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HARITH JABBOURI LOQA for the MASTER OF SCIENCE
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Title: EFFECTS OF SULFUR ON YIELD AND PLANT SULFUR OF SUBTERRANEAN CLOVER

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Dr. Murray D. Dawson

Subterranean clover was grown in nutrient solution and S deficient Steiwer soil in the greenhouse. Plants were harvested at 15, 30, 45 and 60 days after emergence.

When subterranean clover was grown in nutrient solution significant yield response to each increment of added sulfate (SO_4-S) occurred in 15, 30, 45 and 60 day old plants. Yield response to SO_4-S fertilized Steiwer soil also occurred at each rate of applied gypsum except in the 15 day old plants.

The increase in yield of subterranean clover grown in the nutrient solution due to added SO_4-S was similar for plants of different ages compared with the control. In the Steiwer soil the highest percentage yield increase due to added SO_4-S was in 45 day old plants.

The plant S content decreased as the plant matured in both nutrient solution and in the Steiwer soil. In the nutrient solution the

correlation coefficients for plant S content in the tops with subterranean clover yield increase at all stages of maturity were highly significant. The correlation coefficients were 0.952, 0.908, 0.729, 0.997 for 15 to 60 day old plants respectively. In the Steiwer soil the correlation coefficient of 0.99 for plant S content in the tops with yield increase was highly significant for both 15 and 30 day old plants.

As subterranean clover matured SO_4-S content in plants grown in nutrient solution decreased except at the high level of S. However SO_4-S in Steiwer grown plants remained rather constant.

There was a significant correlation (0.99 and 0.95) between plant SO_4-S and yield increase in 15 and 60 day old plants grown in nutrient solution. A similar significant correlation (0.829) occurred with 30 day old plants grown in the Steiwer soil.

The critical plant S content of subterranean clover ranged between 1.2 to 1.8 mg. S/g. of dry matter, while critical plant SO_4-S between 375 to 458 ppm. The critical plant N-S ratio ranged between 9.2:1 to 13.3:1.

At a rate equivalent to 40 lbs. S/acre some evidence of S mineralization occurred as the result of the addition of gypsum to the Steiwer soil.

Effects of Sulfur on Yield and Plant
Sulfur of Subterranean Clover

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Harith Jabbouri Loqa

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In Charge of Major

Head of Department of Soils

Dean of Graduate School

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INTRODUCTION

Sulfur has been known from the time of Liebig to be one of the elements required for plant growth, but only recently has this element received the attention it deserves as a plant nutrient. The fact that over the past several years crop deficiencies of S throughout the world have been reported with increasing frequency has focused greater attention on the importance of this element in plant nutrition.

As a major plant nutrient a close third to nitrogen (N) and phosphorus (P) in California, S must be applied in many instances to maintain crop production. Recent studies carried out both in the United States and abroad indicates that plant tissue analysis may be useful in predicting the need for S fertilization. The most promising correlation appears between S and N content of the plant. Analysis of plant parts to determine the critical values for the N:S ratio, in the plant, sulfate sulfur (SO_4-S) and total plant S have been suggested for use as guides to the S fertilization of certain legumes.

Some investigations were designed to obtain information which would enable one to predict the probability of a yield response of subterranean clover to applied S on the basis of various measures of the soil S status.

But only limited information is available which relates the uptake of S during the early stages of growth with the S supply in the soil.

Furthermore, few investigators appear to have studied the soil-plant S budget, amount of S applied to the soil removed by the plant in terms of the supply of S already present in the soil.

Objectives

The objectives of the present investigation were:

1. To study the effect of different levels of S in soil and in Hoagland solution on the growth and nutrient content of subterranean clover (*Trifolium subterraneum*).
2. To determine the influence of S levels in soil and in nutrient solution on the growth and plant S status as a function of time.
3. To investigate the effect of applied S on the soil S status as a function of time.

LITERATURE REVIEW

Historical

Since the days of Liebig, the essentiality of S as a plant nutrient was known. Gypsum was used as a fertilizer by Reverend Meyer in Switzerland's Canton of Berne in 1768 and by others in France and Germany during the last part of the 18th Century. Gypsum was used in England and the United States shortly afterwards as it was reported by Crocker (1945).

The use of S as a fertilizer began as early as 1913 in Oregon, and systematic field and laboratory experiments, which were conducted by Powers (1923) over a period of years showed responses to applied S on a variety of crops grown on different soils. According to Powers (1923) in 1913 a substantial yield response to S was obtained with field peas in one trial in Oregon.

There has been a renewed awareness of the practical importance of S in the past 10 years. The development and use of high-analysis, low-S fertilizers in the United States created problems of S nutrition. The widespread occurrence of S deficiency was noted by Conard (1950). In California the common occurrence of S response in both legumes and grasses was emphasized by Martin (1966); Jordan and Reisenauer (1957) prepared a map to indicate the areas of responses to S in the United States. In most of the western and southern states significant responses to S have been reported. Responses to S were noted in certain areas of Brazil by McClung and Martens (1959), in parts of Australia by Stephens and Donald (1958), and in New Zealand by McNaught and Chrisstoffels (1961). Recently it has been shown by Walker and Adams (1958) in New Zealand, and by Jones (1964) in California that the grasses in a pasture mixture compete so vigorously for S supplied by the soil that legumes often make little growth under conditions of low S supply in the natural grass lands.

Yield Response to Sulfur

Rending (1956) reported studies with alfalfa in California which showed that the annual yield could be approximately doubled by an application of 200 lbs. of gypsum per acre. He showed that for each of five successive cuttings of alfalfa, the yield was increased by S fertilization. Moreover slightly higher yields were produced when 400 rather than 200 lbs. of gypsum was applied per acre; the yield difference was signficant for only the first two cuttings. White clover was grown in the greenhouse on seven surface soils in Mississippi (Bardsley and Jordan, 1957). In the absence of applied S, yields for the third crop on three of the soils were lower than when S was applied; this was true for all soils in the fifth crop, and the seventh crop was virtually a failure.

Begg (1963) reported that subterranean clover grown on S-deficient soil responded to S applied in the form of sodium sulfate (Na_2SO_4). Jones (1964) indicated that 20 lbs. of S per acre supplied as gypsum increased the yield of subterranean clover grown in the greenhouse. Furthermore, he demonstrated that during the first year subterranean clover produced near maximum yield where 40 lbs. of S per acre was applied as gypsum (Jones, 1964). The carry-over in the second year indicated that the effect from 80 lbs. of S was roughly equivalent to 20 lbs. of S applied the first year.

Plant Sulfur Uptake and Requirements of Forage Crops

Although many field fertilizer experiments were conducted so that the S content of forage plants could be determined at various stages of growth, more information is needed on the actual S needed for the optimum yield of this type of crop. It is apparent in the published reports that the amount of S removed by forage crops at harvest may vary widely, depending to a degree upon the amount of S fertilizer applied to the soil.

Alfalfa grown experimentally in California, was harvested at five successive cuttings each time it reached the early bloom stage of maturity (Rending, 1956). When no S was applied he reported that the uptake of S in alfalfa for the five cuttings ranged from 1 to 3 lbs. per acre. Where S was applied to the soil at the rate of 72 lbs. of S per acre as gypsum the plant uptake of S for the corresponding harvests ranged from 6 to 13 lbs. per acre. The removal of S amounted to 5 to 6 lbs. per ton of hay. Pumphrey and Moore (1965a) carried out field trials at several locations in eastern Oregon. They reported that when 0, 10, 20, 40 lbs. of S per acre was applied to soil as gypsum, values for the uptake of S by the crop were 2.98, 5.38, 8.35, 9.79 lbs. per acre respectively. Moreover they reported that the removal of S in the hay amounted to only 3.6 to 3.8 lbs. per ton.

Williams, McKell, and Reppert (1964) indicated that the removal of S by rose clover varied with the year, but at most amounted to only about 4 lbs. of S per acre or about 2 lbs. per ton of forage produced.

Jones (1964) reported the uptake of S by competing pasture species which were grown under conditions of adequate rainfall. Thus, where no S was applied to a grass-clover seeding, the grass and clover each removed nearly equivalent amounts of S (1.4 and 1.2 lbs. per acre respectively). When the rate of S application was increased, a larger proportion of the applied S was found in the clover than in the grass.

Total Plant Sulfur

Harward, Chao, and Fang (1962b) reported a critical level of

S in alfalfa as 0.22 percent based on greenhouse experiments. Oregon field experiments conducted by Pumphrey and Moore (1965a) indicated that stage of maturity as well as the rate of S fertilization alter the S concentration in alfalfa. They showed that the percentage of S decreased steadily throughout the growth cycle and that the extent of decrease depends upon the level of S fertilization.

Martin and Walker (1966) showed that S deficient clover contained a somewhat lower total S content than did S deficient alfalfa.

Nitrogen Sulfur Ratio in Plants

Several researchers (Pumphrey and Moore, 1965a; Walker, Adams, and Orchiston, 1956; Jorden and Bardsley, 1958) have suggested that N:S ratio might provide a useful means to evaluate the S status of herbage. Ratios of N:S remain relatively constant at different growth stages as shown by Pumphrey and Moore (1965a). In their experiments which were located in fields in eastern Oregon, N:S ratios ranging from 15 to 25 indicated progressively more acute S deficiency, while N:S ratios below 11 indicated an adequate S supply.

Walker, Adams and Orchiston (1956) have considered N:S ratio to evaluate the S status of clover and grass in New Zealand. They interpreted an N:S ratio of 15 to indicate acute S deficiency, and values less than 11 indicated an adequate S supply. Dijkshoorn

(1960) proposed the use of the N:S relationship based upon the mole ratios of N and S as a mean of evaluating the S assay status of herbage. He showed that when values for the total S assay in the herbage were lower than 0.027 Kjeldahl-N, a true condition of S deficiency existed.

Plant Sulfate Sulfur

Several workers (Ulrich, 1961; Jones, 1962; Jones and Martin, 1964) have suggested that the amount of SO_4-S in certain plants is a sensitive indicator of the adequacy of the S supply for the requirement of the plant. Jones (1962) showed little difference between leaves, petioles, and stems, in critical values. Approximately 170 ppm SO_4-S appeared critical regardless of the portion of plant sampled. Jones and Martin (1964) showed that on mixed pasture stands 200 ppm SO_4-S was critical. Under conditions of low N supply, SO_4-S values in grass tended to be higher, since N, not S, limited growth. Jones (1962) has suggested that the SO_4-S of legume appears to measure the S needs of the plant community under field conditions.

Soil Sulfur Status

Organic Bound Sulfur

In soil S exists in both organic and inorganic forms. Freney, Barrow, and Spencer (1962) indicated that organic S was generally estimated as the difference between total S and SO_4-S. Moreover they indicated that organic S accounts for a major fraction of the total soil S present in the surface horizon of most soils. Evans and Rost (1945) attempted to measure directly the organic S content of some Minnesota soils, and their results supported the contention that the major portion of the S in soil is organic. They reported that the organic-S contents ranged from 216 to 428 ppm. Lowe (1964) working with soils from Quebec indicated that 53 to 90 per-cent of the S is organic. It was reported by Nelson (1964) that most of the S in the surface horizon of soils is in the organic form and bears a definite relationship to the amount of organic carbon (C) present. According to Nelson (1964) these soils had an average C:N:S ratio of 126:10:1, and the amounts of organic C, total N and total S were positively correlated. Walker and Adams (1958a) who studied grassland soils formed from widely different parent materials reported C:N:S organic-P ratios of 120:10:1.3:2.7. Harward, Chao, and Fang (1962b) reported that the bulk of the S of surface soils is in the organic form.

Little is known of the nature of the organic S. According to Starkey (1950) a great variety of organic S compounds are present in the soil organic matter as proteins and other compounds produced by higher plants, animals, and microorganisms.

In soil most of the S containing amino acids in soil appear to be bound in some manner to the mineral and humus fractions. According to Bremner (1965) cystine, cysteic acid, methionine, methionine sulfoxide and methionine sulfone have all been reported in soil organic matter. Freney (1961) and others (Lowe and de Long, 1961; Lowe, 1964) have shown that a significant fraction of the total S in soil ranging from 24 to 77 percent, could be reduced to hydrogen sulfide with hydroiodic acid, or hydrolyzed to SO_4-S by acid or alkali. This S may exist, at least in part, in the form of organic S, such as phenolic-SO_4, choline-SO_4, and SO_4-esters of carbohydrates and lipids. Lowe and de Long (1963) reported that another fraction of the organic S appears to be directly bonded to carbon. Moreover they (Lowe and de Long, 1963) estimated that up to 58 percent of the total S in some Canadian soils occurred in C-S linkages. According to Freney and Stevenson (1966) the C-bonded fraction includes S-containing amino acids, such as cysteine and methionine.

Microbial Oxidation of Sulfur

Bacteria are responsible for oxidation of most of the organic S in soil. Starkey (1966) reported that thiobacilli oxidized sulfide, elemental S, thiosulfate, tetrathionate, thiocyanate, and sulfite to SO_4-S as the final product.

Inorganic Sulfur

Most soils contain significant amounts of SO_4-S. Freney, Barrow, and Spencer (1962) indicated that the SO_4-S may be present in the soil solution as SO_4 ion or precipitate as SO_4 salts of calcium (Ca) and magnesium (Mg). The coprecipitated ion of SO_4 with calcium carbonate is not uncommon. Williams and Steinberg (1962) in Australia showed that on calcareous soils, inorganic bound SO_4 comprises an important portion of the total S. Insoluble S may also be present as barium sulfate ($BaSO_4$) or pyrite (Freney, Barrow, and Spencer, 1962; Cairns and Richer, 1960).

Sulfate Sorbed Sulfur. Numerous workers have shown that soils differ in their ability to retain SO_4-S. Kamprath, Nelson, and Fitts (1956) showed that soils have the capacity to adsorb significant amounts of SO_4 ion. Neutral or slightly acid soils sorb less SO_4 than strongly acid soils. Moreover, Kaolinite clay minerals with a 1:1 lattice that are saturated with aluminum (Al) sorb more SO_4 than montmorillonitic clay minerals with a 2:1 lattice (Kamprath, Nelson, and Fitts, 1956).

Harward (1962a) suggests that SO_4 retention by soils may involve several mechanisms including:

(1) Anion exchange with hydroxyl ion on hydrous iron and aluminum oxides or on the crystal edges of clays, especially kaolinite, at low pH values.

(2) Retention of SO_4 ions by hydroxy-aluminum complexed by coordination.

(3) Salt adsorption resulting from attraction between the surface of soil colloids and the salt.

(4) Amphoteric properties of soil organic matter which develops positive charges under certain conditions.

Gains and Losses of Soil Sulfur

The atmosphere frequently provides a source of S especially in heavily industrialized countries. Plants may adsorb sulfur dioxide (SO_2) by gaseous diffusion into the leaves. Another source of soil S is from insecticides, although the more recent synthetic organic materials are relatively S free.

Williams and Steinbergs (1958) indicated that S from precipitation ranged to 120 lbs. per acre per year.

Crop removal and leaching account substantially for SO_4-S removal from the soil. Bertramson, Fried, and Tisdale (1950) indicated legumes remove 20 lbs. per acre per year. Loss of soil S by leaching may be extensive. Jordan and Ensminger (1958), in studies conducted using lysimeters, have shown that 55 lbs. of S per acre per year may be leached from sandy soils.

Sulfate Soil Analysis

In recent years attention has been given to soil analysis as a means of predicting plant-available S. Several different extracting solutions have been used in an attempt to assay the soil for plant-available S. Spencer and Freney (1960) used water as extracting solutions. They obtained good correlation between the S uptake by plants and the cold water extractable SO_4-S. Williams and Steinbergs (1959) found moderate correlation (r = 0.78) between the amounts of SO_4-S extracted with solutions of dilute electrolyte (0.15% $CaCl_2$ and 0.2 to 6.0% NaCl) and the S found in the above ground portion of plants grown in samples of the corresponding soils in the greenhouse. Bardsley and Lancaster (1960) reported that SO_4-S extracted with dilute acetic acid was not significantly correlated with S uptake by white clover. They reported that reserve S (largely organic S) was significantly correlated with plant S uptake. Bardsley and Lancaster (1960) and William and Steinbergs (1959) reported that the total S content of a soil does not normally correlate well with S uptake by plants.

Ensminger and Freney (1960) reported that extractants consisting of phosphate (PO_4) salts in solution were effective for estimating the plant-available S in soils which contain appreciable amounts of adsorbed SO_4-S. Spencer and Freney (1960) showed that on a wide range of S-deficient soils, PO_4-extractable SO_4-S correlated well with S uptake by plants. A significant correlation was obtained by Kilmer and Nearpass (1964) between S extractable by 0.55 M sodium bicarbonate ($NaHCO_3$) at pH 8.5 and uptake by plants.

One form of "heat-soluble" S (SO_4-S extractable in hot water) was used by several workers as an index of available S (Spencer and Freney, 1960; Williams and Steinbergs, 1959). The S extractable from soil after mild hydrolysis of the sample at 50^oC or after drying at 100^oC (also designated "heat-soluble" S) is thought to consist of the soluble SO_4-S plus the more labile organic SO_4-S released by mild hydrolysis. Williams and Steinbergs (1959) reported that the "heat-soluble" SO_4-S of soil was significantly correlated with S uptake by plants in the greenhouse.

MATERIALS AND METHODS

Two distinct experiments were conducted in the greenhouse. The first experiment was carried out by growing subterranean clover (*Trifolium subterraneum*) variety (Mount Barker) in nutrient solution containing different SO_4-S levels. The second experiment was conducted using an S deficient soil to which increasing increments of S were added in the SO_4 form.

Hoagland's Nutrient Solution Experiment

Dry quartz sand which had been previously washed with 0.5 N HCl and leached with distilled water was placed in plastic containers which were painted black. In each container was placed 2,800 g. of washed sand and 80 inoculated subterranean clover seeds. After emergence the seedlings were thinned so that a uniform number of 50 plants were established in each container. A nutrient solution containing the different levels of S was added to the sand to provide the S treatments. The nutrient solution was prepared using a 1:2 dilution of the original solution of Hoagland (Rosell and Ulrich, 1964). Further, the S content of the nutrient solution was adjusted to the appropriate level by adding the amounts of SO_4-S indicated in Table 1.

The pots were flushed daily with the appropriate nutrient solution to maintain as closely as possible the initial nutrient levels.

Table 1. Nutrient solutions used in the experiment and their respective sulfate contents.

Solution number	Nutrient solution composition	S added to nutrient solution mg./pot	meq. SO_4/ℓ.
1	0.5N Hoagland Solution with no sulfur	0	0.0000
2	0.5N Hoagland Solution with 1/16	1	
	Normal Sulfur		0.3125
3	0.5N Hoagland Solution with 1/8	2	
	Normal Sulfur		0.6250
4	0.5N Hoagland Solution with 1/2	8	
	Normal Sulfur		2.5000

The greenhouse temperature was controlled at 16-21°C and day length of 16 hours.

At selected time intervals (15, 30, 45 and 60 days) entire subterranean clover plants (tops plus roots) were removed from the appropriate container. The plant material was weighed wet and then dried at 65°C for three days for dry weight determination.

A complete block design with four replications was used. The four nutrient solutions with different SO_4-S concentrations were arranged at random within each replication on the greenhouse bench.

Growth of Subterranean Clover in Sulfur-Deficient Soil

In the second experiment conducted in an adjacent greenhouse subterranean clover was grown in an S deficient soil. Surface soil to a depth of 6 in. was taken at random from a field experimental site that had previously indicated response by subterranean clover to applied gypsum. The soil was classified as a Steiwer silt loam and the chemical characteristics are recorded in Table 2.

Table 2. Chemical characteristics of the sample of the Steiwer soil used for growing subterranean clover.*

Chemical Determination	Recorded Value
pH (1:2, soil:water suspension)	5.8
$NaHCO_5$ extractable P	3 ppm
Cation exchange capacity and extractable cations [1 N ammonium acetate (CH_3 - CO_2 - NH_4), pH 7]	
CEC	17.9 meq./100 g.
K	0.52 meq./100 g.
Ca	9.1 meq./100 g.
Mg	2.5 meq./100 g.
Hot water-extractable boron (B)	0.51 ppm
Total N by Kjeldahl method	0.194%
Organic matter (Walkley & Black, 1934)	3.7%

*Chemical determinations by the Oregon State University Soil Testing Laboratory.

SO_4-S was extracted with a solution of sodium dihydrogen phosphate (NaH_2PO_4) using a modified Chesnin-Yien method suggested by Bardsley and Lancaster (1960).

The soil was air dried and passed through a two mm sieve. A blanket fertilizer application was made to the bulk soil sample and thoroughly mixed to supply the following nutrients with materials and rates indicated in Table 3.

Table 3. The plant nutrients added to the sample of the Steiwer soil used for growing subterranean clover in the greenhouse.

Plant nutrient	Fertilizer material	mg/pot	Equiv. lb/AFS*
(1) P	KH_2PO_4	15	100
(2) K	KCl	15	100
(3) Ca and Mg	$CaMg(CO_3)_2$	600	4000
(4) molybdenum (Mo)	$Na_2MoO_4 \cdot 2H_2O$	0.15	1
(5) B	H_3BO_3	0.75	5
(6) zinc (Zn)	$ZnCl_2$	0.45	3

*AFS or acre furrow slice is equivalent to 2 million lbs. of soil.

After treatment and mixing, 300 g.of soil was weighed into each plastic container (11 cm. diameter). A hole was made at the base of the container to permit drainage. The hole of the container was lined with glass wool to prevent the loss of soil. On March 28, 1966 the subterranean clover seeds were innoculated and 70 seeds were placed uniformly over the surface of the soil in each pot. These seeds were lightly covered with dry soil in each container.

After seeding S was applied in solution to appropriately labelled containers at rates equivalent to 0, 10, 20, 40 lbs. S per acre.

After emergence the plants were thinned to 50 plants per container. Moisture was maintained at approximately field capacity by watering daily with distilled water. Any drainage water was collected and recycled through the container.

Greenhouse temperature was controlled at 16-21^{o}C night and day and daylength of 16 hours. At selected time intervals of 15, 30, 45, 60 days entire subterranean clover plants (tops plus roots) were removed from appropriate containers. The plant material was weighed wet and then dried at 65^{o}C for 3 days for dry weight determination.

Plant Sulfur Analysis

The plant samples were dried, ground, and then digested with HNO_3 and $HClO_4$. The plant digests were analyzed for total S using the method described by Butters and Chenery (1959). The method of Walker and Bentley (1961) was used to determine SO_4-S in the plant tissue. For the SO_4-S determination, the plant samples which were previously dried and ground were extracted by adding 70% ethanol to the sample container and then placing the container on an electrical shaker for 30 minutes. Prior to SO_4-S analysis, the total S content of the ethanol extract was determined.

Total N was determined using the Kjeldahl method (Chapman and Pratt, 1961). Organic S was determined by the difference of total and SO_4-S (Johnson and Ulrich, 1959).

Soil Sulfur Analysis

Total S was determined by turbidimetric assay with barium chloride ($BaCl_2$) as described by Butters and Chenery (1959) after oxidation in the muffle furnace with magnesium nitrate [$Mg(NO_3)_2$]. Total S was also determined gravimetrically using sodium peroxide and sodium carbonate fusion followed by barium sulfate ($BaSO_4$) precipitation as described by Johnson and Nishita (1952). The two methods when compared (see Appendix Table 1, page 70) were found to be in close agreement.

Organic carbon was determined using the Allison method (1935). Total N was determined using the Kjeldahl method (Alban and Kellogg, 1959). Total phosphorus was determined using the method described by Saunders and Williams (1955). Inorganic phosphorus was determined by the method described by Saunders and Williams (1955). Organic phosphorus was determined by subtraction of inorganic phosphorus from total phosphorus as it is described by Saunders and Williams (1955). Organic S was determined by subtraction of SO_4-S from total S as it is described by Freney, Barrow and Spencer (1962).

A complete block design with four replications was used for greenhouse experiment. The pots consisting of five rates of S were arranged at random within each replication on the greenhouse bench.

RESULTS AND DISCUSSION

Growth of Subterranean Clover in Nutrient Solution

Subterranean Clover Yield Response to Sulfur

Plants subjected to various levels of S in nutrient solution and harvested at different time intervals produced yields recorded in Table 4. There was a considerable increase in yield as the plant matured. When yields were plotted against SO_4-S added in nutrient solution as in Figure 1 somewhat different response patterns emerged. After the first harvest increasing the level of S from 0.625 to 2.5 me SO_4/ℓ resulted in about 150 percent yield increase relative to the control (Solution 1). It is evident that in sufficient S was supplied for optimum growth in nutrient solution at least until solution number 4 was used.

Plant Sulfur Content

Figure 2 shows the relationship between plant S content and relative yield. The actual plant S content obtained in the nutrient solution experiment is recorded in Table 5. Data presented in Table 5 suggest that after the 15 days the plant supplied with nutrient solution 4 removed about 10 mg. S/g. of dry matter produced. The plant S content tended to decrease as the plant matured regardless of the amount of S supplied in the nutrient solution. These

Table 4. Yield at the indicated intervals after plant emergence for subterranean clover grown in nutrient solution containing different levels of SO_4-S (means of four replications).

Nutrient Solution number	S applied to sand mg./pot	Plant yield at day intervals after emergence: 15 g./pot	15 Relative to control	30 g./pot	30 Relative to control	45 g./pot	45 Relative to control	60 g./pot	60 Relative to control
1	0	0.412	100	0.648	100	1.265	100	2.744	100
2	1	0.445	108	0.741	120	1.392	111	3.677	134
3	2	0.519	126	0.840	136	1.847	146	3.814	139
4	8	0.567	137	1.235	150	2.530	150	5.380	151
LSD 5%		0.0167		0.0167		0.0678		0.170	
1%		0.0241		0.0241		0.0975		0.244	

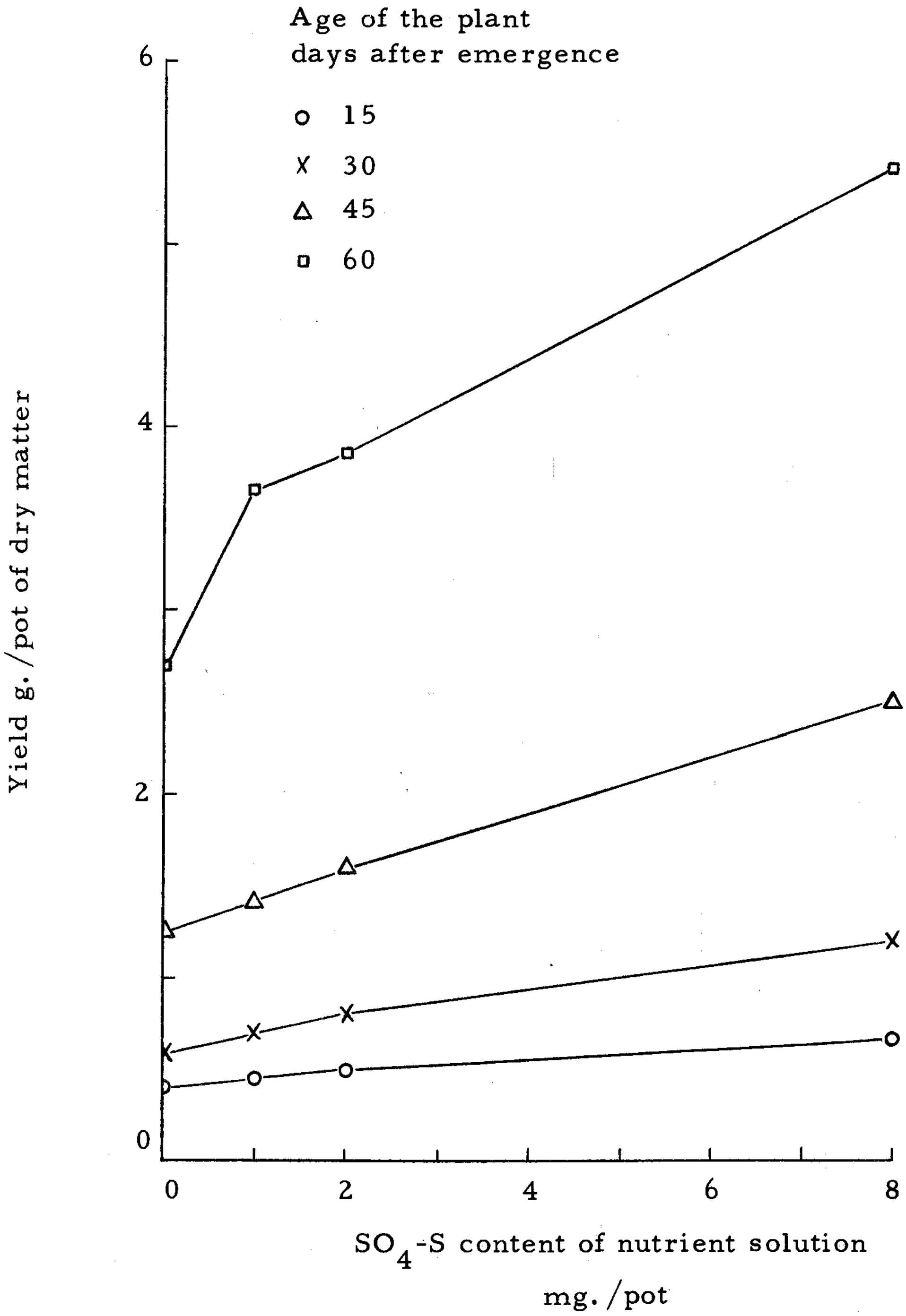

Figure 1. The relationship between subterranean clover yield and SO_4-S added to the nutrient solution.

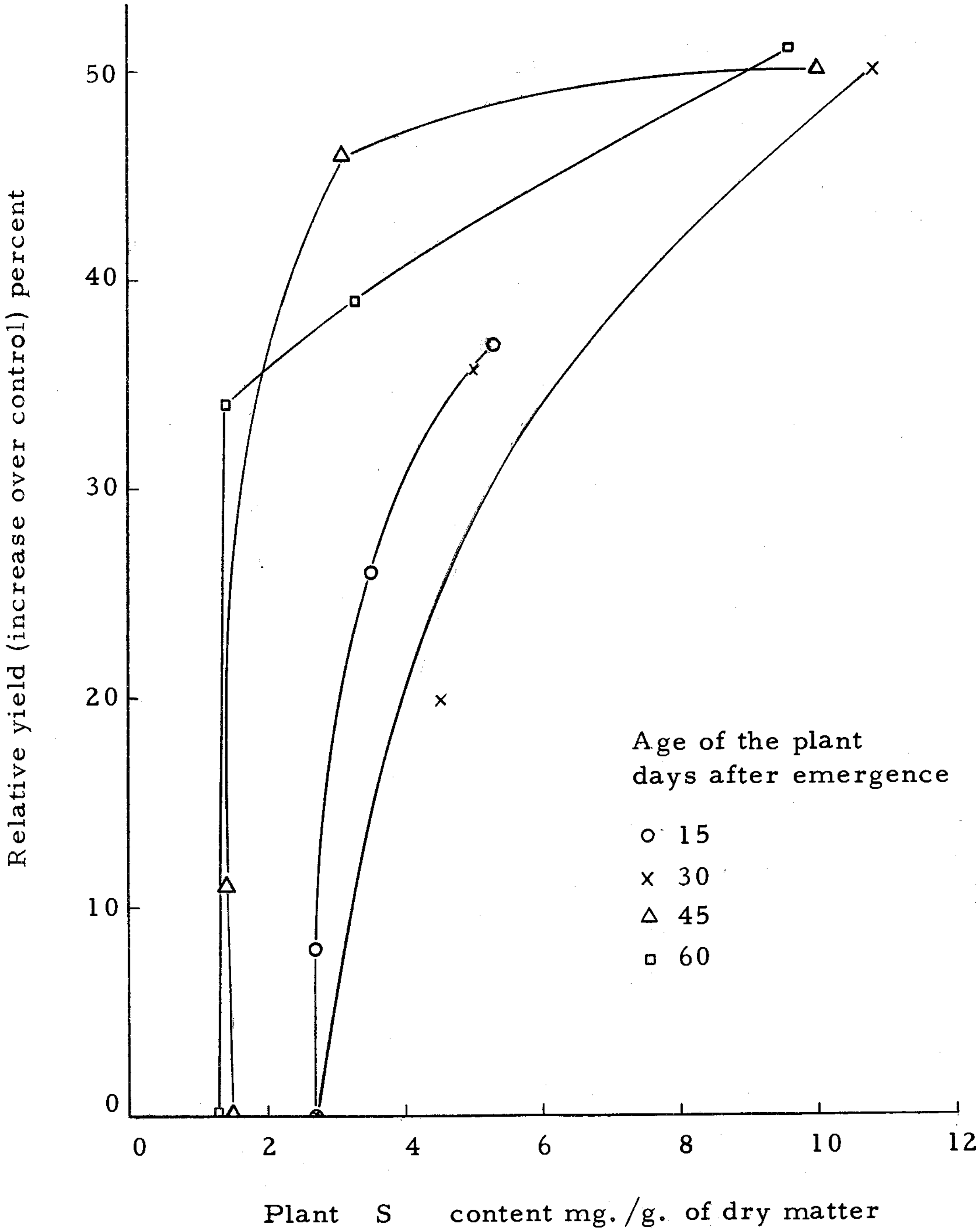

Figure 2. The relationship between plant S content and relative yield in nutrient solution.

Table 5. Plant sulfur content of subterranean clover grown in nutrient solution at 15 day intervals (means of two replications).

Nutrient Solution number	Plant age at day intervals after emergence											
	15			30			45			60		
	Plant S Content mg./g.	Plant Organic S Content mg./g.	Plant SO_4-S Content mg./g.	Plant S Content mg./g.	Plant Organic S Content mg./g.	Plant SO_4-S Content mg./g.	Plant S Content mg./g.	Plant Organic S Content mg./g.	Plant SO_4-S Content mg./g.	Plant S Content mg./g.	Plant Organic S Content mg./g.	Plant SO_4-S Content mg./g.
1	2.7	2.4	0.3	2.7	2.3	0.4	1.5	1.5	0.02	1.3	1.3	0.01
2	2.7	2.3	0.4	4.5	4.0	0.5	1.4	1.2	0.20	1.4	1.4	0.02
3	3.5	3.0	0.5	5.0	4.4	0.6	3.1	2.7	0.4	3.4	3.2	0.2
4	5.3	4.7	0.6	10.8	8.8	1.5	10.0	9.0	1.0	9.6	7.8	0.18

Table 6. Total plant sulfur uptake by subterranean clover grown in nutrient solution at 15 day intervals (means of two replications).

		Plant age at day intervals			
		15	30	45	60
Nutrient Solution number	S added mg./pot	Total plant S uptake mg./pot	Total plant S uptake mg./pot	Total plant S uptake mg./pot	Total plant S uptake mg./pot
1	0	1.08	1.65	1.94	3.66
2	1	1.19	3.31	1.85	5.14
3	2	1.83	4.20	5.78	12.70
4	8	3.01	12.75	25.27	51.24

findings agreed with the work done by Jones (1963) who reported that the concentration of plant S in subterranean clover decreased as the season advanced. Pumphrey and Moore (1965a) reported that plant S content was higher in the young herbage than in old herbage. The relation between plant S content and SO_4-S in the nutrient solution shown in Figure 3 indicates the marked increase in plant S content when supplied with nutrient number 4.

For all harvests the correlation coefficients obtained when plant S content in the tops was related to the increase in yield of subterranean clover were highly significant. The correlation coefficients were 0.952, 0.908, 0.729, 0.997 for first to fourth harvest intervals respectively.

Total Plant Sulfur Uptake. Total plant S uptake data presented in Table 6 represent the product of plant S content multiplied by yield. The increased S content of the nutrient solution significantly increased the total S uptake of the crop. This was particularly pronounced in subterranean clover grown in nutrient solution number 4 (2 to 4 fold over nutrient solution number 3). The relationship between the total plant S uptake and SO_4-S added in the nutrient solution (Figure 4) indicates the marked increase in total plant S uptake when supplied with nutrient solution number 4.

Plant Sulfate Content. After the 15 day old plant SO_4-S decreased as the plant matured (Table 5). There appeared to be much

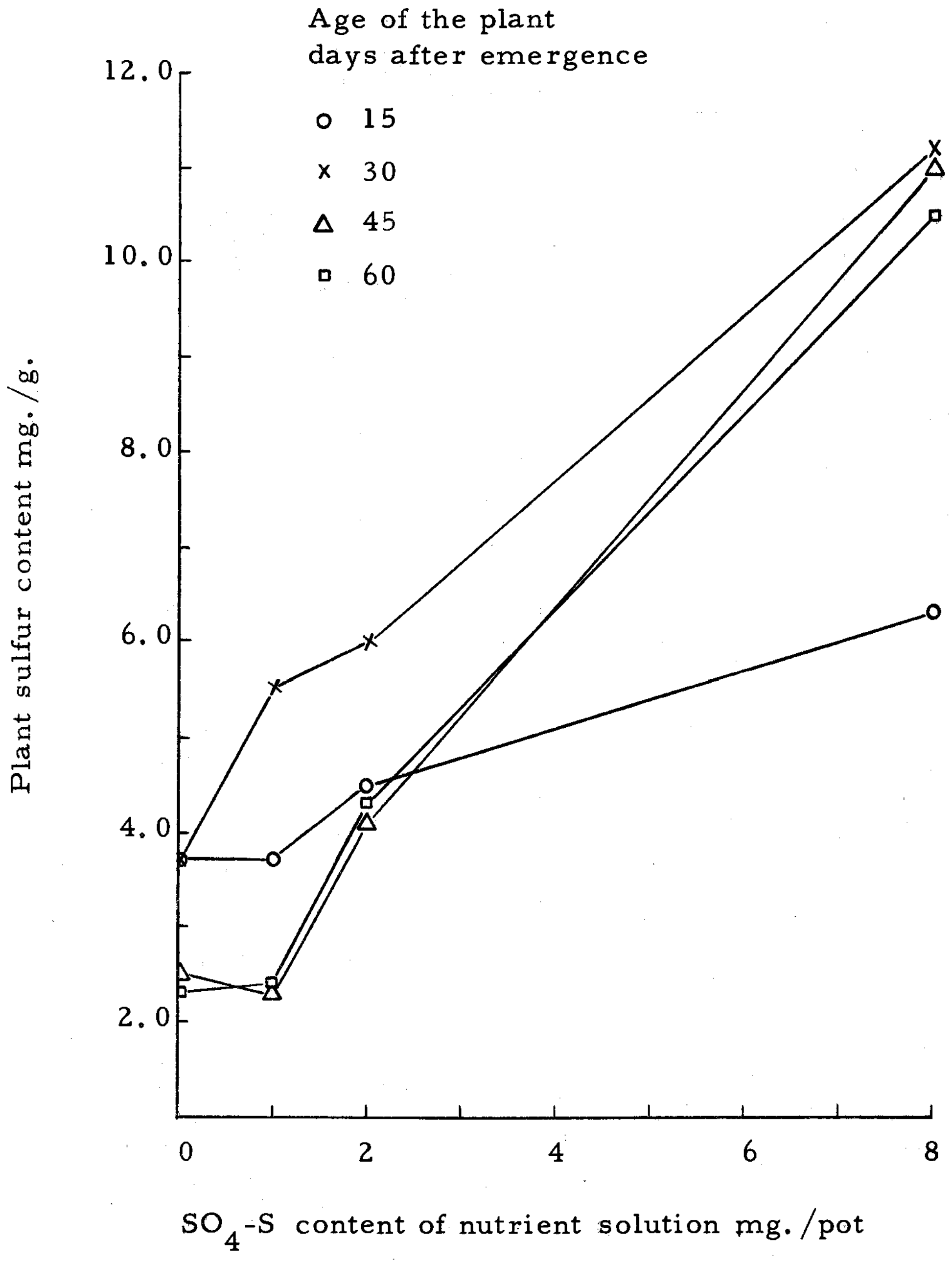

Figure 3. The relationship between plant S content and SO_4-S added to nutrient solution.

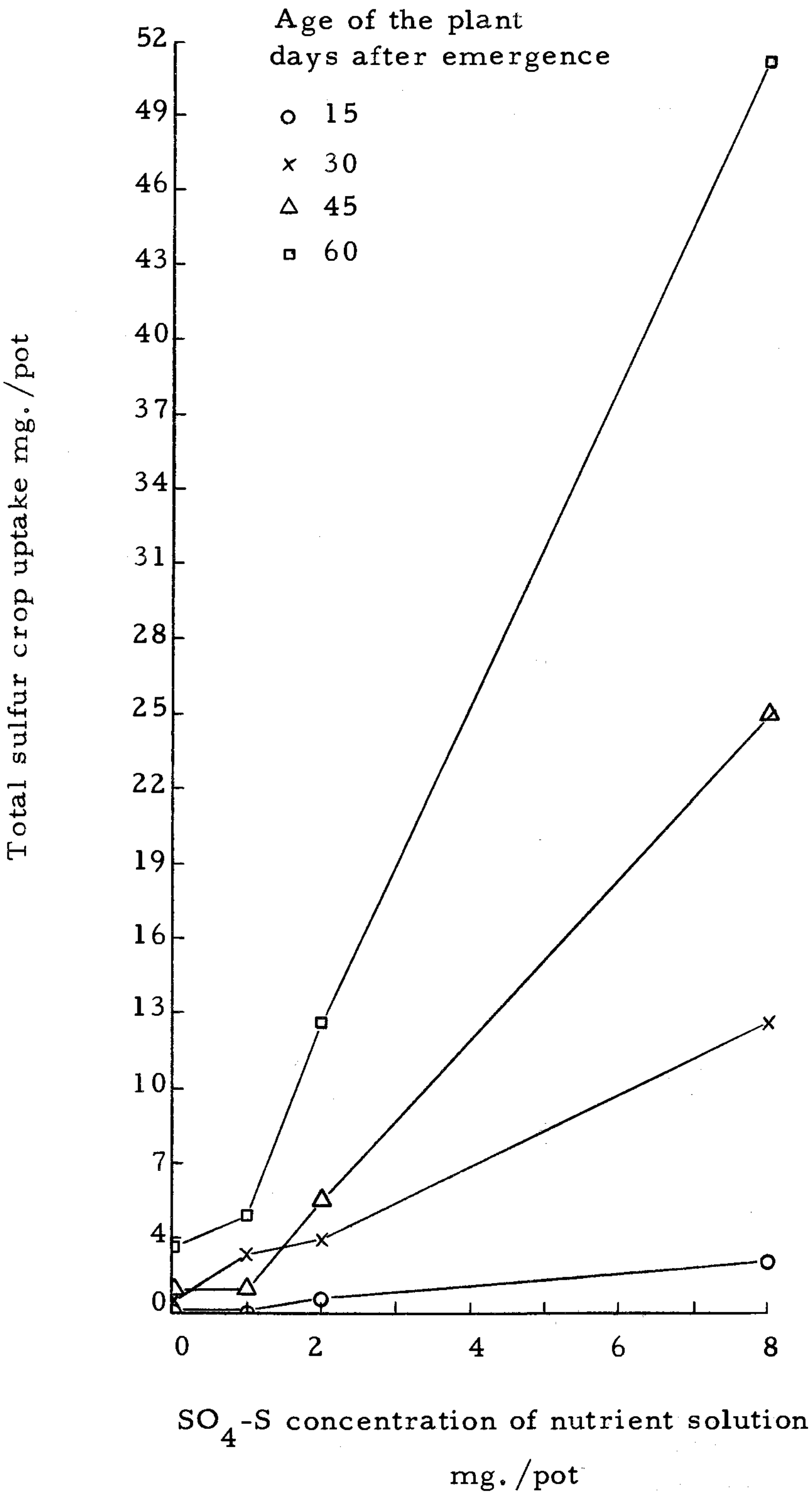

Figure 4. The relationship between total plant S uptake and SO_4-S added to nutrient solution.

less effect of S nutrient level on plant SO_4-S at the 15 day old plant (0.3 to 0.6 mg./g. of dry matter) than at the 60 day old plants (0.01 to 0.18 mg./g. of dry matter). Results obtained in this study suggest that at about 45 days (third harvest interval) the plant sulfate decreased.

In the 15 and 60 day old plants correlation coefficients, (0.99 and 0.95 respectively) indicative of the relationship between plant SO_4-S content and yield increase for the various S treatments were highly significant, but not for the 30 and 45 day old plants.

Plant Organic Sulfur Content. The plant organic S content varied with both the level of S in the nutrient solution and with the stage of maturity (Table 5). After 15 days the plant organic S remained reasonably constant at between 8 to 9 mg./g. of dry matter for plants growing in the nutrient solution with the highest S level (solution number 4). But at lower S levels the concentration of plant organic S decreased as the plant matured (4.0 to 1.4 mg./g. dry matter).

Plant Organic Sulfur to Plant Sulfur Ratio

As noted in Table 7 and Figure 5 little change occurred in the plant organic S to plant SO_4-S ratio in the young plant with increasing S supplied in the nutrient solution. But in 45 and 60 day old plants marked changes occurred in the organic S:SO_4-S ratio

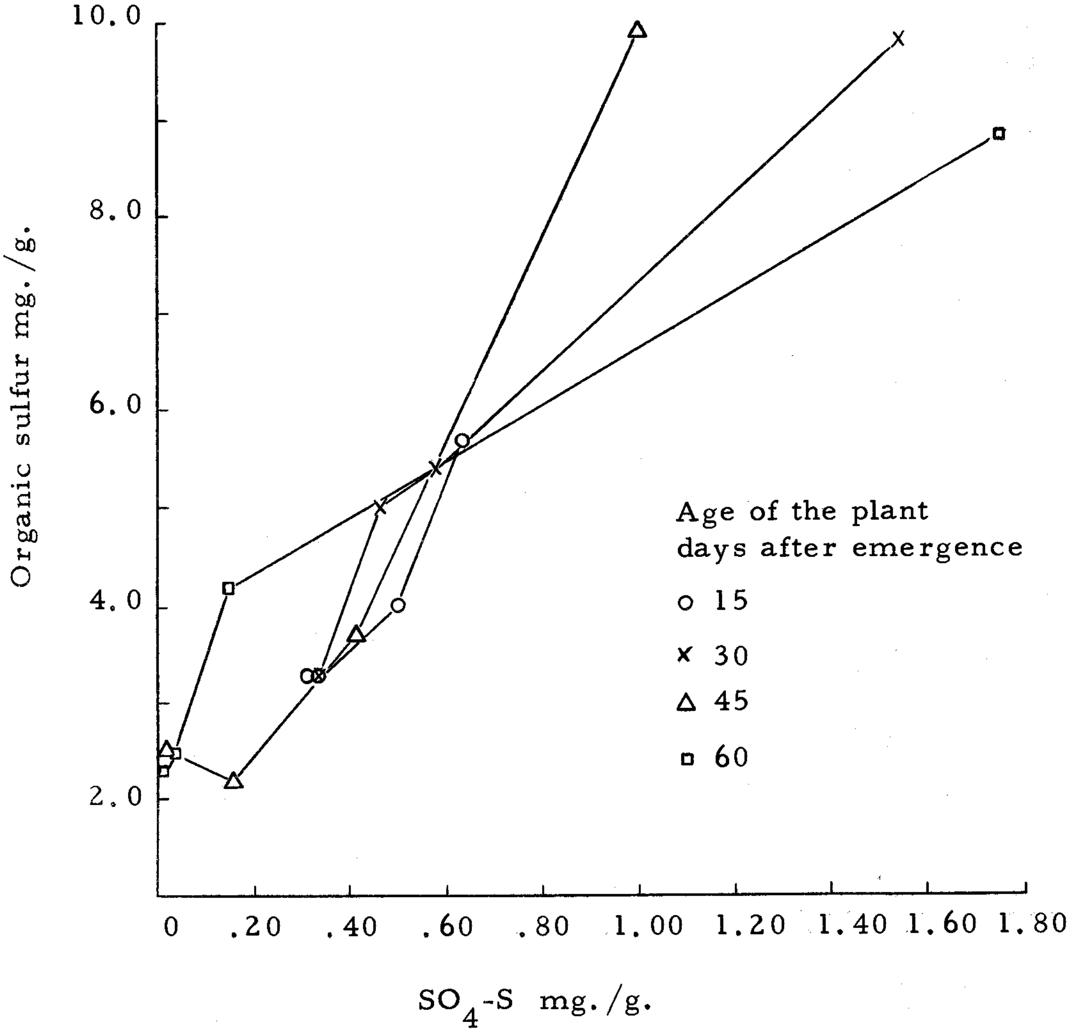

Figure 5. The relationship between plant organic S and plant SO_4-S of subterranean clover grown on nutrient solution.

especially for plants grown in solutions with the lower S levels. For instance in 60 day old plants there was more than a 30-fold difference in organic S:SO_4-S ratio (Table 7) as shown by comparing plants grown in solutions 1 and 4. This would suggest that in 45 and 60 day old plants where levels of supplied S were less than nutrient solution number 4 most of the plant SO_4-S was used in protein synthesis.

Table 7. Plant organic sulfur to plant sulfate ratio of subterranean clover grown in nutrient solution (means of two replications).

Nutrient Solution number	Plants 15 days of age Organic S / SO_4-S	Plants 30 days of age Organic S / SO_4-S	Plants 45 days of age Organic S / SO_4-S	Plants 60 days of age Organic S / SO_4-S
1	8:1	6.8:1	75.0:1	130.0:1
2	6.8:1	8.0:1	60.0:1	70.0:1
3	6.0:1	7.3:1	6.8:1	16.0:1
4	7.8:1	5.8:1	9.0:1	4.3:1

Plant Nitrogen Content

Plant N content appeared to decrease as the plant matured, and this was more marked in plants receiving less SO_4-S (Table 8). This decline in plant N content with growth where no S was supplied to the nutrient solution was from 4.80 to 3.24 mg./g. of dry matter, while the decline in plant N content where nutrient solution number 4 was applied was from 3.91 to 2.59 mg./g. of dry matter. This decline in total N with growth was similar to that reported by

Pumphrey and Moore (1965a). They reported that plant N content was highest in the earliest sampled herbage and lowest in the herbage obtained at the last sampling of the experiment done in the field. Moreover this decline in plant N content at advanced stages of growth was similar to that reported by other workers (Kust and Smith, 1961).

Total Nitrogen Uptake

Total N uptake presented in Table 8 represents the product of plant N content multiplied by yield. Levels of supplied S had relatively little effect on total N uptake at the first and second harvest intervals. However by the fourth harvest interval the S level supplied in nutrient solution markedly affected total N uptake (88.9 and 139.3 mg./g. of dry matter for no-S and high-S treatments respectively). Total uptake increased up until the fourth harvest interval.

N:S Ratio

An examination of the data of Table 5 and 8 revealed that increasing the S content of the nutrient solution markedly influenced the plant S content relative to plant N content at each harvest interval. The N to S ratio were widest (18.0:1, 17.8:1, 20.8:1, 24.3:1 for the 15, 30, 45 and 60 day old plants respectively) where

Table 8. Plant nitrogen content, total nitrogen uptake, and N:S ratio of subterranean clover grown in nutrient solution (means of two replications).

Nutrient Solution number	Plant age at day intervals after emergence											
	15			30			45			60		
	Plant N Content mg./g.	Total N uptake mg./pot	N:S ratio	Plant N Content mg./g.	Total N uptake mg./pot	N:S mg./pot	Plant N Content mg./g.	Total N uptake mg./pot	N:S mg./pot	Plant N Content mg./g.	Total N uptake mg./pot	N:S ratio
1	4.80	19.7	18.0	4.75	29.4	17.8	3.19	40.4	20.8	3.24	88.9	24.3
2	4.64	20.6	17.4	4.11	30.5	9.2	2.44	34.0	18.3	2.50	91.9	17.9
3	5.93	30.8	16.7	3.34	28.1	6.6	4.94	91.2	15.7	3.57	136.2	11.6
4	3.91	22.1	7.4	2.56	31.6	3.5	2.31	58.4	2.3	2.59	139.3	2.7

little or no S was supplied in the solution and lowest at the highest S level (7.4:1, 3.5:1, 2.3:1, 2.7:1 for the 15, 30, 45 and 60 day old plants respectively). These results agree with the findings of Pumphrey and Moore (1965a) who reported that N to S ratio were widest where little or no S was applied and lowest at the highest S level. In the nutrient solution number 2 and 3 plant N:S ratio dropped in 30 day old plants from 17.4:1, 16.7:1 to 9.2:1, 6.6:1 respectively and then increased again as the plants reached 45, 60 days (Table 8). This low N:S ratio in 30 day old plants was associated more closely with an increase in plant SO_4-S content than a change in plant N content. After 15 days, N:S ratio remained fairly constant (3.5:1 to 2.3:1) in plants grown in the highest level of supplied S.

Growth of Subterranean Clover in Sulfur-Deficient Soil

Subterranean Clover Yield Response to Sulfur

Subsequent to the first harvest interval subterranean clover responded significantly to SO_4-S applied to the Steiwer soil (Table 9). This response for the second and third harvest intervals was significant at the 0.01 level of probability and at the 0.05 level for the 60 day old plants. Figure 6 shows some of the growth responses observed in the greenhouse from applied S. In the case of 15 and 30

Table 9. Yield of subterranean clover grown on the Steiwer soil as influenced by applied gypsum (means of four replications).

Gypsum applied lbs. S/acre	Plant yield at day intervals after emergence 15 Yield g./pot	15 Relative to control	30 Yield g./pot	30 Relative to control	45 Yield g./pot	45 Relative to control	60 Yield g./pot	60 Relative to control
0	0.54	100	1.45	100	1.41	100	2.89	100
5	0.63	117	2.04	141	2.47	175	2.96	102
10	0.66	122	2.11	146	2.91	206	4.30	149
20	0.62	115	2.21	152	2.47	174	3.90	135
40	0.74	137	2.08	143	2.92	207	4.02	139
LSD 5%	n.s.		0.46		0.532		0.822	
1%	n.s.		0.64		0.732		n.s.	

Figure 6. The effect of applied gypsum to Steiwer soil in subterranean clover growth (45 day old plants).

day old plants a rate of S equivalent to 5 lbs./acre significantly increased yields by 141 and 175 percent respectively over the control. A significant yield increase (48.7%) at the fourth harvest interval occurred up to a rate of applied SO_4-S equivalent to 10 lbs./acre. Figure 7 presents the pattern of yield response to SO_4-S applied to the Steiwer soil. It is interesting to note that the yield of dry matter tended to level off in 30, 45, 60 day old plants after 1.5 mg. S/pot (10 lbs. S/acre) was added to the pots. After the 15 days subterranean clover yield responded to a rate of applied SO_4-S equivalent to 10 lbs. S/acre. This response ranged between 146 and 206 percent of the control (Table 9). The greatest yield increase of subterranean clover occurred at the third harvest interval where more than a doubling in yield was recorded (1.41 to 2.92 g./pot) upon the application of gypsum.

Plant Sulfur Content

Plant S content increased markedly due to S fertilization (Table 10) in 15 day old plants (2.8 mg./g. to 6.2 mg./g. of dry matter). Subsequently the rates of S fertilization appeared to have little affect on plant S content at any given harvest interval. Indeed, after 15 days (Figure 8) the S content remained rather constant within each harvest interval regardless of the SO_4-S applied to the Steiwer soil. It is interesting to note that the plant S content

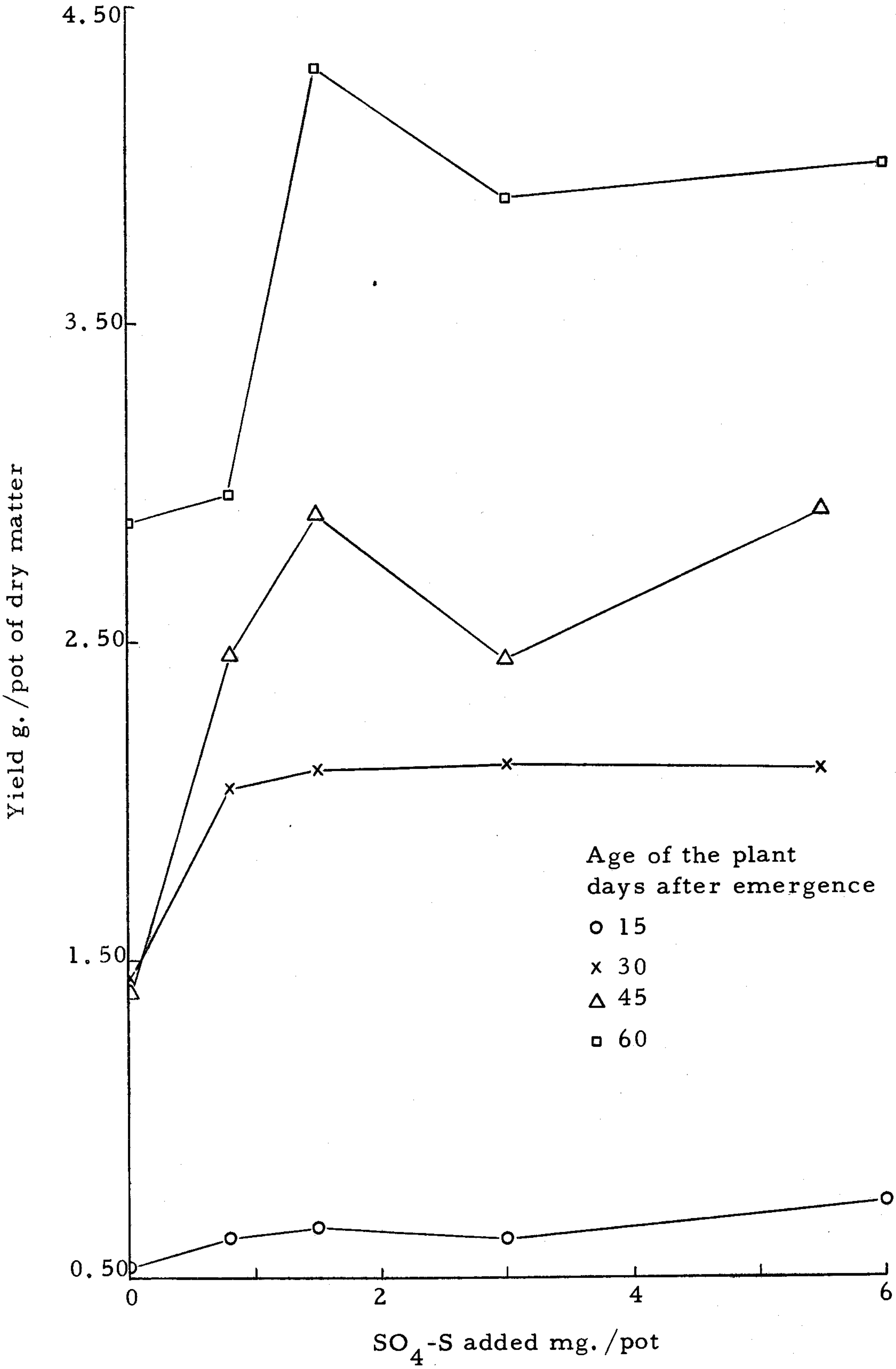

Figure 7. The relationship between subterranean clover yield and SO_4-S added to the Steiwer soil.

Table 10. Plant sulfur, plant organic sulfur, plant sulfate content of subterranean clover grown on Steiwer soil as influenced by age and applied gypsum (means of two replications).

	Plant age at day intervals after emergence											
	15			30			45			60		
Gypsum applied lbs. S/acre	Plant S content mg./g.	Plant organic S content mg./g.	Plant SO_4-S content mg./g.	Plant S content mg./g.	Plant organic S content mg./g.	Plant SO_4-S content mg./g.	Plant S content mg./g.	Plant organic S content mg./g.	Plant SO_4-S content mg./g.	Plant S content mg./g.	Plant organic S content mg./g.	Plant SO_4-S content mg./g.
0	2.8	2.3	0.541	1.8	1.4	0.446	1.8	1.3	0.499	1.1	0.6	0.458
5	3.8	3.2	0.582	2.4	2.0	0.416	2.0	1.6	0.374	1.2	0.9	0.300
10	3.9	3.3	0.582	2.0	1.5	0.458	1.8	1.4	0.416	1.2	0.8	0.375
20	4.7	4.1	0.634	2.1	1.6	0.458	2.1	1.5	0.603	1.3	0.9	0.374
40	6.2	5.6	0.634	2.2	1.7	0.458	2.6	1.8	0.770	1.5	1.0	0.499

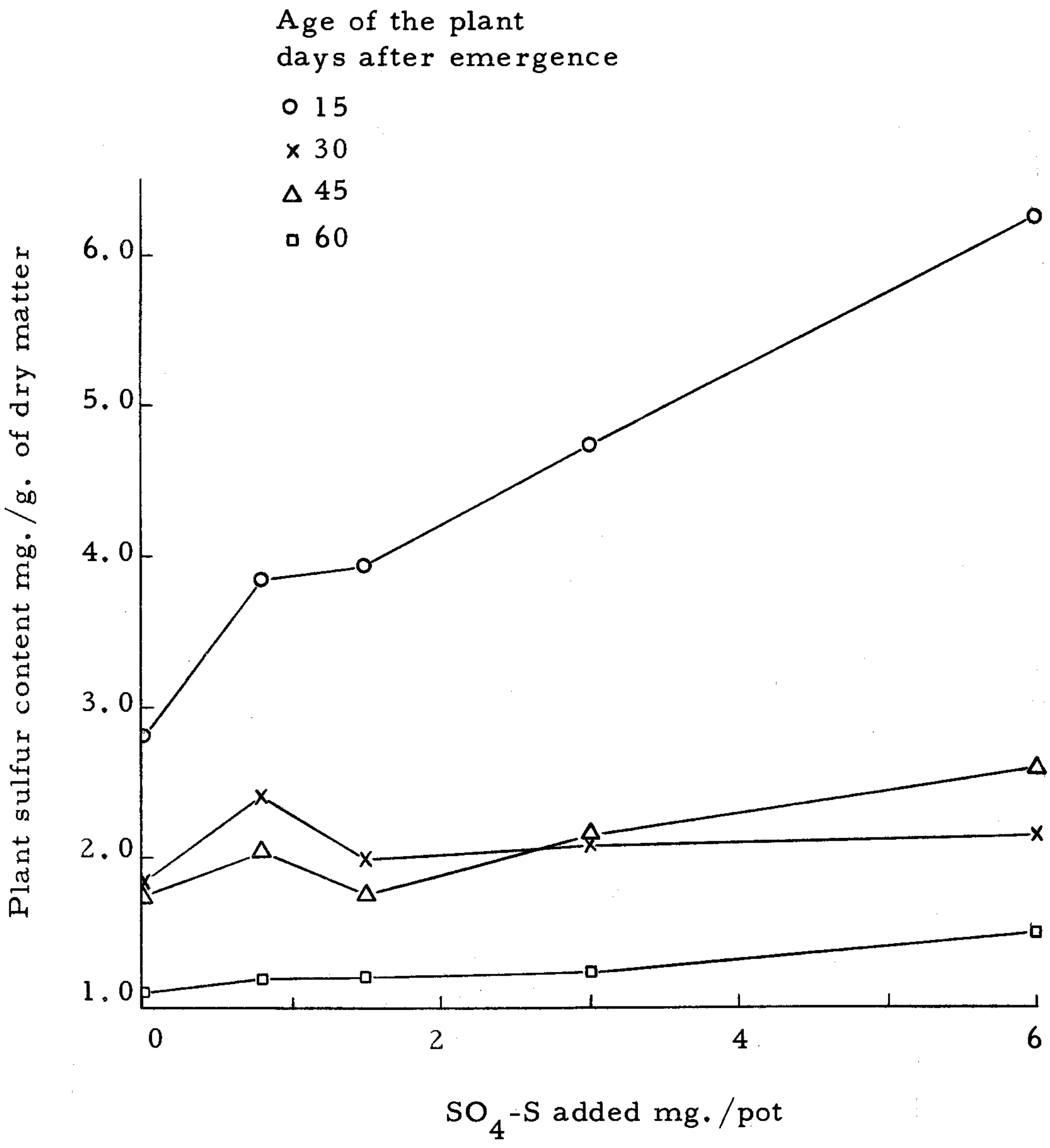

Figure 8. The relationship between plant S content and SO_4-S added to the Steiwer soil.

dropped after 15 days at all levels of applied S. This drop in plant S content was most marked when comparing 15 day old plants (6.2 mg. S/g. of dry matter) with 60 day old plants (1.5 mg. S/g. of dry matter) at 40 lbs. of S/acre.

The correlation coefficients obtained when plant S content in the tops was related to the yield increase were highly significant at the first and second harvest interval. They were for the first and second harvest intervals 0.990, 0.990 respectively.

Total Plant Sulfur Uptake. Data obtained from the product of plant S multiplied by yield appears in Table 11. As shown in Figure 9 except for 30 day old plants, the total plant S uptake was increased with S fertilization. Where S was not applied total plant S uptake increased as the plant matured (1.5 to 3.0 mg./pot). Where S was applied the total plant S uptake increased only until the third harvest interval and reached a maximum of 7.2 mg./pot when S was applied at a rate of 40 lbs. per acre. There was evidence particularly at the higher rates of applied gypsum that total plant S uptake decreased in 60 day old plants. This was due to a decrease in plant S content rather than a decrease in yield of dry matter. Jones (1962) reported that subterranean clover S content decreased with maturity (1962).

Plant Sulfate Content. It is of interest to note that only a slight increase in plant SO_4-S content occurred with S fertilization (Table

Table 11. Total plant sulfur uptake by subterranean clover grown on the Steiwer soil as influenced by age and applied gypsum (mean of two replications).

		Plant age at day intervals after emergence			
		15	30	45	60
gypsum applied lbs. S/acre	S added mg./pot	Total plant S uptake mg./pot	Total plant S uptake mg./pot	Total plant S uptake mg./pot	Total plant S uptake mg./pot
0	0	1.5	2.6	2.8	3.0
5	0.8	2.7	4.3	4.4	3.5
10	1.5	2.6	4.5	5.0	4.8
20	3.0	3.0	4.4	5.0	4.6
40	6.0	4.6	4.2	7.2	5.7

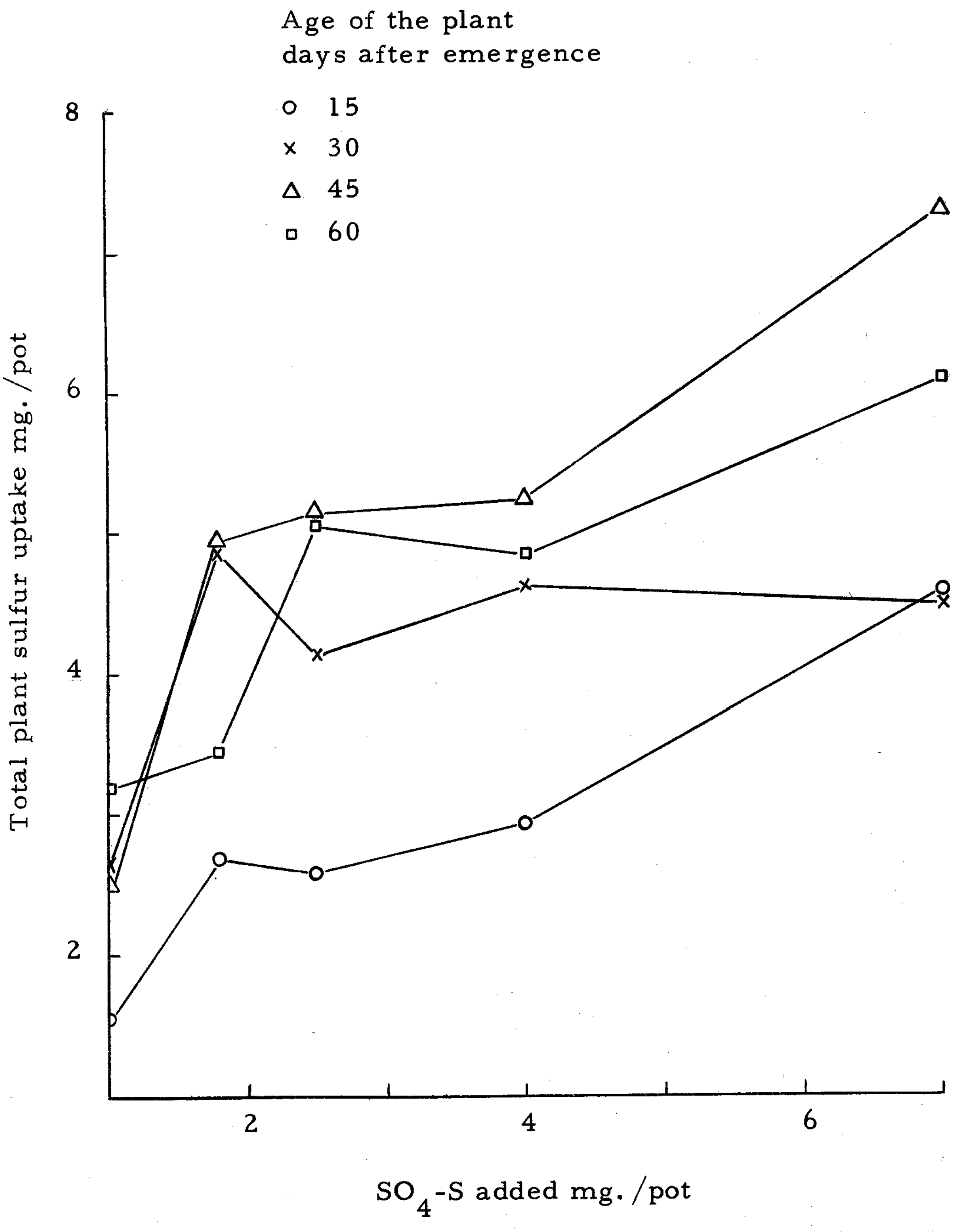

Figure 9. The relationship between total plant S uptake and SO_4-S added to the Steiwer soil.

10). Plant SO_4-S content varied little with the stage of plant development. The correlation coefficient between plant-SO_4-S content with yield increase was significant at the 0.05 level ($r = 0.829$) for 30 day old plants.

Plant Organic Sulfur Content

Plant organic S content was highest in 15 day old plants at all levels of applied gypsum (Table 10). A gradual decrease in plant organic S followed through 45 and 60 day plants. For the no-S treatment plant organic S decreased from 2.3 to 0.6 mg./g. and for the S treatment consisting of an application equivalent to 40 lbs. per acre organic S decreased from 5.6 to 1.0 mg./g. of dry matter.

Moreover the effect from rate of applied gypsum on plant organic S was pronounced in 15 day old plants and it appeared to have little influence on plant organic S at other harvest intervals.

Plant Organic Sulfur to Plant Sulfate Ratio. Upon comparing the plant organic S content to plant SO_4-S content (Table 12) two trends emerge. In the first place, levels of S fertilization affect this ratio only in 15 day old plants 4.6:1 to 9.3:1. Secondly the plant organic S to plant SO_4-S ratio decreased as the plant matured at all levels of gypsum applied to the soil. These results suggest that at the early stages most of the S applied went into the organic fraction of the plant. As the plant matured a corresponding smaller

Table 12. Ratio of plant organic sulfur to plant sulfate sulfur in subterranean clover grown on the Steiwer soil as influenced by applied gypsum (means of two replications).

Gypsum applied lbs. S/acre	Plant yield at day intervals after emergence			
	15	30	45	60
	$\frac{\text{Organic S}}{SO_4\text{-S}}$	$\frac{\text{Organic S}}{SO_4\text{-S}}$	$\frac{\text{Organic S}}{SO_4\text{-S}}$	$\frac{\text{Organic S}}{SO_4\text{-S}}$
0	4.6:1	3.5:1	2.6:1	1.2:1
5	5.3:1	5.0:1	4.0:1	3.0:1
10	5.5:1	3.0:1	3.5:1	2.0:1
20	6.8:1	3.2:1	2.5:1	1.0:1
40	9.3:1	3.4:1	2.3:1	2.0:1

fraction of the plant S was found in an organic form. The rate of applied gypsum in plants beyond 15 days had little if any effect on this ratio.

Plant Nitrogen Content

It is noteworthy from data presented in Table 13 that applied S had little effect on the plant N content. The highest N content was obtained in 15 day old plants harvested at the first harvest interval. At this harvest interval about 3.0 mg. N/g. of dry matter was obtained. As the plant matured the level of plant N decreased at each level of S fertilization until in 60 day old plants harvest interval 1.6 to 1.7 mg. N/g. was obtained. This decline in plant N content with growth was similar to that reported by other workers (Pumphrey and Moore, 1965a).

Total Nitrogen Uptake

The data on total N uptake presented in Table 13 represents the product of plant N content multiplied by yield. Levels of supplied S had relatively little effect on total N uptake in 15 day old plants (16.3 to 22.7 mg./g. of dry matter). In 30, 45 and 60 day old plants S fertilization considerably influenced the total N uptake. Thus in 30 day old plants the increase 27.4 to 44.7 mg. N/pot, and 45 day old plants (32.4 to 68.9 mg. N/pot of dry matter). Total

Table 13. Plant nitrogen content, total nitrogen uptake, and N:S ratio of subterranean clover grown on the Steiwer soil as influenced by age and applied gypsum (means of two replications).

	Plant age at day intervals after emergence											
	15			30			45			60		
Gypsum applied lbs. S/acre	Plant N content mg./g.	Total uptake mg./pot	N:S ratio	Plant N content mg./g.	Total uptake mg./pot	N:S ratio	Plant N content mg./g.	Total uptake mg./pot	N:S ratio	Plant N content mg./g.	Total uptake mg./pot	N:S ratio
0	3.1	16.7	10.9:1	1.9	27.4	10.3:1	2.3	32.4	13.0:1	1.6	46.8	14.7:1
5	2.6	16.3	6.7:1	1.8	37.5	7.7:1	2.2	54.3	10.9:1	1.7	50.0	14.4:1
10	3.2	21.1	8.1:1	1.8	37.6	9.0:1	2.1	61.1	11.9:1	1.6	67.9	13.3:1
20	2.9	17.8	6.0:1	1.9	42.7	9.2:1	2.0	50.0	9.5:1	1.7	65.1	13.3:1
40	3.1	22.7	4.9:1	2.2	44.7	9.9:1	2.4	68.9	9.1:1	1.6	64.7	10.6:1

nitrogen uptake increased until plants were 60 days old. The increase in total nitrogen uptake due to S fertilization suggests the beneficial effect that the applications of gypsum to the Steiwer soil had upon N fixation. Pumphrey and Moore (1965a) have noted the effect that S had upon symbiotic fixation in leguminous plants.

N:S Ratio

An examination of the data of Tables 10 and 13 revealed that increasing the rate of S application influenced the plant S content relative to plant N content. The N to S ratios were widest (10.9:1, 10.3:1, 13.0:1, 14.7:1 in 15, 30, 45 and 60 day old plants respectively) where little or no S was supplied. In contrast they were lowest at the highest S level (4.9:1, 9.9:1, 9.1:1, 10.6:1 for for 15, 30, 45 and 60 days respectively). These results agree with the findings of Pumphrey and Moore (1965a) who reported that N to S ratios of alfalfa were widest where little or no S was applied and lowest at the highest S level.

The N:S ratios decreased with S fertilization. Thus in 15 day old plants the N:S ratio dropped from 10.9:1 to 4.9:1 and at the fourth harvest interval from 14.7:1 to 10.6:1. These results agree with the findings of Pumphrey and Moore (1965a) who reported that N:S ratios of alfalfa decreased with sulfur fertilization. After 15 days, the N:S ratios remained fairly constant (9.9:1 to 10.6:1) for plants grown on the Steiwer soil which had the high rate of applied gypsum.

Critical Level of Sulfur in the Plant

Several workers (Rendig, 1956; Harward, Chao, and Fang, 1962b; Jones, 1962; Pumphrey and Moore, 1965b) have used critical levels to determine the point on a yield curve where some component of the nutrient status of plant can be used to predict the adequacy of a given nutrient. Thus Rendig (1956) showed the yield curve of alfalfa, breaking sharply at the critical level (0.22% plant S content) or point of adequacy beyond which yield remained constant and plants exhibited luxury uptake of S. Jones (1962) determined the critical level in terms of plant SO_4-S content, which was 170 ppm, of 132-146 day old subterranean clover grown in the greenhouse. Pumphrey and Moore (1965b) showed that S deficient alfalfa more than 4 months old had a critical level in terms of N:S ratio of more than 15, while alfalfa with adequate S had N:S ratio of less than 11. Below the critical level the plant would be S deficient and above it the plant would be S efficient.

Using the data obtained in this study the critical level was determined for 30, 45 and 60 day plants relating the maximum yield with corresponding plant S content, plant SO_4-S content, and N:S ratio. It was difficult to determine the critical level for 15 day old plants because the maximum yield was obtained at the highest level of applied gypsum (40 lbs. S/acre). It might be that the true maximum yield at this interval would be obtained at S rates which exceed the highest gypsum used in this study.

In 30, 45 and 60 day plants the critical level in terms of plant S content were 2.1, 1.8, 1.2 mg./g. of dry matter (Table 14). For these same aged plants the critical level in terms of plant SO_4-S content were 458, 416, 375 ppm respectively. The critical level in terms of N:S ratio in 15, 30, 45 and 60 day plants were 9.2:1, 11.9:1, 13.3:1 respectively.

As the plants matured from 15 days to 60 days the critical level in terms of plant S and plant SO_4-S content decreased. The critical N:S ratio during this period increased.

Soil Sulfur Status

Results obtained for total, organic and soil SO_4-S appear in Table 15. Total soil S increased slightly with S fertilization. Organic S however decreased with S fertilization. For instance in 15 day old and 60 day old plants the soil organic S decreased from 41.2 to 31.3 mg./pot and from 39.3 to 33.9 mg./pot respectively as the rate of applied gypsum increased. It is interesting to note that the changes in the soil SO_4-S status occurred at the highest rate of applied gypsum. Thus when 40 lbs. of S/acre was applied to

Table 14. Critical level in terms of plant sulfur content, sulfate content, N:S ratio and maximum yield of subterranean clover grown on the Steiwer soil (means of two replications).

Plant age after emergence	Plant S content mg./g.	Plant SO_4-S content ppm	N:S ratio	Maximum yield mg./pot
30	2.1	458	9.2:1	2.21
45	1.8	416	11.9:1	2.91
60	1.2	375	13.3:1	4.30

Table 15. Total soil sulfur, organic soil sulfur, sulfate soil sulfur in the Steiwer soil as influenced by applications of gypsum (means of two replications).

	Plant age at day intervals after emergence											
	15			30			45			60		
Gypsum applied lbs. S/acre	Total S mg./pot	Organic S mg./pot	SO_4-S mg./pot	Total S mg./pot	Organic S mg./pot	SO_4-S mg./pot	Total S mg./pot	Organic S mg./pot	SO_4-S mg./pot	Total S mg./pot	Organic S mg./pot	SO_4-S mg./pot
0	41.7	41.2	0.5	39.6	39.5	0.1	39.2	39.0	0.2	43.2	39.3	0.3
5	40.5	40.0	0.5	38.8	38.6	0.2	38.8	38.5	0.3	44.0	39.9	0.3
10	41.0	39.8	1.2	39.4	38.8	0.6	38.4	38.1	0.3	44.7	39.5	0.3
20	42.2	40.5	1.7	41.4	40.3	1.1	39.6	39.0	0.6	46.2	41.3	0.3
40	43.6	31.3	12.3	44.6	34.1	10.5	42.6	33.6	9.0	49.0	33.9	8.7

the Steiwer soil there was not a marked change in total soil S but a relative by large redistribution in the proportions of organic S and SO_4-S present in the soil sample.

Mineralization of Phosphorus

Table 16 presents C: N: S: organic P of the soil on which subterranean clover was grown. After 15 and 30 days soil organic phosphorus ratio increased as the rate of applied gypsum increased. Barrow (1960) suggested that in some Australian soils where C/P ratio exceeds 40: 1 immobilization of phosphorus decreased with S fertilization. In Table 15 the C: organic P ratios reported in Table 16 tend to support Barrow's findings namely that when C/P ratios exceeded 40: 1 immobilization of P in an organic form decreased.

Mineralization of Soil Sulfur

An inventory of the Steiwer soil S status and plant S uptake is presented in Table 17. Upon comparing the amount of SO_4-S added to the soil with the total plant S uptake and soil SO_4-S some interesting observations emerge. The original soil organic S content of 43. 3 mg. /pot might be assumed to be quite high. Evidence that organic S mineralization occurred after SO_4-S had been applied to the soil appeared. Moreover, the amount of SO_4-S mineralized appeared greatest at the highest rate of S fertilization.

Table 16. C:N:S:P (organic) ratio in the Steiwer soil as influenced by applications of gypsum (means of two replications).

Gypsum applied lbs. S/acre	Soil sampled after 15 days				Soil sampled after 30 days			
	C:	N:	S:	Organic P	C:	N:	S:	Organic P
0	142:	10:	0.75 :	4.2	127:	10:	0.60 :	2.7
5	145:	10:	0.75 :	3.8	134:	10:	0.65 :	1.4
40	140:	10:	0.80 :	1.3	139:	10:	0.80 :	1.3

Gypsum applied lbs. S/acre	Soil sampled after 45 days				Soil sampled after 60 days			
	C:	N:	S:	Organic P	C:	N:	S:	Organic P
0	140:	10:	0.70 :	1.2	137:	10:	0.75 :	1.6
5	132:	10:	0.70 :	1.3	132:	10:	0.70 :	1.5
40	139:	10:	0.70 :	1.1	132:	10:	0.70 :	1.0

Table 17. Soil sulfur status after application of gypsum and total plant sulfur uptake at different stages of maturity (means of two replications).

Gypsum applied	SO_4-S added in soil	Total SO_4-S present + added in soil	Total plant S uptake	SO_4-S in soil at end	Total plant S uptake + SO_4-S in soil at end	S mineral-ized
lbs. S/acre	mg./pot	mg./pot	mg./pot	mg./pot	mg./pot	mg./pot
			Plants 15 days of age			
0	0.0	0.9	1.5	0.5	2.0	1.1
5	0.8	1.7	2.7	0.5	3.2	1.5
10	1.5	2.4	2.6	1.2	3.8	1.4
20	3.0	3.9	3.0	1.7	4.7	0.8
40	6.0	6.9	4.6	12.3	16.9	10.0
			Plants 30 days of age			
0	0.0	0.9	2.6	0.1	2.7	1.8
5	0.8	1.7	4.3	0.2	4.5	2.8
10	1.5	2.4	4.5	0.6	5.1	2.7
20	3.0	3.9	4.4	1.1	5.5	1.6
40	6.0	6.9	4.2	10.5	14.7	7.8
			Plants 45 days of age			
0	0.0	0.9	2.5	0.2	2.7	1.8
5	0.8	1.7	4.9	0.3	5.2	3.5
10	1.5	2.4	5.1	0.3	5.4	3.0
20	3.0	3.9	5.3	0.6	5.9	2.0
40	6.0	6.9	7.5	9.0	16.5	9.6
			Plants 60 days of age			
0	0.0	0.9	3.2	0.3	3.5	2.4
5	0.8	1.7	3.5	0.3	3.8	1.9
10	1.5	2.4	5.1	0.3	5.4	3.0
20	3.0	3.9	4.9	0.3	5.2	1.3
40	6.0	6.9	6.1	8.7	14.8	7.9

Thus at the 40 lbs. S/acre data presented in Table 17 revealed that 10.0, 7.8, 9.6 and 7.9 mg. S/pot were mineralized after 15, 30, 45 and 60 days respectively. This finding is substantiated by Barrow (1960) who reported that when the amount of S was varied moderate amounts of phosphorus and relatively large amounts of S were mineralized.

Furthermore, Freney's studies on five different Australian soils, suggested that mineralization of soil organic S occurred following S fertilization when subterranean clover was planted. He attributed the S mineralization to better subterranean clover root growth and a greater concentration of soil microorganisms. These conditions favored a stimulatory effect upon the heterotrophic soil microbial population resulting in secretions which probably produced an increased breakdown of soil organic matter and a consequent release of SO_4-S.

Nutrient Solution Compared with Fertilized Steiwer Soil

The experiment involving subterranean clover grown in nutrient solutions containing different S levels provided an interesting comparison with S response on an S deficient Steiwer soil. Thus where no S was added to either soil or nutrient solution comparable plant yields occured at all stages of maturity except the second. When S was added to either the nutrient solution or soil the subterranean clover response in terms of grams per pot was greatest in the soil medium (Figure 10). However, by 60 days subterranean clover grown in nutrient solution containing S produced as well as subterranean clover grown in soil. Each increment of added S to nutrient solution resulted in rather similar percentage yield increase. At the high level of S this amounted to 50% increase in dry matter over the check. In case of the soil the percentage increase from applied S over the control increased until the plants were harvested at 45 days. At this time the increase in subterranean clover growth at the 40 lbs. S/acre rate was 107 percent greater than the control.

Where no S was added to either soil or nutrient solution the plant S content was similar (2.8 mg. S/g. of dry matter) in 15 day old plants and gradually declined to a little over one mg. S/g. of dry matter in 60 day plants. The greatest difference in plant S content between the nutrient solution and Steiwer soil grown subterranean clover occurred at the highest level of applied S. Thus after 15 days when the high rates of applied S were compared plant S removed below three mg. S/g. of dry weight when the subterranean clover was grown in the Steiwer soil. However plant S content removed about 10 mg. S/g. of dry matter when grown in nutrient solution number 4.

In general the same trends in plant organic S occurred as with

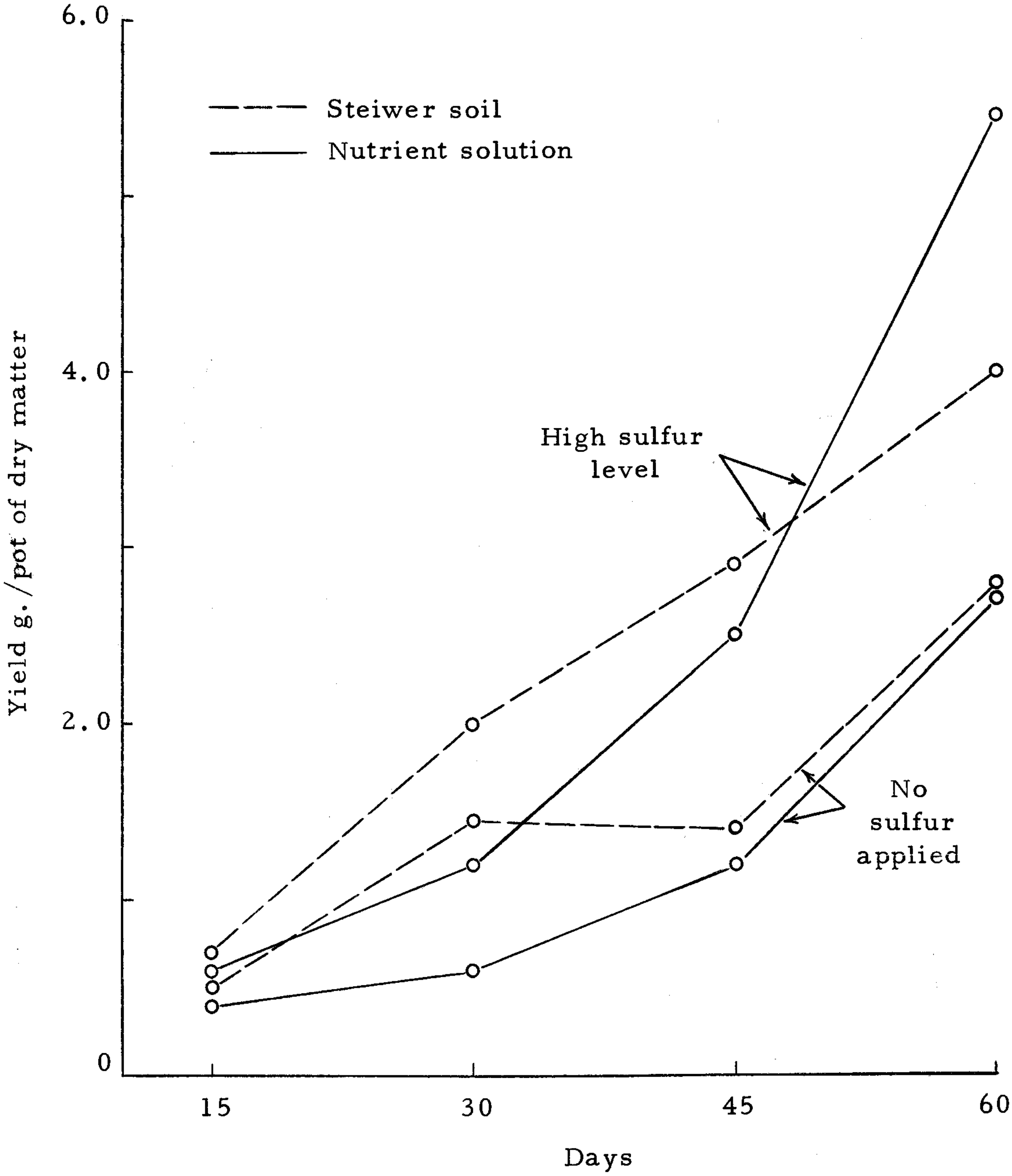

Figure 10. Subterranean clover yield for both nutrient solution and the Steiwer soil grown plants at low and high S levels.

plant S content when the Steiwer soil and nutrient solution grown subterranean clover were compared. Thus when the highest level of S was added to either nutrient solution or soil the plant organic S content was greater in nutrient solution. After 15 days plant organic S grown in the nutrient solution varied between about 8 and 9 mg. S/g. of dry matter. Yet plant organic S grown in the Steiwer soil at the high rate of applied S remained below 2 mg. S/g. of dry matter.

Where little or where no S was applied plant SO_4-S values in both nutrient solution and Steiwer soil were similar in 15 day old plants. Plant SO_4-S increased slightly with the addition of S to their respective growth media. The most noted change in plant SO_4-S between the nutrient solution and Steiwer soil occurred at the high level of applied S. Apparently nutrient solution at the high level was more effective in promoting plant SO_4-S accumulation than was the Steiwer soil.

Where no S was added to either medium N:S ratio in plants grown nutrient solution were higher than grown in Steiwer soil. Plant N:S ratios in nutrient solution ranged from 18:1 to 24:1 while in soil they ranged from 10:1 to 15:1. At the high rate of applied S the plant N:S ratio decreased in both media. But the Steiwer soil grown plants had a higher N:S ratio than those grown in nutrient solution. Doubtless the greater accumulation of plant S in the nutrient grown subterranean clover largely accounted for the narrower N:S ratio.

However it is interesting to note that at the highest level of S in the nutrient solution N:S ratio dropped markedly. This was not the case when plant N:S ratios between the 20 lbs. S/acre and 40 lbs. S/acre applied to the soil were compared. One plausible explanation of this phenomena is that more than adequate S was supplied in nutrient solution number 4.

SUMMARY

The effects of applied S to nutrient solution and the Steiwer soil on yield, plant S content, plant organic S content, and plant SO_4-S content of subterranean clover was investigated. The experiment was conducted in a greenhouse where reasonably constant conditions of light at 300 foot candle for 16 hours and temperature at 60-70° F were maintained. Plant samples were taken at 15, 30, 45, 60 days.

Significant subterranean clover yield response to each increment of added SO_4-S occurred at all harvest intervals when grown in the nutrient solution. Yield response to SO_4-S fertilized Steiwer soil also occurred at each rate of applied gypsum except in the 15 day old plants.

In the Steiwer soil the highest percentage yield increase due to added SO_4-S was in 45 day old plants. However, at any given SO_4-S level when age of plants were compared the greatest response occurred between 15 and 30 days. In the nutrient solution grown subterranean clover the increase due to added SO_4-S was similar for plants of different ages compared with the control.

In the nutrient solution the highest plant S content occurred in 30 day old plants and in the Steiwer soil in 15 day old plants. In both nutrient solution and the Steiwer soil the plant S content dropped as

the plant matured. In the nutrient solution the correlation coefficients for plant sulfur content in the tops with subterranean clover yield increase at all stages of maturity were highly significant. The correlation coefficients were 0.952, 0.908, 0.729, 0.997 for 15, 30, 45 and 60 days respectively. In the Steiwer soil the correlation coefficient of 0.99 for plant S content in the tops with yield increase was highly significant for 15 and 30 day old plants.

Plant organic S tended to decrease after 30 days as the subterranean clover matured. Where the clover was grown in nutrient solution an increase in plant organic S occurred at the high S level at all stages of maturity. However, in Steiwer soil grown clover after 15 days increased applications of gypsum appeared to have little influence in the plant organic content.

Stage of development had little effect in plant SO_4-S content in clover grown on the Steiwer soil, however, it decreased in plants grown in nutrient solution except at the high level of added SO_4-S.

Significant correlations between plant SO_4-S content with yield occurred in 15 day old plants grown in the Steiwer soil (0.829) and in nutrient solution grown plants in 15 and 30 day old plants (0.99 and 0.95).

In both nutrient solution and the Steiwer soil N:S ratios increased with supplied S. In nutrient solution the N:S ratios were widest where little or no S was supplied and lowest at the highest S level. In the Steiwer soil the N:S ratios were widest where little or no S was supplied and lowest at the highest S level at all stages of maturity except for the 30 day old plants.

After 15 days critical levels were determined for plant S content, plant SO_4-S content and N:S ratio. Critical plant S content of subterranean clover ranged between 1.2 to 1.8 mg S/g of dry matter, while critical plant SO_4-S ranged between 375 to 458 ppm. The critical plant N:S ratio ranged between 9.2:1 to 13.3:1.

Some evidence of S mineralization occurred as the result of the addition of gypsum to the Steiwer soil at a rate equivalent to 40 lbs. S/acre.

BIBLIOGRAPHY

Alban, L. A. and Mildred Kellogg. 1959. Methods of soil analysis as used in the Oregon State University soil testing laboratory. Corvallis. 8 p. (Oregon Agricultural Experiment Station. Miscellaneous paper no. 65)

Allison, L. E. 1935. Organic carbon by reduction of chromic acid. Soil Science 40:311-320.

Bardsley, D. E. and H. V. Jordan. 1957. Sulfur availability in seven southeastern soils as measured by growth and composition of white clover. Agronomy Journal 49:310-312.

Bardsley, C. E. and J. D. Lancaster. 1960. Determination of reserve sulfur and soluble sulfates in soils. Soil Science Society of America Proceedings 24:265-268.

Barrow, N. J. 1960. The effects of varying the nitrogen, sulfur and phosphorus contents of organic matter on its decomposition. Australian Journal of Agricultural Research 11:317-330.

Begg, J. E. 1963. Comparative responses of indigenous, naturalized, and commercial legumes to phosphorus and sulphur. Australian Journal of Experimental Agriculture and Animal Husbandry 3:17-19.

Bertramson, B. R., M. Fried and S. L. Tisdale. 1950. Sulfur studies of Indiana soils and crops. Soil Science 70:27-41.

Bremner, J. M. 1965. Soil organic nitrogen. In: Soil nitrogen, ed. by W. V. Bartholomew and F. E. Clark. Madison, Wisconsin. p. 93-149. (American Society of Agronomy. Agronomy monograph no. 10)

Butters, B. and E. M. Chenery. 1959. A rapid method for the determination of total sulfur in soils and plants. Analyst 84: 239-245.

Cairns, R. R. and A. C. Richer. 1960. A comparative study of a sulfur responsive and a non-responsive Grey wooded soil. Canadian Journal of Soil-Science 4:246-254.

Chapman, H. D. and P. F. Pratt. 1961. Methods of analysis for soils, plants, and waters. (Berkeley), University of California Division of Agricultural Sciences, 309 p.

Chesnin, L. and C. H. Yien. 1950. Turbidimetric determination of available sulfates. Soil Science Society of America Proceedings 15: 149-151.

Conard, J. P. 1950. Sulfur fertilization in California and some related factors. Soil Science 70: 43-54.

Crocker, William. 1945. Sulfur deficiency in soils. Soil Science 60: 149-155.

Dijkshoorn, W., J. E. M. Lampe and P. I. J. VanBurg. 1960. A method of diagnosing the sulfur nutrition status of herbage. Plant and Soil 13:227-241.

Ensminger, L. E. and J. F. Freney. 1960. Diagnostic technique for determining sulfur deficiencies in crops and soils. Soil Science 101:283-290.

Evans, C. A. and C. O. Rost. 1945. Total sulfur and humus sulfur of Minnesota soils. Soil Science 59:125-137.

Freney, J. R. 1958. The determination of water soluble sulfates in soils. Soil Science 86:241-244.

Freney, J. R. 1961. Some observations on the nature of organic sulfur compounds in soil. Australian Journal of Agricultural Research 12:424-432.

Freney, J. R. and K. Spencer. 1960. Soil sulfate changes in the presence and absence of growing plants. Australian Journal of Agricultural Research 11:339-345.

Freney, J. R. and F. J. Stevenson. 1966. Organic sulfur transformations in soils. Soil Science 101:307-316.

Freney, J. R., N. J. Barrow and K. Spencer. 1962. A review of certain aspects of sulphur as a soil constituent and plant nutrient. Plant and Soil 17:295-308.

Harward, M. E., T. T. Chao and S. C. Fang. 1962a. Soil properties and constituents in relation to mechanisms of sulphate adsorption. In: Symposium on the use of radioisotopes in soil-plant nutrition studies, Bombay, 1962. Vienna, International Atomic Energy Agency. p. 93-114.

Harward, M. E., T. T. Chao and S. C. Fang. 1962b. The sulfur status and sulfur supplying power of Oregon soils. Agronomy Journal 54: 101-106.

Jackson, M. L. 1958. Soil chemical analysis. Englewood Cliffs, N. J., Prentice-Hall. 498 p.

Johnson, C. M. and H. Nishita. 1952. Micro estimation of sulfur in plant materials, soils, and irrigation waters. Analytical Chemistry 24: 736-742.

Johnson, C. M. and A. Ulrich. 1959. Analytical methods for use in plant analysis. Berkeley. p. 54-58. (California Agricultural Experiment Station. Bulletin 766)

Jones, M. B. 1962. Total sulfur and sulfate sulfur content in subterranean clover as related to sulfur responses. Soil Science Society of America Proceedings 26: 482-484.

Jones, M. B. 1963. Effect of sulfur applied and date of harvest on yield, sulfate sulfur concentration and total sulfur uptake of five annual grassland species. Agronomy Journal 55: 251-254.

Jones, M. B. 1964. Effect of applied sulfur uptake of various California dryland pasture species. Agronomy Journal 56: 235-237.

Jones, M. B. and W. E. Martin. 1964. Sulfate sulfur concentration as an indicator of sulfur status in various California dryland pasture species. Soil Science Society of America Proceedings 28: 539-541.

Jordan, H. V. and C. E. Bardsley. 1958. Response of crops to sulfur on south eastern soils. Soil Science Society of America Proceedings 22: 254-256.

Jordan, H. V. and L. E. Ensminger. 1958. The role of sulfur in soil fertility. Advances in Agronomy 10: 408-482.

Jordan, H. V. and H. N. Reisenauer. 1957. Sulfur and soil fertility. In: U.S. Department of Agriculture Yearbook, 1957. p. 107-111.

Kamprath, E. J., W. L. Nelson and J. W. Fitts. 1956. The effect of pH, sulfate and phosphate concentrations on the adsorption of sulfate by soils. Soil Science Society of America Proceedings 20: 463-471.

Kamprath, E. J., W. L. Nelson and J. W. Fitts. 1957. Sulfur removed from soil by field crops. Agronomy Journal 49: 289-293.

Kilmer, V. J. and D. C. Nearpass. 1964. The determination of available sulfur in soils. Soil Science of America Proceedings 24: 337-340.

Kust, C. A. and D. Smith. 1961. Influence of harvest management on level of carbohydrate reserves, longevity of stands, and yields of hay and protein from vernal alfalfa. Crop Science 1: 267-269.

Lowe, L. E. 1964. An approach to the study of the sulfur status of soils and its application to selected Quebec soils. Canadian Journal of Soil Science 44: 176-179.

Lowe, L. E. and W. A. Delong. 1961. Aspects of the sulfur status of three Quebec soils. Canadian Journal of Soil Science 41: 141-146.

Lowe, L. E. and W. A. Delong. 1963. Carbon bonded sulfur in selected Quebec soils. Canadian Journal of Soil Science 43: 151-155.

Martin, W. E. 1958. Sulfur deficiency wide spread in California soils. California Agriculture 192: 10-12.

Martin, W. E. and T. W. Walker. 1966. Sulfur requirements and fertilization of pasture and forage crops. Soil Science 101: 248-257.

McClung, A. C. and L. M. Martins de Freitas. 1959. Sulfur deficiency in soils from Brazilian Campos. Ecology 40: 315-317.

McNaught, K. J. and F. J. E. Christoffels. 1961. Effect of sulfur deficiency on sulfur and nitrogen levels in pasture and lucerne. New Zealand Journal of Agriculture Research 4: 177-196.

Nelson, L. E. 1964. Status and transformation of sulfur in Mississippi soils. Soil Science 97:300-306.

Powers, W. L. 1923. Sulfur in relation to soil fertility. Corvallis. 45 p. (Oregon Agricultural College. Experiment Station. Bulletin no. 199)

Pumphrey, F. V. and D. P. Moore. 1965a. Sulfur and nitrogen content of alfalfa herbage during growth. Agronomy Journal 57:237-239.

Pumphrey, F. V. and D. P. Moore. 1965b. Diagnosing sulfur deficiency of alfalfa from plant analysis. Agronomy Journal 57:364-366.

Rendig, V. V. 1956. Sulfur and nitrogen composition of fertilized and unfertilized alfalfa grown on a sulfur deficient soil. Soil Science Society of America Proceedings 20:237-240.

Rosell, R. A. and Albert Ulrich. 1964. Critical zinc concentrations and leaf minerals of sugar beet plants. Soil Science 97: 152-167.

Saunders, W. M. H. and E. G. Williams. 1955. Observation on the determination of total organic phosphorus in soils. Journal of Soil Science 6:254-267.

Spencer, K. and J. R. Freney. 1960. A comparison of several procedures for estimating the sulfur status of soils. Australian Journal of Agricultural Research 11:948-959.

Starkey, R. L. 1966. Oxidation and reduction of sulfur composition in soils. Soil Science 101:297-306.

Stephens, C. G. and C. M. Donald. 1958. Australian soils and their responses to fertilizers. Advances in Agronomy 10: 168-253.

Ulrich, A. 1961. Plant analysis in sugar beet nutrition. In: Plant analysis and fertilizer problems, ed. by W. Reuther. Washington, D. C. p. 190-211. (American Institute of Biological Sciences. Publication no. 8)

Vacharotagan, Sorasith. 1962. Correlation of phosphorus soil tests and forms of inorganic soil phosphorus with crop response and phosphorus uptake from Aiken soils. Ph.D. thesis. Corvallis, Oregon State University. 201 numb. leaves.

Walker, T. W. and A. F. R. Adams. 1958a. Studies on soil organic matter: I. Influence of phosphorus content of parent materials on accumulations of carbon, nitrogen, sulfur and organic phosphorus in grassland soils. Soil Science 85:307-318.

Walker, T. W. and A. F. R. Adams. 1958b. Competition for sulfur in a grass-clover association. Plant and Soil 9:353-366.

Walker, D. R. and C. F. Bentley. 1961. Sulphur fractions of legumes as indicators of sulfur deficiency. Canadian Journal of Soil Science 41:164-168.

Walker, T. W., A. F. R. Adams and H. D. Orchiston. 1956. Effect of level of Calcium sulphate on yield and composition of a grass and clover pasture. Plant and Soil 7:290-300.

Walkley, A. and I. A. Black. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Science 37:29-38.

Williams, W. A., C. M. McKell and J. N. Reppert. 1964. Sulfur fertilization of an annual-range soil during years of below normal rainfall. Journal of Range Management 17:1-4.

Williams, C. H. and A. Steinbergs. 1958. Sulfur and phosphorus in some eastern Australian soils. Australian Journal of Agricultural Research 9:483-491.

Williams, C. H. and A. Steinbergs. 1959. Soil sulfur fractions as chemical indexes of available sulfur in some Australian soils. Australian Journal of Agricultural Research 10:340-352.

Williams, C. H. and A. Steinbergs. 1962. Evaluation of plant available sulfur in soils. Plant and Soil 17:279-294.

APPENDIX

Appendix Table 1. A comparison of the turbidimetric method with the gravitimetric method in the determination of total soil sulfur (means of two replications).

	Plant age at day intervals after emergence							
	15		30		45		60	
	Total S		Total S		Total S		Total S	
Gypsum applied lbs. S/acre	Turbidimetric method mg./pot	Gravimetric method mg./pot	Turbidimetric method mg./pot	Gravimetric method mg./pot	Turbidimetric method mg./pot	Gravimetric method mg./pot	Turbidimetric method mg./pot	Gravimetric method mg./pot
	43.2	44.1						
0	41.7	38.6	39.6	38.0	39.2	38.0	43.2	38.0
5	40.5	39.2	38.8	38.6	38.8	38.8	44.0	38.6
10	41.0	41.3	39.4	40.7	38.8	40.7	44.7	40.5
20	42.2	41.6	41.4	41.6	39.6	41.9	46.2	41.6
40	43.6	44.6	44.6	44.3	42.6	44.3	49.0	44.3