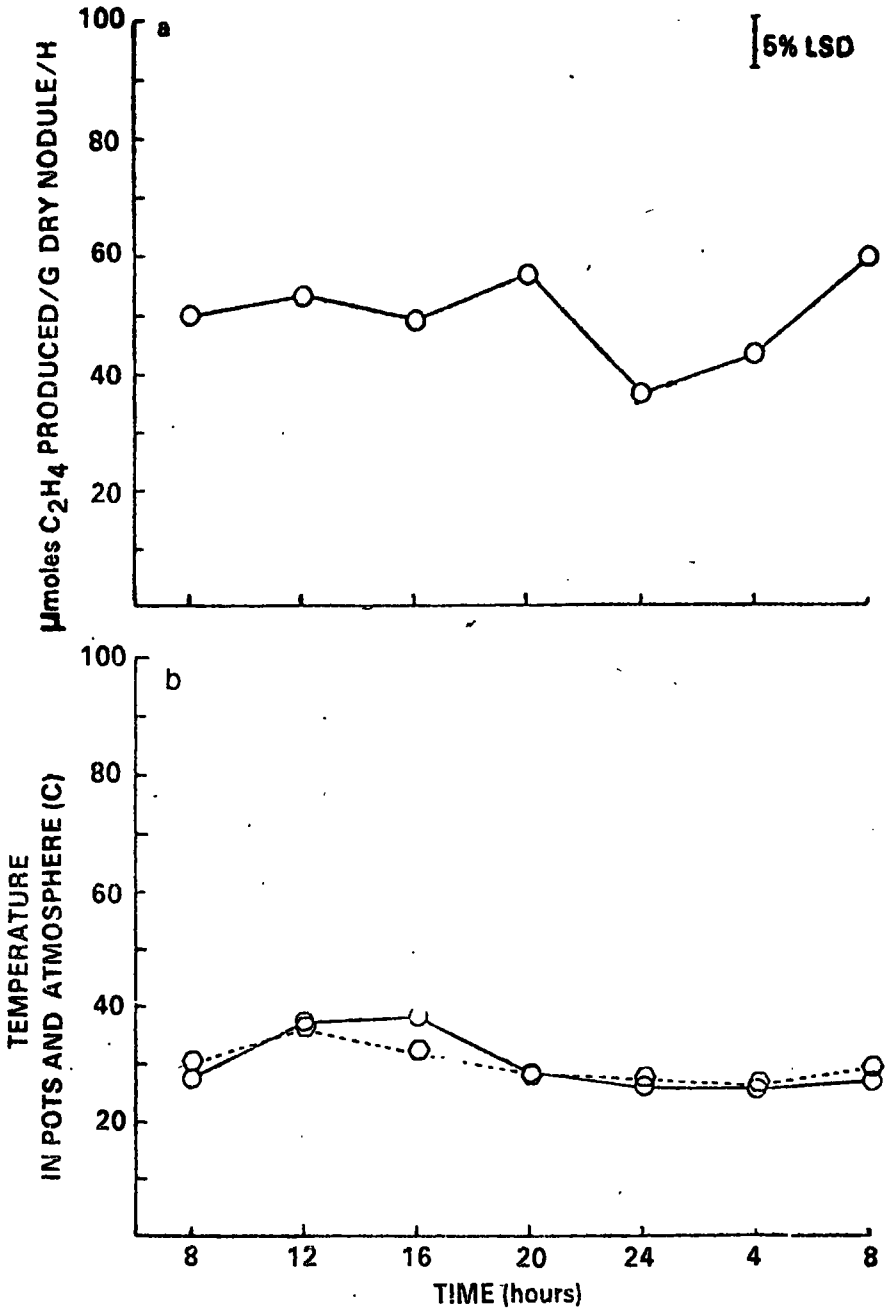

Figure 2 Acetylene reduction by
D. ovalifolium (a) temperature of pots (bO) and atmosphere (bo) in relation to time of day.

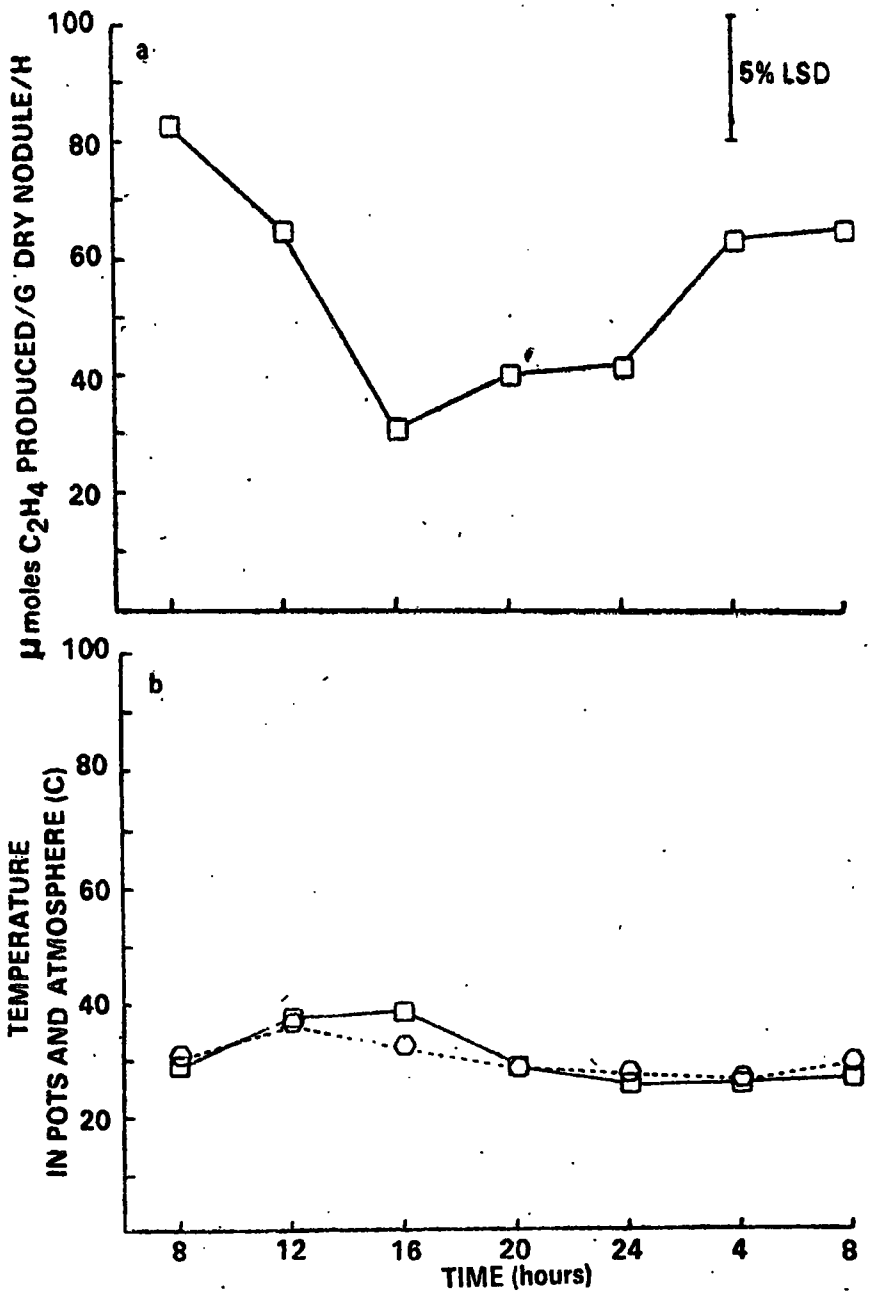

Figure 3 Acetylene reduction by M invisa (a) temperature of pots (bo) and atmosphere (bo) in relation to time of day.

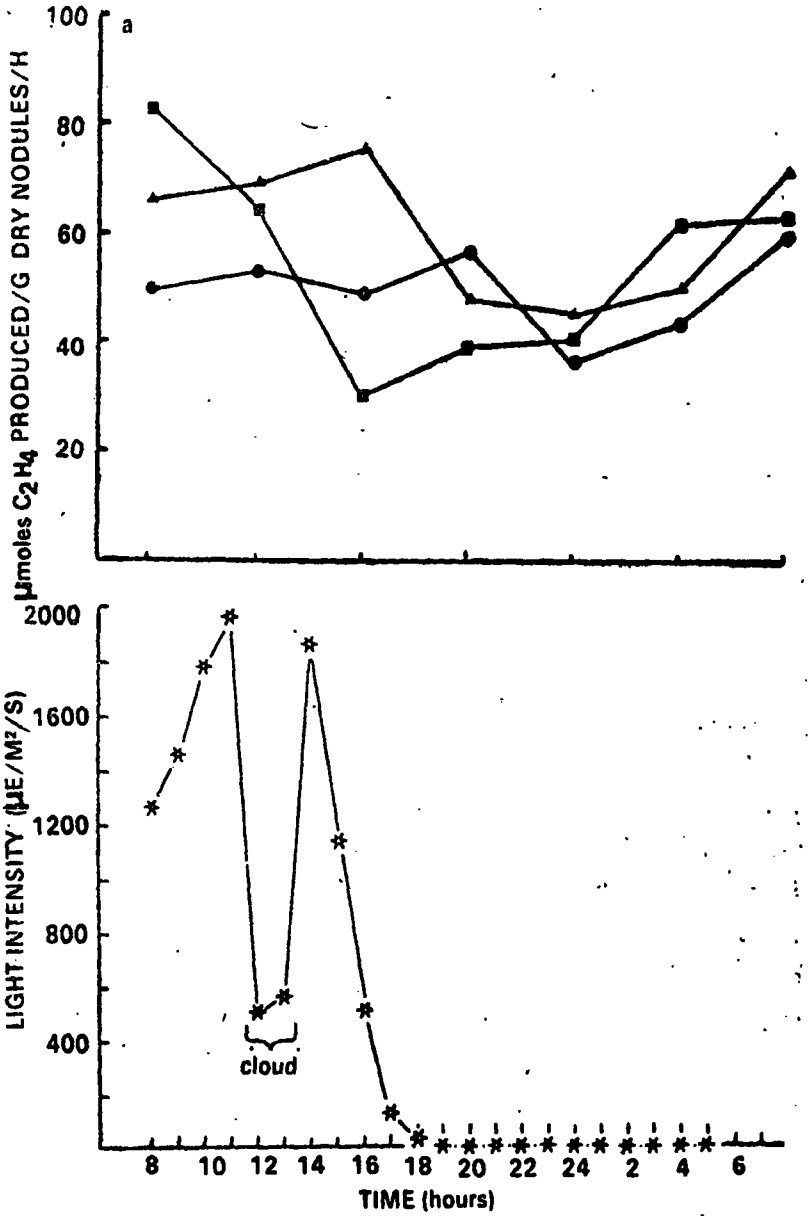

Figure 4 Acetylene reduction by P. phaseoloides S (a▲), D, ovalifolium (a●) and M invisa (a■), and light intensity (6) in relation to time of day.

Note: clouds appeared only for a few minutes during the time of measuring light intensity

The maximum air temperature (36oC) was recorded around 12. 00 noon, and the maximum soil temperature (38°C) In the pots was recorded around 4. 00 pm for all three crops (*Fig. 1b*, *2b* and *3b*).

The significant ($P < 0.05$) drop in acetylene reduction activity in *Mimosa* around 4 00 pm could be attr,buted to its sensitivity to high temperatures. It is worth noting that soil temperature was around 38 °C from 12. 00 noon to 4 .00 pm.

Nitrogenase activity in all three species continued in the dark at 50 to 60% of the maximum activity, possibly indicating that photosynthetes accumil ated during the day sustained a considerable degree of nitrogen fixation at night, However, acetylene reduction activity significantly increased ($P < 0.05$) during the day for all three experimental plants, which were actively fixing nitrogen by 8. 00 am. Therefore, it can be concluded that acetylene reduction activity of *P. phaseoloides*, *D. ovalifolium* and *M. invisa* positively correlated with diurnal rhythm in light intensity. These results are generully in agreement with the findings for *Glycine max* (Hardy et al., 1968: Bergersen, 1970; Mague and Burris, 1972; Sloger et al., 1975), *Cicer arietinum* (Anon, 1978) and *Arachis hypogaea* (Anon, 1978).

The reason for this increased activity during day time is probably because carbohydrate and ATP are more readily available to the plant during the time of photosynthesis as discussed in introduction,

However, it is ineresting to note that diurnal variation in temperature can also effect nitrogenase activity in certain legume species. As mentioned above, the exceptional behaviour in *M. invisa* at 4.00 pm was most probably due to its high sensitivity to temperature (38°C). It is unfortunate that there are no reports of similar studies on the three experimental crops studied here so that comparisons could be made on their behaviour.

Contrary to the above findings Teleker and Kuo (1979) reported that acetylene reduction activity was significantly greater during the night than the day in *Vigna radiata*. Trinick *et al.*, (1976) found that there was no marked diurnal fluctuation in acetylene reduction activity in *Lupinus* spp. Further it has been shown that *Vigna unguiculata* had two peaks in nitrgoen fixation, a major peak at 12. 00 noon and minor peak at 12. 00 mignight (Zary and Miller, 1980).

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