Jl. Rubb. Res. Inst. Sri Lanka (1990) 70, 1–10

SEASONAL VARIATIONS IN NODULE ACTIVITY OF *PUERARIA PHASEOLOIDES* (Roxb) Benth.

By

C. K. JAYASINGHE, C. A. PARKER* AND S. A. KULASOORIYA**

SUMMARY

The age of *Pueraria phaseoloides* plants had a marked effect upon the rate of acetylene reduction in their nodules. Acetylene reduction activity increased with the age of the plant and maximum efficiency of nodule activity was detected 60 days after sowing, followed by a marked decline on the 120th day and continued at a low rate. Dry matter yield and total nitrogen content increased with time but the percentage nitrogen content in plants was more or less constant through out the assay period.

Key Words: nitrogenase activity, seasonal variations, nodule activity, dry matter, nitrogen content, *Pueraria*

INTRODUCTION

The use of leguminous covers is a standard practice in rubber plantations of Sri Lanka and the most popular and widely grown cover crop is *Pueraria phaseoloides* (Roxb) Benth. It serves as a cover crop in about 90% of the new planting and roplanting areas which are estimated to be more than 24,000 hectares.

Generally the total amount of nitrogen fixed per plant increases with age. But significant variation may occur in specific nitrogenase activity (μ moles ethylene/g nodule dry weight / h) due to the physiological status of the plant and the changes in environmental conditions such as soil moisture and temperature. These seasonal variations have been extensively studied for legumes such as *Glycine max* (Hardy *et al.*, 1971; Weber *et al.*, 1971; Mague and Burris, 1972; Sloger *et al.*, 1975 Ham *et al.*, 1976: Patterson and LaRue, 1983): *Arachis hypogaea* (Hardy *et al.*, 1971: Anon, 1978, 1980); *Vigna radiata* (Talekar and Kuo, 1979); *Lupinus* app. (Trinick *et al.*, 1976); *Cicer* (Anon, 1980, 1981); *Vigna unguiculata* (Zary and Miller, 1980) and *Phaseolus vulgaris* (Graham and Rosas, 1977), but no literature is available on any of the legumes which are grown as cover crops in Sri Lankan rubber plantations.

*University of Western Australia, Perth, Australia.
**University of Peradeniya, Peradeniya, Sri Lanka.

It is widely reported that symbiotic nitrogen fixation is initiated several weeks after planting, continues at a low rate during the initial stages of vegetative growth, while the major portion of nitrogen is fixed between the flowering and pod-filling stages (Hardy *et al.*, 1968: Hardy *et al.*, 1971; Graham and Rosas, 1977: Zary and Miller, 1980). The host is thereby supplied with the nitrogen it needs for seed production. However, with regard to *Vigna radiata* (Talekar and Kuo, 1979) and *Glycine max* (Ham *et al.*, 1976) it was found that although the acetylene reduction activity was maximum during flowering it dropped drastically during pod formation period. This was interpreted as competition for limited photosynthates with developing pods and seeds (Ham *et al.*, 1976).

A detailed study on the seasonal variation of specific acetylene reduction activity in *Lupinus* spp. has been carried out (Trinick *et al.*, 1976) showing that specific activity of *Lupinus* nodules rapidly increased after initiation of nitrogen fixation to a maximum at age of five weeks.

Then a marked decline was observed and it continued until the death of the plant. The total amount of nitrogen fixed also varied throughout the life cycle. But changes in nodule efficiency or in total amount of nitrogen fixed were not reflected in flower initiation, pod setting or pod filling. It was suggested that increased specific activity was due to improved soil conditions and warm weather, and reduced activity was due to excessive water remainig on the soil surface after heavy winter rains.

The following experiments were conducted to investigate changes in nodulation and nitrogenase activity in *P. Phaseoloides* with age, up to the flowering of the plants.

MATERIALS & METHODS

1 *Plant culture*

Pueraria phaseoloides was grown in 0.6 x 0.6 m plots lined with concrete slabs in the field. Although the experiment was conducted in fertile soil (total N 0.236% and Org. C 1.867%), basal nutrients without nitrogen (Brockwell, 1980) were added to ensure that fertilizer requirements were met.

Acid-treated seeds (Waidyanatha and Ariyaratna, 1976) were sown and plants were thinned two weeks after planting to give three uniform plants per plot.

11 *Plant assay*

Plants in four randomly selected plots were harvested at 30 day intevals from October 15th, 1985 to determine plant dry matter accumulation percentage nitrogen content, nodulation, nitrogenase activity and available soil nitrogen. Nitrogen content was detected using semi-micro Kjeldahl distillation and available soil nitrogen was determined on the potassium chloride extract by semi-micro Kjeldahl distillation. Acetylene reduction activity was measured in root systems with intact nodules. All plants were harvested around 8.00am on each harvesting date and a two-hour incubation period was used for acetylene reduction assay. 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.

RESULTS & DISCUSSION

Nodule formation in *P. phaseoloides* was initiated before 30 days after sowing. Nodule dry matter yield continued to increase thoreafter with a marked increase on the 120th day where the maximum nodulation was also observed. At the final harvesting date (150th day) nodulation was more or less similar to that of the 120 day harvest (Fig. 1a).

Little nitrogenase activity per plant was detected on the 30th day, after which it increased rapidly until the 150 day harvest (Fig. 1b) when flowering was also initiated in *Pueraria phaseoloides.*

When the acetylene reduction activity is expressed per unit nodule dry weight (specific activity), the efficiency of the nodules was already high by 30 days and rapidly increased to a maximum at 60 days after sowing, followed by a marked decline on the 120th day and continued at a low rate until the final harvest (Fig. 1c).

Dry matter yield and total nitrogen content increased with time (Fig. 2a 2c and Table 1) as expected. The percentage nitrogen content in plants was more or less constant throughout the assaying period (Fig. 2b), available total soil nitrogen in plots decreased significantly (P–0.05) with time and reached a very low concentration by 120th day after sowing (Fig. 3).

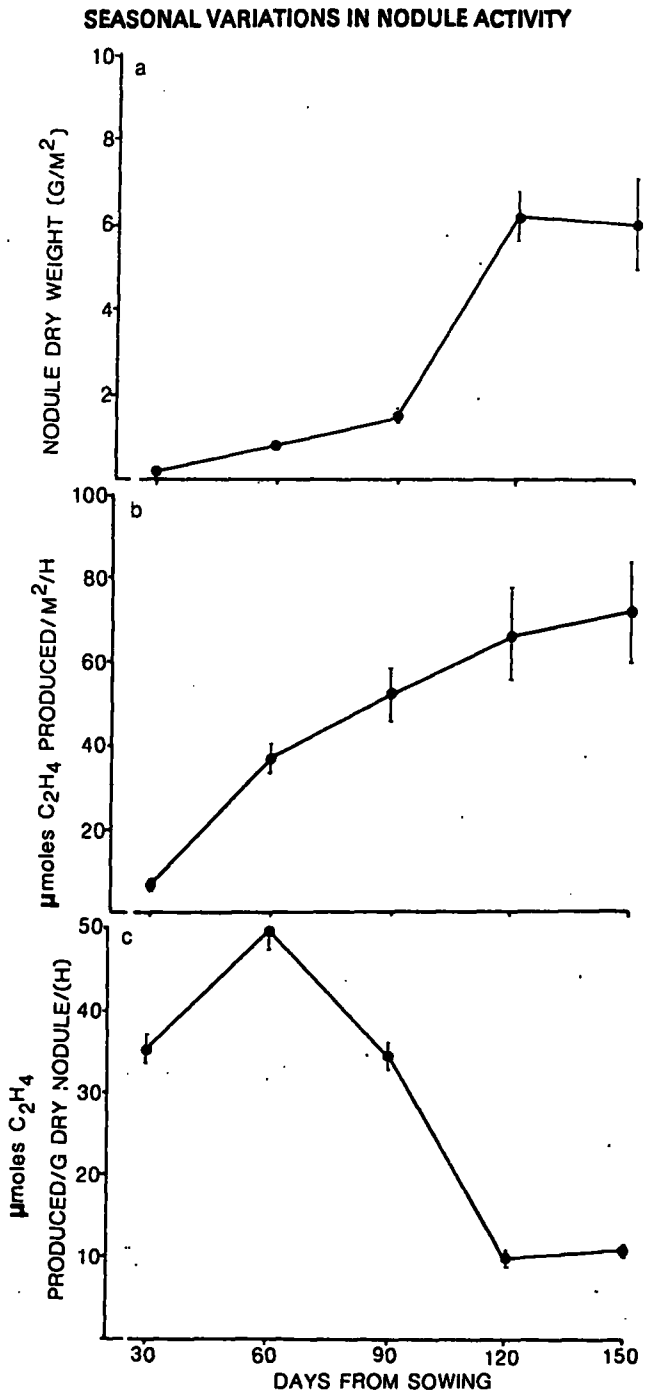

Figure 1. Effect of plant age on nodulation (a) total acetylene reduction (b) and specific acetylene reduction (c) by P. phaseoloides.

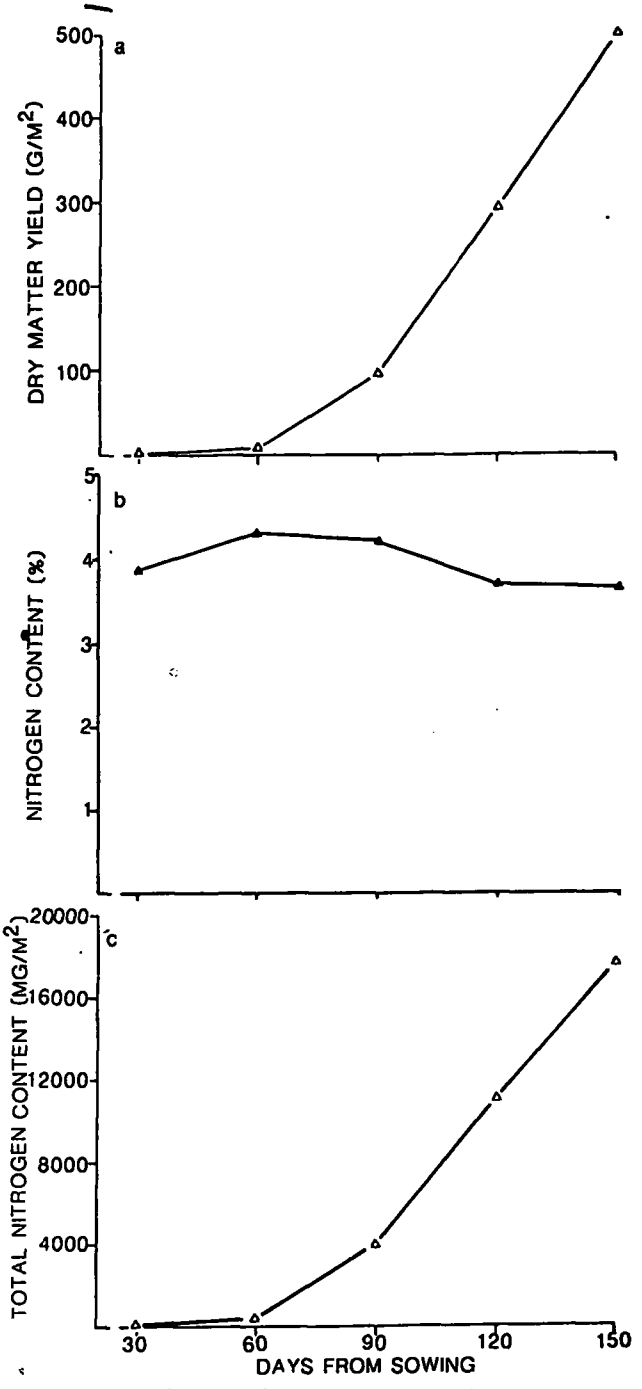

Figure 2. Effect of plant age on dry matter yield (a) percentage nitrogen content (b) and total nitrogen (c) of P. phaseoloides

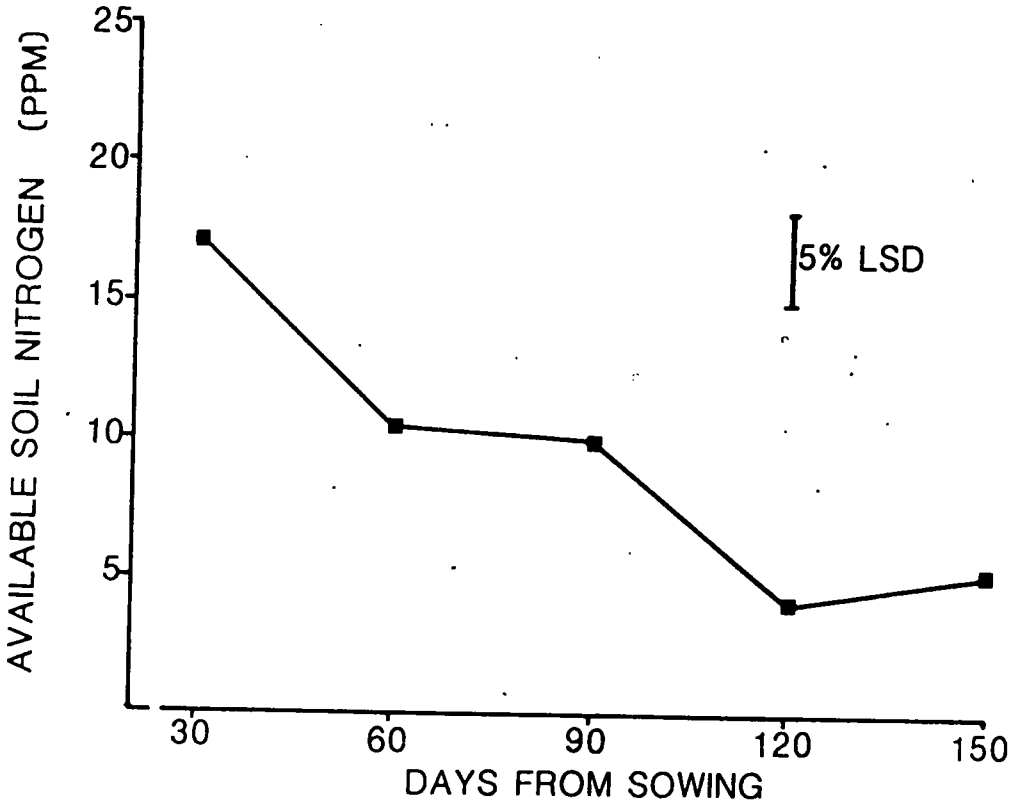

Figure 3. Variation in available soil nitrogen in plots with time.

Table 1. Effect of plant age on growth and total nitrogen in *P. phaseoloides*.

Days from sowing	Total N of plants (mg/m2) (log transformed)	Dry matter yield (g/m2) (log transformed)
30	1.933	0.3490
60	2.573	0.9386
90	3.592	1.9675
120	4.013	2.4463
150	4.243	2.6990
Significance of treatment differences	***	***
LSD 5%	0.267	0.2242

*** $P < 0.001$

Detection of more or less the same amount of nodulation on the 150th day as on the 120th day may be due to lower nodule initiation and senescence on older nodules after 120 days.

In agreement with other workers using different legumes such as *Glycine max* (Hardy *et al.*, 1971 and Ham *et al.*, 1976); *Arachis hypogaea* (Hardy *et at.*, 1971); *Vigna radiata* (Talekar and Kuo, 1979); *Vigna unguiculata* (Zary and Miller, 1980) and *Phaseolus vulgaris* (Graham and Rosas, 1979) we found total nitrogenase activity per plant increased to a maximum at flower initiation. We did not measure changes in nitrogenase activity during pod filling and maturation stages as the experiment was terminated on the 150 th day.

SEASONAL VARIATIONS IN NODULE ACTIVITY

The maximum efficiency of nodule activity (specific activity) detected 60 days after sowing differs from the findings of Ham *et al.,* (1976) for *Glycine max.* They reported that increase in nitrogenase activity per plant during flowering was also followed by specific nitrogenase activity. However, our results are in accordance with work of Trinick *et al.,* (1976) for *Lupinus* spp. and Dart *et al,* (1976) for *Cicer arietinum.* Maximum specific activity for *Lupinus* spp. was detected at 35 days after sowing whereas flower initiation occurred much later than that (Trinick *et al.,* 1976). Furthermore, they observed a minor peak at 105th day which they thought may be due to improved soil conditions and warmer weather. With regard to *Cicer arietinum* maximum specific nitrogenase activity was detected before four weeks and maximum nitrogenase activity occurred on the seventh week after sowing (Dart *et al.,* 1976). However, they observed flowering in between the fourth and seventh weeks.

Sprent (1973) suggested that the fall in specific activity may be due to a progressive shortage of photosynthate or an increase in nonactive nodule tissue under the experimental conditions. The possible reason for the significant ($P < 0.05$) reduction in specific activity after 60 days in *P. phaseoloides* may be due to an increase in nonactive nodule tissue as it seems unlikely that photosynthates are limiting under our tropical climatic conditions.

The marked fall in available soil nitrogen (Fig. 3) which continued throughout the assaying period can be attributed to two factors; utilization of soil nitrogen by *Pueraria* during its vegetative growth and leaching of nitrates due to high rainfall experienced during this period (rainfall from September 12, 1985 to February 14, 1986 was 2110 mm).

ACKNOWLEDGEMENT

Authors wish to thank Mr. D. S. Wettasingho for his technical assistance and Mr. W. N. Wickramasinghe, Biometrician for the Statistical analysis of the data.

REFERENCES

Anon, 1978. Annual Report 1977 – 1978. ICRISAT, India.

Anon, 1980. Annual Report 1979 – 1980. ICRISAT, India.

Anon, 1981. Annual Report 1981 ICRISAT, India.

Brockwell, J. 1980. Experiments with crop and pasture legumes – principles and practice In *'Methods for Evaluting Biological Nitrogen Fixation'*(F. J. Bergelsen ed.) John Wiley and Sons, Chichester. pp. 417 – 488.

Dart, P. J., Day, J. M. Islam, R. and Johanna Doberciner. 1976. Symbiosis and tropical grain legumes; Some effects of temperature and the composition of the rooting medium. In *'Symbiotic Nitrogen Fixation in Plants'.* (P. S. Nutman ed.). Cambridge University Press, London pp. 361–383.

Graham, P. H. and Rosas, J. C. 1977. Growth and development of indoterminate bush and climbing cultivars of *Phaseolus vulgaris* L. inoculated with *Rhizobium.*
J. Agric Sci. **88**: 503–508

Ham, G. E., Lawn, R. J. and Brun, W. A. 1976. Influence of inoculation, nitrogen fertilizers and photosynthetic source – sink manippulations of field-grown soybeans, In *'Symbiotic Nitrogen Fixation in Plants'.* (P. S. Nutman ed) Cambridge University Press, London. pp. 239 - 253

Hardy, R. W. F., Burns, R. C., Holsten, R. D. Jackson, E. K. 1371. Biological nitrogen fixation; A key to world protein.
Plant & Soil Special Volume 1971: 561 – 590.

Hardy, R. W. F., Holsten, R. D., Jackson, E. A. and Burris, R. C. 1968. The acetylene ethylene assay for measurements of nitrogen fixation; laboratory and field evaluation.
Plant Physiol. **43**: 1185 – 1207.

Mague, T. H. and Burris, R. H. 1972. Reduction of acetylene and nitrogen by field grown soybeans.
New Phytol. **71**: 275 – 286.

Patterson, T. G. and LaRue, T. A. 1983, Nitrogen fixation by soybeans; seasonal and cultivar effects, and comparison of estimates.
Crop Sci. **23**: 488 – 492.

Sloger, C., Bezdicker, d., Millberg, R. and Boonkerd, N. 1975. Seasonal and diurnal variations in N2 (C2H2) fixing activity in field grown soybeans. In *'Nitrogen Fixation by Free-living Microorganisms'.* (W.D.P. Stewart ed.) Cambridge University Press; pp. 271 – 284.

Sprent, J. I. 1973. Water deficits and nitrogen fixing root nodules. In *'Water Deficits and Plant Growth'*. (T. T. Koztowski ed.) Academic Press, Now York. pp. 291 – 315.

Talekar, N. S. and Kuo, Y. C. 1979. Acetylene reduction (N. – fixation) studies with mungbean.
Trop. Grain Leg. Bull. 15:9 – 14.

Trinick, M. J., Dilworth, M. J. and Grounds M. 1976. Factors affecting the reduction of acetylene by root nodules of *Lupinus* species.
New Phytol 77: 359 – 370.

Waidyanatha, U. P. de S. and Ariyaratne, W. A. 1976. Breaking dormancy in seeds of cover legumes.
J. Rubb. Res. SL. 53:8 – 16.

Weber, D. F., Caldwell, B. E., Sloger, C. and Vest, H. G. 1971. Some USDA studies on the soybean *Rhizobium* symbiosis. In *'Biological Nitrogen Fixation in Natural and Agricultural Habitats'*.
Plant & Soil Special Volume 1971. pp. 293 – 304.

Zary, K. W. and Miller, J. C. 1980. The influence of genotype on diurnal and seasonal patterns of nitrogen fixation in southern pea (*Vigna unguiculata* (L) Walp.).
J. Am. Soc. Hort. Sci. 105: 699 – 701.