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# Sulphur accumulation and redistribution in wheat (*Triticum aestivum*): a study using stable sulphur isotope ratios as a tracer system

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## ABSTRACT

**Wheat plants were grown hydroponically and fed with two sulphate sources differing in stable isotope composition, one having a $\delta^{34}$S of 13·7‰ and the other 4·1‰. Plant sulphur (S) isotope ratios were determined using an on-line continuous flow-isotope ratio mass spectrometer. This method greatly simplified the procedure for the measurement of S isotope ratios, and was found to be precise for samples containing > 1 mg S g$^{-1}$ dry weight. The $\delta^{34}$S values of plant shoots, which had been grown on a single sulphate source, were very close to the source values, suggesting little isotope fractionation during sulphate uptake and transport from roots to shoots. By changing the sulphate sources at different growth stages, it was possible to estimate S accumulation and redistribution within different plant parts. At maturity, wheat grain derived 14, 30, 6 and 50% of its S from the accumulation during the following successive growth stages: between emergence and early stem extension, between stem extension and flag leaf emergence, between flag leaf emergence and anthesis, and after anthesis, respectively. It was estimated that 39, 32 and 52% of the S present in the flag leaves, older leaves and stems, respectively, at anthesis, was exported during the postanthesis period. These results demonstrate considerable cycling of S within wheat plants, and highlight the importance of S uptake after anthesis to the accumulation of S in grain under the experimental conditions employed.**

*Key-words*: $\delta^{34}$S; redistribution; stable isotopes; sulphur; uptake; wheat.

## INTRODUCTION

Recent studies have demonstrated the importance of sulphur (S) supply to grain yield of winter wheat (*Triticum aestivum* L.) in the UK and other European regions. This is increasingly relevant as a consequence of the large decrease in atmospheric S inputs over the last three decades (McGrath, Withers & Zhao 1996). In particular, maintaining both a sufficient level of S and a balance between nitrogen (N) and S in grain is essential for the breadmaking quality of wheat (Randall & Wrigley 1986; Zhao *et al*. 1997). It is, therefore, important to understand the mechanisms of S accumulation in grain and the factors controlling this process.

S deficiency symptoms appear first in young leaves whilst older leaves remain green, suggesting that S is relatively immobile in mature leaves. However, this is an overgeneralized conclusion, and recent studies have shown very different mobility of S in different pools/compartments. Insoluble S (e.g. protein-S) in mature leaves is generally immobile even under conditions of S deficiency (Adiputra & Anderson 1995; Sunarpi & Anderson 1996), but its mobility is enhanced by N deficiency (Sunarpi & Anderson 1997). Sulphate stored in mesophyll vacuoles is also relatively immobile, and net export of this sulphate in times of S deficiency is slow (Clarkson, Hawkesford & Davidian 1993). Bell, Cram & Clarkson (1994) demonstrated *in vitro* that mature leaves of *Macroptilium atropurpureum* export vacuolar sulphate 100 times more slowly than root cells, at a rate that could not sustain normal plant growth. However, there is strong evidence that a large proportion of sulphate delivered from roots to shoots via xylem does not mix with the vacuolar sulphate pool in the mature leaves, but cycles rapidly to phloem and redistributes to young leaves and roots (Smith & Lang 1988; Larsson *et al*. 1991; Clarkson *et al.* 1993; Herschbach & Rennenberg 1994; Adiputra & Anderson 1995). These studies, all using the radioactive tracer $^{35}$S, have focused mainly on S uptake and redistribution in young plants over relatively short periods of time, and have not addressed the question of S redistribution towards maturing grain.

Using $^{35}$S for tracer studies of nutrient uptake and redistribution, over a full growing season with a hydroponic growing system, is impractical due to the necessary safety cautions associated with the use of radioactive compounds. An alternative solution is to use stable isotopes. There are four stable S isotopes, occurring naturally with average atom percentages of: $^{32}$S (95·02%); $^{33}$S (0·75%); $^{34}$S (4·21%) and $^{36}$S (0·02%). Because of their higher abun-

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dance, $^{32}S$ and $^{34}S$ are usually studied in S isotope analyses. The $^{34}S/^{32}S$ ratio of a sample is usually expressed as the parts per thousand deviation from that of the reference standard, Vienna Cañon Diablo Meteorite (VCDT), in the following standard $\delta$-notation (Coplen & Krouse 1998):

$$\delta^{34}S\ (‰) = (R_{sample}/R_{VCDT} - 1) \times 1000$$

where $R = {}^{34}S/{}^{32}S$.

Unlike $^{15}N$, highly enriched $^{34}S$ compounds are not widely available. However, naturally occurring S compounds can have a relatively wide range of $\delta^{34}S$, and it is possible to use a combination of materials with different $\delta^{34}S$ as 'natural tracers'. This approach has been used recently to investigate the fate of S applied to forest ecosystems (Giesemann, Jäger & Feger 1995; Prietzel *et al*. 1995) but not to the redistribution of S already accumulated. Two conditions must be satisfied for this approach to be successful in tracer studies. First, the difference in $\delta^{34}S$ between tracer sources, or between tracer and the background must be sufficiently large; second, isotopic fractionation, which may occur in the processes studied, must be small relative to the difference between the tracers.

In this paper, a detailed description of S isotope ratio measurement using on-line mass spectrometry is presented. Variations of $\delta^{34}S$ in different plant parts and in whole shoots, at different growth stages, were evaluated in wheat plants supplied with a single S source in a hydroponic experiment. The final objective of this study was to estimate the proportion of S in the grain at maturity derived from S accumulated in the whole plant biomass during different growth stages, using two S sources of differing $\delta^{34}S$ value.

## MATERIALS AND METHODS

### Hydroponic culture

The experiment was conducted in a greenhouse during 1996 and 1997. Winter wheat (*T. aestivum* cv. Hereward) was grown to maturity in hydroponic culture. No additional lighting was supplied but heating ensured that the temperature did not fall below freezing during the winter period, while allowing vernalization to proceed. From 15 April 1997 onwards, when plants were at GS 31 (Zadoks, Chang & Konnzak 1974), the greenhouse was covered with shading sheets to prevent overheating. Seeds were sown in $3 \times 3 \times 6$ cm rock wool blocks (Grodania A/S, Hedehusene, DK) on 16 December 1996. The rock wool blocks were placed in two plastic trays half-filled with perlite (Gem Gardening, Leeds, UK), with 50 blocks per tray. The blocks were watered with – S nutrient solution sufficient to give a 2 cm depth of solution in the tray. Fifteen days after emergence (15 DAE) when the roots were extending ≈ 15 cm from the base of the rock wool block, 60 plants were selected for uniformity. The rock wool blocks were trimmed, without damaging the roots, and two plants were placed in a $13 \times 13 \times 20$ cm square pot filled with perlite that had been wetted with deionized water. Six pots were placed on top of an upturned seed tray in each of five 23 $dm^3$ opaque polyethylene vessels. Each vessel was aerated by a single Elite 800 aquarium pump (Rolf C. Hagen Ltd, Castleford, UK) which had an air output of 1·2 $dm^3\ min^{-1}$. The aerating tube was fixed under the seed tray to ensure uniform circulation of the aerated solution. Each vessel contained the plants of one treatment only. The vessel was then filled with 13 $dm^3$ of – S nutrient solution at the required strength, and the S source was added to each vessel dependent on treatment. The plant pots were about 75% immersed in the solution, ensuring that the roots were sufficiently submerged to absorb water whilst maintaining the proper moisture status of the seed crown. The pots were arranged such that a gap of 10 cm separated the two adjacent pots down the centre of the vessel. This gap and the sides of the vessels were covered with aluminium foil and strips of foil were laid over the top of the pots such that they allowed the free growth of the plants.

Following germination with 25% of the full strength nutrient solution (Table 1), the strength of the nutrient solution was altered according to the following schedule [after Heberer & Below (1989)]: 50% at 28 DAE, 75% at 49 DAE and 100% at 70 DAE, 75% at 154 DAE, 50% at 168 DAE and 25% at 182 DAE. The increases in nutrient concentration simulated field conditions, where nutrients are added as fertilizer during the early growing stages. The reductions in nutrient concentrations after 154 DAE also simulated field conditions, when reducing soil moisture and depleting nutrient reserves lead to a reduction in available nutrients (Gregory, Crawford & McGowan 1979). Additionally, production of late tillers was minimized. When all the main tillers had reached full maturity the plants were harvested. During the experiment, the nutrient solution was changed weekly. The pots were removed from the experimental vessels and placed in similar vessels containing deionized water, to prevent the roots drying. Whilst in these vessels the pots were watered with deionized water to remove any residual nutrient solution. The pots were then returned to their experimental vessels, which had been scrubbed clean with deionized water and filled with new nutrient solution. Samples of the old and new nutrient solution were taken from each experimental vessel at this time and were analysed by inductively coupled atomic emission spectrometry (ICP-AES) to monitor fluctuations in the concentration of all nutrients except N. Although the concentration of certain elements was difficult to monitor (e.g. boron and molybdate), the remaining nutrients in the old nutrient solution

**Table 1.** The composition of the nutrient solution used

| Macronutrient | mol $m^{-3}$ | Micronutrient | mmol $m^{-3}$ |
|---|---|---|---|
| $NH_4NO_3$ | 2·75 | Fe-EDTA | 13·97 |
| KCl | 1·97 | $ZnCl_2$ | 1·17 |
| $MgCl_2 \cdot 6H_2O$ | 0·15 | $MnCl_2 \cdot 4H_2O$ | 1·40 |
| $CaSO_4$ | 0·12 | $CuCl_2 \cdot 2H_2O$ | 0·24 |
| $Ca(H_2PO_4)_2$ | 0·38 | $H_3BO_3$ | 0·71 |
| $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ | 0·01 | | |

(including S) never depleted to less than 25% of the concentration in the new solution, before replenishment.

## Experimental treatments

Two sources of $CaSO_4$ with different $^{34}S/^{32}S$ ratios were used: one with high $\delta^{34}S$ (13·7‰) and the other with low $\delta^{34}S$ (4·1‰). The high $\delta^{34}S$ source was a commercially available horticultural gypsum (Chempak Ltd, Hoddesdon, UK); and the low $\delta^{34}S$ source was produced by reaction of equimolar amounts of $Ca(NO_3)_2$ with a laboratory $Na_2SO_4$ (BDH, Poole, UK) found to have a low $\delta^{34}S$ value. The resultant $CaSO_4$ precipitate was washed repeatedly with deionized water and dried at 120 °C. A preliminary experiment using sand culture has shown that this difference in $\delta^{34}S$ was sufficiently large to study the uptake and redistribution of S within plants (Monaghan, Evans & Scrimgeour 1997).

Prior to GS 12 no treatment received any S. The experiment consisted of five treatments. Treatments A and B received only the high and low $\delta^{34}S$ after GS 12, respectively. Treatments C–E received high and low $\delta^{34}S$ sources at different growth stages (Fig. 1). Immediately before the S source was changed from high to low $\delta^{34}S$ in treatments C–E, three pots were selected randomly and removed from that experimental vessel. These sequential samples, together with the samples of treatment A at maturity, had received high $\delta^{34}S$ source only, and were used to evaluate overall plant growth and development, and the pattern of S uptake and isotopic fractionation. The three remaining pots in treatments C–E were then placed to one side of the experimental vessel on a half-width stand. A sealed polythene bag containing six house bricks was placed in the vessel, halving the volume of nutrient solution.

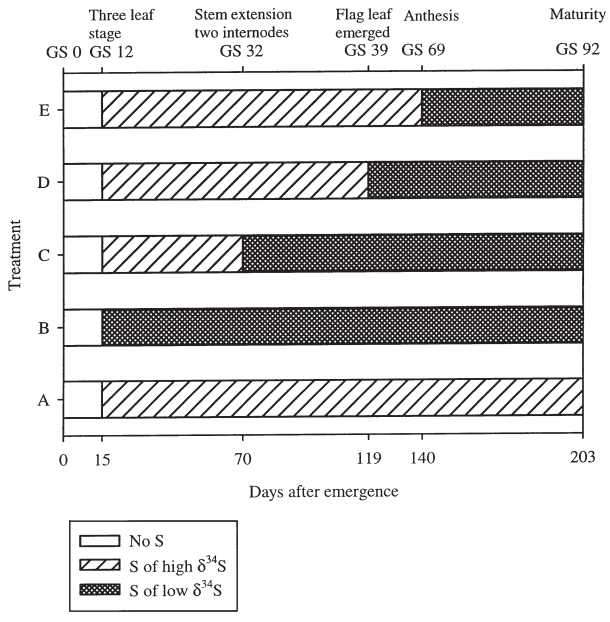


**Figure 1.** Schematic diagram of experimental treatments.

Consequently, treatments C–E had the same volume of nutrient solution per plant as treatments A and B, which had six pots per vessel throughout. Analysis of the nutrient solution indicated that the depletion of nutrients followed similar patterns in all treatments. In the experimental vessel that was sampled, all the tillers that were at the required growth stage (e.g. GS 32 in treatment C) were tagged with coloured wool and defined as main tillers, enabling their identification at harvest; the remaining tillers were classified as late tillers. Whereas six plants were grown to maturity in treatments C–E, 12 plants were grown to maturity in treatments A and B. For correct comparison between treatments, three pots (six plants) were randomly selected from both treatments A and B at maturity, and the remainder were discarded.

## Sampling and analysis

The six plants sampled from the same treatment on each occasion were treated separately as six replicates. Plants sampled at GS 32 were taken as whole plants; at GS 39 the plants were separated into stem, flag leaves, and older leaves (all leaves but flag leaf); at GS 69 the plants were separated into stem, flag leaves, older leaves, and ears. At maturity all remaining plants were initially split into main tillers and late tillers and then separated into stem, older leaves, flag leaves and ears. All plant parts were dried to constant weight at 80 °C. Dried ears harvested at maturity were threshed, giving chaff and grain. The biomass of the plant fractions of the main tillers and late tillers was measured separately for all treatments. Whole plant biomass (main and late tillers) was the sum of the two values for each plant fraction.

About 1 g of material from each plant fraction of each plant was ground into a fine powder in a Glen Creston ball mill, using stainless steel containers and ball bearings, giving six replicates per treatment. The concentration of total S and the ratio of $^{34}S/^{32}S$ were determined using mass spectrometry (see below). S isotope results were calculated as $\delta^{34}S$ in ‰ deviation from the VCDT standard. Sulphate in the plant fractions from sequential samples was extracted with deionized water, and determined using ion chromatography (Dionex 2000; AS9C column). All analyses were carried out in duplicate.

Analysis of variance was performed to assess the significance of treatment effects ($P < 0{\cdot}05$) for all measured variables at maturity. Student's *t*-tests ($P < 0{\cdot}05$) were used to compare differences in the $\delta^{34}S$ values between the S source and the S accumulated in plant fractions.

## Analysis of S isotope ratios using continuous flow-isotope ratio mass spectrometry (CF-IRMS)

The successful coupling of an elemental analyser to an IRMS for measuring $^{34}S/^{32}S$ ratios was reported over 10 years ago (Pichlmayer & Blochberger 1988; Haystead 1991). However, it is only recently that this technique has been reassessed and used (Giesemann *et al*. 1994), and, it has been more commonly applied to mineral S prepared

from biological samples rather than to intact plant or animal samples. However, by paying particular attention to the operation of the elemental analyser and the IRMS interface it was possible to use CF-IRMS in S analysis of plant samples containing ≥ 1 mg S $g^{-1}$ dry weight.

The underlying principles of S isotope analysis by CF-IRMS are very similar to the widely used ANCA-MS of carbon (C) and N isotopes. However, the conditions for satisfactory conversion of samples to $SO_2$ are rather different, and required the elemental analyser to be set up specifically for S analysis. $SO_2$ has a reputation as a 'sticky' gas in dual inlet IRMS systems (i.e. prone to adsorption on the metal surfaces of the inlet system, leading to memory effects), which are often operated with heated inlet systems when handling $SO_2$. However, most of the problems in analysing S arise from sample conversion in the elemental analyser, and *once dry,* $SO_2$ is easily handled in a conventional CF-IRMS system. Most biological samples produce at least 100 times more water and $CO_2$ than $SO_2$, and the water and $CO_2$ have to be removed completely before the $SO_2$ enters the IRMS.

The schematic layout of the CF-IRMS used in this study is shown in Fig. 2, and the features that distinguish it from an ANCA-MS are discussed below. The instrument was based on an ANCA-SL sample converter and 20–20 IRMS (both Europa Scientific Ltd, Crewe, UK).

There was a combined oxidation and reduction tube, with tungstic oxide as the oxidant and copper at the bottom of the tube to convert any $SO_3$ to $SO_2$. Tungstic oxide on zirconia was quite satisfactory, and cheaper than pure tungstic oxide. The copper was from a freshly opened vial.

A removable ash collection tube was used. This was essential for efficient use of the combustion tube packing, as severe tailing of the $SO_2$ peak occurred if too much ash was present. The removable quartz liner allowed the ash to be easily and completely removed after each completed run of ≈ 100 samples.

PTFE couplings and tubing were used between the combustion tube and the Nafion™ drying tube. The combustion products did not come in contact with metal surfaces in the presence of water.

The Nafion™ drying tube was a sulphonated PTFE membrane with a high affinity with water, but impervious to other gases. As water was produced only as a short pulse after each sample was combusted, the waste helium flow from the IRMS open split could be used to purge the water from the membrane. Magnesium perchlorate was not used as a drying agent as, once a little water had been trapped, variable and tailing $SO_2$ peaks were observed.

The Porapack QS gas chromatography (GC) column achieved a good separation of $N_2 + CO_2$ from $SO_2$ (the $SO_2$ eluting later). For complete separation of the $SO_2$ from

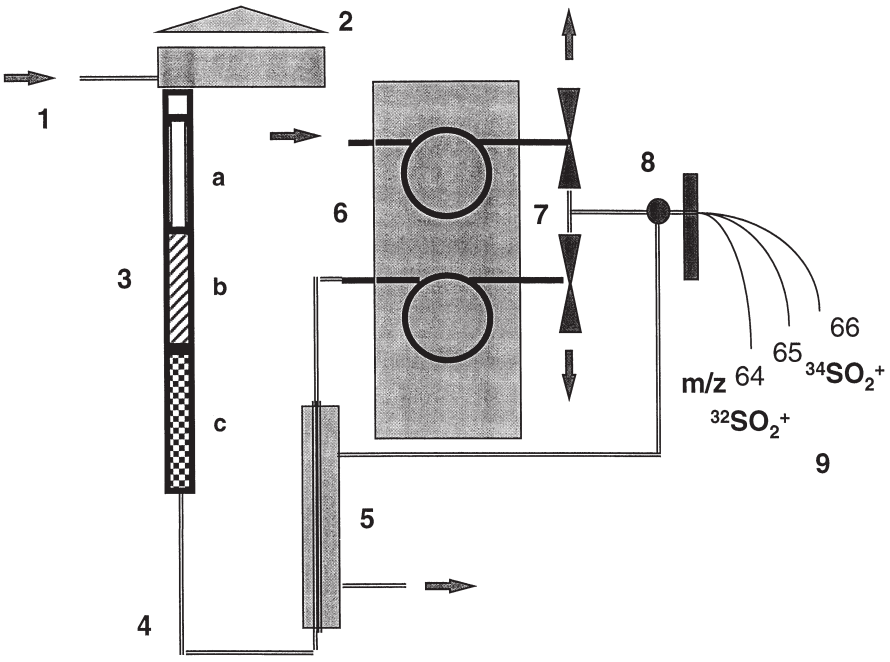


**Figure 2.** Schematic diagram of the continuous flow-isotope ratio mass spectrometer (CF-IRMS) system for $^{34}S$ measurement. (1) Continuous flow of helium into the elemental analyser and autosampler. (2) Autosampler holding solid samples in tin foil balls. (3) Combustion/reduction tube at 1000 °C. The top of the tube contains the removable ash tube (a), the middle section is packed with tungstic oxide on zirconia (b), and the bottom contains copper wires (c). (4) PTFE connections and tubing to the Nafion™ drying trap. (5) Nafion™ drying trap, purged with the waste helium flow from the open split. (6) Dual column gas chromatograph oven at 35 °C with a Porapak QS column in-line. (7) Gas-switching valves to direct the standby flow from the other column to the open split, except when $SO_2$ is eluting from the Porapak QS column. (8) Open split. (9) IRMS. The arrows show helium flow into and out of the system. Valves (7) are operated to maintain flow past the open split throughout the analysis.

$CO_2$ the column was operated near to room temperature. The instrument used had two GC columns which could be switched for C and N or S analysis.

The dual column arrangement also allowed sample gas switching to prevent $CO_2$ entering the IRMS. This was achieved by switching the Porapak QS column effluent to the IRMS only when the $SO_2$ was eluting, and directing the standby flow from the other column to the IRMS at all other times.

Complete and consistent sample conversion was aided by adding vanadium pentoxide to the plant samples. This was added to the samples as they were being weighed out, putting in twice the sample weight of vanadium pentoxide. Sample size was a compromise between signal size and efficient combustion without too much ash being formed. Ten milligram samples were the maximum used (with an additional 20 mg vanadium pentoxide), but samples below 5 mg were preferred. Where there was sufficient S ($\approx$ 5 mg S $g^{-1}$ sample) the samples were weighed out to contain 25 $\mu$g S, otherwise (down to 1 mg S $g^{-1}$ sample), 10 $\mu$g S was used. Samples much below 1 mg S $g^{-1}$ were not suitable for direct analysis.

Pairs of ammonium sulphate working standards were run after each 10 samples. These were freeze-dried from solution in tin cups, in amounts to match the S content of the samples. No vanadium pentoxide was required (which also minimized ash formation). The working standards were calibrated against the silver sulphide standards S1 and S2 from IAEA (Vienna, Austria) and had a $\delta^{34}S_{VCDT}$ of 7·4‰.

## RESULTS

### Biomass and S accumulation over time

The morphology, colour and harvest index (grain weight/total biomass) of the hydroponically grown plants were similar to those of normal field-grown plants (Hocking 1994). A notable difference was that plants grown in this hydroponic experiment developed more late tillers, probably due to adequate nutrient supply and little shading at the base of the plant after anthesis. There were no significant differences between the five treatments in total biomass, biomass of the plant parts, S accumulation or S concentration in the samples taken at maturity. The accumulation of biomass appeared to be slower between GS 32 and GS 39 than between GS 39 and GS 92 (Fig. 3a). Over half (53%) the total biomass at maturity was accumulated after anthesis (GS 69), although net accumulation in vegetative organs ceased by anthesis (Fig. 3a).

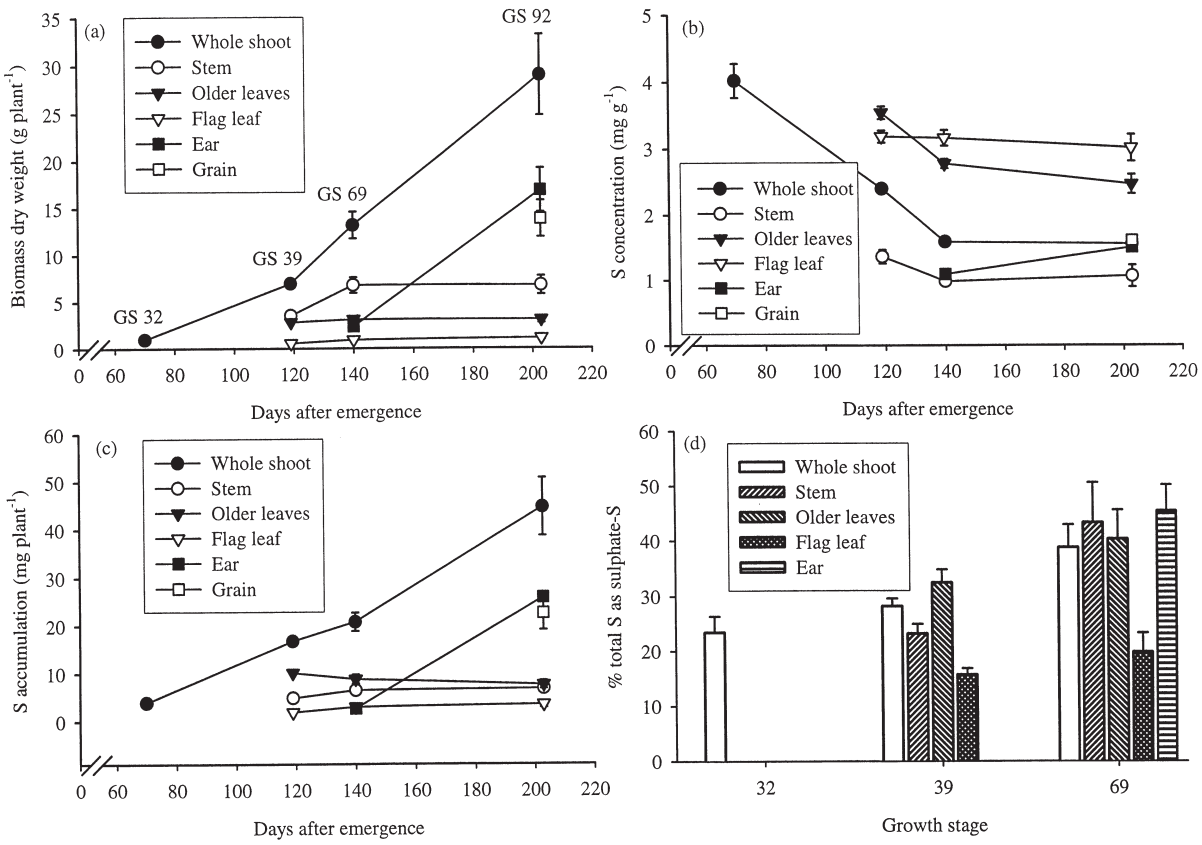


**Figure 3.** (a) Accumulation of biomass in different plant parts and whole shoots. (b) Changes in the concentrations of sulphur (S) in different plant parts and the whole shoots during different growth stages. (c) Accumulation of S in different plant parts and in the whole shoots. (d) Sulphate as a percentage of the total S in different plant parts and whole shoots. Vertical bars represent ± standard errors.

The concentrations of S in the whole plants decreased significantly from GS 32 to GS 69, but remained relatively constant from GS 69 to GS 92 (Fig. 3b). The concentrations of S in older leaves and stems also decreased between GS 39 and GS 69, whereas it remained relatively stable in the flag leaves. In contrast, the concentration of S in ears increased between GS 69 and GS 92.

Plant shoots accumulated S almost linearly between GS 32 and GS 92 (Fig. 3c). Similar to biomass accumulation, 54% of S in the shoots at maturity was taken up after anthesis, and 50% of the total S was in the grain. Interestingly, the amount of S accumulated in the stem, older leaves and flag leaves remained relatively constant from GS 39 to GS 92. The distribution pattern of S in different plant parts at maturity was similar to that observed in field-grown wheat (F. J. Zhao *et al*., unpublished).

The proportion of $SO_4$-S relative to total S in the whole shoots increased from 24% at GS 32 to 40% at GS 69 (Fig. 3d), suggesting that the proportion of the S taken up by plants which was assimilated into organic S decreased with growth stage. Among all plant fractions, flag leaves had the smallest percentage of $SO_4$-S in total S. For vegetative tissues, these values are comparable to field-grown wheat (Spencer & Freney 1980; Zhao *et al*. 1996). However, immature ears had a high proportion of sulphate (≈ 50%), which was much higher than has been reported in mature grain (< 10%; Byers, McGrath & Webster 1987).

## Differences in $\delta^{34}$S between different plant parts and changes over time

The mean $\delta^{34}$S values for whole shoots of the plants receiving only the high $\delta^{34}$S source (treatment A) increased slightly from 13·1‰ at GS 32 to 13·9‰ at GS 92 (Fig. 4). Overall, the $\delta^{34}$S values for shoots were very similar to the $\delta^{34}$S value of the S source (13·7‰). At the four different growth stages examined, only the GS 32 samples had a $\delta^{34}$S value significantly different from that of the source ($P < 0{\cdot}05$ in *t*-test). The $\delta^{34}$S value of the whole shoots from treatment B at maturity was 0·6‰ higher than that of the low $\delta^{34}$S source ($P < 0{\cdot}05$ in *t*-test) (Fig. 4).

There were significant ($P < 0{\cdot}05$) differences in the $\delta^{34}$S values between different plant parts (Fig. 4), the maximum differences being 1·4‰ in treatment A and 2·3‰ in treatment B at GS 92. However, these differences were not consistent at different growth stages. For example, in treatment A stems had a higher $\delta^{34}$S than leaves at GS 39, but the opposite was observed at GS 92. Comparing treatments A and B at GS 92 (Fig. 4), it is also clear that there was no consistent pattern in the differences in $\delta^{34}$S between plant parts. The variations in $\delta^{34}$S within the stems (GS 39 and GS 69) and the ears (GS 69) were larger than those observed in other tissues, and this may be explained by the lower concentrations of S in these samples compared with leaves and grain (see Fig. 3b), and

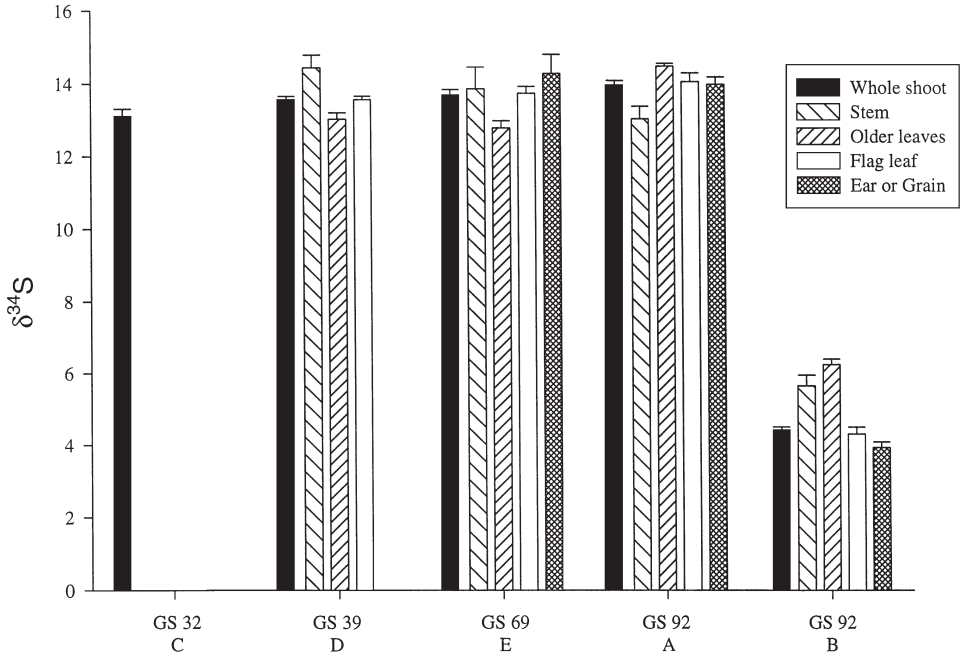


**Figure 4.** $\delta^{34}$S values in different plant parts and whole shoots at different growth stages for treatments C, D and E (following uninterrupted supply of high $\delta^{34}$S source); and at GS 92 for treatments A and B. The $\delta^{34}$S values for the whole shoots at GS 32 were determined directly, and the values for the whole shoots at GS 39, GS 69 and GS 92 were calculated from the $\delta^{34}$S and the sulphur (S) contents of individual plant parts. The $\delta^{34}$S values for chaff at GS 92 were not determined, but this plant part contained < 6% of the total S in the shoots. Capped bars represent standard errors.

hence a poorer precision in the determination of the isotope ratios. For flag leaves and ears/grain, deviations of $\delta^{34}S$ from the source value were ≤ 0·3‰, whereas for stems and leaves the deviations varied between 0·1 and 2·2‰.

### Differences in $\delta^{34}S$ between treatments and estimation of S redistribution

The $\delta^{34}S$ values of all plant parts sampled at GS 92 increased in the order of treatments B, C, D, E and A (Fig. 5), reflecting the relative lengths of exposure to the two S sources. The effects of treatments were highly significant ($P < 0·001$) for all plant parts. The differences between treatments D and E were small, which was expected due to the short period between GS 39 and GS 69 (21 d, Fig. 1) and consequent small S uptake (Fig. 3c). In treatments C–E, where both S sources were given to plants for differing durations, stems, flag leaves and grain appeared to have similar $\delta^{34}S$ values, whereas older leaves behaved very differently in having a considerably higher $\delta^{34}S$ value (Fig. 5).

Assuming that there was little isotopic fractionation during S uptake and redistribution, the differences in $\delta^{34}S$ between treatments can be used to calculate the contributions of S accumulated in the biomass between different growth stages to the total S in each plant part at maturity. For example, the proportion of S in the grain at maturity derived from S accumulated in the plants before GS 32 was calculated as follows:

$$\%\text{ of the S in grain derived from uptake before GS } 32 = \{(\delta^{34}S_{Cg} - \delta^{34}S_{Bg})/(\delta^{34}S_{Ag} - \delta^{34}S_{Bg})\} \times 100$$

where the subscripts A, B and C denote treatment codes and the subscript g denotes grain. Similarly, the percentage contribution of the S accumulated in the biomass between GS 32 and GS 39 to the S in grain at maturity was calculated as follows:

$$\%\text{ of the S in grain derived from uptake between GS 32 and GS } 39 = \{(\delta^{34}S_{Dg} - \delta^{34}S_{Cg})/(\delta^{34}S_{Ag} - \delta^{34}S_{Bg})\} \times 100.$$

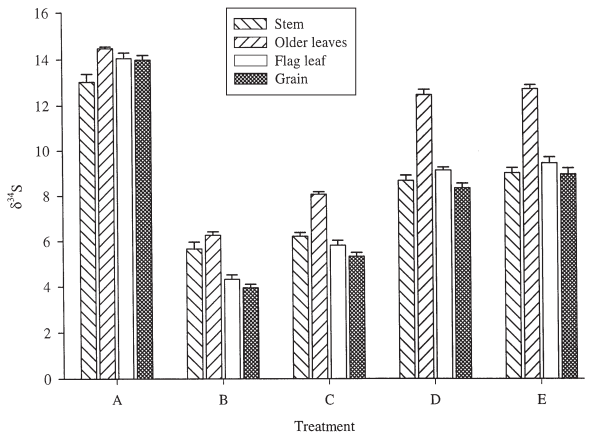


**Figure 5.** $\delta^{34}S$ values in different plant parts at maturity (GS 92) in different treatments. Capped bars represent standard errors.

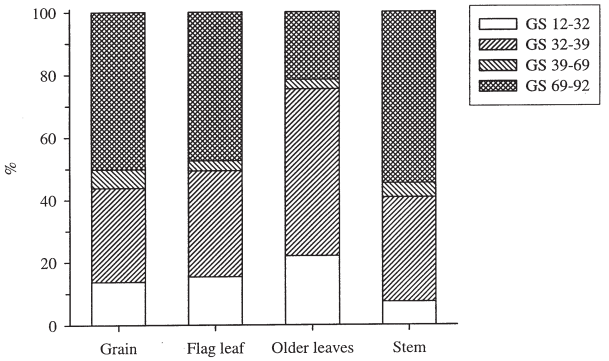


**Figure 6.** Percentages of sulphur (S) in grain, flag leaves, older leaves or stems derived from the accumulation during different growth stages.

This calculation was repeated for the remaining growth stages and plant parts, and the results are presented in Fig. 6. It emerged from these calculations that grain, stems and flag leaves followed a broadly similar pattern in terms of the origin of S, whereas older leaves showed a distinctive pattern. About 50% of the S in grain, stems and flag leaves at maturity came from S accumulated after anthesis (GS 69), and 7–15%, 30–34% and 3–6% was derived from that accumulated between GS 12 and GS 32, GS 32 and GS 39, and GS 39 and GS 69, respectively. In contrast, older leaves derived much less S from accumulation after anthesis (22%), but more from accumulation between GS 12 and GS 32, and GS 32 and GS 39 (22 and 53%, respectively). The contribution of S accumulated between GS 39 and GS 69 to all plant parts at maturity was small, again due to the short period of time between the two growth stages and the resulting small net uptake of S.

## DISCUSSION

The natural variation in stable S isotope ratios has been widely used to delineate sources of S in different ecosystems (Krouse 1977; Nriagu, Coker & Barrie 1991; Stam *et al*. 1992; Zhao *et al*. 1998). More recently, Prietzel *et al*. (1995) and Giesemann *et al.* (1995) have used $^{34}S$-enriched sulphate to trace the movement and transformation of S in forest ecosystems. This potentially powerful tool has been little exploited in plant nutrition studies. Trust & Fry (1992) attributed this to the intensive sample preparation and large sample sizes required in the determination of the S isotope ratios using conventional off-line mass spectrometry. The on-line method, using a directly linked elemental analyser and mass spectrometer, greatly simplifies sample preparation, and reduces the amount of sample required (Giesemann *et al*. 1994). This study shows that the method can be reliably used to analyse plant samples with S concentrations > 1 mg $g^{-1}$, which includes most plant materials.

For isotopes to be used as a tracer to gain quantitative information, there must be minimal isotope fractionation in the processes studied. The present study showed that the

isotope ratios of the whole plant shoots, which had been supplied with only one S source, were very close to the $\delta^{34}S$ values of the sources, with a maximum deviation of ± 0·6‰, suggesting that there was little isotope fractionation during sulphate uptake and transport from roots to shoots. This is in general agreement with other reports in the literature. Krouse, Stewart & Grinenko (1991), and Trust & Fry (1992) concluded in their reviews that, with higher plants, either negligible fractionation or an average depletion of $^{34}S$ by 1–2‰ in the organic S, compared with the sulphate source, occurs. No attempt was made in the present study to determine the $\delta^{34}S$ values of sulphate or organic S. Some differences in $\delta^{34}S$ between plant parts were noticeable, but they were more likely to be due to random errors than isotope fractionation, as there was no consistent pattern in the differences. Because the absolute deviations of the $\delta^{34}S$ values of grain and flag leaves from the source values in treatments A and B were much smaller than those for the stems and older leaves, it may be expected that the calculations of S distribution (Fig. 6) would be more accurate for grain and flag leaves than for stems and older leaves. The difference in $\delta^{34}S$ between the two S sources used in this study was relatively small (9·7‰). Under the conditions of the experiment, most calculations are likely to have errors of < 10%. Accuracy can be improved by using larger differences in the $\delta^{34}S$ values of S sources.

By supplying the plants with S of contrasting $\delta^{34}S$ values at different growth stages, it was possible to estimate S redistribution within plants. The contributions of the S accumulated before and after anthesis to the grain at maturity were about equal. At anthesis there was only a very small amount of S in the ears, and probably a negligible amount in the developing grain. Consequently, the 50% of the S in the grain of preanthesis origin must have been redistributed (remobilized) from other plant parts. Part of the S derived from postanthesis accumulation was probably delivered first to the stems and leaves, and then redistributed to the grain. There was little change in the total S content of both flag leaves and stems from anthesis to maturity, yet S derived from accumulation after anthesis in these two plant parts accounted for 48–55% of total S. This again indicates a continuous cycling of S between plant parts. Taking into account the actual change in the total S contents from anthesis to maturity, it can be estimated that 39 and 52% of the preanthesis S in the flag leaves and stems, respectively, was exported after anthesis. The older leaves were less active in the S cycling, containing only 22% of postanthesis-derived S at maturity, and exporting 32% of their preanthesis S after anthesis. This may be due to the fact that at anthesis the bottom leaves were senescing or had already senesced.

Collectively, these results show the intermediate extent of S redistribution (remobilization) in wheat during reproductive growth, being less than that for N and P (Marschner 1995). Furthermore, S derived from accumulation in the biomass, before and after anthesis, is equally important in the accumulation of S in wheat grain. Caution must be taken when extrapolating these results to field-grown wheat. In particular, availability of S and water in soils may be lower in the post- than in the preanthesis period, thus affecting the contribution of postanthesis S uptake to grain S accumulation.

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