Indexes of Sulfur Deficiency in Alfalfa. II. Plant Analyses[1]

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ABSTRACT

Both soil and plant analysis are diagnostic tools used in identifying S deficiencies; however, soil tests evaluating soil S availability levels are not always successful and deficiencies must then be identified by plant analysis. In addition, the diagnostic tool must be correlated to crop responses under various growing conditions to be useful. Identification of S deficiency on alfalfa (*Medicago sativa* L.) in southern Idaho permitted the collection of correlation data for both S soil tests and plant analyses. The S soil test correlation data has been previously reported (Agron. J. 66:578-581, 1974). This paper reports the relationships found between the plant S indexes of total S, SO_4-S and total (N/S) ratio, and the response of alfalfa to S fertilization. All data were evaluated by correlation analyses.

The total S and SO_4-S concentrations and the total (N/S) ratio were all found to be satisfactory indexes of S deficiency in whole alfalfa at early bloom. Maximum forage yields were obtained when the tops contained between 0.15 to 0.20% S or 0.05% SO_4-S. Total S and SO_4-S were related and readily interchangeable as indexes. Increases in total S above 0.14% S resulted from the accumulation of SO_4-S. Yield responses to S fertilization were obtained when the total (N/S) ratio was greater than 17 to 18. Total N and total S were not related, but protein N increased linearly as protein S increased. The protein (N/S) ratios were not constant and increased from 17 to 23 as the degree of S deficiency increased.

Additional index words: **Total S, SO_4-S, Total (N/S), Protein (N/S), Lucerne, *Medicago sativa* L.**

IDENTIFYING nutrient deficiencies by plant analyses is based on the concept that plant growth will not be restricted when the concentration of an essential element exceeds a critical level. This concept is useful since the critical nutrient level should be a constant for a given plant part, at a given stage of development of a species grown over a wide range of soil and climatic conditions. Two general classes of plant analyses may be used: (a) total analysis, which measures both the assimilated and unassimilated nutrient concentrations, and (b) tissue test analysis, which measures only the latter or some chemical form of the nutrient.

Total S and SO_4-S, both expressed as percentages of the dry matter, have been used as indexes of S sufficiency in alfalfa (*Medicago sativa* L.). Critical concentrations of total S in alfalfa are dependent upon stage of development (16), but generally group around 0.20 to 0.22% S in whole tops at early bloom (5, 11, 13). Approximately 150 μg/g SO_4-S in the second to fourth mature leaf, and 40 μg/g SO_4-S in the midstems at the bloom stage are critical concentrations under greenhouse conditions (21). However, S-deficient plants contained 250 to 300 μg/g SO_4-S, and S-fertilized plants contained over 700 μg/g SO_4-S in a field study, suggesting a critical level of whole plant tops at early bloom near 500 μg/g SO_4-S (12). The use of SO_4-S concentrations in plant materials is attractive for determining S status, since SO_4-S can be determined rapidly and accurately without preliminary dry or wet-ashing.

Several researchers (3, 4, 10, 19) have also suggested that the N concentration of the plant be considered in a (N/S) ratio in evaluating S deficiencies. When this concept is used, the (N/S) ratio in the protein fraction (alchol-insoluble) of a plant species is assumed to remain constant, but the total N/total S ratio, $(N/S)_T$, increases above the protein N/protein S ratio, $(N/S)_P$, under S deficiency. This occurs because (a) protein synthesis is limited by S deficiency and nonprotein N increases, and (b) nonprotein S decreases. This concept also suggests that an increase in protein N is accompanied by a proportional increase of protein S. The $(N/S)_P$ ratio has been found to range from 13.6 in Gramineae plants to 17.5 in Leguminoseae plants (4, 19).

The use of a $(N/S)_T$ ratio should overcome some of the problems associated with nutrient changes in maturing plants since it is nearly constant in alfalfa at different stages of development (16). In an Oregon study, $(N/S)_T$ ratios below 11 in alfalfa tops indicated an adequate S supply, whereas values from 15 to 25 indicated increasing severity of S deficiency (17). Good relationships between the $(N/S)_T$ ratio and S status for ryegrass (*Lolium perenne* L.) (8), coastal bermudagrass (*Cynodon dactylon* (L.) Pers.) (11), and sugarbeets (*Beta vulgaris* L.) (10) have also been reported. However, not all studies are in agreement (2, 18, 22), and recently the constancy of the $(N/S)_P$ ratio has been questioned (9, 15).

The first paper in this series reported the relationships found between soil SO_4-S levels and alfalfa response to S fertilization (23). This paper presents the relationship found between the total S, SO_4-S, and $(N/S)_T$ ratio in whole alfalfa tops at early bloom and the plant response to S fertilization, and the effect of S deficiency on the (N/S) ratio in the alcohol-insoluble (protein) fraction.

METHODS AND MATERIALS

Thirteen field experiments were conducted in 1970 and 1971 in the mountain valleys found in the Idaho counties of Camas, Custer, and Teton. These valleys are at elevations of 1,520 to 1,850 m, have 60 to 90 frost-free days, and receive 180 to 380 mm annual precipitation. Soil moisture was not limiting for the first harvest. A description of the soils at each location have been given previously (23). In general, these soils are classified in the Mollisol, Entisol, or Aridisol orders. All experimental locations had been seeded to the cultivar 'Ranger' for 2 to 10 years. Sulfur, as $CaSO_4 \cdot 2H_2O$ was applied to the soil surface at the rate of 22 to 67 kg S/ha. Plant analyses indicated that all other essential plant nutrients were adequate.

Only the plant yield and nutrient concentration data from the control and maximum yielding S treatments for the first harvest at early bloom are discussed. Each datum point for yields and chemical analysis represents an average of four replications. All plant materials were oven-dried at 55 C and ground to pass a 40-mesh screen before chemical analysis. The yield data have been expressed as relative yield, i.e., relative yield (RY) = (yield without S ÷ yield with S) × 100. Curvilinear

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regression equations were used when they gave a significantly better fit than linear equations. All indexes of determination (R^2) and coefficients of determination (r^2) reported were significant at the 1% level.

Total S was determined turbidimetrically (20) after wet-ashing, and total N and protein N by the semi-micro Kjeldahl procedure modified to include nitrate (1). The $(N/S)_T$ ratio was then calculated by using the total N and total S concentrations. Soluble SO_4-S was extracted from the plant materials by 2% acetic acid in a 1 100 ratio with carbon black added and determined turbidimetrically (20) on the filtrate after filtering through Whatman No. 50 filter paper.[3]

To determine the $(N/S)_P$ ratio, a 0.5 g plant sample in 33 ml of 70% (v/v) ethanol was placed in a hot water bath at 80 C and digested for 9 min after the ethanol started to boil. This extract was filtered through a No. 41 Whatman filter paper and washed with the 70% ethanol until approximately 100 ml of filtrate were collected. The residue was dried and separate portions were used for N and S analysis. For protein S, a weighed portion of the residue (ca. 0.25 g) was placed in a 50 ml beaker and 3 ml of 50% (w/w) $Mg(NO_3)_2$ were added, dried at 105 C, and ashed for 2 hours in a muffle furnace at 550 C. After cooling, 10 ml of 3.8 *N* HCl were added to the ash and warmed slightly. This solution was then diluted to 25 ml and filtered through a No. 50 Whatman filter paper. Protein S was determined on a 10 ml aliquot of the filtrate by the turbidimetric procedure (20). The $(N/S)_P$ ratios are based on the protein N and protein S concentrations, which are expressed as percentages of the alcohol-insoluble residue.

RESULTS

Sulfur fertilization significantly increased forage yields on 9 of the 13 experimental sites where the control (without S fertilization) yields ranged from 0.94 to 4.46 metric tons/ha. Yields on the S-fertilized treatments ranged from 1.68 to 5.57 metric tons/ha.

An excellent relationship was obtained between relative yield and percent S at early bloom (Fig. 1). Percent S ranged from a low of 0.065 to a high of 0.211 on the control treatments. Maximum yields were obtained when the tops contained between 0.15 and 0.21% S. Percent S in the tops of the S-fertilized plants ranged from 0.175 to 0.245. Only one experimental site produced forage with less than 0.20% S after S fertilization.

Maximum yields were obtained when the tops contained at least 0.05% SO_4-S (Fig. 2). This concentration is identical to that found in whole alfalfa tops by Rendig as reported by Martin and Walker (12). This relationship also closely follows that predicted by the Mitscherlich equation if one growth unit is 0.01% SO_4-S (dashed line, Fig. 2). The SO_4-S concentration of the whole tops appears to be nearly as good as index of S deficiency as total S (Fig. 1 vs. Fig. 2); however, SO_4-S has been found to be superior when only the second to fourth mature leaf is used for analysis (20). The S indexes, percent S and SO_4-S, are readily interchangeable (Fig. 3), although the relationship is curvilinear. These data also indicate that above 0.14% S the increase in total S results from the accumulation of SO_4-S.

The relative yield was inversely related to the $(N/S)_T$ ratio of the control treatments (Fig. 4). The regression line indicates that maximum yields were obtained when the $(N/S)_T$ was approximately 12, but no responses to S fertilization were measured when this ratio was less than 17-18. Ratios increased

[3] The use of trade names or proprietary products does not imply its approval by USDA to the exclusion of other products that may also be suitable.

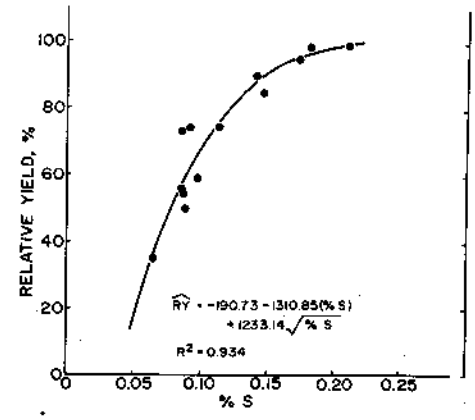

Fig. 1. The relationship between percent S (total S) and relative yield of alfalfa. The R^2 is significant at the 1% level.

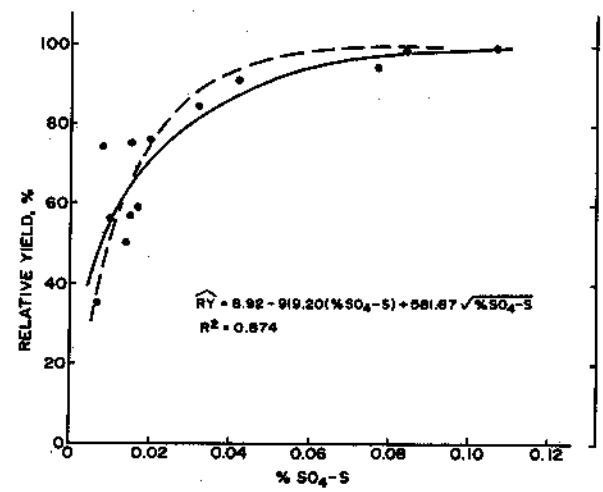

Fig. 2. The relationship between percent SO_4-S and relative yields of alfalfa. The R^2 is significant at the 1% level. Dashed line is Mitscherlich's equation using 0.01% SO_4-S as one growth unit.

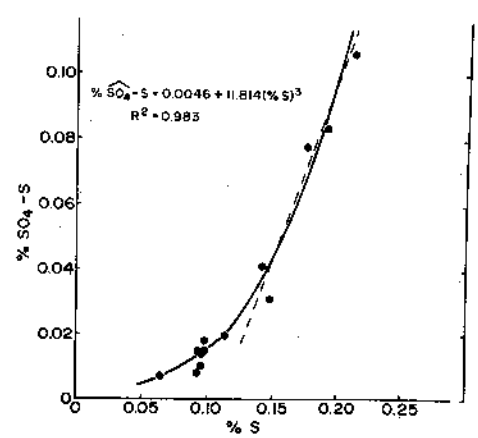

Fig. 3. The relationship between percent S and percent SO_4-S in whole alfalfa tops at early bloom. The R^2 is significant at the 1% level. Dashed line is drawn freehand.

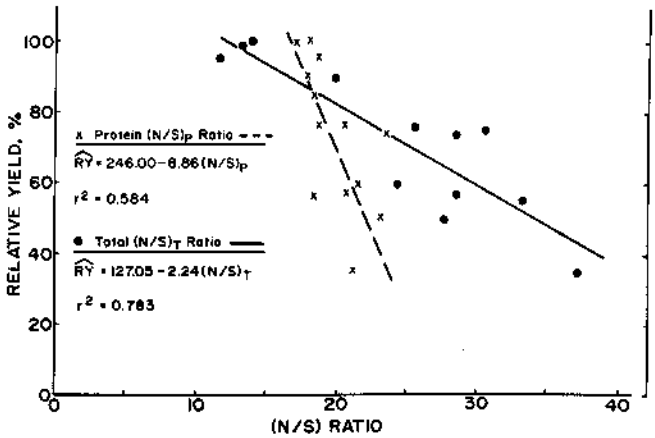

Fig. 4. The relationship between the $(N/S)_P$ and $(N/S)_T$ ratios, and relative yields of alfalfa. Both r^2's are significant at the 1% level.

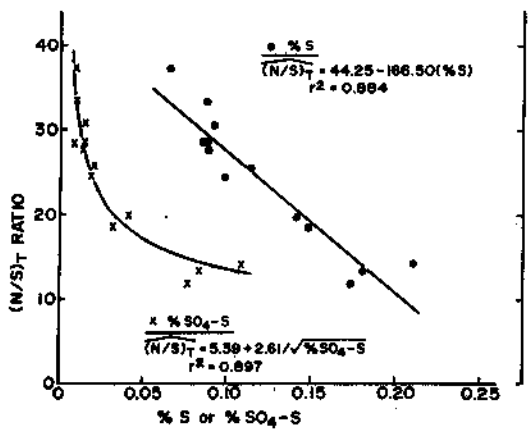

Fig. 5. The relationship between the percent S and SO_4-S, and the $(N/S)_T$ ratio. Both r^2's are significant at the 1% level.

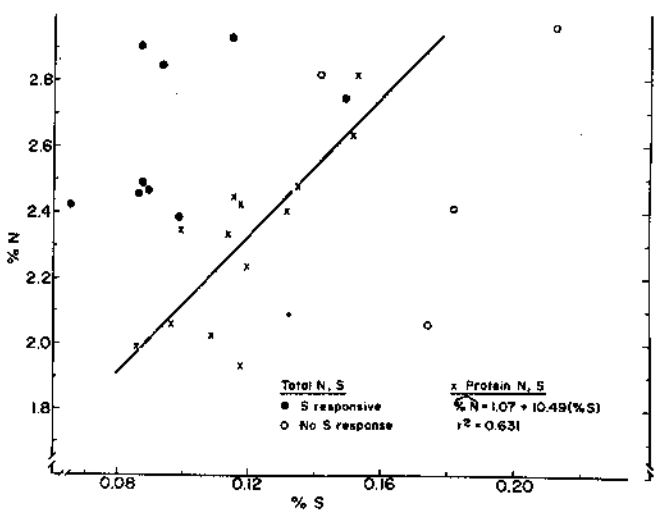

Fig. 6. The relationship between protein N and S, compared with total N and S. The r^2 for the protein N-S relationship is significant at the 1% level.

to greater than 30 as the severity of S deficiency increased. The $(N/S)_T$ ratio decreased as the percent S increased in the alfalfa tops (Fig. 5), indicating that the $(N/S)_T$ ratio largely reflects the S nutritional status of the plant. No relationships were found between the percent N and the $(N/S)_T$ ratio or relative yield. In comparison, the $(N/S)_T$ ratio decreased rapidly as the level of SO_4-S increased to about 0.05% and then changed very little at SO_4-S concentrations greater than 0.05%. That point also corresponds to a $(N/S)_T$ ratio of 17 to 18.

The relationship between the $(N/S)_P$ ratio and the relative yield level is also shown in Fig. 4 This ratio increased from about 17 to 18 at adequate S levels to near 23 under S deficiency. An increase in the $(N/S)_P$ ratio with increasing severity of S deficiency has also been observed in studies with barley (*Hordeum distichum* L.) (6), white clover (*Trifolium repens* L.) and ryegrass (*Lolium perenne* L.) (15), and a tropical legume (*Stylosanthes humilis* L.) (9). Other studies indicate that the $(N/S)_P$ ratio is a constant, near 17.5 for Leguminoseae (4, 19). The $(N/S)_P$ ratio of randomly selected S-fertilized treatments was also 17 to 18 in the study reported here.

If all of the $(N/S)_P$ ratios in Fig. 4 were similar, then the slope of a protein N vs. protein S relationship would be approximately 17 to 18, provided that the regression line goes through the origin. The slope of the calculated regression line for the protein N and S data from the control treatments in this study is 10.49 (Fig. 6), indicating that the $(N/S)_P$ ratio changes with S status. This relationship may become curvilinear and go through the origin with a wider range of data points. When the total N and S concentrations of the same treatments are plotted (Fig. 6), the protein N-S line identifies the experimental sites that did not respond to S fertilization. The total N and S points above and to the left of the protein N-S line would have $(N/S)_T$ ratios greater than that in the proteinaceous material, indicative of S deficiency, whereas S is not lacking on those points below and to the right of the protein N-S line. All but one point is correctly identified. No relationship was found between total N and total S (Fig. 6).

DISCUSSION

The (N/S) ratio of a specific protein is constant since the sequence and number of amino acids in the polypeptide chain are determined by genetic information. Therefore, the (N/S) ratio of the proteinaceous material of a plant varies only when changes occur in the relative proportions of the individual proteins formed. Mertz and Matsumato (13) found that the electrophoretic protein patterns of normal and S-deficient alfalfa leaves showed a change in the relative proportions of the cytoplasmic proteins, even though no significant changes occurred in the relative amino acid composition. The $(N/S)_P$ ratio was also shown to decrease in plants as they mature (4, 9). This has been attributed to an increase in the proportion of chloroplastic protein (24), which can contain 70% of the protein S in leaves (7).

Mertz and Matsumoto (13) found that protein N is distributed about equally between particulate and cytoplasmic proteins, even though S deficiency de-

creased the total protein N in alfalfa leaves. The $(N/S)_P$ ratios caculated from their amino acid composition data show very little effect of S deficiency on the ratio in the particulate proteins, but an increase in the ratio from 18.9 to 21.6 in the cytoplasmic proteins. This indicates that the $(N/S)_P$ ratio of the plant could increase as a result of S deficiency. The leaf/stem ratio has also been observed to increase in S-deficient alfalfa (14). This would cause a further increase in the $(N/S)_P$ ratio of whole plants and may help explain our results and those reported by others (6, 9, 15). In contrast, data reviewed by Dijkshoorn and Van Wijk (4) showed that the concentration of cytoplasmic proteins decreased faster than that of the chloroplastic proteins, thereby causing a decrease in the $(N/S)_P$ ratio in S-deficient barley leaves. These differences may be due to differences in the extraction and analytical methods for the proteinaceous material, differences between plant species, or developmental differences within a plant species.

Metson and Collie (15) gave evidence that the nonprotein S was not completely extracted by a single hot-ethanol treatment and subtracted a reducible-S fraction. However, even after making this correction, their $(N/S)_P$ ratios were higher in treatments of lower S status. Increasing the extraction time from 2 to 30 min in the study reported here increased the amount of alcohol-soluble N and S extracted, but did not change the $(N/S)_P$ ratios. This indicates that the nonprotein N and S forms were being extracted by the procedures used. Checks on the analytical procedures for both N and S with the amino acids cystine and methionine gave satisfactory recoveries and reproducibilities for each element.

In conclusion, total S, SO_4-S, and the $(N/S)_T$ ratio in whole alfalfa tops at early bloom were all found to be satisfactory indexes of S deficiency in alfalfa. Total S and SO_4-S concentrations are related, and increases in total S above 0.14% S resulted from the accumulation of SO_4-S. The $(N/S)_P$ ratio increased from 17 to 23 as the degree of S deficiency increased; however, this did not change the interpretation of the $(N/S)_T$ ratio data. These and other reported data suggest that a re-evaluation of the effect of S deficiency on the relative amounts of the different proteins in plants is needed.

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