

NODULATION, NITROGEN FIXATION, LEAF AREA,
AND SUGARS CONTENT IN *LABLAB PURPUREUS* AS
AFFECTED BY SULFUR NUTRITION

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

*Department of Land, Air and Water Resources, Hoagland Hall,
University of California, Davis, California U.S.A.*

KEY WORDS

Dolichos Growth Greenhouse *Lablab* Leaf area Nitrogen fixation Nodulation
Sand culture Sugars Sulfur

SUMMARY

In order to explore interrelations between S nutrition, soluble sugars, leaf area, nodulation and N₂ fixation, greenhouse experiments were done with several levels of S added to perlite-sand cultures or to a moderately S-deficient soil. Sulfur had indirect effects on nodulation and N₂ fixation, possibly by improving sugars supply and N metabolism.

In perlite-sand culture, leaf area increased with concentrations of supplied S up to 50 and 200 μM for symbiotic and N-treated plants respectively, then decreased at higher concentrations. Plant yield and total sugars content (mg per plant) for the N-treated plants behaved similar to leaf area in response to added S but in the symbiotic plants maximum values were obtained at 100 μM S. In soil, Mo had no effect on growth but interacted significantly with S in affecting total sugars content. High levels of S depressed sugars content at low Mo but raised it at high Mo.

Sulfur increased the N content of soil-grown plants. It increased the N content of plants grown in perlite-sand culture except at very high levels of S. There was little effect on concentration of N in the shoots. Nitrogen content correlated significantly with leaf area and sugar content, and highly significantly with S concentration in the shoots.

INTRODUCTION

Supply of photosynthate to the nodule is an important factor restricting nodule development and activity^{5,6,7,11,12,18,21}. The nodules require photosynthate for exporting fixed N besides the requirements for growth and respiration^{6,18,21}.

Sulfur nutrition in legumes influences nodulation^{2,14,19,20} and N metabolism^{2,17}. Also, according to Chen⁸, S deficiency decreased the chlorophyll content and rate of CO₂ assimilation in alfalfa leaves. Correction of S deficiency improved sugar concentration of the roots²³ and leaves⁸ of alfalfa. S interacted

* Now with Department of Range Management, P.O. Box 199, Khartoum, Sudan.

with P in enhancing nodulation and increasing soluble sugars content of the roots and stubble of the tropical legume *Clitoria ternatea* L.²⁶.

To our knowledge no investigation has been done with legumes to simultaneously relate effects of S nutrition on leaf area and sugar content, nodulation, nodule activity and N gain by the plant. The study reported here is an attempt to explore these relationships.

EXPERIMENTAL

Influence of S nutrition in perlite-sand culture

Six S levels and two N levels were combined factorially (Table 1) to give 12 treatments, replicated three times in randomized blocks. Perlite-sand mixture at the ratio of 2:1 (by volume) was moistened and placed in drained pots fitted with paper wicks. The mix in each pot was rinsed with distilled water, then with nutrient solution (Table 1), and allowed to drain. Then each pot was suspended in a glazed crock containing one liter of nutrient solution corresponding to a particular S treatment, the wick dipping into the solution. All assemblies were autoclaved for one hour.

Seeds (commercial seed obtained from the Department of Range Management, Khartoum, Sudan), were surface-sterilized in 30% H₂O₂ for five minutes, rinsed generously with sterilized water and planted four to a pot (February 1978). Inoculated zero-N pots received one ml (10⁴ cells) per seed of a suspension of Rhizobium strain TAL 309 (= CB756). Following emergence, seedlings were removed except for two uniform plants per pot.

Nutrient-solutions were changed every three days during the first four weeks, then every second day. Nitrogen was supplied to the + N pots starting two weeks after emergence. These pots received two ml of 1 M NH₄NO₃ with each change of nutrient solution.

Harvesting was done six weeks from emergence. Tops were oven-dried at 70°C for 24 hours. Roots were washed gently, blotted, then used for acetylene reduction activity measurements. They were

Table 1. Composition of nutrient solution[†]

Nutrient element	Compound used and its concentration	ml of the original/liter	g/liter of original solution
Phosphorus	0.5 M KH ₂ PO ₄	0.6	
Calcium	1.0 M CaCl ₂	1.0	
Magnesium	1.0 M MgCl ₂	0.25	
Nitrogen*	1.0 M NH ₄ NO ₃	2.0	
Iron	0.1 M FEDTA	1.0	
Micronutrients		1.0	
Boron	H ₃ BO ₃		0.572
Manganese	MnCl ₂ ·4H ₂ O		0.362
Zinc	ZnCl ₂		0.100
Copper	CuCl ₂ ·2H ₂ O		0.050
Molybdenum	NaMoO ₄ ·H ₂ O		0.020

[†] Sulfur was added as 0.1 mM K₂SO₄ to give: 25, 50, 100, 150, 200 and 250 μM of S.

* Nitrogen was added to plus N pots only.

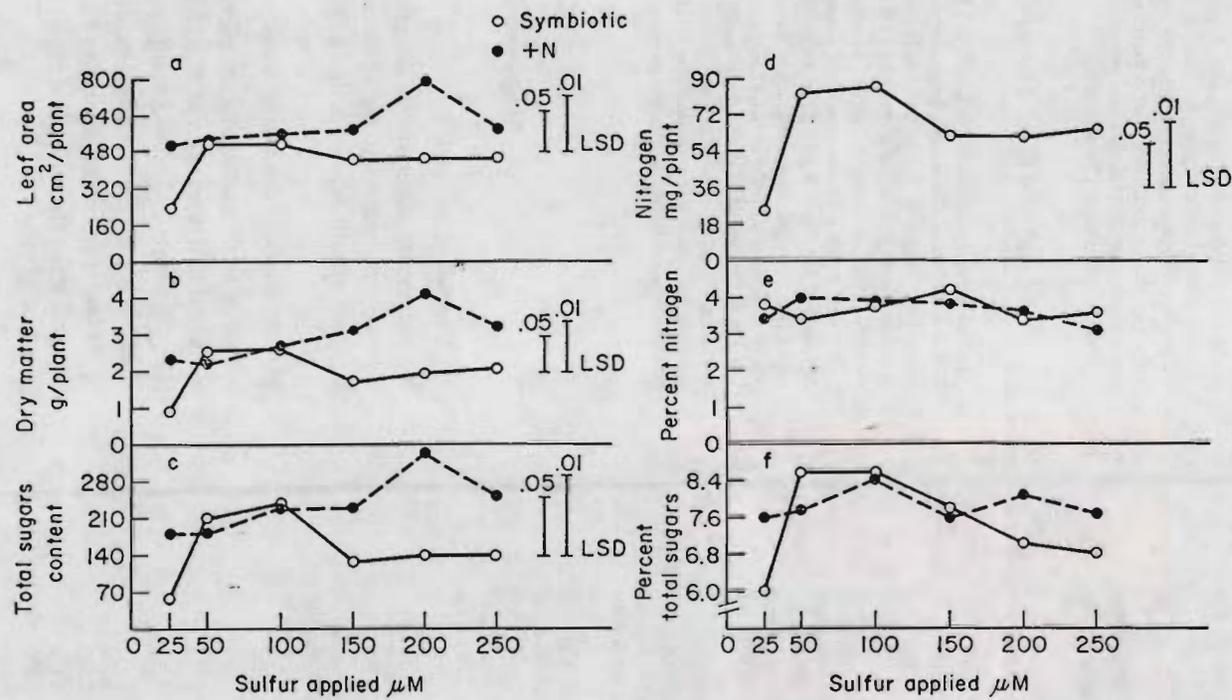


Fig. 1. Effects of S nutrition on: (a) leaf area, (b) dry matter yield, (c) total sugars content, (d) nitrogen acquired by symbiotic fixation, (e) N concentration, and (f) total sugars concentration.

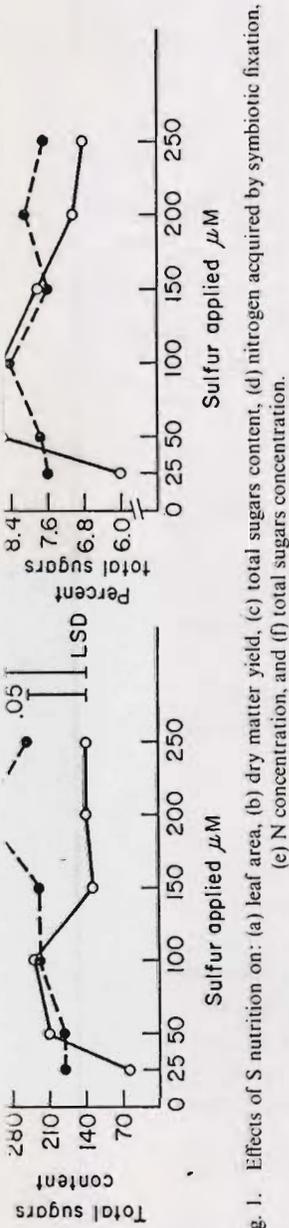


Fig. 1. Effects of S nutrition on: (a) leaf area, (b) dry matter yield, (c) total sugars content, (d) nitrogen acquired by symbiotic fixation, (e) N concentration, and (f) total sugars concentration.

c) *Total sugars content (mg/plant)*

In symbiotic plants, sugars content increased with increasing S to the 100 μM concentration then dropped at higher levels of S (Fig. 1c). Sulfur enhanced sugars content in + N plants and the maximum was reached at 200 μM S. Chen⁸ has reported increase in sugars concentration of alfalfa leaves when S deficiency was corrected. Plants receiving inorganic N had higher sugars than inoculated plants at the higher levels of S and at the lowest level, and in two instances the difference was significant (Fig. 1c).

d) *Nitrogen*

Nitrogen acquired by fixation (Fig. 1d) was calculated by subtracting seed N from total plant N. Like leaf area and total sugars, it increased to a maximum at 100 μM S and then declined at higher S. The decline was associated with only slight lowering of N concentration in the shoot (Fig. 1e). The N content of inoculated plants acquired by fixation correlated significantly ($P < 0.01$) with both leaf area ($r = 0.96$) and sugars content ($r = 0.98$). Correlation between nodule fresh weight and N content was significant only at the 5% level of probability ($r = 0.89$). Acetylene reduction capacity of nodules and S concentrations in the tops did not correlate significantly with N acquired by fixation.

Soil experiment with Mo and S

a) *Nodulation*

Nodule number per plant was affected slightly by S and Mo, but the means were not significantly ($P < 0.05$) different. Significant changes in nodule weight occur-

Table 2. Effects of addition of sulfur on nodule fresh weight, ethylene production and nitrogen yield of *Lablab purpureus* grown in soil

Sulfur applied ppm	Nodule fresh weight g/plant	Ethylene formed μmol/g nodule fresh weight/hour	Nitrogen yield mg/plant
0	1.42	5.45	65.8
10	2.02	5.02	98.1
20	2.06	11.27	102.5
40	2.13	10.90	108.7
LSD _{t,0.05}		5.81	29.7
LSD _{t,0.01}	0.53		41.0

Table 3. Effects of addition of sulfur and molybdenum on sugars concentration and total sugars content of *Lablab purpureus* tops

Sulfur applied ppm	Percent total sugars		Total sugars content	
	100 µg Mo/kg soil	200 µg Mo/kg soil	100 µg Mo/kg soil	200 µg Mo/kg soil
0	4.71	3.15	124.6	88.0
10	4.48	4.97	146.3	151.2
20	4.42	4.00	148.2	131.9
40	3.17	5.77	98.1	193.5
LSD _{t,05}	0.90		49.9	
LSD _{t,01}	1.24		68.8	

red (Table 2) without change in nodule number. Neither nodule weight nor ethylene production was influenced significantly by Mo treatment, hence the values were pooled at a given S level. The first increment of S significantly increased nodule weight. Sulfur deficiency has been reported to reduce number and weight of nodules^{1,2,19,21}.

Acetylene reduction activity, like nodule weight, was increased by S (Table 2). The correlation coefficient ($r = 0.74$) for the relation between ethylene formed and S concentration in the plant tops was significant ($P < 0.05$).

b) Dry matter and nitrogen yield

Neither S nor Mo affected plant yield significantly. Likewise, N content of the tops was not affected significantly by Mo treatment. Accordingly, the data in Table 2 represent pooled values for the two Mo levels. Increasing S supply improved yield of N to the highest S level. This was reflected in a highly significant correlation between N content and S concentration in the plant tops ($r = 0.90$)³. Plants containing only 66 mg N were chlorotic and slow in growth. From 0–10 ppm S, nodule activity was reduced slightly while N yield increased rapidly. Although S in the range 10–20 ppm doubled ethylene production it caused slight increases in N yield.

c) Sugars content (mg/plant)

Both S and S × Mo interaction affected sugars content significantly (Table 3). At the first level of Mo, S increased the sugars content to the 20 ppm S level. At high Mo, sugars content was increased up to the highest S level (40 ppm).

sugars concentration and total sugars
s tops

Total sugars content

100 μ g Mo/kg soil	200 μ g Mo/kg soil
124.6	88.0
146.3	151.2
148.2	131.9
98.1	193.5
49.9	
68.8	

DISCUSSION

Nodulation and nodule activity

The beneficial effects of S on nodulation and nodule activity are in agreement with results of other workers^{10, 14, 23}. In sand culture, the 50 μ M S supplied was apparently sufficient for nodule formation and activity. This is supported by results of Rendig and McComb²³ where S concentrations above 0.8 ppm but less than 8 ppm increased N content of alfalfa through improvement in nodulation and nodule activity. The present results differ in that they indicate that increasing S supply to 100 μ M did not significantly increase nodule weight and activity; but it did increase N yield, leaf area and sugars content, supporting the claim that S requirements for metabolism outside the nodule exceed those within the nodule²⁴. The S requirement for nodule formation was small compared to the requirement by the plants to attain maximum N content.

For soil-grown plants, the higher S requirement for nodule formation and activity at the higher Mo level suggests competition between the similar molybdate and sulfate ions for absorption and/or preutilization sites^{13, 22}.

Leaf area, sugars content and N content

Addition of S increased the sugars content of the plants as a result of improved plant yield and higher sugars concentration (Figs. 1b, 1f). The sugars concentration was increased probably by the enhancement of leaf area. Data from other studies indicate that S improved the rate of CO₂ assimilation by alfalfa leaves⁸. Reduction of leaf area at S concentration above 100 μ M can possibly be explained by the simultaneous 25% reduction in number of trifoliolate leaves per plant that was observed. This curtailment of photosynthetic tissue was ultimately reflected in lower sugars concentration. The drop in total sugars content was possibly due to the decline in both plant yield and sugars concentration as S supply was elevated above 100 μ M. Although photosynthesis was not measured, it appears that it was disrupted at levels above 100 μ M, because the decline in total sugars content (Fig. 1c) cannot be entirely attributed to reduction in leaf area or plant yield (Figs. 1a, b). Impairment of sugars concentration at these S levels (Fig. 1f) may support such interference with photosynthesis.

Limitations of growth by very low or high S were probably not in this case due to inadequate N supply, because N concentration in the plant tops remained high (Fig. 1e). Any effects of S on nodulation and nitrogen fixation were due mainly to differences in plant vigor, photosynthesis and N metabolism^{2, 17}.

er. Neither nodule weight nor
y by Mo treatment, hence the
st increment of S significantly
een reported to reduce number

it, was increased by S (Table 2).
ation between ethylene formed
icant ($P < 0.05$).

tly. Likewise, N content of the
ment. Accordingly, the data in
Mo levels. Increasing S supply
his was reflected in a highly
concentration in the plant tops
e chlorotic and slow in growth.
lightly while N yield increased
doubled ethylene production it

nent significantly (Table 3). At
it to the 20 ppm S level. At high
est S level (40 ppm).

Acetylene-reducing activity of nodules at harvest related poorly to yield of N. In the soil experiment, the increase in N yield without a corresponding increase in nodule activity at 10 ppm S could be due to the increase in nodule weight. The doubling of acetylene reduction activity at 20 and 40 ppm S did not reflect increased yield of N. This may indicate that high S promotes partial devotion of nitrogenase activity to the reduction of protons to hydrogen⁹; or that the high activity of nodules at high S expressed an effect of nodule age brought about by delay in nodulation. Such delay has been observed for greenhouse-grown peanut plants (M. G. Zaroug, unpublished).

Some workers have reported that S deficiency reduced soluble sugars concentration of alfalfa leaves⁸ and roots²³. In contrast, Anderson and Spencer² found that decrease of nodulation brought about by S deficiency was associated with an increase in percentage carbohydrate. In the present study, reduction in total sugars at the first level of Mo and high (40 ppm) S might have been due to interference of high sulfate with photosynthesis or a process related to it. Both plant yield and sugars concentration were reduced at this level of S, the reduction being significant for sugars concentration only (Table 3). Addition of more Mo moderated the effect of high sulfate to some extent as suggested by improved sugars concentration at 40 ppm S.

In another study, with *Clitoria ternatea* L.²⁶, total sugars concentration was higher for symbiotically-grown than N-treated plants. The symbiotic plants grew better. In the present study, with *Lablab purpureus*, the lower sugars content of the symbiotic plants was a consequence of their poorer growth. Also, in the *Clitoria ternatea* L. study, sugars were determined in roots and stubble as opposed to shoot material of *Lablab purpureus* here.

Improved S nutrition increased leaf area and has been shown in other studies to enhance the rate of CO₂ fixation⁸. There is reason to suppose that much of its influence on nitrogen fixation is intimately related to provision of sugars (Fig. 1f) and other photosynthetic products. Proper S nutrition should be particularly important in forage, range and pasture legumes, where leaf area is frequently reduced by cutting or grazing.

ACKNOWLEDGMENT

This investigation was partly supported by the U.S. Agency for International Development through the University of Hawaii NifTAL Project. We gratefully acknowledge advice and comment from Miss Elizabeth McComb, Dr. V. V. Rendig and Dr. D. A. Phillips.

Received 30 January 1979

st related poorly to yield of N. but a corresponding increase in increase in nodule weight. The and 40 ppm S did not reflect promotes partial devotion of hydrogen⁹; or that the high nodule age brought about by for greenhouse-grown peanut

reduced soluble sugars contrast, Anderson and Spencer² by S deficiency was associated the present study, reduction in (m) S might have been due to a process related to it. Both at this level of S, the reduction (table 3). Addition of more Mo as suggested by improved

total sugars concentration was ts. The symbiotic plants grew, the lower sugars content of poorer growth. Also, in the ed in roots and stubble as e.

been shown in other studies n to suppose that much of its o provision of sugars (Fig. 1f) rition should be particularly where leaf area is frequently

International Development through wledge advice and comment from ps.

REFERENCES

- 1 Anderson, A. J. and Spencer, D. 1949 Molybdenum and sulfur in symbiotic nitrogen fixation. *Nature* **164**, 273-274.
- 2 Anderson, A. J. and Spencer, D. 1950 Sulfur in nitrogen metabolism of legumes and non-legumes. *Aust. J. Sci. Res.* **B 3**, 431-449.
- 3 Andrew, C. S. 1977 The effect of sulfur on the growth, sulfur and nitrogen concentrations and critical sulfur concentration of some tropical and temperate pasture legumes. *Aust. J. Agric. Res.* **28**, 807-820.
- 4 Association of Official Chemists. *Methods of analysis*, 11th Ed. Washington D.C. (1970).
- 5 Bach, K. M., Magee, W. E. and Burris, R. H. 1958 Translocation of photosynthetic products to soybean nodules and their role in nitrogen fixation. *Plant Physiol.* **33**, 118-124.
- 6 Bergersen, F. J. 1977 Physiological chemistry of dinitrogen fixation by legumes. *In A Treatise on Dinitrogen Fixation III*. Eds. R. W. F. Hardy and W. S. Silver. John Wiley and Sons, Inc., New York.
- 7 Bethlenfalvay, G. J. and Phillips, D. A. 1977 Photosynthesis and symbiotic nitrogen fixation in *Phaseolus vulgaris* L. *In Genetic Engineering for Nitrogen Fixation*. Eds. A. Hollaender *et al.* Plenum Publishing Corporation, New York.
- 8 Chen, C. L. H. 1967 Assimilation of ¹⁴CO₂ by *Medicago sativa* leaves in relation to sulfur nutrition. Ph.D. Thesis, University of California, Davis.
- 9 Dixon, R. O. D. 1978 Nitrogenase-hydrogenase interrelationships in Rhizobia. *Biochimie* **60**, 233-236.
- 10 Gates, C. T. 1974 Nodule and plant development in *Stylosanthes humilis* H.B.K.: Symbiotic response to phosphorus and sulfur. *Aust. J. Bot.* **22**, 45-55.
- 11 Gibson, A. H. 1977 Limitation to dinitrogen fixation by legumes. *In Proceedings of the First International Symposium on Nitrogen Fixation I*. Eds. W. E. Newton and C. J. Nyman. Washington State University Press.
- 12 Graham, P. H. and Halliday, J. 1977 Inoculation and nitrogen fixation in the genus *Phaseolus*. *In Exploiting the Legume-Rhizobium Symbiosis in Tropical Agriculture*. Eds. J. M. Vincent, A. S. Whitney and J. Base. College of Tropical Agriculture Miscellaneous Publications 145, Department of Agronomy and Soil Science, University of Hawaii.
- 13 Gupta, U. C. and MacLeod, L. B. 1975 Effects of sulfur and molybdenum on the molybdenum, copper and sulfur concentrations of forage crops. *Soil Sci.* **11a**, 441-447.
- 14 Janssen, K. A. and Vitosh, M. L. 1974 Effect of lime, sulfur, and molybdenum on nitrogen fixation and yield of dark kidney beans. *Agron. J.* **66**, 736-740.
- 15 Johnson, C. M. and Ulrich, A. 1959 Analytical methods for use in plant analysis. *Calif. Agric. Exp. Stn. Bull.* **766**.
- 16 McKenzie, H. A. and Wallace, H. S. 1954 Kjeldahl determination of nitrogen: a critical study of digestion conditions. *Aust. J. Chem.* **7**, 55-70.
- 17 McNaught, K. J. and Christoffels, P. J. E. 1961 Effect of sulfur deficiency on sulfur and nitrogen levels in pastures and lucerne. *N.Z.J. Agric. Res.* **4**, 177-196.
- 18 Minchin, F. R. and Pate, J. S. 1973 The carbon balance of a legume and the functional economy of its roots nodules. *J. Exp. Bot.* **24**, 259-271.
- 19 Munns, D. N. 1977 Mineral nutrition and the legume symbiosis. *In A Treatise on Dinitrogen Fixation IV*. Eds. R. W. F. Hardy and A. H. Gibson. John Wiley and Sons, Inc. New York.
- 20 Oke, O. L. 1969 Sulfur nutrition of legumes. *Exp. Agric.* **5**, 111-1116.
- 21 Pate, J. S. 1977 Functional biology of dinitrogen fixation by legumes. *In A Treatise on Dinitrogen Fixation III*. Eds. R. W. F. Hardy and W. S. Silver. John Wiley and Sons, Inc. New York.
- 22 Reisenauer, H. M. 1963 The effect of sulfur on the absorption and utilization of molybdenum by peas. *Soil Sci. Soc. Am. Proc.* **27**, 535-555.

- 23 Rendig, V. V. and McComb, E. A. 1959 Effect of nutritional stress on plant composition. I. The interaction of added nitrogen with varying sulfur supply. *Soil Sci. Soc. Am. Proc.* **23**, 377-380.
- 24 Robson, A. D. 1979 Mineral nutrients limiting nitrogen fixation in legumes. *In Mineral Nutrition of Legumes in Tropical and Subtropical Soils*. Eds. C. S. Andrew and E. J. Kamprath. CSIRO, Melbourne, Australia. *In press*.
- 25 Steinbergs, A., Iismaa, O., Freney, J. R. and Barrow, N. J. 1962 Determination of total sulfur in soil and plant material. *Anal. Chim. Acta* **27**, 158-164.
- 26 Zaroug, M. G. and Munns, D. N. Effects of phosphorus and sulfur nutrition on soluble sugar and growth in *Clitoria ternatea* L., *In preparation*.