Total Sulfur and Sulfate Sulfur Content in Subterranean Clover as Related to Sulfur Responses[1]

MILTON B. JONES[2]

ABSTRACT

Subclover was grown on sulfur deficient soil in the greenhouse and in the field with various rates of S applied. Plants were harvested in the greenhouse when the first, third, and fifth flower per stem appeared, and the plant material was separated into leaflets, petioles and stems. The total S and SO_4-S concentrations in the plant did not change significantly over the three stages of growth.

The first 20 pounds per acre increased yield and the organic-S concentration, but did not change the SO_4-S concentration in the plant. Rates of S > 20 pounds per acre did not increase the yield, but increased the $SO\text{-}_4S$ more than the organic S fraction in the plant. More SO_4-S accumulated in the stems than in the petioles or leaflets at high rates of applied S, but at low levels of S there was little difference between the SO_4-S concentration of the three plant parts. There was more organic-S in the leaflets than the other plant parts at all levels of applied S.

The SO_4-S concentration was determined in whole subclover tops grown in the greenhouse on 13 soils fertilized with sulfur and phosphorus. Eight of the soils produced clover with SO_4-S concentrations of 170 ppm. or less when unfertilized and each of these soils produced more clover when sulfur was applied if adequate phosphorus was available. The SO_4-S concentration in whole subclover plants showed the same relation to yield in the field as in the greenhouse. The SO_4-S concentration in subclover identified plants deficient in S, but did not indicate the degree of S deficiency.

SUBCLOVER (*Trifolium subterranean L.*) was introduced into California by W. W. Mackie in 1933 (7), and since that time it has become an important species in the wetter areas of the state below 2,000 feet. Recent tests (3) in coastal counties have indicated that S deficiency is widespread and that many yield increases attributed to phosphorus were due to the SO_4-S content of the phosphorus carrier.[3] In view of the importance of subclover in an area of widespread S deficiency, some effects of S supply on plant growth, as related to total, organic and SO_4-S concentrations within the plant parts at various growth stages were studied.

PROCEDURE

Cole loam known to be deficient in available sulfur was taken from the 0- to 8-inch depth from a site 3 miles east of Middletown, California. The soil was passed through a 5-mm. screen, mixed, oven-dried, and 850 g. placed into each of seventy-two 5-inch painted clay pots. Each pot was fertilized with 0.16 g. KH_2PO_4 in 10 ml. of solution, plus the assigned sulfur treatment. The pots were seeded to Mt. Barker variety of subclover on Jan. 26, 1960. The KH_2PO_4 gave an equivalent of 88 pounds of P and 110 pounds of K per acre. The S was applied as Na_2SO_4 at rates of 0, 20, 40, 80, 160, and 200 pounds S per acre. There were 12 pots for each S treatment. Four pots from each treatment were clipped on each of three dates, June 6, 13, and 20 when the average number of flowers per runner was approximately 1, 4, and 7, respectively. The plants were separated into leaflets, petioles, and stems and each component was oven-dried at 70°C., weighed, and ground in a Wiley mill. Total S and SO_4-S were determined by Johnson and Ulrich's method (2).[4]

The plot area for the field experiment was a Laughlin gravelly loam, and was seeded to sub, rose, and crimson clovers and soft chess and Harding-grass in September, 1956. After seeding, the plot was fertilized with varying rates of N and P. By the spring of 1958 there was a good stand of each of the seeded species except crimson clover. However, visual symptoms of S deficiency were noted. In October 1958 the plots, arranged in a 4 by 4 Latin square, were fertilized with gypsum at the rates of 0, 20, 40, and 80 pounds S per acre. Subplots in each of the sulfur treatments were given 0, 17, 35, or 70 pounds per acre P, applied as treblesuperphosphate containing about 3% S. All of the plots were fertilized with 160 pounds of N per acre to reduce the variability from N applied in previous years. The plots were clipped February 2, 1959, after which they were closely grazed, and then clipped again May 1, 1959. Samples of subclover were taken from each plot for chemical analysis just before the last harvest. For chemical determinations, plant samples from the field were handled in the same way as greenhouse plants, except that whole plants were used.

A second greenhouse experiment was started in early February, 1960. In this experiment, 13 different soils in 5-inch pots were seeded to subclover, after the following treatments had been made within each soil type: *(1)* Zero S and P; *(2)* 44 pounds P per acre; *(3)* 50 pounds S per acre; and *(4)* 44 pounds P per acre plus 50 pounds S per acre. Sources for these two elements were Na_2SO_4 and NaH_2PO_4. Each treatment was replicated 3 times. The plants were harvested May 31, before flowering had commenced. The whole plants were dried, weighed, and ground for SO_4-S determinations.

The data from the three experiments were analyzed statistically.

RESULTS AND DISCUSSIONS

In the greenhouse study the clover was harvested at three dates. Yields increased as the season advanced, but the SO_4-S content of the clover did not change over the period of time the plants were harvested.

The results shown in figure 1 are means of the three harvest dates. In the pots which were not fertilized with S the SO_4-S concentration of the three plant parts was very nearly equal, ranging between 90 and 140 ppm. The average yield was 3.6 g. per pot. When 20 pounds per acre S was applied the yield increased to 9.4 g. per pot, however, the SO_4-S concentration increased only slightly with values ranging from 110 to 160 ppm. With each additional increment of sulfur above 20 pounds per acre, there was very little increase in yield, but there was a large increase in the SO_4-S concentration. More SO_4-S accumulated in the stems than in the petioles or leaflets at the high rates of sulfur application.

The effects of increasing amounts of applied S on the total S concentrations of the three plant parts are given in figure 2. There was no significant change in total S concentration in the clover over the flowering period of the clover studied. Therefore, the values of the three harvest dates were combined. Total S in the petioles and stems was about 1,000 ppm. where no S was applied and increased only slightly when 20 pounds S per acre was applied. About 1,600 ppm. total S was in the leaflets in

[1]Contribution of the Agronomy Department, University of California, Davis. Received Nov. 24, 1961. Approved Jan. 3, 1962.

[2]Assistant Agronomist, University of California, Hopland Field Station, Hopland, Calif.

[3]Unpublished observations of test plots in Lake and Mendocino Counties by Milton Jones in cooperation wtih Farm Advisors W. C. Lusk and W. E. Brooks.

[4]The author wishes to acknowledge the assistance of Joseph Ruckman, Agronomy Department, University of California, Davis, for his services in carrying out the chemical determinations.

the unfertilized plants, and application of 20 pounds S per acre increased the total S in the leaflets to 2,000 ppm. Additions of S above 20 pounds per acre increased the total S concentration in all the plant parts rapidly, but the stems accumulated more than the other plant parts. Rendig and McComb (4), working with alfalfa in solution culture, found that three levels of sulfate had no significant effect on total S content of the stems, but increasing the level caused an increase in the S concentration in the leaves. Rendig and McComb's results with alfalfa agree substantially with the total S levels in subclover where 20 pounds of S per acre was applied.

Johnson and Ulrich (2) indicated that organic S may be obtained by subtracting SO_4-S from total S. Most of the first increment of S added went into the organic fraction of the plant, but with additional increments of S the SO_4-S fraction increased much more rapidly than the organic fraction (figure 3). Concentrations of SO_4-S were more nearly equal in the three plant parts than the organic S values where S was deficient. The leaves were highest in organic S throughout the curve and the stems were highest in SO_4-S where large amounts of S were added.

Because there was little difference between SO_4-S values of the three clover parts in S-deficient plants, SO_4-S concentrations were determined in whole plants taken from the field. Figure 4 shows the relation of total forage yield in the field to SO_4-S concentrations in subclover with increasing amounts of applied sulfur. Where no sulfur was applied, 3,400 pounds of forage per acre were produced. Subsequent tests indicate that applications of P at the site of the field experiment did not increase plant growth. It was therefore concluded that the 3% S content of the treblesuperphosphate increased the yield to about 4,500 pounds of forage per acre when 3 and 6 pounds S per acre was applied. There was no significant difference in yield between plots receiving from 12 to 90 pounds S per acre. The SO_4-S concentration in the clover was from 70 to 120 ppm. as the rate of applications increased from 0 to 12 pounds S per acre. Additions of S at rates $>$ 12 pounds per acre increased the SO_4-S concentration rapidly.

When S alone was applied to 13 soils in the greenhouse (table 1) clover growth was increased on 5 (Cole No. 1, Konokti, Manzanita, Pinole No. 1, and Rincon No. 2). The unfertilized subclover growing on each of these soils had SO_4-S concentrations of 170 ppm. or less.

Both S and P were required to increase yields on 4 of the soils (Hugo, Klamath, Laughlin, and Rincon No. 1). The unfertilized clover on these 4 soils had SO_4-S concentrations of 90, 860, 100 and 90 ppm., respectively. Fertilization with P alone reduced the SO_4-S concentra-

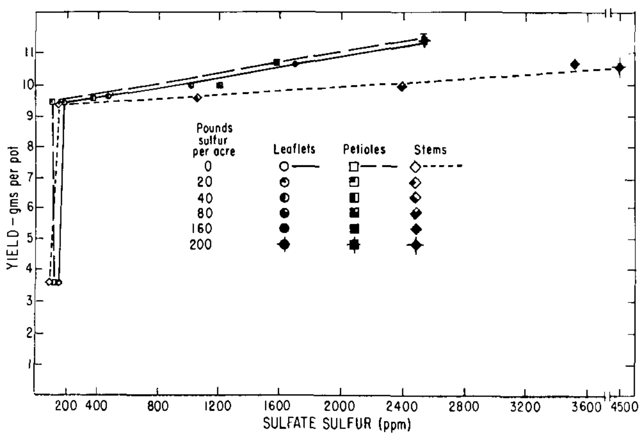

Figure 1—Relation of yield to the SO_4-S concentration in the leaflets, petioles, and stems of flowering subclover plants receiving increasing amounts of S.

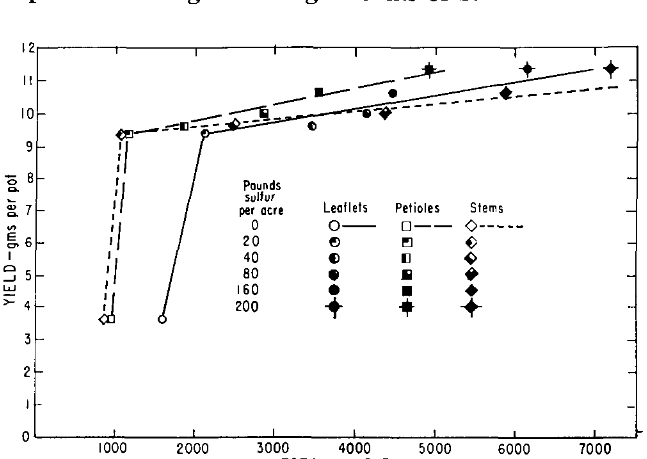

Figure 2—Relation of yield to total S concentration in the leaflets, petioles and stems of flowering subclover plants receiving increasing amounts of S.

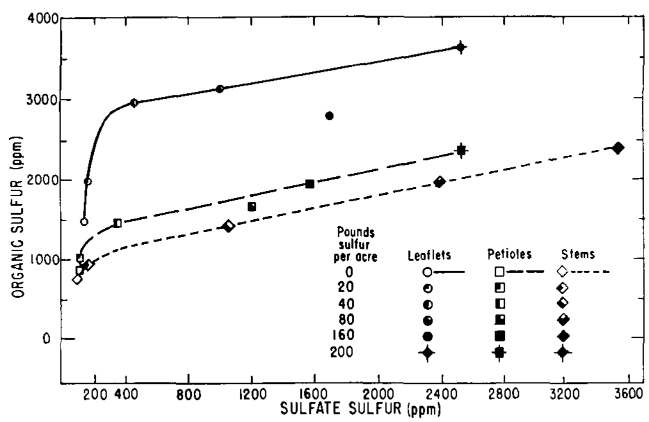

Figure 3—Relation of SO_4-S to organic S in the leaflets, stems and petioles of subclover plants fertilized with increasing rates of S.

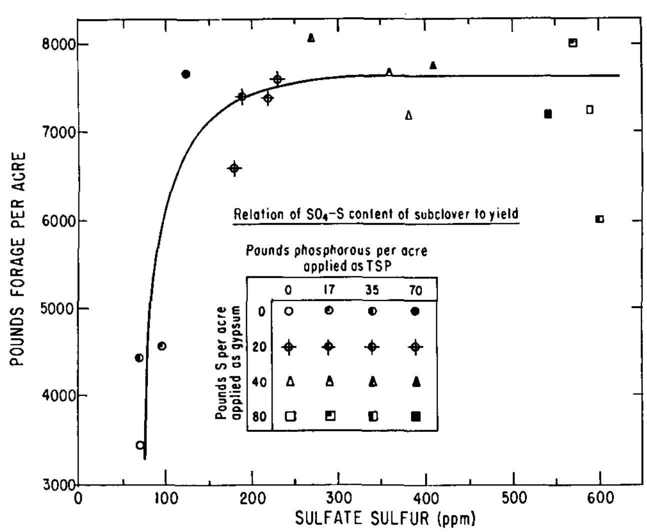

Figure 4—Relation of total forage production to the SO_4-S content of subclover plants on plots fertilized with increasing amounts of S.

Table 1—Effect of phosphorus and sulfur on the yield and SO_4-S content of subclover at preflowering in the greenhouse.

No.	Soil series	Treatments				LSD 5%	Treatments			
		P_0S_0	$P_{44}S_0$	P_0S_{50}	$P_{44}S_{50}$		P_0S_0	$P_{44}S_0$	P_0S_{50}	$P_{44}S_{50}$
		Yield, g./pot					SO_4-S concentration, ppm.			
1	Aiken	5.8	7.7	4.7	4.0	3.1	180	250	3130	2500
2	Cole #1	3.0	3.2	10.7	9.6	1.7	110	110	810	1150
3	Cole #2	1.5	10.5	1.4	9.9	2.2	700	130	2470	1370
4	Hugo	7.0	6.6	9.4	10.6	3.6	90	110	1090	960
5	Klamath	1.2	3.7	1.2	5.1	1.0	860	110	2040	740
6	Konokti	3.5	2.9	8.6	7.0	1.1	110	110	360	370
7	Laughlin	1.9	2.9	3.1	6.7	1.5	100	80	1100	1390
8	Manzanita	4.8	5.2	8.8	13.0	1.6	150	160	2050	940
9	Newville	0.5	2.2	0.5	1.8	0.6	780	100	300	1910
10	Pinole #1	3.0	3.6	5.2	7.0	1.0	170	130	1680	1640
11	Pinole #2	1.2	3.9	0.6	4.3	2.2	3410	1350	5440	2930
12	Rincon #1	3.2	4.5	3.5	7.5	3.2	90	150	2020	1130
13	Rincon #2	4.6	4.8	8.6	7.4	1.4	90	90	230	600

tion of the Klamath from 860 to 110 ppm. Although S was adequate when P was not applied, S became deficient when P was adequate in the Klamath soil. All of the clover growing on the 4 remaining soils (Cole No. 2, Newville, Pinole No. 2 and Aiken) had SO_4-S concentrations of 180 ppm. or greater when no fertilizer was applied and no increase in yield resulted when S was applied. When P only was applied the SO_4-S concentrations of clover growing on Cole No. 2 and Newville dropped to 130 and 100 ppm., respectively, indicating that S supply was inadequate. Yet there was no response to S. The soil structure of Newville was extremely poor and probably accounted at least in part for the low yield. The Cole No. 2 soil produced 10.5 g. of clover per pot when 44 pounds per acre P was applied. Either more P was needed or some other factor was limiting growth more than S on Cole No. 2.

Ulrich (6) defined critical concentration as that nutrient concentration which is just deficient for maximum growth. He indicated that the critical concentration of SO_4-S for sugar beet leaf blades is approximately 250 ppm. Dijkshoorn (1) stated that S deficiency occurs in perennial ryegrass when SO_4-S concentration is 0.01 g. atom per kg. (320 ppm.). Subclover with SO_4-S concentrations $>$ 170 ppm. did not increase in growth when S was applied to the soil, and subclover growth with SO_4-S concentrations $<$ 170 ppm. was increased by the application of S to the soil if no other factors were limiting. Therefore, the critical SO_4-S concentration for subclover appears to be about 170 ppm. Certainly more work will be required to determine the critical value over a wide range of conditions for field use.

LITERATURE CITED

1. Dijkshoorn, W., J. E. M. Lampe, and P. F. J. Van Burg. A method of diagnosing the sulfur nutrition status of herbage. Plant and Soil 13:227-241. 1960.
2. Johnson, C. M., and A. Ulrich. Analytical methods for use in plant analysis. California Agr. Exp. Sta. Bull. 766:54-58. 1959.
3. Martin, W. E. Sulfur deficiency widespread in California soils. Calif. Agr. 12(11):10-12. 1958.
4. Rendig, V. V., and E. A. McComb. 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. 1959.
5. Spencer, K. Growth and chemical composition of white clover as affected by sulfur supply. Australian J. Agr. Res. 10:500-509. 1959.
6. Ulrich, A. Plant analysis in sugar beet nutrition. Am. Inst. of Biol. Sci. Publ. No. 8. pp. 190-211. 1961.
7. Williams, W. A., R. M. Love, and L. J. Berry. Production of range clovers. California Agr. Exp. Sta. Ext. Serv. Circ. 458. 1957.