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YIELD AND BREADMAKING QUALITY RESPONSES OF WINTER WHEAT TO SULPHUR FERTILISER

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YIELD AND BREADMAKING QUALITY RESPONSES OF WINTER WHEAT TO SULPHUR FERTILISER

by

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ABSTRACT

Sulphur deficiency has become more common in wheat as a result of decreased S inputs from atmospheric deposition. This project was initiated to evaluate the responses of grain yield and breadmaking quality to the additions of S fertilisers under field conditions (Part I.), and to investigate the physiological basis of the S nutrition of wheat in terms of the critical phases of S supply and re-distribution of S to wheat grain (Part II.).

In Part I., twelve field experiments were carried out at Bridgets (Hampshire), Raynham/Barsham (Norfolk), Woburn (Bedfordshire) and in the Scottish Borders over the three growing seasons from 1994 to 1997. The winter wheat variety Hereward was used in the first two seasons, and three breadmaking varieties in the third season, Hereward, Rialto and Spark, were compared. The experiments in the first two seasons also compared applications of S as gypsum in early spring versus foliar applications of ammonium sulphate at the milky ripe stage. Complete data sets for yield and S uptake were obtained in 11 experiments, and for breadmaking quality parameters in 10 experiments. The main findings can be summarised as follows:

1). Significant yield increases in response to S additions in early spring were obtained in 3 out of 11 field experiments over the three seasons from 1994 to 1997. In addition, one experiment showed S deficiency symptoms during stem elongation, although the grain yield response to S did not reach a significant level. The responsive sites were a shallow calcareous soil at Bridgets and a sandy soil at Woburn. In the responsive experiments, yield increases due to S varied between 0.43 and 1.34 t ha^{-1}, or between 8.7 and 26.5% on the relative basis. Most of the yield increase was obtained from the application of the first 20 kg S ha^{-1}.

2) Applications of S in early spring increased loaf volume significantly in six out of the ten experiments that produced suitable grain samples for breadmaking tests. All 4 sites showed responses in one or two seasons, suggesting that breadmaking quality response to S was more common than yield response. Increases in loaf volume typically varied between 40 and 100 ml. In addition, S also improved crumb score in two experiments. Three breadmaking varieties, Hereward, Rialto and Spark, appeared to respond similarly to S. In comparison, increasing the

amount of N applied from either 180 to 230 kg ha^{-1} in nine experiments, or from 230 to 280 kg ha^{-1} in one experiment, increased loaf volume significantly only in one case, even though this increased grain protein significantly in most experiments.

3) Loaf volume correlated more closely with grain S concentration than with grain N (grain protein). These results indicate that, within the range of grain protein concentration obtained in this series of experiments (8.5-14.3%), the concentration of crude protein was not as limiting a factor as the concentration of S in grain to breadmaking performance. Because grain S concentration correlated with loaf volume in a linear pattern, it was difficult to derive a critical value of grain S for breadmaking quality. In many cases, a low loaf volume was associated with a grain N:S ratio of greater than 16:1. These results confirm that grain S status is important for breadmaking quality of wheat.

4) There were significant effects of S on dough rheology, and the amount and elastic modulus of gel protein. Sulphur addition in general increased gel protein content, but decreased its elastic strength. Sulphur also decreased dough resistance, and increased dough extensibility. The effects of S on dough rheology and the elastic strength of gel protein could be explained by the positive influence on the ratio of LMW/HMW subunits of glutenin. Despite their different rheological properties, Hereward, Rialto and Spark responded similarly to S.

5) Compared to the spring applications of gypsum, foliar applications of ammonium sulphate at the milky ripe stage were not effective in correcting S deficiency for grain yield. In some cases, foliar applications resulted in scorching and yield losses. In terms of the effects on grain S concentration and breadmaking quality parameters, foliar applications of S produced inconsistent results. It was concluded that the best practice at present was to apply S, in a sulphate form, in spring.

6) It was established that winter wheat crops generally require >15 kg S ha^{-1} to ensure S sufficiency. The harvest index for S was much lower than that for N, even under S deficient conditions, indicating that the re-utilisation of S within plants was less efficient than of N. Analysis of plant samples at early stem

elongation (GS 31-32) was useful in predicting S deficiency, with a critical value of 2 mg g^{-1} of total S in the whole plant shoots.

7) An extractable sulphate-S concentration in the soil profile of greater than 3 mg kg^{-1} in early spring appeared to indicate a sufficient S supply for grain yield. However, S deficient sites could not be predicted reliably even when soil extractable sulphate-S was less than 3 mg kg^{-1}. In this series of field experiments, breadmaking quality responses were not related to soil extractable S in early spring.

In Part II, pot experiments were conducted to investigate the effects of S deficiency and the timing of S addition on yield and yield components, and to quantify the re-distribution to grain of the S accumulated in wheat plants at different growth stages. A method was developed to use different S sources varying in their natural abundance of the stable isotope ^{34}S as a tracer system for the quantification of S re-distribution under hydroponic conditions. The breadmaking variety Hereward was used in both experiments. Main findings are summarised as follows:

1) Severe S deficiency decreased grain yield markedly by affecting the number of ears and the number of grain per ear, whereas single grain weight was little affected. Compared to the S deficient control, ear number was increased significantly by the additional S given to the S-deficient plants at pre-stem elongation and stem elongation stages, but not by the additional S given after stem elongation. This indicates that S supply before and during stem elongation is important for the initiation and survival of tillers. In contrast, the critical phases for the number of grain per ear appeared to be the stem elongation and pre-anthesis ear development stages. Additional S given to the S deficient plants after anthesis did not correct the deficiency significantly.

2) Grain S concentration appeared to be influenced more by the S supply after stem elongation. Additional S given to the S deficient plants at the pre- and post-anthesis ear development stages restored the concentration of S in grain to levels similar to or above that found in the S sufficient control. Increasing proportion of

low molecular weight gluten polymer was found to be associated with increasing grain S concentration.

3) 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 elongation, between stem elongation 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 post-anthesis 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.

Overall, the results suggest that the stem elongation stage is the most critical phase of S supply for grain yield, whereas S supply after anthesis is important for achieving a high concentration of S in grain to give a quality benefit.

PART I. YIELD AND BREADMAKING QUALITY RESPONSES OF WINTER WHEAT TO SULPHUR UNDER FIELD CONDITIONS

1. Introduction

Although the essential role of sulphur (S) for plant growth and development has long been recognised, deficiency of S in agricultural crops was rare in the UK until about a decade ago. A massive decrease in the inputs of S from atmospheric deposition since the early 1970s, coupled with increased crop yields and a change from the use of S-containing fertilisers to S-free fertilisers, has contributed to increased S deficiency over the last decade (McGrath *et al.*, 1996). A previous project funded by HGCA (McGrath *et al.*, 1995) reported yield increases in cereals in response to the addition of S fertilisers in several field experiments. In addition, the concentrations of S in British wheat grain samples had decreased substantially from the early 1980s to the early 1990s, whereas the N:S ratio had increased (Zhao *et al.*, 1995). The need for S fertilisers is predicted to increase in the future as atmospheric deposition of S is likely to decrease much further (McGrath and Zhao, 1995).

For wheat, deficiency of S can result not only in yield losses, but also in low breadmaking quality. In the 1980s, Australian researchers demonstrated that S deficiency in wheat had a profound effect on the composition of gluten proteins in wheat grain, with increased synthesis of S-poor proteins (ω-gliadins and high molecular weight (HMW) subunits of glutenin) at the expense of S-rich proteins (α- and γ-gliadins and low molecular weight (LMW) subunits of glutenin) (Moss *et al.*, 1981; 1983; Wrigley *et al.*, 1984). These compositional changes were associated with decreased extensibility and increased elasticity of dough. Field experiments conducted in England before 1990 showed that breadmaking quality, as measured by loaf volume, was not affected significantly by the applications of S fertilisers (Salmon *et al.*, 1990; Kettlewell *et al.*, 1998), probably because S deficiency was rare at that time. However, there was some evidence that when a large amount of N was applied late to wheat, the quality of gluten proteins for breadmaking deteriorated due to an imbalance of N and S (Timms *et al.*, 1981). Since S deficiency in wheat has

become more common in the 1990s in the UK, it is important that the effects of S nutrition on the breadmaking quality of field grown wheat are fully understood.

2. Objectives

There have been no systematic studies on the responses of winter wheat in terms of breadmaking quality parameters to the additions of S fertilisers in the UK. The objectives of Part I of this project were:

1). To evaluate responses of yield and breadmaking quality of winter wheat to the additions of S. This would also answer the question as to whether quality can be affected when there is no yield response to sulphur application.

2). To quantify the S requirement of breadmaking wheat.

3). To identify efficient fertiliser application practices which increase grain S concentration, yield and breadmaking quality.

4). To evaluate the relationships between grain S concentration or N:S ratio and yield or breadmaking quality, and to establish the critical values for S concentrations and N:S ratios in grain if possible.

3. Materials and Methods

3.1. Field experiments

Field experiments were carried out at four sites: Bridgets (Hampshire), Woburn (Bedfordshire), Raynham/Barsham (Norfolk) and WarK Common/Ellingham near Kelso (the Scottish Borders), in the 1994-95, 1995-96 and 1996-97 seasons. These sites were chosen because they were located in the high or medium risk areas of S-deficiency for cereals (McGrath and Zhao, 1995). Large variations between plots in crop growth and yield occurred in the experiment at Raynham in 1995-96, which were probably caused by variations in the degree of water shortage. This experiment was therefore excluded from further analysis.

In 1994-95 and 1995-96, the breadmaking variety Hereward was grown at all sites. Crops were sown in autumn between mid September and early November. The experimental design was the same for all sites in each season. The main treatments were factorial combinations of two N rates (180 and 230 kg ha^{-1}) and six S rates. The rates of S were 0, 20, 40, 60, 80 and 100 kg ha^{-1} in 1994-95, and 0, 10, 20, 40, 70 and 100 kg ha^{-1} in 1995-96. Nitrogen was applied as ammonium nitrate in two dressings in March and April, and S was applied as gypsum (18% S) in March. In both seasons, four extra treatments testing foliar sprays of urea and ammonium sulphate at a late stage (GS 75, milky ripe) were also included, with constant foliar N (50 kg ha^{-1}) and different amounts of S. The amounts of S applied to the foliar treatments were 0, 20, 40, and 20 soil + 20 foliar (kg ha^{-1}) in 1994-95, and 0, 10, 20, and 20 soil + 20 foliar (kg ha^{-1}) in 1995-96. All treatments were replicated in three plots in a randomised block design. Plot size varied between 36 and 50 m^2 at different sites, of which about 6 m^2 at one end of the plot was used for sequential crop sampling. Herbicides, fungicides and insecticides were applied according to standard practices.

The objective of the field experiments in 1996-97 was to compare the responses of three breadmaking varieties to S addition. There were 18 treatments in each experiment at the four sites, consisting of all factorial combinations of three varieties of winter wheat, three S levels and two N levels. The varieties were Hereward, Rialto and Spark, all being of good breadmaking potential. The S treatments were 0, 20 and 100 kg ha^{-1} S. The N treatments were 180 and 230 kg ha^{-1} for the Woburn and Borders sites, and 230 and 280 kg ha^{-1} for the Bridgets site. Higher rates of N were used at Bridgets because field experiments at the same site in the two previous seasons showed consistently low protein concentrations in grain. All treatments were replicated in three plots in a randomised block design. Other experimental details were similar to those for the 1994-95 and 1995-96 seasons.

Grain yields were determined using plot combine harvesting. Grain samples were collected for the determination of moisture content and for chemical and quality measurement.

3.2. Soil and crop sampling

Soil samples were taken from 0-30, 30-60 and 60-90 cm depths in autumn, spring before fertiliser application, and summer after harvest from the S_0 and S_{100} plots. Soil cores were combined for each block for the autumn and spring samples, but kept separately for each plot for the samples taken

after harvest. Samples were air-dried and ground to <2 mm for the analysis of extractable S. Fresh samples collected in spring were used for the analysis of soil mineral N as soon as possible after sampling.

In the first two seasons, crop samples were taken on five occasions at the beginning of April, May, June, July and August. The first four samples were collected from six rows each of 0.5 m length, and the last from a 1 m^2 quadrat for the measurement of harvest index and S uptake. In 1996-97, three crop samples were taken from quadrats at stem extension (GS 32), flag leaves (GS 39) and full maturity. Samples were dried at 80°C for 16 hours and dry matter determined. All plant samples were ground to <0.5 mm for chemical analysis.

3.3. Chemical analysis

For the determination of soil extractable S, air-dried soils were extracted in 1:5 ratio with 0.016 M potassium di-hydrogen phosphate, and the extracted S was determined with ion chromatograph (IC). Mineral N (nitrate and ammonium) in the fresh soils were extracted with 2 M potassium chloride and determined colourimetrically using a continuous-flow analyser. Both mineral N and extractable S concentrations are expressed on an oven-dried soil basis. Bulked soil samples collected in spring were used for the determination of total C and N using a Dumas combustion method (LECO CNS 2000). Soil pH was determined with glass electrode in a suspension of soil and water (1:2.5).

Plant samples were digested using $HNO_3/HClO_4$, and the concentrations of total S determined using ICP (Zhao *et al.* 1994). Tissue sulphate was determined in a selection of plant samples by IC. Total N was determined in selected samples using a combustion method (LECO CNS analyser). Grain protein concentration was calculated from the N concentration by multiplying by a factor of 5.7. The concentrations of N and S are expressed on dry matter basis, whereas grain protein concentration was calculated on an 86% dry matter basis. Grain N:S ratio was calculated from the N and S concentrations.

3.4. Milling and breadmaking quality measurements

Before milling, the Hagberg Falling Numbers (HFN) of all grain samples were determined. Milling and breadmaking tests were performed only on the grain samples from selected treatments

with HFN greater than 220. This criterion excluded all samples from the Barsham experiment in Norfolk, and many Hereward samples from Bridgets, in the 1996-97 season.

Grain samples were milled on a Buhler MLU 202 mill to produce straight-run white flour. A Buhler MLU 203 impact finisher was then used to remove adhering endosperm from the bran and offal fractions obtained during the initial milling. The additional flour produced was blended with the straight-run white flour for quality testing. Flour protein concentration and moisture content were measured by Near Infrared Reflectance (NIR), which had been calibrated with the Kjeldahl protein values. HFN of the white flour samples was also determined.

The water-absorbing capacity of each flour sample was measured using the Brabender Farinograph working to the 600 BU line. This test provides a measure of the water required to mix a dough to a fixed consistency which is used subsequently in test baking. A standard laboratory-scale Chorleywood Bread Process (CBP) baking test was used to produce 400 g white loaves (FMBRA, 1992). The recipe used for the CBP bread was (all as a proportion of flour weight): 2.5% yeast; 2% salt; 1% hard fat; 0.01% ascorbic acid; water as determined by Farinograph 600 BU line; mixing work input 39.6 kJ kg^{-1}. Each test bake was carried out in duplicate. Loaf volume was measured by displacement of seed. The quality of crumb structure was assessed visually by an expert. A high score (maximum 10) for crumb cell structure was awarded for a close and uniform structure of small, thin-walled cells.

3.5. Dough rheology and gel protein

Dough resistance and extensibility of the flour samples from the 1995-96 and 1996-97 harvests were determined using a Brabender Extensograph according to manufacturer's instructions.

The amount of gel protein in white flour and its elastic modulus were determined by the method of Pritchard and Brock (1994). Flour (10 g) was defatted with 25 ml petroleum ether (b.p. 40-60°C) for 1 hour, filtered and dried. Defatted flour (5 g) was stirred with 90 ml of 1.5% sodium dodecyl sulphate for 10 min at 10°C before being centrifuged at 40,000 g for 40 min. The gel protein layer was removed and weighed. The elastic modulus (G') of gel protein was measured using a small strain oscillatory rheometer (Bohlin VOR), after a 30 minute relaxation period at 10° C.

3.6. Size distribution of flour proteins and glutenin subunit composition

Selected white flour samples from the 1994-95 and 1995-96 harvest were used to extract and fractionate proteins on the basis of size according to the method of Batey *et al.* (1991). Flour samples were extracted with 0.5% (w/v) SDS in 50mM Na-phosphate buffer (pH 6.9) with sonication, and then resolved into three fractions using size exclusion high performance liquid chromatograph (SE-HPLC, Beckman System with TSK Gel 3000SW column, mobile phase containing 50% acetonitrile and 0.06% TFA). The replicates of the SDS extract gave consistent readings of absorbance at 280 nm, with the coefficient of variation varying between 4 and 6% in 10 replicates. To identify the proteins present in these peaks, the three fractions were collected, freeze-dried, and then separated by SDS-PAGE under non-reducing conditions and also after reduction of disulphide bonds.

Selected white flour samples from the 1996-97 harvest were used to for the determination of glutenin subunit distribution. Duplicate 0.5 g samples of flour were defatted by stirring for 1 hour at room temperature with 10 ml of water-saturated butan-l-ol followed by centrifugation at 8000 g for 5 min, the procedure being repeated once. This was followed by two extractions under similar conditions with 10 ml 0.5M aq. NaCl followed by water to remove salt-soluble proteins (albumins + globulins), followed by two extractions with 70% (v/v) aqueous ethanol to extract gliadins. Subunits present in alcohol-insoluble glutenin polymers were then extracted with 55% (v/v) aq. propan-l-ol containing 2% (v/v) 2-mercaptoethanol and 1% (v/v) acetic acid, the extraction being repeated twice. The combined supernatants were dialysed against distilled water and lyophilized. 10 mg protein was dissolved in 0.063M Tris/HCl buffer, pH 6.8, containing 2% (w/v) SDS, 5% (w/v) 2-mercaptoethanol, 10% (v/v) glycerol and 0.001% bromophenol blue and aliquots separated on 13% acrylamide Laemmli gels. Gels were stained with Coomassie BBR250 and duplicate separations quantitized using a BioRad Gel Doc 1000 image analysis system with Molecular Analyst software.

3.7. Data analysis

Analysis of variance (ANOVA) was performed on all data sets in two steps: first to test the significance of effects of N and S treatments at each site; and then data from all sites in the

same season were pooled to test the effects of sites, N and S treatments. There was no evidence of variance heterogeneity in the second step ANOVA, indicating that pooling the data from all sites was statistically valid. Data from all sites in each year were combined in correlation and regression analyses. Factors such as site, N and S treatments were not accounted for in these regression analyses. The statistical package Genstat 5 was used (Genstat 5 Committee, 1993).

4. Results and Discussion

4.1 Site information

Some of the soil properties are presented in Table 1. The soil at Bridgets is a shallow chalky clay loam, containing about 32% $CaCO_3$. The soils at the other sites are non-calcareous and light textured. A different field was used in each year at the same site, and this is reflected in the variations in the concentrations of mineral N and extractable sulphate-S between seasons. The Bridgets and Borders sites had considerably larger amounts of mineral N in spring in all three seasons than the Woburn and Raynham/Barsham sites, probably because the formers contained more organic matter. Soil extractable S, determined in early spring using ion chromatography, was below 3 mg kg^{-1} in 7 out of the 11 field trials (Table 1). The concentration of extractable S was generally uniform in the soil profile between 0 and 90 cm in early spring. The Bridgets soil used in the 1995-96 trial had considerably higher concentrations of extractable S than in the other seasons, because the field had received S fertiliser in the previous year.

Soil extractable S tended to vary during the growing season. In most cases, extractable S in the top soils decreased over the winter period, indicating leaching losses (Figure 1a). From early spring to the end of growing season (early August), extractable S in the top soils tended to increase, even though the crop had also taken up considerable amounts of S over the same period. This increase can only be explained by the mineralisation of organic S in the soils. Applications of 100 kg S ha^{-1} as gypsum in early spring left substantial amounts of S in the soil profiles, particularly in the top 30 cm layer (Figure 1b), although the amount of extractable S left over from the additions of S fertiliser varied with sites and seasons. Because leaching occurs predominantly over the late autumn-early spring, a large proportion of the residual S at the end of experiment may be lost by next spring.

Table 1. Soil properties, concentrations of available N and S, and previous cropping at each site

	Bridgets			Raynham/Barsham		Woburn			Borders		
	1994-95	1995-96	1996-97	1994-95	1996-97	1994-95	1995-96	1996-97	1994-95	1995-96	1996-97
Previous cropping	W. oat	Oilseed rape	W. wheat	Pea	Rape	W. oat	Lupin	S. barley	W. wheat	Oilseed rape	Linseed
Soil series	Andover	Andover	Andover	Barrow	Barrow	Cottenham	Cottenham	Cottenham	Nupend	Nupend	Nupend
Soil texture	Clay loam	Clay loam	Clay loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Loamy sand	Loam	Loam	Loam
Organic C (%)[a]	3.82	3.49	3.5	1.38	0.85	1.03	1.35	0.63	2.87	2.48	2.5
Total N (%)[a]	0.29	0.31	0.31	0.09	0.09	0.06	0.08	0.06	0.16	0.16	0.16
pH[a]	8.3	7.9	7.9	8.3	7.7	6.9	7.4	7.0	6.2	6.2	6.2
Mineral N (mg kg^{-1})[b]											
0-30 cm	21.7	12.0	23.6	4.1	4.1	1.5	3.5	2.5	14.7	9.4	8.0
30-60 cm	15.3	6.8	9.1	1.6	1.7	2.0	3.0	3.0	3.3	5.6	8.0
60-90 cm	7.0	5.4	8.1	1.4	4.9	1.6	1.9	5.0	3.2	2.8	5.6
Extractable S (mg kg^{-1})[b]											
0-30 cm	2.1	6.5	2.6	1.2	3.3	1.3	2.1	2.1	0.9	4.4	5.4
30-60 cm	2.1	9.8	1.9	0.8	3.1	2.7	2.8	3.2	1.6	3.8	6.4
60-90 cm	1.9	6.8	1.5	0.9	4.8	3.2	1.8	2.2	3.0	6.2	5.6

[a] Analyses were done on the topsoil (0-30 cm) samples collected in autumn before sowing. Organic C and total N were determined using a LECO CNS analyzer. pH was determined in a soil and water suspension with a glass electrode.

[b] Soils were collected in spring before fertiliser additions.

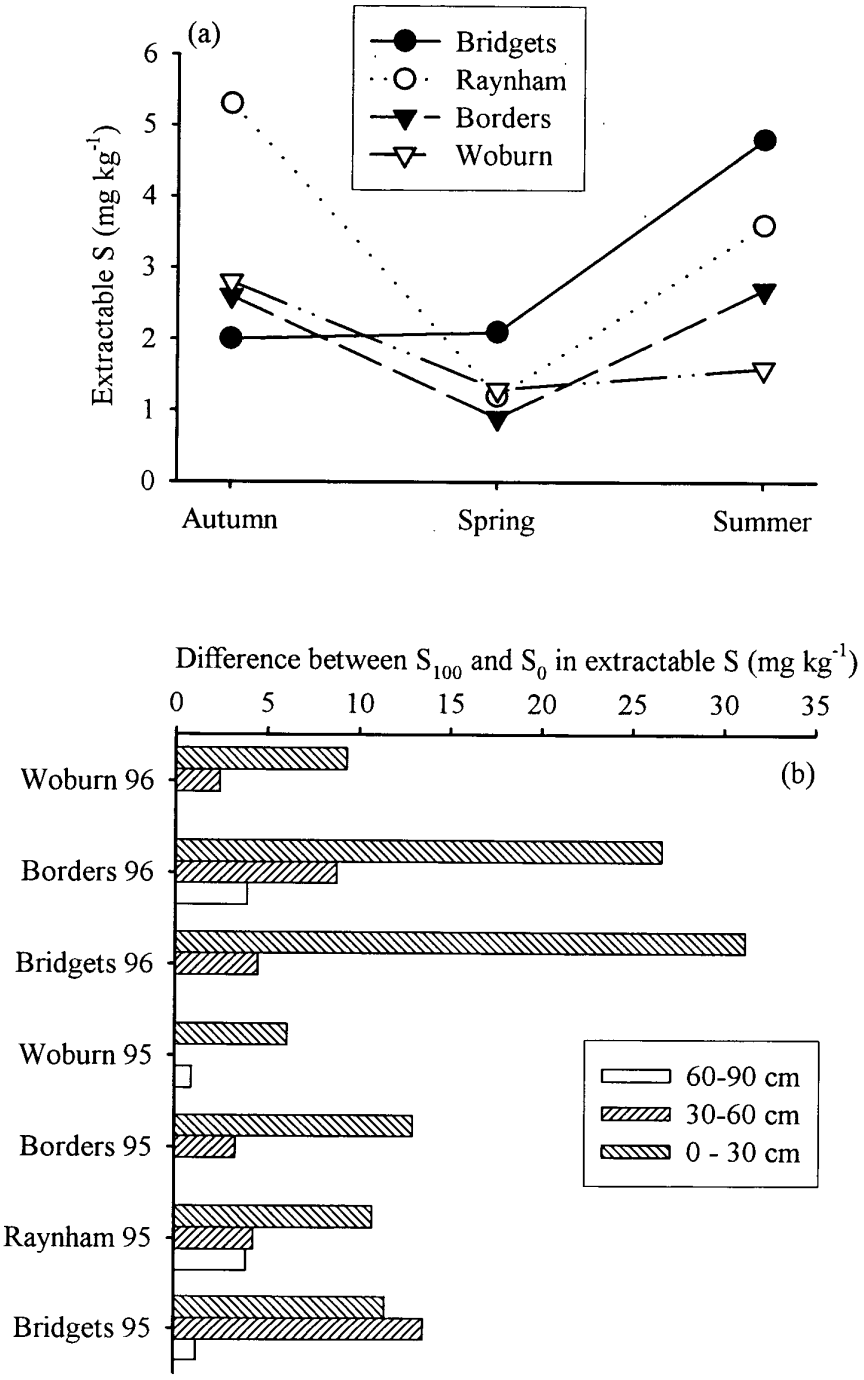

Figure 1. (a) Changes in the concentration of extractable SO_4-S in the top soils (0-30 cm) over the growing season in 1994-95. (b) Differences between the S100 and S0 treatments in extractable SO_4-S after crop harvest.

4.2 Yield responses

Effects of S application in early spring

Valid data sets of grain yield were obtained in 11 out of the 12 field experiments carried out over the three growing seasons between 1994 and 1997 (Appendices 1-11). Three experiments gave significant yield responses to the applications of S in early spring. These are Bridgets and Woburn in 1994-95, and Bridgets in 1996-97. The yield responses in these experiments are shown in Figure 2.

The responses at Bridgets in 1994-95 were very large. Averaged across all treatments with added S, yield increases due to S at N180 and N230 were 0.95 and 1.34 t ha^{-1}, or 11.5 and 15.7% on a relative basis, respectively. A greater response to S at the higher N rate suggests a positive interaction between N and S, although this was not statistically significant. Furthermore, about 80% of the yield increases were obtained from the application of the first 20 kg S ha^{-1} (Figure 2a).

Yield increases due to S applications were also substantial at Woburn in 1994-95 (Figure 2b), being 0.62 and 0.95 t ha^{-1}, or 16.0 and 26.5% on the relative scale, for the means of all plus S treatments in N180 and N230, respectively. Drought conditions at Woburn gave rise to a relatively large experimental error (CV=12.9%). In particular, the S_{80} treatments produced yields smaller than would otherwise be expected from the overall response pattern (Figure 2b). This was at least partly due to the fact that all replicate plots of the S_{80} happened to be in the poorer part of the experimental area as a result of randomisation. The response pattern suggests that an application of 20 kg S ha^{-1} was sufficient to achieve the maximum yield at the site.

The effect of S on grain yield was confirmed in all three breadmaking varieties tested in 1996-97 at Bridgets (Figure 2c). Applications of S increased the yields of Hereward, Rialto and Spark by 17.1, 18.3 and 8.7%, respectively. Despite a smaller increase in Spark, there was no significant interaction between varieties and S. For the variety Spark, there were considerable yield increases due to S applications with N230, but a lack of responses with N280. Therefore, the apparent difference between Spark and the other two varieties is probably due more to the experimental error than to a true genetic difference in the responsiveness to S.

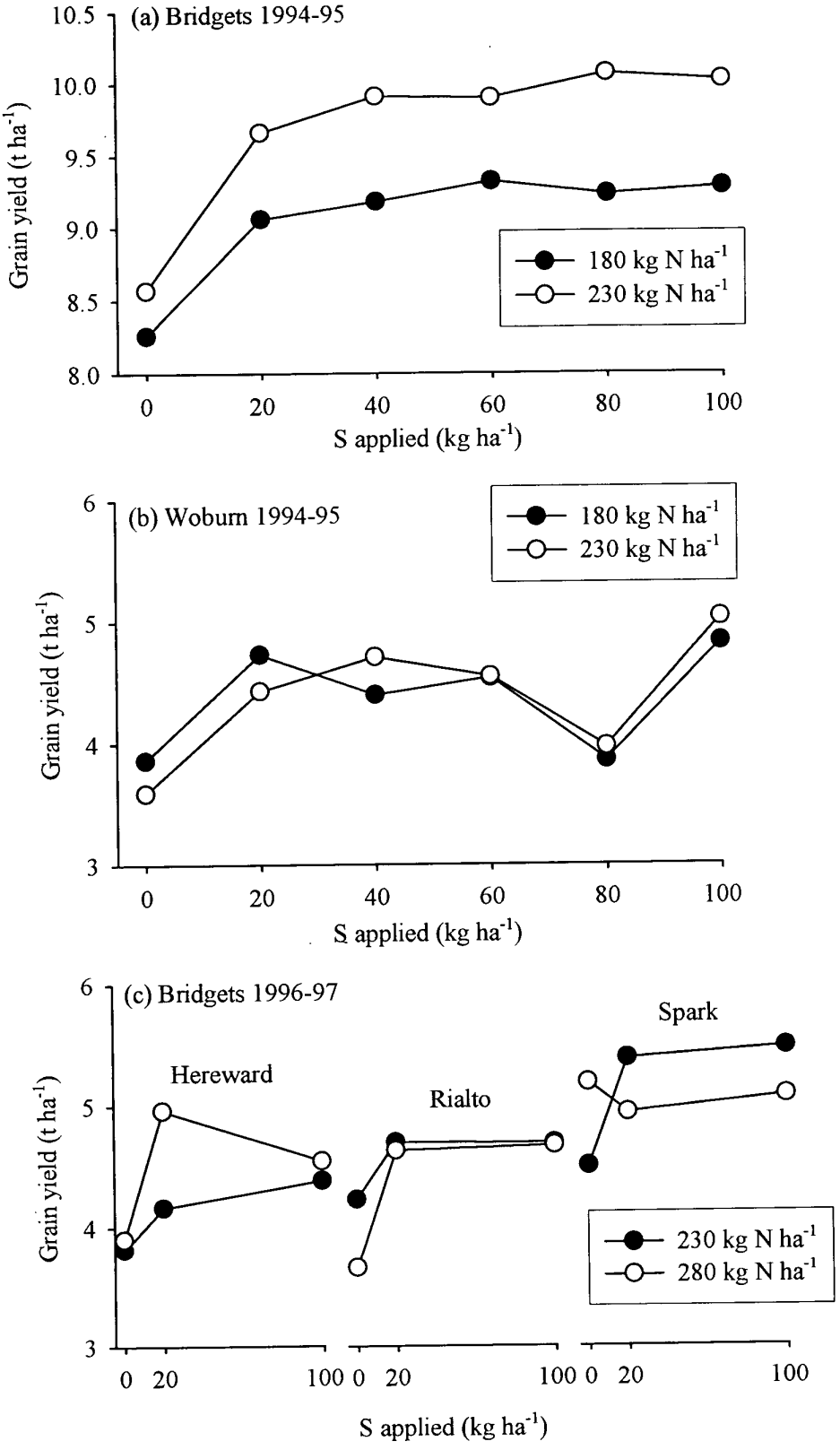

Figure 2. Yield responses to S applied in early spring. (a) Bridgets 1994-95. (b) Woburn 1994-95. (c) Bridgets 1996-97.

Apart from the above responsive experiments, S deficiency symptoms were observed during the stem elongation stage at Woburn in 1995-96. These symptoms, characterised by a lighter green colour in the young fully developed leaves, largely disappeared by the anthesis stage (Figure 3), and the final yield increases due to S applications did not reach a statistically significant level.

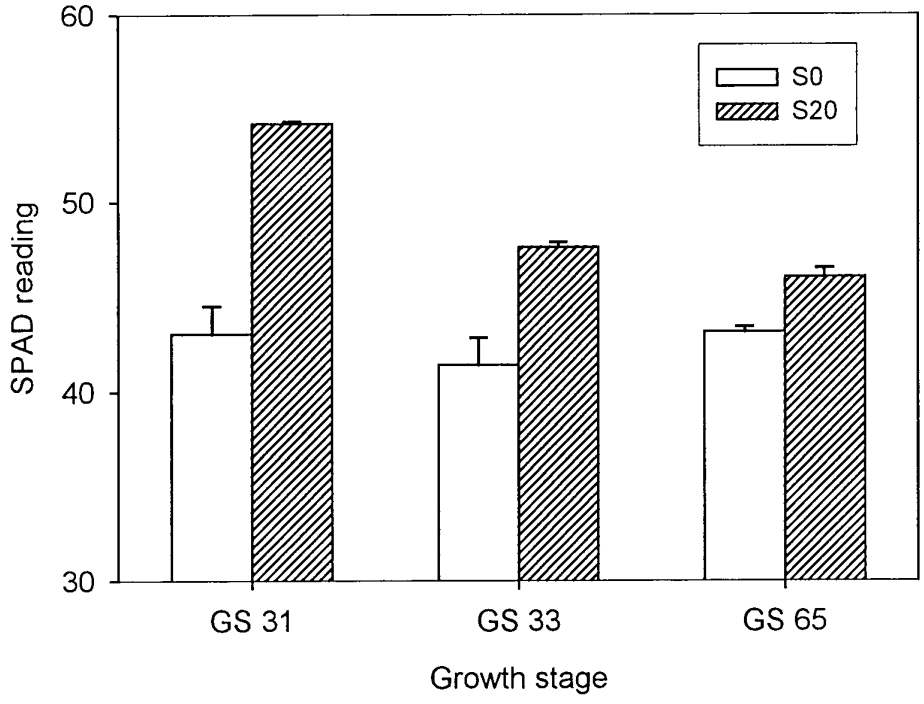

Figure 3. Effect of S treatments on leaf chlorophyll content (SPAD) at Woburn in 1995-96.

Comparison of yield response data in the three seasons at Bridgets shows that the non-responsive season, 1995-96, was associated with much higher concentrations of extractable SO_4-S in the soil profile than the other two responsive seasons. However, when all 11 experiments are compared, the relationship between soil extractable SO_4-S and yield response to S (or occurrence of S deficiency symptoms) becomes rather unclear. On one hand, it is true that no significant yield response to S, or occurrence of S deficiency symptoms, was obtained when soil extractable SO_4-S

was greater than 3 mg kg^{-1} in early spring. On the other hand, several experiments in this series showed no significant yield responses, or occurrence of S deficiency symptoms, even though the soils contained less than 3 mg kg^{-1} extractable SO_4-S. These results indicate that soil analysis can predict non-responsive sites reliably when extractable SO_4-S is high, but cannot predict S deficient site accurately when extractable SO_4-S is low or borderline.

In all 11 experiments, applying an extra 50 kg N ha^{-1} in spring increased grain yield significantly in 3 experiments (Bridgets in 1994-95 and 1995-96, Raynham in 1994-95), decreased grain yield significantly in 1 experiment (Borders 1995-96), and had no significant effect in the others (Appendices 1-11). Whether an extra 50 kg N ha^{-1} in spring increased yield appeared to be dependent on the yield potential of the site, rather than the concentration of mineral N in soil profile measured in early spring.

Effects of post-anthesis foliar application of S

Foliar application of ammonium sulphate at a late stage (milky ripe) did not prevent yield losses due to S deficiency at Bridgets in 1994-95 (Figure 4). Only in the treatments where half of the S was applied as solid (gypsum) in early spring was yield significantly higher than the control. At Woburn in 1994-95, where symptoms of S deficiency were clearly visible and yield response to soil applied S was obtained, foliar treatments produced inconsistent results because the replicates were variable (Appendix 4). In several experiments, including Raynham, Borders and Woburn in 1994-95, and Bridgets in 1995-96, foliar applications of ammonium sulphate decreased grain yield, probably as a result of leaf scorching (Appendices 1-7).

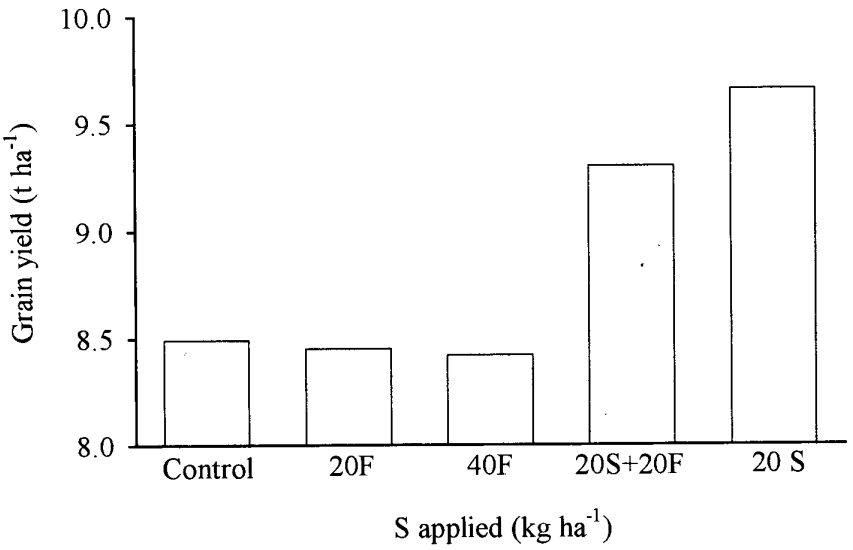

Figure 4. Effects of foliar appications of ammonium sulphate versus spring applications of gypsum on grain yield at Bridgets in 1994-95.

Thousand grain weight and specific weight

Applications of S in early spring decreased thousand grain weight significantly in 5 out of the 11 experiments, but had no significant effect in the other experiments (Appendices 1-11). The data from the 5 sites showing a significant effect are shown in Figure 5a. The decrease in thousand grain weight was most pronounced in response to the additions of the first 40 kg S ha^{-1}, which resulted in decreases of 2-3 g. Furthermore, the effect of S on thousand grain weight did not correspond to the effect on grain yield. Because grain yield was not decreased by S in any of these experiments, and in two of the experiments yield was actually increased by S (Bridgets 1994-95 and 1996-97), the negative effect of S on thousand grain weight can only be explained by increased tillers or increased number of grain per ear in response to S, through a compensatory mechanism. The number of tillers and the number of grain per ear were not determined in this study.

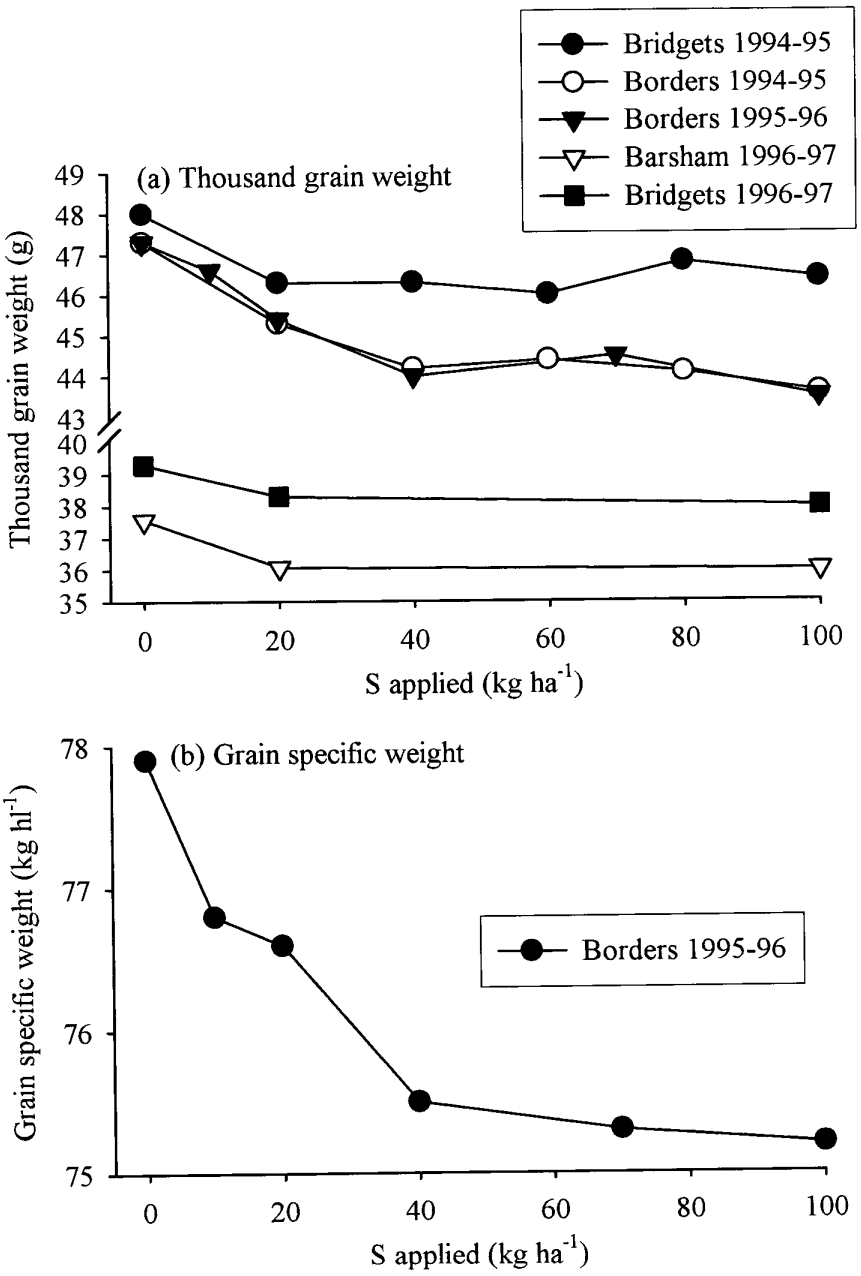

Figure 5. Effects of S on thousand grain weight (a) and on grain specific weight (b).

Additions of S had no significant effect on grain specific weight in general, except in one experiment (Borders 1995-96) where S additions decreased specific weight by up to 2.7 kg hl^{-1} (Figure 5b). The reason for this is not clear.

4.3. Sulphur uptake and distribution

Patterns of S uptake by wheat

In both 1994-94 and 1995-96 seasons, wheat crops were sequentially sampled on five occasions at the beginnings of April-August. The main factors influencing total S uptake were site, crop growth and S treatment. Figures 6 and 7 show S uptake patterns for the S0 and S20 treatments (means of the two N rates) in the two seasons. In general, S uptake increased linearly from April to July, and thereafter either increased slightly, levelled off or decreased slightly, depending on sites. At maturity, total S uptake ranged from 8.5 to 23.5 kg ha^{-1} in the S0 treatment, and from 18 to 24 kg ha^{-1} in the S20 treatment (Appendices 1-11). Applications of S fertiliser in early spring increased crop S uptake in most experiments. The scale of the increase appeared to relate to soil S availability. In S deficient sites (Bridgets 1994-95, and Woburn 1994-95 and 1995-96), the effect of S application on crop S uptake was generally greater than in the non-deficient sites (Figures 6 and 7). In the S0 treatment, there was a general trend for total S uptake at maturity to increase with final grain yield (Figure 8). Figure 8 also includes the S uptake data in the S0 treatment from a previous HGCA-funded project (McGrath *et al.*, 1995). This shows that most of the S-deficient wheat crops had a total S uptake of less than 15 kg ha^{-1}, whereas most of the S-sufficient crops contained between 15 and 25 kg S ha^{-1} at maturity.

In the 1996-97 season, total S uptake by the three breadmaking varieties of winter wheat was measured on three occasions (see Appendices 8-11 for the S uptake at maturity). Figure 9 compares the S uptake of the three varieties in the S0 and S20 treatments (means of the two N rates) at maturity. In all four experiments, there were no significant differences between varieties, although applications of S increased the crop S uptake significantly at all sites.

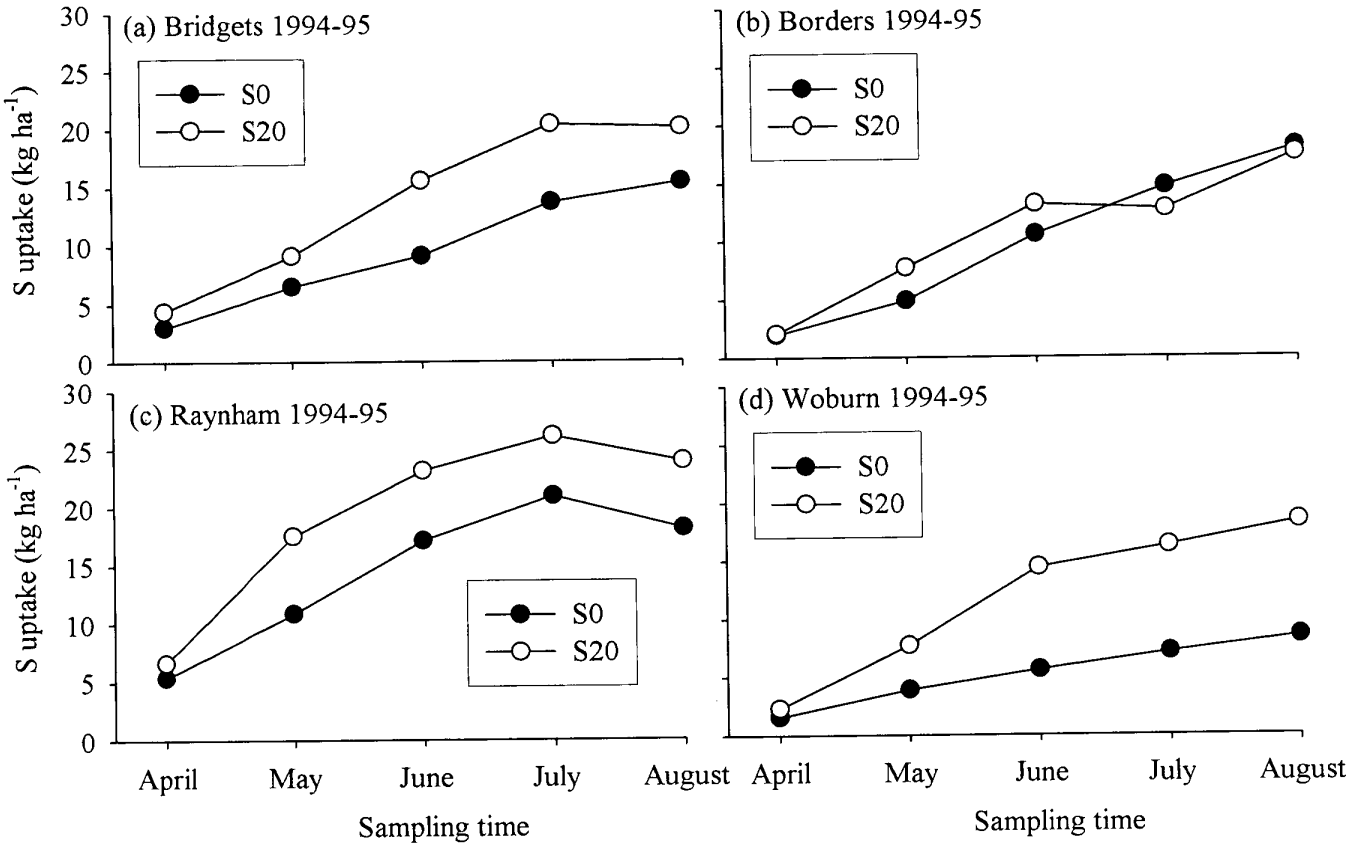

Figure 6. Sulphur uptake by wheat in the four experiments in 1994-95.

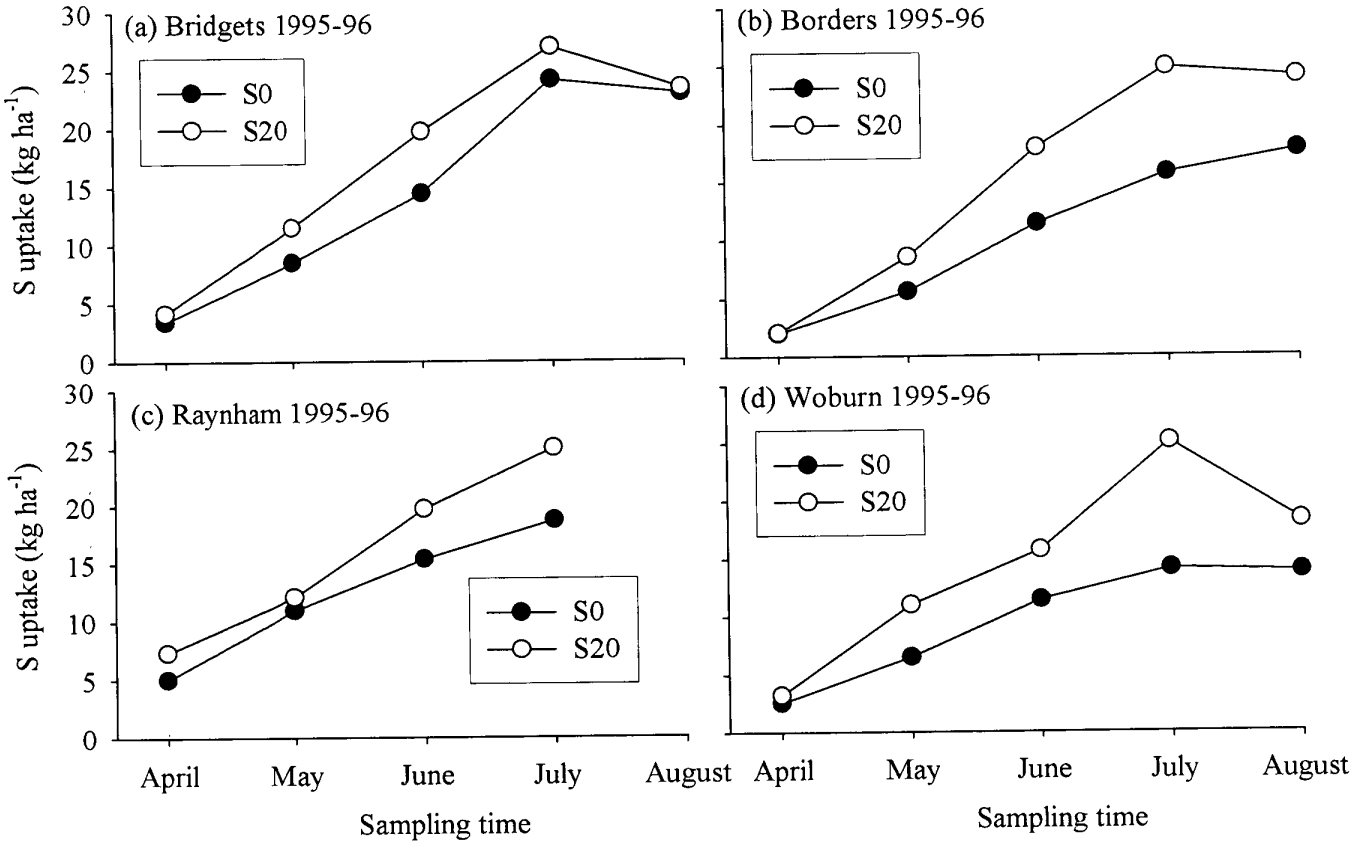

Figure 7. Sulphur uptake by wheat in the four experiments in 1995-96.

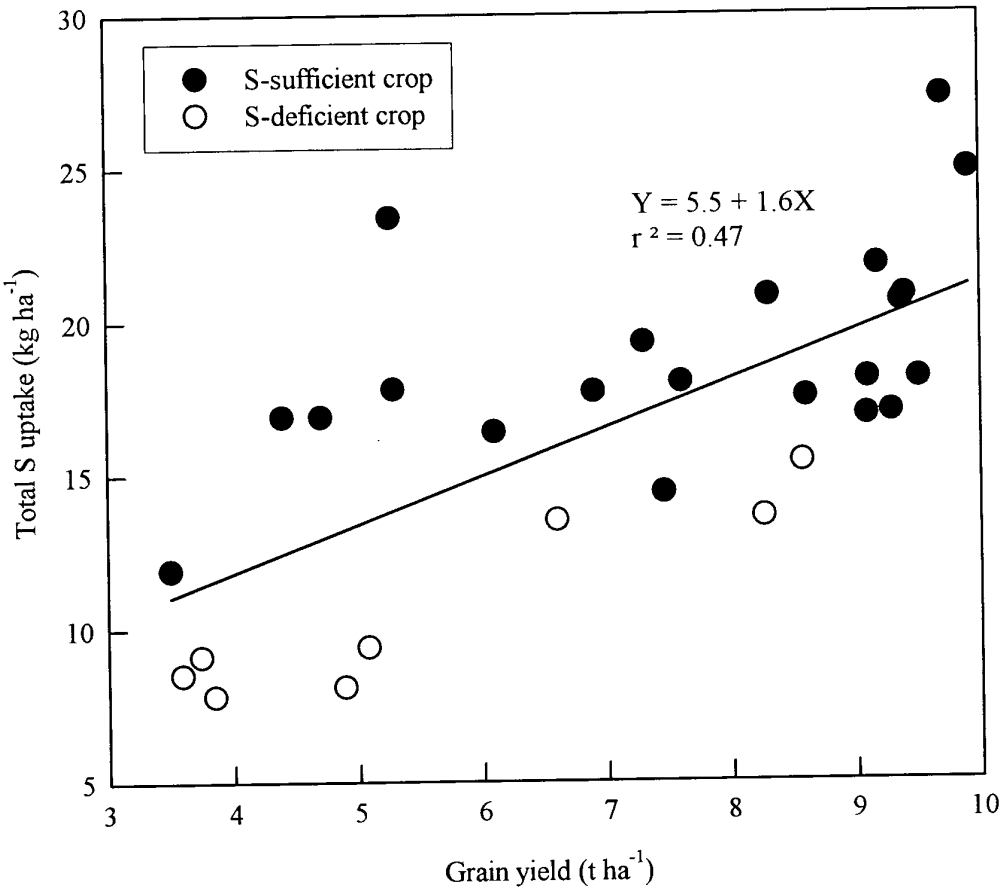

Figure 8. Relationship between grain yield and total S uptake of winter wheat at maturity.

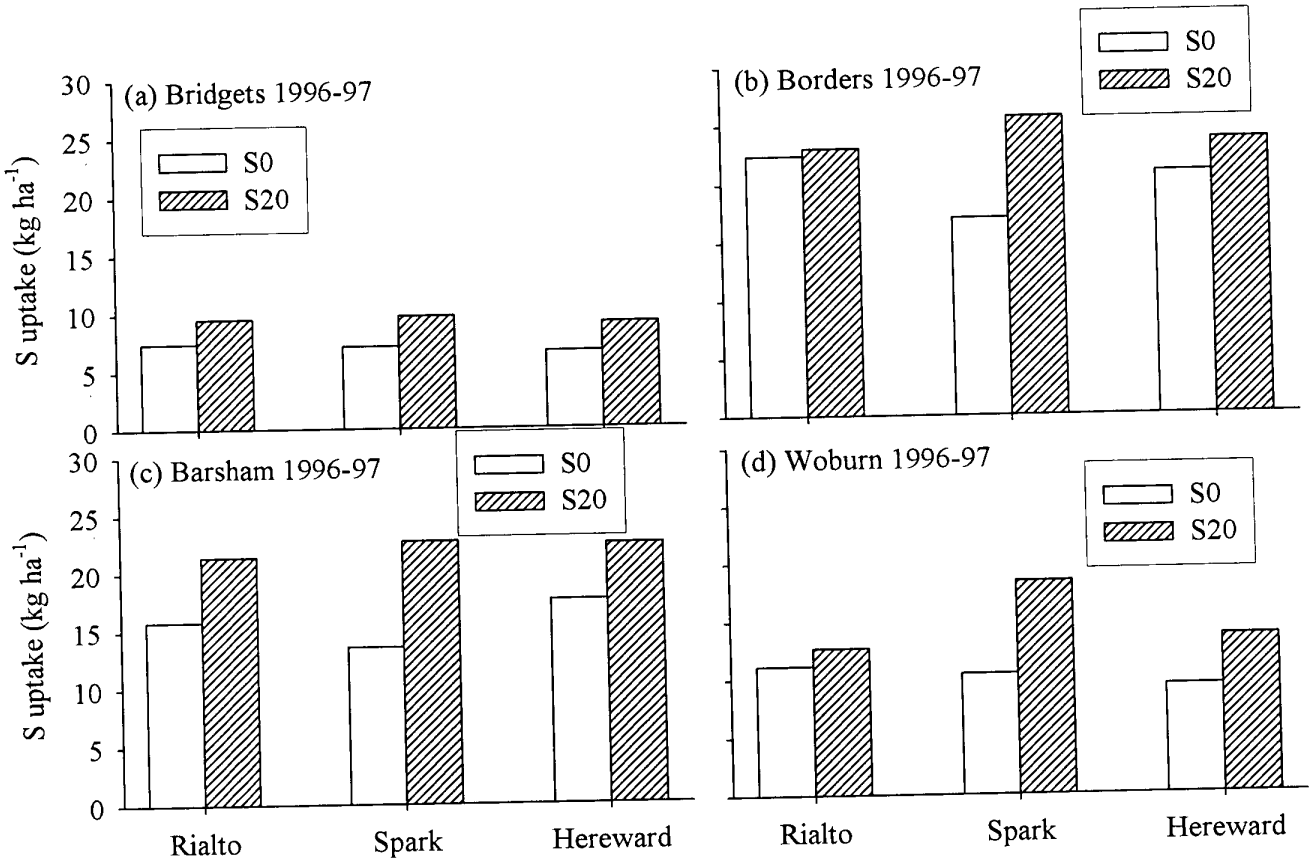

Figure 9. Sulphur uptake by three wheat varieties at maturity in 1996-97.

The harvest indices of N and S are defined as the proportions of the total contents of N or S in the whole crop at maturity which are distributed to the grain. Figure 10 shows two examples (Bridgets and Woburn in 1994-95) of the harvest indices of dry matter (DM), N and S for the variety Hereward. It is clear that the N harvest index was much larger than that for DM or S. Also, S application did not affect the harvest indices of DM and N in both experiments which showed significant yield responses to S. In contrast, S harvest index decreased with the increasing rate of S addition. Sulphur harvest index was larger than that for DM in the S0 treatment, but tended to approach the level of DM harvest index when S supply was sufficient. These results suggest that wheat crop is much more efficient in re-mobilising N to grain than S.

Similar values of DM and S harvest indices were again obtained in the 1996-97 seasons with three varieties (Table 2). These were substantially smaller than the N harvest index. There were also significant differences between Hereward, Rialto and Spark in the N and S harvest indices (Table 2). In general, Hereward appeared to have the lowest N and S harvest indices among the three varieties.

Table 2. Harvest indices for dry matter, N and S of three wheat varieties in 1996-97 (means of all treatments)

Variety	Bridgets	Borders	Barsham	Woburn
		DM harvest index		
Rialto	0.60	0.52	0.44	0.41
Spark	0.57	0.48	0.41	0.42
Hereward	0.58	0.48	0.42	0.42
		N harvest index		
Rialto	0.80	0.78	0.74	0.60
Spark	0.84	0.79	0.74	0.68
Hereward	0.80	0.75	0.71	0.62
		S harvest index		
Rialto	0.68	0.55	0.47	0.44
Spark	0.72	0.51	0.44	0.48
Hereward	0.68	0.48	0.41	0.44

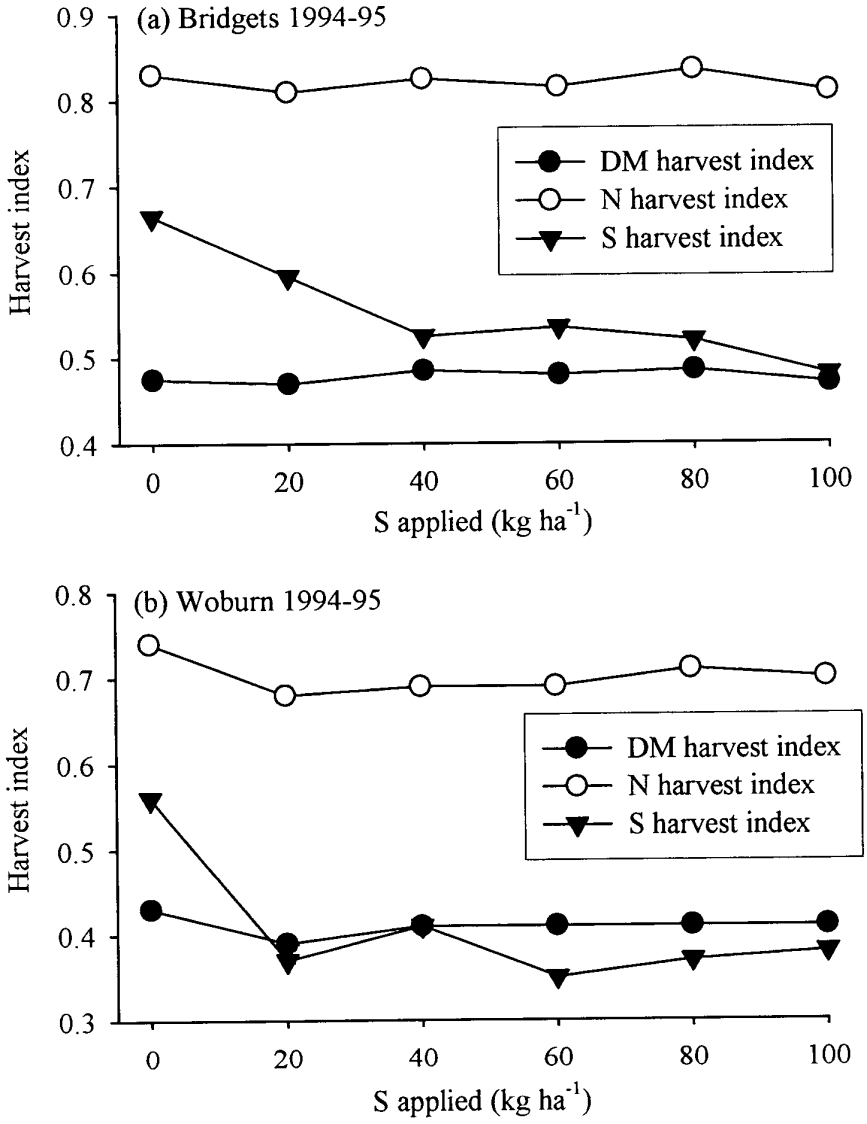

Figure 10. Effects of S applied in early spring on the harvest indices for dry matter, N and S. (a) Bridgets 1994-95. (b) Woburn 1994-95.

The concentration of S in plant tissues and the N:S ratio changed considerably during crop growth. Therefore, diagnosis of S deficiency using plant tissue analysis will need to be targeted at a precise growth stage. Because S deficiency symptoms usually start to occur at the early stage of stem elongation as a result of accelerating growth and demand for S, sampling and analysing plants at this stage should offer reasonable reliability in diagnosis and yet allow corrective action to be taken before too late. Table 3 shows the concentrations of total S and sulphate-S, and the N:S ratio in the whole plants (excluding roots), sampled at GS 31-32, in the S0 treatments from all 11 experiments in the three seasons. The S-deficient sites are those showing significant yield increases in response to S applications, or where clear deficiency symptoms were visible. McGrath *et al.* (1996) suggested critical values for winter wheat at the early stage of 2.0 mg g^{-1} for total S, 0.25 mg g^{-1} for sulphate-S and 17:1 for N:S ratio in the whole plant shoots. The results shown in Table 3 validate the above critical values by and large. For example, total S and sulphate-S concentrations in the S-deficient sites were all lower than 2.0 and 0.25 mg g^{-1}, respectively, whereas in all but one S-sufficient sites total S was greater than 2.0 mg g^{-1}. In the case of the N:S ratio, three of the four S-deficient sites, as well as one of the seven S-sufficient sites, had a value of greater than 17:1. It is thus advisable to use several indices together, rather than one in isolation.

An important aspect about tissue diagnosis that is often ignored is that it does not predict whether the crop will recover from deficiency later on. This point is very well illustrated by the experiment at Woburn in 1995-96. All indices showed that the crop was deficient at that growth stage, and this was confirmed by the visible symptoms and chlorophyll measurement (Figure 3). Yet towards anthesis, the crop recovered from S-deficiency, and the yield increases due to S did not reach a significant level. Recovery from S deficiency could be due to more favourable climatic conditions that enhance S mineralisation in soils, or utilisation of subsoil S reserves.

Table 3. Total S, N:S ratio and sulphate-S concentration in whole plants at stem elongation (GS 31-32). Values are means of all S0 treatments at each experiment

Site	Season	Total S (mg g^{-1})	N:S ratio	Sulphate-S (mg g^{-1})
S-deficient sites				
Bridgets	1994-95	1.81	18.6	0.12
Bridgets	1996-97	1.63	12.3	-
Woburn	1994-95	1.98	21.5	0.14
Woburn	1995-96	1.78	19.7	0.15
S-sufficient sites				
Bridgets	1995-96	2.60	14.9	-
Borders	1994-95	2.46	19.0	0.30
Borders	1995-96	2.67	16.0	0.49
Borders	1996-97	2.28	14.9	-
Raynham	1994-95	1.92	13.5	0.32
Barsham	1996-97	2.91	12.9	-
Woburn	1996-97	2.83	13.4	-

Grain S concentration and N:S ratio

The concentrations of S in grain were above 1.2 mg g^{-1} at all sites in 1994-95 (Figure 11a, means of the two N rates). The differences between sites in the grain S concentrations from the S_0 treatments were relatively small, although those from Bridgets were slightly lower than from other sites. Application of S as gypsum in early spring increased the concentration of S in grain significantly. The responses were the greatest at Woburn and the least at the Borders site. Maximum increases in grain S were 16, 18, 43 and 11% at Bridgets, Raynham, Woburn and the Borders site, respectively. As would be expected, these were obtained with application rates of 80-100 kg S ha^{-1}. An application of 20 kg S ha^{-1} increased grain S by 31% at Woburn, and between 3-6% at the other sites. Increasing the N rate from 180 to 230 kg/ha increased grain S significantly at Bridgets, Raynham and the Borders site, but had no significant effect at Woburn (Appendices 1-4). Grain N:S ratios for the 1994-95 samples are shown in Figure 11b (means of the two N rates). As expected, application of S decreased the ratio. This was most evident at Woburn and Bridgets, the two responsive sites in terms of grain yield. Without the application of S, the N:S ratios were well above 17:1 at Woburn, and above 16:1 at the other sites. Increasing N rate increased the N:S ratio, although the N effects were significant only at Bridgets and Woburn (Appendices 1-4).

The responses of grain S concentration to S addition in 1995-96 (Figure 12a) were similar to those in the previous year. All samples from the three experiments had greater than 1.2 mg g^{-1} total S in the grain. Both absolute and relative responses to S additions were the greatest at Woburn and the least at Bridgets. Maximum increases in grain S were 26, 20 and 12% at the Woburn, Borders, Bridgets and Raynham sites, respectively. These were obtained with the application rate of 100 kg S ha^{-1}. An application of 20 kg S ha^{-1} (as gypsum) increased grain S by 6-9% at the Borders and Woburn, but only by 1.2% at Bridgets. Grain N:S ratios are shown in Figure 12b. Without the application of S, the N:S ratios were between 16-17:1 at the Woburn and Borders sites, but well below 16:1 at Bridgets. The grain N:S ratios were lower than those obtained in the 1994/95 seasons. Application of S as gypsum decreased the ratio significantly. This was most evident at Woburn because of the large response in the grain S concentration to the S addition.

Experiments in both 1994-95 and 1995-96 also tested the effects of foliar applications of ammonium sulphate. Foliar applications at the milky ripe stage generally increased grain S concentrations (Appendices 1-7). Compared to the early spring applications of gypsum, foliar applications were less effective in raising grain S concentration in some experiments (Woburn in 1994-95 and all three experiments in 1995-96), but more effective in the other experiments (Borders, Bridgets and Raynham in 1994-95).

In 1996-97, there were no significant differences between Hereward, Rialto and Spark in grain S concentration in all four experiments (Figure 13a-d). Application of S increased grain S concentration significantly at all sites, the response being much greater at Bridgets and Woburn than at Borders. At Bridgets, there were significant interactions between variety and S on grain S concentration, with Spark and Rialto showing greater increases than Hereward. Grain N:S ratio rarely exceeded 16:1 in the Barsham and Borders samples, and was not significantly affected by S addition at the Borders site (Figure 13). In contrast, most of the grain samples from the S0 treatments at Bridgets and Woburn had an N:S ratio greater than 16:1, and S addition decreased N:S ratio significantly. Significant differences between varieties were found only at Borders, with Hereward and Spark having higher N:S ratios than Rialto. Grain N:S ratio was increased by increasing the N application at the Borders and Woburn sites (Appendices 8-11).

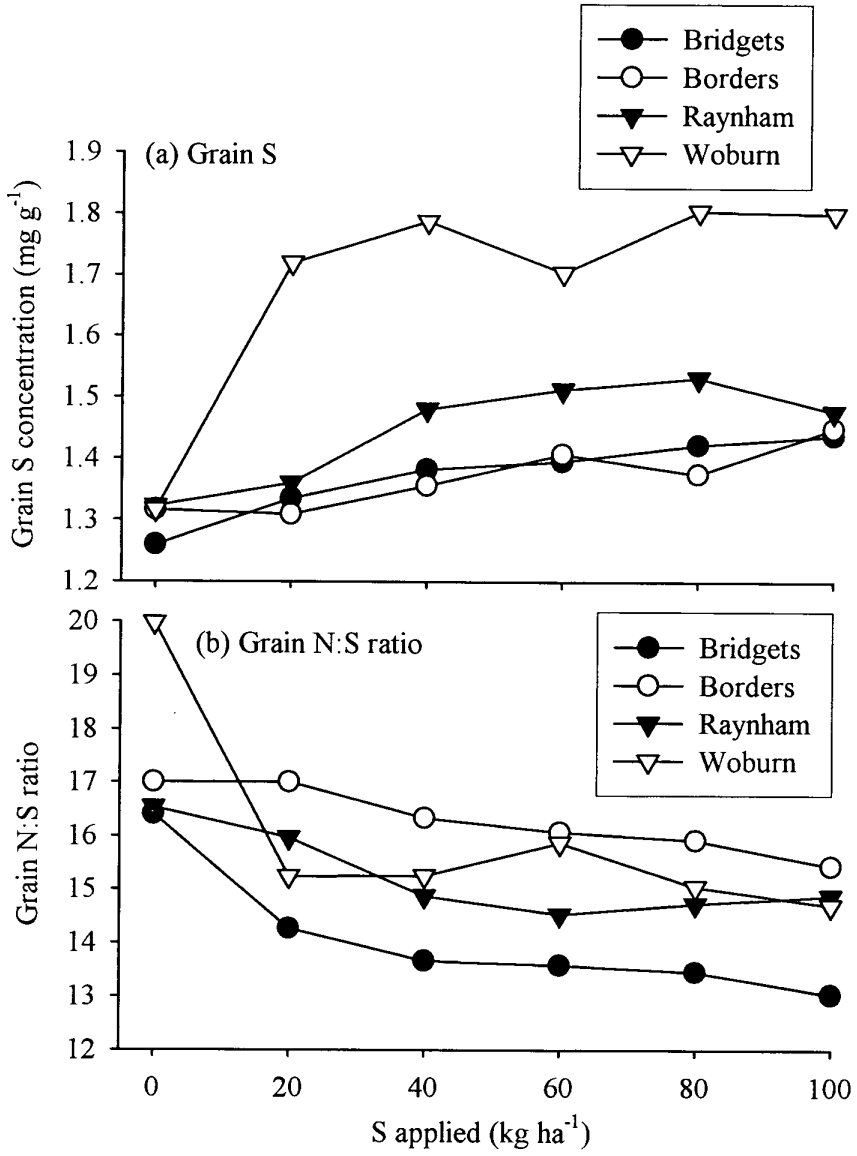

Figure 11. Effects of S applied in early spring on grain S concentration (a) and grain N:S ratio (b) in 1994-95.

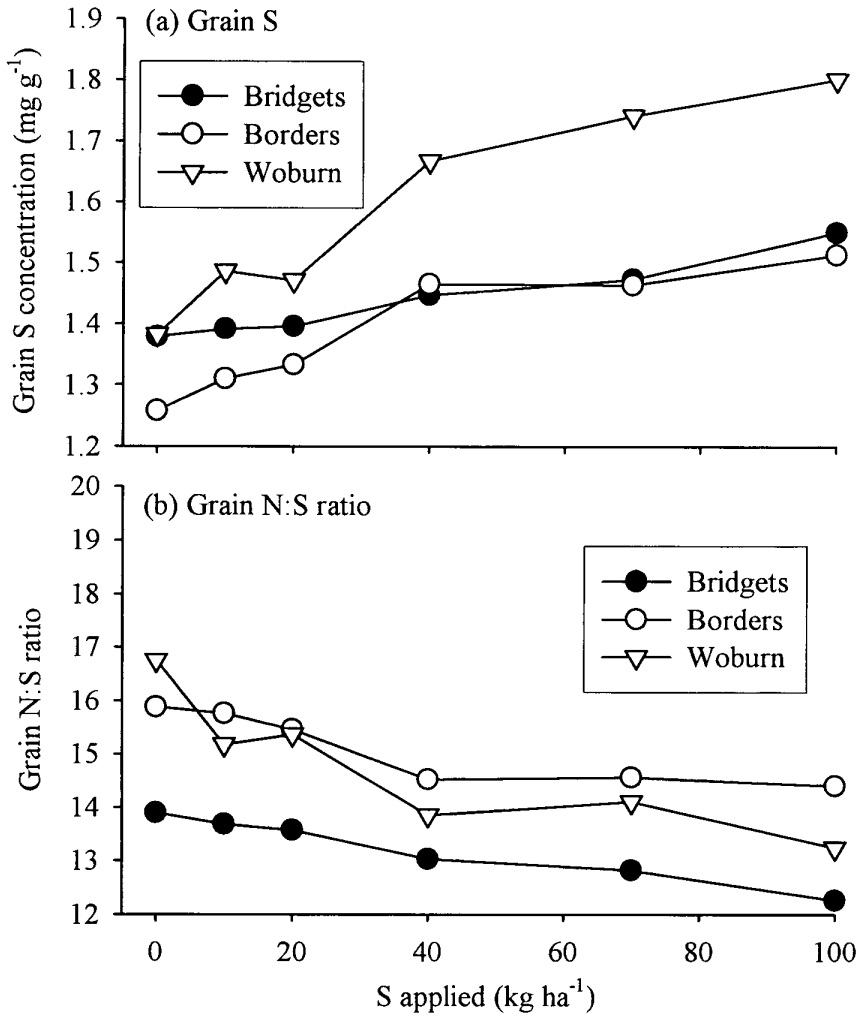

Figure 12. Effects of S applied in early spring on grain S concentration (a) and grain N:S ratio (b) in 1995-96.

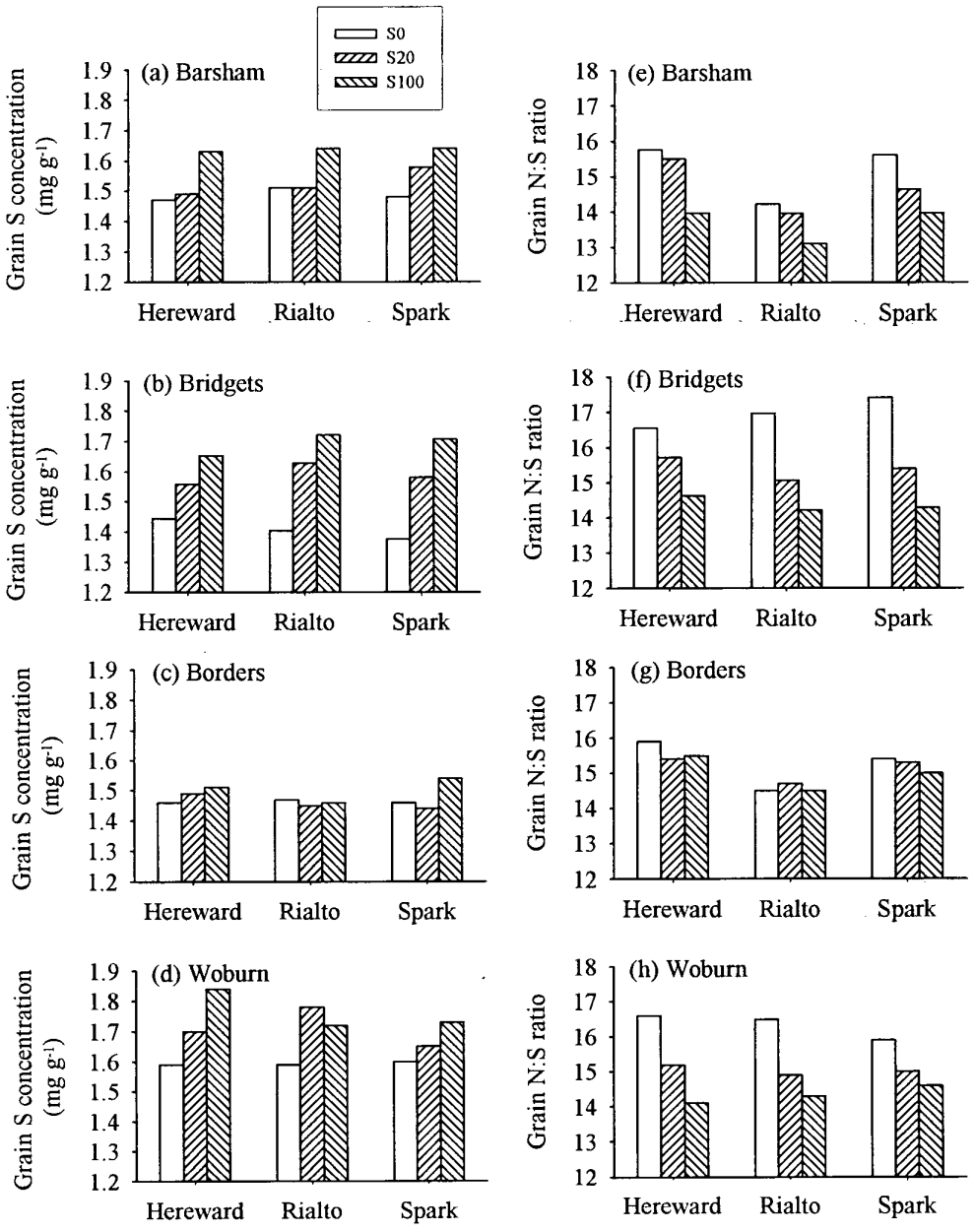

Figure 13. Effects of S applied in early spring on grain S concentration (a-d) and grain N:S ratio (e-h) of three varieties in 1996-97.

There have been very few reports on the concentration of sulphate-S in wheat grain. The mature wheat grain samples from the experiments in 1994-95 were analysed for sulphate concentration. Figure 14 shows that sulphate accounted for 2-6% of the total S in grain, with the percentage increasing with the amount of S fertiliser applied. These results indicate that, unlike vegetative tissues which contain between 10-40% of the total S as sulphate-S, mature wheat grain has only small concentrations of sulphate-S.

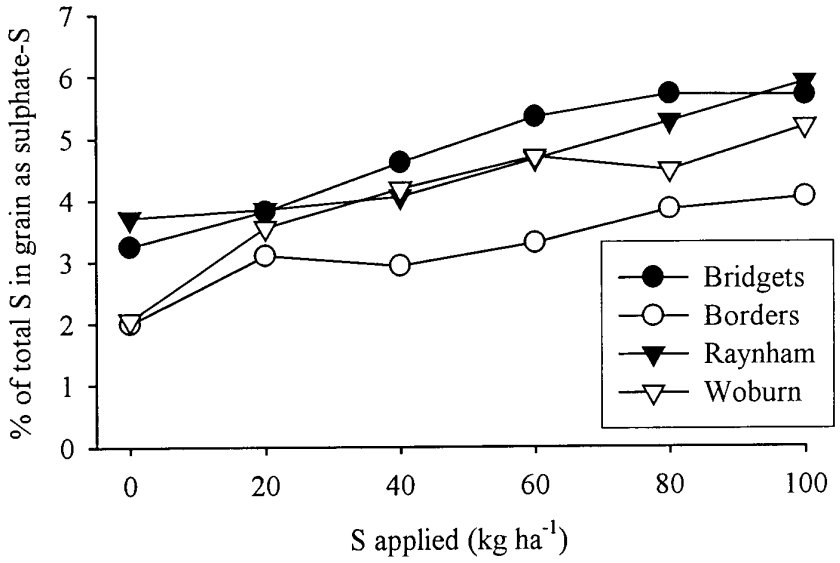

Figure 14. Effects of S applied in early spring on grain SO_4-S concentration in 1994-95.

4.4 Grain and breadmaking quality

Protein concentration

In 1994-95, mean protein concentrations of grain were 9.3, 10.7, 12.9 and 10.8 % (based on 86%DM) at Bridgets, Raynham, Woburn and the Borders site, respectively. The threshold of 11% protein in grain was exceeded in all treatments at Woburn, and in the N230 treatments at Raynham and the Borders site, but not exceeded in all treatments at Bridgets (Appendices 1-4). The low

protein concentrations at Bridgets were likely due to the high yields, whereas low yields at Woburn resulted in high protein concentrations in grain. Increasing the N rate from 180 to 230 kg/ha increased grain protein concentration by 0.8-1.1% (P<0.001) (Figure 15a, means of all S treatments).

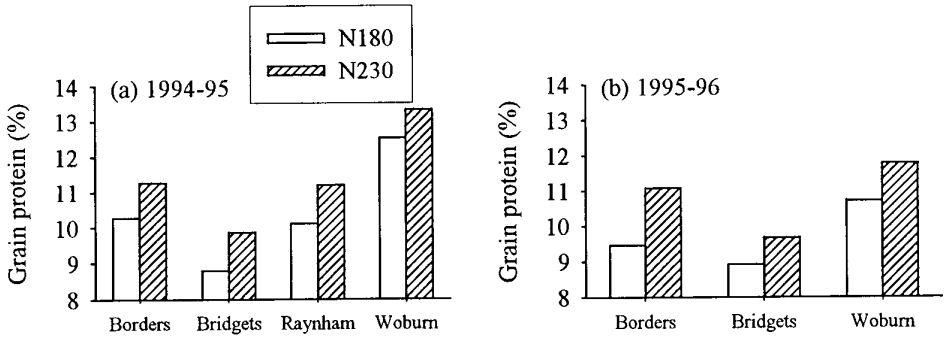

Figure 15. Effects of N on grain protein concentration in different experiments in 1994-95 (a) and 1995-96 (b).

In 1995-96, mean protein concentrations of grain were 11.2, 10.3 and 9.3 (based on 86% DM) at Woburn, the Borders and Bridgets, respectively. The threshold of 11% protein in grain was exceeded in the N230 treatments at Woburn and some of the N230 treatments the Borders site, but not exceeded in all treatments at Bridgets (Appendices 5-7). Similar to the results of the 1994-95 season, increasing the N rate from 180 to 230 kg ha^{-1} increased grain protein content by 0.6-1.6% (p<0.001) (Figure 15b).

In 1996-97, mean concentrations of grain protein were 11.9, 11.0, 11.0 and 12.6% for Bridgets, Barsham, Borders, and Woburn, respectively. Increasing the N rate either from 180 to 230 kg ha^{-1} (Barsham, Borders and Woburn), or from 230 to 280 kg ha^{-1} (Bridgets), increased grain protein concentration significantly (Table 4), although the effect was much smaller at Bridgets than at the other three sites. Significant differences between the three varieties, in the order of Hereward = Spark >Rialto, were observed at Barsham and Borders, but not at Bridgets and Woburn.

Table 4. Effects of variety and N treatment on grain protein concentration (%) in 1996-97

	Bridgets	Borders	Barsham	Woburn
Variety				
Hereward	11.85	11.38	11.25	12.76
Rialto	11.89	10.42	10.49	15.57
Spark	11.87	11.06	11.29	12.36
N treatment				
N1*	11.76	10.48	10.58	12.04
N2*	11.98	11.42	11.44	13.09

* N1 was 180 kg ha^{-1} for Borders, Barsham and Woburn, and 230 kg ha^{-1} for Bridgets.
N2 was 230 kg ha^{-1} for Borders, Barsham and Woburn, and 280 kg ha^{-1} for Bridgets.

In the total of 11 experiments over the three seasons, the effects of S on grain protein concentration were significant only in two experiments, at Bridgets in 1994-95 and 1996-97. Applications of S in early spring, particularly of the first 20 kg S ha^{-1}, decreased grain protein concentration in the 1994-95 season (Figure 16a), but increased protein concentration significantly in 1996-97 (Figure 16b). Total amounts of protein in grain were calculated for both seasons (Figure 16). These show that S additions actually increased the total amount of protein produced in both seasons. Therefore, the negative effect of S on grain protein concentration observed in 1994-95 was attributable to a dilution effect due to a large response in grain yield (Figure 2a).

Flour protein concentrations (Appendices 1-11) correlated closely with grain protein concentrations, but were 0.5-0.9% lower. Approximately 0.2% of this difference was attributable to the difference between the Dumas method used for grain samples and the Kjeldahl-calibrated NIR method used for flour samples.

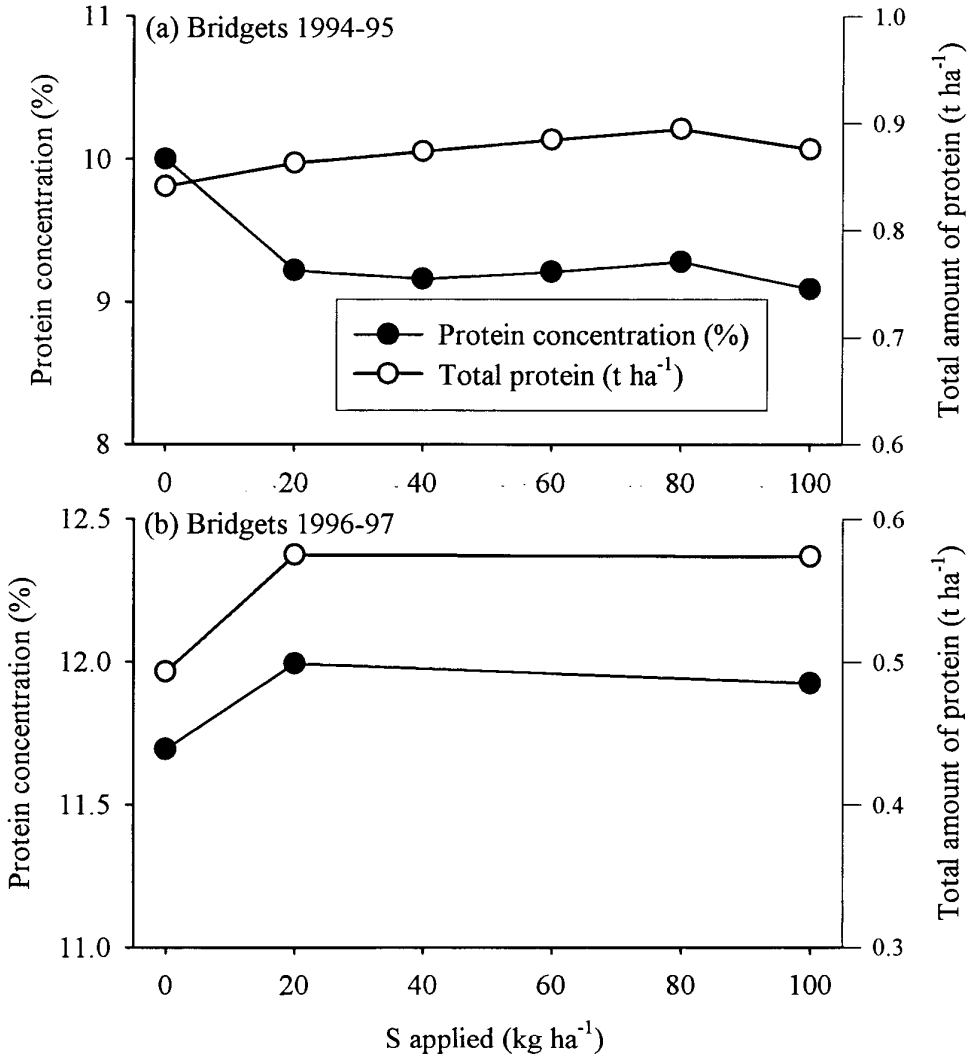

Figure 16. Effects of S applied in early spring on grain protein concentration and total amount of protein produced in grain at Bridgets in 1994-95 (a) and 1996-97 (b).

Hagberg Falling Number (HFN)

High HFNs were obtained in all grain samples in both 1994-95 and 1995-96, with values mostly greater than 300 (Appendices 1-7). The N and S treatments had relatively small effects on grain HFN.

In 1996-97, grain HFN differed widely between sites (Appendices 8-11). HFNs greater than 350 were obtained for all varieties at Woburn. In contrast, there were major differences between varieties in grain HFN at both Bridgets and Borders, where Hereward had much lower grain HFNs than Rialto and Spark. At Barsham, averaged HFNs were 120, 210 and 209 for Hereward, Rialto and Spark, respectively, with most grain samples having HFNs<220. At Bridgets, averaged grain HFNs were 240, 339 and 335 for Hereward, Rialto and Spark, respectively. At the Borders site, averaged grain HFNs were 230, 264 and 293 for Hereward, Rialto and Spark, respectively. Because of low HFN, all of the samples from Barsham and the Hereward samples from Bridgets were not used for milling and breadmaking tests.

Loaf volume

Flour yield varied slightly between sites and seasons, but was not significantly influenced by the N and S treatments, except at Borders in 1996-97 where S application increased the flour yield by about 0.2%.

The ranges of loaf volume in 1994-95, 1995-96 and 1996-97 were 1269-1538, 1481-1783 and 1534-1816, respectively (Appendices 1-11). The values in the last two seasons fell within the normal range, whereas those in 1994-95 were substantially lower than normal. The crumb scores in 1994-94 were also lower than in the two later seasons (Appendices 1-11). It is not clear why the loaf volumes and crumb scores in 1994-95 were much lower than in the two other seasons. Low loaf volumes in 1994-95 could not be explained by factors such as grain specific weight, HFN, grain protein concentration, or flour yield.

Breadmaking tests were not performed on the grain samples from Barsham in 1996-97 because of low HFN. In the total of 10 experiments where loaf volume data were obtained, increasing the N rate from 180 to 230 kg ha^{-1}, or from 230 to 280 kg ha^{-1} at Bridgets in 1996-97, improved loaf volume significantly only in one experiment (Bridgets 1995-96), even though

increasing the N rate increased grain and flour protein concentrations significantly in all experiments.

In contrast, application of S in early spring increased loaf volume significantly in six experiments. These were: Bridgets in all three seasons, Raynham in 1994-95, Borders in 1995-96 and Woburn in 1996-97 (Figure 17). Typically, the increases in loaf volume in these experiments ranged from 40 to 100 ml. The largest response occurred in the Borders experiment in 1995-96, which showed an increase in loaf volume of more than 100 ml as a result of the application of 100 kg S ha^{-1}, representing a relative increase of 6.7%. Proportionally, application of the first 20 kg S ha^{-1} produced a larger response than the further dose of S in the first two seasons. In 1996-97, increasing the rate of S to from 20 to 100 kg S ha^{-1} had little further effect on loaf volume. When all data in each season were combined in a single ANOVA, site and S treatment were the highly significant factors affecting loaf volume, whereas N treatment had no significant effect.

In the four experiments where increases in loaf volume in response to S additions were not statistically significant, two (Borders 1994-95 and 1996-97) also showed very small increases in the concentration of S in grain. Limited effects of S applications on grain S concentration in these two experiments probably explained the lack of responses in loaf volume to S. The other two non-responsive experiments were Woburn in 1994-95 and 1995-96, even though the site was clearly deficient in S in both seasons and the increases in grain S concentration due to S applications were the greatest among all experiments. The reason for the anomalous results at Woburn in the two seasons is not clear, but may be partly due to a lack of water during the grain filling period. Total amount of rainfall during the active growth period between 1st April and 31st July in 1995 and 1996 was 99 and 123 mm, respectively, both of which were considerably smaller than the 30 year average of 200 mm at the site.

It is clear from comparison with Table 1 that the responsiveness to S in terms of loaf volume was not related the concentration of soil extractable S. In another words, analysing soil sulphate concentration could not predict reliably whether S application would benefit breadmaking quality.

In addition to the increases in loaf volume, crumb structure was also improved significantly by S in two experiments (Borders 1995-96 and Bridgets 1996-97. Appendices 1-11). Overall, these results indicate that the responses in breadmaking quality to S were more common than yield responses.

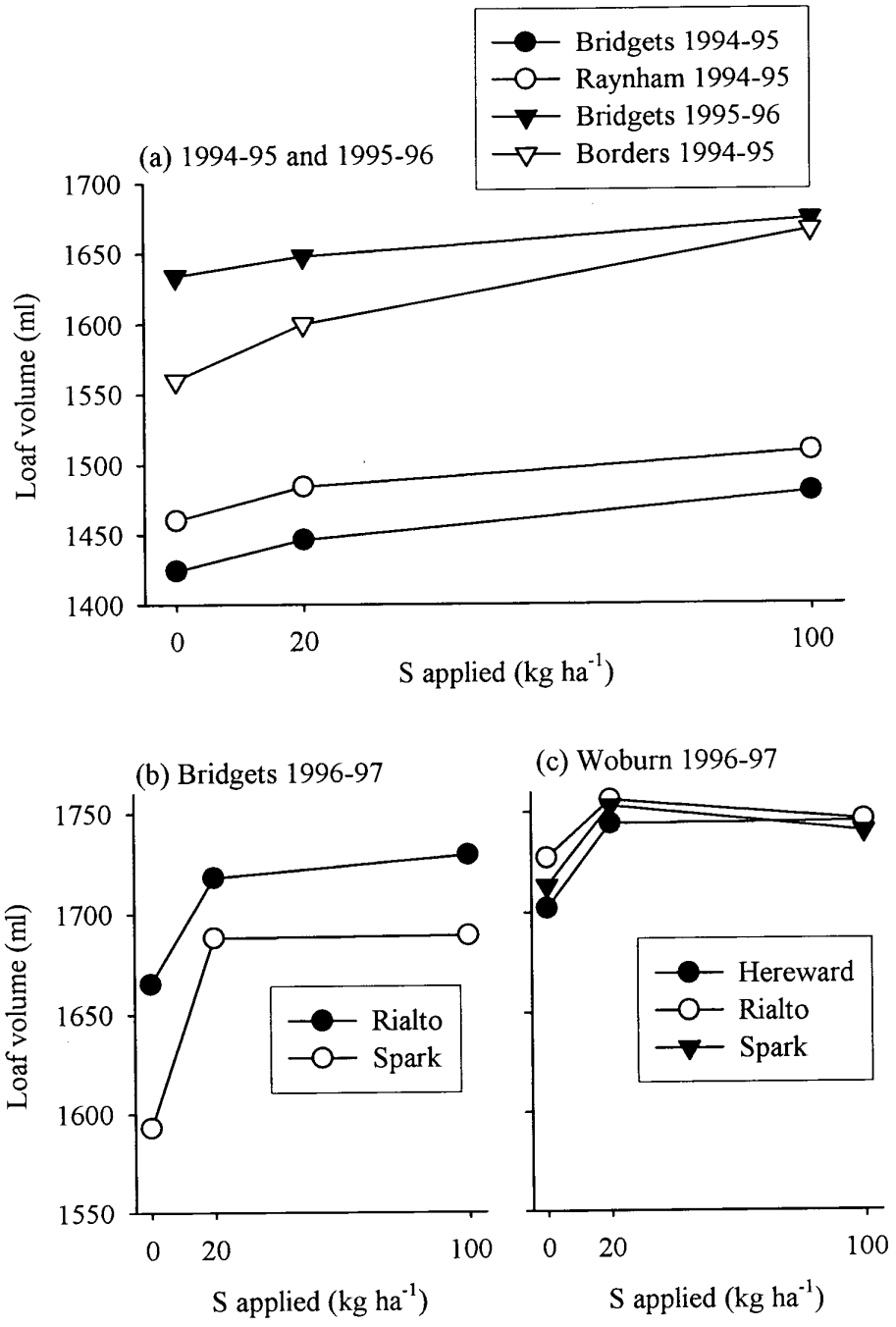

Figure 17. Effects of S applied in early spring on loaf volume in 1994-95 and 1995-96 (a), Bridgets 1996-97 (b), and Woburn 1996-97 (c).

The experiments in 1996-97 compared three breadmaking varieties. The differences between varieties were not significant at Woburn, but were significant at the other two sites. At the Borders site, Hereward had the highest mean loaf volume, followed by Rialto and Spark. At Bridgets, Rialto also gave higher loaf volumes than Spark (samples of Hereward were not used in breadmaking tests because of low HFN values). There were no significant interactions between variety and S at any site, suggesting that the three varieties in general responded similarly to S.

There were significant differences between the three varieties in crumb score at all sites (Appendices 8-11). Mean crumb scores of Hereward and Spark were similar, and higher than those of Rialto at both Borders and Woburn. At Bridgets, Spark produced a higher mean crumb score than Rialto. Applications of S fertiliser improved crumb score significantly at Bridgets, but not at the other two sites. Again, the N treatment did not influence crumb score significantly at any site.

Relationships between loaf volume and grain N and S concentrations

All data from each season, including both responsive and non-responsive sites, were used in correlation and regression analysis. The correlations between loaf volume and grain N or S concentrations were generally poor in 1994-95, possibly because of the atypical low values of loaf volume. There was a negative correlation between loaf volume and grain N:S ratio ($p<0.001$; Figure 18a), suggesting that a high N:S ratio, particularly if >16:1, was associated with low loaf volume.

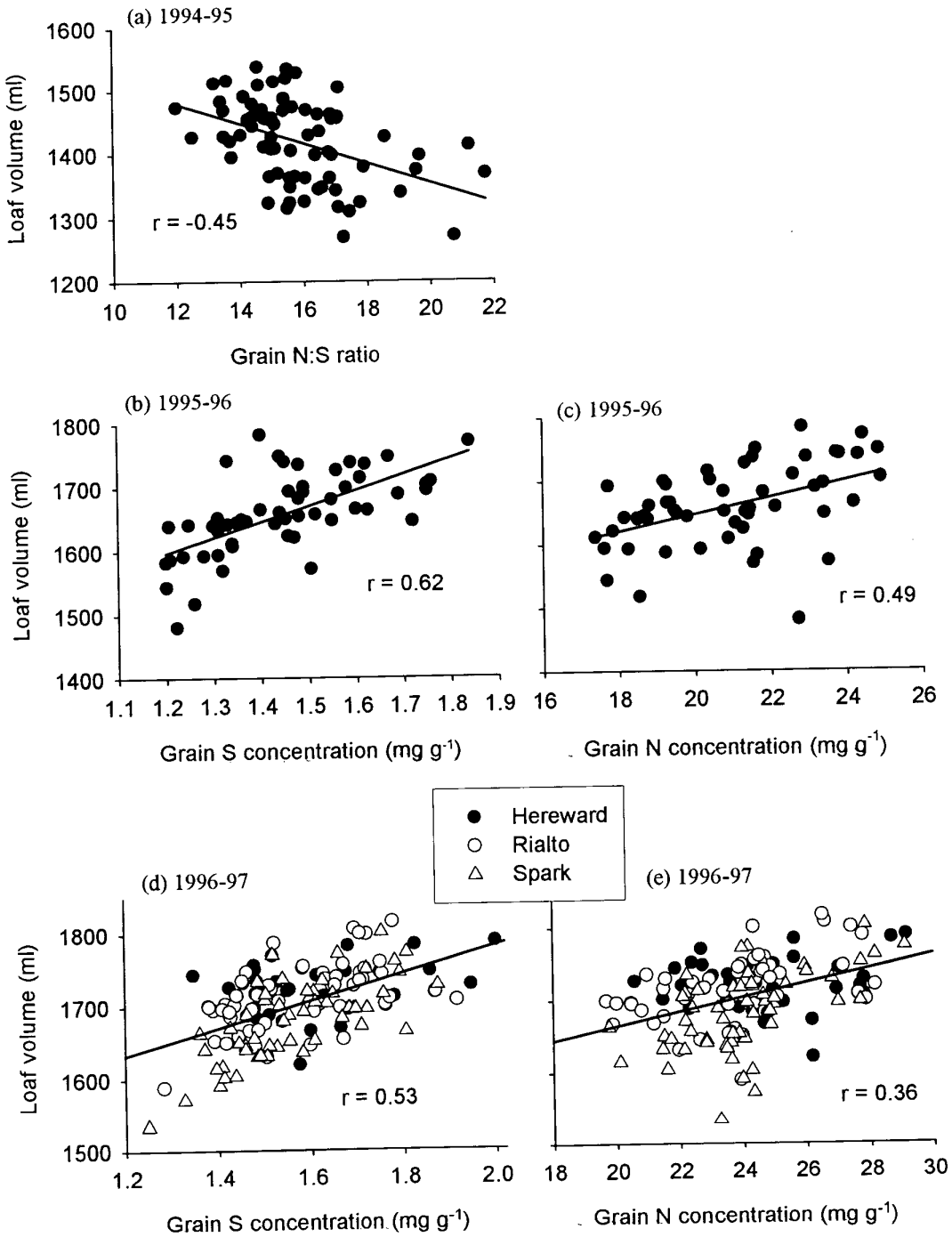

Figure 18. Correlations between loaf volume and grain N:S ratio, grain S concentration and grain N concentration in different seasons.

In both 1995-96 and 1996-97, when normal loaf volumes were obtained, loaf volume was found to correlate more closely with grain S concentration than with grain N concentration (the equivalent of grain protein concentration). The closer relationship between loaf volume and grain S concentration than between loaf volume and grain N concentration is further demonstrated in Figure 18b-e. In 1995-96, grain S concentration alone explained 39% of the variation in loaf volume, whereas grain N concentration explained only 19% of the variation. In 1996-97, the percentage of variance accounted for by the linear regression of loaf volume against grain S concentration was 29%, and this increased to 42.7% when the three varieties were separated in the regression. In comparison, grain N concentration only accounted for 16% of the variance in its linear regression with loaf volume, and 29% when the three varieties were separated. Also in 1996-97, the correlation coefficient between loaf volume and grain N:S ratio was negative ($p<0.001$).

These results indicate that, within the range of grain protein concentration obtained in this series of experiments (8.5-14.3%), the concentration of crude protein was not as limiting a factor as the concentration of S in grain to breadmaking performance. Because about 95% of the total S in wheat grain is bound in organic forms (Figure 14), mainly as proteins, it is not surprising that the concentration of S correlated strongly with grain protein concentration. Apart from being an indicator of the quantity of proteins in grain, the S concentration also reflects the quality of proteins. This is also demonstrated by the profound influence of S nutrition on the composition of gluten proteins and on the rheology of dough (see later). An imbalance of N and S in wheat grain, as indicated by a grain N:S ratio of >16:1, affects the breadmaking quality adversely. A slightly higher critical N:S ratio of 17:1 was reported by Randall and Wrigley (1986).

Dough extensibility and resistance

Extensograph measurements were performed only on the samples from the experiments in 1995-96 and 1996-97. In 1995-96 with the variety Hereward, increasing the rate of N application increased the dough extensibility significantly in two experiments (Bridgets and Borders), but had no significant effect on dough resistance (Figure 19). In contrast, application of S increased dough extensibility significantly only in one experiment (Borders), and decreased dough resistance significantly in two out of the three experiments (Bridgets and Borders; Figure 19). When data

from all three experiments in 1995-96 were pooled in ANOVA, it is clear that N had the most significant effect on dough extensibility, whereas S had the most significant effect on dough resistance. Figure 20 shows the relationships between grain S and N concentrations with dough resistance and extensibility, using all data from the three experiments in 1995-96.

In 1996-97, there were significant differences between varieties in dough rheology. In general, the Hereward dough was weaker in the Extensograph resistance and more extensible than the other two varieties (Figure 21). Application of S decreased dough resistance significantly at Bridgets and Woburn, but not at Borders (Figure 21). In contrast, dough extensibility was increased significantly by S only at Bridgets. Although increasing N application had no significant effect on dough resistance, it increased dough extensibility significantly at all sites (Appendices 8-11). These results again indicate that the main effect of S was to decrease dough resistance, whereas the main effect of N was to increase dough extensibility.

Gel protein weight and elastic modulus

The gel protein fraction comprises mainly glutenin polymers and previous work has shown that the amount and rheological properties of this fraction correlated with measures of breadmaking quality (Pritchard and Brock, 1994). The flours from the experiments in 1994-95 had higher concentrations of gel protein (expressed as the fresh weight of gel protein in 5 g flour) than in 1995-96 (Figure 22), which was consistent with the higher total protein concentrations in the first season. Increasing the N rate increased gel protein concentration significantly in five out of the seven experiments in the first two seasons (Appendices 1-7). Application of S also tended to increase gel protein concentration (Figure 22), with the effect being significant in three experiments (Bridgets and Woburn in 1994-95, and Borders in 1995-96). The S effect was most apparent for the first 20 kg ha^{-1}, beyond which little further increase was observed. The elastic modulus (G') of the gel protein was decreased significantly by the S treatment in all but one experiment in the first two seasons (Figure 22), the effect being more pronounced in 1994-95 than in 1995-96. The influence of the N treatment was not consistent in the two seasons. Increasing the N rate increased elastic modulus in two experiments in 1994-95, but had no significant effect in the second season (Appendices 1-7).

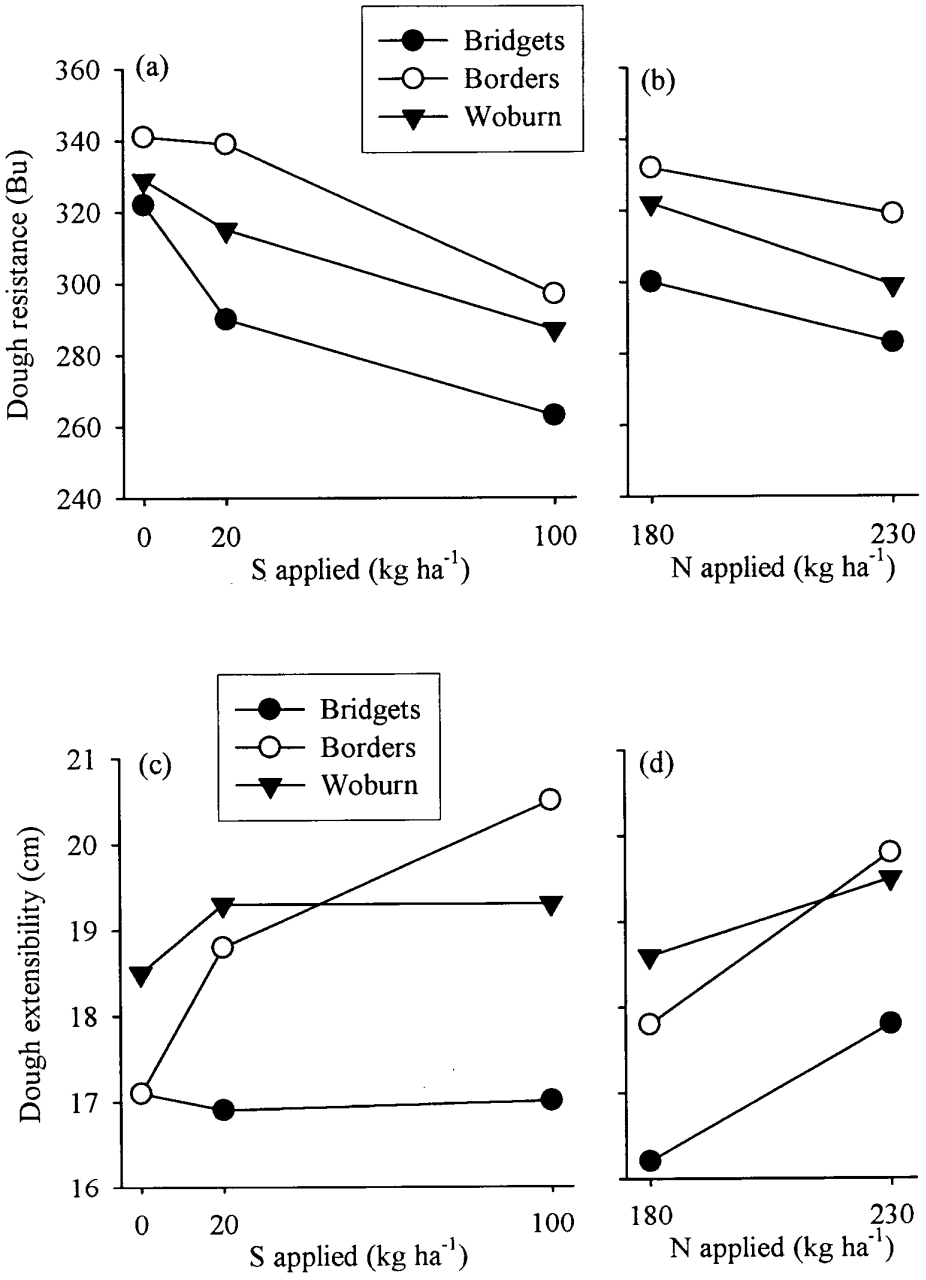

Figure 19. Effects of S and N applied in early spring on dough resistance (a-b) and dough extensibility (c-d) in 1995-96.

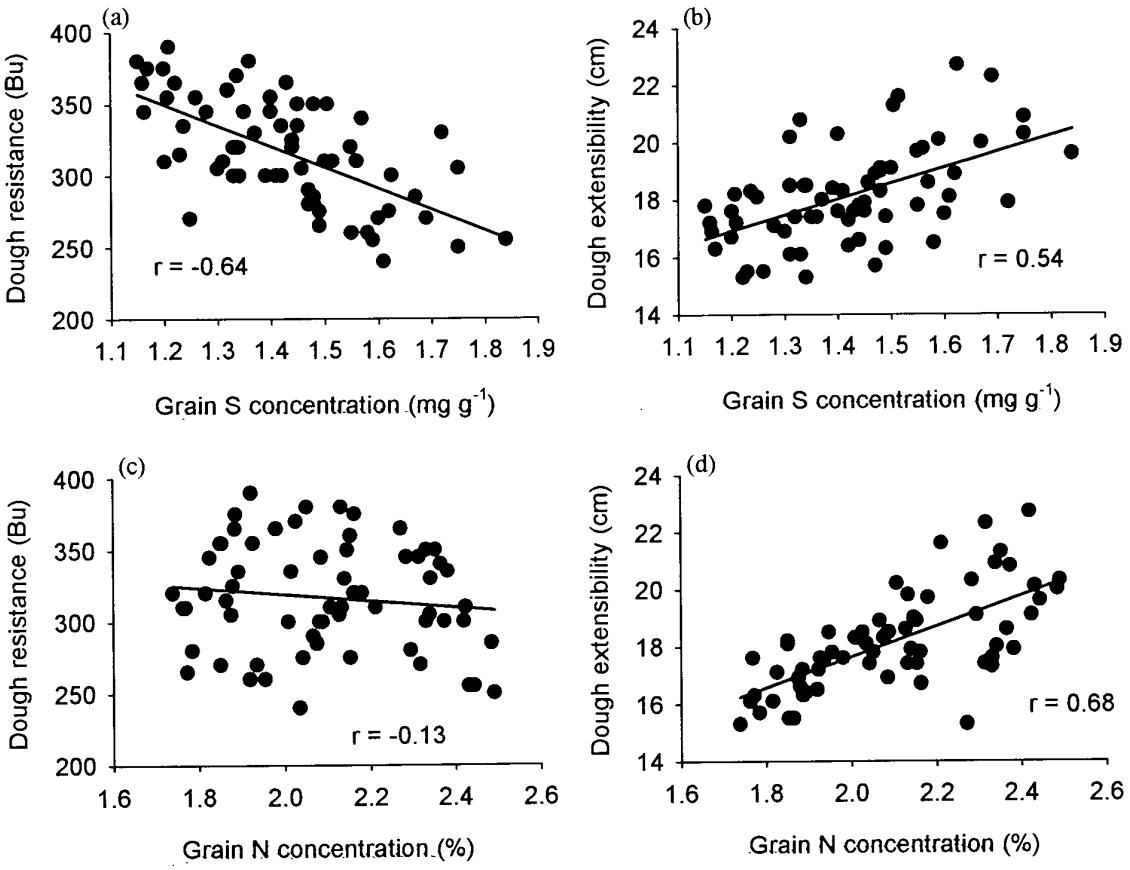

Figure 20. Correlations between dough resistance and grain S or N concentration (a, c), and between dough extensibility and grain S or N concentration (b, d) in 1995-96.

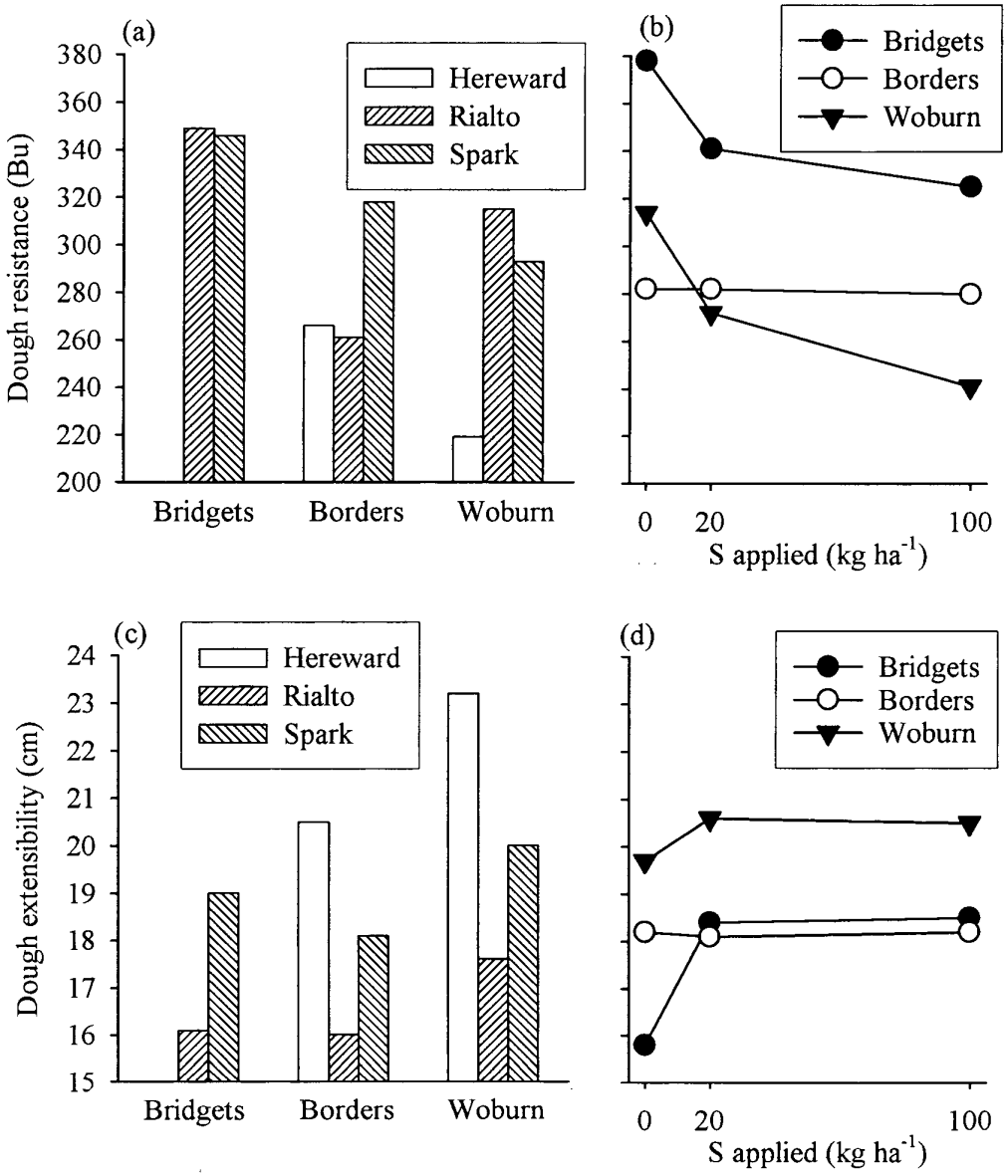

Figure 21. Differences between varieties in dough resistance and extensibility (a, c), and effects of S on dough resistance and extensibility in 1996-97.

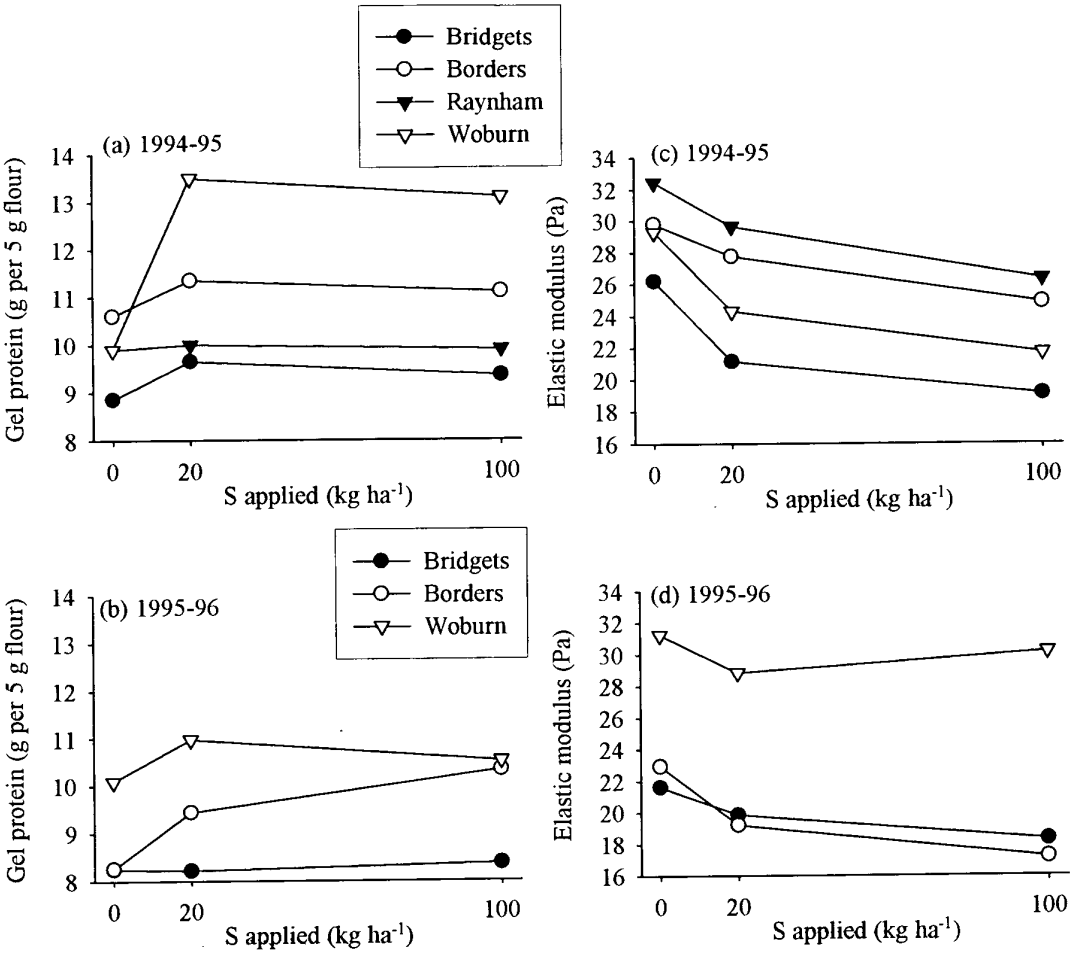

Figure 22. Effects of S on gel protein content (a, b) and the elastic modulus of gel protein (c, d) in 1994-95 and 1995-96.

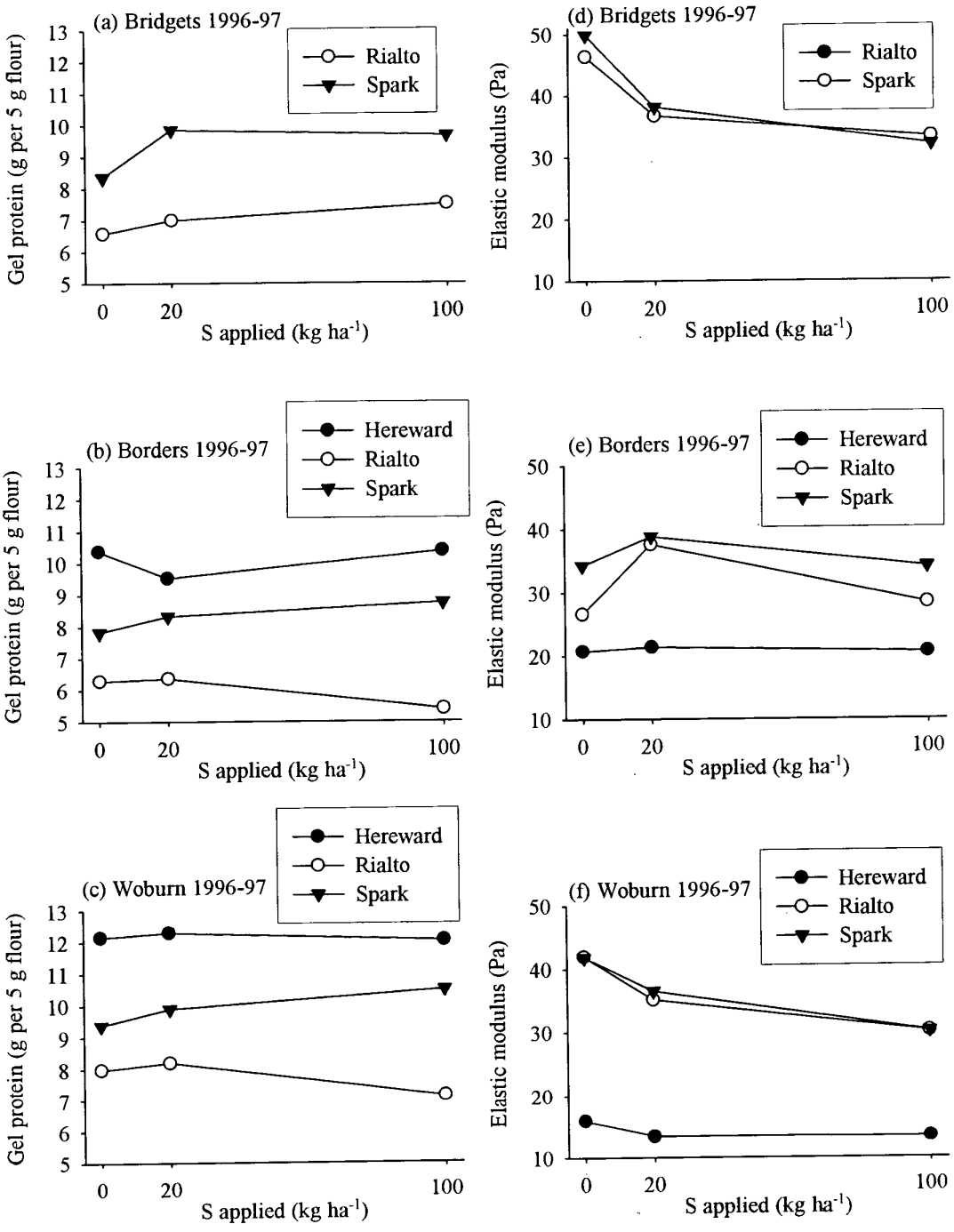

Figure 23. Effects of S on gel protein content (a-c) and its eleastic modulus (d-f) in three varieties in 1996-97.

In 1996-97, gel protein weight differed significantly between varieties, in the order of Hereward>Spark>Rialto (Figure 23). The ratio of gel protein weight to flour protein concentration also followed the same order (data not shown). The effect of S on gel protein content was positive and significant at Bridgets, but was not significant in the other two sites. The gel protein of the three varieties also differed markedly in the elastic modulus (G'), with Hereward having much lower G' values than Rialto and Spark (Figure 23). The differences between Rialto and Spark were small. Sulphur addition decreased G' significantly at all sites, particularly Bridgets and Woburn. The effect of S was consistent in all three varieties with similar relative decreases in G' in response to S. Nitrogen gave a significant increase in the G' value of gel protein at Woburn, but not at the other two sites.

Size distribution of gluten proteins and analysis of glutenin subunit composition

It has been established that glutenin polymers play a vital role in the breadmaking performance (Weegel *et al.*, 1996). Size exclusion-HPLC was used to determine the size distribution of total protein fractions extracted from flour of the Woburn and Bridgets samples from 1994-95 and the Woburn and Borders samples from 1995-96. The proteins were resolved into three peaks, with peak 1 corresponding mainly to glutenin polymers, peak 2 to a mixture of medium M_r polymers and monomers and peak 3 to mainly monomers with some low M_r polymers. Nitrogen had no significant effects on the relative proportions of the three peaks in all four sets of samples. However, there were significant effects of the S treatment on the relative proportions of peaks 1 and 2 in the Woburn 1994-95 and Borders 1995-96 samples. These were the two experiments having the most significant effects of S on gel protein concentration (Figure 22). In both experiments, application of S increased the relative proportion of peak 1 and decreased that of peak 2, but had little effect on peak 3. Results for peaks 1 and 2 are shown in Figure 24.

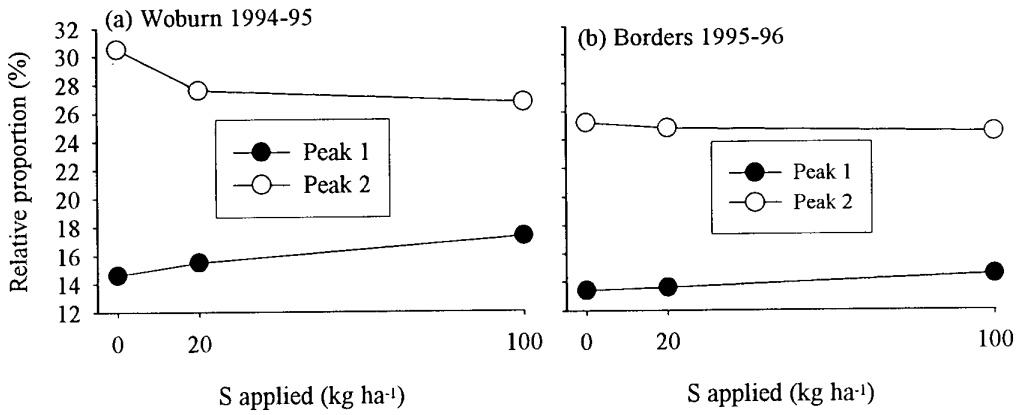

Figure 24. Effects of S on the relative proportions of Peak 1 and Peak 2 in SE-HPLC. (a) Woburn 1994-95. (b) Borders 1995-96.

Glutenin polymers consist of HMW and LMW subunits, which are linked through inter-chain disulphide bonds. LMW subunits are rich in S, and contain cysteine residues that form intra- and inter-chain disulphide bonds, whereas HMW subunits are relatively poor in S (Shewry and Tatham, 1997). Glutenin fractions were prepared from the S0 and S100 samples of Rialto (with N280) grown at Bridgets in 1996-97 and their subunit compositions compared by SDS-PAGE and quantified by image analysis. This showed that the combined proportion of HMW subunits decreased from 21.5% in the samples grown without additional S to14.7% in the sample with additional S, with corresponding increases in the proportions of total LMW subunits (from 68.2% to 72.8%).

Overall, these results showed that increasing S availability to wheat increased the S-rich LMW subunits of glutenin at the expense of HMW subunits, resulting in a larger LMW/HMW ratio. Because the LMW subunits are the major components of glutenin, the net effect of increasing grain S concentration would be to increase the total amount of polymeric proteins. This was confirmed by increased gel protein content and the proportion of peak 1 proteins in SE-HPLC, in response to S additions. MacRitchie and Gupta (1993) also showed that the percentage of polymeric proteins in the total protein increased with S concentration. Increased LMW/HMW ratio would be expected to result in a decrease in the molecular size of glutenin polymers (Field *et*

al., 1983), which probably explains a marked decrease in the elastic modulus of gel protein and dough resistance in response to S. Differences between the three breadmaking varieties may also be, at least partly, explained on this basis. Hereward contains considerably more gel protein fraction than the other two varieties, but its gel protein fraction is substantially weaker in the elastic modulus. It is likely that the LMW/HMW ratio of glutenin in Hereward would be higher than in the other two varieties, resulting in more but smaller glutenin polymers and hence weaker gel protein. Regardless of the significant differences in the gel protein content and elastic strength, all three varieties appeared to respond to S similarly in terms of breadmaking performance.

5. Summary

This series of field experiments constituted a systematic study of the effects of S nutrition on yield and breadmaking quality of winter wheat. It was the most comprehensive study on the topic that has ever been carried out in the UK. The study has produced many interesting results that are of not only practical but also scientific importance. The main findings are summarised as fellows:

1) Significant yield increases in response to S addition in early spring were obtained in three out of eleven field experiments over the three seasons from 1994 to 1997. In the responsive experiments, yield response to S varied between 0.43 and 1.34 t ha^{-1}, or between 8.7 and 26.5% on the relative basis. Most of the yield increase was obtained from the application of the first 20 kg S ha^{-1}.
2) Application of S in early spring increased loaf volume significantly in six out of the ten experiments that produced suitable grain samples for breadmaking tests, suggesting that breadmaking quality response to S was more common than yield response. Increases in loaf volume typically varied between 40 and 100 ml. In addition, S also improved crumb score in two experiments. Three breadmaking varieties, Hereward, Rialto and Spark, appeared to respond similarly to S. In comparison, increasing the amount of N applied from either 180 to 230 kg ha^{-1} in nine experiment, or from 230 to 280 kg ha^{-1} in one experiment, increased loaf volume significantly only in one case, even though this increased grain protein significantly in most experiments.
3) Loaf volume correlated more closely with grain S concentration than with grain N (grain protein). These results indicate that, within the range of grain protein concentration obtained in this series of experiments (8.5-14.3%), the concentration of crude protein was not as limiting a factor as the concentration of S in grain to breadmaking performance. Because grain S concentration correlated with loaf volume in a linear pattern, it was difficult to derive a critical value of grain S for breadmaking quality. In many cases, a low loaf volume was associated with a grain N:S ratio of greater than 16:1.
4) There were significant effects of S on dough rheology, and the amount and elastic modulus of gel protein. Sulphur addition in general increased gel protein content, but decreased its elastic strength. Sulphur also decreased dough resistance, and increased dough

extensibility. Despite different rheological properties, Hereward, Rialto and Spark responded similarly to S.

5) Compared to the spring applications of gypsum, foliar applications of ammonium sulphate at the milky ripe stage was not effective in correcting S deficiency for grain yield. In some cases, foliar applications resulted in scorching and yield losses. In terms of the effects on grain S concentration and breadmaking quality parameters, foliar applications of S produced inconsistent results. It was concluded that the best practice was to apply S, in a sulphate form, in spring.
6) It was established that a winter wheat crop required >15 kg S ha^{-1} to ensure S sufficiency. The harvest index for S was much lower than that for N, even under S deficient conditions, indicating that the re-utilisation of S within plants was less efficient than of N. Analysis of plant samples at early stem elongation (GS 31-32) was useful in predicting S deficiency, with a critical value of 2 mg g^{-1} of total S in the whole plant shoots.
7) An extractable sulphate-S concentration in the soil profile of greater than 3 mg kg^{-1} in early spring appeared to indicate a sufficient S supply for grain yield. However, S deficient sites could not be predicted reliably even when soil extractable sulphate-S was less than 3 mg kg^{-1}. In this series of field experiments, breadmaking quality responses were not related to soil extractable S in early spring.

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PART II. EVALUATION OF THE CRITICAL PHASES OF SULPHUR NUTRITION AND SULPHUR RE-DISTRIBUTION IN WHEAT

1. Introduction

The importance of sulphur (S) supply to both grain yield and grain quality in winter wheat has been demonstrated in Part I. Results shown in the Part I also demonstrate that the majority of S is taken up by wheat during the period of stem extension. Under field conditions, crops showing mild S deficiency symptoms at the stem extension stage may recover after anthesis, possibly because roots have reached the S reserve in the subsoil, or mineralisation of soil organic S has increased due to increasing soil temperature. Whether plants can recover from S deficiency will depend on the degree of deficiency, as well as how soon the additional S supply is made available to the plants. It is therefore important to investigate the critical phases of S supply that have the greatest influence on various yield components. By altering the timing of S application to pot-grown winter wheat, it is possible to study the response of grain yield and grain quality; an understanding of which will aid the efficient targeting of S fertiliser applications to field crops.

Because grain S concentration has an important influence on breadmaking quality, it is therefore important to understand the mechanisms of S accumulation in grain and the factors controlling this process. Sulphur 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 over-generalised 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 and Anderson, 1995; 1996), but its mobility is enhanced by N deficiency (Sunarpi and 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 *et al.*, 1993). In contrast, 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 re-distributes to young leaves and roots (Larsson *et al.* 1991; Clarkson *et al.*, 1993; Adiputra and Anderson, 1995). These studies, all using radioactive tracer ^{35}S, have focused mainly on S uptake and

re-distribution in young plants over relatively short periods of time, and have not addressed the question of S re-distribution towards maturing grain.

For tracer studies of nutrient uptake and re-distribution, it is desirable that the half-life of a radioactive tracer is longer than the duration of the experiment. This makes ^{35}S (half-life 87 days) unsatisfactory for studies involving the whole growth cycle of plants such as winter wheat. Safety regulations on the use and disposal of radioactive tracers also make the use of ^{35}S difficult in long–term studies involving large tanks of nutrient solutions or other growth media. 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 abundance, ^{32}S and ^{34}S are usually studied in S isotope analysis. The $^{34}S/^{32}S$ ratio of a sample is usually expressed as per thousand deviation from that of the reference standard, Canyon Diablo Troilite (CDT), in the following standard δ-notation:

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

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 (Prietzel *et al.* 1995; Giesemann *et al.*, 1995). Two conditions must be satisfied for this approach to be used in tracer studies. Firstly, the difference in $\delta^{34}S$ between tracer sources, or between tracer and the background must be sufficiently large; secondly, isotopic fractionation, which may occur in the processes studied, must be small relative to the difference between the tracers. This technique has not been applied to studies on S uptake and redistribution in higher plants.

The second part of this project aimed to gain a better understanding of the physiological basis of the S nutrition of wheat. Specifically, pot experiments were carried out to evaluate the effects of supplying S to wheat at different stages on grain yield and yield components, and to quantify the contributions of S from vegetative tissues to grain through re-distribution. The second objective was achieved by using two S sources differing in $\delta^{34}S$ value.

2. Materials and Methods

2.1. Experiment 1

This experiment was carried to investigate the effects of S addition at different growth stages on grain yield, yield components and grain protein composition. The experiment was established at Cockle Park Experimental Farm, Northumberland. The experiment was carried out in a poly-tunnel, with a roof of transparent plastic sheeting to prevent any input of rainwater S. Mesh side walls allowed the free passage of air, keeping the plants at ambient temperatures during the season. Thirty pots, each of 22 cm diameter, were filled with 10 kg of sharp sand. Approximately 20 litres of de-ionised water was used to 'flush' any sulphate from the sand in each pot. The pots were then placed on saucers. The variety Hereward was used. The growth stages were split into four periods:

- sowing until the initiation of stem extension (GS 0-31) (Zadoks *et al.*, 1974)
- the initiation of stem extension until flag leaf emergence (GS 31-37)
- flag leaf emergence until anthesis (GS 37-69)
- anthesis until maturity (GS 69-92)

These treatments were termed pre-stem extension, stem extension, pre-anthesis ear development and grain-filling, respectively. At the end of each period the saucers were removed and all the pots were 'flushed through' with about 20 l of de-ionised water, the saucers were replaced and the treatments were applied. After flushing, the leachate contained approximately 2 mg l^{-1} S. During each period all the pots received S free nutrient solution (Haneklaus *et al.*, 1995) and 20 mg S, applied as calcium sulphate solution. The S adequate treatment received an additional 80 mg S at each period (as calcium sulphate solution); the S timing treatments received an additional 80 mg S at one period only (Table 5).

At maturity (GS 92) all plants were harvested and separated into stem, leaves, chaff and grain. Nitrogen was determined using a LECO FP428 analyser (Leco Ltd, Missouri, USA) and S by X-Ray Fluorescence. The data were analysed using ANOVA and minimum significant difference (MSD) was calculated for each variable. Grain proteins were extracted from the bulked grain of each treatment, by single stage extraction (Batey *et al.*, 1991). Extracts were separated by size exclusion high-performance liquid chromatography (SE-HPLC) using a Beckman system (166 detector, 126 solvent module and 507e autosampler).

This analysis was repeated five times for each bulked grain sample to allow statistical analysis by ANOVA. Significance between treatments was analysed using MSD.

Table 5. Treatments used in Experiment 1

	Period of S addition (growth stage)			
Treatment	0-31	31-37	37-69	69-92
	(mg S pot^{-1})			
Pre-stem extension	100	20	20	20
Stem extension	20	100	20	20
Pre-anthesis ear development	20	20	100	20
Grain-fill	20	20	20	100
Adequate S control	100	100	100	100
deficient S control	20	20	20	20

2.2. Experiment 2

This experiment was set up to investigate the re-distribution of S in wheat using two S sources differing in the stable S isotope ratio. The experiment was conducted in a greenhouse during 1996 and 1997. Winter wheat (*Triticum 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, yet allowing vernalisation to proceed. From 15 April 1997 onward, when plants were at growth stage 31, the greenhouse was covered with shading sheets to prevent overheating in the greenhouse. Seeds were sown in rock wool blocks (Grodan, DK) on 16 December 1996. The rock wool blocks were placed in two plastic trays half filled with perlite (Gem Gardening, UK), with 50 blocks per tray. The blocks were watered with -S nutrient solution sufficiently for a 2 cm depth of solution in the tray. Fifteen days after emergence (15 DAE) when the roots were extending about 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 cm square pot filled with perlite that had been wetted with de-ionised water, so that the roots of the young plants were at the bottom of the pot. Six pots were placed on top of an upturned seed tray in each of five 23 l opaque polyethylene vessels. Each vessel was aerated by a single Elite 800 aquarium pump (Rolf. C. Hagen Ltd, UK) which had

an air output of 1.2 l 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 l 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 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 on 25% of the full strength solution (Table 6), the strength of the nutrient solution was altered according to the following schedule: 50% at 28 DAE, 75% at 49 DAE and 100% at 70 DAE, 75% at 154 DAE (14 days after anthesis), 50% at 168 DAE and 25% at 182 DAE. When all the main tillers had reached full maturity the nutrient solution was no longer replenished. During the experiment, the nutrient solution was changed weekly. The pots were removed from the experimental vessels and placed in similar vessels containing de-ionised water, to prevent the roots drying. Whilst in these vessels the pots were watered with de-ionised water to remove any residual nutrient solution. The pots were then returned to their experimental vessels, which had been scrubbed clean with de-ionised 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.

Table 6. The composition of nutrient solution used in Experiment 2.

Macro-nutrient	mol m^{-3}	Micro-nutrient	mmol m^{-3}
NH_4NO_3	2.75	Fe-EDTA	13.97
KCl	1.97	$ZnCl_2$	1.17
$MgCl_{2.6}H_2O$	0.15	$MnCl_{2.4}H_2O$	1.40
Ca_2SO_4	0.12	$CuCl_2.2H_2O$	0.24
$Ca(H_2PO_4)_2$	0.38	H_3BO_3	0.71
		$(NH_4)_6Mo_7O_{24.4}H_2O$	0.01

Experimental treatments

Two sources of Ca_2SO_4 with different $^{34}S/^{32}S$ ratios were used: one with high $\delta^{34}S$ (13.7 ‰) and the other with low $\delta^{34}S$ (4.05 ‰). The high $\delta^{34}S$ source was a commercially available horticultural gypsum (Chempak Ltd, 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) and was found to have a low $\delta^{34}S$ value. The resultant Ca_2SO_4 precipitate was washed repeatedly with deionised water and dried at 120°C. A preliminary experiment using sand culture has shown that this difference of $\delta^{34}S$ was sufficiently large to study the uptake and redistribution of S within plants.

Prior to GS 12 (three leaf stage) no treatment received added S. Five treatments were studied. 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 (Figure 25). 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. Consequently, treatments C-E had the same volume of nutrient solution per plant as treatment A and B, which had six pots per vessel throughout. Sequential analysis of the nutrient solution indicated that the depletion of nutrients followed similar patterns in different treatments. In the experimental vessel that was sampled, all the main tillers (MT) that were at the required growth stage were tagged with coloured wool, enabling their identification at harvest; the remaining tillers were classified as late tillers (LT) Whereas six plants were grown to maturity in treatments C-E, twelve 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.

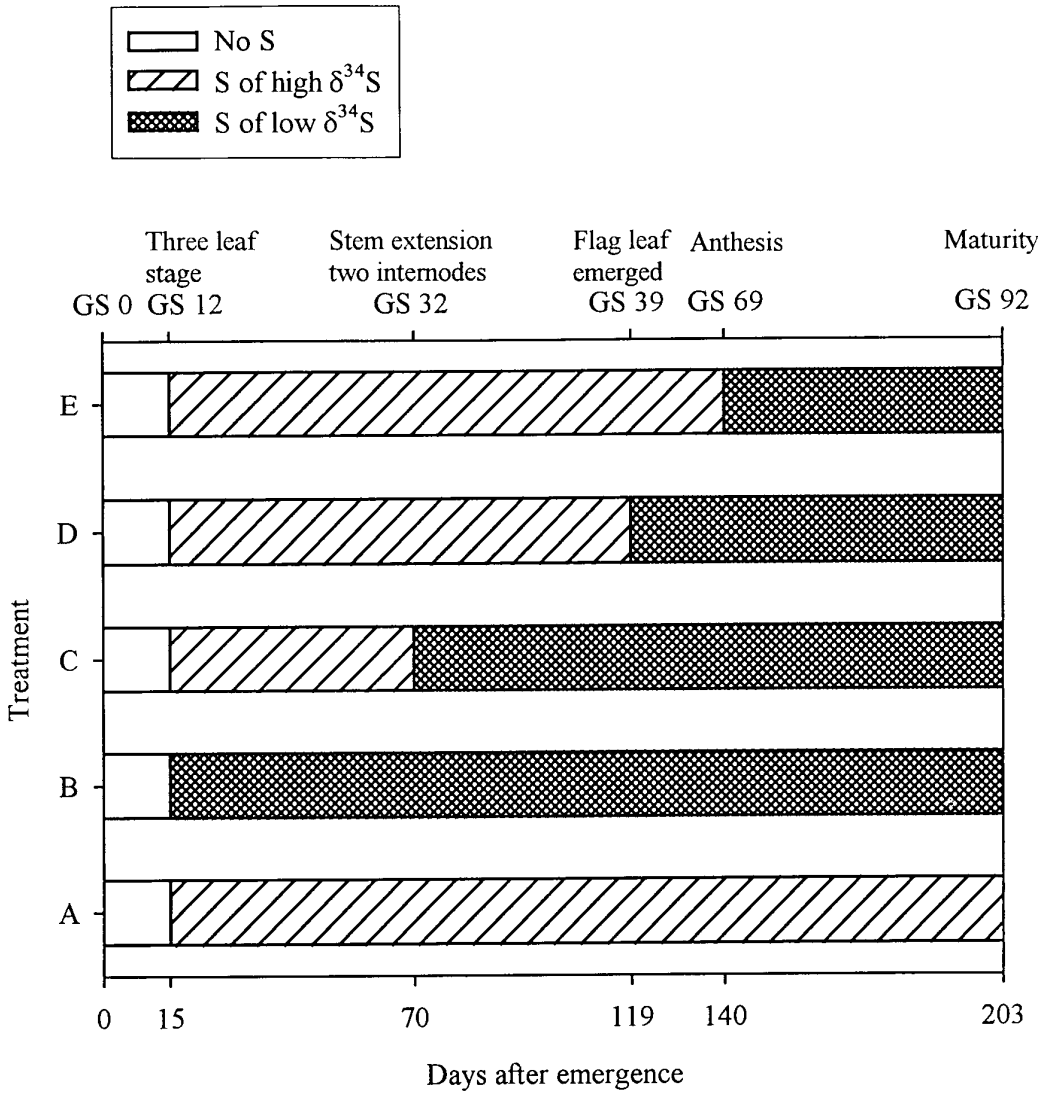

Figure 25. Design of treatments in Experiment 2.

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; at GS 69 plants were separated into stem, flag leaves, older leaves, and ears. At maturity all remaining plants were initially split into

main tillers (MT) and late tillers (LT) and then separated into stem, older leaves (all leaves but flag leaf), 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 1g of material from each plant fractions was ground into a fine powder in a Glen Creston ball mill, using stainless steel containers and ball bearings. The concentration of total S and the ratio of $^{34}S/^{32}S$ were determined using continuous flow isotope ratio mass spectrometry. Sulphur isotope results were calculated as $\delta^{34}S$ in ‰ deviation from the Canyon Diablo Troilite standard. Sulphate in the plant fractions from sequential samples was extracted with de-ionised water, and determined using ion chromatography (Dionex 2000; AS9C column). All analyses were done in duplicate.

Analysis of variance was performed to assess the significance of treatment effects. Student's *t*-tests were carried out to compare the differences in the $\delta^{34}S$ values between the S source and the S accumulated in plant fractions.

3. Results and Discussion

3.1. Experiment 1

Biomass accumulation

Plants receiving adequate S throughout the whole experiment were comparable with field grown wheat in appearance. However, the plants in the S deficient control and the S timing treatment were visibly smaller. During the period of S addition the new leaves produced by the plants were a darker green. Plants grown with an inadequate supply of S produced fewer ears and grains, and a smaller grain, leaf and stem biomass, when compared to plants grown with an adequate supply of S (Table 7). Additional S during the period before stem extension significantly increased the number of fertile tillers produced by the plant, presumably through increased tiller initiation. The number of fertile tillers decreased the later additional S was applied. The addition of S during the period of stem extension, when the rate of biomass

accumulation is highest, increased grain number, leaf and grain biomass compared to the S deficient control plants. Grain number was also significantly increased by additional S supply during the period between flag leaf emergence and anthesis, but grain biomass was unaffected, suggesting that additional S promoted spikelet formation but, due to the previous S deficiency and consequent limitation of leaf biomass and hence photosynthesis, the grains formed were smaller on average. The plants that received additional S during the period of grain development had the same grain, leaf and stem biomass as the plants that received no additional S at any time. This may be because the processes determining tiller number, leaf biomass and grain number were all completed by anthesis.

Concentrations of N and S in the biomass

The concentration of N in the grain and leaf biomass did not differ between any of the treatments (Table 7). All the treatments received 2000 mg pot^{-1} N in total and the plants grown with adequate S accumulated an average of 1800 mg N in the above ground biomass, suggesting that N was not limiting in any of the treatments. However, it was notable that significant difference was observed in the concentration of N in the stem biomass. The plants that received an adequate supply of S or additional S during either stem extension or pre-anthesis ear development all had significantly smaller concentrations of N in the stem biomass. This response was not detected in the leaf biomass. These three treatments all produced a significantly larger amount of grain, and the reduced stem N concentration may be the result of re-distribution of stem N reserves to developing grains. The concentration of S in the grain and leaf biomass was significantly higher in the plants grown with an adequate supply of S, compared to those grown with a deficient S supply. The S deficient control produced grain with a grain S concentration of 1.2 mg g^{-1} and grain N:S ratio of 25:1. The accepted critical values for wheat are 1.2 and 17:1, respectively (McGrath *et al.*, 1996), indicating that S supply was inadequate. Of particular interest was the response of grain S concentration to the timing of S addition. The later the period of S addition the higher the grain S concentration. The plants that received additional S after anthesis had the highest concentration of S in the grain, significantly higher than all other treatments, including the adequate S control. This may be due to the higher S supply per unit grain biomass in the plants that received additional S after anthesis and/or an increase in the rate of S

accumulation following prolonged S deficiency. However, it is clear that the capacity for S accumulation is maintained after anthesis, and may be source limited.

Table 7. *Treatment mean values for the variables measured at maturity.*

			Timing of S addition				
Variable	S deficient control	S adequate control	GS 0-31	GS 31-37	GS 37-69	GS 69-92	MSD (df = 15)
Ear number	17	32	28.3	27	21.8	15.3	10
Grain number	583	1570	1047	1233	1254	615	482
Grain dry wt (g)	25.2	69.8	48.3	59.7	49.0	30.4	28.3
Grain N (%)	2.89	2.42	2.44	2.55	2.75	3.08	0.55
Grain S (mg g^{-1})	1.2	1.5	1.1	1.3	1.5	1.9	0.3
Grain N:S	25.1	16.7	23.2	19.2	18.0	16.3	3.03
Leaf dry wt (g)	11.9	18.4	14.5	17.8	11.5	13.3	4.8
Leaf N (%)	1.23	1.23	0.76	0.77	0.88	1.36	0.64
Leaf S (mg g^{-1})	1.2	3.9	1.1	2.0	3.2	2.4	0.9
Leaf N:S	11.0	2.0	6.8	3.8	2.8	5.5	2.78
Stem length (cm)	318.3	387.8	388.9	331.8	347.6	319.1	104.5
Stem dry wt (g)	15.2	26.6	21.2	23.2	18.8	16.0	8.2
Stem N (%)	1.59	0.27	1.05	0.38	0.43	1.11	0.63
Stem S (mg g^{-1})	0.5	0.4	0.1	0.2	0.4	0.5	0.06
Stem N:S	51	12	72	26	13	27	48

Grain protein composition

The total grain proteins were extracted with buffer containing SDS and separated by size exclusion HPLC on a TSK 3000SW column. This resolves three peaks corresponding to high molecular weight (HMW) glutenin polymers, low molecular weight (LMW) glutenin polymers and oligomers + monomers. No difference was observed in the proportion of HMW polymers in the grain protein extract (Table 8). However, significant differences were observed in the proportion of both LMW polymers and oligo/monomers in the grain protein extract. The S deficient control plants had a significantly lower proportion of LMW polymers than two treatments, and the highest proportion of oligo/monomers, significantly greater than three treatments. The response became clear when the proportion of either protein fraction

were compared to grain S concentration (Figure 26). The proportion of LMW polymers in the grain extract increased with grain S concentration, whereas the proportion of oligo/ monomers decreased, indicating differential regulation of the synthesis and/or assembly of the two protein fractions.

Table 8. *SE-HPLC results of the bulked grain samples showing the proportion of each fraction in the grain protein extract.*

Timing of S addition	HMW polymers (%)	LMW polymers (%)	Oligo/ monomers (%)
Pre-stem extension	23.67	49.08	26.49
Stem extension	22.75	52.13	25.12
Ear development	22.89	52.53	24.53
Grain-fill	22.81	52.96	24.23
S adequate control	22.60	51.05	26.35
S deficient control	22.16	49.88	27.95
Mean	22.81	51.27	25.78
MSD (df = 24)	3.08	2.51	2.23

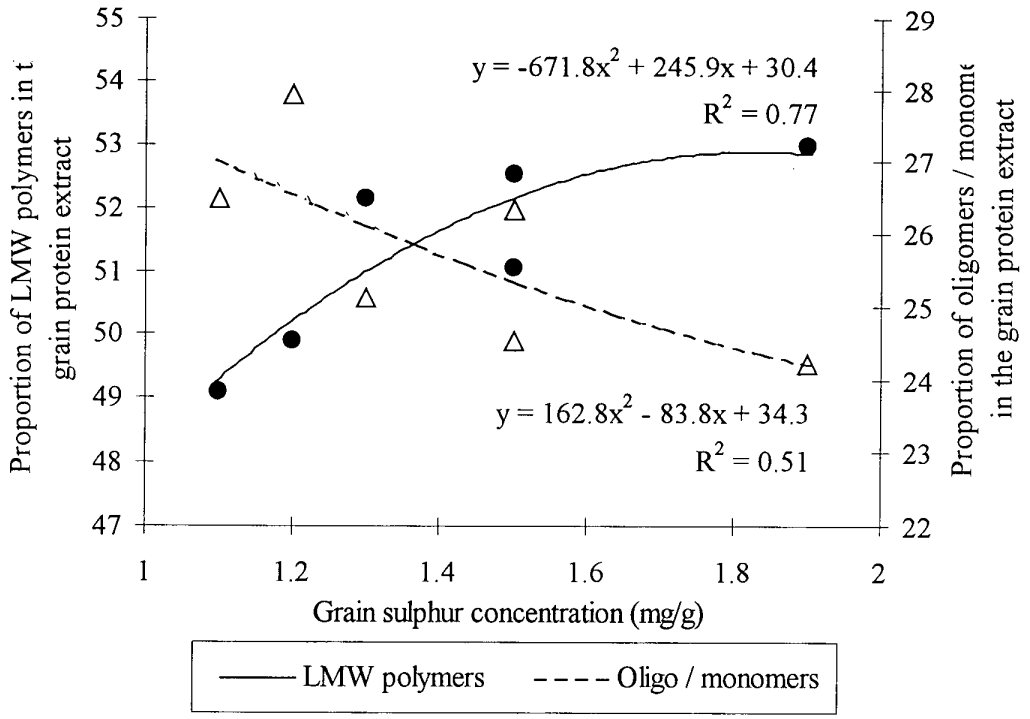

Figure 26. Relationship between grain S concentration and the relative proportions of LMW polymers (represented as •) and oligomers/monomers (represented as Δ) in the grain protein extract.

3.2. Experiment 2

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. A notable difference was that plants grown in this hydroponic experiment developed more late tillers (LT), 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 at maturity. Accumulation of biomass appeared to be slower between GS 32-39 than between GS 39-92 (Figure 27a). Over half (53%) of the total biomass at maturity was accumulated after anthesis (GS 69), although net accumulation in vegetative organs ceased by anthesis (Figure 27a).

The concentrations of S in the whole plants decreased sharply from GS 32 to GS 69, but remained relatively constant from GS 69 to GS 92 (Figure 27b). The concentrations of S

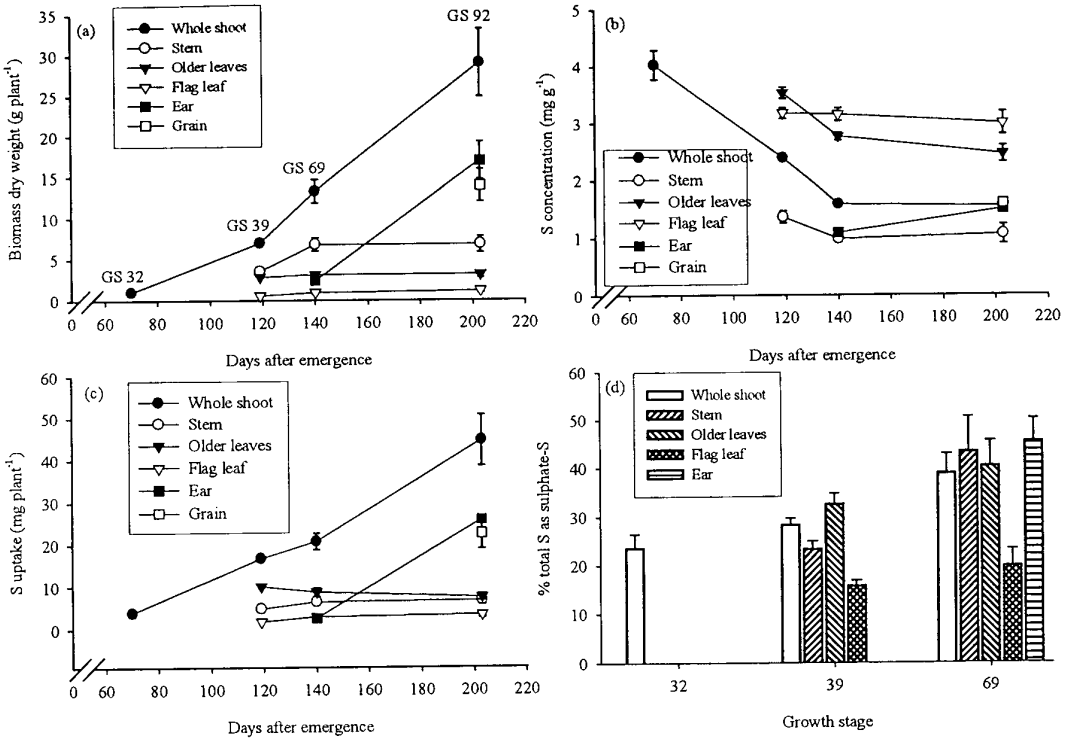

Figure 27. Dry matter accumulation (a), changes in total S concentration in different tissues (b), S uptake and distribution (c) and changes in SO_4-S concentration (d) in Experiment 2.

in older leaves and stems also decreased considerably between GS 39-69, whereas it remained relatively stable in the flag leaves. In contrast, the concentration of S in ears increased between GS 69-92.

Plant shoots accumulated S almost linearly between GS32-92 (Figure 27c). 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. The distribution pattern of S in different plant parts at maturity was similar to that observed in field-grown wheat (Part I). From GS 39 to GS 92, older leaves lost S, whereas all other plant fractions gained S. From GS 69 to GS 92, relative changes in the amounts of S in stems, older leaves and flag leaves were 5, -14 and 17%, respectively.

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 (Figure 27d), 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.

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 (Figure 28). Overall, the $\delta^{34}S$ values for shoots were very similar to the $\delta^{34}S$ value of the S source (13.7‰). In the four different growth stages examined, only the GS 32 samples had a $\delta^{34}S$ value significantly different from that of the source ($p<0.05$ 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 S source ($p<0.05$ in *t*-test) (Figure 28).

There were significant ($p<0.05$) differences in the $\delta^{34}S$ values between different plant parts (Figure 28), 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 (Figure 28), 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 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 to leaves and grain (see Figure 27b), and 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 0.1-2.2‰.

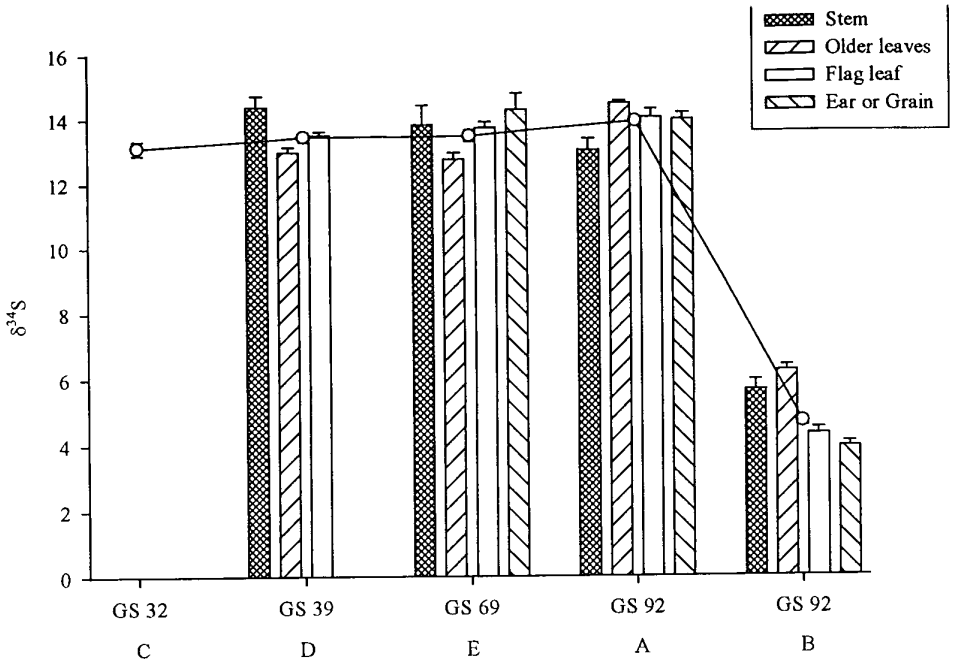

Figure 28. $\delta^{34}S$ values in different plant parts and whole shoots at different growth stages for treatments C, D and E (following unaltered supply of high $\delta^{34}S$ source); and at maturity for treatments A and B.

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 *et al.* (1991) 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 to 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.

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 (Figure 29), 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. Differences

between treatments D and E were small, which was expected due to the short period between GS 39 and GS 69 (21 days, Figure 25) and consequent small S uptake (Figure 27c). 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 (Figure 29).

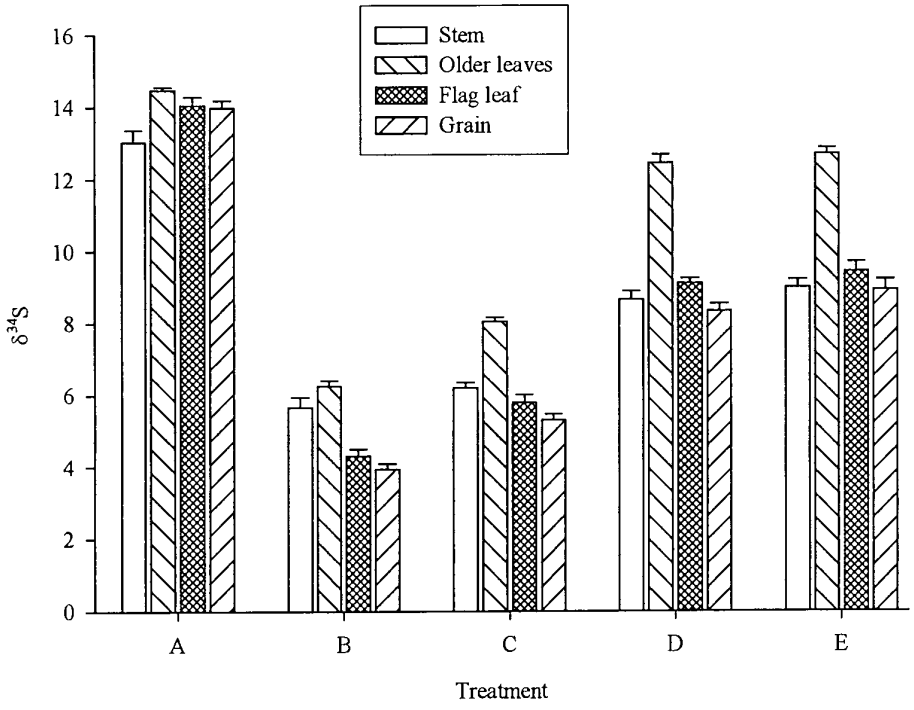

Figure 29. $\delta^{34}S$ values in different plant parts at maturity in different treatments.

Because there was little isotopic fractionation during S uptake and re-distribution, 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:

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

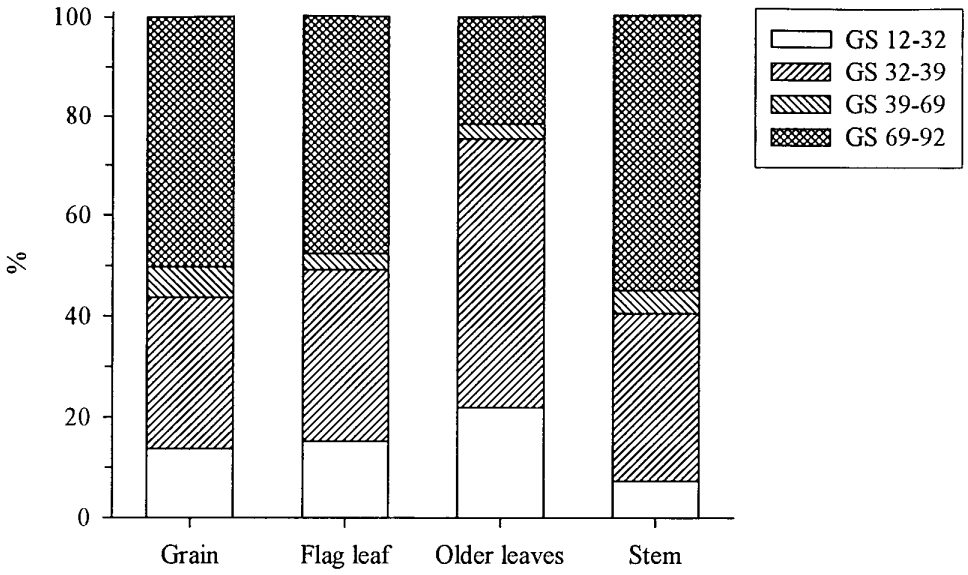

Figure 30. Percentages of S in grain, flag leaves, older leaves or stems derived from the accumulation during different growth stages.

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

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

This calculation was repeated for the remaining growth stages and plant parts, and the results are presented in Figure 30. 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 12-32, GS 32-39, and GS 39-69, respectively. In contrast, older leaves derived much less S from accumulation after anthesis (22%), but more from accumulation between GS 12-32 and GS 32-39 (22, and 53%, respectively). The contribution of S accumulated between GS 39-69 to all plant parts at maturity was small, again due to the short period of time between the two growth periods and the resulting small net uptake of S.

These results suggest that the contributions of the S accumulated before and after anthesis to the grain at maturity were about equal. Because at anthesis there was only a very small amount of S in the ears, and probably a negligible amount in the developing grain, the 50% of the S in the grain of pre-anthesis origin must have been re-distributed (re-mobilised)

from other plant parts. Part of the S derived from post-anthesis accumulation was probably delivered first to the stems and leaves, and then re-distributed 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 pre-anthesis 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 post-anthesis derived S at maturity, and exporting 32% of their pre-anthesis 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 re-distribution (re-mobilisation) in wheat during reproductive growth, being less than that for N and P (Hocking, 1996). 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 pre-anthesis period, thus affecting the contribution of post-anthesis S uptake to grain S accumulation.

4. Summary

The results from the two experiments are summarised as follows:

1) Severe S deficiency decreased grain yield markedly by affecting the number of ears and the number of grain per ear, whereas single grain weight was little affected. Compared to the S deficient control, ear number was increased significantly by the additional S given to the S-deficient plants at pre-stem elongation and stem elongation stages, but not by the additional S given after stem elongation. This indicates that S supply before and during stem elongation is important for the initiation and survival of tillers. In contrast, the critical phases for the number of grain per ear appeared to be the stem elongation and pre-anthesis ear development stages. Additional S given to the S deficient plants after anthesis did not correct the deficiency significantly.

2) Grain S concentration appeared to be influenced more by the S supply after stem elongation. Additional S given to the S deficient plants at the pre- and post-anthesis ear development stages restored the concentration of S in grain to levels similar to or above that found in the S sufficient control. Increasing proportion of low molecular weight gluten polymer was found to be associated with increasing grain S concentration.

3) 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 elongation, between stem elongation 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 post-anthesis 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.

Overall, the results suggest that the stem elongation stage is the most critical phase of S supply for grain yield, whereas S supply after anthesis is important for achieving a high concentration of S in grain for quality benefit.

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LIST OF PUBLICATIONS BASED ON THIS PROJECT

1. Zhao, F.J., McGrath, S.P., Salmon, S.E., Shewry, P.R., Quayle, R., Withers, P.J.A., Evans, E.J. and Monaghan, J. 1997. Optimising sulphur inputs for breadmaking quality of wheat. In: *Aspects of Applied Biology 50, Optimising Cereal Inputs: Its Scientific Basis, Eds. M.J. Gooding and P.R. Shewry*. The Association of Applied Biologists, Wellesbourne, UK. 199-205.
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APPENDICES 1-11

Appendix 1: Bridgets 1994-95

N applied (kg/ha)	S applied (kg/ha)	Grain yield (t/ha)	S uptake (kg/ha)	N uptake (kg/ha)	Grain S concentration (mg/g)	Grain N concentration (%)	Grain N:S ratio	Grain protein (%)	Grain HFN	Grain specific wt (kg/hl)	T.G.W. (g)	Flour protein (%)	Flour HFN	Flour water absorption (%)	Loaf Volume (ml)	Crumb Score	Gel Protein weight (g/5g)	Elastic modulus (G', Pa)
180	0	8.26	13.6	173.0	1.25	1.95	15.6	9.56	349	83.8	47.8	8.43	396	57.1	1456	6.7	8.8	24.1
	20	9.06	17.7	184.7	1.29	1.83	14.1	8.95	327	83.6	46.1	8.20	381	55.2	1432	6.5	8.9	20.7
	40	9.18	21.8	182.5	1.33	1.78	13.4	8.71	344	83.0	46.1							
	60	9.32	20.4	169.2	1.33	1.74	13.0	8.51	333	82.9	45.9							
	80	9.23	23.3	188.2	1.37	1.81	13.2	8.87	323	83.0	46.2							
	100	9.28	24.5	194.2	1.41	1.78	12.7	8.74	326	82.9	45.9	7.90	378	54.6	1457	6.0	8.5	17.9
230	0	8.57	15.4	209.0	1.27	2.18	17.2	10.67	354	84.3	48.3	9.93	410	60.9	1393	6.2	8.9	28.3
	20	9.66	20.1	210.4	1.37	1.98	14.4	9.71	357	83.4	46.5	9.07	387	57.0	1460	6.8	10.4	21.6
	40	9.91	24.9	220.2	1.44	2.00	14.0	9.82	328	83.4	46.4							
	60	9.90	24.8	234.5	1.46	2.06	14.1	10.11	348	84.3	46.1							
	80	10.07	25.9	228.5	1.47	2.02	13.7	9.90	323	83.9	47.2							
	100	10.02	28.9	218.9	1.47	1.97	13.4	9.64	340	84.3	46.8	9.07	381	56.4	1504	6.7	10.2	20.3
180 + 50F	0	8.49			1.32	2.14	16.2	10.51	339	85.2	49.2	9.83	413	60.2	1440	6.0	9.8	29.0
180 + 50F	20F	8.45			1.51	2.16	14.3	10.59	352	84.3	49.0	9.80	387	59.8	1458	6.0	10.7	23.5
180 + 50F	40F	8.42			1.50	2.03	13.5	9.93	349	84.1	48.3							
180 + 50F	20S + 20F	9.30			1.48	1.98	13.3	9.71	346	83.7	46.2							
ANOVA significance level:																		
	N	***	***	***	**	***	*	***	***	***	NS	***	NS	***	NS	NS	***	NS
	S	***	***	NS	***	**	***	**	*	NS	**	***	*	***	***	NS	*	***
	N x S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	***	*	*	NS
	NS not significant, * p <0.05, ** p <0.01, *** p <0.001																	

Appendix 2: Borders 1994-95																		
N applied (kg/ha)	S applied (kg/ha)	Grain yield (t/ha)	S uptake (kg/ha)	N uptake (kg/ha)	Grain S concentration (mg/g)	Grain N concentration (%)	Grain N:S ratio	Grain protein (%)	Grain HFN	Grain specific wt (kg/hl)	T.G.W. (g)	Flour protein (%)	Flour HFN	Flour water absorption (%)	Loaf Volume (ml)	Crumb Score	Gel Protein weight (g/5g)	Elastic modulus (G', Pa)
180	0	7.46	14.4	181.9	1.27	2.19	17.2	10.7	335	85.5	47.8	10.0	407	57.7	1325	5.0	10.3	29.5
	20	6.87	17.7	193.3	1.34	2.20	16.5	10.8	336	85.3	45.7	10.5	396	57.9	1334	4.8	11.2	28.3
	40	6.90	15.5	153.7	1.30	2.05	15.8	10.0	332	83.6	45.0							
	60	6.91	21.9	198.8	1.34	2.11	15.7	10.3	347	84.2	44.8							
	80	7.18	21.8	196.0	1.27	2.03	16.0	10.0	346	85.8	45.7							
	100	6.88	20.7	188.2	1.41	2.15	15.2	10.6	335	84.5	44.2	9.9	401	57.5	1360	5.0	10.0	24.2
230	0	7.60	18.0	214.0	1.36	2.29	16.8	11.2	345	86.1	46.8	11.1	416	59.9	1312	4.7	10.9	30.1
	20	7.21	17.4	193.7	1.28	2.23	17.5	10.9	350	84.4	44.9	10.7	417	58.5	1332	4.8	11.5	27.2
	40	7.34	19.3	195.3	1.41	2.39	16.9	11.7	341	84.7	43.5							
	60	7.07	22.0	226.5	1.47	2.41	16.4	11.8	358	84.6	44.0							
	80	6.98	22.8	209.3	1.47	2.34	15.9	11.5	343	83.3	42.6							
	100	7.12	21.0	194.7	1.49	2.32	15.7	11.4	360	83.2	43.0	11.3	407	58.7	1328	5.0	12.2	25.5
180 + 50F	0	7.37			1.39	2.46	17.7	12.1	360	85.6	45.4	11.8	430	61.3	1304	4.3	11.7	31.4
180 + 50F	20F	7.41			1.41	2.41	17.1	11.8	343	85.0	44.5	11.8	411	60.4	1309	4.8	12.0	29.5
180 + 50F	40F	6.81			1.67	2.45	14.7	12.0	350	84.7	43.5							
180 + 50F	20S + 20F	6.29			1.69	2.36	14.0	11.6	356	84.0	43.2							
ANOVA significance level:																		
	N	NS	NS	***	***	***	NS	***	***	NS	**	*	*	*	NS	NS	*	NS
	S	NS	***	*	*	NS	NS	NS	*	NS	**	NS	NS	NS	NS	NS	NS	**
	N x S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	NS not significant, * p <0.05, ** p <0.01, *** p <0.001																	

Appendix 3: Raynham 1994-95																		
N applied (kg/ha)	S applied (kg/ha)	Grain yield (t/ha)	S uptake (kg/ha)	N uptake (kg/ha)	Grain S concentration (mg/g)	Grain N concentration (%)	Grain N:S ratio	Grain protein (%)	Grain HFN	Grain specific wt (kg/hl)	T.G.W. (g)	Flour protein (%)	Flour HFN	Flour water absorption (%)	Loaf Volume (ml)	Crumb Score	Gel Protein weight (g/5g)	Elastic modulus (G', Pa)
180	0	9.09	16.9	214.6	1.26	2.06	16.4	10.11	333	81.7	41.7	9.3	395	57.6	1442	5.8	9.3	30.3
	20	9.32	20.0	206.2	1.30	2.07	15.9	10.15	339	81.3	40.7	9.2	389	56.6	1481	6.0	9.1	26.9
	40	9.37	20.6	226.2	1.39	2.08	15.0	10.21	341	81.2	41.1							
	60	9.44	22.6	214.6	1.45	2.08	14.3	10.18	339	81.3	40.1							
	80	9.15	29.3	250.6	1.46	2.14	14.7	10.51	334	81.4	41.4							
	100	9.24	26.1	231.7	1.44	2.08	14.5	10.20	323	81.0	40.6	9.0	388	56.4	1490	6.3	8.9	24.7
230	0	9.51	18.1	232.1	1.39	2.31	16.7	11.32	340	81.6	41.1	10.5	406	59.3	1478	6.0	10.5	34.6
	20	9.77	23.9	260.3	1.42	2.26	16.0	11.08	335	81.5	41.3	10.5	401	59.2	1486	6.5	10.9	32.4
	40	9.70	27.3	258.5	1.57	2.32	14.8	11.36	336	81.6	40.6							
	60	9.56	26.8	268.1	1.57	2.32	14.7	11.37	339	81.5	39.8							
	80	9.58	26.2	256.0	1.60	2.36	14.7	11.55	340	81.1	39.8							
	100	9.53	34.1	264.9	1.52	2.32	15.3	11.36	337	81.4	39.4	10.6	406	58.9	1528	6.2	10.9	28.0
180 + 50F	0	9.30			1.33	2.29	17.3	11.24	346	82.2	42.4	10.9	417	60.6	1423	5.8	10.3	38.2
180 + 50F	20F	9.09			1.54	2.35	15.3	11.54	338	82.0	41.4	10.7	406	60.5	1487	6.0	10.1	28.6
180 + 50F	40F	8.69			1.59	2.29	14.4	11.23	338	81.2	38.8							
180 + 50F	20S + 20F	8.12			1.57	2.19	13.9	10.72	337	80.4	37.9							
ANOVA significance level:																		
	N	**	*	***	***	***	NS	***	NS	NS	NS	***	NS	***	NS	NS	***	*
	S	NS	**	NS	***	NS	***	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	*
	N x S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	NS not significant, * p <0.05, ** p <0.01, *** p <0.001																	

Appendix 4: Woburn 1994-95																		
N applied (kg/ha)	S applied (kg/ha)	Grain yield (t/ha)	S uptake (kg/ha)	N uptake (kg/ha)	Grain S concentration (mg/g)	Grain N concentration (%)	Grain N:S ratio	Grain protein (%)	Grain HFN	Grain specific wt (kg/hl)	T.G.W. (g)	Flour protein (%)	Flour HFN	Flour water absorption (%)	Loaf Volume (ml)	Crumb Score	Gel Protein weight (g/5g)	Elastic modulus (G', Pa)
180	0	3.86	7.8	120.0	1.36	2.59	19.1	12.68	372	80.4	41.4	12.3	451	62.9	1379	5.5	10.4	29.4
	20	4.73	13.8	127.1	1.67	2.53	15.1	12.39	374	79.9	39.3	12.2	447	61.3	1409	6.0	12.9	22.3
	40	4.40	16.9	140.8	1.77	2.67	15.1	13.10	376	79.3	40.3							
	60	4.54	19.4	156.3	1.75	2.55	14.6	12.52	373	79.1	39.9							
	80	3.86	18.2	133.2	1.78	2.64	14.8	12.94	357	78.6	35.4							
	100	4.83	17.6	135.3	1.71	2.53	14.9	12.42	370	79.5	40.4	12.1	438	60.7	1427	6.0	13.3	19.8
230	0	3.59	8.5	132.1	1.28	2.66	20.9	13.06	355	79.0	38.4	12.8	458	65.1	1392	6.0	9.4	29.2
	20	4.43	18.3	150.5	1.77	2.71	15.3	13.30	361	78.6	39.8	13.5	454	62.8	1433	5.8	14.1	26.3
	40	4.71	16.9	154.1	1.80	2.78	15.4	13.61	376	79.8	39.9							
	60	4.55	18.2	158.0	1.66	2.82	17.1	13.82	374	79.1	39.3							
	80	3.97	18.9	149.5	1.83	2.79	15.3	13.69	371	79.0	39.4							
	100	5.03	21.6	167.9	1.89	2.74	14.5	13.43	368	79.2	38.0	13.4	468	62.4	1410	5.8	12.8	23.6
180 + 50F	0	4.79	8.2	123.1	1.33	2.47	18.7	12.09	370	81.2	41.9	12.0	455	62.1	1417	6.2	10.4	30.7
180 + 50F	20F	4.56	9.2	112.0	1.56	2.57	16.5	12.58	369	80.8	40.7	12.0	447	63.1	1430	5.7	11.8	26.8
180 + 50F	40F	3.54	9.0	114.2	1.50	2.63	17.6	12.91	379	79.1	39.4							
180 + 50F	20S + 20F	4.40	15.6	152.5	1.71	2.70	15.8	13.24	359	79.1	38.3							
ANOVA significance level:																		
	N	NS	NS	***	NS	***	*	***	NS	NS	NS	***	NS	**	NS	NS	NS	*
	S	**	***	**	***	NS	***	NS	NS	NS	NS	NS	NS	**	NS	NS	***	***
	N x S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	NS not significant, * p <0.05, ** p <0.01, *** p <0.001																	

Appendix 5: Bridgets 1995-96																			
N applied (kg/ha)	S applied (kg/ha)	Grain yield (t/ha)	S uptake (kg/ha)	Grain S concentration (mg/g)	Grain N concentration (%)	Grain N:S ratio	Grain protein (%)	Grain HFN	Grain specific wt (kg/hl)	T.G.W. (g)	Flour protein (%)	Flour HFN	Flour water absorption (%)	Loaf Volume (ml)	Crumb Score	Gel Protein weight (g/5g)	Elastic modulus (G', Pa)	Dough resistance (Bu)	Dough extensibility (cm)
180	0	9.18	18.5	1.32	1.84	13.91	9.00	294	78.4	43.3	8.15	331	52.3	1627	7.8	7.6	21.6	307.5	16.5
	10	9.12	21.4	1.36	1.85	13.61	9.05	278	78.6	43.3									
	20	9.19	22.5	1.37	1.81	13.22	8.88	290	78.3	43.4	7.88	327	52.3	1639	7.8	7.7	20.0	321.7	16.0
	40	9.18	21.6	1.39	1.80	12.93	8.81	290	78.4	44.1									
	70	9.01	22.7	1.43	1.81	12.67	8.85	290	78.2	42.7									
	100	9.29	25.5	1.51	1.83	12.05	8.95	292	78.2	43.0	7.97	320	52.5	1671	7.5	7.8	19.9	268.3	16.2
230	0	9.57	23.0	1.44	1.99	13.88	9.78	306	78.7	42.5	8.91	345	53.7	1664	7.8	8.9	21.6	335.0	17.8
	10	9.34	23.7	1.43	1.96	13.75	9.59	308	78.5	42.9									
	20	9.60	23.4	1.42	1.97	13.91	9.67	301	78.8	43.0	8.77	349	53.8	1677	7.8	8.7	19.7	255.0	18.0
	40	9.58	28.2	1.50	1.97	13.12	9.66	300	78.8	43.0									
	70	9.44	26.7	1.52	1.97	12.97	9.65	296	78.2	42.0									
	100	9.49	28.3	1.59	1.98	12.46	9.68	302	78.4	41.8	8.79	336	52.4	1676	8.0	9.0	16.7	256.7	17.8
180 + 50F	0	9.34		1.29	1.95	15.13	9.57	290	79.2	44.3	8.57	346	53.6	1627	8.2	8.6	19.1	337.5	17.4
180 + 50F	10F	8.96		1.37	1.96	14.32	9.62	293	79.1	42.9									
180 + 50F	20F	8.85		1.38	1.94	14.16	9.52	298	78.5	41.6	8.67	343	53.4	1654	7.8	8.7	23.0	313.3	16.9
180 + 50F	20S + 20F	8.83		1.50	1.96	13.09	9.61	291	78.4	42.9									
ANOVA significance level:																			
	N	***	***	***	***	NS	***	*	*	*	***	NS	***	**	NS	***	NS	NS	***
	S	NS	*	***	NS	***	NS	NS	NS	NS	NS	NS	NS	**	NS	NS	*	**	NS
	N x S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS
	NS not significant, * p <0.05, ** p <0.01, *** p <0.001																		

Appendix 6: Borders 1995-96

N applied (kg/ha)	S applied (kg/ha)	Grain yield (t/ha)	S uptake (kg/ha)	Grain S concentration (mg/g)	Grain N concentration (%)	Grain N:S ratio	Grain protein (%)	Grain HFN	Grain specific wt (kg/hl)	T.G.W. (g)	Flour protein (%)	Flour HFN	Flour water absorption (%)	Loaf Volume (ml)	Crumb Score	Gel Protein weight (g/5g)	Elastic modulus (G', Pa)	Dough resistance (Bu)	Dough extensibility (cm)
180	0	8.81	17.3	1.22	1.82	14.9	8.9	317	77.7	47.8	8.6	377	53.1	1567	7.7	8.2	22.6	340.0	17.1
	10	8.85	22.1	1.22	1.89	15.5	9.3	297	77.4	47.0									
	20	8.77	18.5	1.24	1.92	15.5	9.4	300	77.1	46.1	8.7	360	52.2	1591	8.3	9.0	19.6	356.7	17.5
	40	8.61	25.1	1.40	1.97	14.1	9.7	325	76.1	44.3									
	70	7.98	23.7	1.38	1.93	14.0	9.4	294	75.8	44.8									
	100	8.78	25.2	1.42	2.04	14.4	10.0	316	75.8	44.2	8.7	363	52.2	1662	8.0	9.3	15.1	295.0	18.9
230	0	8.39	17.8	1.29	2.17	16.8	10.6	298	78.1	46.8	9.3	379	54.0	1553	8.0	8.3	23.3	341.7	17.1
	10	8.15	24.6	1.40	2.24	16.0	11.0	316	76.1	46.3									
	20	8.35	24.0	1.42	2.20	15.4	10.8	307	76.0	44.8	10.2	397	53.3	1610	9.0	9.9	18.8	321.7	20.0
	40	8.35	30.0	1.53	2.29	15.0	11.2	328	74.9	43.8									
	70	8.38	30.6	1.55	2.35	15.1	11.5	330	74.8	44.3									
	100	7.85	27.5	1.61	2.32	14.4	11.4	312	74.6	42.7	10.8	382	54.8	1670	8.0	11.3	19.3	293.3	22.2
180 + 50F	0	8.69	16.2	1.16	2.01	17.3	9.8	302	77.4	45.8	8.9	377	53.8	1574	8.0	8.0	21.5	363.3	17.3
180 + 50F	10F	8.84	23.6	1.38	2.05	14.9	10.1	307	77.0	45.3									
180 + 50F	20F	8.78	21.7	1.38	2.01	14.6	9.9	309	77.5	44.7	9.0	380	53.0	1603	8.0	8.6	20.3	335.0	18.4
180 + 50F	20S + 20F	8.85	21.3	1.32	1.94	14.7	9.5	316	77.2	45.1									
ANOVA significance level:																			
	N	*	*	***	***	**	***	NS	*	*	**	NS	**	NS	NS	*	NS	NS	*
	S	NS	**	***	NS	**	NS	NS	***	***	NS	NS	NS	**	*	**	**	*	**
	N x S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	NS not significant, * p <0.05, ** p <0.01, *** p <0.001																		

Appendix 7: Woburn 1995-96

N applied (kg/ha)	S applied (kg/ha)	Grain yield (t/ha)	S uptake (kg/ha)	N uptake (kg/ha)	Grain S concentration (mg/g)	Grain N concentration (%)	Grain N:S ratio	Grain protein (%)	Grain HFN	Grain specific wt (kg/hl)	T.G.W. (g)	Flour protein (%)	Flour HFN	Flour water absorption (%)	Loaf Volume (ml)	Crumb Score	Gel Protein weight (g/5g)	Elastic modulus (G', Pa)	Dough resistance (Bu)	Dough extensibility (cm)
180	**0**	6.17	13.1	167.9	1.38	2.24	16.3	11.0	356	79.3	41.5	9.7	378	53.4	1657	8.2	9.7	32.3	336.7	18.1
	10	7.34	14.9	161.6	1.44	2.08	14.5	10.2	349	78.9	39.4									
	20	6.63	15.4	163.2	1.47	2.19	15.0	10.8	361	79.5	40.5	9.7	380	53.6	1753	8.0	10.6	26.6	325.0	19.3
	40	6.78	21.6	180.8	1.65	2.20	13.3	10.8	366	78.7	40.0									
	70	6.25			1.59	2.23	14.1	10.9	360	79.5	41.2									
	100	6.68	24.6	192.3	1.70	2.19	12.9	10.7	359	79.4	41.3	9.9	380	54.8	1691	7.8	9.7	30.5	302.5	18.4
230	**0**	7.04	13.8	190.4	1.38	2.37	17.2	11.6	367	79.7	41.8	10.0	386	54.1	1710	8.0	10.5	30.2	321.7	18.9
	10	6.39	14.7	176.5	1.53	2.43	15.9	11.9	359	79.1	40.4									
	20	6.94	18.2	198.1	1.55	2.31	14.9	11.3	356	79.3	41.2	10.1	387	54.3	1713	7.8	11.3	31.1	305.0	19.4
	40	6.60	20.7	182.5	1.69	2.41	14.4	11.8	356	79.1	40.8									
	70	6.20			1.74	2.46	14.2	12.1	359	78.8	39.7									
	100	6.98	23.7	192.0	1.78	2.43	13.6	11.9	358	78.9	40.5	11.0	375	55.5	1723	8.3	11.3	29.9	270.0	20.3
180 + 50F	**0**	6.44			1.41	2.37	16.8	11.6	357	79.3	41.4	10.3	376	55.9	1626	7.3	9.8	36.4	327.5	18.3
180 + 50F	**10F**	7.00			1.46	2.39	16.3	11.7	362	79.5	40.0									
180 + 50F	**20F**	6.23			1.48	2.34	15.8	11.5	363	80.0	42.6	10.2	376	55.4	1652	7.8	9.6	33.4	330.0	17.8
180 + 50F	**20S + 20F**	7.06			1.67	2.31	13.9	11.3	355	79.3	39.5									
ANOVA significance level:																				
	N	NS	NS	*	*	***	*	***	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	S	NS	***	NS	***	NS	***	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	N x S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	NS not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$																			

Appendix 8: Bridgets 1996-97

Variety	N applied (kg/ha)	S applied (kg/ha)	Grain yield (t/ha)	S uptake (kg/ha)	N uptake (kg/ha)	Grain S concentration (mg/g)	Grain N concentration (%)	Grain N:S ratio	Grain protein (%)	Grain HFN	Grain specific wt (kg/hl)	T.G.W. (g)	Flour protein (%)	Flour HFN	Flour water absorption (%)	Loaf Volume (ml)	Crumb Score	Gel Protein weight (g/5g)	Elastic modulus (G', Pa)	Dough resistance (Bu)	Dough extensibility (cm)
Hereward	180	0	3.81	6.6	96.1	1.43	2.39	16.7	11.7	240	75.8	39.8									
		20	4.16	9.0	115.2	1.55	2.44	15.7	12.0	248	75.5	39.2									
		100	4.38			1.63	2.38	14.6	11.7	242	75.8	39.6									
	230	0	3.90	7.0	99.8	1.45	2.38	16.4	11.7	234	75.6	41.1									
		20	4.96	9.3	125.1	1.56	2.46	15.7	12.0	233	75.9	39.6									
		100	4.54			1.68	2.45	14.6	12.0	245	75.7	39.4									
Rialto	180	0	4.23	7.4	107.7	1.41	2.37	16.8	11.6	339	72.9	40.8	10.1	384	63.6	1665	7.5	6.5	45.7	383.3	14.9
		20	4.70	9.5	121.2	1.61	2.43	15.1	11.9	352	72.0	39.6	10.4	379	61.3	1710	7.7	6.8	37.7	338.3	16.3
		100	4.69			1.71	2.42	14.1	11.8	347	72.4	39.1	10.6	370	61.1	1738	7.7	6.9	35.1	345.0	16.3
	230	0	3.66	6.2	96.9	1.40	2.39	17.2	11.7	322	72.7	40.9	10.2	397	63.5	1665	7.5	6.6	47.0	415.0	14.7
		20	4.63	9.5	123.0	1.65	2.48	15.1	12.1	336	72.3	40.6	10.8	373	61.9	1725	7.8	7.2	35.7	316.7	16.7
		100	4.67			1.73	2.47	14.3	12.1	340	71.7	39.5	10.9	369	61.6	1720	7.5	8.1	31.5	301.7	17.7
Spark	180	0	4.50	7.1	107.5	1.36	2.37	17.5	11.6	326	74.9	36.2	10.3	388	63.3	1584	7.5	8.6	50.3	343.3	16.4
		20	5.39	9.7	128.1	1.56	2.40	15.4	11.8	326	74.4	35.1	10.4	393	63.2	1687	8.0	9.4	32.3	346.7	19.7
		100	5.48			1.68	2.39	14.2	11.7	340	74.7	35.4	10.4	386	63.7	1696	8.0	9.9	30.5	323.3	19.6
	230	0	5.19	8.2	125.3	1.40	2.41	17.3	11.8	340	75.7	37.0	10.4	388	62.6	1603	7.5	8.1	49.5	373.3	17.1
		20	4.94	9.8	126.0	1.60	2.47	15.5	12.1	334	74.8	35.7	10.7	377	61.5	1689	8.3	10.3	43.9	363.3	20.8
		100	5.08			1.73	2.48	14.3	12.2	344	74.5	34.9	10.8	386	64.2	1682	8.0	9.4	33.7	328.3	20.5
ANOVA significance level:																					
		Variety	**	**	NS	NS	NS	NS	NS	***	***	***	NS	NS	*	***	*	***	NS	NS	***
		N	NS	NS	NS	NS	***	NS	***	NS	NS	*	**	NS	NS	NS	NS	NS	NS	NS	*
		S	*	***	***	***	***	***	***	NS	NS	***	***	NS	*	***	*	*	***	**	***
		Variety x N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
		Variety x S	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS	NS	NS	NS	*
		N x S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$

Appendix 9: Borders 1996-97																					
Variety	N applied (kg/ha)	S applied (kg/ha)	Grain yield (t/ha)	S uptake (kg/ha)	N uptake (kg/ha)	Grain S concentration (mg/g)	Grain N concentration (%)	Grain N:S ratio	Grain protein (%)	Grain HFN	Grain specific wt (kg/hl)	T.G.W. (g)	Flour protein (%)	Flour HFN	Flour water absorption (%)	Loaf Volume (ml)	Crumb Score	Gel Protein weight (g/5g)	Elastic modulus (G', Pa)	Dough resistance (Bu)	Dough extensibility (cm)
Hereward	180	0	8.34	20.8	211.1	1.40	2.19	15.6	10.7	216	70.4	46.9	10.2	287	57.2	1721	8.2	9.4	17.0	268	20.0
		20	8.41	22.6	200.3	1.46	2.18	14.9	10.7	230	70.4	46.1	10.2	297	57.2	1722	8.0	8.7	24.1	282	19.6
		100	8.38			1.50	2.29	15.3	11.2	246	70.3	46.1	10.1	312	56.3	1729	7.8	10.2	23.3	248	20.7
	230	0	8.24	20.9	227.2	1.52	2.46	16.2	12.1	220	69.1	43.3	11.0	297	58.1	1712	7.5	11.3	24.5	280	20.8
		20	8.19	23.7	234.0	1.52	2.42	15.9	11.9	229	68.8	45.6	10.8	293	57.8	1697	7.7	10.3	18.7	248	21.2
		100	8.60			1.52	2.39	15.8	11.7	229	70.0	47.0	10.6	299	57.0	1699	7.5	10.6	17.8	267	20.5
Rialto	180	0	9.19	20.9	211.4	1.44	2.06	14.3	10.1	269	66.8	45.3	9.1	320	56.9	1701	7.7	5.2	25.7	238	15.9
		20	9.18	20.0	191.1	1.40	2.04	14.5	10.0	266	67.3	46.7	9.0	323	56.8	1691	7.5	6.9	41.1	265	15.8
		100	9.32			1.44	2.03	14.1	9.9	262	67.8	47.1	8.8	326	55.9	1686	7.3	4.9	24.4	242	14.9
	230	0	9.44	22.4	248.2	1.50	2.21	14.8	10.8	258	67.6	47.7	9.9	318	58.1	1675	7.2	7.3	27.5	268	17.1
		20	9.41	23.0	230.1	1.49	2.23	14.9	10.9	261	68.1	47.2	9.9	338	57.6	1711	7.5	5.9	34.1	268	16.4
		100	9.52			1.48	2.19	14.8	10.7	268	66.7	45.7	10.0	329	56.9	1696	7.3	5.9	32.3	287	16.0
Spark	180	0	8.65	19.7	205.1	1.40	2.16	15.4	10.6	280	69.8	43.4	9.3	347	59.0	1645	7.7	7.7	33.3	312	16.9
		20	8.22	19.3	183.7	1.43	2.11	14.8	10.3	288	69.2	41.2	9.2	351	58.1	1623	7.3	8.2	42.0	320	17.7
		100	8.24			1.51	2.20	14.5	10.8	292	69.4	40.5	9.6	347	58.7	1641	7.8	8.0	30.9	290	18.4
	230	0	8.27	17.1	181.8	1.52	2.34	15.4	11.5	287	68.2	39.6	10.4	372	59.3	1663	7.8	7.9	35.1	325	18.7
		20	8.44	25.7	227.8	1.46	2.31	15.8	11.3	315	69.5	42.5	10.5	349	60.1	1648	7.8	8.5	35.6	313	18.0
		100	8.14			1.56	2.42	15.5	11.8	298	69.7	39.9	10.7	371	59.6	1704	7.8	9.5	37.3	345	18.8
ANOVA significance level:																					
		Variety	***	NS	**	NS	***	***	***	***	***	***	***	***	***	***	*	***	***	***	***
		N	NS	*	***	***	***	***	***	NS	NS	NS	***	NS	***	NS	NS	**	NS	NS	***
		S	NS	**	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	*	NS	
		Variety x N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS	NS	NS	NS
		Variety x S	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
		N x S	NS	**	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS
		NS not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$																			

Appendix 10: Barsham 1996-97																					
Variety	N applied (kg/ha)	S applied (kg/ha)	Grain yield (t/ha)	S uptake (kg/ha)	N uptake (kg/ha)	Grain S concentration (mg/g)	Grain N concentration (%)	Grain N:S ratio	Grain protein (%)	Grain HFN	Grain specific wt (kg/hl)	T.G.W. (g)	Flour protein (%)	Flour HFN	Flour water absorption (%)	Loaf Volume (ml)	Crumb Score	Gel Protein weight (g/5g)	Elastic modulus (G', Pa)	Dough resistance (Bu)	Dough extensibility (cm)
Hereward	180	0	6.43	14.3	166.0	1.44	2.26	15.7	11.1	122	73.1	38.3									
		20	6.67	19.9	167.7	1.49	2.24	15.1	11.0	118	72.4	36.5									
		100	6.76	28.5	182.1	1.61	2.22	13.8	10.9	129	72.4	37.1									
	230	0	6.82	17.6	196.8	1.50	2.37	15.8	11.6	114	72.8	37.5									
		20	6.46	22.2	190.3	1.48	2.36	15.9	11.6	123	71.8	36.7									
		100	6.71	28.2	192.3	1.64	2.33	14.2	11.4	114	72.1	36.6									
Rialto	180	0	7.24	16.0	170.3	1.51	2.08	13.7	10.2	223	70.8	40.0									
		20	6.86	18.2	151.3	1.46	2.00	13.7	9.8	221	70.3	37.9									
		100	7.38	25.9	171.8	1.58	1.99	12.6	9.8	225	68.2	37.9									
	230	0	7.02	15.4	181.2	1.50	2.20	14.7	10.8	220	70.3	38.8									
		20	7.46	21.4	192.1	1.57	2.23	14.2	10.9	206	70.1	38.3									
		100	6.74	28.8	194.6	1.70	2.30	13.6	11.3	172	68.9	38.1									
Spark	180	0	6.21	11.7	141.7	1.45	2.18	15.0	10.7	215	73.9	35.0									
		20	6.37	22.0	166.0	1.55	2.23	14.4	10.9	219	69.4	33.7									
		100	6.05	27.4	155.7	1.55	2.19	14.1	10.8	204	72.5	32.9									
	230	0	6.70	13.6	183.7	1.50	2.44	16.2	12.0	211	73.0	35.8									
		20	6.40	22.7	182.4	1.61	2.39	14.9	11.7	200	72.7	33.4									
		100	6.34	30.0	175.8	1.73	2.38	13.8	11.7	205	72.4	33.3									
ANOVA significance level:																					
		Variety	***	NS	**	NS	***	***	***	***	***	***									
		N	NS	*	***	***	***	***	***	*	NS	NS									
		S	NS	***	NS	***	NS	***	NS	NS	NS	***									
		Variety x N	NS	NS	NS	NS	*	NS	*	NS	NS	NS									
		Variety x S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS									
		N x S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS									
		NS not significant, * p <0.05, ** p <0.01, *** p <0.001																			

Appendix 11: Woburn 1996-97

Variety	N applied (kg/ha)	S applied (kg/ha)	Grain yield (t/ha)	S uptake (kg/ha)	N uptake (kg/ha)	Grain S concentration (mg/g)	Grain N concentration (%)	Grain N:S ratio	Grain protein (%)	Grain HFN	Grain specific wt (kg/hl)	T.G.W. (g)	Flour protein (%)	Flour HFN	Flour water absorption (%)	Loaf Volume (ml)	Crumb Score	Gel Protein weight (g/5g)	Elastic modulus (G', Pa)	Dough resistance (Bu)	Dough extensibility (cm)
Hereward	180	0	3.46	13.0	155.1	1.58	2.55	16.2	12.5	369	75.7	40.4	12.0	411	59.8	1727	7.5	12.2	15.9	261.7	22.0
		20	3.17	13.4	135.2	1.66	2.53	15.2	12.4	369	75.9	41.1	11.8	410	59.5	1731	7.7	12.1	12.5	205.0	24.0
		100	3.28			1.80	2.54	14.1	12.4	356	75.6	41.4	12.0	395	59.3	1736	7.3	12.3	11.7	205.0	23.5
	230	0	3.23	9.3	117.9	1.60	2.70	16.9	13.2	379	75.3	40.6	12.7	412	61.0	1678	7.2	12.1	15.9	248.3	22.7
		20	4.14	13.6	136.9	1.74	2.64	15.2	12.9	371	75.5	40.3	12.5	408	60.3	1757	7.5	12.5	14.2	213.3	23.6
		100	4.44			1.87	2.66	14.1	13.0	368	76.3	38.7	12.6	405	60.3	1755	7.2	11.8	14.9	181.7	23.3
Rialto	180	0	3.92	12.5	154.4	1.55	2.47	16.0	12.1	356	75.4	44.9	11.5	369	59.6	1711	7.3	7.2	39.7	348.3	16.3
		20	3.76	15.1	153.1	1.72	2.50	14.6	12.3	331	74.3	42.8	11.6	372	60.1	1764	6.8	7.8	29.7	283.3	17.7
		100	3.99			1.74	2.42	13.9	11.8	360	74.8	43.1	11.3	374	60.0	1730	7.2	7.3	25.1	222.5	17.9
	230	0	3.41	11.1	146.5	1.63	2.76	17.0	13.5	371	73.6	43.3	12.7	376	61.3	1744	7.2	8.7	44.2	393.3	18.4
		20	3.40	12.6	134.4	1.84	2.78	15.1	13.6	347	73.3	42.7	12.9	382	61.2	1741	6.8	8.6	40.6	326.7	18.1
		100	6.07			1.69	2.47	14.6	12.1	361	75.1	40.2	11.6	381	59.8	1762	7.0	7.0	35.1	296.7	17.2
Spark	180	0	5.24	12.0	140.2	1.50	2.35	15.7	11.5	342	78.9	37.6	10.8	385	59.4	1712	7.3	8.3	39.3	275.0	18.9
		20	5.03	15.7	145.2	1.61	2.42	15.0	11.9	355	77.9	36.8	11.2	381	60.3	1750	7.7	9.7	34.1	257.5	19.4
		100	5.18			1.64	2.33	14.3	11.4	331	78.4	38.5	10.8	373	59.8	1731	7.3	9.8	29.9	246.7	19.5
	230	0	3.16	10.4	129.5	1.70	2.75	16.2	13.5	361	75.7	38.7	12.7	403	62.4	1715	7.8	10.5	44.5	300.0	20.0
		20	5.32	18.4	182.4	1.69	2.55	15.1	12.5	367	75.7	36.1	12.1	392	60.5	1756	7.5	10.1	39.0	300.0	20.7
		100	3.79			1.83	2.73	15.0	13.4	369	76.9	36.0	12.6	380	61.3	1749	7.7	11.2	30.4	260.0	21.8
ANOVA significance level:																					
		Variety	*	NS	NS	NS	NS	NS	NS	*	***	***	*	***	NS	NS	***	***	***	***	***
		N	NS	NS	NS	**	**	*	***	**	*	NS	***	NS	***	NS	NS	*	***	NS	*
		S	NS	**	NS	***	***	***	NS	NS	NS	NS	NS	NS	NS	**	NS	NS	***	***	NS
		Variety x N	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
		Variety x S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
		N x S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$