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# EFFECTS OF PHOSPHORUS AND SULFUR NUTRITION ON SOLUBLE SUGARS AND GROWTH IN *CLITORIA TERNATEA* L.

**by M. G. ZAROUG and D. N. MUNNS**

*Land, Air and Water Resources University of California, Davis, CA 95616 U.S.A.*

**KEY WORDS**

Nodulation Regrowth Root reserves Symptoms

**SUMMARY**

Effects of P and S on growth and regrowth were assessed in a greenhouse trial in which Clitoria was grown through three cuttings on a deficient soil with a factorial combination of 4 levels of P and 3 levels of S.

Phosphorus deficiency caused stunting, flowering delay, and a rusty appearance of leaflets. Sulfur deficiency caused stunting and chlorosis. With successive cuttings, P deficiency became less severe and S deficiency more severe.

Nodulation, observed at the end of the trial, was influenced more by S than by P. Maximal nodulation was achieved with the highest S level, at any of the highest three P levels.

Root and stubble dry weight, and food reserves evaluated by etiolated regrowth and by soluble sugar in roots and stubble, responded positively to both P and S, and correlated with each other and with shoot growth preceding the final cutting. For maximal etiolated regrowth, the S requirement was higher than the P requirement.

## INTRODUCTION

Phosphorus and S interact on growth of a variety of legumes when they are grown in soils deficient in both nutrients [2,3,10,19]. Different authors have reported increase in S requirement in forage legumes with successive cuttings[11,22] ; perhaps not only due to depletion of S, since species differ[11]. Phosphorus requirement sometimes decreases with time after establishment[7,16]. Therefore the present investigation was done to evaluate P and S interaction with time on *Clitoria ternatea L.*

*Clitoria ternatea is* grown and managed as a perennial forage plant. In perennial forages, the unharvested portions of the plants are particularly important as food reserves used during dormancy and subsequent regrowth [12]. Hence another aim of this investigation was to assess effects of P and S on food reserve



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accumulation. These effects were measured as soluble sugar concentration in stubble and roots and by determining mass of shoot material regenerated in the dark (‘etiolated regrowth’).

## MATERIALS AND METHODS

Seed of *Clitoria ternatea* was supplied by the Department of Range Management, Ministry of Agriculture, Khartoum, Sudan. Up to 60% of the seed normally is hard. For this reason, it was treated for 15 minutes with concentrated $H_2SO_4$ and rinsed with distilled water, with a resulting increase in percent germination from 38% to 98%.

The soil, surface (0-20 cm) material from a Josephine silt loam (Typic Hapludult), came from the Univ. of California Field Station at Hopland. It is slightly acid (pH 6.0 in saturation paste), and known to be both S and P deficient (Milton B. Jones, personal communication). Soil was air-dried, screened, mixed and weighed in 3 kg portions into plastic undrained pots with polyethylene liners. Micronutrients were applied to every pot at rates corresponding to 8 kg/ha each of $CuCl_2$, $ZnCl_2$, $MnCl_2$ and $H_3BO_3$. Sodium molybdate was added at 0.5 kg/ha.

Three levels of S (0, 20, and 40ppm referred to as $S_1$, S2, and $S_3$ respectively) were combined factorially with 4 levels of P (0, 50, 100 and 200 ppm, referred to as $P_1$. $P_2$, $P_3$ and $P_4$). Each treatment was replicated 6 times. Sulfur was added as $K_2SO_4$ solution, and P as $CaHP0_4$ powder. A solution of KCl was added to equalize K in all treatments.

Five seeds, inoculated with a Rhizobium isolate from *Clitoria ternatea* L. were planted per pot and thinned to 3 plants. Pots were watered to field capacity (29% by weight) daily with distilled water. Four weeks after planting 50 ppm N as $NH_4NO_3$ solution was applied to all pots because the foliage was yellow-green. The N corrected the symptom. Greenhouse air temperature (June-July) was kept in the range 21 to 32°C. No supplemental light was provided.

Tops were harvested 3 times, at 7,12, and 17 weeks after planting. Ten percent flowering in the $P_4S_3$ treatment was used as criterion for harvesting throughout the experiment. In the first harvest plants were cut directly above the second node, taking the cotyledons' attachment as the first node. In the second and third harvests only the new growth was clipped. Tops were dried at 70°C for 24 hours and weighed. After the third harvest, stubble and roots from 3 randomly chosen pots of each treatment were used for soluble sugar determination, and the other 3 pots were used for measurement of etiolated regrowth.

For etiolated regrowth, pots were watered the evening before the third harvest. The following morning, tops were harvested. Cotyledons and unifoliate leaves remaining below the second node were detached. Each pot then received 10 ppm S as $K_2SO_4$ solution. Pots were left in the dark for one week, when etiolated regrowth was removed, dried and weighed.

For soluble sugar determination, roots and stem bases were washed in tap water and blotted. Nodules were detached and their size and number recorded. The remaining roots and stubble were dried at 70°C for 24 hours, ground to pass a 40-mesh screen, and stored in tight plastic containers. Total and reducing sugars were determined by a Shaffer-Somogyi copper reduction procedure[s] and nonreducing sugar was obtained by difference.

## RESULTS

### *Growth, flowering and nodulation*

Phosphorus-deficient plants were stunted and had small leaves, which reached the pinnate stage (5 leaflets) a week later than in other treatments. Similar observations have been made with *Macroptilium atropurpureum*[23] and *Stylosanthes humilis*[8]. Some plants in the $P_1$ treatments had leaves with rusty appearance, more pronounced when no S was applied. Senescence of cotyledons and unifoliate leaves began early in P-deficient plants, but when P was supplied, cotyledons remained present up to the final harvest and some were still green. Growth of the $P_1$ plants progressively improved with succeeding cuttings.

Symptoms of S deficiency did not appear until the second growth period, when plants without added S developed chlorotic leaflets. This agrees with observations on *Trifolium repens* [22]. During the third period, leaflets of S deficient plants became brittle.

Flowers appeared, during the first growth period, at 5 weeks in the $P_4$ treatment, 3 days later in the $P_3$ and $P_2$ treatments, and not at all in the $P_1$ treatment. A similar flowering pattern occurred in the second growth period, except that flower buds now appeared in the $P_1S_2$ and $P_1$S3 treatments. At the third harvest, all treatments had some flowers except the $P_3S_1$ treatment.

Table 1. Effect of phosphorus and sulfur nutrition on number and size of nodules of *Clitoria ternatea*

| Treatment | | Number of nodules per plant | Nodule size (diameter mm) |
|---|---|---|---|
| P ppm | S ppm | | |
| 0 | 0 | < 10 | < 1 |
| 0 | 20 | 10–15 | 3–5 |
| 0 | 40 | 10–15 | 3–5 |
| 50 | 0 | 10–15 | 1–3 |
| 50 | 20 | 15–20 | > 5 |
| 50 | 40 | 20–25 | > 5 |
| 100 | 0 | 10–15 | 1–3 |
| 100 | 20 | 15–20 | 3–5 |
| 100 | 40 | 20–25 | > 5 |
| 200 | 0 | 10–15 | 1–3 |
| 200 | 20 | 20–25 | 3–5 |
| 200 | 40 | 20–25 | > 5 |

Nodule number and size were reduced by deficiency of S or P (Table 1). Addition of S increased nodule number and size more than addition of P and the greatest nodulation was in treatments with highest yield at the final harvest. This contrasts with observations that in *Stylosanthes humilis* S had little effect on early nodulation but P had a large effect[9].

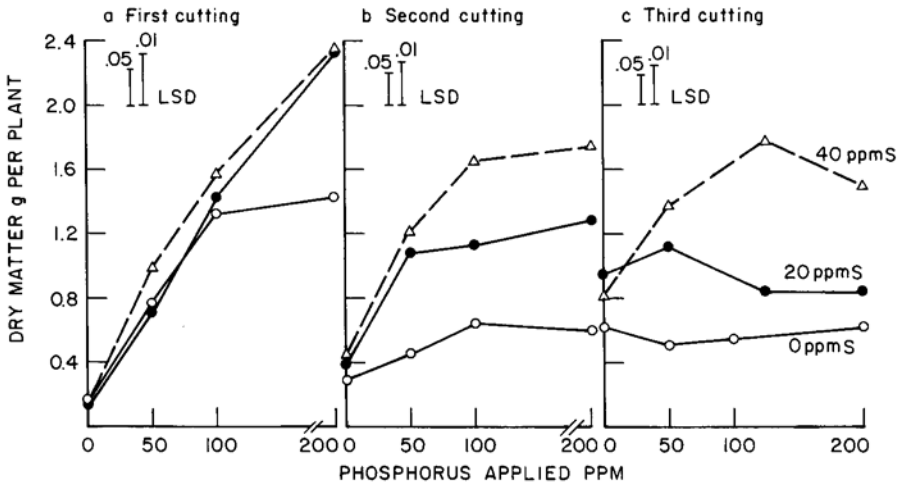


Fig. 1. Phosphorus and sulfur interactions on dry matter yield of Clitoria. a) first harvest, b) second harvest, c) third harvest.

*Shoot dry matter yield*

During the first period of growth, addition of P linearly increased yield in the presence of S (Fig. 1a). Sulfur had no effect except in the $P_4$ treatment. Similar response has been obtained with *Stylosanthes humilis* [19, 21]. In the second period, both P and S increased the yield (Fig. 1b). The first increment of P produced greater increase in yield than the second or third. In the third growth period, the effect of S increased, but the effect of P diminished: there was virtually no difference between various P levels at $S_1$ and $S_2$; and at $S_3$ the highest level of P reduced the yield (Fig. 1c). The increasing effect of S with time agrees with results for *Macroptilium atropurpureum*[11] and *Trifolium repenS*[22].

*Roots and stubble; sugars and regrowth*

Increasing either P or S gave larger roots + stubble (Table 2), both variables having greater effect the higher the level of the other. Maximal root + stubble weight was attained in the $P_4S_3$ treatment, whereas maximal shoot weight was attained in the $P_3S_3$ treatment.

Etiolated regrowth was increased by only the first increment of S or P (Table 2). Etiolated regrowth correlated highly significantly with both roots + stubble dry weight *(r = 0.874)* and total sugar content (mg/plant) in roots + stubble *(r*

Table 2. Effects of phosphorus and sulfur applied to Typic Hapludult soil on root and stubble dry weight, etiolated regrowth and soluble sugar concentration of *Clitoria ternatea*

| Treatment | | Root and stubble dry weight g/plant | Etiolated regrowth mg/plant | Percent total sugar |
|---|---|---|---|---|
| P ppm | S ppm | | | |
| 0 | 0 | 0.91 | 13.4 | 4.76 |
| 0 | 20 | 1.21 | 11.8 | 5.05 |
| 0 | 40 | 0.92 | 11.1 | 5.68 |
| 50 | 0 | 1.67 | 19.6 | 4.47 |
| 50 | 20 | 2.13 | 28.2 | 5.80 |
| 50 | 40 | 2.17 | 28.1 | 6.42 |
| 100 | 0 | 2.04 | 18.5 | 5.59 |
| 100 | 20 | 2.34 | 26.2 | 7.12 |
| 100 | 40 | 2.65 | 26.3 | 7.59 |
| 200 | 0 | 2.08 | 21.1 | 6.06 |
| 200 | 20 | 2.70 | 26.4 | 6.37 |
| 200 | 40 | 3.30 | 27.3 | 7.12 |
| $LSD_{t.05}$ | | 0.58 | 8.01 | 1.03 |
| $LSD_{t.01}$ | | 0.79 | 10.86 | 1.39 |

= 0.839). Root and stubble weight may be the most influential single factor determining regeneration of *Clitoria ternatea L.*

Both P and S increased total sugar concentration (Table 2) but had no significant effect on reducing sugar. Non-reducing sugar behaved in the same manner as total sugar. Reducing sugar is less subject to fluctuation in concentration than total sugar under varying nutritional regimes[17]. Sugar concentration was not significantly correlated with dry matter yields for the first or second harvests, but did correlate with dry matter yield in the third harvest ($r = 0.736$, $p < 0.01$) and with root + stubble dry weight ($r = 0.782$, $p < 0.01$).

## DISCUSSION

Phosphorus supply was most critical during the first period of growth. Sulfur at first was probably supplied by seed and soil reserves in amounts adequate for all except the high-yielding $P_4$ treatment. The sharp drop in dry matter of $P_4S_2$ treatment in the second and third harvests (Fig. 1) may be attributed to removal of S by vigorous plant growth. However, the decline in yield in the highest S treatment cannot be attributed to depletion of S. Less than 45 mg out of the 120 mg S applied per pot would have been removed by the 3 cuttings, which totalled about 19 g dry weight (3 plants) and contained about 0.21% S. Likewise, for the same reason, depletion of S cannot explain the diminution in P response with successive cuttings.

The influence of P on yield might have diminished in the second and third harvests because of a lowering of the external P requirement following establishment, as observed for other species[17, 16]. The effect was partly due to improvement in yield of plants receiving no applied P. This may have resulted from better root development, which explored a larger volume of soil. Development of mycorrhizae at the lower P levels cannot be overruled[4].

The main effect of P and S was possibly to improve photosynthesis, by increasing leaf area (M. G. Zaroug, unpublished), by enhancing $C0_2$ fixation per unit leaf area[6], and by improving N metabolis[1,2] . All these effects in turn could also have resulted in higher concentrations of soluble sugars in the storage organs. Rendig and McComb[18] indicated that the chlorotic condition of leaves of S-deficient plants was visual evidence of disturbance of photosynthesis; and suggested that deficiency severe enough to disrupt normal photosynthesis would ultimately reflect in changes in the kinds and amounts of carbohydrates.

Sulfur addition enhanced nodulation. S may have increased nodulation by increasing growth and nitrogen demand[2, 13.] Nodule size and number were greatest in the treatments that gave the highest yield and sugar concentration. On the other hand, increased nodule mass, if the nodules remained efficient, could have improved nitrogen nutrition of the plants with resulting improvement in the concentration of soluble sugar[14] as well as growth. Work is required to verify possible influence of P and S on the relation between N-fixation and sugars.

Discrepancies between the present observations on nodulation and those of Gates[9] with *Stylosanthes humilis* may be due to any of the following reasons: (i) the species difference; (ii) the possibility that his large effect of P may have arisen from delay in nodulation associated with early limitation of plant growth at low P[20]; (iii) the fact that nodulation in the present experiment was observed only after the third cutting, when the influence of S on plant growth was most clear and the effect of P had diminished.

The lack of response in etiolated regrowth to P supplied above 50 ppm may indicate that the P requirement was small because the plants were not photosynthesizing. Likewise, the lack of difference in etiolated regrowth between the $S_2$ and $S_3$ treatments may have been due to similarity of soluble sugar content in contrast with the low sugar of treatment $S_1$.

The overall effect of P and S was to improve plant growth and nodulation, and to increase concentrations of soluble sugar in storage organs. Part of the increase in soluble sugar may have been channeled to nitrogen fixation with consequent accumulation of N reserves, which in perennials can be used to foster early resumption of growth after dormancy[15]. Since soluble sugar correlated with dry matter yield from the third cut but not the earlier cuts, it is likely that carboydrate storage in Clitoria is mainly a function of the immediately preceding growth period.

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