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THE SULPHUR REQUIREMENTS OF PLANTS AS EVIDENCED BY THE SULPHUR–NITROGEN RATIO IN THE ORGANIC MATTER

A REVIEW OF PUBLISHED DATA

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INTRODUCTION

When more sulphate is transported to plant leaves than can be metabolized it accumulates in them as both sulphate and organic sulphur. If no more sulphate is supplied, the sulphate in the tissues falls progressively to a very low concentration by dilution and metabolism. This eventually results in the appearance of sulphur deficiency; because of a shortage of S, protein synthesis stops and the leaves turn a paler green.

Even when sulphate is removed from the supply, it may take a considerable time for S-deficiency symptoms to develop [17] since the metabolism continues to draw on residual sulphate still present in the tissues. Because of the low rate of translocation of sulphate, the upper, younger leaves turn yellow first; on the branches of S-deficient trees, the chlorotic terminal growth can be readily distinguished from the older, greener leaves below [8].

Since the organic forms of S and N are involved to a large extent in the synthesis of proteins, the ratio of organic-S to organic-N in the plant should lie close to that in its proteins. In perennial ryegrass a content of 0.027 gram atoms organic-S per gram atom of organic-N was found in the foliage, independent of the total-S and total-N contents [16]. This ratio would appear to be of value in assessing the sulphur requirements and also provides a means for calculating from the total-S and organic-N contents the contribution of sulphate to the ionic balance [13]. The above value has been used as a basis for calculating the organic-S contents of a number of plant species from their organic-N contents [11 51]. However, in view of possible variations in this ratio, it would appear more appropriate to use a value based on data for a greater range of plant species.

The purpose of this article is to extract from the literature values for organic-S and organic-N, to see whether there is any similarity in the ratios for different plant species that may give some confidence in a particular value, and to show that this ratio is of real significance in the study of protein synthesis and the sulphur requirements for growth.

All data have been recalculated as gram atoms S and N in their various forms per kg dry plant material. Organic-N has been calculated from total-N minus nitrate-N; errors due to incomplete recovery of nitrate-N in the determination of total-N have been ignored since these are presumed small. In the absence of nitrate and where ammonium was supplied in solution, total-N has been considered equal to organic-N; this has also been done where the nitrate content was not recorded, but where other evidence suggested its presence in insignificant amounts.

RESULTS OF REVIEW

Sulphate and organic sulphur

Total-S is usually determined by ashing and subsequent extraction as sulphate, sulphate-S by hot water extraction of the dry, powdered plant material in the presence of HCl, and organic-S by difference. Incomplete extraction of sulphate will result in too high a value for organic-S; this may account for the abnormally high ratio of organic-S to organic-N obtained from the data of Hall [23] for alfalfa hay. The presence of ethereal sulphates [35] was inferred from a greater recovery of sulphate after prolonged treatment of the plant material with hot dilute acid, but their nature and the extent of their occurrence remains uncertain [54]. A typical discrepancy was found in Eaton's results[17] for soybean. In the sulphur-deficient plants, the ratio, total-S to organic-N appeared to be 0.025, a value typical of the ratio of organic-S to organic-N, and these plants must have contained very little sulphate. The records show as much as 0.03 gram atoms sulphate-S per kg dry matter; it would seem that the quoted values for sulphate-S were too high.

The absence of sulphate in sulphur-deficient plants has been observed by Peterson [39] in the case of rape, radish and clover growing in a sulphur deficient soil with an abundant supply of other nutrients, utilizing all available sulphate in the tissues and leaving none as sulphate. For diagnostic purposes however, it is desirable to specify more exactly what is meant by the absence of sulphate in S-deficient plants. A slow rate of redistribution and the localization of metabolic consumption may mean that some sulphate is not readily accessible for metabolism, and a more detailed analysis may reveal sulphate in crops otherwise designated as free from sulphate.

The order of magnitude of the very low sulphate contents of S-deficient plants may be evaluated from the data of Walker and Bentley [52]. All plants designated as deficient according to their yield response to sulphate application were those with sulphate-S contents below 0.006 gram atoms per kg dry matter. From their quoted values, the organic-S contents have been calculated and plotted in Fig. 1 against the total-N contents. The line relating these two quantities indicates an organic-S : total-N ratio of 0.023. Since this corresponds to the normal ratio of organic-S to organic-N, it suggests a very low nitrate content with total-N being practically equivalent to organic-N.

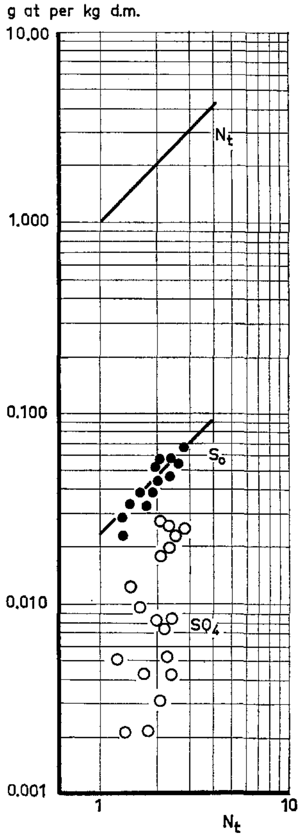

Fig. 1

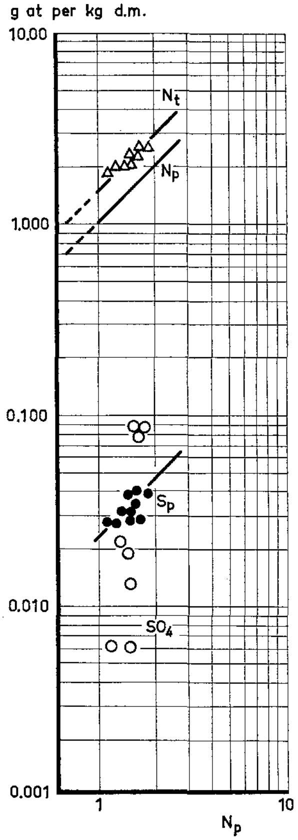

Fig. 2

Fig. 1. Organic sulphur (solid dots) and sulphate sulphur (open circles) in field grown legumes in relation to the total nitrogen content N_t. Data of Walker and Bentley [52].

Fig. 2. Total nitrogen (N_t), protein sulphur (S_p), protein nitrogen (N_p) and sulphate sulphur (SO_4) in red clover. Data of Miller [33].

In Fig. 2, the data of Miller [33] for red clover have been presented in a similar way, in this case plotting the content of sulphate-S, total-N, protein-S against protein-N. At harvest none of the plants had sulphate-S contents below 0.006 gram atoms per kg dry matter and none responded to sulphate added in the fertilizer. Protein-N constituted about 70 per cent of the total-N and the line drawn through the points for protein-S indicates a S : N ratio of 0.023 in the proteins.

In general, organic-S contents of plants vary proportionally with the organic-N contents. Depending on the species, manurial conditions and stage of growth, organic-N can vary from, say, 1 to 4 gram atoms per kg dry matter. Sulphate-S contents depend on the supply of mineral ions and on the ability of the species to accumulate sulphate in excess of metabolic requirements. A summary of the data on both forms in vegetation (Fig. 3) shows that the content of sulphate-S varies to a much greater extent than that of organic-S.

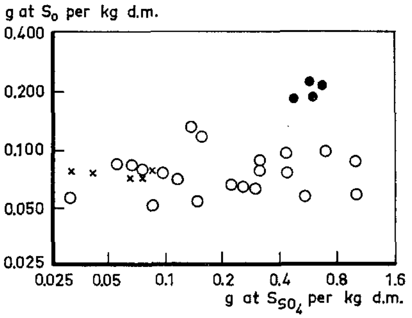

Fig. 3

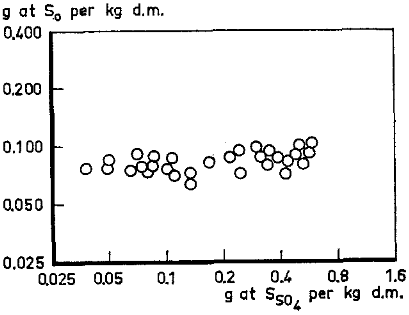

Fig. 4

Fig. 3. Organic sulphur and sulphate sulphur in various plant species. From data of Thomas *et al.*[48], the solid dots are for Tamarix. The crosses are for 5 grass species from data of Aitken [1].

Fig. 4. Organic sulphur (ordinates) and sulphata sulphur (abscissae) in alfalfa fumigated with sulphur dioxide. Data of Thomas *et al.*[47].

The data presented in Fig. 4 for the variation in the sulphate content of alfalfa induced by fumigation with sulphur dioxide are of particular interest. In spite of a more than ten-fold increase in the content of sulphate-S in the tissues, that of the organic-S remained constant. The absorbed sulphur dioxide, after oxidation to sulphur trioxide, is combined with water from the tissues to form sulphuric acid.

Up to about 300 mmol sulphuric acid per kg dry matter was introduced by fumigation, and titration of the sap indicated a buffer capacity of about 200 me per unit pH per kg dry matter within the pH range 4 to 6. In spite of the large excess of sulphuric acid, the pH of the sap of the fumigated plants remained at pH 5.9, the same as that in the control plants; the only difference was that the buffer capacity of the sap of the fumigated plants within the range of pH 5 to 3 was smaller. Since in this range the acids of the sap soluble organic salts are the main buffering agents [7], fumigation must have caused a decrease in the organic anion content of the leaves. The sap from 1 kg dry weight from the fumigated plants required about 300 me less acid to change the pH from 6 to 3 than that from the non fumigated plants. On the alkaline side, between pH 6 and 9, no effect of fumigation on the buffer capacity of the sap was observed, and since within this range the amino acids act as buffer agents, these do not appear to have been affected by fumigation. Considerably more sulphate and less carbonate was found in the leaf ash of fumigated than in that of non fumigated plants, but the inorganic cations were present in about the same amounts.

The above findings suggest that sulphur dioxide applied to leaves accumulates as the sulphates of the inorganic cations in the leaves. The alkali required to neutralize the sulphuric acid introduced by fumigation comes from the decarboxylation of their organic salts in the leaves, and the sulphates accumulate at the expense of the salts of organic plant acids. This is similar to the mechanism of accumulation of inorganic anions, absorbed by the root system in association with hydrogen ions [14 15 51]. The conclusion of Thomas *et al.*, that neutralization of the sulphuric acid 'was largely accomplished by organic bases such as amino groups and other nitrogenous compounds' does not agree with their experimental results.

Fig. 5 summarizes the unpublished results of Vertregt for the organic-N, organic-S and sulphate-S of the alga Scenedesmus, the line indicating a gram atom ratio of 0.027 for the organic matter. Sulphate varied, but always well above the deficiency level.

Release of sulphate from organic-S has been observed during starvation in the dark; Fig. 6 illustrates this effect in detached leaves. The total-S remained unchanged and a varying fraction of the protein-S released by proteolytic breakdown was recovered as sulphate-S. It may be argued from this that metabolic reoxidation of organic-S can take place.

Plants supplied with ammonium salts may contain greater amounts of sulphate than those given nitrate. In the latter case a

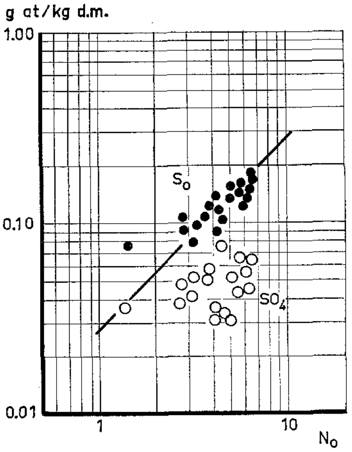

Fig. 5

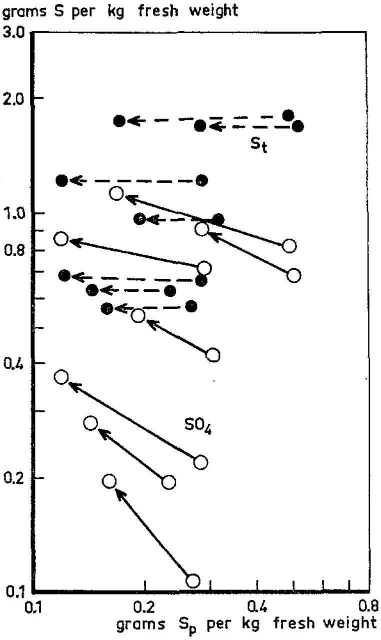

Fig. 6

Fig. 5. Organic sulphur (filled dots) and sulphate sulphur (open circles) in relation to organic nitrogen (N_0) in the alga *Scenedesmus* spec. Data of Vertregt (unpublished).

Fig. 6. Total sulphur (solid dots) and sulphate sulphur (open circles) in detached leaves of Phaseolus. The origins of the arrows indicate the initial contents, the points those found after some days in darkness. Protein sulphur (abscissae) decreased by proteolysis and sulphate increased at a constant total sulphur content. Figures in grams per kg initial fresh weight. Data of Mothes [34].

considerable proportion of the anion uptake is concerned with nitrate and the change from nitrate to ammonium allows more of the anion uptake capacity to be occupied by other salt anions [51]. The only exception appears to be a change from nitrate to ammonium when nitrate in the supply is replaced by chloride. In this case chloride may be a more effective inhibitor of sulphate accumulation than nitrate so that the sulphate content of plants supplied with both ammonium and chloride may be lower than that of plants supplied with nitrate, but no chloride. Heiserich [25] attempted to explain this on the basis of competition between nitrate and sulphate for reducing metabolites, but this theory confuses two entirely different phenomena. The competative factor thought to be localized in the metabolic utilization system is merely a case of salt ion competition in the accumulating system. Rosz [43] and Mothes [34] correctly attributed Heiserich's findings to his introduction of chloride in the salts supplied with ammonium. Bauer, who reported on sulphate and nitrate in a number of plant species, also criticized the conclusions of Heiserich, but, her records should not be considered in this connection.

Protein sulphur and protein nitrogen

Most of the organic S and N in plants is used in protein synthesis and the composition of the proteins determines to a large extent the ratio of organic-S to organic-N in the plant material. Data supporting this are presented in Fig. 7 in which the lines relating S and N contents indicate similar ratios of 0.033 for both organic and protein material. It is clear from the left-hand figure that the sulphate-S content (S_t minus S_0) was very much higher than the critical level of 0.006 gram atoms per kg dry matter, and this agrees with the fact that the varying supply of sulphate had not affected the yield.

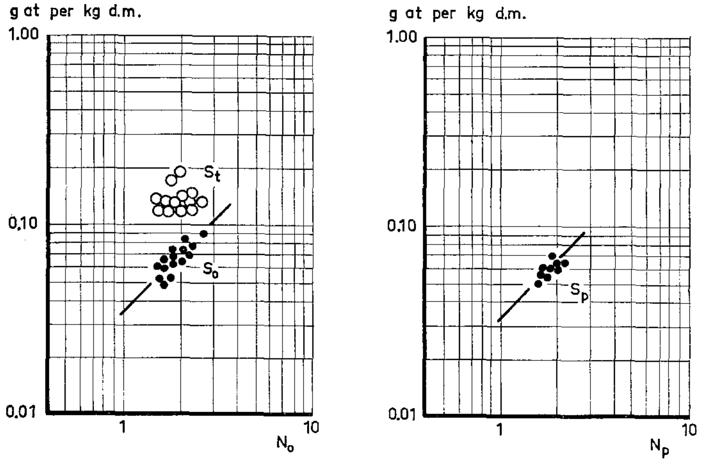

Fig. 7. Total sulphur (open circles), organic sulphur (S_o) and protein sulphur (S_p) plotted against organic nitrogen (N_o) or protein nitrogen (N_p) in the dry matter of *Phalaris tuberosa*. Data of Wood and Barrien [54].

Results for *Lolium subulatum* are summarized in Fig. 8 The N. content varied according to the plant organ sampled and the duration of a starvation period in the dark. The ratio of protein-S to protein-N remained at 0.031.

The data of Nightingale *et al.*[36] gave variable values for the ratio of organic-S to organic-N in tomato, well outside the normal range, and it is likely that the analyses were unreliable.

Lugg [27] [28] reported on the ratio of S to N in proteins prepared from the leaves of a number of plant species ranging in age from young to quite old and also gave the protein contents of the materials used. In Fig. 9 the protein-S contents have been plotted against the protein-N contents of the dry plant material. The average value of

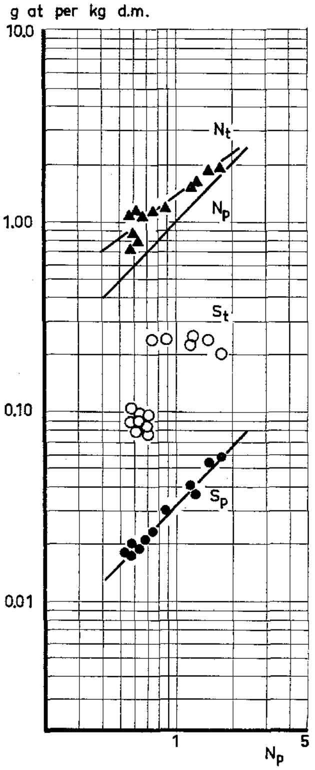

Fig. 8. Total nitrogen (N_t), protein nitrogen (N_p), total sulphur (S_t) and protein sulphur (S_p) in *Lolium subulatum*. Data of Wood and Barrien [55].

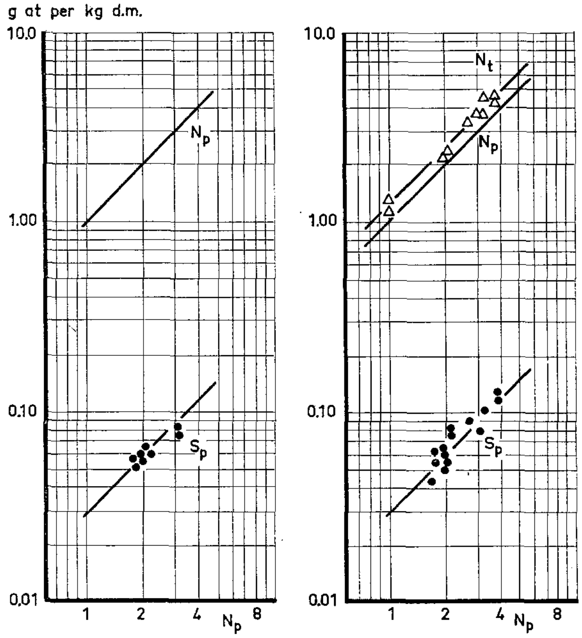

Fig. 9. Protein sulphur (S_p), protein nitrogen (N_p) and total nitrogen (N_t) in some gramineous plants and legumes. Data of Lugg [27] [28].

the ratio of these two quantities was 0.030; the data reported suggest that the ratio was about 0.032 in gramineous plants and about 0.025 in legumes.

The different kinds of proteins that occur in plant material may differ from one another in the S : N ratio. The data of Osborne [38] for proteins from wheat kernel suggest that the gluten proteins have a ratio of 0.030; the embryo albumins of 0.035 and the globulins of 0.015. Proteins associated with the nucleins had much higher ratios of 0.05 to 0.06.

Data on the varying S : N ratio of leaf proteins is provided by the work of Wood and his associates on *Andropogon sudanensis*: the results for plants of different age and for isolated chloroplasts are summarized in Fig. 10. As expected, the protein-N content decreased with increasing age of the plants; during the early growth stages with high protein content, the ratio, protein-S : protein-N was about 0.028, but increased gradually with increasing age to about 0.038. The chloroplast proteins had a constant S : N ratio of

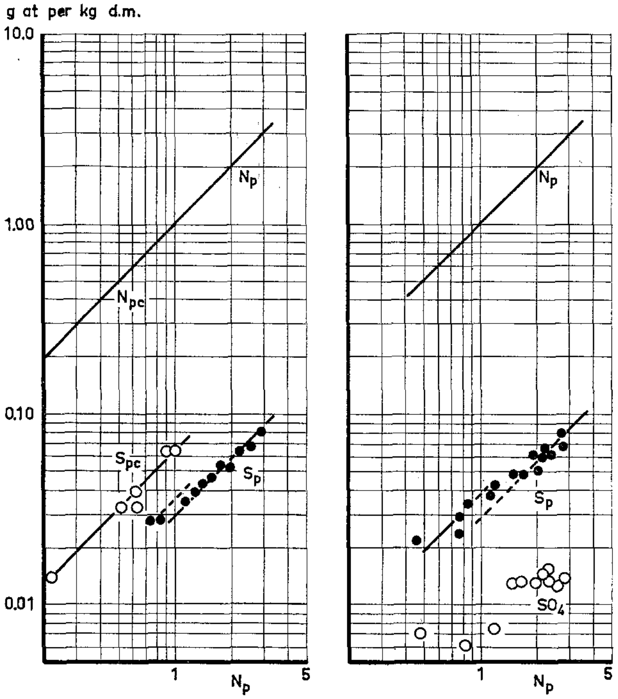

Fig. 10. Protein sulphur (S_p), chloroplast protein sulphur (S_{pc}), protein nitrogen (N_p) and chloroplast protein nitrogen (N_{pc}) in the dry material of *Andropogon sudanensis* of varying age. Data of Hanson *et al.*[24] and Barrien and Wood[3].

about 0.065. The right-hand figure shows more distinctly a similar increase in the S : N ratio of the protein with increasing age and decreasing protein-N content.

Fig. 11 shows that with increasing age there was a continuous increase in the proportion of chloroplast protein in the total protein, and this would appear to account for the corresponding increase in the organic-S : organic-N ratio in the plant material. Although the effect of age on this ratio may be small, statements that it remains constant during plant development are not necessarily correct.

Some workers have investigated the distribution of S and N in different particulate fractions of plant tissues. Mertz and Matsumoto[30] separated cytoplasmic protein and a green particulate fraction from the leaves of alfalfa. From analysis for amino acids S : N ratios of 0.013 and 0.016, respectively can be calculated, and these values are unexpectedly low compared with other data on plant proteins.

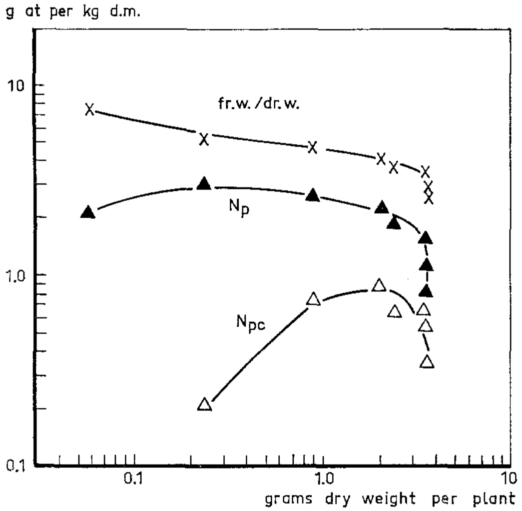

Fig. 11. Changes in fresh weight per unit dry weight, in protein nitrogen (N_p) and in chloroplast-protein nitrogen contents with plant weight and age in *Andropogon sudanensis*. Data of Wood [53].

From barley leaves, Fujiwara and Torii [20] prepared a coarse fraction (A) of cell walls, nuclei and cell debris, a green particulate sediment (B); cytoplasmic protein (C) and a fraction D consisting of cytoplasmic non-protein material. The plants used were grown with and without sulphate in the supply. The results, expressed in gram atoms per kg. dry matter, are summarized in Fig. 12. The solid symbols are for nitrate, and the open symbols for ammonium in the supply; the lengths of the vertical lines correspond to a S : N ratio of 0.025.

It will be observed from Fig. 12 that the S : N ratio in the dry matter of the fractions B and C was higher when ammonium was replaced by nitrate in the supply. This is contrary to what might be expected in view of other findings which showed that form of nitrogen supply had generally no effect on the ratio, organic-S : organic-N in the tissues. There is some indication that the S : N ratio is higher in the green particulate material (B) than in the cytoplasmic protein (C), but this applies only to the plus-sulphur plants and the difference is small. In the soluble, non-protein cytoplasmic fraction (D), the ratio is lower in the —S plants than in the +S plants, presumably because of the absence of sulphate in the former.

Sulphur shortage is also associated with a lower dry weight of the cytoplasmic protein fraction (C), but not of the green particulate fraction (B). It would appear, therefore, that the fall in protein content characteristic of sulphur deficiency is associated mainly with the cytoplasmic proteins and not with the proteins of the chloroplasts. This may explain the increase in the ratio, protein-S : protein-N brought about by S-deficiency in cotton [19] and discussed in detail in a previous article [16].

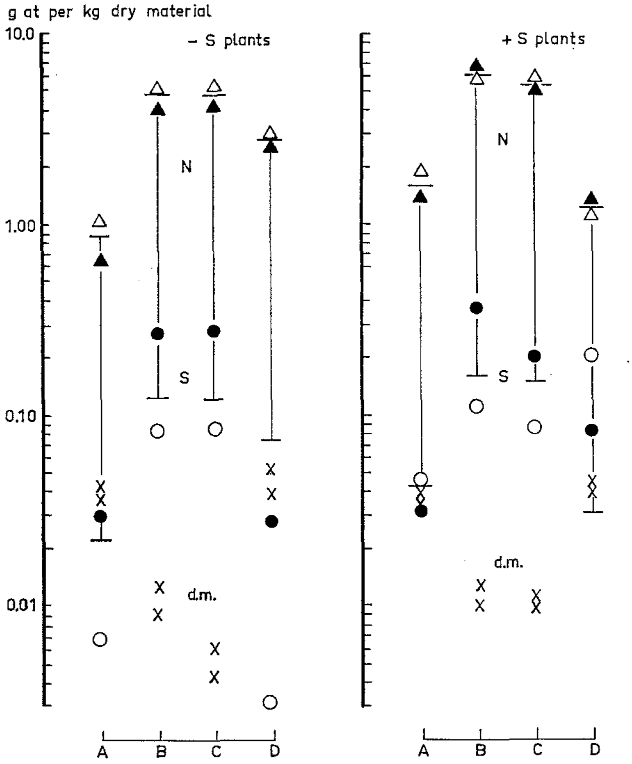

Fig. 12. Nitrogen (triangles), sulphur (circles) and dry matter (crosses) in particulate fractions (A and B), cytoplasmic protein (C) and cytoplasmic non-protein material (D) from the leaves of barley. The dry matter is given as fraction of total dry weight × 1/10. Data of Fujiwara and Torii [20].

Methionine-S and cystine-S represent the sulphur present in proteins. For instance, data from analysis for methionine and cystine contents of gliadine preparations [12] gives a ratio of cystine-S plus methionine-S to N of 0.030 in gliadine, which is very close to Osborne's values for gliadine derived from elemental analysis.

By microbiological assay, Mertz *et al.*[31] determined all the amino acids commonly recognized as normal constituents of plant proteins, in hydrolysates of alfalfa with and without S-deficiency. The results are summarized in Fig. 13 in terms of gram moles per kg dry weight of plant material hydrolyzed; the data include both free amino acids and those from the proteins. The values for methionine and cystine are both expressed as gram atoms of sulphur present in these forms.

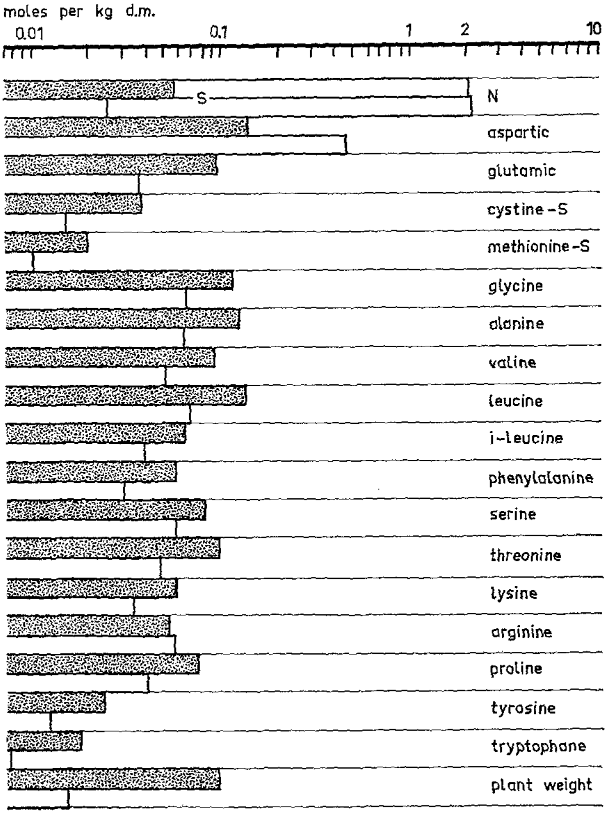

Fig. 13. Amino acids in hydrolyzate of alfalfa with adequate sulphur (shaded columns) and deficient in sulphur (unshaded columns). The upper columns give the total gram atoms N and S in the amino acids, the lower columns plant weights in arbitrary units. Data of Mertz *et. al.* [31].

The two upper histograms represent the total-N and total-S of the amino acids. In the normal plants, with the shaded column for S, the ratio S : N is 0.027; this agrees very closely with the S : N ratio of alfalfa proteins prepared by Lugg. In the S-deficient plants the ratio is only 0.013.

A comparison between the deficient and the normal plants shows that cystine and methionine did not decrease out of proportion to the decrease in the content of the other amino acids as a result of S-deficiency. It was therefore assumed that in the deficient plants protein synthesis was limited by the amount of cystine and methionine in the tissues. However, the total amino acid nitrogen content remained unchanged, because of a compensatory increase in aspartic acid in the deficient plants; in these plants 78 per cent of the aspartic acid could be removed by dialysis and was thus not an integral constituent of the proteins.

Sulphur and protein synthesis

In this connection, reference to the work of Tottingham [50] occurs in the literature. Red clover was grown at varying proportions of nitrate and sulphate in the supply. The nitrate and nitrogen contents of the plants were the same, independent of the amount of nitrate supplied, so that none of them were deficient of nitrogen. The increase in yield with increasing sulphate supply could, therefore, be entirely attributed to an improvement of the sulphur status, with a corresponding increase in dry matter and protein production. However, this investigation merely confirms the necessity of sulphate for growth and shows with an adequate N-supply the amount of N utilized increases with the yield. Any factor which affects growth other than the N-supply will give the same result. Again, the findings of Tolman and Stoker [49] that all increases in seed yields of sugar beet following the application of S were obtained when N was applied and that responses to N were obtained with additional S, can be applied to any combination of essential factors and therefore do not express the S : N relationship in the present context.

Protein synthesis may presumably be limited by a shortage of sulphuretted amino acids in the tissues and this forms the basis of the close relationship between S and N utilization by growing plants. When sulphur deficiency begins, the conversion of nitrate and ammonium into organic-N may continue and a cessation of protein synthesis makes free amino acids to accumulate in the tissues. The results of Mertz *et al.*[31], previously discussed, already showed that non-protein organic-N accumulates in alfalfa in the form, giving aspartic acid on hydrolysis.

Fig. 14 shows that in S-deficient alfalfa amide-N accumulates in large amounts. The amide-N was shown by chromatography to be mainly asparagine. In this particular experiment total-N was not determined and since the S-deficient plants were smaller their N-contents may have been higher because of reduced exhaustion of the N supplied. In spite of this it would appear reasonable to conclude that the increase in asparagine was the direct result of a reduced protein synthesis, in view of the fact that it became more than ten fold.

Fig. 15 illustrates the accumulation of free, non protein amino acids in

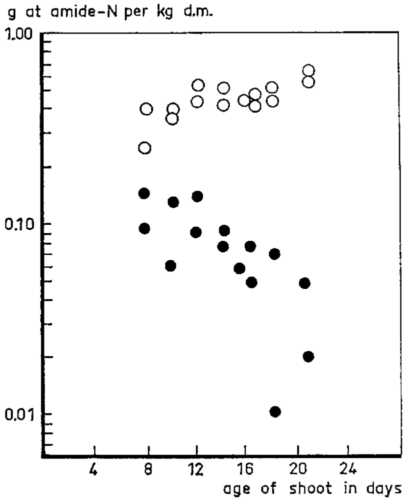

Fig. 14

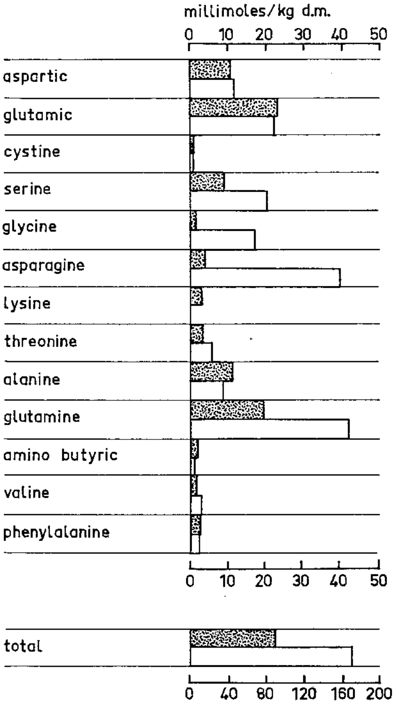

Fig. 15

Fig. 14. Amide nitrogen in petioles and stems of normal (solid dots) and of sulphur deficient (open circles) alfalfa. Data of Rendig and McComb [42].

Fig. 15. Free amino acids in leaves of barley with adequate sulphur (shaded columns) and with sulphur shortage (unshaded columns). Data of Coic *et al.*[9].

normal and S-deficient barley leaves [9]. Sulphur-deficient plants gave a lower yield, but the total-N content of 2 gram atoms per kg dry matter was unaffected; on the other hand the concentration of free amino acids was about twice that in normal plants, the increase being mainly in asparagine, glutamine, glycine and serine. Using a similar technique Coleman [10] also found an increase in free amino acids such as asparagine, glutamine, and arginine in a number of plant species; the type of amino acid or amide showing the greater increase varied somewhat between species.

With a more severe S-deficiency the organic-S : organic-N ratio may fall to a subnormal value because of the relative increase in free amides and amino acids low in sulphur. An example of this is shown in Fig. 16. With one exception, the ratio of organic-S to organic-N

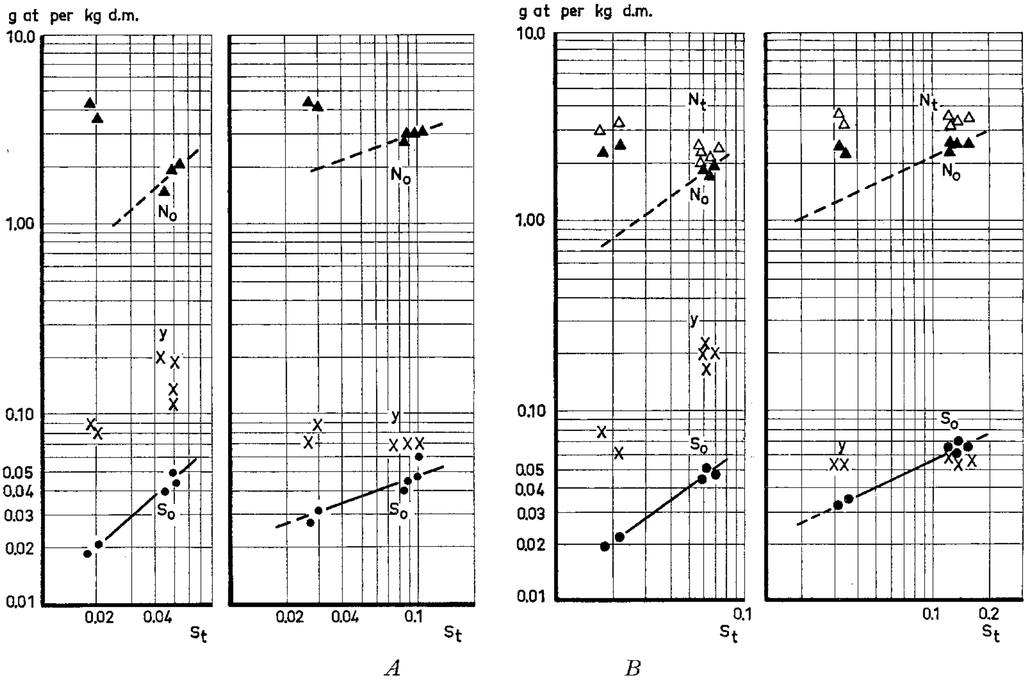

Fig. 16. Effect of raising the total sulphur content (S_t) from the deficient to the normal status on total nitrogen (N_t) organic nitrogen (N_o) and organic sulphur (S_o) in maize seedlings supplied with nitrate or ammonium as the nitrogen source. The crosses denote dry weight per seedling in 10 g units Data of Rosz [43].

was 0.025 in the non S-deficient plants which contained sulphate besides organic sulphur. In the S-deficient plants, sulphate was absent and the ratio fell to an abnormally low value between 0.005 and 0.010.

Sulphur and proteolysis

The behaviour of S during proteolysis, *e.g.* during the mobilization of reserve proteins in seeds and cotyledons, is of particular interest. Some of the results of Mothes [34] dealing with this problem are summarized in Fig. 17. Seeds of *Pisum sativum* were germinated on sulphate free nutrient solutions and assayed at intervals for protein-N, protein-S, total-S and sulphate-S in the seeds plus seedlings. In one set, raised in the dark, the protein content decreased

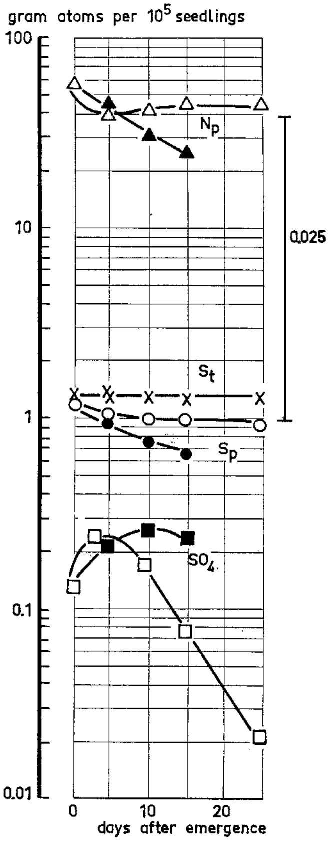

Fig. 17

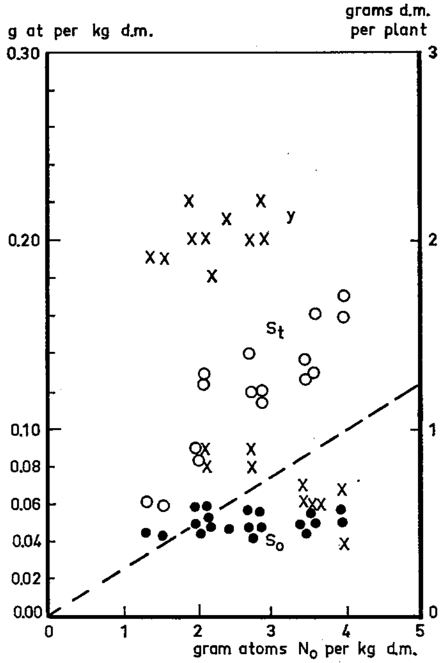

Fig. 18

Fig. 17. Protein nitrogen (N_p), protein sulphur (S_p), total sulphur (S_t) and sulphate sulphur (SO_4) in the seeds plus seedlings of *Pisum sativum*, grown on sulphate-free nutrient solution. Open symbols – plants in the light; solid symbols – plants in the dark. Data of Mothes [34].

Fig. 18. Total sulphur (S_t), organic sulphur (S_o), organic nitrogen (N_o) and plant weights (crosses), in maize seedlings with sufficient Fe (around 2 g dry weight) and deficient in Fe (around 0.7 g dry weight). Data of Rosz [43].

continuously during the 15 days after emergence, the total-S content remained unchanged whilst sulphate increased at the expense of organic-S; the last indicates oxidation of organic-S to sulphate-S during proteolysis. In the light, during the first 5 days, there was a slight decrease in protein-N and protein-S, and some increase in sulphate; thereafter however, the protein content was maintained by *de novo* synthesis in the seedlings and the sulphate content fell rapidly through metabolic consumption. It appears from the graph that in all samples the ratio, protein-S : protein-N was maintained at a value of 0.025; this again supports the contention that the requirement for sulphur is determined essentially by its stoichimetric relation with nitrogen in the make up of proteins.

Similar results were obtained in a dark starvation experiment on *Lolium subulatum*. The constancy of the S : N ratio in the protein has already been discussed (Fig. 8) and in Fig. 19, the contents have been plotted on a time basis. Proteolysis was most pronounced in the leaves, the organic-S diminishing in proportion to protein-N. That organic-S decreased without any change in total-S suggests some conversion of organic-S into sulphate.

According to the available data it appears that although the S : N ratio remains unchanged in the residual proteins, the non protein

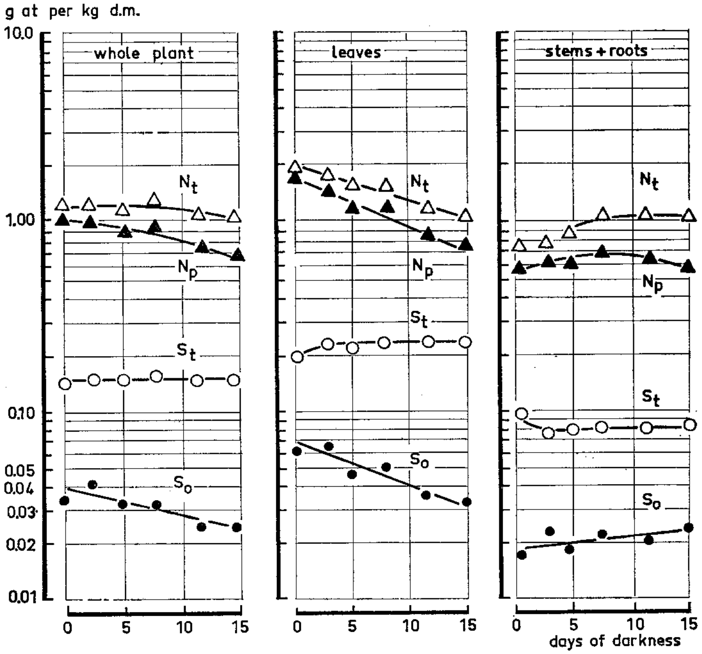

Fig. 19. Nitrogen and sulphur fractions in *Lolium subulatum* during starvation in the dark. Plant dry weights remained constant at 10 g of which 4.5 g were leaves. Data of Wood and Barrien [55].

substance produced by proteolysis loses organic-S by conversion into sulphate. If this proceeds to an appreciable extent, it may give rise to subnormal values for the organic-S : organic-N ratio in the presence of sufficient sulphate in the tissues. Such conditions may occur in small seedlings drawing to a large extent on seed reserves, the proteins of which undergo proteolysis prior to translocation, and in darkened plants.

Fig. 20 illustrates the results of Rosz [43] who studied the N- and S-fractions of small seedlings of *Lupinus albus* grown on nutrient solutions with two levels of sulphate supply. In intact seedlings the organic-S : organic-N ratio was abnormally low (0.013) although there was sufficient sulphate in the tissues to preclude S-deficiency. In seedlings from which the cotyledons were removed, the organic nitrogen content was lower and the organic-S : organic-

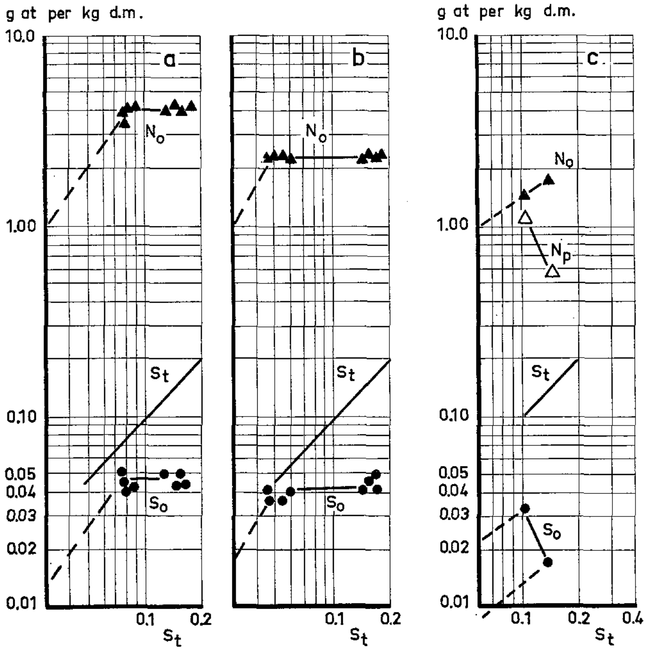

Fig. 20. Organic nitrogen (N_o), protein nitrogen (N_p), total sulphur (S_t) and organic sulphur (S_o) in small seedlings of *Lupinus albus* (0.3 g dry weight per plant) grown at two sulphur levels. *a;* intact seedlings; *b;* cotyledons removed. *c;* same for field grown plants 50 days old, before (higher N_p content) and after (lower N_p-content) a dark period of several days. The broken lines are drawn to facilitate reading of the S : N ratios in the organic substance. Data of Rosz [43].

N ratio was 0.019. In a field grown plant, 50 days after emergence, the ratio of organic-S to organic-N was 0.023, close to the normal value. When a similar plant was kept in the dark for several days, the protein-N content fell considerably due to proteolysis; the organic-S content decreased in proportion to the protein-N content causing a fall in the organic-S : organic-N ratio to the very low value of 0.008. Since in both normal and darkened organic-S occurred mainly as protein-S, proportionality between it and protein-N was nearly maintained. The organic-S released by proteolysis was oxidized to sulphate-S.

Fig. 18 refers to maize seedlings grown with and without an iron supply, the resulting dry weights being about 2 and 0.6 g. respectively; the latter was very small considering that the seed weight was about 0.25 g. The points representing the organic-S contents of the plants supplied with iron and giving the higher yields lie close to the line corresponding to 0.025 times the organic-N content and thus indicate a normal ratio between these quantities; in the iron-deficient plants the ratio was much lower, of the order of 0.015.

Since the iron-deficient plants had dry weights only about twice that of their seed, they may have stored a much greater proportion of the nitrogen from their seed as proteolytic products with a low S-content than did the non-deficient plants. The increased growth brought about by applying iron may have increase the proportion of synthesised or resynthesised proteins in the leaves with a normal S : N ratio.

The above provides a fair amount of indirect evidence relating the effect of iron to the growth response. Seedlings may acquire a normal organic-S : organic-N ratio only if further growth permits protein synthesis to occur to a sufficient extent. If growth is retarded the seedlings may have a subnormal ratio characteristic of utilization of N supplied by the seed for a much longer period than faster growing plants.

Sulphur and growth

The practical consequences of S-deficiency in certain regions of the U.S.A. were recognized in the early twenties [37 40]. In Nyassaland the disease of the tea bush known as 'yellows' has been identified as due to a deficiency in sulphur [46].

Field crops may remove from 15 to 30 pounds per acre from the soil [26]. Much attention has been paid to the contribution of atmospheric S, in relation to distance from industrial centers [29]. In Minnesota, S from the air was from 5 in the north to over 100 pounds per acre per annum near Mineapolis [2]; and in Indiana, from 20 to 30 pounds per acre per annum [6]. Atmospheric S, together with that in superphosphate and ammonium sulphate has generally been considered sufficient for crops. However, there has been a shift towards the use of high-analysis fertilizers containing less sulphur so that one must be on one's gard against further neglect of sulphur in deficient regions [21].

In earlier agronomic investigations, those areas in which S application gave increases in crop yield and recovery from symptoms were classified as sulphur deficient, but few plant analyses were made [37]. Occasionally, total-S was determined, but data on S alone are not enough to asses the sulphur status since the amount required is proportional, not to the weight of the plant, but to the amount of organic nitrogenous substance, mainly protein, to which the S contributes in a fixed proportion. A more reliable basis for diagnosis should, therefore, be provided by the ratio of S to organic nitrogen [16].

Many agronomic investigations supply data on total S and total N; if the normal organic-S : organic-N ratio is known, and nitrate is low, the S requirement may then be calculated from the total-N value.

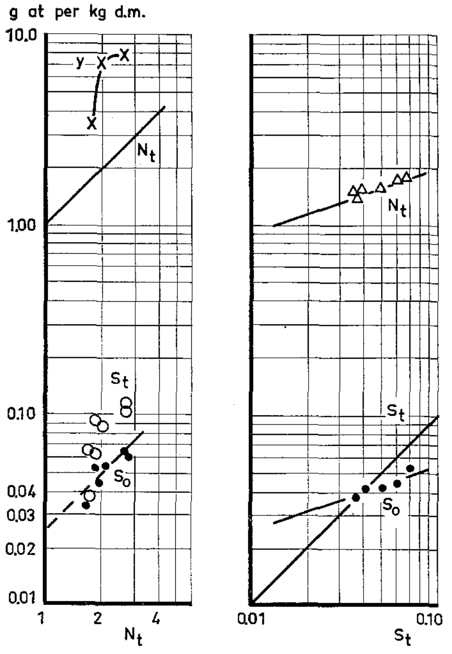

Fig. 21

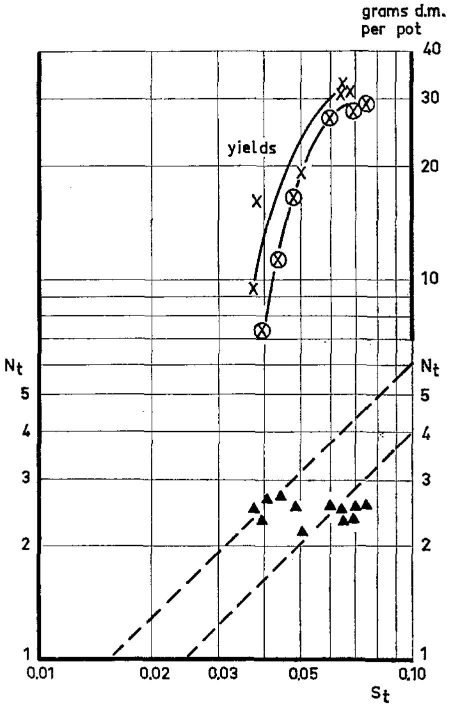

Fig. 22

Fig. 21. Total nitrogen, total sulphur and organic sulphur in alfalfa. Graph on the *left*: data of Rendig [41], yield (y, crosses) in units of 1000 pounds per acre. Graph on the *right*: data of Miller [32].

Fig. 22. Total sulphur, total nitrogen and yields of two strains of alfalfa. The broken lines indicate S : N ratios of 0.015 and 0.025. Data of Bertramson *et al.*[6].

Data on the S and N contents of alfalfa have been plotted in Fig. 21. In this, lines of best fit have been drawn visually corresponding to an organic-S : organic-N ratio of 0.027, as suggested by the data of Lugg [27] [28] for alfalfa protein. There is little doubt that in this case the nitrate content must have been sufficiently low for total-N to be practically equal to organic-N.

Data on alfalfa, fertilized with varying amounts of sulphate have been summarized in Fig. 22 in which lines have been drawn at S : N ratios of 0.015 and 0.025. When total-S rose above 0.025 × N, no further increase in yield was obtained with increasing S; this limit, therefore, represents the actual S requirement for growth. In the S-deficient plants, showing reduced growth, the ratio fell to the subnormal value of about 0.015.

The yield and composition of sugar beet and turnip foliage, as affected by the application of sulphate to a soil low in sulphur, has been summarized in Fig. 23; the experiment was made at three different levels of N-application indicated as 1, 2 and 3. Data on the foliage contents of organic-N (calculated from the difference between total-N and nitrate-N), nitrate and dry weight of the tops are plotted against the total-S contents.

In the sugar beet grown with low nitrogen (1), no response in dry matter was obtained by increasing the total-S content by increasing the sulphate supply; in this case the S-content of the low-S plants was above the deficiency level represented by the broken line. These leaves must have contained inorganic sulphate and there was no need for additional S. Because of the nitrogen shortage however, the nitrate contents and yields were low.

At the highest nitrogen level (3) the N-content was very much higher and the low-S plants were clearly deficient in S because it coincides with the broken line. With increasing total-S content beyond this deficiency level there was a marked increase in the yield since the S-deficiency disappeared and there was sufficient N to support the extra growth. The decrease in the N content was a dilution effect brought about by the increased growth; the more advanced exhaustion of the N-supply with increasing yield was reflected in the steep fall in nitrate content. The results at nitrogen level (2) were intermediate between these and (1) and (3).

Similar results were obtained with turnip, but even at the lowest N-level (1) the low-S plants were deficient in sulphur because the

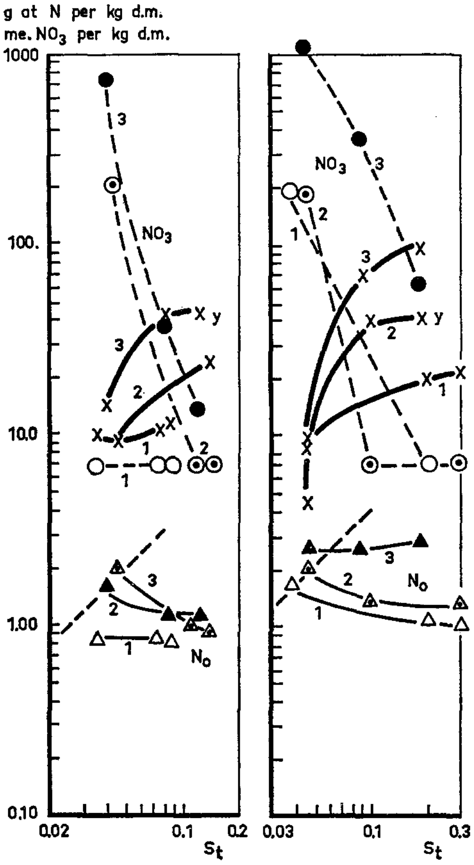

Fig. 23. Effect of increasing the total sulphur content (S_t) on the yield (crosses, heavy lines), the nitrate and the organic nitrogen (N_0) in leaves of sugar beet (left) and turnip (right) grown at three nitrogen levels (1, 2 and 3). The broken lines indicate S:N = ratios of 0.025 Data of Gunnarson [22].

point coincides with the broken line. The yield response to added sulphate was greater at the higher N levels again, because the extra growth increased the need for additional nitrogen.

These experiments provide good evidence for the significance of the S-N relationship and, assuming that the S-deficient plants had no sulphate in the tissues, they confirm that the organic-S : organic-N ratio is approximately 0.025 for sugar beet and turnip.

Recalculation of Schmalfusz's [44] data on the S and N fractions

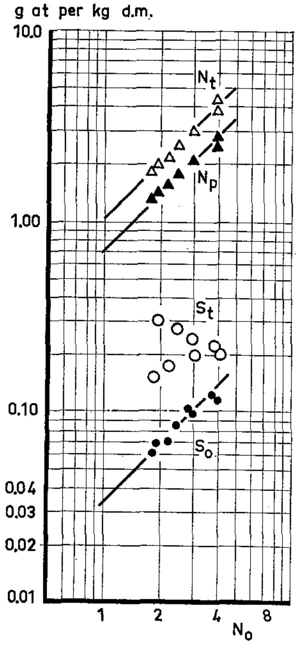

Fig. 24

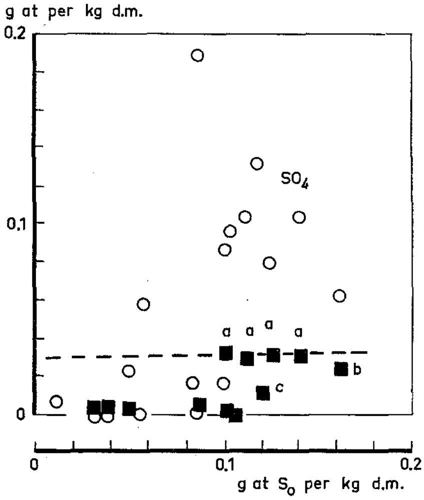

Fig. 25

Fig. 24. Nitrogen and sulphur fractions in the leaves of spinach. The nitrogen content varied as a result of differences in plant weight, being due to varying K, at a limited nitrogen supply. Data of Schmalfusz and Reinicke [45].

Fig. 25. Volatile sulphur (solid squares), sulphate and organic sulphur (plus volatile sulphur) in alfalfa, sugar beet, clover, radish (c), cabbage (b) and rutabagas (a). Data of Peterson [39].

in sugar beet, gave organic-S : organic-N ratios ranging from 0.006 to 0.023, possibly because of analytical errors; his data on maize and oats also yield abnormal ratios. Fig. 24 illustrates data on spinach which indicated a ratio of 0.033.

Other forms of organic sulphur

The Brassica form a group of plants containing a relatively high proportion of their organic-S as glycosides which on hydrolysis yield organic iso-thiocyanates: these and certain other S-compounds are commonly referred to as 'volatile sulphur'.

A detailed study of volatile S in different species was made by Peterson [39]; fresh samples of the plant material were placed in a tube kept at 100°C, air was passed through the tube and volatile S trapped by hot copper oxide followed by KOH solution. The CuO was extracted with water, the S in the KOH oxidized with bromine, and the whole of the volatile S determined as barium sulphate. The test material in the tube was subsequently analyzed for sulphate-S and organic-S.

Data on the contents of sulphate-S, volatile S and non volatile organic-S have been summarized in Fig. 25. In alfalfa, sugar beet, and clover volatile-S did not exceed 5 per cent of the organic-S so that it can be safely neglected in the sulphur balance. In the Brassica species, however, the amount of volatile S present corresponded to about 0.03 gram atoms S per kg dry matter, *i.e.* up to about 30 per cent of the organic-S.

By heating with dilute acid to hydrolyze the glycosides, adding salt to raise the boiling point to 140°C and distilling into saturated bromine water, followed by precipitation as barium sulphate, Eaton[18] found up to about 0.03 gram atoms of volatile-S per kg of dry matter in leaves of black mustard.

Estimates of organic-S contents based on the difference between total-S and sulphate-S will include volatile sulphur. If the non-volatile organic-S consists mainly of cystine and methionine in proportions characteristic of proteins, the presence of volatile S will mean a higher than normal ratio of organic-S to organic-N.

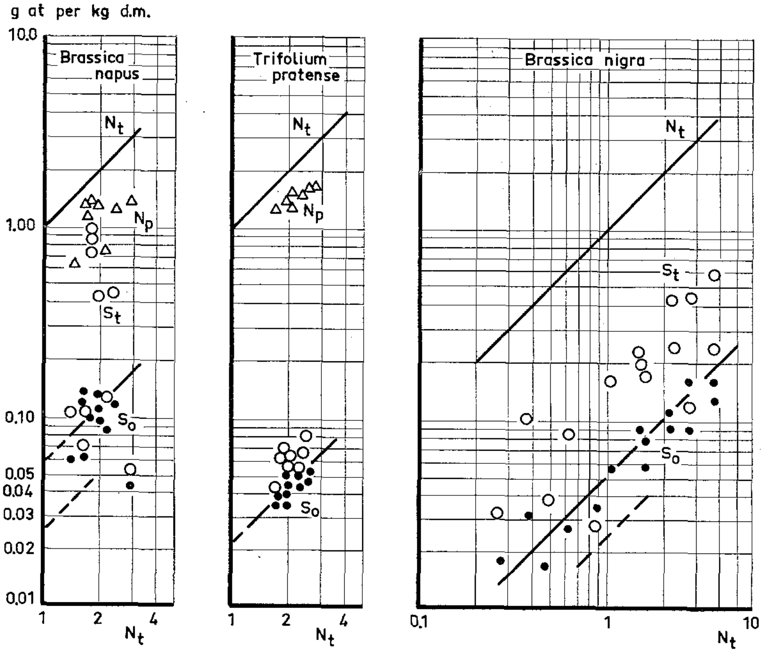

Fig. 26. Sulphur and nitrogen in rape and red clover. Data of Miller[33]. Same for black mustard. Data of Berthelot[5].

In Fig. 26 data are presented for the S- and N-fractions in two Brassica species, and in red clover. In the latter the organic-S : organic-N ratio was about 0.023, but in the former about 0.055. There is, however, no evidence that volatile-S occurs on such constant proportions as to make up for the latter value in Brassica under different conditions. All that can be said is that in these Brassica species, the ratio of organic-S to organic-N is much higher than that of the proteins because of the presence of organic-S compounds not related to the protein metabolism.

SUMMARY

From a review of published data on S- and N-fractions it has been shown that on a gram atom basis these elements occur in organic forms in a ratio ranging from 0.025 (legumes) to 0.032 (gramineous plants) and that this is the same as the S : N ratio in the proteins which constitute about 80 per cent of the organic S and N present.

In plants deficient in sulphur, the ratio organic-S : organic-N becomes less than normal due to an increase in the proportion of non-protein organic-N compounds low in sulphur. With sulphur deficiency the cytoplasmic proteins decrease out of proportion to the chloroplast proteins of a higher S content so that the ratio of protein-S to protein-N tends to increase. A similar change may occur during the fall in tissue protein content with increasing plant age.

Sulphur-deficient plants show a subnormal ratio of organic-S to organic-N in the absence of sulphate in the tissues. Plants in which the protein content is decreased by proteolysis may have subnormal organic-S : organic-S ratios in the presence of sulphate in the tissues, *i.e.* without S shortage. This may occur during dark starvation or in seedlings drawing upon reserve proteins in the seeds.

The S-requirements for growth are reflected in the normal ratio of organic-S to organic-N.

In most species investigated, organic-S occurs mainly as cystine and methionine and is directly related to protein metabolism. Because of the presence of other forms of organic-S, the organic-S : organic-N ratio in the Brassica species is higher.

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