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INFLUENCE OF PHOSPHORUS AND SULFUR NUTRITION ON COMPOSITION OF *CLITORIA TERNATEA* L.

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KEY WORDS

Nodulation Nitrate N-fertilization Plant P, S, N Sugar

SUMMARY

A greenhouse experiment on a silt loam surface soil (Typic Hapludult) was done to investigate effects of P and S on yield, quality aspects and sugar reserves in the tropical forage legume *Clitoria ternatea* L. Four levels of P and two N treatments (NH_4NO_3 vs symbiotic) were arranged in a factorial design with four replications. After the first cutting two levels of S were imposed on this design.

Phosphorus enhanced dry matter yield in the first cutting. Its effect was smaller in the second cutting. Amount of P required to produce maximum plant yield dropped from 200 mg/kg soil (or more) at the first cutting to 50–100 mg/kg at the second. Added S improved growth at suboptimal levels of P. At optimal P and S, symbiotic and + N plants yielded alike.

Phosphorus and S fertilization caused several changes in plant composition. Nitrogen concentration was raised by S treatment and lowered by P. Combined addition of P and S lowered plant nitrate content. In symbiotic plants, soluble sugar concentrations were higher than in N-treated plants, and were increased by P and S treatment. In N-treated plants, neither P nor S increased reducing sugar concentration, but they increased total sugar.

INTRODUCTION

Addition of S to legumes grown at low S conditions is known to affect N metabolism and protein synthesis[1]. Increased concentrations of P may improve early establishment and function of nodules[9], probably because of greater seedling growth[18]. Fertilization of forage legumes with P and S can improve not only yield but also quality aspects such as concentrations of P, S and protein N[1,2,3,17], and sugar concentration[6,15,16].

In a previous study with the forage legume *Clitoria ternatea* L., P and S nutrition were found to affect nodulation, growth, soluble sugars and etiolated regrowth[22]. Response to P was prominent in early plant growth while response to S was greatest during regrowth after cutting. As both establishment and

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regeneration after cutting are essential features for management of perennial forage plants, a better understanding of these nutrient effects is pertinent.

The aim of the experiment reported here was to assess effects of P and S nutrition on composition of *Clitoria ternatea* L., and relate these effects to differences in plant growth and regeneration after cutting.

MATERIALS AND METHODS

The experimental was initially a 4 × 2 factorial design in randomized blocks with four replicates for each treatment. There were four levels of P (0, 50, 100 and 200 ppm, referred to as P_1, P_2, P_3, P_4, respectively) and two N treatments (inoculated with no N fertilizer, or 200 ppm N as NH_4NO_3). All treatments received a basal dressing of Zn and Mo. The soil was a Josephine loam (Typic Hapludult), 0–30 cm, as described previously[22]. *Clitoria ternatea* seed were obtained from Sudan Ministry of Agriculture, Khartoum.

Five seeds, scarified with sandpaper, were planted in each pot containing 2 kg soil. In the inoculated treatment, each seed received 1 ml of a suspension containing 10^4 viable cells of Rhizobium strain TAL173, obtained from the University of Hawaii NifTAL Project. Pots were watered to field capacity by weight with distilled water as needed. One week after emergence, plants were thinned to three per pot, and 100 ppm N was added to the + N pots. Another 100 ppm N was added 10 days later.

Eight weeks from planting, tops were cut, oven-dried and weighed. One day after cutting, half of the replicates received 15 ppm S as K_2SO_4 solution, and all the + N pots received another 200 ppm N. Then all pots were watered to field capacity. Five weeks from the first cutting, all pots were harvested again.

Tops were ground for analysis after drying and weighing. Percent N was determined by a microKjeldahl procedure[12], nitrate with phenol disulphonic acid[11], S by sulfate reduction[11] after dry ashing with silver oxide[20], P by a molybdate blue procedure[11], and total and reducing sugars by A.O.A.C. procedure[4].

RESULTS AND DISCUSSION

Dry matter accumulation

In both cuttings, P treatment increased yields of both symbiotic and + N plants (Tables 1 and 2). The increases were progressive and significant over most of the range of P addition in the first cutting. In the second cutting, the amount of P required for maximal yield diminished to a level between 50–100 ppm. Similar findings have been reported for some other legumes[7,14]. The shift in added P requirement was not accompanied by decrease in plant P and was not due to exhaustion of S. It is possible that the root system became well established during the first period of growth, hence the plant requirement for P was mainly for shoot growth. In many instances yield responses were paralleled by responses in P concentration in the shoot tissue (Table 1 and 2).

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Table 1. Effect of phosphorus supplied on dry matter yields (g/plant), NO_3-N and phosphorus concentration of inoculated and plus nitrogen plants (first cutting)

Phosphorus supplied, ppm	Dry matter yield	% NO_3-N	% Phosphorus
Inoculated			
0	0.13	0.64	0.09
50	0.27	0.51	0.14
100	0.32	0.48	0.17
200	0.54	0.62	0.19
Plus nitrogen			
0	0.13	1.17	0.10
50	0.24	0.96	0.14
100	0.35	0.68	0.18
200	0.51	0.66	0.20
$LSD_{t.05}$	0.14	0.18	0.02
$LSD_{t.01}$	0.20	0.25	0.03

Table 2. The effect of phosphorus and sulfur on dry matter yield (g/plant), NO_3-N and phosphorus content of inoculated and plus nitrogen plants (second cutting)

Phosphorus supplied ppm	Dry matter yield		% NO_3-N		%Phosphorus	
	without S	with S	without S	with S	without S	with S
Inoculated						
0	0.25	0.47	0.47	0.58	0.14	0.17
50	0.58	0.52	0.53	0.32	0.19	0.23
100	0.49	0.57	0.47	0.38	0.25	0.26
200	0.63	0.53	0.34	0.30	0.21	0.21
Plus nitrogen						
0	0.27	0.25	2.30	1.49	0.16	0.20
50	0.33	0.62	1.79	1.34	0.27	0.31
100	0.47	0.54	1.71	0.82	0.30	0.27
200	0.52	0.54	1.18	0.78	0.35	0.27
$LSD_{t.05}$	0.15		0.80		0.05	
$LSD_{t.01}$	0.21		1.09		0.06	

Sulfur deficiency symptoms (chlorosis) were observed in the first growth period only in the highest-yielding, P_4, treatment, and the relationships between yields and S concentrations were inverse (Table 1 and Fig. 1). In the second cutting, S deficiency symptoms appeared at all P levels, especially in + N plants. Yield responded to S only at certain suboptimal levels of P.

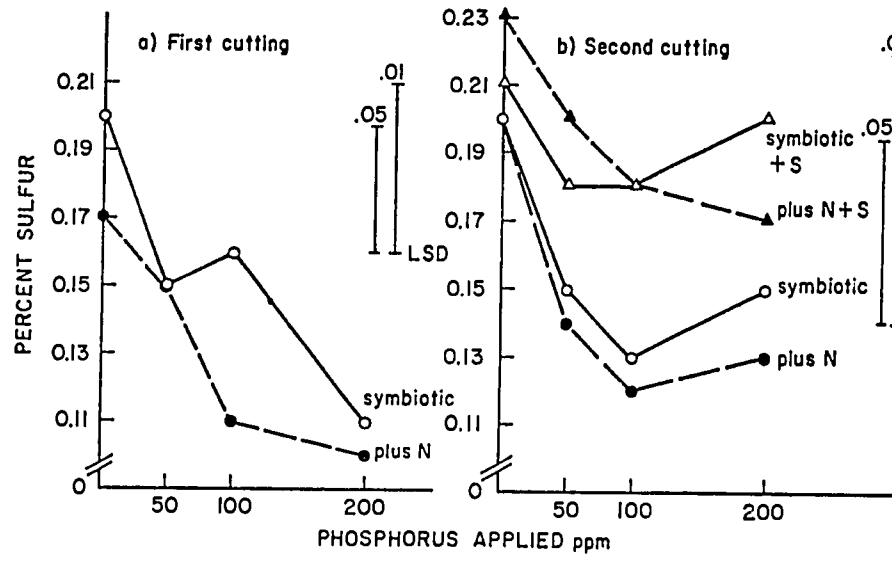


Fig. 1. Sulfur concentration in shoot dry matter, at first cutting (a), and second cutting (b).

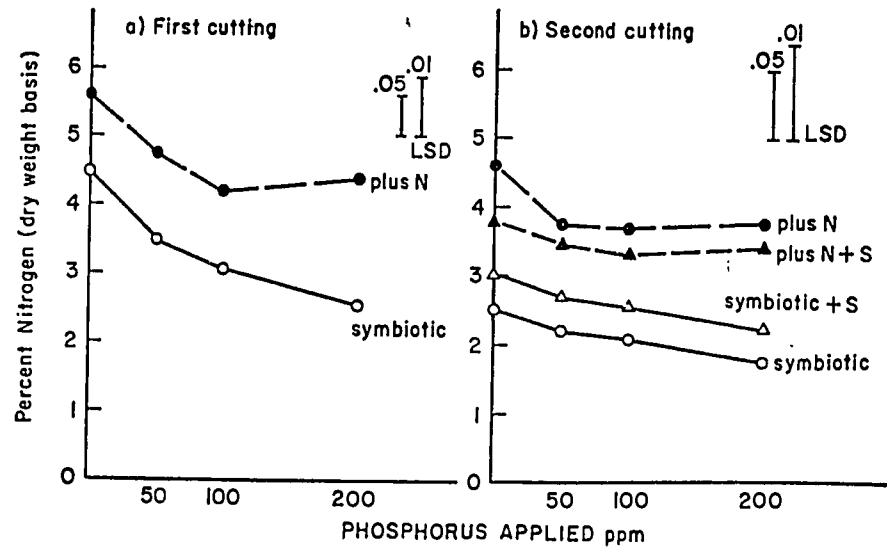
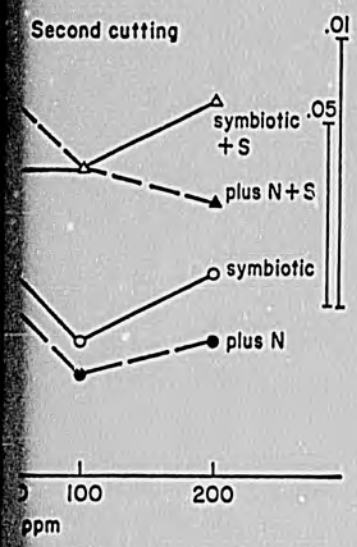


Fig. 2. Nitrogen concentration in shoot dry matter, at first cutting (a), and second cutting (b).



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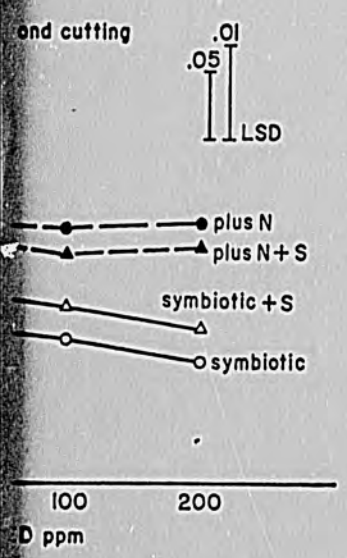

cutting (a), and second cutting (b).

Inorganic N addition did not increase yields of dry matter over that from symbiotic plants. Indeed, without added S the symbiotic plants outyielded the +N plants at most P levels supplied (Table 2).

Total and nitrate N

Total N declined gradually with increasing P supplied to both symbiotic and +N plants (Fig. 2). This contrasts with results of Andrew and Robins[2], where N concentrations in several legumes were increased by P fertilization. *Clitoria ternatea* L. might have a tendency to accumulate N under growth-limiting stress.

Nitrate was much higher in +N plants than in symbiotic plants. Adding P to +N plants consistently depressed their nitrate concentration (Table 1, 2).

In the regrowth, S addition caused marked differences in total and nitrate N (Fig. 2 and Table 2). Total N concentration in symbiotic plants increased in accordance with other findings[1,3,13]. Nitrate N was slightly lowered. In +N plants, S caused a large decrease in nitrate, enough to account for the decrease in total N. Accumulation of nitrate in S-deficient plants has been reported[1,13].

Sulfur and phosphorus

In both cuttings, increasing the supply of P lowered the S concentration in the shoots (Fig. 1a, b). Correlations between plant S and plant P were negative ($r = -0.91$, $P < 0.01$ in first cutting, $r = -0.54$, $P < 0.05$ in second cutting). Addition of S in the second period increased plant S; but S concentration was highest in the stunted zero P plants.

Phosphorus concentration in the tops increased with each increment of P supplied in the first cutting, but only with the first one or two increments in the second cutting (Table 2). There were no significant differences in P concentrations between symbiotic and +N plants at the four rates of P in the first cut, but in the second cut with addition of P the +N plants generally had higher P concentration than symbiotic plants, and any plants of the first cutting. This is consistent with the suggestion that the plants become, with age, better able to acquire soil P.

Soluble sugar

Concentrations of reducing sugar were low, less than 0.5%, in roots and stubble of N-treated plants, and were little affected by P or S. By contrast, reducing sugar was higher in symbiotic plants, and here it increased with addition of S and with

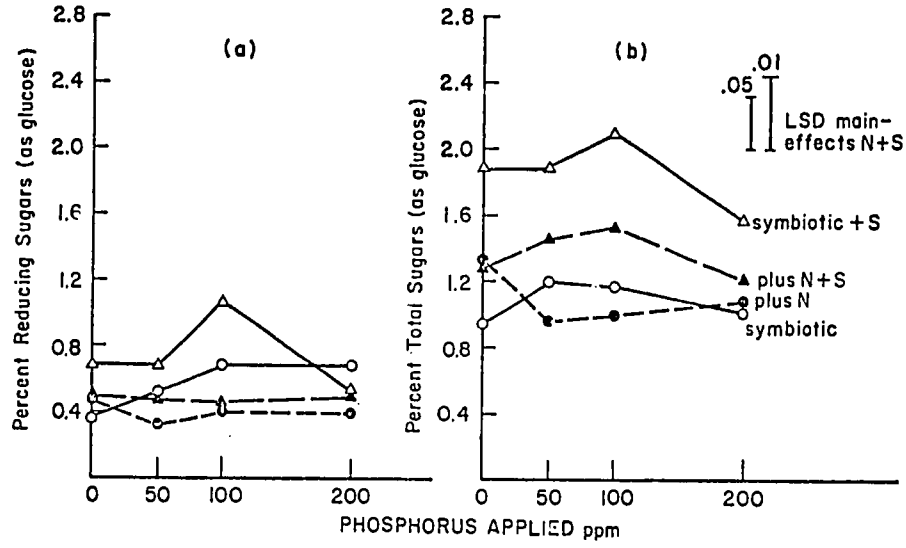

Fig. 3. Percent reducing sugars (a), and total sugars (b) in roots + stubble from second cutting.

addition of P up to level P_3 (Fig. 3a). Rendig and McComb[15] found no significant difference in the concentrations of reducing sugars in shoots of alfalfa grown with various S levels, but in a separate study[16] they found that shortage of S decreased the concentration of reducing sugar especially glucose in the stems. The latter effect was detected through use of chromatographic techniques rather than the regular Somogyi method used previously.

Total and non-reducing sugar behaved alike, hence, only total sugar is presented in Fig. 3b. Addition of S increased total sugar content (Fig. 3b), agreeing with evidence that it increased incorporation of $^{14}CO_2$ into glucose, fructose and sucrose[6]. Dry matter yield and S content for the second cutting correlated significantly ($P < 0.05$) with total sugar concentration ($r = 0.60$ and $r = 0.49$, respectively). Given S, symbiotic plants had almost 40% more total sugar than + N plants at any P level supplied. These differences between the two N levels were significant (Fig. 3b). They might imply that large net consumption of photosynthate for N_2 fixation[10] is not universal to all legumes under various growth conditions.

Symbiotic performance

Similarity of dry matter yields of symbiotic and + N plants in the first cutting and general superiority of symbiotic plants in the second cutting, suggests that

the symbiosis was highly effective or that *Clitoria ternatea* L. had difficulty utilizing mineral N for better growth even when S was supplied. The accumulation of nitrate in the tissue may indicate the latter. It precludes the possibility that N supply to the plants was limiting in the + N treatments, especially since uptake was never greater than 25% of the N added to the soil, and there was no leaching. High symbiotic effectiveness of *Clitoria ternatea* L. was confirmed in a separate study, where plants inoculated with each of four Rhizobium strains (TAL173, 29B2, TAL200NA, TAL305) all outyielded plants given 200 ppm N as NH_4NO_3 (M. G. Zaroug, unpublished).

Significance of effects on composition

Concentrations of P in the shoots reflected the dominant effects of P-supply on growth. The nitrogen concentration data argue against any important direct effect of P on nodulation (Fig. 2). For a given level of P supplied, the concentration of P in the plant was greater in the second cut, probably because of better exploration of the soil. Nitrogen and S noticeably lowered plant P at levels P_3 and P_4 in the second cut. This may indicate some antagonistic effect in uptake. It was not a result of differential onset of flowering, as found by Robinson and Jones[17] for Stylosanthes, because all P treatments flowered within a three day period when S was supplied.

The highest P level, P_4, lowered total sugar concentration (Fig. 3b). No firm explanation can be offered, but the effect has been observed before[22]. Most other effects of P were probably the consequence of dilution by increased dry matter. These include the decreases in total N, nitrate and S.

Both S and N influenced soluble sugars. The increase in sugars due to S addition was possibly simply a consequence of greater photosynthesis[6]. The reduction in sugar level by N-addition may have been due to increased root production and consequently a greater sink for carbohydrate[19]. Whatever the reason for lower sugar in + N than symbiotic plants, it is inconsistent with the supposition that N_2 fixation lowers and is limited by carbohydrate supply[10].

However, in *Clitoria ternatea* L. as in other legumes, carbohydrate reserves in nodules and roots may be important for sustaining nitrogenase activity during the night and during periods of low light intensity[5]. The reserves are also important for regeneration after cutting and for competitive efficiency in mixed stands. This may emphasize the need for enhancing food reserves of storage organs by judicious fertilization and management.

The increase in total N when symbiotic plants were supplied with S probably related to enhanced protein synthesis[1] and nodulation[1,9]. In + N plants, S

lowered N concentration in the plant, probably because it eased a restriction on protein synthesis[1,8,15] and lowered the accumulation of soluble N[8,13,16], including nitrate in this case.

Reduction of nitrate levels when P or S were added might also be partly attributable to higher total sugar, as suggested by Wright and Davidson[21], in addition to the major effect of dilution by the stimulated growth. Accumulation of nitrate in + N plants suggests that rate of assimilation had not kept pace with rate of uptake. The values of nitrate in the top material from the + N plants could be hazardous to livestock; they compare with lethal values of 0.6 to 3.5% NO_3-N quoted for other plants[21].

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