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EFFECT OF COMBINED NITROGEN ON GROWTH AND NODULE FUNCTION OF *Pueraria phaseoloides*

C. K. Jayasinghe & D. S. Wettasinghe

SUMMARY

Nodulation and nitrogen fixation rates of most of the legumes are reported to be severely affected when they are grown in soils high in available nitrogen. Furthermore, this phenomenon may become more critical when legumes are grown in multiple cropping systems like *Pueraria phaseoloides* in rubber plantations where nn legume counterpart is fertilized with nitrogenous fertilizer.

Results of experiments conducted in seedling agar to find the effect of nitrogen on growth and nodule function of *P. phaseoloides* showed that nodulation of *P. phaseoloides* was inhibited at a concentration between 24.4 and 44.8 ppm nitrogen in the form of NH_4 NO_3. In pot experiments, both nodulation and nitrogenase activity of *P. phaseoloides* declined significantly ($P < 0.05$) beyond the addition of 70 ppm nitrogen as NH_4 NO_3 to the Agalawatta series soils (pH 4.3; org C 2.22%; Total N 0.25%) and completely inhibited at a concentration around 140 ppm N. However, dry matter production continued at a maximum rate of 140 ppm N depending totally on available soil nitrogen indicating that combined nitrogen and nitrogen fixation were complementary in meeting the nitrogen requirements of *P. phaseoloides*. It was also pointed out that Soil Chemists should be cautious when recommending nitrogenous fertilizer to *Hevea* plantations as there is no advantage from nitrogenous fertilizer in promoting nitrogen fixation of *P. phaseoloides*. On the other hand, high levels of nitrogen had an inhibitory effect.

Key Words:

Pueraria, nitrogen fixation, combined nitrogen, inhibition.

INTRODUCTION

The need to conserve the soil against erosion and to assist in maintaining the fertility led to the traditional practice of introducing leguminous cover crops to the rubber plantations in Sri Lanka, the most popular and widely grown cover crop being *Pueraria phaseoloides* (Roxb) Benth.

Inorganic (or available) nitrogen, applied as fertilizer or present in the soil itself through mineralization is referred to as combined nitrogen. The effect of combined nitrogen on nitrogen fixation has been discussed by several investigators (Franco, 1977; Gibson, 1976; Norris and Date, 1976; Kanehiro et al., 1983), and from their findings it could be concluded that there are three possible results of the inorganic nitrogen supply on nitrogen fixation viz. stimulatory effects, neutral effects and negative effects.

Low levels of combined nitrogen, especially when applied as a "starter" fertilizer at germination, frequently stimulates plant growth and enhances nodule mass and nitrogen fixation (Pate and Dart, 1961; Dart and Wildon, 1970; Dart *et al.*, 1976; Summerfield *et al.*, 1977; Agboola, 1978; Huxley, 1980; Minchin *et al.*, 1981; Eaglesham *et al.*, 1983).

On the other hand when most of the legumes are grown in soil high in available nitrogen, the nitrogen fixation rate is severely affected. This has been demonstrated as far back as 1917 (Wilson, 1917, cited by Franco, 1977) and more recently by Munns (1968), Dart *et al.* (1976) Eaglesham *et al.* (1983), Miller *et al.* (1982) and Graham and Scott (1984).

Inhibitory effects of high levels of combined nitrogen are significantly important in tropical soils as warm temperatures and moist conditions generally favour rapid nitrification if soil pH is favaourable (Kanehiro *et al.*, 1983). Some tropical soils with a high level of NO_3–N ranging from 18 to 400 ppm have a marked inhibitory effect on nodulation of legumes (Norris and Date, 1976). Furthermore the phenomenon of high background soil nitrogen may become more critical when legumes are grown in mixed or multiple cropping systems where non-legume counterparts are fertilized with nitrogenous fertilizers. For instance, approximately 100 kg of N $ha^{-1}Y^{-1}$ is recommended for Sri Lankan rubber plantations during the immature period of tree growth (first 4 years) where legume cover crops are also grown extensively.

The degree of inhibition appears to vary with several factors, but the most critical factor is the concentration of combined nitrogen. For example, it has been shown with plants which were grown in agar that 6 ppm nitrogen as nitrate (Norris and Date, 1976) and 15mM nitrate (Dazzo and Truchet, 1984) inhibited the nodulation of clover and 20 mM nitrate inhibited the nodulation of pea (Diaz *et al.*, 1981) Dart and Wildon, 1970 showed that nitrogen concentrations such as 240 ppm pot $^{-1}$ reduced the nitrogen fixation of *Bradyrhizobium* legume symbioses. Further it has been reported that there was no advantage in the application of nitrate in promoting nodule mass or acetylene reduction activity in three cultivars of cowpea in Texas (Miller *et al.*, 1982). With regard to *Vigna radiata* and *Vigna mungo*, nitrogen fixation of both crops was reduced with the addition of combined nitrogen, resulting in virtually no fixation at 80–100 kg of N ha^{-1} as NH_4 NO_3.

The investigations reported in this paper were designed to find (a) whether the fixation process under the influence of indigenous microbiota and inherent soil nitrogen can fulfil the nitrogen requirements for maximum growth of *P. phaseoloides* and (b) effect of fertilizer nitrogen on nodulation and nitrogen fixation of *P. phaseoloides* during early growth.

MATERIALS AND METHODS

Experiments in Enclosed Tubes

I Test plants and medium:

P. phaseoloides was used as the test plant and grown completely within cotton–plugged test tubes (4×21 cm) on 40 ml agar slopes under sterile conditions.

II Preparation of medium and planting

Seedling agar was prepared as indicated by Jensen, 1942 and Gibson 1980 and different concentrations of ammonium nitrate were added to obtain final concentrations of 0, 0.7, 1.4, 2.8, 5.6, 11.2, 22.4 44.8, 89.6, 179.2, 358.4, 716.8 and 1433.6 ppm N. Tubes were autoclaved (15psi, 120°C at 20 mnts) and sloped with the top of the agar half way up the tube.

Acid–treated seeds (Waidyanatha & Ariyaratne, 1976) were germinated on water agar and introdouced on to the slopes. There were four replicates per treatment.

III Inoculation and maintenance:

Each seedling was inoculated with one ml (around 10^8 bacteria ml^{-1}) of 7-day old *Bradyrhizobium* CB 756 culture and tubes were maintained in the glass house.

IV Harvestin :

The plants were harvested approximately 6 weeks after establishment. The tops were dried in an oven at 80°C for two days and weighed. Wet weights of the nodules of *P. phaseoloides* were recorded as the dry nodules were too small to weigh.

Experiments in Pots

I Test plants and soils:

P. phaseoloides, were grown in Agalawatta series soils (pH 4.3; Org C 2.22%; Total N 0.252%).

II Preparation of pots

Test plants were grown in 5 kg of soil using polythenelined pots. Basal nutrients (brockwell, 1980) were pipetted on the soil surface and allowed to dry. Different concentrations of nitrogen were obtained by adding solutions of NH_4 NO_3 equivalent to 0, 17.5, 35.70, 140, 280, 560 and 1120 ppm N. (Beyond concentrations of 140 ppm, NH_4 NO_3 was added in smaller quantities as 140 ppm N once a week to reach the required concentration). When all the solutions had dried the nutrients were mixed throughout the pot by shaking 50 times in a polythene bag. Distilled water was added to bring the pots up to 80% field capacity. There were eight treatments for each test species and each treatment was replicated four times to give 32 pots.

III Sowing

About 10 acid-treated seeds were planted in each pot at about 2 cm depth.

IV Maintenance

Pots were maintained in the glass house (28 ± 4° C & 70–80%RH) in a complete randomized design. Two weeks after, they were thinned to two plants per pot.

V Harvesting

Plants were harvested approximately 2½ months after sowing. Tops were removed and dry matter yield was determined after oven drying the samples at 80°C for 2 days. Nitrogen content was measured using semi-micro Kjeldahl distillation.

Acetylene reduction activity was measured in root systems with intact nodules using four hour incubation period as some treatments yielded a very low nodule mass. Separation of acetylene and ethylene was achieved on a Porapak N glass colomn (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 test plant.

RESULTS AND DISCUSSION

Experiments in Enclosed Tubes:

It was found that nodulation of *P. phaseoloides* was inhibited at a concentration between 24.4 and 44.8 ppm N as NH_4NO_3 in seedling agar (Table 1).

Plant growth continued at an increasing rate up to 179.2 ppm and growth was reduced thereafter with toxic symptoms (Table 1).

Effect of Fertilizer Nitrogen on *P. phaseoloides* Grown in Pots

The maximum dry matter yield was produced when the plants were supplied with 70 ppm N as NH_4NO_3. Thereafter, the yields continued to drop with the further supply of nitrogen, and at a concentration of 280 ppm N a significant ($P<0.05$) reduction was observed compared to the optimum nitrogen concentration for plant growth. The dry matter yield was drastically reduced when plants were supplied with 1120 ppm N (Fig. 1a).

The addition of nitrogenous fertilizer to the experimental soil had no effect either stimulatory or inhibitory on both nodulation and nitrogenase activity until a concentration of about 35 ppm N was added as NH_4NO_3. However, nodulation and nitrogenase activity declined significantly ($P<0.05$) beyond the addition of 70 ppm N and completely inhibited at a concentration around 140 ppm N (Fig. 1b & 1c).

It is interesting to note that even though nodulation and nitrogenase activity were negligible around 140 ppm N, the dry matter production continued at a maximum rate depending totally on available soil nitrogen indicating combined nitrogen and nitrogen fixation were complementary in meeting the nitrogen requirements of *P. phaseoloides* (Fig. 1).

There was no significant effect on nitrogen content of *Pueraria* until 280 ppm N was added to the experimental soils. Beyond this level the percentage nitrogen in the plant responded positively to the addition of nitrogenous fertilizer (Fig. 2a).

The results of the experiments have shown that exisitng symbiosis under inherent combined soil nitrogen in experimental soil was functioning at a maximum capacity, and it could not be further stimulated by addition of "starter" nitrogen. These observations differ from the findings of Dart and Wildon 1970, Dart *et al.*, (1976) and Eaglesham *et al.*, (1983) that low levels of supplemental nitrogen may enhance nitrogen fixation. The discrepancy might be due to the type of host plant and to different concentrations of nitrogen in experimental soils.

However, the findings of this study clearly indicates that there is no advantage from nitrate application in promoting nitrogenase activity in *P. phaseoloides*; rather, high levels had an inhibitory effect. Hence, scientists should be cautious when recommending nitrogenous fertilizer to *Hevea brasiliensis* in Sri Lankan rubber plantations if efficient atmospheric nitrogen fixation is expected from the cover crop, *Pueraria phaseoloides*. The same trend was observed for some cultivars of *Vigna unguiculata* by Miller *et al.* 1982. The inhibitory effects of heavy doses of fertilizer nitrogen have been shown by several investigators for bradyrhizobia symbioses including Dart and Wildon (1970), Ezedinma (1964), Dart *et al.*, (1976) and Eaglesham *et al.*, (1983). It is difficult to make a comparison on inhibitory or stimulatory concentrations from available literature as most of the investigators have not stated the existing combined nitrogen levels of the experimental soils.

Although the dry matter yield was significantly ($P < 0.05$) reduced beyond 280 ppm when compared to maximum growth, the total nitrogen of the plant was not affected even at a concentration of 560 ppm N (Fig. 2b). The high nitrogen content in plant tissue at these concentrations must have contributed to this. However, it appears that a concentration around 1120 ppm N as NH_4NO_3 was toxic to *P. phaseoloides*.

Table 1. *Effect of nitrogen on growth and nodulation of* P. phaseoloides *grown in seedling agar.*

N content (ppm)	*Dry matter yield (mg/plant)*	*Nodule wet weight (mg/plant)*
0	35.3	4.8
0.7	37.5	5.1
1.4	31.1	5.8
2.8	36.0	4.4
5.6	36.7	5.9
11.2	37.2	5.2
22.4	46.8	3.4
44.8	46.5	NN
89.6	44.6	NN
179.2	53.2	NN
358.4	42.8	NN
716.8	VP	NN
1433.6	—	NN

* Significance of treatment difference

LSD 5% 11.7

VP very poor growth;–could not establish the plants; NN no nodules;
*P> 0.05.

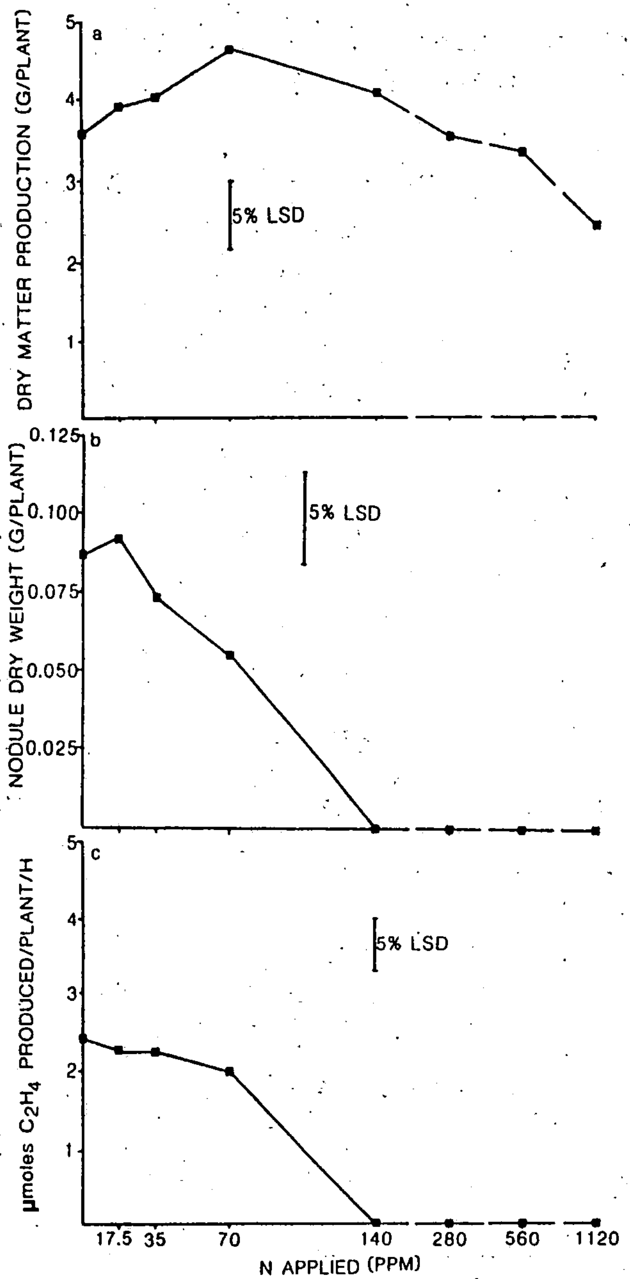

Figure 1. Effect of fertilizer nitrogen on growth (a), nodulation (b) and acetylene reduction (c) by P.phaseoloides grown in pots

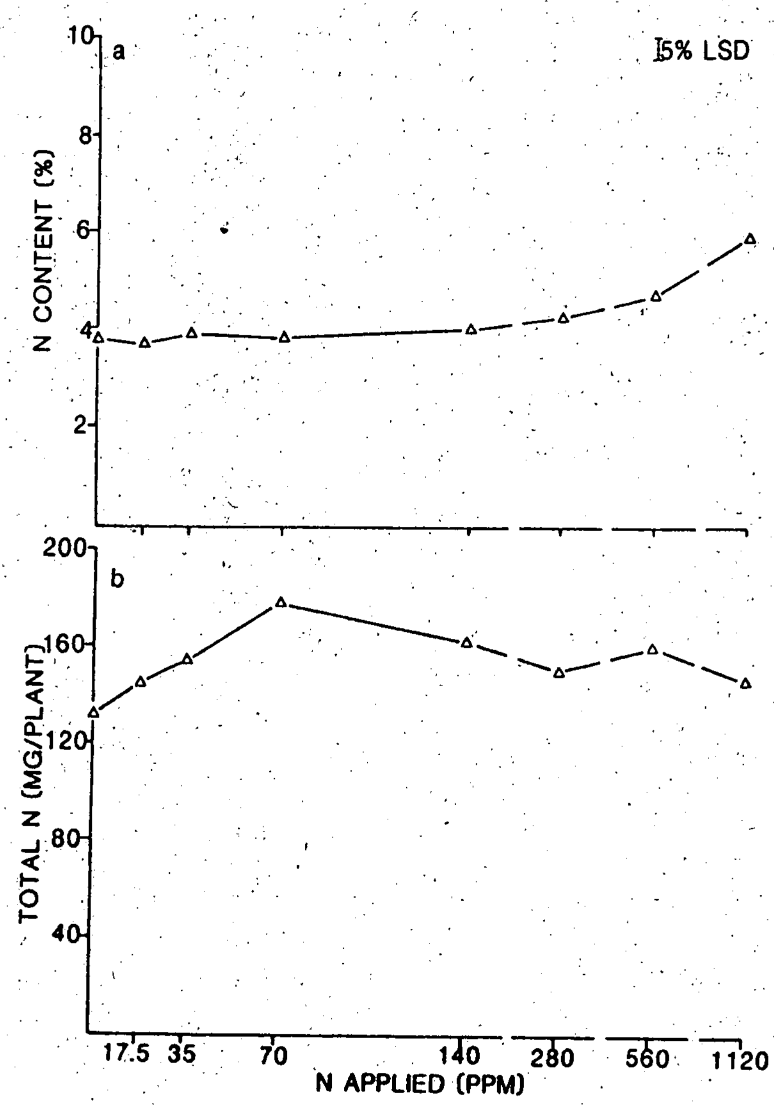

Figure 2. Effect of fertilizer nitrogen on percentage nitrogen content (a) and total nitrogen (b) in P. phaseoloides grown in pots

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